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Rare neuroendocrine neoplasms

Edited by
Barbara Altieri, Antongiulio Faggiano and Enzo Lalli

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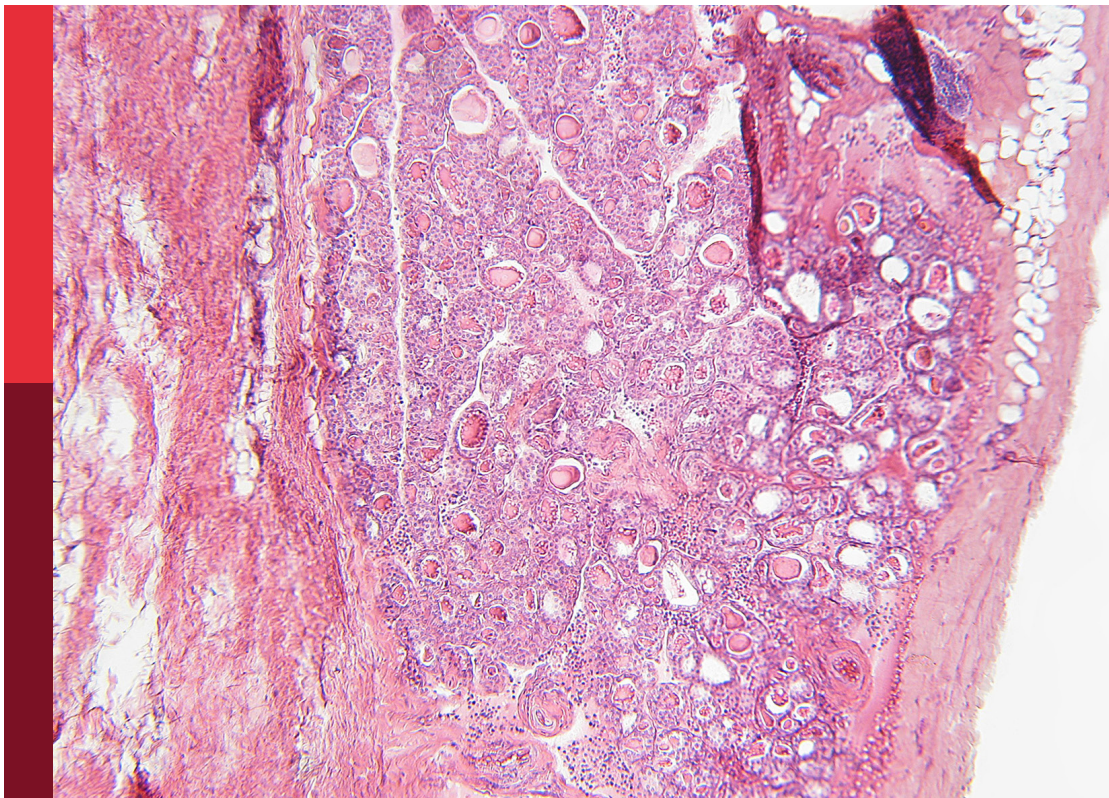

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Rare neuroendocrine neoplasms

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CASE REPORT
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Pineal Gland Metastasis From Poorly Differentiated Carcinoma of Unknown Primary Origin

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Pineal metastasis is an exceedingly rare finding in patients with systemic malignancies. Such lesions are typically the manifestation of a primary lung cancer; nonetheless, a variety of malignancies have been reported to disseminate to the pineal gland including gastrointestinal, endocrine, and skin cancers, among others. However, to our knowledge, pineal gland metastasis without a primary origin has yet to be described. Carcinoma of unknown primary origin is a heterogeneous group of cancers characterized by the presence of metastatic disease without an identifiable primary tumor on metastatic workup. Here, we present a case of a 65-year-old male found to have a heterogeneously enhancing lesion of the pineal gland as well as an enhancing lesion of the left cerebellar hemisphere. Comprehensive metastatic workup demonstrated multifocal metastatic adenopathy without an identifiable primary lesion. Stereotactic biopsy of the pineal lesion revealed poorly differentiated carcinoma with an immunophenotype most consistent with gastrointestinal origin. To our knowledge, this is the first case to describe a pineal gland metastasis without a primary origin. We discuss the relevant literature on pineal gland metastases as well as carcinoma of unknown primary origin.

Keywords: pineal gland, neuroendocrinology, cancer endocrinology, carcinoma of unknown primary (CUP), metastasis

BACKGROUND

Pineal region tumors are a rare entity constituting approximately 1% of all intracranial tumors in the adult population (1). Although metastasis to the brain is common in the setting of primary malignancies, metastasis specifically to the pineal region is an exceedingly rare phenomenon accounting for 0.4–3.8% of all intracranial metastases (1, 2). Metastasis to the pineal gland is most commonly a derivative of a primary lung malignancy; nevertheless, there are reports of a variety of primary tumors that have metastasized to this neuroendocrine secretory circumventricular organ

Abbreviations: CUP, carcinoma of unknown primary origin; CT, computed tomography; PET, positron emission tomography; MRI, magnetic resonance imaging.

including esophageal, stomach, liver, colon, pancreas, kidney, bladder, prostate, thyroid, breast, melanoma, myeloma, and leukemia (3, 4). However, to the best of the authors' knowledge, pineal gland metastasis without a primary origin has yet to be described.

Carcinoma of unknown primary origin (CUP) is a heterogeneous group of cancers defined by the presence of metastatic disease without an identifiable primary tumor on metastatic workup (5, 6). CUP has been reported to constitute 2–5% of all cancer cases and, remarkably, represents up to 15% of all patients with brain metastases (5, 6). Here, we present a case of a 65-year-old male found to have a heterogeneously enhancing lesion of the pineal gland as well as an enhancing lesion of the left cerebellar hemisphere. Metastatic workup including computed tomography (CT) of the chest, abdomen, and pelvis as well as whole-body positron emission tomography (PET) scan demonstrated multifocal metastatic adenopathy without an identifiable primary lesion. Stereotactic biopsy of the pineal lesion revealed poorly differentiated carcinoma with an immunophenotype most consistent with gastrointestinal origin. To our knowledge, this is the first case to describe a pineal gland metastasis from CUP. We discuss the relevant literature on pineal gland metastases as well as carcinoma of unknown primary origin.

CASE PRESENTATION

A 65-year-old gentleman presented to our emergency department with two weeks of progressively worsening headaches and fatigue. Physical examination was unremarkable. The patient was without any relevant past medical history or cancer diagnoses. He admitted to a 60-pack year smoking history. CT of the head demonstrated a partially calcified hyperdense pineal lesion causing obstructive hydrocephalus with marked supratentorial ventricular dilatation. Subsequently, magnetic resonance imaging (MRI) revealed a heterogeneously enhancing lesion of the pineal gland with mass effect and compression of the cerebral aqueduct resulting in supratentorial ventricular dilatation with periventricular white matter T2 hyperintensity consistent with transependymal flow (**Figure 1**). Moreover, MRI revealed a second enhancing lesion of the left cerebellar hemisphere. Metastatic workup demonstrated multifocal metastatic adenopathy including the supraclavicular, prevascular mediastinal, paratracheal, hilar, and internal mammary regions. However, there was no evidence of a primary malignancy or other metastatic disease in the chest, abdomen, or pelvis. Tracheal aspirates retrieved *via* bronchoscopy demonstrated alveolar histiocytes, mixed inflammatory cells and reactive pneumocytes without evidence of malignant cells. The patient was started on dexamethasone and medically optimized for surgical intervention to address his symptomatic obstructive hydrocephalus and obtain tissue diagnosis.

An endoscopic third ventriculostomy in conjunction with biopsy of the pineal lesion was performed with stereotactic navigation and intraoperative neuromonitoring (7). The patient tolerated the procedure well and without complication. Pathology was consistent with poorly differentiated carcinoma (**Figure 2**). The tumor consisted of nests and singly dispersed cells. A small minority of tumor cells exhibited signet ring features. The tumor cells were strongly and diffusely reactive for cytokeratin AE1/AE3, cytokeratin 7, and cytokeratin 20 immunohistochemical stains. p40 and PAX8 highlighted rare tumor cells. Cytokerain 5/6, napsin A, thyroid transcription factor-1, melan-A, prostate-specific antigen, CDX2, synaptophysin, glial fibrillary acidic protein and GATA3 immunostains were negative in all tumor samples. As such, the immunophenotype was most consistent with upper gastrointestinal or pancreatobiliary origin of a poorly differentiated carcinoma.

Post-operatively, the patient received CyberKnife radiosurgery directed at the pineal and left cerebellar metastases utilizing nine gray fractions over three treatment days for a total dose of 27 Gy. Prior to consideration of chemotherapeutic initiation, a PET scan was recommended for identification of the primary malignancy as well as staging of his disease. Imaging demonstrated hypermetabolism of the known multifocal metastatic adenopathy; however, no evidence of primary malignancy was revealed (**Figure 3**). Given the immunophenotype of the lesion and a small minority of tumor cells exhibiting signet ring features, the patient was referred to a gastroenterologist who recommended esophagogastroduodenoscopy with possible biopsy of any identifiable abnormal tissue. The patient declined further workup and treatment. He expired 3 months from the time of diagnosis due to respiratory issues. A basic diagnostic workup and treatment flowchart of a solitary pineal lesion without additional intracranial lesions on imaging is depicted in **Figure 4**. Our diagnostic workup and treatment plan of the case described herein (*i.e.*, suspected pineal region metastasis) is depicted in **Figure 5**.

DISCUSSION

The pineal gland, or epiphysis cerebri, represents an exceptionally uncommon site of systemic metastasis (1, 2). Indeed, metastasis to the pineal gland is most commonly a derivative of a primary lung malignancy; however, numerous other primary malignancies have been reported to metastasize to the epiphysis cerebri including esophageal, stomach, liver, colon, pancreas, kidney, bladder, prostate, thyroid, breast, melanoma, myeloma, and leukemia (3, 4). Considered one of the neuroendocrine secretory circumventricular organs, capillaries of the pineal gland are mostly permeable to solutes in the blood (8). As such, metastases are thought to spread hematogenously given the lack of the blood–brain barrier of the pineal region (9). Lesions of the pineal gland typically remain clinically silent until they meet a threshold in size to compress critical surrounding neuroanatomic structures causing diverse clinical symptomatology. Compression of the posterior aspect of the third ventricle and cerebral aqueduct may cause obstructive hydrocephalus and increased intracranial pressure manifesting clinically as progressive headaches, fatigue, and, ultimately, coma and death if left untreated. Moreover, compression of the dorsal midbrain, specifically the superior

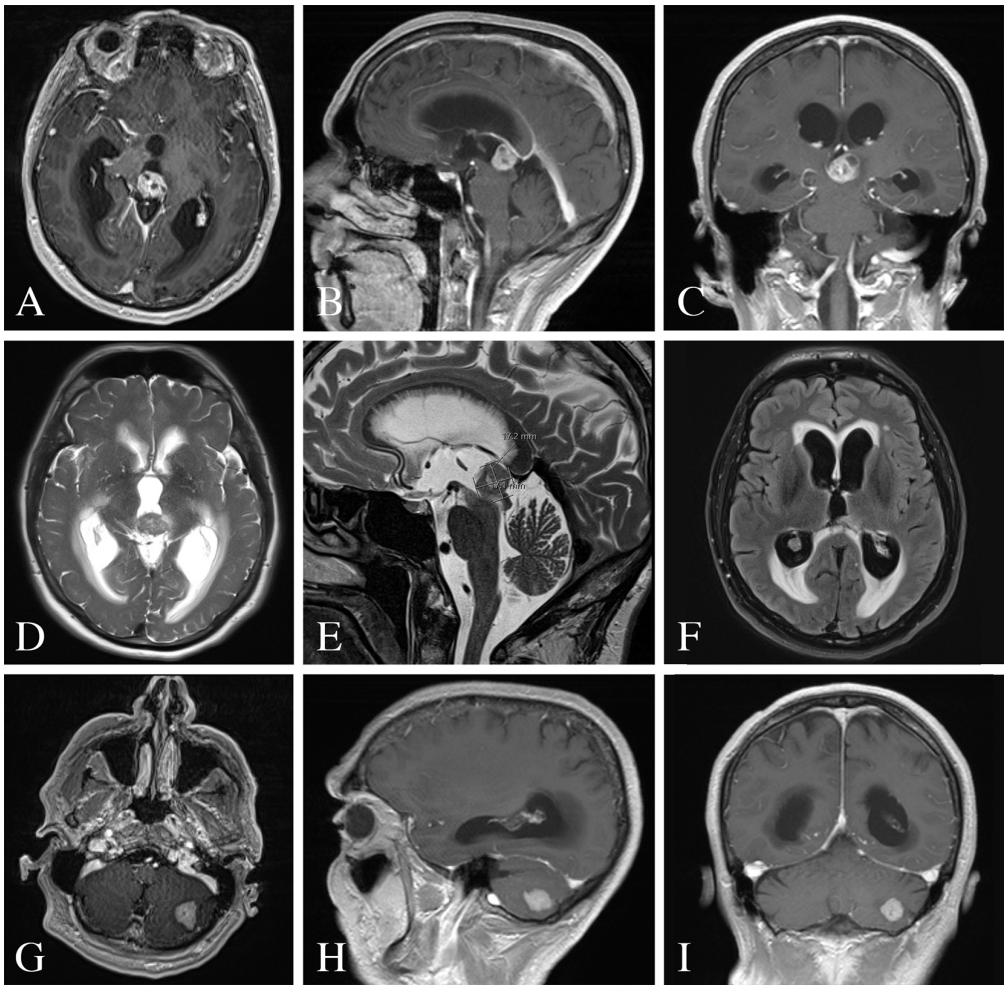

FIGURE 1 | Pre-operative MRI of the brain. **(A–C)** MRI T1-weighted images with contrast demonstrating a heterogeneously enhancing lesion of the pineal gland. **(D, E)** MRI T2-weighted images with a T2 hypointense lesion with mass effect and compression of the cerebral aqueduct resulting in supratentorial ventricular dilatation and periventricular white matter signal abnormality. **(F)** MRI FLAIR image demonstrating periventricular transependymal flow of cerebrospinal fluid indicative of acute hydrocephalus. **(G–I)** MRI T1-weighted images with contrast demonstrating an enhancing lesion of the left cerebellar hemisphere.

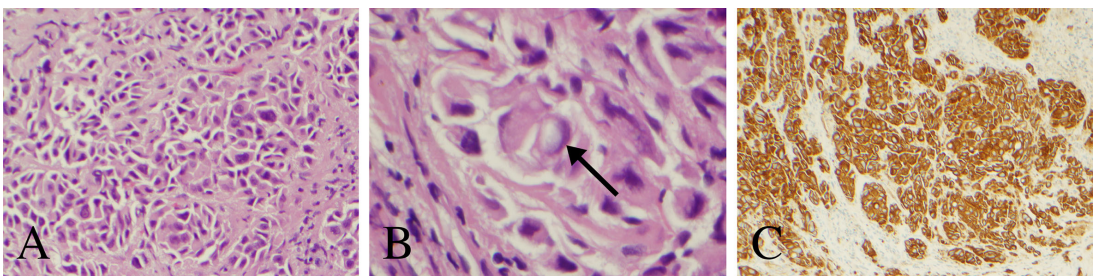

FIGURE 2 | Histopathology and immunohistochemical analysis of the pineal lesion. **(A)** Hematoxylin and eosin stain with nests and singly dispersed cells with a high nuclear to cytoplasm ratio (200× magnification). **(B)** Hematoxylin and eosin stain demonstrating a signet ring-like cell (arrow) (600× magnification). **(C)** Cytokeratin AE1 immunostain demonstrating strong reactivity (100× magnification).

FIGURE 3 | Whole-body PET scan. Numerous hypermetabolic lesions identified including the pineal region and left cerebellar hemisphere, bilateral cervical chain adenopathy, mediastinal/hilar adenopathy, bilateral axillary adenopathy, left cardiophrenic lymph node and left adrenal nodule. No evidence of a primary lesion was depicted.

colliculus and rostral interstitial nucleus of medial longitudinal fasciculus, translates clinically into Parinaud's syndrome characterized by upward gaze paralysis, pseudo-Argyll Robertson pupils, convergence-retraction nystagmus, eyelid retraction and conjugate downgaze in the primary position. Our patient presented with clinical sequelae attributable to hydrocephalus including progressive headaches and fatigue.

Cancer of unknown primary origin is defined as a group of metastatic tumors for which the standardized metastatic workup fails to discover the site of origin. The pathobiology of CUP

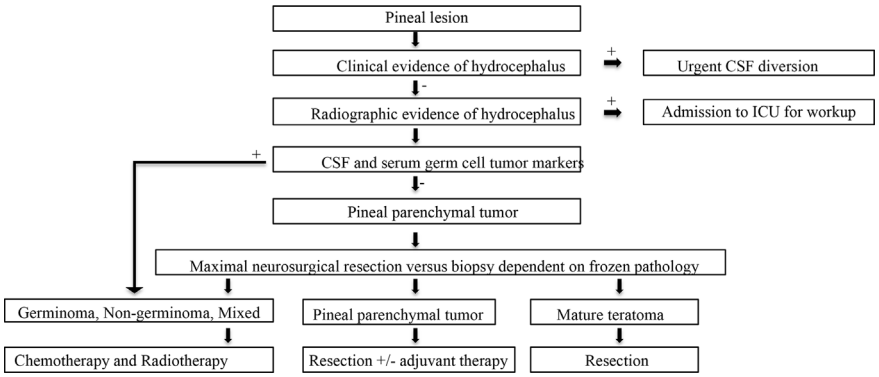

FIGURE 4 | Basic diagnostic workup and treatment flowchart of a solitary pineal lesion.

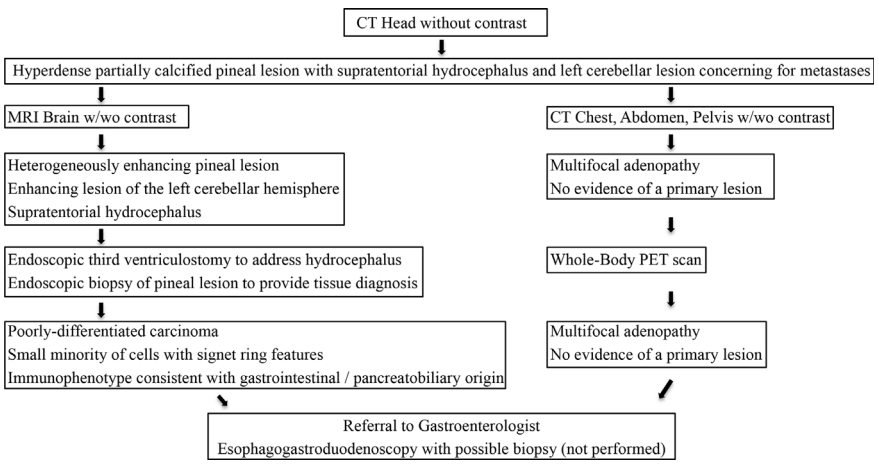

FIGURE 5 | Our diagnostic workup and treatment plan of the case described herein (*i.e.*, suspected pineal region metastasis).

remains to be elucidated; however, two hypotheses have been described to explain their inception. The first theory establishes that a tumor can develop without a premalignant lesion or primary tumor (10, 11). The second theory postulates that metastatic progression occurs parallel to development of the primary lesion emphasizing that CUP metastases may be a premature event in tumorigenesis (10, 11). Regardless of etiology, histological confirmation of a metastatic tumor is the fundamental basis of a diagnosis of CUP. Subsequent to light microscopy and immunohistochemistry, CUP can be classified in one of five morphological subtypes including: (i) well- or moderately differentiated adenocarcinoma, (ii) poorly differentiated adenocarcinoma or undifferentiated carcinoma, (iii) squamous-cell carcinoma, (iv) undifferentiated neoplasms, or (v) carcinomas with neuroendocrine differentiation (11). Subsequently, tumors with specific treatments must be excluded, such as lymphomas, germ-cell tumors, melanoma, or sarcoma. Further immunohistochemical analysis is then carried out on CUP cases. Our case was strongly and diffusely reactive with cytokeratin AE1/AE3 consistent with carcinoma and strongly positive for cytokeratin 7 and cytokeratin 20 typically consistent with upper gastrointestinal or pancreatobiliary origin. Further immunohistochemical stains including cytokerain 5/6, napsin A, thyroid transcription factor-1, melan-A, prostate-specific antigen, CDX2, synaptophysin, glial fibrillary acidic protein and GATA3 were negative. As such, the immunophenotype was most consistent with upper gastrointestinal or pancreatobiliary origin of a metastatic poorly differentiated carcinoma. The immunophenotype would not have been classic for melanoma, lung, prostate, kidney, bladder or lower gastrointestinal origin. Moreover, signet ring features are classically associated with signet ring cell carcinoma originating from the stomach, which further raised suspicion of a gastrointestinal origin of the pineal lesion (12).

CUP has traditionally been classified into two broad clinicopathologic groups with distinct outcomes. The first

group includes patients with a favorable risk profile, more responsive to chemotherapy and long-term disease control (11, 13, 14). This group includes women with serous papillary adenocarcinoma of the peritoneal cavity, women with isolated axillary nodal adenocarcinoma, patients with midline poorly differentiated carcinoma, neuroendocrine carcinoma of unknown primary, squamous cell carcinoma involving cervical or inguinal lymph nodes, colonic type adenocarcinoma and men with prostate-specific antigen positive osteoblastic metastases (11, 13, 14). Comparatively, a second group exists who exhibit multiple visceral metastatic deposits, chemotherapy resistance and typically succumb to their disease within 6 months (11, 13, 14). This group, representing 80% of all CUP cases, encompasses metastatic adenocarcinoma to the liver, lungs, brain, or other viscera, non-papillary peritoneal adenocarcinoma and multiple prostate specific antigen null bony deposits (11, 13, 14). Despite the identification of these two clinicopathologic subgroups, the heterogeneity of CUP represents unpredictable objective responses to known chemotherapeutics (13).

Several studies have associated prognostic factors with poor patient survival in CUP including: male sex, Eastern Cooperative Oncology Group performance status >1, high comorbidities, age greater than 64 years of age, history of smoking (greater than 10 pack-years), weight loss, lymphopenia, low serum albumin, and elevated alkaline phosphatase and lactate dehydrogenase concentrations (11, 13, 14). Petrakis et al. examined factors from 311 patients with CUP and developed a novel prognostic scoring algorithm known as I-SCOOP (Ioannina Score for CUP Outpatient Oncologic Prognostication) based on clinicopathologic CUP subgroup, performance status, and presence or absence of leukocytosis (13). The clinicopathologic parameter encompassed three subgroups including (i) serous peritoneal, axillary nodal and squamous head and neck (zero points), (ii) nodal, neuroendocrine and mucinous peritoneal (one point), or (iii) visceral (two points) (11). The second parameter provided one point for leukocytosis (>10,000/mm^3) and zero points for a normal white-blood cell count (<10,000/mm^3) (13). The third parameter entailed performance status with one point for a performance status of one or greater (symptomatic and ambulatory, cares for self) and zero points for a performance status of zero (normal activity without restrictions) (13). Scores of zero, one, two, three, and four were associated with a median overall survival of 36, 14, 11, 8, and 5 months, respectively (13). The patient we present here was given a score of three based on this algorithm (two points for visceral, zero points for leukocytosis, one point for performance status) translating into a median overall survival of 8 months. Our patient expired 3 months after diagnosis.

Eighty percent of patients diagnosed with CUP exhibit a poor prognosis with a median overall survival of 6 months regardless of intervention (15). Empiric treatment of CUP includes combinatory chemotherapeutics, including platinum/taxane or platinum/gemcitabine, which has translated into response rates of approximately 20% with a median survival of 9 months (11, 13–15). A treatment regimen with carboplatin and paclitaxel has been used as first-line therapy with or without maintenance therapy with erlotinib and bevacizumab with a reported response rate of 53% and an overall survival of 13 months (15). In addition to chemotherapeutics, radiosurgery plays an integral role in the treatment of CUP. In our case, radiosurgery was offered for adjuvant treatment of the two intracranial lesions despite the lack of identification of a primary lesion. Han et al. retrospectively evaluated 540 patients who underwent gamma knife radiosurgery and demonstrated that identification of a primary tumor prior to the initiation of gamma knife radiosurgery did not affect patient outcomes (6). This emphasizes that radiosurgery can be an effective adjuvant treatment modality for brain metastases in cases without a primary lesion. Moreover, initiation of radiation should not be delayed pending identification of a primary source.

CONCLUSIONS

Here, we describe the first case of a pineal gland metastasis from CUP. Albeit exceedingly rare, the presence of a pineal lesion in individuals with a known systemic malignancy should raise clinical suspicion for metastatic disease dissemination. However, as depicted by the present case, the pineal gland can also be a location for metastatic disease deposition with an unknown primary origin. With each reported case, we gain a better understanding of the natural history and therapeutic treatment options of carcinoma of unknown origin.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JC, MK: primary authors of the manuscript. JC, MK, MB, RJ, CR, MW, EM: provided substantial contributions to the conception and design of the manuscript. JC, MK, MB, RJ, CR, MW, EM: contributed to manuscript revision, read and approved the submitted version. JC, MK, MB, RJ, CR, MW, EM: agree to be accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are investigated and resolved. All authors contributed to the article and approved the submitted version.

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REVIEW
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Clinical Epigenetics of Neuroendocrine Tumors: The Road Ahead

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Neuroendocrine tumors, or NETs, are cancer originating in neuroendocrine cells. They are mostly found in the gastrointestinal tract or lungs. Functional NETs are characterized by signs and symptoms caused by the oversecretion of hormones and other substances, but most NETs are non-functioning and diagnosis in advanced stages is common. Thus, novel diagnostic and therapeutic strategies are warranted. Epigenetics may contribute to refining the diagnosis, as well as to identify targeted therapy interfering with epigenetic-sensitive pathways. The goal of this review was to discuss the recent advancement in the epigenetic characterization of NETs highlighting their role in clinical findings.

Keywords: epigenetics, neuroendocrine neoplasms, trials, biomarkers, neuroendocrine, neuroendocrine tumor

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INTRODUCTION

Neuroendocrine neoplasms (NENs) are a heterogeneous group of malignancies originating from neuroendocrine cells diffuse throughout the body. The gastroenteropancreatic (GEP) tract and the bronchopulmonary system represent the main site of origin. NENs are mostly sporadic, but in 10–30% they can arise within the context of familial syndromes, mainly multiple endocrine neoplasia type 1 (MEN1) (1). Incidence and prevalence of NENs have markedly increased in the last decades, irrespective of stage and grade (2). Clinical presentation and prognosis of NENs may widely vary. NENs can be functional when they release biologically active hormones that cause distinct clinical syndromes or more often may be non-functional, thus diagnosed incidentally or due to mass effect. Delayed diagnosis is common, as well as the detection of metastases, mainly to the liver, already at diagnosis. Patients with localized disease have a better prognosis, with 5-year survival ranging from 78 to 93%, while in metastatic disease, the 5-year survival is worse (19–38%), although improved over the past years (3). The improvement of survival rates may be the consequence of the availability of effective therapies, as well as earlier and more accurate clinical and pathologic diagnoses with relative downstaging. NENs have usually an indolent course and patients need life-long therapy.

Abbreviations: CAPTEM, Capecitabine and temozolomide; cfDNA, Cell-free DNA; CIMP, CpG island methylator phenotype; CNV, Copy number variation; CTC, Circulating tumor cell; ctDNA, Circulating tumor DNA; ddPCR, Droplet digital PCR; GEP, Gastroenteropancreatic; MEN1, Multiple endocrine neoplasia type 1; NEN, Neuroendocrine neoplasm; NET, Neuroendocrine tumor; NEC, Neuroendocrine carcinoma; NF1, Neurofibromatosis type 1; PNEC, Pancreatic neuroendocrine carcinoma; PNEN, Pancreatic neuroendocrine neoplasm; PNET, Pancreatic neuroendocrine tumor; SI-NET, small intestinal neuroendocrine tumors; VHL, Von Hippel Lindau.

Notably, the landscape of the therapeutic options in NENs has considerably expanded in the last decades. The current systemic therapies for locally advanced or metastatic NENs include somatostatin analogs (SSAs), molecular targeted therapy with mTOR inhibitors (Everolimus), or anti-angiogenesis (Sunitinib), peptide receptor radionuclide therapy (PRRT) with either 90Yttrium (90Y) or 177Lutetium (177Lu) and chemotherapies with temozolomide, capecitabine or platinum-based regimens. These options can be used in sequence or association with surgery, locoregional treatments (e.g., radiofrequency ablation, cryoablation, chemoembolization, and radioembolization), and/or other drugs used as supportive therapies (e.g., telotristat, diazoxide and proton pump inhibitors) (4, 5). In this review we will focus on well or moderately differentiated neuroendocrine tumors (NETs), excluding neuroendocrine carcinomas (NEC) for their peculiar pathology and treatment.

EPIGENETIC MODIFICATIONS AND NEUROENDOCRINE TUMORS

Epigenetic changes, such as DNA methylation and histone modification, are critical for regulating genes and non-coding RNA expression. Genomic alterations and gene mutations which are involved in the pathogenesis NETs, as MEN1, VHL-hypoxia-inducible factor, RASSF1A, have a consequence on the aberrant placement of epigenetic markers and related pathways (6–10).

Epigenetic mechanisms can modify gene expression altering DNA methylation status, histones post trascriptional modifications, and influencing the expression of non-coding RNAs. Hypermethylation of a promoter is a mechanism that determined gene silencing, while hypomethylation can lead to chromosomal instability and consequently influences gene expression (9, 10). Histone modifications involves the addition of methyl, acetyl, phosphorylation at different aminoacid residues of histone proteins. These modifications alter chromatin accessibility to transcription factors and lastly gene expression. MicroRNAs (miRNAs) and long noncoding RNAs are other layers of epigenetic regulation. They are small, or long sequences of non-coding RNAs regulating gene expression post-transcriptionally, considered to be a cancer-associated epigenetic mechanism (11).

METHYLATION PATTERNS RELEVANCE IN THE PATHOGENESIS OF NETS AND CLINICAL FINDINGS

The pathogenesis of NETs is further to be elucidated, as in most other solid tumors. Nevertheless, epigenetic studies have improved our knowledge. Pancreatic neuroendocrine tumors (PNETs) account for 1 to 2% of all pancreatic tumors and most of them are sporadic and non-functioning, 5–7% arise within inherited syndromes, including MEN1, Von-Hippel Lindau (VHL) syndrome, neurofibromatosis type 1 (NF1), and tuberous sclerosis. The majority of familial PNETs are caused by germline inactivating mutations in the MEN1 gene, suggesting a key role in PNETs tumorigenesis. MEN1 gene encodes the transcription factor MENIN, ubiquitously expressed, and involved in many biological functions. MENIN, plays an essential role in chromatin remodeling and gene expression recruiting the H3K4me3 histone methyltransferase on mixed-lineage leukemia (MLL1) complex, regulating the expression of the cyclin-dependent kinase inhibitors, and influenced the epigenetic regulation of several genes (12). MEN1 mutations or loss of function deregulated cell growth in 75% cases of PNETs favoring hypermethylation of several tumor suppressor genes including RASSF1A (13), HIC-1, MLH1, CDKN2A, and MGMT (6, 7). Characteristics of the sporadic form of PNETs are mainly gene mutations in DAXX (death-domain-associated protein) or ATRX (alpha thalassemia/mental retardation syndrome X-linked) (12). Both DAXX and ATRX are chromatin remodellers and are involved in the incorporation of the histone variant H3.3 at the telomeres and pericentric heterochromatin necessary (14). Proteins loss, as well as mutations in DAXX or ATRX, are associated with chromosome instability (CIN), reduced genomic H3K9me, and aggressive PNET phenotype (12, 15). Increased risk of PNET was also associated with loss of chromosome 11q containing the genes Men1, but also DNA repair pathway genes as BRCA2 and ATM, and amplification region activating PIK3CA and mTOR pathway. In some cases associated with MENIN loss were also found mutation affecting VHL tumor suppressor gene that determined a constitutive hypoxia transcription factors (HIF) activation and uncontrolled angiogenesis (16, 17), suggesting that MENIN loss or mutation is a key initiator in PNET tumorigenesis (15, 18–21). In pulmonary NET in addition to MEN1 mutations affected also as histone lysine methyltransferase (SETD1B2, SETDB1), histone acetylation modifiers (BRWD3 and HDAC5) and ATP-dependent chromatin remodeling SMARCA1 indicating a key pathogenic role (22). Genomic profile of small intestinal NET (SI-NET) identify two different groups, one characterized by loss of chromosome 18, and another one characterized by the presence of chromosome 18 but with clustered gains on chromosomes 4, 5, 7, 14, and 20 (23). Correlation of loss of chromosome 18 and RASSF1A promoter hyper-methylation and hypo-methylation of long intergenic element 1 (LINE1) and ALU sequences was found in SI-NETs (24) although not associated with grade and tumor size (25).

In hereditary SI-NET causative role was attributed to germline mutations in IPMK (inositol polyphosphate multikinase) p53 activity and MutY DNA glycosylase genes (26) affecting the oxidative pathway. Above mentioned studies emerged that in SI-NET epigenetic machinery is not causative however the uncontrolled pathways of oxidative stress and genomic rearrangement activated several epigenetic modifications (27).

METHYLATION PATTERNS RELEVANCE AS DIFFERENTIAL DIAGNOSTIC BIOMARKERS

The DNA methylation profile of sporadic PNET, VHL and MEN1-related PNETs, and pancreatic islets were analyzed by

Illumina array (850k array) with the goal to find novel diagnostic markers. The study identified a distinct cluster of methylation genes associated with VHL, sporadic and MEN1-related PNETs, indicated that mutations in these genes influence the epigenetic pathway and clinical presentation of diseases (28, 29). Differential methylation patterns were also reported among GEP-NETs (24, 30). Indeed, the analysis conducted in 60 tumors selected a pool of 807 genes. These gene sets were able to distinguish NETs in functional GEP-NETs (insulinoma, gastrinoma) and, non-function subtypes underlying the clinical and histological characteristics. Gastrinomas showed hypomethylation of genes including metalloproteinases (MMP1, MMP3, TIMP2, TIMP3), the serpin family (SERPINA5, SERPINB5), and oncogenes (IL2, MCF2, and MOS), whereas hypermethylation was reported for tumor suppressors (SMARCB1, CASP8, and NBL1) (24, 25, 30). Promoter hypermethylation of the IGF2 pathway was characteristic of insulinomas shedding a light on signaling responsible for their differentiation from a common origin (31). A study on SI-NET identified TCEB3C gene hypermethylation to be specific for this histology. Interestingly, treatment of SI-NET cell lines with the de-methylating agent decitabine and the histone methyltransferase inhibitor 3-deazaneaplaoncin A-induced TCEB3C re-expression, confirming an epigenetic regulation of this gene (32). Followed this stem study, Verdugo et al. and then Karpathakis et al. identified hyper-methylation of the gastric inhibitory polypeptide receptor (GIPR) as another specific marker of SI-NETs and reported hyper-methylation in several genes. They selected on chromosome 18 as laminin alpha 3 (LAMA3), serpin peptidase inhibitor clade B member 5 (SERPINB5), and factor receptor superfamily member 11a NFKB activator (RANK or TNSFRSF11A), suggesting that epigenetic silencing could be the possible second step in tumor development upon chromosome 18 loss (33–35).

METHYLATION PATTERNS: RELEVANCE IN PROGNOSIS AND RESPONSE TO THERAPY

Since epigenetic changes play a key role in the progression of PNETs the finding to select an epigenetic prognostic factor, is crucial (34). In particular, some epigenetic changes area correlated to DAXX or ATRX protein loss because this complex regulates H3K9me and influenced DNA methylase. Indeed, promoter hypermethylation of RASSF1A and p57Kip2 in PNENs was responsible for NAP1L1 overexpression associated with the metastatic phenotype (35–38). Additionally, a peculiar group of PNETs named (CIMP) showed hypermethylation of CpG islands including tumor suppressor genes, such as RASSF1A, hMLH1, and hypomethylation of LINE-1 sequence. These peculiar epigenetic pathways were associated with poor prognosis and advanced stage of PNETs (39). While hypermethylation ofCDKN2A was associated with early tumor recurrence and poor outcomes of GEP-NETs (40). A general decrease in methylation levels was observed in SI-NET metastases compared to the primary tumors. In particular, differential methylation of AXL, CRMP1, FGF5, CXXC5, and APOBEC3C genes were detected in primary tumors compared to metastases (34). However, no validation of these markers was reported in the study population. In a follow-up of primary SI-NET and liver metastases, it was selected a panel of epigenetically dysregulated genes that were progressively methylated or demethylated from the primary tumor to metastases (33, 41), suggesting their potential use as markers. Recently dysregulation of TET1/TET2 enzymes that catalyze DNA demethylation was observed in SI-NETs open a potential novel class of drug treatment (42, 43). Differential methylations of specific gene promoters were also associated with response to therapy. **Table 1** shows the most representative observational studies involving epigenetic biomarkers. One example is O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme removing alkyl groups from an alkylguanine. Retrospective studies have found an association between methylation of MGMT and response to treatment with temozolomide (an alkylating agent) making it a promising marker (44–47) (**Table 1**). A prospective trial confirmed this correlation (48).

MiRNAS RELEVANCE IN DIFFERENTIAL DIAGNOSIS AND PROGNOSIS

MicroRNAs (miRNAs) are small (19–24 nt) regulatory RNA molecules that can also be used to classify cancer because of their abundance, cell-type, and disease-stage specificity which support their possible use to predict clinical outcomes and differential diagnosis. Multiple miRNA profiling studies have been performed on NET pathological types using different RNA isolation, detection, and analysis methods. Although these differences complicate inter-study comparisons, miRNAs still hold much promise as markers. A set of 10 miRNAs (miR-99a, 99b, 100, 125a, 125b-1, 125b-2, 129-2, 130a, 132, and 342) was selected as a potential tool to differentiate pancreatic NEN from pancreatic acinar cell carcinoma (49), while miR-21a was selected as potential biomarker for GEP-NETs (50). Moreover, in another study in insulinomas, miR-204 was the unique miRNA selectively overexpressed while miR-186 showed significantly downregulated in 39 colorectal NET patients (51).

Different sets of miRNAs were identified as predictors of metastases on the base of tissue used as control. Overexpression of miR-21, involved in the regulation of the PI3K/Akt/mTOR pathway, and the Ki-67 proliferation index was significantly associated with liver metastases when pancreatic normal tissue was used as control (52). In contrast proliferation index Ki-67, miR-642, and miR-210 were correlated with metastases of PNETs when pancreatic islets were used as control (49). These data suggest that reference tissue influences the selection of markers. From the comparison of primary tumor and metastasis and then validation in 37 patients, the miRNA-196a was found significantly associated with tumor grade and recurrence (53).

A different approach is the NETest algorithm for the prediction of the clinical status of NETs (54, 55). The test is PCR-based

TABLE 1 | Observational studies on epigenomics and NETs.

Type of intervention	Drugs and targets	Phase	NIH Clinical Trialcode	End points	n. Patients
Use of Blood Biomarkers to Predict Gastric Cancer Risk	blood-based biomarkers analyses	observational	NCT04329299 (2012-2016)	Micro RNAs (miRNAs) and blood-based protein markers in participants	6,862
Tissue Procurement for Gastric Cancer including neuro endocrine cancer,	blood-tissues from any kind of treatment	observational	NCT01416714 (2011-2025)	Blood -tissues collection	1,000
A Collection of Clinical and Epidemiologic Data Combined With Tissue and Blood From Patients With a Diagnosis of Neuroendocrine Tumors GEPNET or NET of unknown primary.	blood- and biopsies collection from any kind of treatment	observational	NCT00745381 (2008-2020)	Blood-based biomarkers molecular testing include evaluation of DNA mutation, alternative splice variants, protein expression and phosphorylation, and immunohistochemistry on sample analyses	500
Community-based Neuroendocrine Tumor (NET) Research Study	Drug: lanreotide target: somatostatin receptor	observational	NCT02730104 (2015-2020)	Data collected will be in accordance with the routine practice of physicians. Blood collection	100
Database ITANET - ENETS Registry	all treatment	observational	NCT04282083 (2020-2022)	Create an Italian database for the collection of data on diagnostic approach, therapy and follow up of patients affected by GEP-NET (gastro-enteric-pancreatic neuroendocrine tumors) and to include these data into a multi-national European registry database, adhering to the ENETS (european neuroendocrine tumor society)-registry project.	3,600
Integrated Cancer Repository for Cancer Research (iCaRe2) including neuro endocrine	all treatments	observational	NCT02012699 (2013-2099)	Register: observational, genetics, biology, early detection, and patient care can collaborate by using the iCaRe2 as a platform for cohort and population studies.	999
SYNERGY-AI: Artificial Intelligence Based Precision Oncology Clinical Trial Matching and Registry	all treatments	observational	NCT03452774 (2018-2021)	Platforms, individual clinical data is extracted, analyzed and matched to a parametric database of existing institutional and non-institutional CT.	1,500
The Lyon Real World Evidence in Metastatic NeuroEndocrine Tumours (LyREMeNet)	advance stage	observational	NCT03863106 (2017-2020)	Clinical characteristics, prognostic factors, treatment patterns, and the overall survival among patients with metastatic GEP and lung NETs.	880
Treatment With Somatostatin Analogues in Patients With Gastroenteropancreatic Neuroendocrine Tumours (STREET)	Drug: somatostatine, targe:t receptor for somatostatine	observational	NCT02788565 (2016-2020)	Direct Cost of Treatment and quality life	156
A Safety and Tolerability Study of INCAGN02390 in Select Advanced Malignancies	all treatments	observational	NCT04028479 (2019-2021)	Diagnostic Test: Testing: Genome Diagnostic Test: Testing: Transcriptome Diagnostic Test: Testing: Proteome Drug: Treatment: CAR-T	10,000
The PIONEER Initiative: Precision Insights On N-of-1 Ex Vivo Effectiveness Research Based on Individual Tumor Ownership (Precision Oncology) (PIONEER)	with or without therapy	observational	NCT03896958 (2019-2021)	Goal of PIONEER is to enable best in class functional precision testing of a patient's tumor tissue to help guide optimal therapy (to date this type of analysis includes organoid drug screening approache	200
Recurrence Rates of Type I Gastric Neuroendocrine Tumors Treated with Long-acting Somatostatin Analogs	Drug: octreotide analog of somatostatine	observational	NCT03812939 (2019-2020)	Clinical symtoms evaluation of miRNAs as, prognostic factors, and treatment patterns	30

measuring 51 individual circulating genes in 1 ml of blood. An algorithmic analysis provides a numeric score of disease status. It can define the completeness of surgical resection, identify residual disease, monitor disease progression, and determine the efficacy of treatment (56–58). NETest was used to evaluate the alteration in genes during the treatment with SSA, PRRT, and following surgery (59–61). In a Dutch cohort of GEP-NET patients, the NETest had good sensitivity but the specificity was relatively low. Thus, NETest would be less suited for screening but could be valuable for the detection of residual disease after therapy (62). Interestingly, the NETtest was successfully used to evaluate efficacy and response to PRRT in metastatic NETs (63) (**Table 1**).

Several miRNAs were also associated with tumor progression of SI-NETs. In the miRNAs study performed by Heverhagen (64), the most promising diagnostic miRNA-biomarker was miR-7-5p higher in pathological tissue compared to control and selected miR-885-5p as predictive of rectal NETs metastases (65). In a cohort study, 3 miRNAs 129-5p, 133a, and 143-3p downregulated were associated with the metastatic phenotype of SI-NETs (64, 66–70), while other upregulated were correlated with SSA treatment

status or tumor stage (71). In another study, 4 differentially expressed miRNAs (miR-21-5p, miR-22-3p, and miR-150-5p) reached a statistical significance (72) underlying the need to add tissue markers, to discriminate NETs and to confirm the findings in annotated sample sets. Two miRNA profiling studies conducted on SI-NETs (66, 69, 73), compared metastatic tumors to primary malignancy, merging the data from both studies (metastasis vs primary) downregulation of miR-133a and upregulation of miR-183 were associated with poor prognosis and the spread of malignancy.

ROLE OF LONG NON-CODING RNAS IN NENS CLINICAL FINDINGS

Long non-coding RNAs (lncRNAs) are non-protein coding RNA transcripts longer than 200 nucleotides that exert multiple types of regulatory functions of all known cellular processes. Increasing evidence supports the role of lncRNAs in NENs development and progression with different mechanisms. In PNETs, tumor hypermethylation and silencing of long noncoding MEG3, determined activation of miR183/BRI3 axis, and cell proliferation due to c-MET oncogene activation (73). The reactivation of MEG3 by demethylating agents suppresses c-MET dependent cell proliferation suggesting that epigenetic targeting of MEG3 may represent an interesting approach in MEN1-PNETs treatment (59).

Moreover, downregulation of noncoding MEG3 and HOX genes has been associated with the development of non-functional pituitary adenomas and parathyroid tumors, respectively (74).

Two other lncRNAs are implicated in the pathogenesis of PNENs, the HOX antisense intergenic RNA chromatin-modifier (HOTAIR) and the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (75). HOTAIR reprograms neuroendocrine differentiation of prostate cancer (76), and its overexpression increases H3K27me and metastatic potential of breast cancer cells (77). Evidence supports the hypothesis that both lncRNAs through epigenetic modification activate downstream pathways Wnt/β-catenin (78) and ERK/MAPK (79) promoting epithelial-mesenchymal transition (EMT). In contrast, the upregulation of both lncRNAs in primary GEP-NETs was associated with less aggressive disease (80), as well as lncRNA, telomeric repeat-containing RNA (TERRA), is necessary to maintain genome integrity (81).

EPIGENETIC MODIFICATIONS ASSESSED IN LIQUID BIOPSIES AS PROGNOSTIC MARKERS

Unlike traditional tissue biopsies, liquid biopsies are faster, less invasive, have the potential to reflect all metastatic sites (i.e. tumor heterogeneity), and can indicate therapeutic response or progression through serial sampling. By considering the potential of genomic analysis, liquid biopsies offer a facilitated means of detecting genomic alterations and can be easily repeated over time. Moreover, cancer-specific circulating DNA (ctDNA) methylation can be used to measure circulating tumor DNA, as well as reveal the methylation patterns in the tumor (10).

In metastatic PNET patients, free circulating DNA carrying oncogenic mutations or methylation have been identified by mutation-specific droplet digital PCR (ddPCR) (82). In particular in a prospective trial ("MGMT-NET"), MGMT hypermethylation was also detectable in ctDNA instead of tissue (83, 84).

The Phase II PAZONET study is evaluating the epigenome modification in circulating tumor cells (CTCs), as potential biomarkers of response to therapy. The same goal was also assessed during SSA treatment in association with PRRT (85–87). This novel approach indicates that epigenetic profiling can identify serum biomarkers with prognostic potential (10).

EPIGENETIC TARGETED AGENTS AND CLINICAL TRIALS

Several clinical studies reported disease control targeting the somatostatin receptor (SSR), overexpressed in 70% of GEP-NETs, supporting the efficacy of both the available SSA octreotide and lanreotid) (88–90). To improve the efficacy and adverse metastatic phenotype, several ongoing trials are evaluating other targets as an inhibitor of angiogenesis, immunotherapy, or combinations of them (**Table 2** and **Figure 1**).

Epigenetics represents a very promising tool in cancer treatment because it can be reverted and epigenetic drugs are in use for the treatment of several cancer types (10, 91).

In vitro studies have already tested DNA methyltransferase inhibitors (DNMTis) because of MEN1 loss increase DNA hypermethylation (92). Promising results in PNET and small intestine cell lines were obtained using inhibitors of DNA methylases and HDAC to reduce cell viability and restoring gene expression (93–97). Interestingly, decitabine increased the expression of SSTR2 and the Ga-DOTATOC uptake also in BON1 tumor-bearing mice, indicating a possible therapy implication (98). However, decitabine has not yet been trialed in humans mainly because this agent targeted the whole methylated genome. Panobinostat, a histone deacetylase inhibitor (HDACi), was used in a completed phase II trial for the treatment of low-grade NENs. Patients showed a high stable disease with the median progression-free survival (PFS) of 9.9 months, and the median overall survival was 47.3 months. However, the low response rates, limitated further investigation (99). Inhibitors of the Bromo and extra terminal domain (BET) protein family, epigenetic readers of histone code, have also tested in experimental models (100). Of particular interest is Rx-001 which acts by blocking both DNMT and HDACs, activity. It showed to induce global epigenetic changes in tumors favoring infiltration of T cells, this histology was correlated with clinical benefit and sensitize tumor microenvironment to chemotherapy (101).

Novel frontier in solid tumor treatment is evaluating a combination of immunotherapy with epigenetic drugs, mainly

TABLE 2 | Clinical trials with drugs interfering with epigenetic pathways.

Hystology	Drugs and targets	Phase	NIH Clinical Trial	End points	n. Patients
Monotherapy					
Solid tumor including adenocarcinoma gastric cancer	Drug: MLN8237 target **aurora kinase**	Phase I/II	EudraCT: 2008-006981-27 (2011 completed)	Safety, tolerability, and efficacy	273
Advanced Neuroendocrine Cancer	Drug: pazopanib target **antiangiogenesis**	Phase II	NCT00454363 (2007-2015)	Disease progression laboratory biomarker	52
Low grade neuroendocrine tumor	Drug: panabinostat target **HDACis**	Phase II	NCT00985946 (2010-2015)	Response to therapy	15
Gastro-enteropancreatic metastatic Neuroendocrine Tumor	Drug: Famitinib target **c-Kit, VEGFR2, PDGFR, VEGFR3, Flt1 and Flt3**	Phase II	NCT01994213 (2015-2019)	Efficacy and molecular testing include evaluation of DNA mutation, and immunohistochemistry	53
First-line treatment in newly-diagnosed patients with Advanced GI Neuroendocrine Tumors.	Drug everolimus, target: **mTor**	Phase II multicenter	NCT01648465 (2012-2019)	Efficacy as first line	25
Gastroenteropancreatic Neuroendocrine Tumor G3	Drug: Anlotinib target: **tyrosin kinase inhibitor** VEGFR2, PDGFR, VEGFR3, Flt1 and Flt3	Phase II	NCT03457844 (2018-2019)	Clinical and molecular data of disease progression	60
A Safety and Tolerability Study of INCAGN02385 in Select Advanced Malignancies	Biological: INCAGN02385 target **LAG3**	Phase I	NCT03538028 (2018-2020)	Safety	40
In Patients With Advanced Neuroendocrine Tumors After Progression on Everolimus (CABINET)	Cabozantinib S-malate, target: **inhibtor tyrosine kinase** VEGFR2 RET MET AXL	Phase III randomized	NCT03375320 (2017-2021) (2018-2021)	Efficacy as first line	395
Treatment of Advanced Adult Solid Tumors including gastric and neuroendocrine	Drug: VMD-928 Capsules target **tyrosine kinase**	Phase I	NCT03556228 (2018-2021)	Safety	54
Select Advanced Malignancies and Neuroendocrine Tumor	Drug: INCAGN02390 target: **antagonize the TIM-3 pathway**	Phase I	NCT03652077 (2019-2021)	Safety	41
Refractory Solid Tumors, Esophageal Carcinoma Gastric (The MATCH Screening Trial)	Drug: Crizotinib **Inhibitor of ALK and ROS1**	Phase II	NCT02465060 (2015-2022)	End-of-treatment biopsy and collection of blood samples for research purposes	6,245
Study of CVM-1118 for Patients With Advanced Neuroendocrine Tumors	Drug: CVM-1118 inhibitor of **vasculogenic mimicry**	Phase II	NCT03600233 (2018- 2022)	Efficacy	30
Unresectable Gastroenteropancreatic Neuroendocrine Tumors (GEP NETs)	Drug: Abemaciclib, target: CDK4/cdk6 inhibitors	Phase II	NCT03891784 (2019-2024)	Disease progression	37
Patients With Grade 2 and Grade 3 Advanced GEP-NET (NETTER-2)	Drug: Lutathera Drug: long-acting octreotide target **receptor somatostatin**	Phase III Multi-center, Randomized	NCT03972488 (2020-2026)	Efficacy of treatment	222
Observational Study Following Neuroendocrine tumor	FT500 **Cellular Immunotherapy** Allogeneic natural killer (NK) cells	Phase I observational	NCT04106167 (2019-2034)	Safety	76
Malignant Esophagogastric Neoplasm MAGE-A4$^{c1\circ32}$T for Multi-Tumor	Autologous genetically modified MAGE-A4$^{c1\circ32}$T cells in subjects who have the appropriate HLA-A2 tissue marker	Phase I	NCT03132922 (2017-2035)	Safety	42
Gastric cancer	Drugs: Atezolizumab target: **PD-L1 immune-checkpoint**	Phase II	EudraCT:2015-000269-30 (2015-ongoing)	Tolerability and efficacy	725
Pancreatic neuroendocrine tumor	Drug: sunitinib target: **tyrosine kinases**	Phase II	EudraCT: 2012-000425-45 (2012-ongoing)	Effects of morning vs evening dosing on the pharmacokinetics and pharmacodynamics of sunitinib	18
Combination therapy					
Patients with unresectable, Neuroendocrine Tumor Metastatic Liver Cancer	Drug: cyclophosphamid **chemotherapy** Drug: poly-ICLC **immuno-stimulatory agent** Radiation	Phase I/II	NCT00553683 (2007-2014)	Safety and efficacy	50
Advanced Metastatic NETs (COOPERATE-1)	Pasireotide target: **somatostatin receptor** Drug: Everolimus Targets: **mTor**	Phase I	NCT01263353 (2010-2016)	Safety	36

(Continued)

TABLE 2 | Continued

Hystology	Drugs and targets	Phase	NIH Clinical Trial	End points	n. Patients
Gastroenteropancreatic Neuroendocrine Tumors (PLANET)	Drug: Somatuline Depot target **receptor somatostatin** Drug: Keytruda anti (PD-1) **immune-checkpoint**	Phasel/II	NCT03043664 (2017-2020)	Clinical and molecular data of disease progression	22
Gastro-enteropancreatic Neuroendocrine Tumor (REGOMUNE)	Drug: Regorafenib targeting **cKIT** Drug: Avelumab anti PD-L1 **immune-checkpoint**	Phasel/II	NCT03475953 (2018-2021)	Disease progression and efficacy	362
Advanced Solid Tumors (DUET-2) Neuroendocrine Tumor	Drug: XmAb20717 target: **bispecific antibody** anti PD-L1/ CTLA-4	Phase I	NCT03517488 (2018-2021)	Safety	154
Advanced Gastrointestinal Neuroendocrine Tumor G3 Type	Drug: Etoposide **chemotherapy** Drug: Irinotecan target **inhibitor of topoisomerase** I.	Phase II	NCT03963193 (2019-2021)	Disease progression	100
Patients With Advanced Solid Tumors and Neuroendocrine Tumor	Drug Pembrolizumab, **target: PD-1 immune-checkpoint** Drug: Sonidegib target: **Hedgehog signaling** PD1 pathway	Phase I	NCT04007744 (2019-2021)	Safety	78
Advanced or Metastatic Cancer (Consortium-IO) Esophageal Cancer Neuroendocrine Tumor	Drug: Nivolumab optidivo **immune-checkpoint** Drug: Vancomycin targeting VE800 **activates CD8 cells**	Pase I/II	NCT04208958 (2019-2022)	Safety and clinical activity	111
Advanced Solid Tumors And neuroendocrine tumor	Drug: **FT500** Drug: **Nivolumab** **Drug: Pembrolizumab** Drug: Atezolizumab Drug: Cyclophosphamide Drug: Fludarabine	Phase I	NCT03841110 (2019-2022)	immunotherapy tolerability	76
Advanced/Metastatic Solid Tumors Gastric Cancer Neuroendocrine Tumor	Drug: SO-C101 target: **super agonist IL15**; Drug: pembrolizumab anti **PD-1**	Phase I	NCT04234113 (2019-2022)	Safety	96
A Study to Evaluate the Safety and Pharmacokinetics of OC-001 in Patients With Locally Advanced or Metastatic Cancers including Neuroendocrine Tumor	Drug: OC-**001** target: **TNF,** Drug: **pembrolizumab anti PD-1**	Phase I/II	NCT04260802 (2020-2022)	Efficacy	80
Locally Advanced or Metastatic Solid Tumors including gastric neuro endocrine	Drug: INBRX-106 - Hexavalent OX40 **agonist antibody TNF** Drug: **Pembrolizumab anti PD-1**	Phase I	NCT04198766 (2019-2023)	Safety	150
Advanced or metastatic cervical cancer, endometrial cancer, gastric cancer, hepatocellular carcinoma	Drug: **INCAGN01876** inhbitor of LAG3 Immune Therapies **PD-1/PD-L1 therapy**	Phase I/II	EudraCT 2016-004989-25 (2017-ongoing)	Safety, Tolerability, and Efficacy	
Gastric Cancer (GC) Pancreatic Cancer (PC)	Drugs: Nivolumab target **PD-1** 1 and Ipilimumab target: **CTLA-4**	Phase I/II	EudraCT: 002844-10 (2013-ongoing)	Safety, Tolerability, and Efficacy	

Targets are in bold.

because some immunosuppressive cancer antigens are regulated by acetylation of their genomic regulative element.

Some trials are testing a combination of agonists of TNF and immunotherapy via checkpoint inhibition (NCT04198766) or antibody with double specificities against PD-L1 and CTLA-4 (NCT03517488). However major interest gained depleting tryptophan enzymes as indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase (ICI). This because tryptophan is able to induce immune suppression within the cancer microenvironment. In tumor cells and nude mice have already targeted tryptophan. The authors by specific inhibitors or by preventing tryptophan promoter acetylation using histone deacetylase inhibitors as BET reported the reduction of immunosuppressive protein expression (10, 102) suggesting a novel therapeutic approach.

CONCLUSIONS AND THE WAY FORWARD

The development of high-throughput techniques and larger datasets (i.e. The Cancer Genome Atlas) have accelerated research even in the field of NENs. Some pioneer studies have used an integrative approach in GEP-NETs (103). EWAS showed that these epigenome profiles can distinguish subtypes with different clinical features (**Figure 2**). The development of the NETest and liquid biopsy, as well as organoids (104), can be used to predict response to therapy and during the clinical follow-up, although not routinely used.

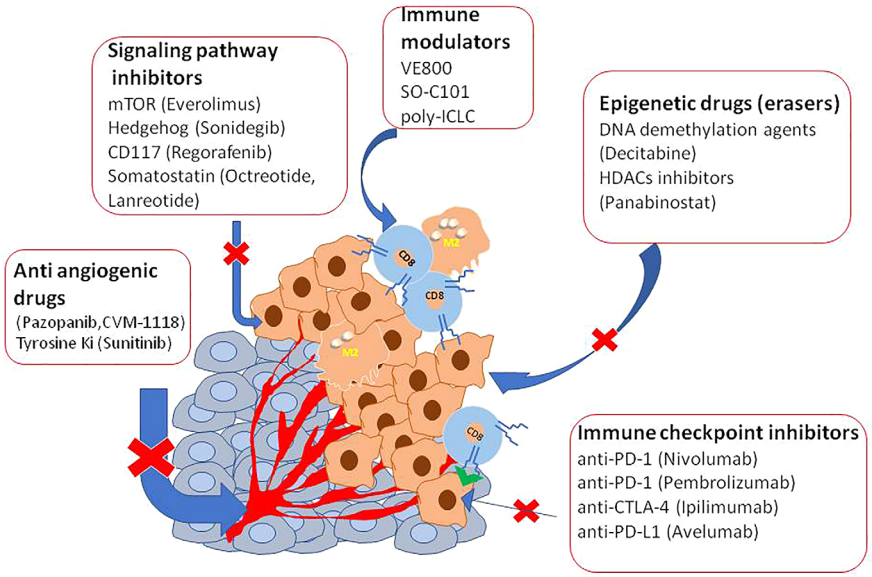

FIGURE 1 | A list of epigenetic agents useful in the therapy of NETs.

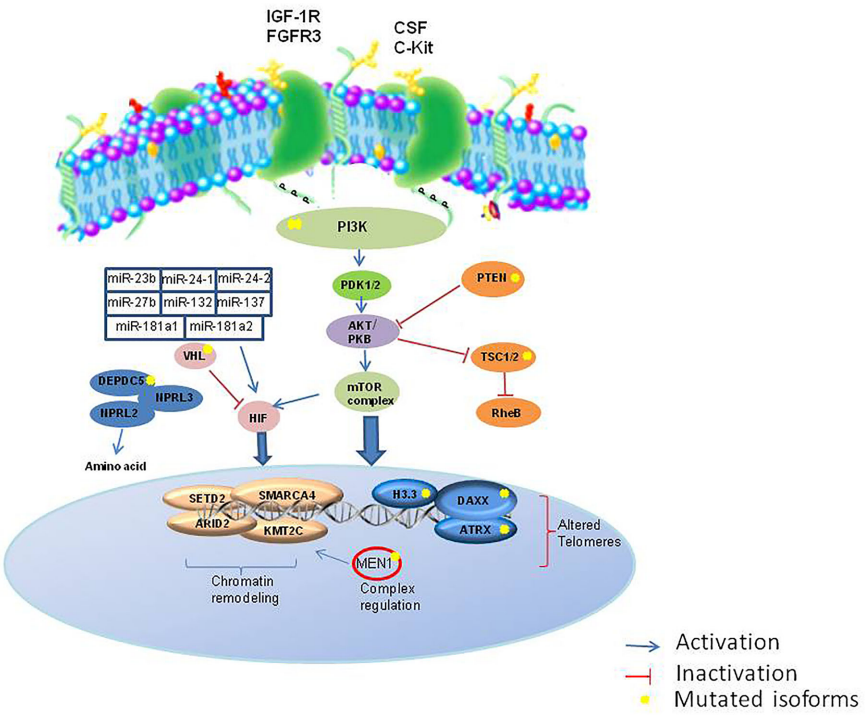

FIGURE 2 | Major epigenetic pathways involved in NETs. IGF1R, insulin growth factor 1 receptor; FGFR, fibroblast growth factor receptor; SCF, colony stimulation factor; c-KIT, c-Kit proto-oncogene; PI3K, phosphatidylinositol 3-kinases; PTEN, Phosphatase and tensin homolog; PDK1/2, protein 3-phosphoinositide-dependent protein kinase-1; TSC1/2, Tuberous sclerosis 1/2; RheB, Ras homolog enriched in brain; HIF, hypoxia factor; RheB, Ras homolog enriched in brain; VHL, Von Hippel-Lindau; DEPDC5, DEP domain containing 5; NPRL3, neuropilin 3.

Recently, it was proposed a bioresponsive drug-delivery depot for a combination of epigenetic modulation and immune checkpoint blockade (105). From the analysis of the clinical trials reported in **Table 1** and **Table 2**, it emerges that the evaluation of the epigenetic pathway as a biomarker of response is of most interest in many studies, involving different kinds of therapies, even in combination (10). NCT03475953 and NCT03841110 ongoing trials are evaluating the therapeutic potential of the combination of direct drugs against tyrosine kinases and immune response pathways such as PD-1/PD-L1 and the opportunity to select from patients' blood epigenetic biomarkers. The major challenge will now be to clinically validate such epigenetic biomarkers, within clinical trials for therapeutics in the new light of precision medicine, as well as network medicine (104, 106–108).

AUTHOR CONTRIBUTIONS

AC, FN, and CN contributed to conception and design of the study. FN, RM and CN analyzed the data and wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Diabetes in Patients With Pancreatic Neuroendocrine Neoplasms

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Objective: Diabetes mellitus (DM) is probably a risk factor for pancreatic neuroendocrine neoplasms (PNENs). However, the prevalence of DM in PNEN patients remains inconclusive. In the present study we observed the prevalence of DM and possible risk factors in PNEN patients.

Methods: After excluding those with insulinoma, a total of 197 patients with PNENs were included. The demographic data, pathological characteristics, and data of blood biochemical tests were recorded. DM was considered if there was evidence of a fasting plasma glucose level of ≥7.0 mmol/L or a 2-h plasma glucose level of ≥11.1 mmol/L, or a history of DM at the time of PNEN diagnosis. Impaired fasting glucose was considered if fasting plasma glucose level was between 6.1 and 7.0 mmol/L.

Results: The prevalence of DM, new-onset DM, and impaired fasting glucose were 17.26, 9.14, and 7.1%, respectively. The prevalence of DM was 26.0% in patients ≥60 years old (19/73) and 12.1% in patients <60 years old. Multivariable logistic regression analysis demonstrated that age, tumor size, and nerve invasion were independent risk factors for DM and impaired fasting glucose + DM ($p < 0.05$). Age, organs and nerve invasion were independent risk factors for impaired fasting glucose. Low high-density lipoprotein (HDL) was also a risk factor for incident of DM (OR = 0.15, 95%CI: 0.03–0.66). G2/G3 was an independent risk factor for DM in women.

Conclusion: Our data shows that the prevalence of DM is 17.26% in patients with PNENs and is 26.0% in patients ≥60 years of age after excluding insulinoma. Age, nerve invasion, tumor size, and HDL are risk factors for DM in PNEN patients.

Keywords: diabetes mellitus, pancreatic neuroendocrine neoplasms, high-density lipoprotein, fasting plasma glucose, tumor grade

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INTRODUCTION

Neuroendocrine neoplasms (NENs) are a group of uncommon lesions that usually occur in the gastroenteropancreatic (GEP) tract or in the bronchopulmonary system (1). The pancreas is one of the most commonly affected organs in NENs (PNENs). PNENs account for 1–2% of pancreatic tumors (2, 3). However, recent studies show that the incidence of PNENs is increasing due to the development in diagnostic techniques (2–4).

The pancreas is also the critical organ for glucose metabolism, as it secretes insulin. Several studies have shown the association between diabetes mellitus (DM) and incident of PNENs (5–8). Those data indicate that DM is probably a risk factor for PNEN occurrences. PNENs may also affect glucose metabolism. Tumor cells may secrete hormones that can affect glucose metabolism or insulin resistance. In addition, tumor mass may influence normal insulin secretion by inducing destruction or atrophy of pancreatic parenchyma (7, 9, 10).

DM has become a challenge for public health in China (11). Few studies have shown the prevalence of DM or impaired fasting glucose in PNENs (5, 12, 13). Ben et al. (5) indicated that the prevalence of T2DM was 16.9% in a Chinese PNEN population. However, poorly differentiated neuroendocrine carcinomas were excluded from that study. A recent study in China demonstrated that the prevalence of type 2 DM (T2DM) was 20.2% (12). In addition, Fan et al. showed that T2DM may be related to the biological behavior of PNENs, such as distant metastases and nerve invasion (12). Insulinoma was included in those two Chinese studies. A recent study showed that 28.3% of PNENs (insulinoma or glucagonoma was excluded) patients had DM or dysglycemia (blood glucose >140 mg/dl) in a German population (13). Insulinoma can decrease glucose levels by excessively secreting insulin and cause hypoglycemia. It would be better to exclude insulinoma from those studies. In addition, the prevalence of DM in PNEN patients is different between studies. Thus, we speculated that the prevalence of DM in PNENs remains inconclusive, and that further study is required. Anti-diabetic drugs may be a potential strategy for adjuvant therapy of PNENs (14, 15). It would be valuable to know the prevalence of DM or impaired fasting glucose in PNEN patients for treatment planning. In the present study we aimed to show the prevalence of DM in PNEN patients after excluding insulinoma and identify possible associated factors.

MATERIALS AND METHODS

Study Population

This cross-sectional study was approved by the Ethics Board of the First Affiliated Hospital, College of Medicine, Zhejiang University. Informed consent was waived because of the retrospective design. We searched our medical record from January 2011 to May 2020. We found a total of 242 surgically or cytologically proven PNEN patients who did not receive medical treatment for their tumor. Subjects with missing information, such as tumor grade, glucose data or medical history, and with a history of malignant disease and chronic pancreatitis were excluded from the final analysis (n = 16). In addition, patients with insulinoma that decreased glucose levels (n = 29) by excessively secreting insulin and type 1 DM (n = 0) were not included in this study. Finally, 197 PNEN patients were included. We recorded the demographic data and data of blood biochemical tests.

Blood Biochemical Tests

Blood biochemical tests included fasting plasma glucose levels, 2-h plasma glucose levels, serum total cholesterol (TC), serum triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). All blood biochemical tests were performed within one week before the operation.

Definition of DM

Definition of DM was based on plasma glucose levels and history of DM. DM was considered if the fasting plasma glucose level was ≥7.0 mmol/L or the 2-h plasma glucose level was ≥11.1 mmol/L during the oral glucose tolerance test. Moreover, DM was considered if a history of DM occurred at the time of PNEN diagnosis. Impaired fasting glucose was considered if the fasting plasma glucose level was between 6.1 mmol/L and 7.0 mmol/L. Patients with normal glucose levels or with no history of DM were regarded as non-diabetic patients. The glucose level was determined within one week before the operation. New-onset diabetes mellitus was defined as DM diagnosed within 2–3 years before a PNEN diagnosis (16).

Histology of PNENs

We recorded the following histological PNEN data: tumor size, location, ki67 index, mitotic count, lymph node invasion, organs invasion, vascular invasion, and nerve invasion. The tumor grades were defined based on the 2017 WHO classification for NENs (17). Grade 1(G1): mitosis count was <2/10 HPF and/or Ki-67 ≤2; Grade 2 (G2): mitosis count was 2–20/10 HPF and/or Ki-67 index was 3–20; Grade 3 (G3): mitosis count was >20 per 10 HPF, Ki-67 index was >20%. G3 neoplasm was not divided into well-differentiated G3 and pancreatic neuroendocrine carcinoma (PNEC) because the separation depended on the genetic backgrounds (p53 and *KRAS* mutations) of the two groups (18) that were not obtained in our population.

Statistical Analysis

Statistical analysis was performed using commercial software SPSS 16.0 (SPSS Inc, Chicago, IL). Data was shown as mean ± standard deviation or number of cases (percentage). Independent sample t test or Mann–Whitney U-test was used for continuous data and Chi-square test or Fisher's exact test was used for categorical variables. Univariable and multivariable logistic regression analyses were used to show the association between high fasting glucose level or DM and demographic data (age, BMI and gender), histological data of PNENs (ki67 index, mitotic count, lymph node invasion, organs invasion, vascular and nerve invasion), tumor size, and location or data of blood biochemical tests (HDL, LDL, TG, and TC). Statistical significance was considered if P value <0.05.

RESULTS

Characteristic of Patients

Characteristics of patients are listed in **Table 1**. There were 93 women and 104 men. The average age was 56.06 years old. The average size was 3.37 cm and ki67 index was 15.2. There were 66 PNEN G1, 85 G2, and 46 G3. The prevalence of DM, impaired fasting glucose, and new-onset DM were 17.26, 7.11, and 9.14%, respectively. Further, 52.9% of DM was new-onset DM.

TABLE 1 | Characteristic of patients.

Variables	Total (n = 197)	Women (n = 93)	Men (n = 104)	p
Age (years)	56.06 ± 11.65	55.82 ± 10.99	56.27 ± 12.27	0.78
Sex	93/104	/	/	
Size (cm)	3.37 ± 2.27	3.36 ± 2.07	3.39 ± 2.45	0.93
ki67 (%)	15.2 ± 23.56	10.04 ± 18.78	19.88 ± 26.06	<0.01
Glucose (mmol/L)	5.53 ± 1.84	5.61 ± 2.15	5.47 ± 1.51	0.63
TG (mmol/L)	1.38 ± 0.84	1.41 ± 0.88	1.35 ± 0.82	0.57
TC (mmol/L)	4.34 ± 1.06	4.43 ± 1.12	4.27 ± 1.00	0.28
HDL (mmol/L)	1.14 ± 0.37	1.19 ± 0.38	1.11 ± 0.35	0.14
LDL (mmol/L)	2.45 ± 0.81	2.50 ± 0.90	2.40 ± 0.73	0.40
Grade (1/2/3)	66/85/46	35/45/13	31/40/33	0.01
Location				0.43
Head-neck	93	46	47	
Body	66	27	39	
Tail	38	23	15	
Diabetes mellitus (yes)	34 (17.26%)	17 (18.28%)	17 (16.34%)	0.72
New-onset diabetes mellitus	18 (9.14%)	10 (10.75%)	8 (7.69%)	0.46
Impaired fasting glucose (yes)	14 (7.11%)	9 (9.68%)	5 (4.81%)	0.18
Impaired fasting glucose + Diabetes mellitus	48 (24.37%)	27 (29.03%)	21 (20.19%)	0.15
Duration of DM (years)*	0.6 (0–4.0)	2.0 (0–4.0)	0.0 (0–5.5)	0.97
Lymph node invasion	18 (9.14%)	9 (9.68%)	9 (8.65%)	0.87
Organs invasion	29 (14.72%)	12 (12.90%)	17 (16.35%)	0.50
Vascular invasion	27 (13.71%)	8 (8.60%)	19 (18.27%)	0.04
Nerve invasion	20 (10.15%)	7 (7.53%)	13 (12.50%)	0.25

TC, Serum total cholesterol; TG, serum triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
**Data was shown as median (interquartile range).*

No6 significant differences were found in the prevalence of DM, new-onset DM and impaired fasting glucose between men and women. The prevalence of DM was 26.0% in patients ≥60 years old (19/73) and 12.1% in patients <60 years old. The G3 PNENs occurred more commonly in men than in women ($p = 0.01$). Consequently, the ki67 index and vascular invasion in men was higher than in women ($p < 0.05$ and $p < 0.01$).

Subsequently, we showed the characteristic of patients based on impaired fasting glucose (**Table 2**) or DM (**Table 3**). The age of patients with impaired fasting glucose or DM was older than those that had none. The sizes of tumors in DM patient were bigger than those that had none ($p = 0.03$). A chi-square for trend analysis showed that the prevalence of DM was increased with tumor grades ($p = 0.10$). No such trends were observed in patients with impaired fasting glucose. Organ invasions were more common in PNENs that had impaired fasting glucose than that had none ($p < 0.01$). Nerve invasion was more common in PNENs that had DM than with none ($p < 0.01$).

The Glucose Levels

The glucose levels in PNENs G2 and G3 were both higher than that in PNEN G1 ($p < 0.05$) (**Figure 1A**). A similar result was found between G1 and G2/G3 ($p = 0.023$) (**Figure 1B**). The prevalence of impaired fasting glucose and DM in G2/G3 tumor was also higher than those of G1 tumors (27.4 *vs* 18.2% and 19.85 *vs* 12.12%), but no significant differences were found.

Associated Factors With Impaired Fasting Glucose and DM

Finally, we adopted a logistic regression analysis to show the potential associated factors of impaired fasting glucose and DM (**Table 4**). Univariable analysis showed that age, organs, TC, and LDL were associated with incidents of impaired fasting glucose (all $p < 0.05$). Moreover, age, size, nerve invasion, HDL, and G3 were associated with DM. Yet still, age, organs and nerve invasions were associated with incident of impaired fasting glucose + DM (all $p < 0.05$). Multivariable analysis demonstrated that age, tumor size

TABLE 2 | Characteristic of patients based on impaired fasting glucose.

Variables	Impaired fasting glucose (n = 14)	Non-Impaired fasting glucose (n = 149)	p
Age (years)	60.53 ± 9.50	54.53 ± 12.00	0.06
Length (cm)	3.18 ± 1.78	3.21 ± 2.00	0.95
ki67(%)	19.67 ± 31.85	14.01 ± 21.82	0.46
Glucose (mmol/L)	6.47 ± 0.19	4.90 ± 0.58	<0.01
TG (mmol/L)	1.48 ± 0.54	1.33 ± 0.88	0.54
TC (mmol/L)	4.84 ± 1.17	4.28 ± 0.97	0.04
LDL (mmol/L)	2.88 ± 1.00	2.39 ± 0.74	0.02
HDL(mmol/L)	1.25 ± 0.43	1.17 ± 0.36	0.43
BMI(kg/m^2)	24.1 ± 4.3	23.6 ± 3.8	0.34
Grade (1/2/3)*	5/6/3	54/64/31	0.99
Location*			0.59
Head-neck	8	67	
Body	3	51	
Tail	3	31	
Lymph node invasion	3	11	0.11
Organs invasion	6	15	<0.01
Vascular invasion	1	21	0.55
Nerve invasion	2	10	0.28

**Chi-square for trend.*
TC, Serum total cholesterol; TG, serum triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

TABLE 3 | Characteristic of patients with and without diabetes mellitus.

Variables	Diabetes mellitus (n = 34)	Non-Diabetes mellitus (n = 163)	p
Age (years)	60.24 ± 8.75	55.06 ± 11.97	0.02
Sex (F/M)	17/17	85/91	0.62
Length (cm)	4.14 ± 3.31	3.22 ± 1.97	0.03
ki67(%)	16.53 ± 23.60	14.92 ± 23.37	0.26
Glucose (mmol/L)	9.33 ± 3.35	5.05 ± 0.73	<0.01
TG (mmol/L)	1.51 ± 0.80	1.35 ± 0.85	0.20
TC (mmol/L)	4.25 ± 1.13	4.36 ± 1.05	0.76
HDL (mmol/L)	2.41 ± 0.92	2.45 ± 0.79	0.90
HDL(mmol/L)	0.98 ± 0.33	1.17 ± 0.36	0.01
BMI (kg/m^2)	24.9 ± 4.6	24.0 ± 4.8	0.16
Grade (1/2/3)*	8/15/11	58/70/35	0.10
Location*			0.39
Head-neck	17	76	
Body	13	53	
Tail	4	34	
Lymph node invasion	4	14	0.54
Organs invasion	8	21	0.11
Vascular invasion	6	21	0.40
Nerve invasion	8	12	<0.01
Hypoglycemic agents			
Insulin	4		
Sulfonylureas	7		
Metformin	5		
Glucosidase inhibitor	2		
Other	1		

**Chi-square for trend*

TC, Serum total cholesterol; TG, serum triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

and nerve invasion were independent associated factors for DM and impaired fasting glucose + DM ($p < 0.05$). Low HDL was an associated factor for DM (OR = 0.15, 95%CI: 0.03–0.66). Age, organ invasion, and nerve invasion were independent associated factors for impaired fasting glucose ($p < 0.05$).

For the female population, we found that age, HDL, and G3 were independent associated factors for DM after adjustment with potential confounders. OR was 1.17 (95%CI: 1.07–1.30), 0.07 (95%CI: 0.07–1.00), and 10.13 (95%CI: 1.30–78.94), respectively.

DISCUSSION

Several case–control studies showed that DM was a risk factor for PNENs (6, 8). However, few data has been reported on the prevalence of DM in PNENs. Capurso et al. (6) showed 10 cases of recent-onset diabetes and 17 cases of DM in 162 PNENs, which indicated a prevalence of 16.7% in the Italian population. Recently, Fan et al. (12) and Ben et al. (5) indicated that the prevalence of DM was 20.2 and 16.9% in two Chinese populations, respectively. However, insulinoma which may cause hypoglycemia is included in the three studies, wherein poorly differentiated neuroendocrine carcinomas were excluded from Fan's study. In the present study insulinoma was excluded and the prevalence of DM and recent-onset diabetes was 17.26 and 9.14%. For patients older than 60 years of age, the prevalence of DM was 26.0%. We also observed that age, tumor size, nerve invasion and HDL levels were independent associated factors for DM in PNEN patients.

The prevalence of DM in China has been reported in several investigations (11, 19). The prevalence of diabetes was 11.5–12.9% and 20.2–20.4% among subjects who were 40 to 59 and ≥60 years of age, respectively. In our study we found that the prevalence of diabetes was 12.1% in PNEN patients <60 years of age, which was similar to the national survey data. However, our data showed that the prevalence of diabetes in PNEN patients ≥60 years (26.0%) was higher than the estimated prevalence of diabetes patients in China. The prevalence of diabetes increases with increasing age (11, 19). Similar data was also found in PNEN patients. We found that age was an independent associated factor of DM in PNEN patients. Atrophy of pancreatic parenchyma is common in old persons. We speculated that the tumor effects of PNENs on pancreatic parenchyma may be more severer in older people. Moreover, we also found that the incident of DM increased when tumor size increased. Gallo et al. (7) reported that the PNEN tumor size in patients with diabetes was greater than those that did not have diabetes. Large tumor mass may induce more severe destruction or atrophy of pancreatic parenchyma (7, 20). However, tumor size was not considered in Fan's study (12).

DM and PNENs may have bidirectional associations (7). DM may be a risk factor for PNEN occurrence (21). However, the specific mechanism linking DM to PNENs has not been clarified. DM-related chronic inflammation and oxidative stress may play an important role (5). Some PNEN secret hormones can induce hyperglycemia and insulin resistance (21), such as glucagonomas or somatostatinomas. In addition, direct tumor effects of PNENs may cause the obstruction of the pancreatic duct and promote atrophy of pancreatic parenchyma, ultimately affecting insulin secretion (10, 21). Surgical treatment or drug therapy, such as pancreatectomy and somatostatin analogs may also affect glucose metabolism (7). Those subjects in our study did not receive any PNEN-related medical therapy before operations.

A few studies have shown the role of DM in the biological behavior of PNENs (12). The risks of nerve invasion and distant metastasis were higher in PNEN patients with diabetes or dysglycemia than those without diabetes (12, 13). Our study found that nerve invasion was associated with impaired fasting glucose and DM, which was consistent with previous studies. Diabetes can induce damage to peripheral nerves (22). Is the damage related to nerve invasion? The mechanism of nerve invasion on occurrence of DM or DM on nerve invasion in PNENs needed further study. In addition, our data showed that impaired fasting glucose was associated with organ invasion. Fan et al. (12) showed that the risk of DM in PNEN patients with distant metastases was higher than without it. A similar association was also observed in PNEN patients with dysglycemia (blood glucose >140 mg/dl) (13). Previous data also indicated that PNENs' grade was associated with DM (12). However, the number of G3 PNENs in Fan's study was small (n = 11). We did not find the association between DM or impaired fasting glucose and PNEN's grade. We speculated that those negative results may be due to the exclusion of insulinoma from our study. Insulinoma usually showed low grade G1 or G2 (23) and low risk of organ invasion. Interestingly, we

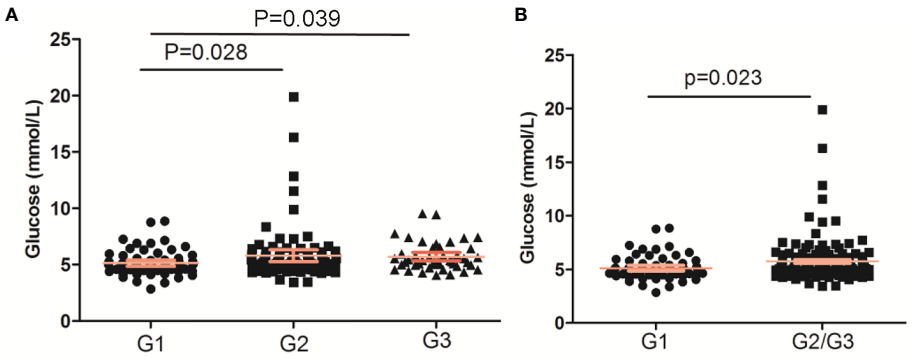

FIGURE 1 | The glucose levels based on WHO grade of pancreatic neuroendocrine neoplasms. **(A)** PNENs were divided into three groups; **(B)** PNENs were divided into two groups.

TABLE 4 | Logistic regression analysis in total population.

	Impaired fasting glucose*		Diabetes mellitus		Impaired fasting glucose + Diabetes mellitus	
	Univariable	**Multivariable**	**Univariable**	**Multivariable**	**Univariable**	**Multivariable**
Variables	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Age (years)	1.06(1.00–1.12)	1.09(1.01–1.18)	1.05(1.01–1.09)	1.06(1.02–1.11)	1.04(1.01–1.08)	1.06(1.02–1.11)
Sex (Male *vs* female)	0.45(0.15–1.42)	0.55(0.13–2.42)	0.87(0.42–1.83)	0.62(0.27–1.45)	0.53(0.27–1.06)	0.51(0.23–1.10)
Tumor size (cm)	0.98(0.73–1.30)	0.97(0.60–1.58)	1.17(1.01–1.35)	1.22(1.00–1.49)	1.13(0.99–1.30)	1.19(1.00–1.44)
Organs invasion (yes *vs* no)	6.70(2.05–21.92)	15.24(2.23–104.1)	2.08(0.84-5.20)	1.16(0.40–3.41)	3.68(1.62–8.35)	2.53(0.95–6.77)
Nerve invasion (yes *vs* no)	2.31(0.45–11.81)	17.28(1.37–218.1)	3.87 (1.44–10.39)	3.05 (1.01–9.60)	2.90(1.12–7.49)	3.51(1.11–11.14)
TG (mmol/L)	1.23(0.73–2.10)	1.81(0.66–4.94)	1.22(0.82–1.80)	1.03(0.56–1.90)	1.27(0.89–1.81)	1.13(0.66–1.99)
TC (mmol/L)	1.78(1.05–3.02)	1.69(0.89–2.86)	1.00(0.71–1.42)	0.64(0.28–1.50)	1.24(0.92–1.68)	0.69(0.29–1.64)
HDL (mmol/L)	1.61(0.37–7.07)	3.56(0.19–65.3)	0.20(0.06–0.61)	0.15(0.03–0.66)	0.44(0.17–1.11)	0.36(0.10–1.31)
LDL (mmol/L)	2.19(1.14–4.20)	13.94(0. 35–551.2)	1.00(0.63–1.57)	2.10(0.75–5.88)	1.35(0.91–2.01)	2.44(0.88–6.80)
Grade						
G1	1	1	1	1	1	1
G2	1.01(0.29–3.50)	0.39(0.05–3.00)	1.81(0.69–4.73)	1.27(0.44–3.67)	1.48(0.67–3.27)	0.72(0.27–1.93)
G3	1.05(0.23–4.67)	0.18(0.01–2.91)	2.98(1.07–8.28)	1.68(0.50–5.67)	2.18(0.91–5.24)	0.72(0.21–2.45)
Location						
Head-neck	1	1	1	1	1	1
Body	0.49(0.12–1.95)	0.31(0.05–1.86)	1.10(0.50–2.45)	1.26(0.50–3.16)	0.76(0.36–1.58)	0.76(0.33–1.79)
Tail	0.81(0.20–3.27)	0.52(0.09–3.25)	0.53(0.17–1.68)	0.61(0.18–2.11)	0.58(0.23–1.49)	0.51(0.18–1.47)

TC, Serum total cholesterol; TG, serum triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Multivariable regression analysis was additionally adjusted with vascular and lymph node invasion.
**Patients with diabetes mellitus were not included.*

found that the higher tumor grade was significantly associated with DM in the female population which was consistent with a recent study (24). There may be gender differences of PNENs in terms of associated comorbidities (24). Our data also supports that G3 PNENs are more common in male patients (18).

Lipid abnormalities, such as high TG and a low concentration of HDL, are also associated with DM (25). However, effects of lipid abnormalities on DM have not been considered in previous studies (12). Our results indicated that low HDL level was also an independent risk factor for DM in PNEN patients.

The present study has several limitations. First, some factors related to development of DM, such as alcohol consumption, cigarette smoking, nutrition, and physical activity were not considered. Second, the data came from a single institution, which weakened generalizability of the results. Third, the tumor stage was not included in the logistic regression analysis. Fourth, the diagnosis of DM was not based on HbA1C because HbA1C was not routinely determined in our institution. Finally, the small sample size might have led to the observed, null associations between some variables and DM.

In conclusion, our data showed that the prevalence of DM and new-onset DM were 17.26 and 8.63% after excluding patients with insulinoma. For patients older than 60 years, the prevalence of DM was 26.0%. The prevalence of DM in PNENs was higher than that of the general Chinese adult population. In addition, we found that age, tumor size, nerve invasion, and HDL levels were potential risk factors for DM in PNEN patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Board of the First Affiliated Hospital, Zhejiang University School of Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XC and CG contributed to conception of the study. XZ, YW, and XC contributed to interpretation of data and preparation of the manuscript. XC and CG performed the data analysis and contributed to the discussion. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Natural History of Renal Neuroendocrine Neoplasms: A NET by Any Other Name?

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Renal neuroendocrine neoplasms are rare, with descriptions of cases limited to individual reports and small series. The natural history of this group of neuroendocrine neoplasms is poorly understood. In this study, we queried the Surveillance, Epidemiology and End Results (SEER) database over a four-decade period where we identified 166 cases of primary renal neuroendocrine neoplasms. We observed a 5-year overall survival of 50%. On multivariate analysis, survival was influenced by stage, histology, and if surgery was performed. We observed that patients managed by operative management had a greater frequency of localized or regional stage disease as well as a greater frequency of neuroendocrine tumor, grade 1 histology; whereas those managed non-operatively tended to have distant disease and histologies of neuroendocrine carcinoma, NOS and small cell neuroendocrine carcinoma. This is the largest description of patients with renal neuroendocrine neoplasms. Increased survival was observed in patients with earlier stage and favorable histologies.

Keywords: kidney, renal, neuroendocrine neoplasm, neuroendocrine tumor, neuroendocrine carcinoma

HIGHLIGHTS

Since the first report of renal carcinoid tumor in 1966, there have been around 100 reported cases in the literature. The early reports are primarily limited to case reports and short series, with descriptions the presentation, imaging characteristics and outcomes of individual patients. More recent reports are still rare but are limited in regards to population based outcomes based on pathology. These reports typically describe these individual cases but fail to describe the overall context of each of these patients in a broader scenario by which these patients present. In our population-based study, 5-year overall survival in primary renal neuroendocrine neoplasm was 50% and was influenced by stage, histology and whether or not surgery was performed. We feel this finding will add additional knowledge to the scarce literature that is in circulation. In addition, this

survey underlines the importance of developing and applying a consistent diagnostic standard, an issue that has plagued many other organ systems and cancer diagnoses.

INTRODUCTION

Neuroendocrine neoplasms (NEN) are rare entities. As a group, their incidence today is about 7 per 100,000 individuals in the United States, with the most common primary sites being lung, gastrointestinal tract, and pancreas (1, 2). Primary renal NENs represent a poorly characterized subset of neuroendocrine neoplasms. The current medical knowledge of this disease is limited to case reports, small series, and pooled studies of reports and series (3, 4). In 2016, the 4th edition of the World Health Organization (WHO) Classification of Tumors of the Urinary System and Male Genital Organs reorganized NENs into well-differentiated neuroendocrine tumors (NET), large cell neuroendocrine carcinoma (LC-NEC), small cell neuroendocrine carcinoma (SC-NEC), and pheochromocytoma (5). These categories are similar to the proposed common classification framework proposed by the International Agency for Research on Cancer (IARC) and WHO in 2018 (6).

While NENs as a group share certain histologic features such as immunohistochemical expression of chromogranin A and synaptophysin, they arise from diverse tissues where resident neuroendocrine cells play various roles depending on their location. In lung and gastrointestinal primary sites, tumors arise from native pulmonary neuroendocrine cells and a diverse group of gastrointestinal neuroendocrine cells that create and secrete bioactive products to local and distant tissues (7, 8). In the kidney, the cell of origin of these neoplasms is not well defined. There are no known native neuroendocrine cells in the renal parenchyma. Because the majority of renal NENs arise from the parenchyma, one hypothesis is that they originate from renal stem cells that develop towards neuroendocrine differentiation (3). Like neuroendocrine tumors from other sites, they have been observed on a case-by-case basis to vary in histologic grade and disease extent. Although published accounts have noted an increased incidence of renal carcinoid tumors in horseshoe kidneys (9), a larger-scale natural history and follow up outcomes of renal NENs as a group has not been attempted before.

Patients with primary NENs of the kidney may present with abdominal or flank pain, a palpable mass, weight loss, or hematuria, although a quarter of patients are asymptomatic at diagnosis (10). Patients with suspected renal NENs are typically evaluated with biochemical testing, such as urinary 5-HIAA and serum Chromogranin A, and imaging is subsequently performed for localization. Cross sectional imaging, including computed tomography (CT) of the abdomen and pelvis may demonstrate a solid, hypodense mass with mild enhancement on venous phase (11). Magnetic resonance imaging may demonstrate heterogeneous signal intensity in T1 and T2-weighted images. On renal ultrasound, the tumor may appear as a hyperechoic mass, but each of these imaging studies typically do not reveal truly distinct features to neuroendocrine tumors (12). Functional imaging with octreotide scinitigraphy of Gallium-68 DOTATATE PET/CT, may be more sensitive and specific study for well-differentiated renal NETs as has been described in NENs in other organs, although this has not been studied in renal NENs (13, 14). Nephrectomy with lymph node dissection is considered standard treatment for localized primary renal NENs. For metastatic renal NENs, long acting somatostatin analogs, tyrosine kinase inhibitors, and peptide-receptor radio nucleotide therapy that are effective in other neuroendocrine tumors are reasonable treatment options as there are no clinical trials to define optimal treatment for renal NENs at any stage (10).

In the present study, we perform the first population-based study to describe the natural history of patients with primary renal NENs. We present a series of 166 cases of primary renal NENs to study patient characteristics, tumor characteristics, and survival.

MATERIALS AND METHODS

Patients

Patients were identified from the Surveillance, Epidemiology, and End Results (SEER) database for the years 1973 to 2014. Inclusion and exclusion criteria are depicted in **Table 1**. We identified patients by the International Classification of Disease for Oncology, morphology codes (ICD-O-3) to include 8240/3 neuroendocrine tumor, grade 1 (NET-G1); 8249/3 neuroendocrine tumor, grade 2 (NET-G2); 8246/3 neuroendocrine carcinoma, NOS (NEC-NOS); 8013/3 large cell neuroendocrine carcinoma (LC-NEC); and 8041/3 small cell neuroendocrine carcinoma (SC-NEC). Other than 8249/3 (NET-G2), these ICD-O morphology codes are the same codes used in the WHO classification. We required that the primary site of tumor to be either in the kidney (C64.9-Kidney, NOS) or the renal pelvis (C65.9-Renal pelvis). We excluded patients who did not have a histologically confirmed diagnosis and those with a prior other

TABLE 1 | Inclusion and exclusion selection criteria identifying renal neuroendocrine neoplasms.

Step	Selection Criterion	No. Remaining
1	SEER data set of patients from 1973-2014	9,675,661
2	Include cases with (1) primary site is "C64.9-Kidney, NOS" or "C65.9-Renal pelvis" AND (2) histologic type ICD-O-3 is 8013/3, 8041/3, 8240/3, 8246/3, or 8249/3	245
3	Exclude patient who did not have a histologically confirmed diagnosis	194
4	Exclude patients with prior cancer	177
5	Exclude patients diagnosed at autopsy or death	166
6	Exclude patients receiving diagnosis while in a nursing home or hospice care	166
7	Exclude cases under the age of 18	166

8240/3 (NET-G1, neuroendocrine tumor, grade 1), 8249/3 (NET-G2, neuroendocrine carcinoma, grade 2), 8246/3 (NEC-NOS, neuroendocrine carcinoma, NOS), 8013/3 (LC-NEC, large cell neuroendocrine carcinoma), 8041/3 (SC-NEC, small cell neuroendocrine carcinoma).

primary cancer. We excluded patients who were diagnosed upon autopsy or death, who were diagnosed while on hospice care or in a nursing home, and patients under the age of 18. Patient demographics included age, gender, race, environment, and year of diagnosis. Tumor characteristics included primary site, laterality, histologic type, stage, grade, and lymph node status. For the purpose of our analysis, age was converted into categorical values.

Statistical Analysis

Patient demographics and tumor characteristics were summarized and compared between operatively and non-operatively managed patients. Pearson $\chi 2$ tests were used to evaluate categorical data. Age was compared across groups with a t-test. Kaplan-Meier analyses were performed to estimate 5-year overall survival (OS) and disease-specific survival (DSS). Survival times used represented time from date of diagnosis to date of death. DSS represented survival time up to death, where cause of death was identified to be due to cancer. Log-rank tests were performed to test equality among groups. A multivariate Cox proportional hazard model was performed for OS and DSS, and hazard ratios and 95% confidence intervals were reported. The Cox proportional hazards model assumptions were tested by calculating scaled Schoenfeld residuals. Analyses were performed using Stata software (StataCorp, College Station, TX). A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 166 patients were identified in the SEER database with a diagnosis of primary renal NENs (**Table 2**). Eighty-five (51.2%) patients were male. The median age at the time of diagnosis was 59 years. The majority of patients were White (136 patients, 81.9%), while 14 (8.4%) were Black and 16 (9.6%) had no recorded ethnicity. Patients with renal NENs were largely identified in large urban communities (97, 59.2%), while a suburban environment (57, 34.7%) being the second most common, and only 10 (6.1%) patients were from rural environments. In the last two 6-year periods from 2003 to 2014, more patients were diagnosed with renal neuroendocrine tumors than in the time period from 1991 to 2006.

We then looked at tumor characteristics (**Table 3**). Seventy patients (42.2%) had documented distant disease, 56 (33.7%) had regional disease, 32 (19.3%) had local disease, and 8 (4.8%) patients had no documented stage. There were slightly more patients with right sided tumors (89, 53.6%) compared to left sided tumors (68, 40.9%). A total of 154 (92.8%) tumors were found in the renal parenchyma and 12 (7.2%) in the renal pelvis. Of histologic types as categorized by SEER, the most common were NET-G1 (56, 33.7%) and SC-NEC (55, 33.1%). There were 51 (30.7%) cases categorized as NEC-NOS. Only two cases each of NET-G2 and LC-NEC were recorded.

From the data available, only 70 of 166 patients had a reported differentiation, with a relatively even distribution of well-differentiated (9.0%), moderately-differentiated (9.0%) and poorly differentiated (10.2%) tumors. Twenty-three (13.8%) tumors were classified as undifferentiated/anaplastic. For the majority of cases in the SEER database (96, 57.8%), there was no recorded tumor differentiation. Most of the tumors were of

TABLE 2 | Patient demographics of patients with renal neuroendocrine neoplasm diagnosed from 1991 to 2014 in the SEER database.

	All patients (n=166)
Age	59
Age Groups	
<50	48 (18.9)
≥50	118 (71.1)
Gender	
Male	85 (51.2)
Female	91 (48.8)
Race/Ethnicity	
White	136 (81.9)
Black	14 (8.4)
Other	16 (9.6)
Community Type	
Urban	97 (59.2)
Suburban	57 (34.7)
Rural	10 (6.1)
Time Period	
1991-1996	22 (13.2)
1997-2002	39 (23.5)
2003-2008	47 (28.3)
2009-2014	58 (34.9)

TABLE 3 | Renal neuroendocrine neoplasm stage and tumor characteristics.

	All patients (n=166)
Stage	
Unknown	8 (4.8)
Local	32 (19.3)
Regional	56 (33.7)
Distant	70 (42.2)
Laterality	
Right	89 (53.6)
Left	68 (40.9)
Undocumented	9 (5.4)
Location	
Renal Parenchyma	154 (92.8)
Renal Pelvis	12 (7.2)
Histologic type	
Neuroendocrine tumor, grade 1 (NET-G1)	56 (33.7)
Neuroendocrine tumor, grade 2 (NET-G2)	2 (1.2)
Neuroendocrine carcinoma, NOS (NEC-NOS)	51 (30.7)
Large cell neuroendocrine carcinoma (LC-NEC)	2 (1.2)
Small cell neuroendocrine carcinoma (SC-NEC)	55 (33.1)
Differentiation	
Well differentiated	15 (9.0)
Moderately differentiated	15 (9.0)
Poorly differentiated	17 (10.2)
Undifferentiated/anaplastic	23 (13.8)
Undocumented	96 (57.8)
Tumor size	
≤2 cm	7 (4.2)
>2 cm and ≤4 cm	21 (12.6)
>4 cm	97 (58.4)
Undocumented	41 (24.7)

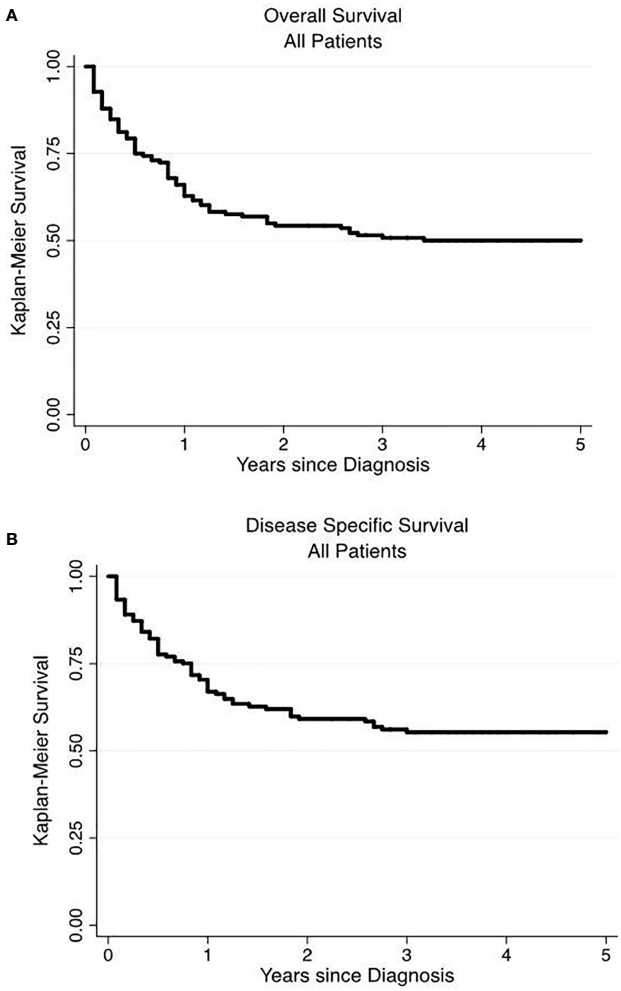

FIGURE 1 | Overall and disease-specific survival of primary renal neuroendocrine neoplasms. Overall **(A)** and disease-specific **(B)** 5-year survival were 50 and 52%, respectively.

>4 cm in size. For 24.7% of patients, primary tumor size was not recorded in the database.

We performed a Kaplan-Meier survivor analysis of all 166 patients, observing a 5-year OS of 50% (**Figure 1A**) and a 5-year DSS of 52% (**Figure 1B**). We then performed a univariate analysis to determine how various factors contributed to overall and disease-specific survival. On univariate analysis, operative management appeared to decrease risk of all-cause mortality (HR 0.22, 95% CI 0.14–0.33) and disease specific mortality (HR 0.19, 95% CI 0.12–0.32) (**Table 4**). Older age, male gender, regional and distant disease, and histology other than NET-G1 were associated with poorer overall survival. In examining disease specific survival, of the listed risk factors, only male gender was no longer associated with poorer survival and White race was associated with increased risk. Large and small cell NECs were associated with the poorest OS and DSS.

We then performed a multivariate analysis using a Cox proportional hazards model (**Table 5**). We included the same patient and tumor characteristics evaluated in the univariate analysis. We found that on both univariate and multivariate analysis, older age, male gender, White race, regional and distant disease, and histology of SC-NEC were associated with increased all-cause and disease-specific mortality. On multivariate analysis, operative management was no longer associated with statistically significant decreased risk of all-cause and disease specific mortality.

To better understand differences in patients managed with operative versus non-operative management, we compared patient and tumor characteristic among these two groups (**Table 6**). We found that operatively and non-operatively managed patients were similar in patient characteristics. There were more patients with distant disease who were managed non-operatively (76.7%) than with localized (3.3%) or regional (11.7%) stage, whereas operatively managed patients had greater proportions of local (28.3%) or regional (46.7%) stage disease (**Table 7**). Histologic types of SC-NEC and NEC-NOS were more frequent among non-operative patients than operative patients.

In our data set, stage described as local, regional and distant disease was the best surrogate measure of extent of disease. We performed Kaplan-Meier analyses, assessing survival among operatively and non-operatively managed patients (**Figure 2**). In patients with local disease, all but two patients underwent operative management and those who did not have surgery eventually died from unrelated pulmonary disease (**Figure 2A**). In patients with regional disease, those managed operatively had a significant advantage in OS ($p<0.0001$) (**Figure 2B**). Seven patients who did not receive surgery had much poorer survival than those who underwent an operation. In those patients with distant disease, 24 patients were operatively managed and 46 patients were non-operatively managed and there was no significant difference in overall survival ($p=0.10$) (**Figure 2C**).

When sorted by tumor histology, the highest OS and DSS were seen in NET-G1 and the lowest in SC-NEC. Those with NEC-NOS showed OS and DSS intermediate to NET-G1 and SC-NEC (**Figures 3A, B**.

DISCUSSION

As a group, neuroendocrine neoplasms are rare. There has been an increasing incidence and prevalence with more frequent detection of early stage disease and improved survival over recent decades (2). Primary renal NENs represent a minority among all NENs. The cumulative knowledge of these rare tumors is composed of case reports and series. This study represents the first population-based investigation of this rare neoplasm.

By querying the SEER database from 1973 to 2014, we identified 166 patients with primary renal NEN. Consistent with the findings of Dasari et al., we observed increased incidence in the last 12 years compared to the previous (2). This has been observed with the increasing incidence of published reports on renal neuroendocrine neoplasms per decade, suggested to be due to the more widespread use of cross sectional imaging (4). In a review of published case reports of renal neuroendocrine neoplasms prior to 2006, Romero et al. observed half of patients to have distant

TABLE 4 | Univariate analysis of mortality (overall survival and disease-specific survival).

Risk factor	Univariate HR OS	95% CI	p-value	Univariate HR DSS	95% CI	p-value
Age						
<50	Ref			Ref		
≥50	1.92	1.18–3.12	0.009	2.26	1.28–3.98	0.005
Sex						
Female	Ref			Ref		
Male	1.62	1.08–2.43	0.02	1.46	0.94–2.29	0.094
Race/ethnicity						
Non-White	Ref			Ref		
White	1.79	1.01–3.18	0.043	2.04	1.05–3.98	0.035
Year diagnosed						
1991–1996	Ref			Ref		
1997–2002	0.73	0.41–1.29	0.279	0.74	0.39–1.39	0.348
2003–2008	0.52	0.28–0.95	0.033	0.55	0.28–1.07	0.077
2009–2014	0.4	0.21–0.76	0.005	0.4	0.19–0.81	0.012
Stage						
Localized	Ref			Ref		
Regional	3.16	1.43–7.02	0.005	2.77	1.09–7.04	0.032
Distant	9.9	4.54–21.59	<0.001	10.47	4.31–25.43	<0.001
Unknown	2.44	0.73–8.13	0.145	3.31	0.93–11.7	0.064
Surgery performed						
No	Ref			Ref		
Yes	0.22	0.14–0.33	<0.001	0.19	0.12–0.32	<0.001
Histology						
NET-G1	Ref			Ref		
NET-G2	6.17	0.80–47.36	0.08	7.02	0.90–54.8	0.063
NEC-NOS	3.1	1.67–5.76	<0.001	3.08	1.54–6.14	0.001
LC-NEC	11.3	2.55–50.11	0.001	13.26	2.92–60.15	0.001
SC-NEC	7.66	4.24–13.83	<0.001	7.48	3.86–14.4	<0.001

TABLE 5 | Multivariate analysis of mortality (overall survival and disease-specific survival).

Risk factor	Multivariate HR OS	95% CI	p-value	Multivariate HR DSS	95% CI	p-value
Age						
<50	Ref			Ref		
≥50	1.25	0.73–2.12	0.418	1.41	0.76–2.63	0.271
Sex						
Female	Ref			Ref		
Male	1.35	0.87–2.09	0.181	1.18	0.73–1.93	0.487
Race/ethnicity						
Non-White	Ref			Ref		
White	1.84	0.99–3.41	0.054	2.15	1.03–4.49	0.041
Year diagnosed						
1991–1996	Ref			Ref		
1997–2002	1.77	0.92–3.41	0.087	1.81	0.86–3.78	0.117
2003–2008	0.95	0.47–1.89	0.879	0.92	0.42–1.99	0.839
2009–2014	0.52	0.26–1.06	0.074	0.49	0.23–1.10	0.085
Stage						
Localized	Ref			Ref		
Regional	2.28	0.98–5.34	0.057	1.83	0.68–4.94	0.231
Distant	4.51	1.86–10.92	0.001	4.39	1.61–11.96	0.004
Unknown	0.99	0.27–3.55	0.985	1.33	0.34–5.18	0.678
Surgery performed						
No	Ref			Ref		
Yes	0.28	0.16–0.48	<0.001	0.27	0.15–0.50	<0.001
Histology						
NET-G1	Ref			Ref		
NET-G2	9.43	1.08–91.85	0.042	10.52	1.15–96.05	0.037
NEC-NOS	2.35	1.19–4.59	0.013	2.23	1.04–4.72	0.037
LC-NEC	5.87	1.12–30.67	0.036	6.63	1.19–36.69	0.03
SC-NEC	7.22	3.56–14.65	<0.001	6.84	3.08–15.18	<0.001

TABLE 6 | Comparing patient demographics of operative versus non-operative management.

	Non-operative (n=60)	Operative (n=106)	p-value
Age group			
<50	12 (20.0)	36 (34.0)	0.057
≥50	48 (80.0)	70 (66.0)	
Sex			
Female	27 (45.0)	54 (50.9)	0.462
Male	33 (55.0)	52 (49.1)	
Marital status			
Married	33 (55.0)	61 (57.5)	0.928
Not Married	25 (41.7)	41 (38.6)	
Unknown	2 (3.3)	4 (3.7)	
Race/Ethnicity			
Non-white	10 (16.7)	20 (18.9)	0.723
White	50 (83.3)	86 (81.1)	
Environment			
Urban	34 (56.7)	64 (60.4)	0.423
Suburban	20 (33.3)	37(34.9)	
Rural	6 (10.0)	11 (4.7)	
Year period			
1991–1996	10 (16.7)	12 (11.3)	0.755
1997–2002	14 (23.3)	25 (23.6)	
2003–2008	15 (25.0)	32 (30.2)	
2009–2014	21 (35.0)	37 (34.9)	

TABLE 7 | Comparing tumor characteristics of operative versus non-operative management.

	Non-operative (n=60)	Operative (n=106)	p-value
Stage			
Localized	2 (3.3)	30 (28.3)	<0.001
Regional	7 (11.7)	49 (46.2)	
Distant	46 (76.7)	24 (22.7)	
Unknown	5 (8.3)	3 (2.8)	
Laterality			
Left	24 (40.0)	44 (41.5)	0.026
Right	29 (48.3)	60 (56.6)	
Unspecified	7 (11.7)	2 (1.9)	
Histology			
NET-G1	8 (13.3)	48 (45.3)	<0.001
NET-G2	1 (1.7)	1 (0.9)	
NEC-NOS	21 (35.0)	30 (28.3)	
LC-NEC	2 (3.3)	0 (0.0)	
SC-NEC	28 (46.7)	27 (25.5)	
Location			
Renal parenchyma	57 (95.0)	97 (91.5)	0.404
Renal pelvis	3 (5.0)	9 (8.5)	
Cause of Death			
Alive	7 (11.7)	62 (58.5)	<0.001
Attributed to primary renal neuroendocrine tumor	26 (43.3)	24 (22.6)	
Other cancer	11 (18.3)	6 (5.6)	
Cardiac and cerebrovascular	1 (1.6)	2 (1.9)	
Pulmonary	8 (13.3)	1 (0.9)	
Other causes	7 (11.7)	11 (10.4)	

metastatic disease. Similarly, most patients in our study had distant disease (42.2%), compared to local and regional disease (19.3 and 33.7%, respectively). This is dissimilar to other types of primary neuroendocrine tumors where local or regional disease is far more frequent than distant disease (1). Our data and those of Romero et al. suggest either a biphasic distribution of NETs versus NECs, or that the anatomic structure of renal NENs predisposes to early hematogenous metastatic spread.

We observed most tumors to be found in the renal parenchyma, with a minority (7.2%) in the renal pelvis. Others have observed similar location of tumor, despite no known native location of neuroendocrine cells in renal parenchyma. It is hypothesized that these tumors arise from neuroendocrine differentiation of pluripotent stem cells present in the parenchyma, misplaced neural crest cells in the kidney from embryogenesis, or development with congenital abnormalities of the kidney. We found a slightly greater frequency of tumors on the right side compared to the left, which has been observed more dramatically in other series (53.6% right) while a more recent series of literature reports found equal right and left sided tumors (10). The SEER database is limited by documenting laterality as right or left. As a consequence, we were unable to confirm previously published findings of increased risk of renal NET in horseshoe kidney (9).

In our study, slightly more than half of the patients were male, which is similar to prior observations. Our population had a median age of 59, which was slightly older compared to prior studies, where the median age was found to be 47, 49, and 52 (3, 4, 10).

The patients in our study were categorized in the SEER database by International Classification of Disease for Oncology, 3rd edition histology codes (ICD-O-3), localized to the kidney or renal pelvis, and required to be identified as the first and primary tumor for each patient across a broad time period. In our series, the earliest identified patient was in 1991. Over the last three decades, the terminology for neuroendocrine tumors has had significant changes. As an overall category, these tumors are NENs and are further divided into NET to include well-differentiated NET-G1 and NET-G2 (ICD-O 8240/3 and 8249/3 respectively), and neuroendocrine carcinoma, which would include NEC-NOS and LC-NEC (ICD-O 8246/3 and 8013/3 respectively).

There is no precise grading system for renal NENs, in part due to their rarity. While some descriptive histology features are correlated with poor prognosis, these features are inconsistently reported and are not recorded in the SEER database. Gastroenteropancreatic (GEP) NENs are graded on a basis of mitotic count and Ki-67 proliferation index, whereas pulmonary NENs are graded by mitotic count and extent of necrosis. The histologic data points of mitotic rate, Ki-67 proliferation index, and degree of necrosis are not discretely documented in the SEER database, which is a limitation of this population-based study.

The SEER database covers a broad timespan, and encompasses wide historical variance in classification styles. We have two possible explanations for the tumor category of NEC-NOS, based on the OS and DSS consistently being in-between that of NET-G1 and SC-NEC. It is possible that NEC-NOS represent what would now be called LC-NEC based on the IARC/WHO consensus proposal. Another possibility is that the NEC-NOS group is composed evenly of NETs (NET-G1 and NET-G2) and SC-NEC, which could represent an average OS and DSS. The paucity of NET-G2 cases, 2 total in 42 years of the database, suggests that NET-G2 is an underdefined category for renal NENs.

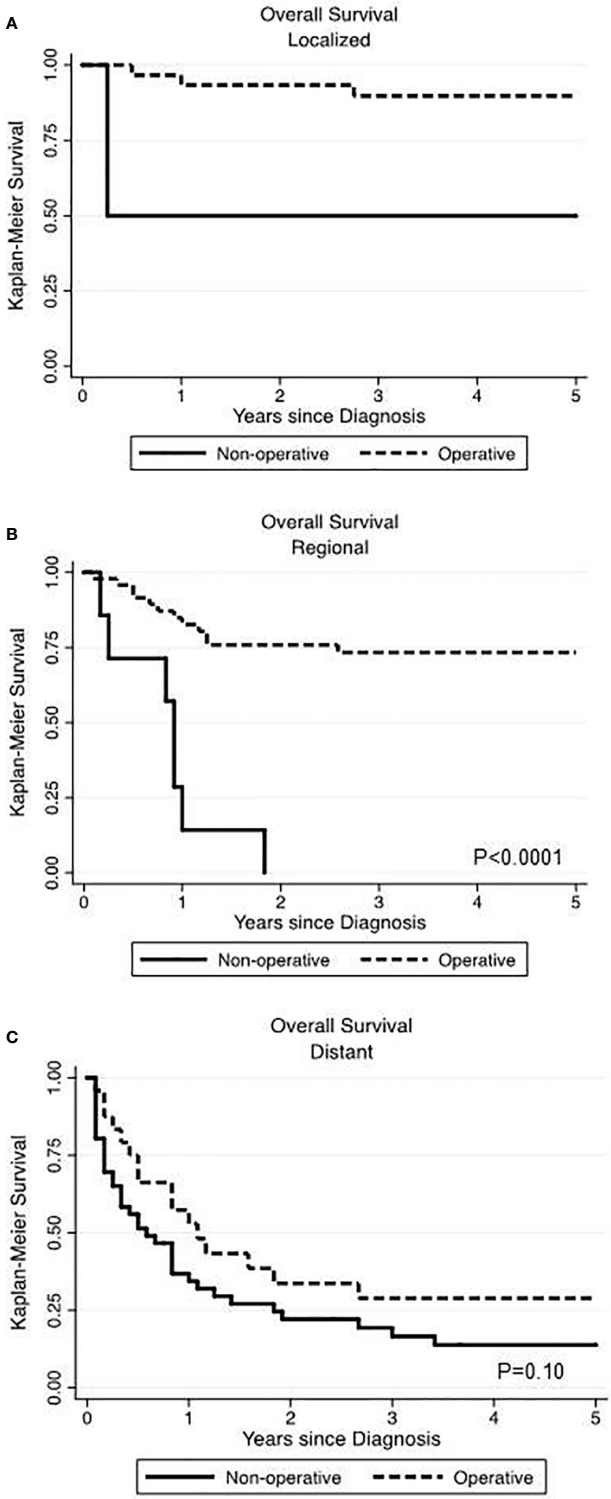

FIGURE 2 | Comparing the impact of operative versus non-operative management on overall survival at various stages of primary renal neuroendocrine neoplasms. Surgery was associated with significantly improved overall survival in localized **(A)** and regional **(B)** disease but not in the setting of distant disease **(C)**.

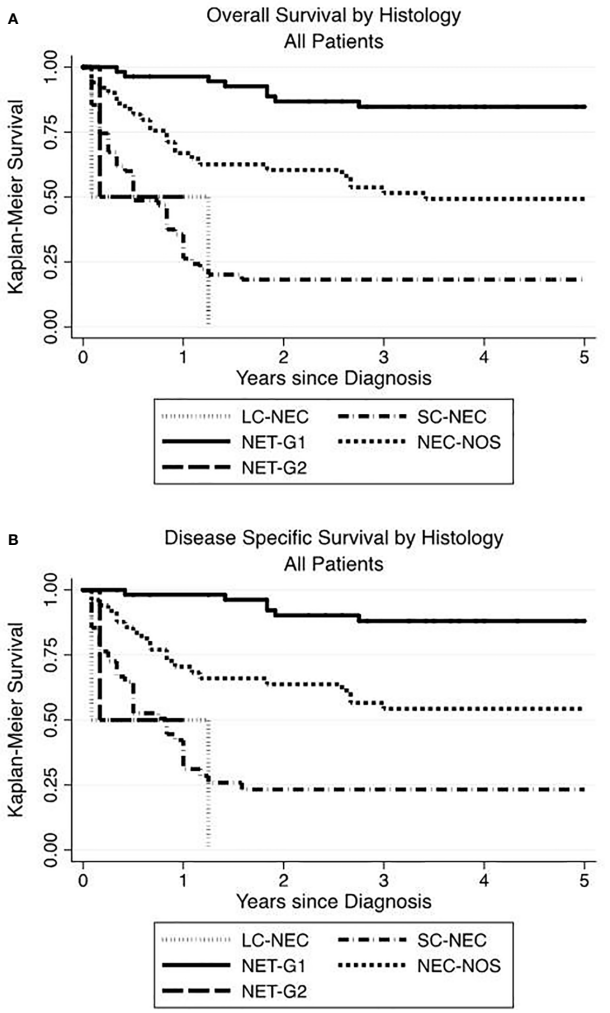

FIGURE 3 | Renal neuroendocrine neoplasm histology influences overall and disease-specific survival. The highest OS **(A)** and DSS **(B)** advantage were seen in low grade NET-G1 and the poorest histologic prognosticator was with small cell neuroendocrine carcinoma.

In our series, we looked at survival across various patient and tumor characteristics. We observed a 5-year OS of 50%. When SC-NEC was excluded, the 5-year OS rose to 62% with a median survival of 8.9 years. On multivariate analysis, more advanced stage was a predictor of poorer survival. Tumors classified as SC-NEC and NEC-NOS were associated with statistically significant hazard ratios of 7.22 and 2.35, respectively. This is consistent with observations in other organ systems that poorer survival is associated with NEC morphology and advanced stage (1). When sorted by tumor histology, the highest OS and DSS were seen in NET-G1 and the lowest in SC-NEC. Those with NEC-NOS showed OS and DSS in between NET-G1 and SC-NEC. Surprisingly, only two cases each were categorized as NET-G2 or LC-NEC, which may reflect historical terminology rather than the natural history of renal NENs. In this study, the paucity of NET-G2 and LC-NEC meant that survival data is underpowered.

Operative management remains the mainstay in curative treatment for NETs. In patients with local or regional disease, a majority underwent an operation. Only 2 of the 32 patients with local disease did not undergo resection and eventually died from pulmonary disease unrelated to the cancer diagnosis. In the patients with regional disease, 7 of 56 patients were not recommended to have an operation, and all died within 22 months of diagnosis. In patients with distant disease, OS and DSS were similar among those had an operation and those who did not. In this group, there may be limited long-term benefit to operative management, and other systemic treatment strategies may be more appropriate for these patients. In future studies, separating NET-G1 from all other categories of renal NENs will provide further insight as to the value of operative management.

Among NENs, those arising from the kidney are incredibly rare. For tumors limited to the kidney and adjacent retroperitoneum, surgery remains the key component to long-term survival for these patients. For patients with distant metastatic disease, systemic therapy may play a greater role in the management of these patients. We observed increased OS and DSS particularly in patients of younger age, earlier stage, and NET morphology. This study highlighted the limitations of studying an uncommon disease through terminology changes over time. Dedication to systematic classification and thorough data collection in SEER and other population databases will lead to more robust conclusions and understanding of these neoplasms. Integrating renal NENs into the larger international NEN dialogue and NEN databases will accelerate our knowledge for proper clinical management and accurate prognosis.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov/data-software/, Epidemiology, and End Results (SEER) database.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AN: Conceptualization, methodology, formal analysis, investigation, visualization, writing—original draft. MO'L: Conceptualization, methodology, writing—review and editing. JA: Conceptualization, methodology, writing—review and editing. PI: Formal analysis, investigation, resources, data curation, writing—review and editing. JK: Conceptualization, supervision, writing—review and editing. DL: Conceptualization, supervision, writing—review and editing. GS: Conceptualization, supervision, writing—review and editing. SC: Conceptualization, visualization, supervision, writing—original draft, writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Endocrinology

CASE REPORT
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Case Report: Pheochromocytoma and Synchronous Neuroblastoma in a Family With Hereditary Pheochromocytoma Associated With a MAX Deleterious Variant

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Duarte DB, Ferreira L, Santos AP, Costa C, Lima J, Santos C, Afonso M, Teixeira MR, Carvalho R and Cardoso MH (2021) Case Report: Pheochromocytoma and Synchronous Neuroblastoma in a Family With Hereditary Pheochromocytoma Associated With a MAX Deleterious Variant. Front. Endocrinol. 12:609263. doi: 10.3389/fendo.2021.609263

Introduction: Pheochromocytomas are rare catecholamine-producing neuroendocrine tumours arising from chromaffin cells of the adrenal medulla or extra-adrenal sympathetic paraganglia. Recent studies have indicated that up to 40% of pheochromocytomas could be attributable to an inherited germline variant in an increasing list of susceptibility genes. Germline variants of the MYC-associated factor (*MAX*) gene have been associated with familial pheochromocytomas and paragangliomas with an autosomal dominant pattern of inheritance, a median age at onset of 33 years and an overall frequency estimated at 1.9%. We describe a deleterious *MAX* variant associated with hereditary pheochromocytoma in a family with four affected individuals.

Case presentation: The first patient presented with bilateral pheochromocytoma in 1995; genetic testing was proposed to his oldest son, when he was diagnosed with a bilateral pheochromocytoma with a synchronous neuroblastoma. Upon the identification of the *MAX* variant c.97C>T, p.(Arg33Ter), in the latter individual, his two siblings and their father were tested and the same variant was identified in all of them. Both siblings were subsequently diagnosed with pheochromocytoma (one of them bilateral) and choose to remain on active surveillance before they were submitted to adrenalectomy. All the tumours secreted predominantly norepinephrine, accordingly to the typical biochemical phenotype ascribed to variants in the *MAX* gene.

Conclusion: This case series is, to our knowledge, the one with the largest number of individuals with hereditary pheochromocytoma with a deleterious *MAX* variant in the same family. It is also the first case with a synchronous pheochromocytoma and neuroblastoma

in carriers of a *MAX* deleterious variant. This report draws attention to some ill-defined features of pheochromocytoma and other malignancies associated with a *MAX* variant and highlights the importance of understanding the genotype-phenotype correlation in hereditary pheochromocytoma and the impact of oriented genetic testing to detect, survey and treat patients and kindreds at risk.

Keywords: MAX gene, pheochromocytoma, hereditary, neuroblastoma, paraganglioma

INTRODUCTION

Pheochromocytomas are rare catecholamine-producing neuroendocrine tumours arising from chromaffin cells of the adrenal medulla (80% to 85% of the cases) or extra-adrenal sympathetic paraganglia (15% to 20% of the cases), the latter also referred as extra-adrenal pheochromocytomas or paragangliomas (1).

In general outpatient clinics, the prevalence of pheochromocytoma in patients with hypertension is 0.1–0.6% (2–4). In recent years there has been an increase in the number of incidentally diagnosed cases, which seems to be related to the greater availability of imaging studies in clinical practice (1). Although epidemiological data on incidence rate is scarce, a recent Dutch study, by Berends et al. (5), found a significant increase in the age standardized incidence rate of pheochromocytomas from 0.29 to 0.46 per 100, 000 person-year between 1995 and 2015.

Recent studies have indicated that up to 40% of pheochromocytomas could be attributable to an inherited germline variant in an increasing list of susceptibility genes (6), which can be grouped into three clusters: pseudohypoxia group (*VHL*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *FH* and *EPAS1*), kinase signalling group (*RET*, *NF1*, *TMEM127*, *MAX* and *HRAS*) and Wnt signalling group (*CSDE1* and *MAML3*) (7). On this basis, international recommendations suggest that it is essential to offer genetic testing to every patient with a pheochromocytoma as a specific inherited mutation impacts surveillance and monitoring for tumour recurrence, therapeutic approaches, and family screening (7–12).

MAX (MYC-associated factor) is a gene associated with regulation of cell proliferation, differentiation, and death (13). Since 2011 (14), germline mutations of the *MAX* gene have been associated with familial pheochromocytomas and paragangliomas with an autosomal dominant pattern of inheritance and a median age at onset of 33 years (range 13-58 years) (7, 11). The overall frequency is estimated at 1.9% and no reliable penetrance estimations are available. The adrenal location is the most common and multifocal tumours are frequent (11, 15). As a kinase signalling pheochromocytoma, it represents a more ultimate cell differentiation with expression of phenylethanolamine N-methyltransferase (PNMT) as the most prominent characteristic, allowing synthesis of epinephrine from norepinephrine (7).

We report a family with hereditary pheochromocytoma carrying a *MAX* deleterious variant in four affected relatives.

CASE REPORT

The patients gave their written consent to sample collection, genetic testing, and the use of genetic test data for the purposes of research. Written informed consent for publication of their clinical details and/or clinical images was obtained from the patients and relative of the patient 2.

Patient 1 – The Index Case

In November 2010, a 27-year old male, the oldest of the three children of patient 2, was admitted to our hospital for evaluation of an inguinal pain. He had no medical or medication history and was a competitive swimmer. He reported a 3 month-history of inguinal pain, resting tachycardia and a weight loss of 10 kg in the last year that he ascribed to intense exercise and diet. At his first observation on the internal medicine ward, his blood pressure (BP) was 200/100mmHg with a resting heart rate of 140bpm (sinus tachycardia with signs of left ventricular volume overload on EKG) but presented an otherwise normal physical examination. Whole body (WB)-CT scan revealed a posterior mediastinal mass (59x80 mm) paravertebral to T9-T11 vertebral bodies, bilateral hypervascular adrenal lesions (46x39 mm and 15.5 mm on the left and 80x110mm on the right) and a retroperitoneal periaortic and peri-common iliac artery lesion (135x185mm) with an extensive encasement of this vessel with a marked compression of the venous structures (**Figure 1**).

A biopsy of the retroperitoneal mass was performed and was suggestive of a paraganglioma (tumour cells reactive with chromogranin and synaptophysin); for this reason, Endocrinology observation was required. The hormonal workup revealed catecholamine hypersecretion (**Table 1**), ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) showed left adrenal and para-aortic and common iliac trace uptake and on echocardiogram there were signs of catecholaminergic cardiomyopathy with severe left ventricular dysfunction (ejection fraction ~20%).

After multidisciplinary review of the case, a metastatic pheochromocytoma and paraganglioma were presumed and surgery was proposed. He needed hospitalization for pre-operative blood pressure and chronotropic control (despite treatment with phenoxybenzamine 100mg, amlodipine 10mg, propranolol 120mg and amiodarone 200mg daily).

He underwent bilateral adrenalectomy with incomplete resection of tumoral mass from the left iliac vessels in February 2011 and pathology was consistent with medullar hyperplasia on the right adrenal gland, and pheochromocytoma with evidence of

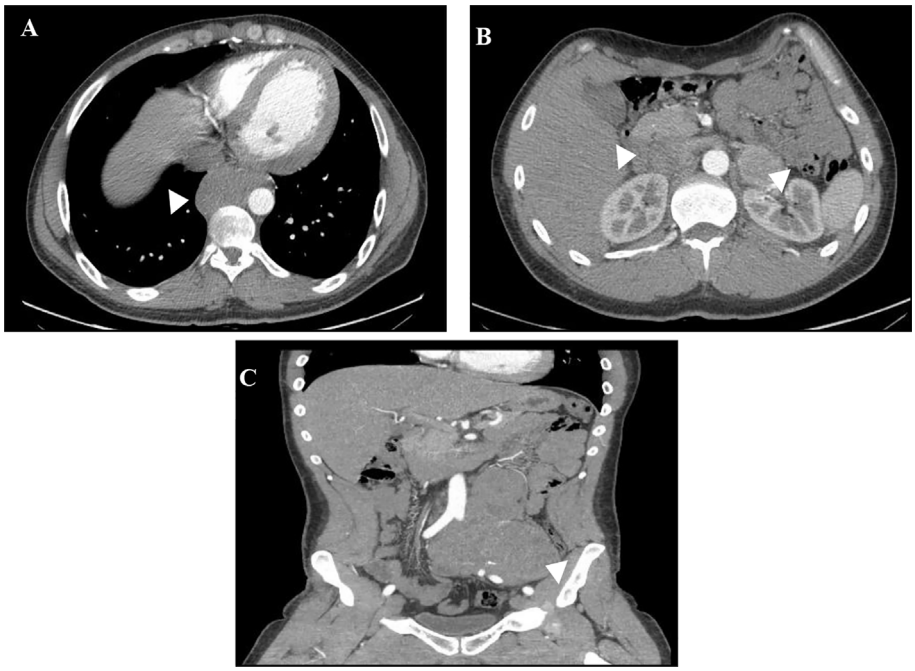

FIGURE 1 | WB-CT scan at diagnosis of patient 1. Posterior mediastinal mass paravertebral to T9-T11 vertebral bodies **(A)**, bilateral adrenal lesions **(B)** and periaortic and peri-common iliac artery lesion with an extensive encasement of this vessel **(C)**.

TABLE 1 | Urinary catecholamines and metanephrines (reference range) at presentation and during follow-up of Patient 1.

		At presentation	Follow-up					
			July 2011	November 2011	August 2012	March 2013	August 2013	December 2013
24-hour urine	Normetanephrines	6506 (480-2424nmol)	1089 (480-2424nmol)	283 (88-444ug)	816 (88-444ug)	1496 (88-444ug)	1067 (88-444ug)	1234 (88-444ug)
	Metanephrines	2618 (264-1729nmol)	78 (264-1729nmol)	<52 (52-341ug)	<52 (52-341ug)	<52 (52-341ug)	<52 (52-341ug)	<52 (52-341ug)

From July'2011 onwards the measurements were performed and kindly provided by the Portuguese Institute of Oncology-Porto.
NM, not measured.

angioinvasion on the left adrenal gland with tumour cells reactive with chromogranin, synaptophysin and S100. The extra-adrenal tumour was suggestive of well differentiated neuroblastoma (tumour cells reactive with neurofilaments, chromogranin, synaptophysin, NSE and S100) with a Ki-67 index of 10%.

On the immediate post-operative period, his blood pressure and his left ventricular ejection fraction normalised and there was no evidence of catecholamine hypersecretion. ^{123}I-MIBG performed on April 2011 showed an intense uptake on his left iliac artery.

Due to significant residual tumoral lesions, the patient was referred to the Portuguese Oncology Institute of Porto (IPOFG, Porto). Next generation sequencing (NGS), using the TruSight Hereditary Cancer Panel (Illumina) identified the *MAX* germline truncating variant c.97C>T, p.(Arg33Ter) (NM_002382.3) in a blood sample. Subsequently, given the unexpected association of neuroblastoma with a variant of the *MAX* gene, the presence of loss of heterozygosity (LOH) was evaluated in a formalin–fixed and paraffin–embedded sample of the neuroblastoma tumour (from a delimited area with >50% tumour cells), using the same NGS panel, and the variant allele frequency (VAF) was 71.2%, which is compatible with LOH. During the follow-up at this centre he presented with recurrence of catecholamine hypersecretion and hypertension. After multidisciplinary team review, it was decided to start palliative chemotherapy for neuroblastoma; despite chemotherapy cycles with cyclophosphamide, doxorubicin, vincristine and topotecan from 2011 to July´2013 there was a disease progression with multiple abdominal and pelvic lymphadenopathies (the largest adenopathic conglomerates with 138mm located by the left iliac vessels and 100x98mm next to renal hilum with bilateral hydronephrosis, severe on the left side). The posterior mediastinal mass' size remained stable and was deemed unresectable; it was assumed as a probable pheochromocytoma metastasis but with no histological evidence.

On March 2014 he was submitted to exploratory laparotomy with debulking; pathology was consistent with poorly differentiated neuroblastoma with no lymph node parenchyma identified. ^{123}I-MIBG on April 2014 documented multiple abdominal and pelvic focus of intense uptake, mainly on the left side. ^{131}I- MIBG therapy was performed on June'2014 with no apparent response on post-treatment MIBG scan.

He was afterwards submitted to right nephrostomy and later, on October 2014, to a double-J stent placement, as a preparation for a new surgical debulking. On the 2nd November 2014 he was admitted to the emergency department with an urosepsis with an acute on chronic kidney disease and severe hyperkalaemia. Despite initiation of broad-spectrum antibiotics, vasopressor support and emergent continuous veno-venous hemofiltration, he died 24 hours later, at the age of 31 years old.

Patient 2

In March 1995, a 32-year old male was referred to our Endocrinology outpatient clinic with complains of daily paroxysms of headaches, diaphoresis, and facial pallor for the last 6 months. A year before he was diagnosed with hypertension, but he had refused anti-hypertensive treatment. At observation he presented a class I obesity (BMI 31.5kg/m^2), his BP was 156/96mmHg with a resting heart rate of 80bpm and an otherwise normal physical examination. He had no signs of Cushing's syndrome, such as rounded face, thin skin, easy bruising, or purple striae.

Our investigation revealed an increase in 24-hour urinary normetanephrines and metanephrines (**Table 2**); 1-mg overnight dexamethasone suppression test did not detect autonomous cortisol secretion. Abdominal-CT and MRI revealed bilateral round shaped adrenal lesions of 25 mm with a high signal intensity on T2-weighted images suggestive of pheochromocytoma and the ^{123}I-MIBG showed bilateral trace uptake. Phosphocalcic metabolism, calcitonin, thyroid function (and ultrasound) were normal.

In May 1995, a right adrenalectomy and subtotal left adrenalectomy was performed. Pathology revealed adrenal hyperplasia and the genetic testing for RET mutation was negative.

At his immediate post-operative follow-up visit he presented normal blood pressure, but 3 months after surgery he relapsed with recurrence of hypertension and paroxysms associated with elevated urinary metanephrines. Abdominal-CT revealed a residual lesion on the left adrenal gland but ^{123}I-MIBG showed no abnormal uptake. After discussing the treatment options with the patient, he opted to resume alpha and beta-block therapy and maintain close clinical, analytical, and imaging surveillance. In June 2000, after clinical worsening with increased frequency of paroxysms associated with retrosternal chest pain, he was submitted to totalization of left adrenalectomy; this time pathology reported a tumoral lesion of 25x15 mm consistent with a pheochromocytoma of low mitotic index. Post-operatively he was started on glucocorticoid and mineralocorticoid hormone replacement and his anti-hypertensive drug was stopped. He has been asymptomatic and with no evidence of catecholamine hypersecretion ever since. After the identification of a deleterious *MAX* variant in the index case (his oldest son, patient 1), he was found to carry the same variant.

Patient 3

In 2012, a 26 years old man without significant medical or medication history was brought to our clinic for screening after identification of a *MAX* germline variant in his father (patient 2) and older brother (patient 1). He was overweight (BMI 29.4kg/m^2) but his physical examination was otherwise normal (blood pressure was 124/74 mmHg and heart rate 90bpm). Biochemical testing revealed a small rise in urinary normetanephrines (see **Table 3**) and the abdominal – CT revealed bilateral adrenal masses (22 mm on the right and 14 mm on the left) both with high density (> 10HU) but without uptake on ^{123}I-MIBG. The genetic testing confirmed that he was a carrier of the *MAX* variant previously identified in the family. The patient refused surgery at the time and decided to remain on surveillance. Until 2018 the patient remained asymptomatic when he reports the onset of episodes of palpitations and is documented an elevation of blood pressure (BP 166/87mmHg) and orthostatic tachycardia requiring treatment with a calcium channel blocker, initiated by his primary care physician. Over the years there was a progressive increase in the levels of plasma and urinary normetanephrines (**Table 3**) and in the dimensions of the adrenal lesions (39x27mm on the right and 30x25mm and left

TABLE 2 | Serum and urinary catecholamines and metanephrines (reference range) at presentation and during follow-up of Patient 2.

		At presentation	Follow-up					
			June 1997	June 1998	March 1999	May2000	September 2000	February 2020
24-hour urine	Normetanephrines	1.36 (<1mg)	*NM*	3090 (480-2424nmol)	3305 (480-2424nmol)	*NM*	2134 (480-2424nmol)	*NM*
	Metanephrines	0.54 (<0.4mg)	*NM*	369 (264-1729nmol)	387 (264-1729nmol)	*NM*	69 (264-1729nmol)	*NM*
Serum	Normetanephrines	–	–	–	–	–	–	<100 (<982.8 pmol/L)
	Metanephrines	–	–	–	–	–	–	757 (<456.3 pmol/L)

Laboratory results from the post-operatively period until 1997 were stated on clinical notes but could not be retrieved. Normal catecholamine secretion since the second post-operative period: the immediate post-op (September'2000) and the most recent hormonal workup are shown. Serum metanephrines were not measured at our lab before 2015.
NM, not measured.

TABLE 3 | Serum and 24-hour urinary metanephrines (reference range), by year of patient 3.

		At presentation	Follow-up							
			March 2014	May 2015	April 2016	June 2017	April 2018	May 2019	October 2019	May 2020
24-hour urine	Normetanephrines (480-2424nmol)	3130	3685	6696	11121	*NM*	38243	2976	2002	*NM*
	Metanephrines (264-1729nmol)	1170	1384	1203	1659	*NM*	3839	50	<25	*NM*
Serum	Normetanephrines (< 982.8 pmol/L)	–	–	1390.4	5700	10398	11290	481	302	606
	Metanephrines (< 456.3 pmol/L)	–	–	304.1	548	753	729	<100	<100	<100

adrenal gland on CT scan, both with enhanced contrast on T2 sequences and no signal intensity loss on the opposed-phased image on the MRI – **Figure 2**). Echocardiogram had normal ventricular wall thickness with preserved ejection fraction, EKG on sinus rhythm with no signs of ischaemia.

At this point the patient accepted surgery and a laparoscopic bilateral adrenalectomy was performed on April'2019 after alpha and beta blockade. Pathology was consistent with bilateral pheochromocytoma with tumour cells reactive with chromogranin, synaptophysin and S100; proliferative index was low (Ki-67<1%).

Post-operatively he was started on glucocorticoid and mineralocorticoid replacement and his anti-hypertensive drug was stopped. Until the last appointment there was no biochemical evidence of catecholamine hypersecretion or relapse of hypertension.

Patient 4

This is the youngest son of patient 2 and in 2012 he was observed at our clinic after the identification of a *MAX* germline mutation in his father and his older brother. He was 14 years old, had a class I obesity (BMI 31.9kg/m2) with no other medical or medication history nor endocrine hypersecretion features and at examination his blood pressure was 132/67mmHg and heart rate of 85bpm with an otherwise normal physical exam. Genetic testing confirmed that he was a carrier of the same *MAX* variant previously identified in the family. From the age of 15 years old, he underwent annual screening with 24-hour urinary metanephrines and abdominal-CT every two years with both remaining normal until 2017 (**Table 4**) when he presented evidence of norepinephrine hypersecretion and the abdominal-CT revealed on the left adrenal gland, a 17mm nodule with a contrast washout >60% with no other lesions seen on the

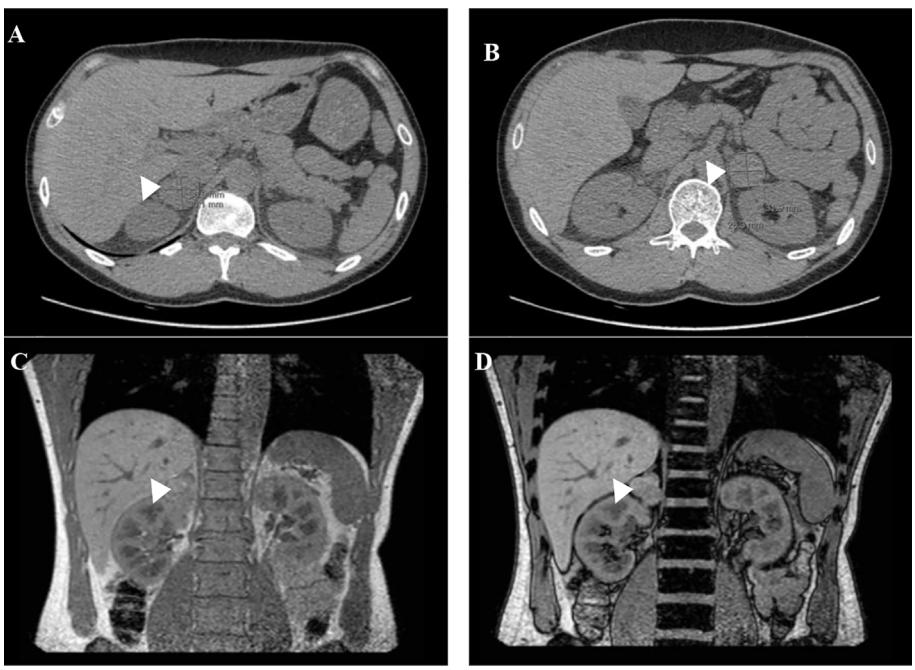

FIGURE 2 | Abdominal-CT **(A, B)** and MRI scan **(C, D)** before bilateral adrenalectomy: adrenal lesions on the right **(A)** and left **(B)** adrenal gland. Coronal in-phase **(C)** and out-of-phase **(D)** MRI images with no loss of signal in the mass.

TABLE 4 | Serum and 24-hour urinary metanephrines (reference range), by year of patient 4.

		Follow-up						
		March 2014	**April 2016**	**October 2017**	**March 2018**	**March 2019**	**March 2020**	**May 2020**
24-hour urine	Normetanephrines (480-2424nmol)	1011	1117	6434	3874	*NM*	*NM*	*NM*
	Metanephrines (264-1729nmol)	688	209	1056	586	*NM*	*NM*	*NM*
Serum	Normetanephrines (< 982.8 pmol/L)	–	644	1347	1399	3724	3383	1118
	Metanephrines (<456.3pmol/L)	–	136	<100	110	209	171	141

Serum metanephrines were not measured at our lab before 2015.
NM, not measured.

contralateral gland (**Figure 3**). ^{123}I-MIBG scan revealed no abnormal uptake but the ^{68}Ga- DOTA-NOC-PET scan documented an uptake on the left adrenal nodule. Like his older brother he chose to remain on active surveillance; although asymptomatic and with normal blood pressure, after considering the unknown risk of malignancy of a deleterious *MAX* variant, he accepted surgery and underwent laparoscopic left adrenalectomy on March 2020 after alpha and beta blockade. Pathology was consistent with pheochromocytoma with tumour cells reactive with chromogranin, synaptophysin and S100; there was also presence of granular cytoplasmatic immunoreactivity for SDHB; proliferative index was low and PASS Score was 0/20.

The immediate post-operative period was uneventful but a month later he reported episodes of headaches with elevated blood pressure and face pallor; serum metanephrines was found slightly elevated. ^{123}I-MIBG scan revealed no abnormal uptake.

DISCUSSION

This case series is, to our knowledge, the one with the largest number of individuals with hereditary pheochromocytoma with a deleterious *MAX* variant in the same family. Although the association between variants in the *MAX* gene and hereditary pheochromocytoma has been established during the past decade, few systematic characteristics are available (16). This report draws attention to some ill-defined features of pheochromocytoma (PPGL) and other malignancies associated with a *MAX* variant.

FIGURE 3 | Abdominal-CT scan before left adrenalectomy showing a 17mm nodule on the left adrenal gland.

Firstly, the occurrence of a potential metastatic pheochromocytoma. *MAX* behaves as a classical tumour suppressor gene that encodes for the MAX protein, which interacts with the MYC proto-oncogene and the MAX dimerization protein 1 (MXD1) family of proteins; this MYC/MAX/MXD1 network regulates cell proliferation, differentiation and apoptosis (14, 17). Alterations in this complex promote hereditary susceptibility to neoplasia (18). Previous studies (11, 14, 16, 18, 19) have presented patients with metastatic PPGL but further research is vital to determine the risk of malignancy associated with *MAX* deleterious variants.

In addition to the frequently bilateral PPGL and the early onset, our patients' tumours secreted predominantly norepinephrine in a greater proportion than epinephrine. This biochemical phenotype is intermediate between the established epinephrine producing tumours due to *NF1* and *RET* variants and the phenotype of norepinephrine tumours harbouring *VHL* or *SDHB/D* variants; this diagnostic phenotype is explained by a significant but limited capacity to produce epinephrine due to the intermediate tissue expression of mRNA for PNMT (11). An interesting aspect is the inconsistent uptake of ^{123}I-MIBG at the adrenal lesions of our four reported cases. Perhaps different levels of differentiation (and consequently PNMT expression) can be held accountable for this varying accumulation.

Second, the synchronous occurrence of a neuroblastic tumour. The association of PPGL and neuroblastic tumours is uncommon and fewer cases had a genetic link. The majority of these reported cases are composite pheochromocytoma-ganglioneuroma and comprised an association with MEN2 (20–24), VHL (25), NF1 (26–28) and, most recently (29), one case of a new *MAX* gene heterozygous variant, c.299G>C (p.Arg100Pro, NM_002382). Our case is, to the best of our knowledge, the first with a simultaneous PPGL and neuroblastoma presenting as two distinct entities in association with a *MAX* pathogenic variant. LOH observed in the neuroblastoma (VAF=71.2%) supports a causal relation between the *MAX* germline pathogenic variant and the origin

of this tumour The embryological common origin and the importance of MYC/MAC/MXD1 network in the development of this neural crest cells tumours are identified as the basis of this association (18). Additionally, evidence suggests that germinative and somatic inactivating *MAX* abnormalities lead to tumour risk, namely renal oncocytoma (18, 19), pituitary adenomas (30–32), pancreatic neuroendocrine tumours (33), small cell lung cancers (34) and Gastrointestinal Stromal Tumours (GIST) (35).

Furthermore, our work highlights the importance of first-degree relatives targeted testing. *MAX* variants present with a parent of origin dependent tumorigenesis and tumour formation occurs almost exclusively through paternal transmission (36). The identification of a germline variant at a PPGL predisposing gene allows the screening of asymptomatic relatives and helps to define a follow-up plan for both mutation carriers and affected individuals (37). Expert recommendations endorse that genetic testing of children is only recommended if they will be offered surveillance during childhood years (11). Although the evidence is limited in *MAX*-related disorders, annual pre-symptomatic biochemical (normetanephrine and metanephrines and methoxytyramine) and biennial imaging (with MRI) surveillance of first-degree relatives (11, 37) should start from five years before the youngest age of onset in the family (11) or no later than 15 years old (38). Patient 4 started his surveillance program by the age of 15 years old but, like his older sibling (patient 3), chose to remain in active surveillance once normetanephrine hypersecretion was confirmed. Due to this mode of inheritance, it is important to analyse the mutation status of patient 3's son (and future offspring of patient 4) to ascertain who would be at risk.

At last, the extent of surgical procedure, in face of an uncertain PPGL malignancy risk, remains controversial. Regarding our patients, patient 3 postponed his surgery for six years since the diagnosis of bilateral pheochromocytoma; patient 4 also chose to remain on active surveillance until now and was submitted to a unilateral adrenalectomy. Laparoscopic total adrenalectomy has been a standard treatment for unilateral or bilateral adrenalectomy; the main sequel is adrenocortical insufficiency with subsequent need for lifelong mineralocorticoid and corticoid supplementation with risk of both Addisonian crises and excessive steroid replacement (39). Cortical sparing adrenalectomy allows more than 50% of patients to maintain normal adrenal function at 10 years with a PPGL recurrence risk estimated between 0-21%, with even lower reported rates (0-3%) with more recent endoscopic approaches (39–41). Individual risk of malignancy must be taken into account when deciding this surgical procedure. Cortical sparing adrenalectomy is increasingly performed in hereditary PPGL like those with *RET* or *VHL* variants who are associated with a low risk of malignancy and high risk of bilateral PPGL (39). Castinetti (41, 42) reported a low recurrence risk with normal glucocorticoid function in more than 50% of the patients at 10 years. However, this approach is not systematically proposed.

In conclusion, we describe a family with a *MAX* variant associated with hereditary PPGL with one of the patients presenting with a bilateral PPGL with synchronous neuroblastoma, the first case reported to our knowledge. Our case highlights the importance of understanding the genotype-phenotype correlation in hereditary PPGL and the impact of oriented genetic testing to detect, survey and treat patients and kindreds at risk.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

DB, LF and CC co-wrote the manuscript. MA was the pathologist responsible for the diagnosis of patient 2. JL, CS and MT carried out the genetic analysis that first demonstrated the presence of the MAX gene deleterious variant for patient 2 (in 2011) and the remaining patients (in 2012). RC is the main physician of the patients; APS was the main physician of patient 1 after his referral IPOFG. LF, APS, MT and MC contributed to the revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Characteristics, Treatment, Outcomes, and Survival in Neuroendocrine G1 and G2 Pancreatic Tumors: Experiences From a Single Tertiary Referral Center

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Purpose: Neuroendocrine tumors of the pancreas (Pan-NETs) are usually hormonally inactive with a capacity to metastasize. Since Pan-NETs are rare, more knowledge is needed.

Methods: We reviewed all patients' medical files with Pan-NET treated at a tertiary center (2006-2019). Grade 1 (G1) and grade 2 (G2) tumors were compared. The latter group was subdivided arbitrarily based on proliferation index into G2a (3-9.9%) and G2b (10-19.9%).

Results: We found 137 patients (76 females, 61 males; G1 n=66, G2 n=42), the median age at diagnosis 61 years (interquartile range (IQR) 50–71), and tumor size 2 cm (1.3–5 cm). The initial surgery was performed in 101 patients. The remaining (n=36) were followed conservatively. Metastatic disease was evident in 22 patients (16%) at diagnosis while new lesions developed in 13 out of 22 patients (59%). In patients without previous metastatic disease, progressive disease was discovered in 29% of G1 vs. 55% of G2 patients (P=0.009), 47% of G2a vs. 75% of G2b patients (NS). Survival was poorer in patients with metastasis at diagnosis vs. those with local disease (P<0.001). During follow-up of 74 months, Pan-NET related death was found in 10 patients. Survival was not different between G1 vs. G2 or G2a vs. G2b, or if tumors were functional. Size ≤2 cm was associated with a better outcome (P=0.004). During the follow-up of small tumors (≤2 cm, n=36) two were resected.

Conclusion: In small non-functional Pan-NETs, active surveillance is reasonable. Progressive disease was more common in G2, but survival was similar in G1, G2 and between G2 subgroups. Survival was poorer in patients with metastasis at diagnosis.

Keywords: neuroendocrine neoplasia, pancreatic, treatment, outcome, survival, Ki-67, size, functionality

INTRODUCTION

The yearly incidence of cases with neuroendocrine pancreatic tumors (Pan-NETs) is 0.5-0.8/100 000 (1). Of these, only a minority is functional, i.e., hormone-secreting, while 60-90% are non-functional (2–4). When non-functional lesions are symptomatic, the most common presenting symptoms are abdominal pain (35–78%), anorexia and nausea (45%), as well as weight loss (20–35%) (5). Symptoms in functional tumors depend on the many variants of hormone-producing Pan-NETs and which hormones are being secreted (6).

Pan-NETs can also be incidental findings when radiology is performed for other reasons, i.e., pancreatic incidentalomas. There is an increase in patients diagnosed with Pan-NETs, mostly in early stages, probably secondary to more frequent imaging (7). Most functioning and non-functioning Pan-NETs occur sporadically, but they can also be diagnosed in the work-up of patients with familial syndromes such as multiple endocrine neoplasia type 1 (MEN1) or von Hippel Lindau disease (vHL). Neuroendocrine lesions in the pancreas are often slowly growing with the potential to metastasize (2). Surgery should generally be performed if tumors are hormonally active and if the tumor is larger than 2 cm. There are controversies whether to operate or not in non-secreting tumors of 1-2 cm in size (8). The best prognostic factor for progression is Ki-67 (9). Neoplasms with neuroendocrine features are graded by proliferation index from G1 (Ki-67 index <3%), G2 (3-20%), and G3 (>20%). G3 tumors can furthermore be classified as either Pan-NET G3 or pancreatic neuroendocrine carcinoma (Pan-NEC). The former category often exhibits well-differentiated histology and mutations in either *DAXX* or *ATRX*, while Pan-NECs display poor differentiation and mutations in *TP53* and/or *RB1* (10).

Pan-NETs are clinically heterogeneous and can exhibit indolent behavior but also progress to more clinically aggressive tumors. The prognostication mainly relies on the Ki-67 proliferation index but also depend on functionality and tumor size. Irrespectively, long-term follow-up of patients with Pan-NETs is required.

This study aimed to describe a sizable institutional series of Pan-NETs from biochemical, surgical, and histopathological features and relate these parameters to patient outcome and survival.

METHODS

This retrospective investigation includes 137 patients with Pan-NETs treated between 2006 and 2019 at the Karolinska University Hospital, Stockholm, Sweden. The catchment area is designated for highly specialized care, including pancreatic surgery of more than 2 million inhabitants (11, 12). All hospital admissions and out-patient visits in Sweden are coded with the International Classification of Diseases version 10 (ICD-10) codes by the attending physician and are stored in both local and national databases (13). All patients with the ICD-10 codes C25.4 (malignant neoplasm of pancreas, islet of Langerhans), C25.9 (malignant neoplasm of pancreas, unspecified), and D13.7 (benign neoplasm of the pancreas) were selected. All the relevant electronic medical files of the patients with Pan-NET were reviewed manually. The date of diagnosis was defined as the time of the multidisciplinary meeting, or the day of surgery if the Pan-NET diagnosis was made first after surgery. We noted radiology, tumor size, biochemical tests, initial and repeated Ki-67 indexes obtained through histopathological investigations in operated patients. The Ki-67 was calculated by counting the percentage of positive tumor nuclei in 2000 cells in hot spot areas. The cohort was divided into G1 cases (tumors with a Ki-67 index of <3%) and G2 group (Ki-67 index between 3–20%). No Pan-NET G3 cases or Pan-NECs were included. As the span in G2 tumors are wide the G2 group was further arbitrarily subdivided into G2a (Ki-67 index 3–9.9%) and G2b (Ki-67 index 10–19.9%). The initial grade could not be evaluated in 36 patients diagnosed by imaging and biochemical testing. These patients were followed clinically with repeated imaging and laboratory tests. Medical treatment before and/or after surgery was registered as the duration of different therapies. Mortality was evaluated secondary to Pan-NET disease, and patients with adenocarcinomas or cystic lesions were excluded. The National Population Register was consulted to find out if the included patients were still alive, and the date of death was retrieved if applicable (14). For subsets of cases in which the original pathology report was devoid of relevant information (tumor size, Ki-67 proliferation index), a histopathological re-evaluation was assessed by one of the authors (CCJ).

The Regional Ethical Review Board in Stockholm approved the study, and due to its retrospective nature, no informed consent was required. However, signed informed consent were obtained from the patients prior to surgery.

STATISTICS

All proportions were calculated, discounting missing values. Median and interquartile range (IQR) were used. Survival was analyzed with the Kaplan-Meier model, and comparisons were made with the log-rank test. Patients who died without local recurrence or related to Pan-NET were censored to the date of death, and patients were censored to the last follow-up if local recurrence or death had not occurred. Further survival analysis

was made with Cox proportional hazard regression. The covariates tumor size, Ki-67 index, and tumor functionality were reported as hazard ratios (HR) with 95% confidence intervals (95% CI). An unpaired two-tailed t-test was also used. Analyses were made using R version 3.6.3 (GUI 1.70 El Capitan build 7735, developed by R Foundation for Statistical Computing, 2016). A P -value <0.05 was considered significant.

RESULTS

During the selected time-period, 137 patients (females n=76) were treated and followed for a Pan-NET at the Karolinska University Hospital. Twelve of these patients were diagnosed before 2006. The median age at diagnosis was 61 years (IQR 50 – 71), and tumor size was 2 cm (IQR 1.3-5 cm). Primary tumor size could be evaluated in 129 patients, 61 had tumors ≤ 2 cm, and 68 were > 2 cm (**Table 1**, **2** and **Figure 1**). Tumors were located in the pancreatic tail (cauda) of 63 patients (46%), 41 (30%) in the head (caput), 16 (12%) in the main pancreatic body (corpus) and 10 (7%) had multiple tumors. No detailed anatomic description was recorded in 3 cases (2%), and the remaining tumors were located in the uncinate process. The diagnosis was made using histopathology according to the criteria laid out by the most recent WHO guidelines at that time. An illustration of some key histopathological and immunohistochemical Pan-NET features is provided in **Figure 2**. The Ki-67 index was available from 108 patients and displayed a median value of 2.0% (IQR 1 – 5%) of which 66 cases were G1 and 42 cases G2 tumors. Metastatic disease was present at diagnosis in 22 patients (16%), of which 9 were in G1 tumors, 9 in G2 tumors (P=0.051), and the Ki-67 proliferation index was missing in three patients. Fifteen patients (11%) fulfilled the clinical criteria for MEN1 (females n=10), of which three were negative on genetic testing. The median age in patients with MEN1 was 46 years (IQR 37 – 58 years) at diagnosis, which was significantly younger than the rest (P<0.001).

TABLE 1 | Summary of basal characteristics of 137 patients with pancreatic neuroendocrine tumors at time of diagnosis and follow-up.

	Subjects (%)	Median (IQR)
Females	75 (56%)	
Males	62 (44%)	
Age (years)		61 (50-71)
Size (cm)		2 (1.3-5)
Stage n=137		
Tx, unknown	7 (5%)	
T 1, <2 cm limited to the pancreas	64 ((47%)	
T 2, 2-4 cm limited to the pancreas	9 (7%)	
T 3, > 4 cm limited to the pancreas	35 (26%)	
T 4, invading adjacent organs	22 (16%)	
MEN-1	15 (11%)	
Functioning	30 (22%)	
Surgery	101 (74%)	
Total pancreaectomy	4 (3%)	
Whipple	28 (19%)	
Partial resection	60 (44%)	
Enucleated	6 (4%)	
Liver procedure	3 (2%)	
Re-operated	11 (11%)	
Conservatively (no surgery initially)	36 (26%)	
Surgery at a later stage	2 (4%)	
Follow-up time (months)		74 (41-110)
Deceased	26 (19%)	
Age at death (years)		73 (66-78)
Age at Pan-NET death (years)		75 (66-76)

IQR, interquartile range; MEN-1, multiple endocrine neoplasia; Pan-NET, pancreatic neuroendocrine tumor.

TABLE 2 | Initial findings in patients with pancreas neuroendocrine tumors and at follow-up.

	n (%)	Median (IQR)
Patients (n), total	137 (100%)	
G1	66 (48.1%))	
G2	42 (30.6%)	
G2a	30 (21.8%)	
G2b	12 (8.7%)	
Tumor size (cm)		2 (1.3-5)
G1		2.3 (1.5 - 5)
G2		4.3 (1.7-6)
G2a		4 (1.6-6)
G2b		5.5 (3.9-7.3)
Age (years)		61 (50-71)
G1		62 (54-72)
G2		67 (47-69)
G2a		56.5 (48-65)
G2b		61 (46-66)
Ki-67 index at first diagnosis	108 (78.8%)	2 (1-5)
G1		1 (1.1.9)
G2		5.1 (3.5-9.9)
G2a		5 (3.3-6.3)
G2b		11.3 (10-13.5)
Ki-67 index at re-evaluation	26 (18.9%)	9.4 (4-13.8)
G1		4 (3-5.5)
G2		12.5 (9-15.5)
G2a		11.4 (7.8-21.8)
G2b		13 (12.8-14)
Follow-up (months)		74 (41-110)
G1		65 (41-120)
G2		69 (50-92)
G2a		50 (46-96)
G2b		62 (48-83)
Functional tumors (n)	30 (21.8%)	
G1	15 (10.9%)	
G2	10 (7.3%)	
G2a	7 (5%)	
G2b	3 (2.2%)	
Metastasis at diagnosis, total	32 (23.3%)	
G1	9 (6.6%)	
G2	9 (6.6%)	
G2a	6 (4.4%)	
G2b	4 (2.9%)	
Progressive, total*	42 (30.6%)	
G1	19 (13.8%)	
G2	23 (16.7%)	
G2a	14 (102%)	
G2b	9 (6.5%)	

*Ki-67 index was evaluated in 108 patients at diagnosis and in 26 during follow-up. All continuous variables are shown as median and interquartile ranges. G1 had a Ki-67 index of <3%, G2 3-20%, G2a 3-9.9% and G2b 10-19.9%. Initial Ki-67 missing in six patients who progressed and by then this index was 10%, 13% and 25%, respectively in three patients. Ki-67 was missing in 5 patients with a functional tumor. F, females. *Patients without initial metastasis. In these patients metastasis developed in median 42 months after surgery (IQR 36-122 months).*

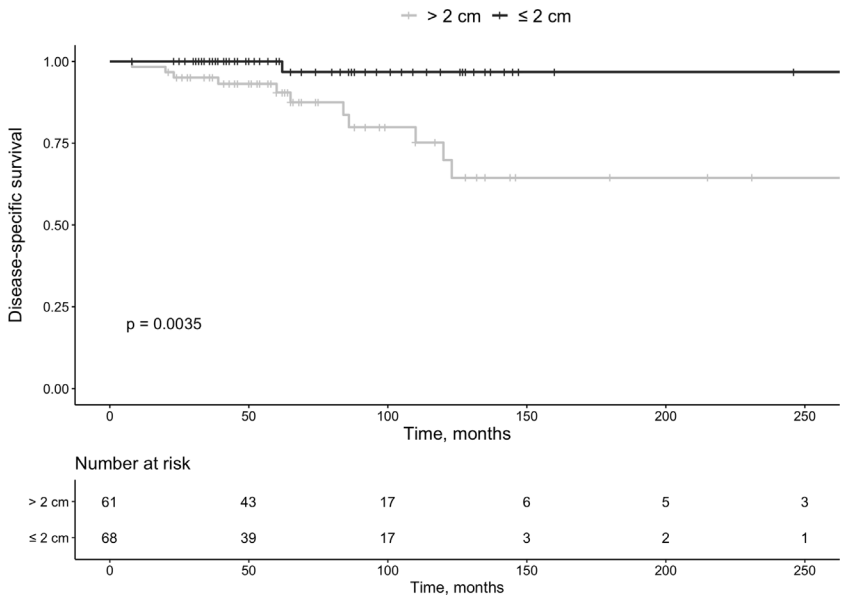

FIGURE 1 | Survival in patients with pan-NETs according to size at diagnosis (<2 cm or >2 cm).

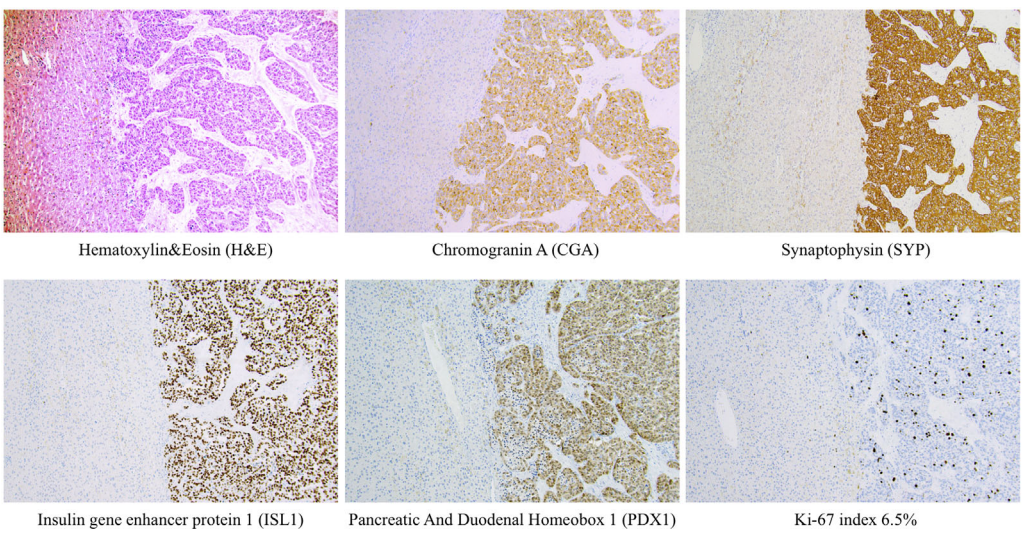

FIGURE 2 | Examples of routine histological and immunohistochemical features of a metastatic pan-NETs WHO grade 2 (Pan-NET G2) from the Karolinska cohort. Metastatic Pan-NETs tissue is evident to the right, while the left section of each image depicts liver tissue. Note the well-differentiated tumoral growth pattern on routine H&E staining. Tumor cells were diffusely positive for markers of neuroendocrine differentiation (CGA, SYP, ISL1) and displayed stainings indicating a pancreatic origin (ISL1, PDX1). The tumor grade was determined to G2, which in this manuscript would translate to the hypothetical G2a category. This patient had been previously diagnosed with a primary pan-NET (data not shown). All photomicrographs were magnified x100.

Initial surgery was performed in 101 patients 74%; partial pancreatic resection n=60, pancreaticoduodenectomy [Whipple´s procedure] n=26, total pancreatectomy n=4, enucleation n=6, and metastatic liver procedure n= 3. The most commonly used medical therapy was somatostatin analogs (SSA), used in 31 patients (**Table 3**).

Preoperatively 3 patients were treated with SSA 3-8 months prior to surgery, 4 were treated with radiofrequency ablation (RF) and 1 with streptozocin-5 FU. After surgery combination therapy was also frequent as three of the 31 somatostatin treated patients were also treated with interferon, three with everolimus, and two with peptide receptor-targeted radiotherapy (PRRT).

TABLE 3 | Pharmacotherapy, ablation, embolization and receptor-targeted therapy in pan-NETs patients.

	Total	Treatment before surgery	Treatment inititated after surgery (median months, IQR)	Duration months (IQR)	Treament initiated in non-operated patients (median months, IQR)	Duration in non-operated patients, (median months IQR)
Somatostatin analogs (n)	31	3 (3 - 8 months)	18 (49, 2-105)	66 (21-98)	12 (43, 21-106)	43 (21–106)
Streptozotocin-5-Fluorouracil (n)	9	1	7 (24, 18-102)	9 (6-14)	1	4
Radiofrequency ablation (RF) (n)	7	3 per-operativ	3 (36, 27–57)		1	
Temozolamide (n)	5		4		1	6
Peptide receptor-targeted radiotherapy (PRRT) (n)	5		2		3	
Cisplatin-Etoposide (n)	2		1		1	6
Everolimus (n)	3		1		1	3
Interferon (n)	3		3			
Transarterial embolization, radioembolization (n)	2		2			

Thirty-one were treated with somatostatin analogs, three of these patients were also treated with interferon, three with everolimus, two with temozolomide, and five with peptide receptor-targeted radiotherapy (PRRT). Other treatments according to the table, see text for details.

The most common cytotoxic agents were streptozotocin in combination with 5-fluorouracil in seven patients, which were further combined during follow-up with RF and SSA in three, with cisplatin-etoposide in one and with temozolomide in four patients (**Table 3**). Twelve non-operated patients were treated with SSA, three also with PRRT, and one of these also with everolimus and cisplatin. One of these non-operated with SSA therapy also received 14 months temozolamide. One further non-operated patient was given streptozotocin-5 FU 4 months and later temozolamide for 6 months and a second patient was treated with RF.

Of all tumors, 30 (22%) were hormonally active (insulinomas n=18 [60%], gastrinomas n=4 [13%], somatostatinomas n=2 [7%], VIPoma n=1 [3%], glucagonoma n=1 [3%], PTHrp related hypercalcemia n=1 [3%] and lesions secreting multiple hormones n=3 [10%, gastrin + glucagon n=1, and insulin + glucagon n=2]). Functional tumors were not smaller than non-functional lesions (P=0.954), and survival was not associated with a functional tumor (P=0.45, **Figure 3**).

Follow-Up

During the median follow-up of 74 months (IQR 41–110 months), 42 (36%) without previous metastatic disease developed metastases, 19 out of 66 (29%) in G1, and 23 out of 42 (55%) in G2 (P= 0.009). All had had primary surgery 45 months (IQR 35-122 months) previously.

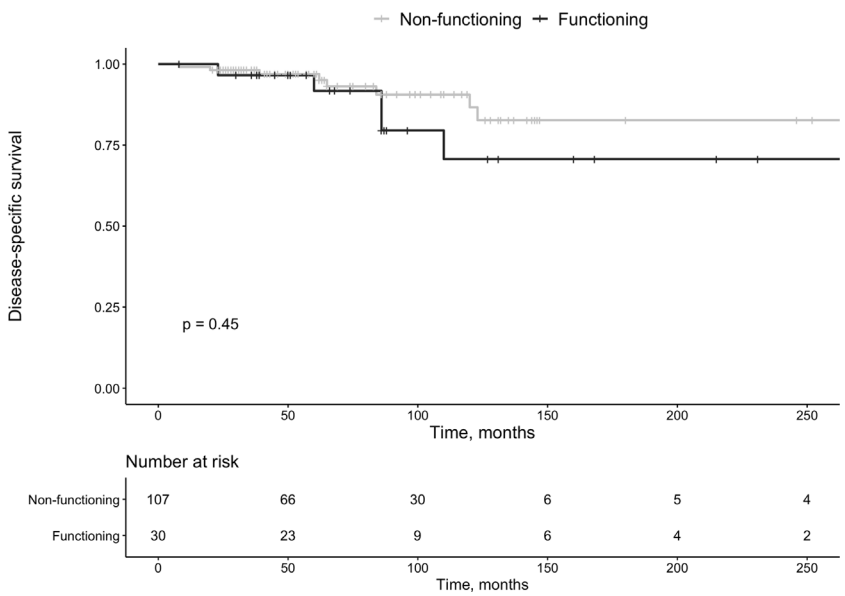

FIGURE 3 | Survival in patients with functioning vs. non-functioning pan-NETs.

Among 22 patients with metastasis at diagnosis, 13 (59%) had further progression during surveillance of 69 months (IQR 37-92 months). Surgery was repeated in 11 of 22 patients 45 months after the initial procedure (IQR 37-92 months). Twenty-six (19%) patients deceased, at a median age of 73 years (IQR 66-78 years) (females, n=16). Ten of 26 deaths (37%) were Pan-NET related (G1 n=2, G2 n=8). Survival was reduced in the patients with metastasis at diagnosis vs. those with localized disease (P<0.001, **Figure 4**). Disregarding other mortality causes, Pan-NET specific death in the whole cohort was 7%. Thus, 111 patients (81%) survived during follow-up. Of the Pan-NET associated deaths, 7 out of 10 (70%) cases exhibited malignant insulinomas. Four of these seven (57%) patients with malignant insulinomas had originally non-functioning tumors, which later transformed into insulin-secreting lesions, which have been described in detail elsewhere (15).

Mortality in Pan-NET disease was associated with primary tumor size (P=0.035) (**Figure 3**). By Cox-regression using tumor size, Ki-67 index and functionality as covariates, tumor size ≤ 2cm was no longer a significant factor for survival with a HR of 0.13 (95% CI 0.02 – 1.03, P=0.053). All patients with MEN1 survived during follow-up (G1 n=7, G2 n=3, and unknown Ki-67 n=5 [small tumors]).

Thirty-six patients of the total cohort (26%) were followed with repeated imaging if they had small non-functioning tumors ≤2 cm (median 1.45 cm, IQR 0.6-1.5 cm). Of these, one had surgery after six years due to radiologic progression from 1.2 to 1.6 cm. Ten patients had no surgery despite a large tumor size (median 5 cm, IQR 3-5.9 cm). Of these, eight had metastatic disease at diagnosis, six were inoperable, one had severe dementia, and one was feeling excellent and did not want any treatment. An 83-year-old patient with an insulinoma hesitant towards surgery was operated two years after diagnosis.

G1, G2 Tumors and Ki-67

The Ki-67 index was clinically re-analyzed in 26 (19%) patients with progressive disease using core needle biopsy material from metastatic lesions and was at that time median 9.4% (IQR 4 – 13.8%) (**Table 2**). The G1 group included 66 patients (females, n=39) with a median tumor size of 2.25 cm (IQR 1.5-5 cm), and a follow-up of median 48 months (IQR 40.4 – 120 months). Nineteen of these had progressive disease (29%). In the G1 tumors <2 cm distant disease was found during follow-up in 4/32 (13%). The Ki-67 index increased in six tumors which reached a G2 grade and in two reaching a G3 grade. Six patients in the G1 group deceased during follow-up, of which two were Pan-NET associated deaths, and four in causes not related to Pan-NET (breast cancer n=1, glioblastoma n=1, cardiovascular disease n=2).

The G2 group consisted of 42 patients (females, n=22). Patients were in median 57 years old (IQR 47-65 years), median Ki-67 index was 5.1% (IQR 3.5-9.9%), and median tumor size 4.3 cm (IQR 1.7-6 cm). Twelve had metastasis at diagnosis, and 23 (56%) developed progressive disease.

In the further analysis of patients with G2 tumors, the G2a group consisted of 30 patients (females n=16), with a median age of 59.5 years (IQR 51-66.5 years), and follow-up of median 74.5 months (IQR 50 – 106 months). Tumor size was median 4 cm (IQR 1.6-6 cm), and the Ki-67 index was median 4.5% (IQR 3-6.8%). In this cohort, 14/30 (47%) developed progressive disease.

In the G2a patients with progressive disease, the new tumor presentation was local in four and 10 (37%) had distant metastasis. The Ki-67 index increased in these G2a patients to 11.4% (IQR 7.8-21.8%), and one patient progressed to G3. Six patients died during follow-up whereof four related to their Pan-NET and two to unrelated causes (glioblastoma n=1, septicemia n=1).

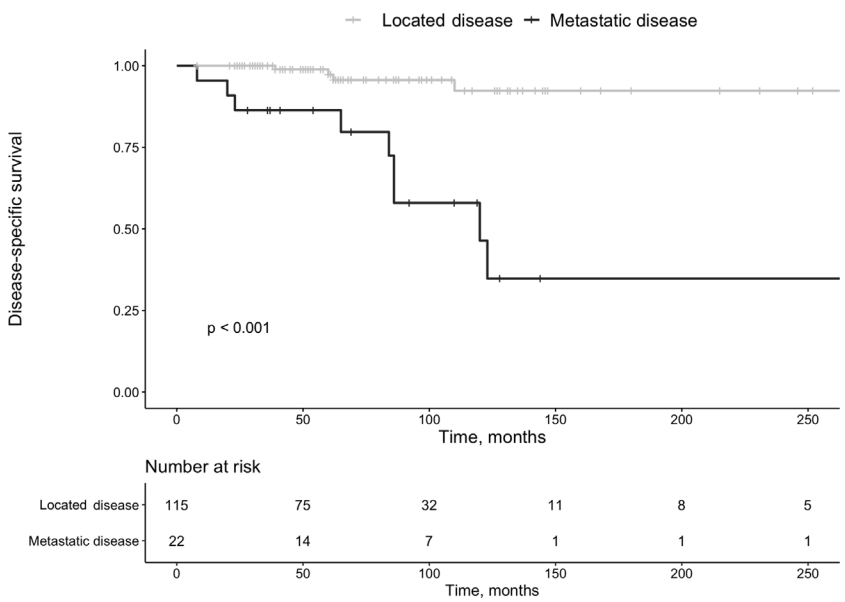

FIGURE 4 | Survival in patients with pan-NETs with localized disease at diagnosis vs. those with metastatic disease at that time point.

Group G2b consisted of 12 patients (females n=6). The median age at diagnosis was 59 years (IQR 46-65 years), and the follow-up was 62.5 months (IQR 48-83 months). Tumor size was 5.5 cm (IQR 3.9-7.3 cm) and the Ki-67 index 11.5% (IQR 10-13.5%). In group G2b, 9 (75%) developed progressive disease and the Ki-67 index in these was 13% (IQR 12.8-14%). No patient progressed to G3. Two G2b patients died during follow-up, of which both deaths were secondary to Pan-NET, combined with liver failure in one. Survival was not statistically different between G1 vs. G2 (*P*=0.065, **Figure 5A**) or G2a vs. G2b (*P*=0.6). Survival in G1 vs. G2 is summarized in **Figure 5A** and in G1 vs. G2a vs. G2b in **Figure 5B**.

DISCUSSION

This retrospective study from a major tertiary referral center, including 137 patients with Pan-NET followed for 74 months, confirms the notion that patients with metastasis at diagnosis had poor survival compared to those with localized disease. Development of metastatic disease was more frequent in G2 than in G1 tumors, but neither Ki-67 index, tumor functionality, or tumor size were alone or together reliable parameters to assess survival.

Boninsegna et al. have previously found that a Ki-67 of more than 5% is a predictor of recurrent disease (9). In a previous

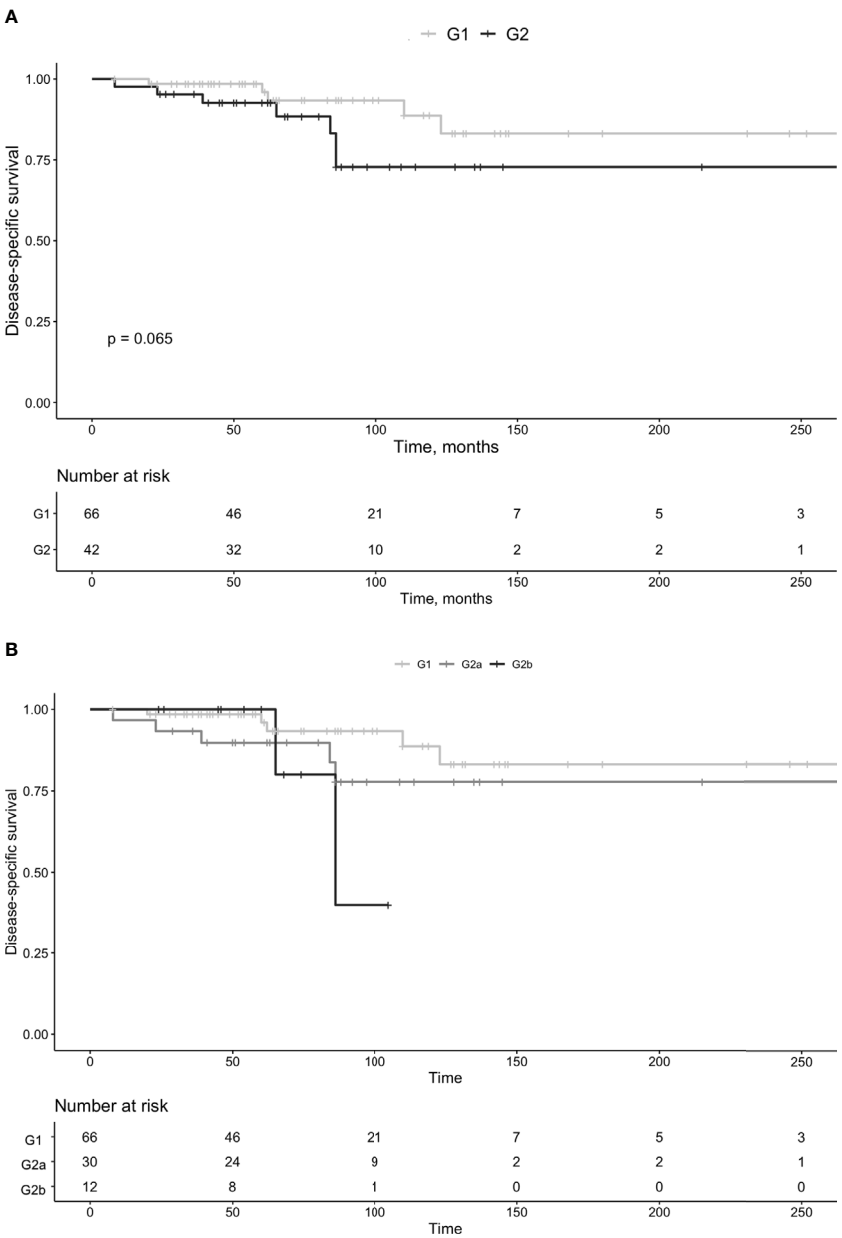

FIGURE 5 | Survival in patients with pan-NETs, divided in G1 and G2 tumors **(A)** and G1, G2a and G2b **(B)**.

study of 24 patients, re-biopsies of tumor relapse displayed increased Ki-67 counts, from median 4% to 11%, of whom four (17%) progressed to G3 lesions (16). Recently, Botling et al. corroborated this when repeated Ki-67 staining was investigated in 45 patients (17), revealing that 55% of patients progressed during a follow-up of 73 months. In our study, we found some support for this as 31% of patients had progressive disease. In those without a previous metastasis of G1 tumors, 29% developed local or distant recurrence versus 55% of patients with G2 tumors. In the cohort with metastasis at initial diagnosis, 59% of patients had progressive disease. However, better survival in all G1 vs. all G2 (i.e., including those with initial metastasis) did not reach statistical significance (P=0.065), probably due to the limited sample size and follow-up time. As the span in proliferation in G2 tumors is wide, ranging from 3-20%, we subdivided this group into hypothetical G2a and G2b groups, but could not display any significant difference in survival after this stratification.

Primary surgery was performed in 74% of patients. Of these, 8% later had repeated surgery due to radiologic progression. Of the remaining, most patients had small non-functional tumors and were followed with clinical and imaging assessment. Seven percent declined follow-up and/or had cardiovascular disease or dementia by which no surgery was performed. There is a controversy whether to operate on patients with tumors between 1-2 cm in size (8), as these often have an indolent behavior (18). However, even small tumors <2 cm can prove to be clinically malignant with distant metastases at diagnosis (19).

During surveillance of small Pan-NETs, a systematic review showed that 0-51% had grown in size during up to 45 months of follow-up, of which 14% of patients had surgery (20). In our investigation, 19 out of 66 (29%) of our patients with G1 tumors developed new local or distant metastasis. In those G1 tumors ≤2 cm in size, this was evident in 4/32 (13%), and the tumor size was missing in four. In two of these small tumors the initial Ki-67 index was lacking. This highlights the importance of adding this information together with radiology and hormone evaluation.

As outlined by Hill and colleagues, surgery is associated with improved survival across all disease stages (21). In that retrospective study, the primary tumor size was not mentioned, but the comparison was made between patient groups that were recommended surgical intervention and had subsequent surgery versus those recommended surgery but did not have surgical intervention (21). When performing surgery in the head of the pancreas, there are risks of gastrointestinal hemorrhage, biliary or gastric outlet obstruction, exocrine and endocrine insufficiency, and the development of a pancreatic fistula (22). These risks have to be considered individually, but also in more advanced disease surgery could be considered to relieve compressive symptoms. Thus, management is controversial in localized tumors in deciding which patients should be recommended surgery. In a retrospective investigation of 125 patients conducted by Phan and co-workers, the best prognosis was observed in patients with clinically indolent disease but also in more aggressive tumors if patients had radical surgery (23). This study differed from ours as 52% of their tumors were functional, and 52% were deemed as malignant. Their median tumor size was 1.9 cm (range 0.3 – 9 cm) in functional tumors and 4 cm (range 0.6 – 18 cm) in non-functional tumors. In other investigations comparing surgery vs. a watchful follow-up in tumors ≤2 cm in Pan-NETs, surgery was not associated with less development of metastasis or death (20, 24).

Larger tumors are more often clinically malignant and have somewhat poorer outcomes, but size alone cannot decide the metastatic potential (25). Tumors >4 cm with invasive characteristics and higher stage have negative prognostic influence, as the 5-year survival in TNM stages I, II, III, and IV were 100%, 93%, 65%, and 35%, respectively (26). In two small investigations (n=9 and n=16, respectively) size was not related to prognosis (27, 28). In contrast, larger tumors were correlated to the development of new lesions in two other investigations (n=108 and n=14, respectively) (29, 30). Recommendations from the National Comprehensive Cancer Network (NCCN) are that tumors ≤2 cm could be considered for observation if discovered incidentally (https://www.nccn.org/professionals/physician_gls/default.aspx, accessed on March 1, 2021). The European Neuroendocrine Tumor Society (ENETS) have proposed intensive observation in non-functional Pan-NETs ≤2 cm (31). In our study, such tumors can progress, but it was rare (13% during 74 months of follow-up). Applying cox-regression, size only showed a trend to be statistically significant. Thus, the approach to have careful surveillance in non-functioning localized tumors ≤2 cm in size seems reasonable. In total, survival varied considerably and of deceased patients, 63% died of causes not related to Pan-NET.

As the Ki-67 index did not accurately pinpoint prognosis in these tumors with heterogeneous activity, clinicians regularly follow these patients. Also, functioning tumors, which are often well-differentiated on histological examination, did not differ from non-functional tumors in terms of survival, and size was not a significant tool using cox-regression. This finding is problematic as non-functioning tumors may change behavior or become functioning (15, 32), and awareness of this is essential if new symptoms or hormone secretion develops.

Other tools, besides the Ki-67 index, tumor size or tumor functionality could be applied. To assess prognosis in NETs, somatostatin receptor PET with low maximum standardized uptake value (SUV_{max}) adds information of less progression-free survival and overall survival in a recent review (33). In contrast, a high SUV_{max} on ^{18}F-FDG-PET correlates with a more advanced grade (34). To improve prognostication protein expression of DAXX/ATRX (35) and tissue-based markers as transcription factors as ATRX and PDX1 assessed by immunohistochemistry (36), hypermethylation (37) or alternative lengthening of telomeres (38, 39), can be of value but warrants further investigations (40).

There are several limitations to this investigation. Even though we included more patients with Pan-NET than most studies, the sample size is still limited, especially in the subgroups. All data on tumor size and Ki-67 index were not available in the 36 non-operated patients, limiting the evaluation.

There is likely a selection bias in those 26 patients with repeated measurements of the Ki-67 index. The 74 months of follow-up is quite long; however, these tumors are slow-growing, and metastasis and death may develop much later, which also may explain difficulties in finding significant findings in the subgroups.

CONCLUSION

Our data support the notion that an active follow-up seems reasonable for non-functional Pan-NETs ≤2cm. Progression was more common in G2 tumors than in G1 lesions. Patients with metastasis at diagnosis had poor survival compared to those with localized disease at initial presentation. Taken together, variables such as tumor size, the Ki-67 proliferation index, and hormonal activity may add prognostic value, but no benefits were found by hypothetically subdividing the G2 group. Better clinical prognostic markers are thus needed to assess Pan-NETs in terms of outcome and survival.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The Regional Ethical Review Board in Stockholm, Sweden approved the study (2018/1909-31), and informed consent was waived due to its retrospective nature. All methods were performed in accordance with relevant guidelines and regulations. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JC investigated the medical files and wrote the first draft of the manuscript and the tables. CJ investigated the histopathologic slides and added **Figure 2**. FC made the statistical analyses and contributed with remaining figures. RB added information on surgical considerations. HF aided with continued manuscript revision. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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Case Report: Re-Treatment With Lu-DOTATATE in Neuroendocrine Tumors

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Peptide receptor radionuclide therapy (PRRT) is an established treatment in advanced neuroendocrine tumors (NETs), which overexpressed somatostatin receptors. However, after progression there are a limited number of available treatments. We want to share a case report about a patient with a NET re-treated with ^{177}Lu-DOTATATE and a literature review about salvage treatment with PRRT. We present a 26-year-old man who started with pelvic pain and after a biopsy of a retro-rectal mass observed in a magnetic resonance was diagnosed with an advanced neuroendocrine tumour. After progression to lanreotide, everolimus and sunitinib, treatment with ^{177}Lu-DOTATATE was initiated, achieving an excellent response with a progression free survival (PFS) of 38 months. At the time of progression, re-treatment with ^{177}Lu-DOTATATE was decided, showing a new partial response, which is currently stable after 15 months. The patient had not presented significant treatment-related toxicity. Although there are no randomized phase III trials or a consensus about the number or dose of cycles, there is evidence about the efficacy and low toxicity of salvage treatment with ^{177}Lu-DOTATATE in NETs. Median progression-free survival ranges from 6 to 22 months. Toxicity is mostly hematologic (anemia and neutropenia), 4-7% grade 3/4.

Keywords: neuroendocrine tumors, Lu-DOTATATE, neuroendocrine neoplasms, peptide receptor radionuclide therapy, case report

CASE REPORT: RE-TREATMENT WITH LU-DOTATATE IN NEUROENDOCRINE TUMORS

INTRODUCTION

Neuroendocrine neoplasms (NENs) are a heterogeneous group of neoplasms, which arise in neuroendocrine cells of the mucous membranes with an incidence of 6-7 cases per 100,000 people in the United States, with an increase in the last years (1). NENs can originate from different organs, although most do so from the lungs, the pancreas, and the gastrointestinal tract.

Based on their histological differentiation and grade that correlate with the proliferation index ki67 and mitotic rate, NENs can be classified in well-differentiated neuroendocrine tumors (NETs) and poorly-differentiated neuroendocrine carcinomas (NECs), which differ in their treatment because of their more aggressive behavior (2). NETs can be divided in low-grade (grade 1) and intermediate grade (grade 2). In addition, there is a subset of NENs that appear histologically well-differentiated with a high proliferation rate. The 2019 WHO classification of NENs recognizes a category of well-differentiated NETs with high-grade (grade 3) (3). Furthermore, NETs can be classified based on their clinical characteristics in functional or nonfunctional tumors, depending on its capacity of secreting hormones, such as serotonin, insulin, gastrin, or glucagon.

The majority of NETs overexpresses somatostatin receptors that are used as a diagnostic and therapeutic target (4). Somatostatin analogs (octreotide and lanreotide) are standard first line of treatment in the advanced disease (5–7). In the last decades there has been an improvement in the knowledge of molecular biology of NETs, and many clinical studies have been launched with targeted therapies involved in tumorigenesis, such as mammalian target of rapamycin (mTOR) inhibitors or tyrosine kinase inhibitors (TKIs), achieving the approval of everolimus and sunitinib (sunitinib only in pancreatic NET) (8–14). Although there are several therapeutic options, limited response rates and significant toxicities make new approaches necessary.

In this context, peptide receptor radionuclide therapy (PRRT) arose as a new targeted option against NETs, delivering radionuclides directly to tumour cells (15). First clinical studies analyzed the efficacy of somatostatin analogs labeled to radionuclides of Yttrium or Indium, but there was an important hematologic and renal toxicity (16, 17). Lately, Lutetium-177(^{177}Lu)-DOTATATE has shown its efficacy with a better safety profile, and it has been established as a valid option in metastatic NETs treatment, with data of clinically relevant long responses. In this context, NETTER-1 is a phase III clinical trial that assessed the activity of ^{177}Lu-DOTATATE compared to high dose of octreotide in patients with advanced midgut NETs (15). ^{177}Lu-DOTATATE increased the objective response rate (ORR) (18% vs 3%; $p < 0.001$) and the progression free survival (PFS) (28.4 vs. 8.5 months; HR 0.21, 95% confidence interval (CI) 0.14–0.33, $p < 0.0001$). Although data is still immature, it showed a trend toward improved overall survival (OS) (median not achieved vs. 27.4 months, HR 0.46, 95% CI 0.14–1.5). European centers of reference have published large series of patients with gastroenteropancreatic and bronchial NETs treated with PRRT (18, 19). These series confirmed the benefit of ^{177}Lu-DOTATATE in NETs of primary tumour sites other than the midgut.

Despite the benefit of systemic treatment in NETs the majority of patients recurs and need a new therapeutic alternative. In this way there is an increasing interest of salvage PRRT with ^{177}Lu-DOTATATE in patients with NETs. Here, we present a patient with a NET treated with salvage ^{177}Lu-DOTATATE and review the literature of salvage PRRT in NETs.

CLINICAL CASE

An 18-year-old Caucasian man without any relevant medical history, surgeries, or medical family history, was evaluated for intense pelvic pain of several months of evolution, refractory to common analgesics. He did not report diarrhea or other symptoms suggesting carcinoid syndrome. Physical examination, including abdomen and pelvis, did not show significant findings. The initial laboratory test did not present biochemical or hematological abnormalities. Due to the lack of clinical or analytical findings and the severity of the pelvic pain, a magnetic resonance was performed, showing a 4,3 x 3,3 x 4 cm retro-rectal mass, and the biopsy revealed a low-grade neuroendocrine tumour.

The patient underwent surgery in June 2012. However, the complete resection was not feasible due to sacrum infiltration. The histology confirmed a neuroendocrine tumour grade 2 and a proliferation index Kinett 67 of 15%, with nodal invasion, pT4pN1Mx (stage IIIB AJCC 8^{a} ed.). Post-surgical computerized tomography (CT) demonstrated tumour persistence. A second surgery was performed in September 2012 achieving a complete resection, followed by adjuvant radiotherapy in December 2012.

In June 2013, a CT scan showed multiple bone metastases. A somatostatin receptor scintigraphy (SRS) demonstrated radiotracer uptake in the occipital bone and the third lumbar vertebrae. Treatment with lanreotide, 120 mg every four weeks, was initiated. Nevertheless, in November 2013 the patient presented new bone progression.

Second line treatment with everolimus, 10 mg daily, was started. Despite initial benefit, in September 2014 a new CT scan and a bone scintigraphy revealed an increase in the number of bone metastases. At that time, sunitinib, 37,5 mg daily, was initiated with stable disease in radiological assessments. However, after two years of treatment progressive disease was observed. In January 2016, the CT scan demonstrated new bone metastases located in the mandible and femur.

In February 2016, the patient was referred to our center and ^{177}Lu-DOTATATE treatment was offered with four doses (7,4 GBq (200 mCi) every eight weeks). The first dose was administered in April 2016. The SPECT-CT, performed after the second dose, demonstrated a decreased number of bone metastases with a lower radiotracer uptake. The treatment was completed in September 2016 with an important clinical and radiological benefit observed in the SRS (**Figure 1**) and in the CT scan by RECIST 1.1. No adverse events related to ^{177}Lu-DOTATATE were observed.

The patient started follow-up with physical examination, laboratory tests, CT scan and SRS every three months, and the disease was controlled until June 2019, when a CT scan showed liver, bone and nodal progression (**Figure 2**). Due to the lack of valid alternative therapeutic options and the excellent previous response, it was decided in a multidisciplinary committee to re-treat with two more doses of 7,29 GBq (197 mCi) of ^{177}Lu-DOTATATE every eight weeks. As in the first treatment, the dose received was assessed by dosimetry after each

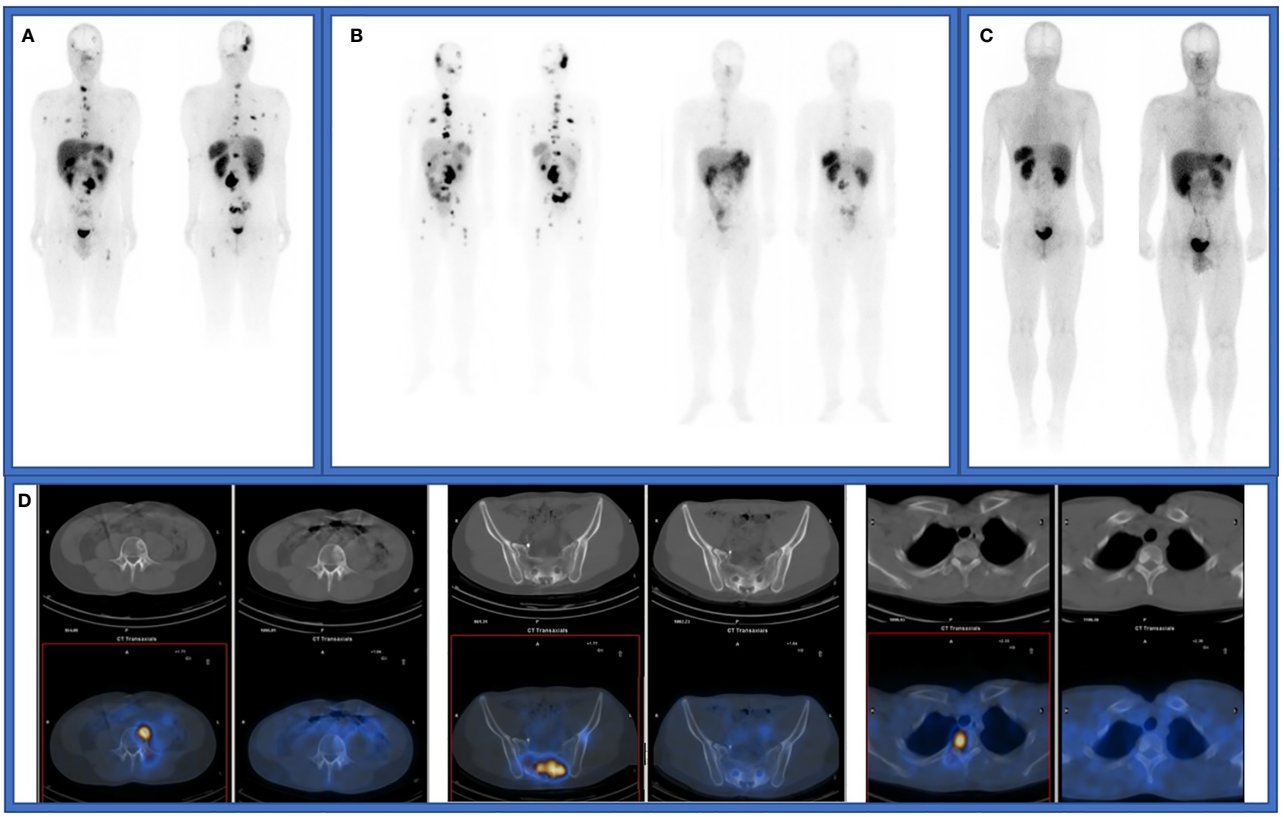

FIGURE 1 | **(A)** Octreoscan imaging in December 2015, before de first treatment with 177Lu-DOTATATE. **(B)** SPECT-CT studies after first and fourth 177Lu-DOTATATE doses. **(C)** Octreoscan imaging in November 2016, after finishing first treatment with 177Lu-DOTATATE. **(D)** SPECT-CT studies in December 2015 and November 2016.

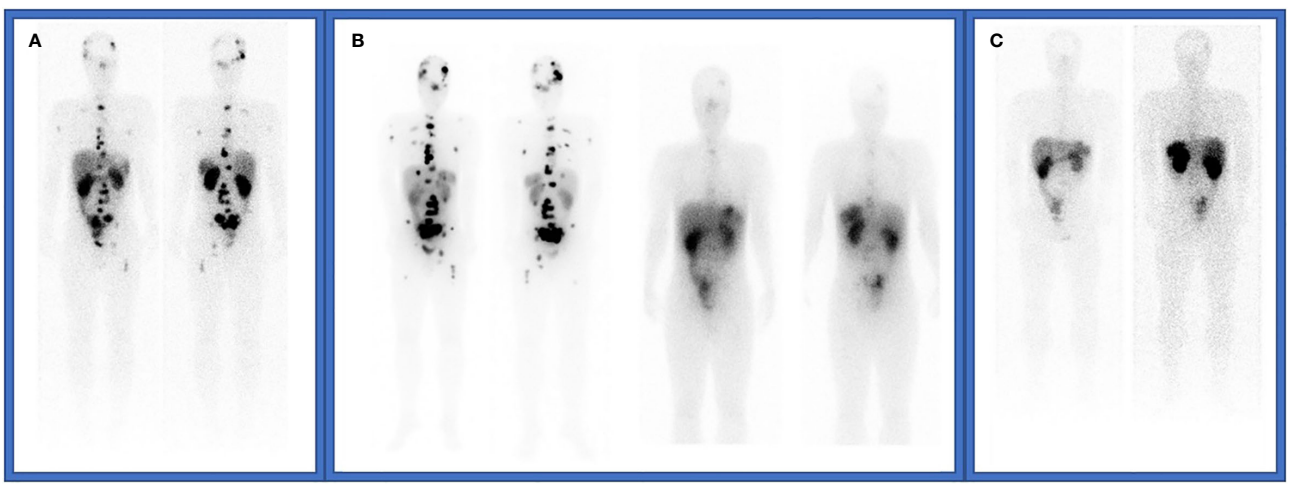

FIGURE 2 | **(A)** Octreoscan imaging at tumor recurrence in June 2019. **(B)** SPECT-CT studies after fifth and sixth 177Lu-DOTATATE doses. **(C)** Octreoscan imaging after complete 177Lu-DOTATATE re-treatment.

administration. The treatment was administered in July and September 2019, achieving again a new response observed in the CT scan by RECIST 1.1 and in the SRS, without clinically relevant hematologic or renal toxicity, and started again follow up. In the current moment, the patient maintains partial response achieved with ^{177}Lu-DOTATATE (**Figure 3**).

DISCUSSION

This case report is a good example of the efficacy and safety of salvage therapy with ^{177}Lu-DOTATATE in heavily pretreated patients after an initial response to PRRT, an especially challenging context with a limited number of alternatives.

Currently, ^{177}Lu-DOTATATE is an established therapeutic option for the treatment of metastatic NETs. However, there is a lack of evidence for salvage therapy. Recently, published studies have shown the efficacy and acceptable tolerance of re-treatment with PRRT (**Table 1**). Nevertheless, these studies are heterogeneous and mostly retrospective, with significant differences between them regarding the patients included, the cumulative dose of PRRT, or the radiolabeled drug used.

Efficacy

Two meta-analysis have been published lately with the aim to evaluate the efficacy and safety of salvage PRRT by Strosberg et al. and Kim et al. (27, 28). These analyses included thirteen and nine articles respectively. Strosberg et al. found a PFS of 12.52 months and an OS of 26.78 months with a disease control rate (DCR) of 71%. Kim et al. reported a PFS of 14.1 months and an OS of 26.8 months and a DCR of 76.9%. However, these meta-analyses present several limitations. These articles found an important heterogeneity between studies, with different doses of salvage PRRT. In addition, the studies also included a small number of patients and presented different criteria regarding toxicity, evaluation of response and outcomes reported.

Van der Zwan et al. published the largest cohort of patients who underwent re-treatment with PRRT with only ^{177}Lu-DOTATATE. 181 patients with bronchial or GEP-NET were included in the analysis after receiving salvage therapy with PRRT. Objective response and stable disease were observed in 26 patients (15.5%) and 100 patients (59.5%), respectively. The median PFS was 14.6 months (95% CI 12.4–16.9). In addition, a control group consisting of patients not undergoing salvage therapy, but in principle qualifying for it, was established for estimating the potential increase in OS. Patients re-treated with ^{177}Lu-DOTATATE had a significantly longer OS than control patients ($p < 0.01$). In fact, in this series some patients were re-treated twice if the patient had reached disease control after the first salvage PRRT. They found 38.5% of partial responses, 53.8% of stable diseases and a median PFS of 14.2 months. In this subgroup the combined OS after the 3 PRRT treatments was 80.8 months (95% CI 66.0–95.6). The study concluded that ^{177}Lu-DOTATATE re-treatment is a suitable option in patients with previous response to PRRT (25).

Other studies have also shown these promising results. The median PFS reported range from 6 to 22 months (22, 26). The short PFS observed by Rudisile et al. compared to other studies could be explained by the late sequence of salvage PRRT, with many intermediate therapies. The DCR was over 50% in the majority of studies, showing more than 80% in the series reported by Severi and Rudisile (22, 26). Yordanova et al. also published overall survival data, achieving 85.6 months against 69.7 months in patients who received only a baseline therapy with PRRT in the same department and time (23).

As expected, efficacy of salvage PRRT is worse than in NETTER-1, with PRRT as first treatment after octreotide analogues (15). There are some reasons, as a lower number of cycles, or the fact that patients have a more advanced disease and worse performance status.

The heterogeneous population included is one of the motives for the variable outcomes. First of all, these studies included NETs from different primary tumor locations: all of them

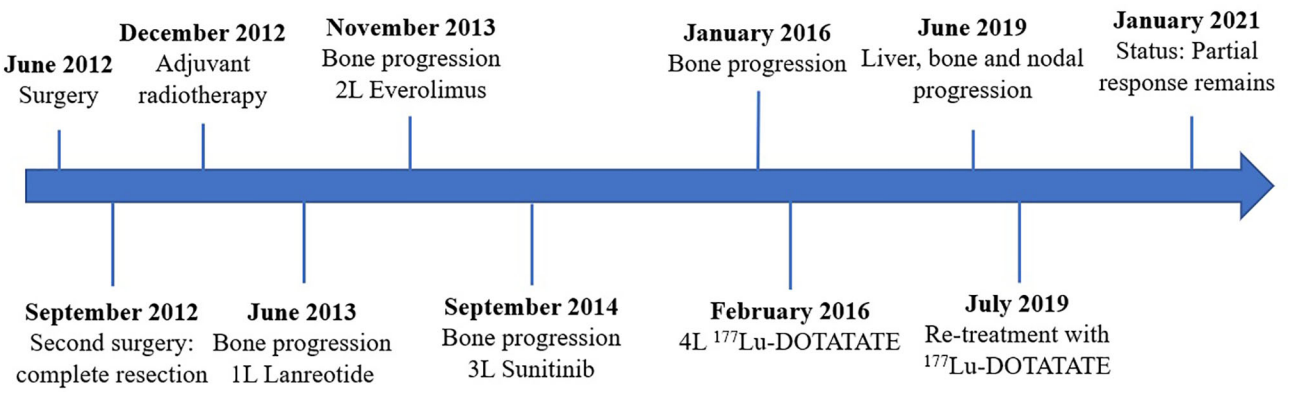

FIGURE 3 | Historic evolution of the patient.

TABLE 1 | Published studies that evaluate salvage with PRRT.

Study (year of publication)	Number of patients (n)	Location (n)	Treatment	Median PFS	Best response	Toxicity grade ≥3
Van Essen et al. (20)	33	Bronchial (3), gastric (1), rectal (1), midgut (15), pancreatic (8), unknown origin (5)	2 cycles of 7.4 GBq ^{177}Lu-DOTATATE	17 months	PR: 6 (18.2%) MR: 2 (6.1%) SD: 8 (24.2%) PD: 17 (51.5%) DCR: 16 (48.5%)	Hematologic: n=5
Sabet et al. (21)	33	Pancreatic (14), foregut (3), midgut (6), hindgut (3), other (7)	2-4 cycles. Mean administered activity during re-treatment: 17.7 GBq ^{177}Lu-DOTATATE	13 months	CR: 1 (3%) PR: 6 (18.2%) MR: 1 (3%) SD: 14 (42.4%) PD: 11 (33.3%) DCR: 22 (66.6%)	Hematologic: n=7
Severi et al. (22)	26	Pancreatic (17), Ileum (5), appendix (1), colon (1), rectum (1), unknown origin (1)	2-5 cycles. Median activity for re-treatment: 16.5 GBq ^{177}Lu-DOTATATE	22 months	CR: 1 (3.8%) PR: 1 (3.8%) SD: 20 (76.9%) PD: 4 (15.4%) DCR: 22 (84.6%)	Renal: n=1. Hematologic: n=1
Yordanova et al. (23)	15	Foregut (8), midgut (3), renal (1), unknown origin (3)	3-6 cycles. Median cumulative activity: 63.9 GBq ^{177}Lu-DOTATATE	18.9 months	NA	Hematologic: n=2
Vaughan et al. (24)	47 Re-retreatment: 44	Midgut (21), pancreatic (15), hindgut (2), lung (3), unknown (2), other (2)	^{90}Y-Dotatoc: 29 patients, 177Lu-DOTATATE: 18 patients.	17.5 months	PR: 10 (21.27%) SD: 37 (78.72%) DCR: 47 (100%) Re-retreatment: PR: 7 (15.9%) SD: 26 (59.1%) PD: 11 (25%) DCR: 33 (75%)	Renal: n=1 Hematologic: n=2 Myelodysplastic syndrome: n=1
Van der Zwan et al. (25)	168 Re-retreatment: 13	Bronchial (13), pancreatic (53), midgut (54)	Re-treatment: 2 cycles (median cumulative dose: 44.7 GBq), re-retreatment: 2 cycles (median cumulative dose: 59.7 GBq)	14.6 months (14.2 months from re-retreatment)	PR: 26 (15.5%) SD: 100 (59.5%) PD: 33 (19,6%) DCR: 126 (75%) Re-retreatment: PR: 5 (38,5%)	Hematologic: n=14 Myelodysplastic syndrome: n=2 Acute myeloid leukemia: n=2

(Continued)

TABLE 1 | Continued

Study (year of publication)	Number of patients (n)	Location (n)	Treatment	Median PFS	Best response	Toxicity grade ≥3
					SD: 7 (53,8%) PD: 1 (7,7%) DCR: 12 (92,3%)	
Rudisile et al. (26)	35	Midgut (23), lungs (5), unknown primary (4), rectal (1), gastric (1), paraganglioma (1)	1-4 cycles. Median cumulative activity 44 GBq ^{177}Lu-DOTATATE	6 months	PR: 1 (3,1%) SD: 26 (81.3%) PD: 5 (15.6%) DCR: 27 (77.1%)	Hematologic: n=1

PR, partial response; MR, minor response; SD, stable disease; PD, progression of disease; DCR, disease control rate; R-PRRT, re-treatment with peptide receptor radionuclide therapy; RR-PRRT, re-retreatment with PRRT.

included gastroenteropancreatic and unknown origin tumours, but some series also included bronchial tumors (20, 26), and other study included even paragangliomes or medullary thyroid carcinomas (24).

These results support the strategy of PRRT re-treatment in patients with NETs. However, there are several limitations in the interpretation of these data due to the heterogeneity between studies and the small number of patients included in these studies.

Toxicity

Hematological and renal toxicity are the main side effects and dose limiting factors for PRRT. However, the safety profiles of ^{177}Lu-DOTATATE and ^{90}Y-DOTATATE are different, particularly renal toxicity is more often reported with ^{90}Y-DOTATATE.

Previously mentioned meta-analysis found a similar toxicity profile between salvage PRRT and initial PRRT. Strosberg et al. described a 5% of grade 3/4 adverse events and 0% of renal toxicity, and Kim et al. found a 10.8% of hematologic toxicity and 0.7% of renal toxicity (27, 28).

In the series, the most common toxicity observed was hematologic with grade 3/4 in 4% to 7% of patients, similar to results with ^{177}Lu-DOTATATE in NETTER-1 (15). In addition, 1% of patients developed late toxicity as acute myeloid leukemia or myelodysplastic syndrome, showed in a study which evaluates not only re-treatment, but also a second re-treatment (25). The data of Sabet et al. stands out because they showed a hematologic toxicity grade 3 or more in 21% of patients, without higher accumulated dose (21) (the mean accumulate activity was 44.3 GBq, while the rest of the studies had a similar range, reaching 63.8 GBq in one series (23)). In a lower number of patients, renal toxicity appears, but it is exclusively observed with ^{90}Y-DOTATATE (up to 4% grade 3/4) (22, 24). Although personalized dosimetry was not used routinely in these studies, the possibility of including this measurement could guide the treatment planning and control the absorbed dose to vulnerable organs (kidneys and bone marrow).

Patient Selection

This is the most important point in PRRT re-treatment. The selection of patients suitable for this strategy will focus our effort to optimize the benefit of PRRT. There are some considerations in which there is more agreement. For example, patients must have a significant clinical benefit after treatment with a previous PRRT to be considered eligible for re-treatment. The controversy is how to measure this benefit. In this way, there are differences in the duration of the clinical benefit: some series request at least 12 months after the last cycle of previous PRRT (20, 22, 24), whereas there is another study that demands at least 18 months (25). The PFS after the first PRRT treatment has been identified as the main factor to predict more durable benefit to salvage ^{177}Lu-DOTATATE (20, 21). Consequently, treatment outcome was less favorable in patients with a short PFS after the first PRRT treatment.

Although there are no other factors clearly associated with response to PRRT re-treatment, some of them have been described as potential predictive markers. However, one of the limitations of this review is that characteristics of patients that receive PRRT re-treatment were not consistently reported across the studies.

The tumour uptake in the somatostatin receptor scintigraphy and ^{68}Ga-DOTATATE PET has a known predictive role to predict response of PRRT in NETs (29, 30). Sufficient radiotracer uptake on SSTR imaging was an indication for PRRT re-treatment in the majority of studies. Van Essen et al. described a higher tumour uptake in patients that received a second treatment with ^{177}Lu-DOTATATE compared with a group of patients treated with the regular therapy (20). However, these findings contrast with other data reported (22), in which the degree of scintigraphy uptake at baseline did not correlate with PFS and OS.

Severi et al. also described a relation between survival after PRRT re-treatment and tumour burden. Patients with an extensive disease, especially those with liver metastases had a shorter OS (22). Despite these data, their potential predictive role is unclear because tumour burden disease and liver involvement are common prognostic factors in oncologic patients.

Tumour dedifferentiation is associated with somatostatin receptor expression. Poorly-differentiated tumors have a lower expression and tumour uptake in the somatostatin receptor scintigraphy with a more aggressive behavior. In this way some authors have described a worse response with PRRT in these patients (20).

The presence of a functional tumor could guide our treatment decision. The current evidence about the efficacy of PRRT regarding control of carcinoid syndrome (CS), showed a symptoms reduction of up to 87,8% (31, 32). The appearance or persistence of an uncontrolled CS could be another factor to consider when evaluating which patients could benefit from the re-treatment.

Schedule of Salvage PRRT

There are also differences in the treatments administered. In this way, different radionuclides have been used as previous treatment or as re-treatment. Severi et al. published a study analyzing 26 patients who received ^{177}Lu-DOTATATE after progression to ^{90}Y-Dotatoc. Median PFS is 22 months with a control disease rate of 84.6% (22). The series reported by Van der Zwan et al. included 181 treated with ^{177}Lu-DOTATATE as initial and salvage PRRT, with a median PFS of 14.6 months (25). Finally, Vaughan et al analyzed retrospectively 47 patients, 45 of them were treated with ^{90}Yttrium (^{90}Y)-DOTATATE and 2 with ^{177}Lu-DOTATATE as initial treatment. The re-treatment was with ^{90}Y-DOTATATE in 29 patients and with ^{177}Lu-DOTATATE in 18 patients. Median PFS was 17.5 months, and no statistically significant differences between both drugs were observed (24).

In addition, there is no consensus about the number or dose of cycles administered: from the fixed two additional cycles in one study (20), up to the six cycles reached in the retrospective study of Yordanova et al. (23). Despite the majority of the studies use a similar per cycle dose of 7.4 GBq, the study of Severi et al. selected a lower dose of 3.7 GBq (22). Furthermore, there is also diversity in the time of salvage therapy. Some studies included patients who receive salvage with PRRT as the first treatment after progression to previous PRRT, but others include extensively pretreated patients with several intermediate treatments (20, 23, 26).

Re-Treatment PRRT in the Guidelines

Treatment guidelines of NETs include the option of salvage PRRT. The Joint International Atomic Energy Agency (IAEA), the European Association of Nuclear Medicine (EANM), and the Society of Nuclear Medicine and Molecular Imaging (SNMMI) accept PRRT re-treatment in patients with previous response, with the same inclusion criteria used in the initial treatment and paying special attention to accumulated doses in bone marrow and kidney (33). The North American Neuroendocrine Tumour Society (NANETS)/SNMMI Consensus Statement on Patient Selection and Appropriate Use of ^{177}Lu-DOTATATE PRRT remark the efficacy and the acceptable toxicity demonstrated by the studies which have evaluated re-treatment (34). In contrast, the latest guidelines of NCCN or ESMO do not propose salvage with PRRT as an option (35, 36).

CONCLUSION

In conclusion, our patient is a good example of re-treatment with PRRT, due to its initial response to ^{177}Lu-DOTATATE, which lasted more than 3 years. In that moment, two additional cycles of PRRT were administered, reaching again partial response without significant toxicity. ^{177}Lu-DOTATATE is an effective therapy in NETs with an excellent safety profile. There is evidence that salvage therapy following progression to PRRT after a long response is an option in these patients, with high disease control rates and acceptable safety profile. Nevertheless, large prospective randomized studies are needed to confirm these findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

EV wrote the manuscript with support from PG, AM, and AS provided the images. All authors provided critical feedback and helped shape the analysis and manuscript, and participated in the management and follow-up of the patient. All authors contributed to the article and approved the submitted version.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Outcome of Patients With Metastatic Lung Neuroendocrine Tumors Submitted to First Line Monotherapy With Somatostatin Analogs

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Objective: Antiproliferative activity of somatostatin analogs (SSAs) has been demonstrated in digestive neuroendocrine tumors (NETs), but few data have been published in patients with pulmonary NETs. We therefore conducted a retrospective study to provide additional data on the outcome of patients with metastatic lung NETs submitted to front line SSAs.

Research Design and Methods: Patients with metastatic lung NET treated with first line SSA-monotherapy (octreotide or lanreotide) in two different reference Institutions were reviewed. Outcome measures were progression-free survival (PFS) overall survival (OS), overall response rate and safety. We also explored prognostic factors associated with PFS.

Methods: The outcome of consecutive patients (pts) with metastatic lung NETs, who underwent first-line treatment with SSAs, recruited from 2014 on 2019 in two Italian reference Institutions, was retrospectively evaluated.

Results: Thirty-one patients entered the study: 14 (45.2%) with typical and 17 (54.8%) atypical carcinoid. Six patients (19.4%) had a carcinoid syndrome. 60.0% of patients had Ki-67 ≤ 10%. Two (6.5%) patients obtained a partial response, 24 (77.4%) disease stabilization while 5 (16.1%) had progressive disease. Median progression free survival (PFS) was 28.6 months, median overall survival (OS) was not attained. Ki-67 ≤ 10%, typical carcinoid histotype and non-functioning disease, were associated with a non-significant PFS prolongation. PFS in patients with atypical carcinoids and in those with Ki-67 >10% was greater than 19 months.

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Conclusions: The long PFS and OS obtained in this case series suggest that SSAs could be effective as first line approach in the management of patients with progressive, metastatic pulmonary NET.

Keywords: lung carcinoid, somatostatin analog, carcinoid syndrome, prognostic factor, distant metastases

INTRODUCTION

Bronchial carcinoids are a rare group of well-differentiated lung neuroendocrine tumors (NETs) with an incidence in western countries ranging from 0.2 to 2/100 000 persons/year (1). They are distinguished from neuroendocrine large and small cell lung carcinomas by histologic features as well as clinical, epidemiologic, genetic and prognostic parameters (2–8). According to WHO histologic criteria (9), lung NETs are divided in typical (low-grade, <2 mitoses/10 HPF and no necrosis) or atypical (intermediate grade, 2-10 mitosis/10HPF and/or foci of necrosis) carcinoids. Surgery is the mainstay of therapy for patients with disease at an early stage. Typical lung NETs have an excellent prognosis following surgical resection while atypical carcinoids are associated with a higher risk of disease relapse and a worse prognosis (10–12).

In patients with unresectable locally advanced or metastatic disease the goals of medical management are to control both hormone-related symptoms and tumor growth (13). Lung NETs frequently express somatostatin receptors, therefore somatostatin analogs (SSAs), such as lanreotide autogel (LAN) and octreotide long-acting release (LAR), are frequently used therapies and recommended by available guidelines (2, 13, 14). These drugs are well tolerated and have demonstrated both anti-secretory and anti-proliferative effects in NET patients. However, the data regarding the efficacy of SSAs in the management of lung carcinoids are very limited and refer to retrospective series (15–19). No prospective study data is currently available. The SPINET trial (NCT02683941), a placebo-controlled randomised phase III study, which was designed to evaluate lanreotide 120 mg in advanced lung carcinoids, was early stopped for insufficient enrolment (20). Most of the published retrospective studies are heterogeneous and included patients with NET of different primaries (21–28). Only 2 studies evaluated patients with lung carcinoids only. One of them enrolled patients undergoing SSAs both as first, and second line approach (21), only one study retrospectively evaluated patients treated with first line SSAs (22).

More data on the efficacy of SSAs in patients with advanced lung carcinoids, not previously submitted to systemic antineoplastic therapies, are therefore needed.

This retrospective study was designed to provide additional data on the efficacy of SSAs as first line therapy in patients with metastatic lung carcinoids.

MATERIAL AND METHODS

We retrospectively reviewed data of consecutive patients with metastatic lung NET, treated with first line SSA monotherapy, from January 2014 to December 2019, at the Medical Oncology Unit of ASST Spedali Civili di Brescia and at the Division of Gastrointestinal Medical Oncology and Neuroendocrine Tumors of the European Institute of Oncology of Milan (IEO). To be included in the study the patients had to meet the following inclusion criteria: histopathological confirmed diagnosis of Lung NET according to 2015 WHO criteria (22), locally advanced or metastatic disease not amenable to radical surgery, absence of prior systemic therapy, at least three months of SSA therapy. Poorly differentiated morphology was an exclusion criterion. A single investigator (EL) collected clinical data from medical records. Database included the following data at baseline conditions: age, gender, tobacco exposure, surgery of primary tumor, histological subtype according 2015 WHO criteria, Ki- 67 index, mitotic count, hereditary or sporadic, primary tumor site, TNM/AJCC (tumor-node-metastasis/American Joint Committee on Cancer 2010 stage) (9), site of metastasis, functional tumor status. The following data were collected before and during the SSA therapy: ECOG PS, disease response at imaging according to RECIST criteria, type of SSA used and dose, date of initiation and completion of SSAs, adverse effects, any local therapies before SSA initiation or during SSA treatment. The study was approved by the institutional review board.

Follow-up visits during SSA therapy were performed approximately every 3 months and included a physical examination, complete blood count and biochemical profile. The evaluation of tumor response was assessed approximately every 6 months. Computed tomography [CT] scan or Positron emission tomography [PET] with Gallium 68 were the imaging techniques employed. The best overall response was defined according to the RECIST 1.1 criteria. Adverse events were evaluated over the whole duration of SSA administration and classified according to CTCAE v4.0.

Treatment with SSAs consisted in an intramuscular injection of octreotide long-acting release (LAR) (at dose of 20 mg or 30 mg every 4 weeks, or 30 mg every 3 weeks) or a subcutaneous injection of lanreotide depot (at dose of 60 mg or 120 mg every 4 weeks).

The primary study end point was progression free survival (PFS), defined as the time elapsing from SSA initiation to disease progression or death whichever occurred first. Patients with no event and alive were censored at the date of the last follow-up. Secondary endpoints were: overall survival (OS), defined as the time from SSA initiation to death from any cause. Survivors were censored at the date of their last follow-up; best overall response under SSA; toxicity.

Statistical Analysis

Patient and tumor characteristics, the type of SSA (duration of treatment) and toxicities were described with conventional

descriptive statistical analysis. Due to the explorative nature of this study, no sample size was determined. Any associations between clinical-pathological features and clinical benefit (partial response and stable disease) to the treatment was tested using the exacted Fisher test. The cut-off date for the analysis was June 2020. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cut-off value of Ki-67 for the prediction of disease progression (**Figure 1**). The PFS and OS curves were calculated with the Kaplan-Meier method and compared with the log-rank test. The prognostic value of age, gender, smoking exposure, histological subtype, Ki-67 category, surgery of primary tumor, functional tumor status, metastatic site, time between diagnosis and treatment start and type of SSA were evaluated in relation to PFS. The Cox regression model was used to estimate the hazard ratios (HR) and relevant 95% confidence intervals (CIs). Two-sided p-values are reported and a p-value of 0.05 was considered statistically significant. The statistical analyses were performed using the SAS software version9.4 (SAS Institute Inc. Cary NC, USA).

RESULTS

Data of thirty-one consecutive patients, meeting the eligibility criteria, were analyzed. The clinical and pathological and characteristics and type of SSAs administered are summarized in **Table 1**.

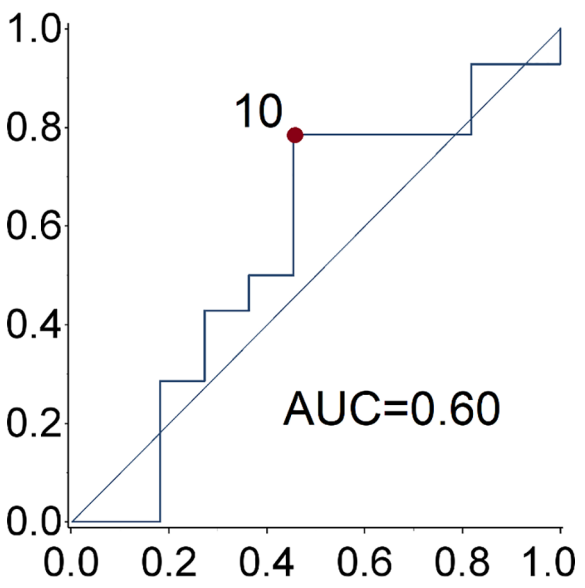

FIGURE 1 | ROC curve of Ki67 for the prediction of disease progression. The optimal ki-67 cut-point maximizing the Youden index for the prediction of disease progression at 28.6 months (median progression free survival) is 10%.

TABLE 1 | Patient Characteristics.

PATIENTS CHARACTERISTICS	n
Patient number	31
Median age at SSA initiation	62 (range 20-82)
Gender	
Female	18 (58%)
Male	13 (42%)
Tobacco exposure	
Yes	1 (3%)
Previous	8 (25%)
Never	11 (32%)
ECOG PS	
0	30 (94%)
1	1 (6%)
TUMOR CHARACTERISTICS	
Histological subtype	
Typical carcinoid	14 (45%)
Atypical carcinoid	17 (55%)
Ki-67	
≤ 2%	5 (20%)
3-20%	19 (76%)
>20%	1 (4%)
Missing	6
Mitotic count	
< 2/2 mm2	8 (57%)
2-10/2 mm2	9 (53%)
Missing	14
Necrosis	
Negative	22 (96%)
Positive	1 (4%)
Missing	8
TTF1	
Negative	9 (29%)
Positive	11 (35%)
Primary tumor site	
Right	18 (58%)
Left	10 (32%)
Bilateral	3 (10%)
Functional status	
Carcinoid syndrome	6 (19%)
Non functioning tumors	25 (81%)
Metastases	
Synchronous	18 (58%)
Metachronous	13 (42%)
Site of metastases	
Liver	8 (26%)
Liver and other sites	11 (35%)
Bone	12 (39%)
Extra-regional nodes	6 (19%)
Lung	6 (19%)
Peritoneal	3 (10%)
Brain	1 (3%)
Others	6 (19%)
Number of metastases sites	
1 organ	16 (52%)
> 1 organ	15 (48%)
Somatostatin receptor positivity at nuclear medicine imaging	
Ga68-PET/CT	13 (42%)
Octreoscan	3 (10%)
TREATMENT CHARACTERISTICS	n (%)
Primary tumor resected	
Yes	18 (58%)
No	13 (42%)

(Continued)

TABLE 1 | Continued

PATIENTS CHARACTERISTICS	n
Somatostatin analogs	
Octreotide LAR	24 (77%)
Lanreotide depot	7 (23%)

CI, confidence interval; LAR, long acting release; OS, overall survival; PFS, progression free survival; RECIST, response evaluation criteria in solid tumors; SSA, somatostatin analogue; LAR, long-acting release.

Thirteen (41.9%) patients were male, median age at SSA initiation was 62 years (range 20-82 years). The majority of patients (93.5%) had an ECOG PS 0.

Fourteen patients (45.2%) had a typical carcinoid, 17 (54.8%) an atypical carcinoid. Six patients had a carcinoid syndrome. One tumor, classified as atypical carcinoid, had Ki-67≥20%. This latter tumor was classified as an atypical carcinoid due to the well-differentiated phenotype. Necrosis was described in only one patient (3.2%). Radiological staging at diagnosis was performed with thoracic and abdominal CT in 17 (55%) patients. PET Gallium or Octreoscan were performed in 16 (51.6%) patients. All disease lesions displayed great uptake of the radiotracers.

Metastases were synchronous in 18 (58.1%) patients and metachronous in 13 (41.9%). Eighteen (58.1%) patients underwent surgery of the primary tumor which consisted in lobectomy in 3 patients (9.7%), lobectomy plus lymphadenectomy in 11 patients (35.5%) and atypical resection in 4 others. Liver was the most frequent metastatic site (61.3%). The other metastatic sites were bone (38.7%), contralateral lung (19.4%), extra-regional nodes (19.4%) and peritoneum (9.7%).

Treatment Administered

Median time from diagnosis of primary neuroendocrine tumor to SSA therapy start was 13 months (range: 3-82). Twenty-seven (87.1%) patients received octreotide LAR, 26 at the standard dose of 30mg/4 weeks (one of them subsequently switched to a dose of 30mg/3 weeks), and one at standard dose of 20mg/4 weeks. Seven (22.6%) patients received lanreotide depot, six at standard dose of 120mg/28 days and one at 60mg/28 days. Three of them received both Octreotide and Lanreotide, in succession. The median treatment duration was 30 months (range: 3-82). Three patients (9.7%) developed mild diarrhea, no other adverse events were noted.

After a median follow up of 44 months (range 11-103 months), 20 patients (64.5%) underwent disease progression and 17 (54.8%) stopped the SSA therapy. Second line therapies were capecitabine plus temozolomide (CAPTEM regimen) in 7 patients, everolimus in 4 patients and peptide receptor radionuclide therapy (PRRT) in 4 patients. The remaining two patients received best supportive care. At the date of the last follow-up examination, 11 (35.5%) patients were free from progression and still under SSAs.

Tumor Response, Progression-Free and Overall Survival

Two patients (6.5%) obtained a partial response (PR), 24 (77.4%) stable disease (SD), whereas five patients (16.1%) had disease progression. After a median follow up of 44 months (range 11-103), the median PFS was 28.6 months (95% confidence interval (CI): 1.5-41.8 months) (**Figure 2**). At the last follow-up examination only two patients died so the median OS was not reached (**Figure 3**). All patients with carcinoids syndrome obtained an efficacious syndrome control (flushing and diarrhea), with complete symptom response in all of them.

A not significant more prolonged PFS was observed in patients with Ki-67 ≤ 10%, typical carcinoid, non-functioning disease, no liver metastases as opposed to their respective counterparts (**Table 2** and **Figure 2**).

None of following parameters were associated with PFS: age (<60 vs ≥60 years), gender, smoking exposure, primary tumor surgery, site of metastasis, time interval between diagnosis and treatment start (<1 vs ≥1 year), SSA administered (octreotide vs lanreotide) (**Table 2**).

DISCUSSION

Somatostatin analogs are standard treatment in the management of well-differentiated neuroendocrine tumors of the gastro-entero-pancreatic tract, both functioning and non-functioning. On the basis of the results of the two prospective randomized studies (29, 30), their administration as first line approach is recommended to patients whose tumor expresses ki-67 in less than or equal to 10% of cancer cells (2). No prospective studies of first-line SSA have been conducted in patients with lung NET.

In this retrospective series the 2 SSAs: octreotide and lanreotide, were shown to be active as first line therapy in patients with advanced/metastatic lung NET with a proportion of partial responses and stable diseases not different from that obtained in the GEP NET setting. The good overall survival should be interpreted considering the short follow-up and the few events observed, that limit the generalization of this result. The 28-month PFS is promising, considering that lung carcinoids, in particular atypical ones, generally have a worse prognosis than GEP NET patients (2). The PFS of the present case series is superior to that of a published retrospective study of lung carcinoids submitted to front line SSAs (22). The difference can be attributed to the patient selection and in particular the higher proportion of typical carcinoids in our series, as compared to that of Bongiovanni A et al. (22).

As expected, atypical carcinoid was associated with a lower PFS than typical carcinoid, although not statistically significant. However, the 19 month PFS of patients with atypical carcinoid is a better outcome compared with historical reports (6). As mentioned in the introduction, the efficacy of SSAs was demonstrated in GEP NETs with Ki67 ≤10%. The observed median PFS of 27 months in lung carcinoid patients with Ki67<10% in the present paper is suggestive of efficacy of these drugs also in this subset. This series however

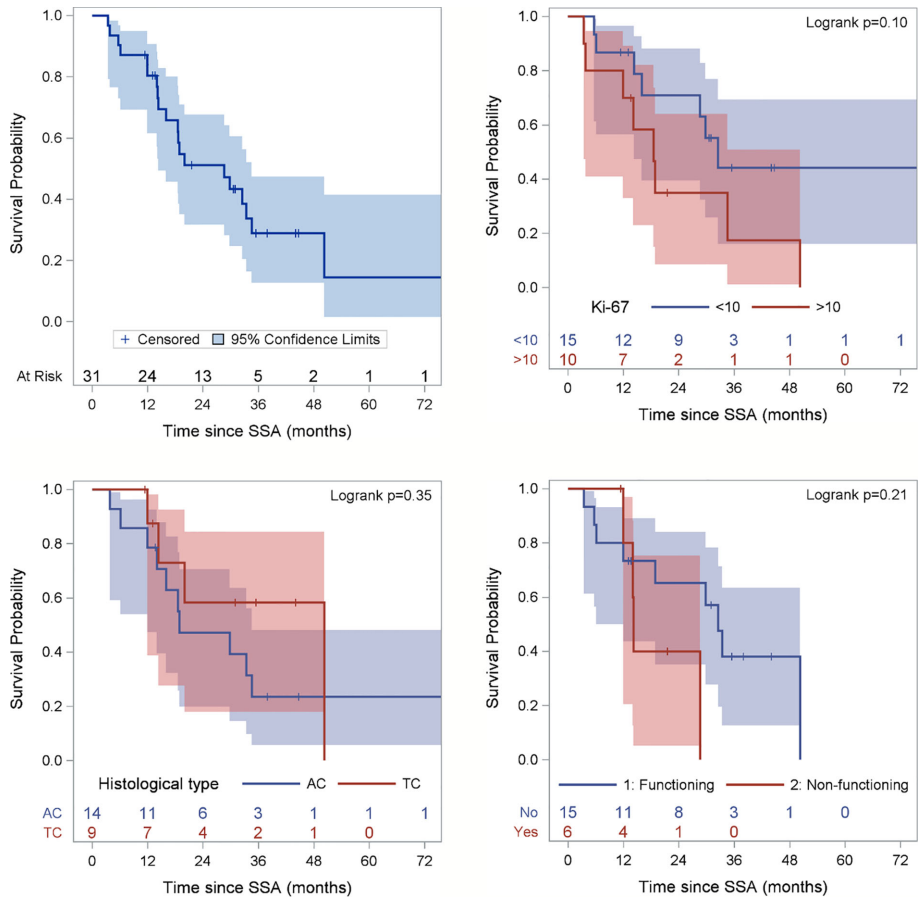

FIGURE 2 | Progression-free Survival (PFS) of the entire population and according to Ki67 category, histological subtype and functioning status. Ki67 is missing for 6 patients and functioning status for 10 patients. Figure for histological type excludes 2 patients with NEN, 2 carcinoid tumors not otherwise specifies (NOS) and 5 well-differentiated NETS.

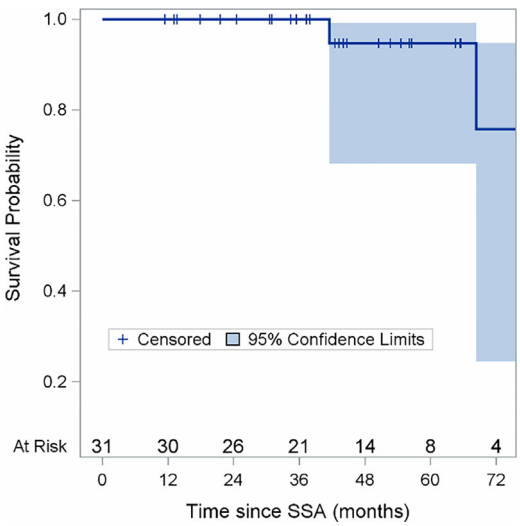

FIGURE 3 | Overall Survival (OS) in the Entire Population.

included 9 patients whose lung carcinoid had a ki67 ranging between 11 and 20% and 1 patient with ki67 >20%. The median PFS of 19.9 months in patients with ki67 >10% is noteworthy and suggests that also selected patients with ki67 >10% could benefit from SSAs administered as frontline therapy. This observation needs confirmation in future prospective studies.

In our series the PFS was negatively influenced by the functional status (although without attaining the statistical significance) confirming a previous observation (22). However, it did not change dividing patients according to the tumor burden and the presence of liver metastases, suggesting that these parameters could not be considered a deterrent in the prescription of SSAs as first-line therapy.

CONCLUSION

In summary, the long PFS obtained in this patient series with progressive, metastatic pulmonary NET, uniformly submitted to first line SSAs suggest that these drugs could be potentially efficacious in this patient setting, confirming previous observations (21, 22).

TABLE 2 | Studies data comparison.

Factor	Median PFS (months)	Hazard Ratio (95% CI)	P
Age			
<60 yr	27.7	0.71 (0.25-1.99)	0.51
≥60 yr	21.4		
Gender			
Male	23.3	1.07 (0.43-2.69)	0.62
Female	24.5		
Smoking exposure			
Yes	22.5	1.45 (0.40-5.25)	0.57
No	28.5		
Ki67			
≤10%	27.6	0.42 (0.15-1.18)	0.23
>10%	19.9		
Histology			
typical	30.6	0.69 (0.27-1.72)	0.16
atypical	19.7		
Status			
Functioning	17.0	2.57 (0.70-9.47)	0.17
Non-functioning	27.3		
Primary tumor surgery			
Yes	22.9	1.07 (0.43-2.65)	0.88
Not	24.1		
Time interval diagnosis -treatment start			
<1 yr	21.9	1.01 (0.43-2.65)	0.89
≥1 yr	24.6		
Liver metastasis			
Yes	21.0	1.63 (0.35-7.63)	0.54
No	21.6		
Metastasis sites			
1	31.9	2.88 (1.13-7.37)	0.19
>1	17.4		
Somatostatin analogs			
Octreotide LAR	27.4	0.96 (0.27-3.50)	0.95
Lanreotide LAR	21.8		

CI, confidence interval; LAR, long acting release; OS, overall survival; PFS, progression free survival; RECIST, response evaluation criteria in solid tumors; SSA, somatostatin analogue.

These findings are relevant in patients with Ki67 ≤10% and typical carcinoids, but are encouraging also patients with greater Ki67 expression and atypical hystotype. Although no definitive conclusions can be drawn from our series of patients and that all our outcomes should be interpreted with caution, the results of our analysis are encouraging and should be considered as hypothesis-generating for further prospective studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee of European Institute of Oncology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

EL, ABe, NF, and FS contributed to conception and design of the study. EL organized the database. PM and AA performed the statistical analysis. AA, EL, and ABe wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Patterns of Lymph Node Metastasis in Patients With T1/T2 Gastroduodenal Neuroendocrine Neoplasms: Implications for Endoscopic Treatment

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Guidelines have differed in their opinion regarding the indications for endoscopic resection of gastric-neuroendocrine neoplasms (g-NENs) and duodenal-NENs (d-NENs). We examined the association between size and lymph node metastasis (LNM) to identify candidates most suitable for endoscopic resection. We identified 706 patients with T1/T2 g-NENs and 621 patients with T1/T2 d-NENs from the SEER database. The prevalence of LNM and risk factors associated with LNM were analyzed. LNM was present in 8.1% of patients with gastroduodenal neuroendocrine tumors (NETs) and 31.6% of patients with neuroendocrine carcinomas (NECs). Multivariate logistic regression indicated that tumor size >10mm, greater invasion depth, and poor differentiation were independently associated with LNM. In addition, the percentage of g-NETs invading submucosa with LNM increased with tumor size (≤10 mm,3.9%;11–20 mm,8.6%;>20 mm,16.1%). However, in contrast to the low LNM risk in patients with small g-NETs (≤10 mm), we found that LNM rate exceeded 5% even for patients with small submucosal-infiltrating d-NETs. Among patients with nodal-negative g-NETs, the cause specific survival (CSS) was similar for those who received surgical resection and endoscopic resection. Among patients with d-NETs, the CSS was better for those who received endoscopic resection. In conclusion, patients with d-NETs had a higher probability of LNM than those with g-NETs. Endoscopic resection can be utilized for curative treatment of submucosa-infiltrating g-NETs and intramucosal d-NETs when the size is 10 mm or less. These results reinforce the need to search for LNM in lesions that are larger than 10 mm.

Keywords: upper gastrointestinal tract, neuroendocrine neoplasm, lymph node metastasis, endoscopic resection, duodenum

INTRODUCTION

Gastroduodenal neuroendocrine neoplasms (NENs), including gastric neuroendocrine neoplasms (g-NENs) and duodenal neuroendocrine neoplasms (d-NENs), account for approximately 10% of NENs within the digestive system (1, 2). With advances in endoscopic techniques, clinicians now incidentally detect an increasing number of gastroduodenal NENs and remove them endoscopically at an early stage (3, 4). Similar to early gastric cancer, the potential risk of lymph node metastasis (LNM) must be considered before endoscopic resection. There are some differences in the guidelines of major European and North American societies regarding the endoscopic management of superficial gastroduodenal NENs (5–7).

Most g-NENs arise from enterochromaffin-like (ECL) cells and multiple guidelines classify them into three types: type 1 (70–80%) is associated with autoimmune gastritis, type 2 (5–6%) results from gastrinoma, and type 3 (14–25%) occurs without hypergastrinaemia (8). The current European Neuroendocrine Tumor Society (ENETS) consensus guidelines consider patients with type 1 g-NENs larger than 10 mm to have an increased risk of metastasis. However, the guidelines of the North American Neuroendocrine Tumor Society (NANETS) recommend that type 1 and 2 g-NENs that are confined to the submucosa, less than 20 mm in diameter, and with no more than six polyps could be resected endoscopically (5). Moreover, the most recent National Comprehensive Cancer Network (NCCN) guidelines suggested that endoscopic resection should be reserved for small (<10 mm), low-grade, and superficial g-NENs (9). Thus, there are differences in whether endoscopic resection should be utilized for curative treatment of g-NENs with diameters of 10 to 20 mm.

More than 90% of d-NENs are in the first or second part of the duodenum, and they are generally small (1.2–1.5 cm) and solitary (8, 10). The ENETS guidelines state that nonampullary d-NENs less than 10 mm in diameter and confined to the submucosal layer are candidates for endoscopic treatment. However, there is no consensus regarding the use of endoscopic or surgical resection for d-NENs that are 10 to 20 mm in diameter (6), and the NANETS and NCCN guidelines do not specifically refer to endoscopic management of d-NENs.

In this study, we examined the indications for endoscopic resection of T1/T2 g-NENs and d-NENs, with a focus on neoplasms that are 10 to 20 mm in diameter, by determining the relationship between the size of gastroduodenal NENs and the prevalence of LNM in a large population.

Abbreviations: NEN, neuroendocrine neoplasm; g-NEN, gastric neuroendocrine neoplasm; d-NEN, duodenal neuroendocrine neoplasm; LNM, lymph node metastasis; ECL, enterochromaffin-like; ENETS, European Neuroendocrine Tumor Society; EUS, endoscopic ultrasound; NANETS, North American Neuroendocrine Tumor Society; NCCN, National Comprehensive Cancer Network; SEER, Surveillance, Epidemiology, and End Results; AJCC, American Joint Committee on Cancer; ICD, International Classification of Diseases; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; CSS, cause-specific survival; OS, overall survival; aOR, adjusted odds ratio; CI, confidence interval; aHR, adjusted hazard ratio; EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; LVI, lymph vascular involvement.

MATERIALS AND METHODS

Study Population

We retrieved clinicopathological data of all patients who were diagnosed with a g-NEN or d-NEN between 2004 and 2015 from National Cancer Institute-sponsored Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov). This registry has research data from 1975 to 2016, and was released in April 2019. The annually-updated SEER database is one of the largest registries in the world, consists of 18 population-based cancer registries, and comprises about 28% of all US cancer cases (11). According to American Joint Committee on Cancer (AJCC) 8th TNM staging system for g-NEN and d-NEN, a T1 tumor is one that has invaded the lamina propria or submucosa and is 10 mm or less in size, and a T2 tumor is one that has invaded the muscularis propria or is more than 10 mm in size (12).

The inclusion criteria were: NEN as the primary tumor; histologically confirmed gastroduodenal NEN with International Classification of Diseases (ICD) histologic codes of 8240 to 8249, 8152 to 8156, 8013, or 8041; primary site ICD code of C16.0 to C16.9 for g-NEN, and C17.0 for d-NEN; and stage T1 or T2. The exclusion criteria were: missing information on tumor size, tumor grade, or lymph node metastasis status; unknown T stage or stage T3 or T4; presence of distant metastasis; receipt of preoperative radiotherapy; and a d-NEN located in the ampulla of Vater.

Definitions of NET and NEC

The term 'NEN' refers to two groups of neoplasms with distinct prognoses: neuroendocrine tumor (NET) and neuroendocrine carcinoma (NEC). This study followed the 2019 WHO classification and grading criteria for tumors of the gastrointestinal tract (13), which defined well or moderately differentiated gastric and duodenal NENs as 'g-NETs' and 'd-NETs', and poorly differentiated gastroduodenal NENs as 'g-NECs' and 'd-NECs'. We referred to the LNM rate of T1a early gastric cancer (14), which is deed as an entity with low risk of LNM, to define the 'low LNM risk' as <5% for gastroduodenal NETs in this study.

Survival Analysis

Surgery of SEER primary site code 20 to 27 was defined as local tumor excision (endoscopic treatment including polypectomy, excisional biopsy, and electrocautery) and code 30 to 90 as open surgery. Nodal involvement was determined by examination of lymph nodes during open surgery or by pre-resection imaging (endoscopic ultrasound, computed tomography scanning, or magnetic resonance imaging) for patients who received endoscopic treatment (15). Cause-specific survival (CSS) was defined as the time from diagnosis to death from gastroduodenal NEN. Multivariate Cox regression analysis for two end points — overall survival (OS) and CSS — was used to assess the prognostic effects of age, sex, tumor size, and treatment method for patients with nodal-negative NETs that were confined to the submucosa. Survival curves were plotted using the Kaplan-Meier method, and the log-rank test was utilized to determine significance of differences for two comparisons:

nodal-negative patients who underwent endoscopic excision *vs.* open surgery and patients with LNM *vs.* no LNM.

Statistical Analysis

Continuous variables were presented as means ± standard deviations, and categorical data as numbers and percentages. Continuous variables with or without normal distribution were compared using Student's *t*-test or the two-sample Mann-Whitney U test, as appropriate. Categorical data were compared using the chi-squared test or Fisher's exact test, as appropriate. Stratified categorical data were compared using the Cochran-Mantel-Haenszel test. A multivariate logistic regression model was employed to identify factors independently associated with LNM. All statistical analyses were performed by IBM SPSS version 22.0 and R version 3.6.1 (https://www.r-project.org/). For all statistical tests, a two-sided *P* value less than 0.05 was regarded significant.

RESULTS

Clinical Characteristics of Patients

We retrospectively examined 1327 patients from the SEER database who had stage T1/T2 gastroduodenal NENs and were diagnosed between 2004 and 2015 (**Tables 1** and **2**). There were 706 patients with g-NENs and 621 patients with d-NENs, and the average age at diagnosis was 59.5 ± 12.9 years for those with g-NENs and 62.2 ± 12.0 years for those with d-NENs. Females accounted for 62.6% of g-NEN patients and 50.5% of d-NEN patients. A total of 95.5% of g-NENs were g-NETs with good differentiation, and 99% of d-NENs were d-NETs. The overall prevalence of LNM was 5.8% among those with g-NENs and 12.1% among those with d-NENs. Analysis of multiple clinicopathological variables indicated LNM had significant associations with male sex, early diagnosis, poor differentiation, deep invasion, and tumor size in patients with g-NENs and d-NENs (all $P < 0.05$). Our comparison of patients with and without LNM also indicated differences in race/ethnicity of those with g-NENs ($P = 0.03$) and age differences in those with d-NEN ($P < 0.001$). However, g-NEN location was not associated with LNM ($P = 0.21$).

Risk Factors for Lymph Node Involvement

We also examined potential risk factors for LNM in patients with gastroduodenal NENs (**Tables 3** and **4**). To avoid the effect of multicollinearity among tumor size, invasion depth, and tumor differentiation ($P < 0.001$ identified by Spearman's correlation analyses, data not shown), we first employed univariate logistic

TABLE 1 | Characteristics of patients with T1/T2 g-NENs (n = 706) with and without LNM.

Variable	No LNM (n = 665)	With LNM (n = 41)	*P*
Age at diagnosis (mean ± SD)	59.3 ± 13.0	62.2 ± 9.8	0.17
Male (%)	238 (35.8%)	26 (63.4%)	<0.001
Year of diagnosis			<0.001
2004-2007	26 (3.9%)	8 (19.5%)	
2008-2011	151 (22.7%)	11 (26.8%)	
2012-2015	488 (73.4%)	22 (53.7%)	
Race/Ethnicity			0.03
Non-Hispanic White	381 (57.3%)	22 (53.7%)	
Black	95 (14.3%)	5 (12.2%)	
Hispanic White	43 (21.5%)	6 (14.6%)	
Asian/Pacific Islanders	32 (4.8%)	7 (17.1%)	
American Indian/Alaska Native	5 (0.8%)	0	
Unknown	9 (1.4%)	1 (2.4%)	
Tumor differentiation			<0.001
Well differentiated	531 (79.8%)	24 (58.5%)	
Moderately differentiated	112 (16.8%)	7 (17.1%)	
Poorly differentiated (NEC)	22 (3.3%)	10 (24.4%)	
Depth of invasion			<0.001
Mucosa	153 (23.0%)	3 (7.3%)	
Submucosa	267 (40.2%)	20 (48.8%)	
Muscularis propria	84 (12.6%)	14 (34.1%)	
T1, NOS	129 (19.4%)	0	
T2, NOS	32 (4.8%)	4 (9.8%)	
Tumor size			<0.001
≤10 mm	452 (68.0%)	9 (22.0%)	
11- 20 mm	136 (20.5%)	10 (24.4%)	
21- 50 mm	68 (10.2%)	14 (34.1%)	
>50 mm	9 (1.3%)	8 (19.5%)	
Location			0.21
Cardia/Fundus	105 (15.8%)	8 (19.5%)	
Body	219 (32.9%)	10 (24.4%)	
Antrum/Pylorus	145 (21.8%)	14 (34.1%)	
Stomach, NOS	196 (29.5%)	9 (22.0%)	

LNM, lymph node metastasis; g-NEN, gastric neuroendocrine neoplasm; SD, standard deviation; NEC, neuroendocrine carcinoma; NOS, Not otherwise specified.

TABLE 2 | Characteristics of patients with T1/T2 d-NENs (n = 621) with and without LNM.

Variable	No LNM (n = 546)	With LNM (n = 75)	*P*
Age at diagnosis (mean ± SD)	63.0 ± 11.9	56.8 ± 11.9	<0.001
Male (%)	280 (51.3%)	29 (38.7%)	0.04
Year of diagnosis			<0.001
2004-2007	19 (3.5%)	6 (8.0%)	
2008-2011	110 (20.1%)	28 (37.3%)	
2012-2015	417 (76.4%)	41 (54.7%)	
Race/Ethnicity			0.39
Non-Hispanic White	290 (53.1%)	49 (65.3%)	
Black	150 (27.5%)	16 (21.3%)	
Hispanic White	61 (11.2%)	4 (5.3%)	
Asian/Pacific Islanders	37 (6.8%)	5 (6.7%)	
American Indian/Alaska Native	2 (0.4%)	0	
Unknown	6 (1.1%)	1 (1.3%)	
Tumor differentiation			0.02
Well differentiated	478 (87.5%)	57 (76.0%)	
Moderately differentiated	64 (11.7%)	16 (21.3%)	
Poorly differentiated (NEC)	4 (0.7%)	2 (2.7%)	
Depth of invasion			<0.001
Mucosa	191 (35.0%)	10 (13.3%)	
Submucosa	280 (51.3%)	26 (34.7%)	
Muscularis propria	51 (9.3%)	36 (48.0%)	
T1, NOS	18 (3.3%)	1 (1.3%)	
T2, NOS	6 (1.1%)	2 (2.7%)	
Tumor size			<0.001
≤10 mm	404 (74.0%)	29 (37.3%)	
11- 20 mm	117 (21.4%)	30 (40.0%)	
21- 50 mm	17 (3.1%)	15 (20.0%)	
>50 mm	8 (1.5%)	2 (2.7%)	

LNM, lymph node metastasis; d-NEN, duodenal neuroendocrine neoplasm; SD, standard deviation; NEC, neuroendocrine carcinoma; NOS, Not otherwise specified.

regression and then used a multivariate model that adjusted for demographic factors (age, sex, and race). The results indicated that tumor size greater than 10 mm, depth of invasion, and poor differentiation were risk factors for LNM in patients with g-NENs in the unadjusted and multivariable logistic regression (all $P < 0.05$). The results were similar for patients with d-NENs.

Our results also indicated that tumor size (stratified as ≤10, 10–20, and >20 mm) had a high correlation with LNM in patients with g-NENs and d-NENs (both $P < 0.001$ for trend). Notably, compared with NENs of 10 mm or less in diameter, the risk of LNM was significantly increased in patients with tumors that were 11 to 20 mm in diameter among those with g-NENs [adjusted odds ratio (aOR): 3.96, 95% confidence interval (CI): 1.54–10.2; $P = 0.004$] and among those with d-NENs (aOR: 4.11, 95% CI: 2.31–7.33; $P < 0.001$).

Prevalence of LNM in NETs With Different Sizes

Overall, 104 of 1289 patients with gastroduodenal NETs (8.1%) and 12 of 38 patients with NECs (31.6%) had LNM. Thus, as expected, the prevalence of LNM was greater in patients with NEC (Chi-square $P <$

TABLE 3 | Logistic regression of factors associated with LNM in patients with g-NENs.

Variable	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*
Tumor differentiation				
Well differentiated	Reference	–	Reference	–
Moderately differentiated	1.38 (0.58-3.29)	0.46	1.37 (0.57-3.27)	0.48
Poorly differentiated (NEC)	10.06 (4.29-23.58)	<0.001	8.38 (3.41-20.58)	<0.001
Depth of invasion*				
Mucosa	Reference	–	Reference	–
Submucosa	3.82 (1.12-13.07)	0.03	3.92 (1.14-13.54)	0.03
Muscularis propria	8.50 (2.38-30.42)	0.001	8.82 (2.44-31.96)	0.001
Tumor size				
≤10 mm	Reference	–	Reference	–
11- 20 mm	3.69 (1.47-9.27)	0.005	3.96 (1.54-10.20)	0.004
>20 mm	14.35 (6.37-32.33)	<0.001	17.38 (7.38-40.92)	<0.001

**Cohort size, n = 541 (data on specific layer of invasion depth were available only in 541 out of 706 g-NEN patients). Logistic regression was utilized to explore the association of tumor differentiation, size, and invasive depth with LNM in model 1 (univariate). Confounding variables of age, sex, and race were adjusted in model 2. LNM, lymph node metastasis; g-NEN, gastric neuroendocrine neoplasm; OR, odds ratio; CI, confidence interval.*

TABLE 4 | Logistic regression of factors associated with LNM in patients with d-NENs.

Variable	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*
Tumor differentiation				
Well differentiated	Reference	–	Reference	–
Moderately differentiated	2.10 (1.14-3.87)	0.02	1.94 (1.02-3.67)	0.04
Poorly differentiated (NEC)	4.19 (0.75-23.40)	0.10	5.71 (0.95-34.19)	0.06
Depth of invasion*				
Mucosa	Reference	–	Reference	–
Submucosa	1.77 (0.84-3.76)	0.14	1.86 (0.87-4.00)	0.11
Muscularis propria	13.48 (6.27-28.99)	<0.001	13.18 (6.00-28.95)	<0.001
Tumor size				
≤10 mm	Reference	–	Reference	–
11- 20 mm	3.70 (2.13-6.44)	<0.001	4.11 (2.31-7.33)	<0.001
>20 mm	9.81 (4.75-20.27)	<0.001	9.73 (4.52-20.95)	<0.001

**Cohort size, n = 594 (data on specific layer of invasion depth were available only in 594 out of 621 d-NEN patients). Logistic regression was utilized to explore the association of tumor differentiation, size, and invasive depth with LNM in model 1 (univariate). Confounding variables of age, sex, and race were adjusted in model 2. LNM, lymph node metastasis; d-NEN, duodenal neuroendocrine neoplasm; OR, odds ratio; CI, confidence interval.*

0.001, **Figures 1A, B**). Analysis of NECs indicated there was no LNM in the 2 patients with T1 d-NEC, but LNM was present in 20.0% of patients with T1 g-NEC, 33.3% of patients with T2 g-NEC, and 50.0% of patients with T2 d-NEC. Analysis of patients with NETs indicated that LNM was present in 1.8% of patients with T1 g-NET, 9.7% of patients with T2 g-NET, 5.4% of patients with T1 d-NET, and 24.4% of patients with T2 d-NET. Thus, patients with T1/T2 gastroduodenal NECs are not optimal candidates for endoscopic resection due to the high prevalence of LNM. We therefore focused on T1/T2 gastroduodenal NETs in our subsequent analysis.

We examined the association of invasion depth, tumor size, and LNM in patients with gastroduodenal NETs for whom data on invasion depth were available (**Table 5**). Among 517 patients with g-NETs, LNM status was detected in 254 patients using cross-sectional imaging or endoscopic ultrasound (EUS) and in 263 patients by open surgery. Analysis of the 517 patients with g-NETs indicated LNM occurred in 1.9% of patients with tumors in the mucosa, 6.4% of patients with tumors in the submucosa, and 12.3% of patients with tumors in the muscularis propria. Among 589 patients with d-NETs, LNM status was detected in

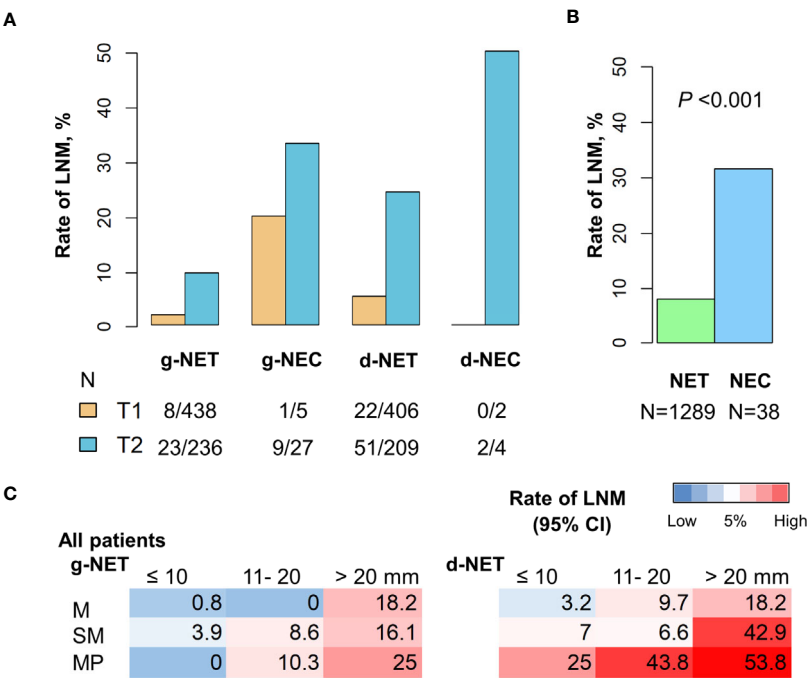

FIGURE 1 | Incidence of LNM in patients with g-NENs and d-NENs. **(A)** LNM rates in T1 and T2 stages stratified by tumor differentiation. **(B)** Comparison of LNM rates in all NETs (n = 1289) and all NECs (n = 38). **(C)** Risk of LNM in NETs with different invasion depths and sizes. g-NEN, gastric neuroendocrine neoplasm; d-NEN, duodenal neuroendocrine neoplasm; LNM, lymph node metastasis; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma.

TABLE 5 | Association of invasion depth, tumor size, and LNM in 1106 patients with gastroduodenal NETs.

Invasion depth	Prevalence of LNM				*P*
	Total	≤10 mm	11-20 mm	>20 mm	
g-NETs (n = 517)					<0.001
Mucosa	3/155 (1.9%)	1/130 (0.8%)	0/14	2/11 (18.2%)	
Submucosa	18/281 (6.4%)	7/180 (3.9%)	6/70 (8.6%)	5/31 (16.1%)	
Muscularis propria	10/81 (12.3%)	0/18	4/39 (10.3%)	6/24 (25.0%)	
d-NETs (n = 589)					<0.001
Mucosa	10/200 (5.0%)	5/158 (3.2%)	3/31 (9.7%)	2/11 (18.2%)	
Submucosa	26/304 (8.6%)	16/229 (7.0%)	4/61 (6.6%)	6/14 (42.9%)	
Muscularis propria	34/85 (40.0%)	6/24 (25.0%)	21/48 (43.8%)	7/13 (53.8%)	

LNM, lymph node metastases; NET, neuroendocrine tumor; g-NEN, gastric neuroendocrine neoplasm; d-NEN, duodenal neuroendocrine neoplasm.

288 patients using cross-sectional imaging or EUS and in 301 patients by open surgery. Analysis of the 589 patients with d-NETs indicated LMN occurred in 5% of patients with tumors in the mucosa, 8.6% of patients with tumors in the submucosa, and 40.0% of patients with tumors in the muscularis propria. Our analysis of the relationship of LNM in each layer with tumor size indicated a high risk for LNM (nodal metastasis rate > 5%) in 8 of the 9 subgroups of patients with d-NETs, but not in the subgroup with mucosal tumors smaller than 10 mm. There was also a high risk for LNM in 5 of the 9 subgroups of patients with g-NETs, but not in the 3 subgroups with tumors smaller than 10 mm or in the subgroup with mucosal tumors that were 11 to 20 mm (**Table 5** and **Figure 1C**).

NEN-Specific Survival According to Tumor Stage/Grade and Treatment Modality

Patients with g-NENs had a median follow-up period of 43.7 months and an overall 5-year survival rate of 84.0%. Patients with d-NENs had a median follow-up period of 43.2 months and an overall 5-year survival of 87.1%. We then determined CSS analysis on four different subgroups for patients with g-NENs and d-NENs: T1 nodal-negative NETs, T2 nodal-negative, nodal-positive NETs, and NECs (**Figure 2**). The results indicated NEN-specific survival for NEC patients was significantly worse than the other 3 subgroups among patients with d-NENs ($P < 0.001$) and among patients with g-NENs ($P = 0.02$). However, the CSS was not significantly different in nodal-negative NET patients with stage T1 or T2. Univariate and multivariate Cox regression analysis indicated that LNM increased the risk for poor OS and CSS in patients with T1/T2 g-NETs (adjusted hazard ratio (aHR): 6.15, 95% CI: 2.67–14.1; $P < 0.001$; **Supplementary Table 1**). In addition, analysis of patients with T1/T2 d-NETs indicated that increased age was associated with worse OS (aHR: 1.05, 95% CI: 1.03–1.08; $P < 0.001$) and nodal involvement was associated with decreased CSS (aHR: 4.25, 95% CI: 1.24–14.5; $P = 0.02$).

Next, we compared the CSS rates of patients who underwent local excision (endoscopic treatment including polypectomy,

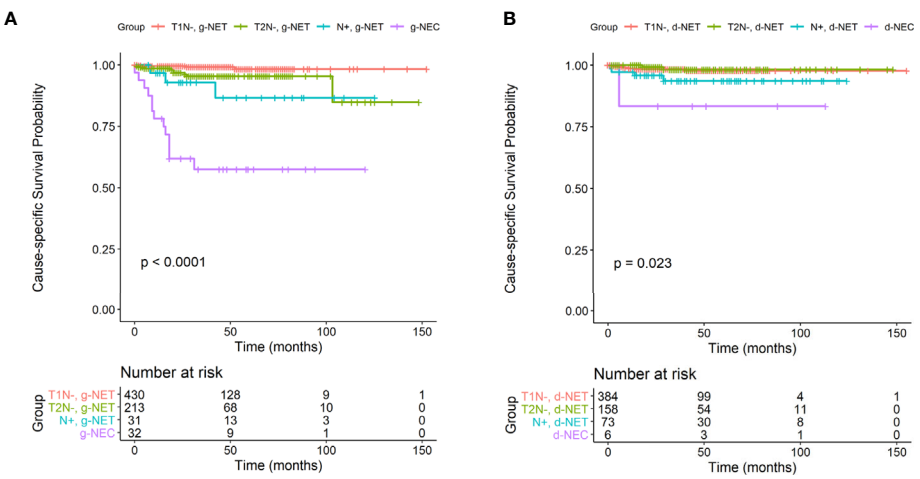

FIGURE 2 | Cause-specific survival in four clinical subgroups (T1 nodal-negative NETs, T2 nodal-negative NETs, nodal-positive NETs, and NECs) among patients with g-NENs **(A)** and d-NENs **(B)**. NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; g-NEN, gastric neuroendocrine neoplasm; d-NEN, duodenal neuroendocrine neoplasm.

excisional biopsy, and electrocautery) or surgical resection. There were 470 patients with nodal-negative g-NETs and 400 patients with nodal-negative d-NETs with tumors confined to submucosal layer for whom information on treatment was available. Among them, 587 patients (67.5%) received local excision and 283 patients (32.5%) received radical surgery. For patients with g-NETs, the unadjusted Kaplan-Meier curves were similar in the two groups (**Supplementary Figure 1A**). However, for patients with d-NETs, those who received surgical resection had a significantly worse CSS (P = 0.004; **Supplementary Figure 1B**). In agreement, Cox regression analysis with adjustment for age, sex, and tumor size also demonstrated that open surgical treatment was associated with poor CSS in patients with nodal-negative d-NETs confined to the submucosa (**Supplementary Table 2**).

DISCUSSION

This large population-based study examined risk factors for LNM in patients who had T1/T2 upper gastrointestinal NENs. We found that tumor differentiation, size, and infiltration depth were significantly associated with LNM. Patients with poorly-differentiated NECs had a high risk of LNM, indicating that endoscopic resection was an inappropriate treatment. Thus we further investigated the association of tumor size, tumor invasion, and LNM in patients with early gastroduodenal NETs to identify the suitability of endoscopic resection.

Our findings indicated that patients with a tumor of 11 to 20 mm had a higher risk of LNM than patients with a tumor than 10 mm for those with early-stage g-NETs or early-stage d-NETs. As such, it should be prudent to perform endoscopic resection of intermediate-sized gastroduodenal NETs (11–20 mm); EUS and abdominal enhancement computed tomography are needed to assess tumor infiltration and LNM for patients with these NETs. Our results suggest that surgical resection is most appropriate when the tumor is larger than 20 mm. In contrast to early gastrointestinal cancer, NETs originate from endocrine cells in the deep mucosa. Previous research showed that cold biopsy forceps polypectomy was inadequate for curative treatment of gastrointestinal NETs because of the high rates of submucosal infiltration. Instead, pathological examination after complete resection by snare polypectomy with electrocauterization, endoscopic mucosal resection (EMR), or endoscopic submucosal dissection (ESD) are appropriate alternatives for biopsy of NETs (16). ESD is a safe and effective procedure that provides accurate pathological assessment and curative treatment for patients with upper gastrointestinal NETs (4, 17, 18). In our opinion, patients with intermediate-sized NETs who are willing to receive endoscopic resection should be informed of a risk for the need of additional open surgery, and diagnostic EMR/ESD can be performed after careful evaluation. As pointed out in ENETS guidelines, EUS should be performed for g-NETs larger than 10 mm before endoscopic excision. Even if curative endoscopic resection is achieved, regular follow-ups are important for patients whose gastroduodenal NETs were larger than 10 mm. If the pathological examination of ESD specimen shows lymphvascular invasion or muscularis propria invasion, additional surgical resection with lymph node dissection is necessary for NET patients, considering worse prognosis of recurrence in regional lymph node (19).

There are five clinical entities of d-NENs, and the two main ones are gastrinomas (non-functional neoplasms with positive neuroendocrine markers) and somatostatinomas. Duodenal gastrinomas are associated with multiple endocrine neoplasia type 1, and somatostatinomas often occur in the periampullary region (10). The term 'd-NEN' in the present study excludes tumors in the ampulla of Vater, because these tumors cannot be resected using endoscopy. A previous study reported that patients with d-NENs had better prognosis but a higher probability of regional LNM than those with g-NENs (20). We also observed higher rates of LNM of d-NETs than NETs from the stomach (5.4% *vs.* 1.8% for T1, 24.4% *vs.* 9.7% for T2). In addition, consistent with previous reports that less than 1 to 3% of d-NENs are poorly differentiated (21), nearly all d-NENs in our study (615 of 621) were well-differentiated. This might be related to the more favorable outcomes of these patients. To date, there is no consensus about the association between tumor size and the prevalence of LNM in patients with NETs in the duodenum due to the rarity of this clinical entity (21). As expected, we found that the incidence of LNM increased as tumor size increased. However, in contrast to the low risk of LNM in patients with small g-NETs, we found that the rate of LNM exceeded 5% even for patients with small d-NETs (≤10 mm) that were in the submucosal layer. In other words, the risk of LNM in patients with d-NETs that are less than 10 mm in diameter must be considered when selecting a treatment.

We also compared the long-term survival of patients with upper gastrointestinal NETs who underwent endoscopic resection *vs.* surgical resection. Our results showed that patients in these two groups had similar survival when they had nodal-negative g-NETs that were confined to the submucosa, but there was improved CSS for patients with d-NETs who underwent endoscopic treatment. A possible explanation for this phenomenon is that some surgical complications resulted from large-scale surgical resection caused worse prognosis in some elderly patients. However, the risk of perforation is higher for endoscopic resection of d-NETs because the bowel wall of the duodenum is thinner (22). A previous study reported a perforation rate of about 6 to 7% (18, 23). As such, although endoscopic resection of d-NETs may improve CSS by reducing the complications resulting from open surgery, considering risk of LNM and perforation, only patients with d-NETs that are small and superficial are candidates for endoscopic treatment by experienced endoscopists (24).

Some limitations should be noted in this SEER-based analysis. The major drawback was missing data on mitotic counts and Ki-67 labeling, which are widely used in clinical practice for assessment of proliferation. According to the newest WHO classification, NETs and NECs are distinguished by tumor cell differentiation, and poorly-differentiated NECs are not formally graded but are considered high-grade by definition (13). Even so, our inability to grade NETs influenced the accuracy and reliability of our findings regarding the patterns of LNM.

Another shortcoming was that we could not classify patients with g-NENs according to clinical subgroups (types 1–3 according

to etiology, in which type 3 lesions have the poorest differentiation and are associated with the poorest clinical outcome). As indicated in ENETS guidelines, open surgery was recommended for type 1 g-NENs with poor differentiation, metastasis, or muscularis propria invasion, and for all type 2 and type 3 g-NENs (6, 7). Because type 1 and type 2 gastric NENs account for the vast majority of g-NETs, and because most type 3 g-NENs are g-NECs (6, 25), we tried to compensate for this limitation by separately assessing LNM in patients with NETs and NECs. Although there were still some type 3 tumors in patients within g-NETs, previous studies found that endoscopic resection of small and well-differentiated type 3 g-NETs can be curative, similar to type 1 and 2 g-NETs (26–28). Besides, the SEER database does not document the exact locations of d-NENs, and we could not exclude the possibility that some d-NENs were in the third and fourth part of the duodenum. Although neoplasms at these sites account for less than 10% of all d-NENs, these regions are inaccessible by upper endoscopy (8). In addition, information of Somatostatin Receptor Scintigraphy was lacking, which might underestimate the presence of small lymph nodal metastases. Also, the SEER database also has no data on lymph vascular involvement (LVI), which is closely associated with LNM. Because LVI is crucial for clinical judgement of curative resection after ESD (29), further studies should examine the relationship between size and grade of NETs and LVI.

In summary, our results showed that LNM was more common in patients with superficial gastroduodenal NETs in which the tumor was more than 10 mm in diameter. In light of the low LNM rate, our results support the use of endoscopic resection for curative treatment of g-NETs that are 10 mm and smaller and confined to the submucosa, as well as intramucosal d-NETs. LNM was more common in patients with d-NETs than g-NETs, and we therefore suggest that the risk of nodal involvement should considered even for submucosa-infiltrating d-NETs that are smaller than 10 mm. Further validation of these findings in a multicenter prospective study is warranted.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Y-JZ and X-BL contributed to study design. Q-WZ, Q-WW, J-NC, Y-JG, and X-YW collected the study data. Y-JZ and Q-WZ contributed to data analysis and interpretation. Y-JZ contributed to manuscript writing. X-BL reviewed the manuscript and contributed to quality control. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.658392/full#supplementary-material

Supplementary Figure 1 | Cause-specific survival of nodal-negative g-NETs **(A)** and d-NETs **(B)** in the submucosal layer in groups that received endoscopic resection or open surgical resection. g-NET, gastric neuroendocrine tumor; d-NET, duodenal neuroendocrine tumor.

Supplementary Table 1A | Cox proportional hazards model assessing factors associated with OS and CSS in T1/T2 g-NETs.

Supplementary Table 1B | Cox proportional hazards model assessing factors associated with OS and CSS in T1/T2 d-NETs.

Supplementary Table 2A | Cox proportional hazards model assessing factors associated with OS and CSS in nodal-negative g-NETs confined to the submucosa.

Supplementary Table 2B | Cox proportional hazards model assessing factors associated with OS and CSS in nodal-negative d-NETs confined to the submucosa.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Multicenter Analysis of Presacral Neuroendocrine Neoplasms—Clinicopathological Characterization and Treatment Outcomes of a Rare Disease

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Background and Aims: Neuroendocrine neoplasms (NENs) of the presacral space are an extremely rare disease entity with largely unknown outcome and no established standard of care treatment. Therefore, we wanted to analyze clinical presentation, histopathological findings, treatment outcomes, and prognosis in a multicentric patient cohort.

Methods: We searched local databases of six German NEN centers for patients with presacral NEN. Retrospective descriptive analyses of age, sex, stage at diagnosis, symptoms, grade, immunohistochemical investigations, biomarkers, treatment, and treatment outcome were performed. Kaplan–Meier analysis was used to determine median overall survival.

Results: We identified 17 patients (11 female, 6 male) with a median age of 50 years (range, 35–66) at diagnosis. Twelve cases presented initially with distant metastases including bone metastases in nine cases. On pathological review the majority of patients had well-differentiated G2 tumors. Immunohistochemical profile resembled rectal NENs. All but one patient had non-functioning tumors. Somatostatin receptor imaging was positive in 14 of 15 investigated cases. Eight patients were treated surgically including palliative resections; 14 patients received somatostatin analogs with limited efficacy. With 14 PRRTs completed, 79% showed clinical benefit, whereas only one patient with neuroendocrine carcinoma (NEC) responded to chemotherapy. Treatment with

everolimus in three patients was not successful, whereas cabozantinib resulted in a disease stabilization in a heavily pretreated patient. During a median observation period of 44.5 months, 6 patients died. Median overall survival was not reached.

Conclusion: Presacral NEN are histopathologically similar to rectal NENs. Presacral NEN should be considered as possible primary in NEN of unknown primary. The majority of tumors is non-functioning and somatostatin receptor positive. PRRT demonstrated promising activity; tyrosine kinase inhibitors warrant further investigations. Further molecular characterization and prospective evaluation of this rare tumor entity are needed.

Keywords: presacral, retrorectal, CUP-NET, neuroendocrine tumor, neuroendocrine carcinoma, carcinoid, PRRT, prognosis

INTRODUCTION

Neuroendocrine neoplasms (NENs) are heterogeneous neoplasms originating from the diffuse neuroendocrine cell system. They are defined by their endocrine phenotype, which is verified by immunohistochemical staining for the small synaptic vesicle-analogue protein synaptophysin and the large dense core-vesicle protein chromogranin A (1). They may originate nearly everywhere in the body, but most often, the primary tumor is located in the gastroenteropancreatic system or in the lung (2). For treatment planning, the knowledge of the primary and the differentiation between primary and metastatic lesion is important. Despite improvement of diagnostic techniques in 8–12% of the NEN patients, the primary remains undetected (CUP-NEN; cancer of unknown primary) (2–5). Somatostatin receptor (SSTR) expression is characteristic for neuroendocrine tumors (NETs) and allows detection of SSTR-expressing NETs by scintigraphy or specific SSTR-PET/CT (68Ga DOTATOC- or DOATATATE-PET-CT) (6). 68Ga-DOTA PET/CTs are particularly important for primary tumor search in CUP-NET, as their sensitivity is superior to other imaging modalities (7).

Specific immunohistochemical stainings including the transcription factors CDX-2 (intestinal primary), TTF-1 (lung/thyroid gland), Islet-1 (pancreas), PDX1 (duodenum, pancreas) (8), and specific hormones may help to identity the primary and are therefore recommended in CUP-NET patients (9). These markers are of very limited use in NEC (9). Prostate-specific acid phosphatase (PSAP) is a glycoprotein-enzyme produced in prostate carcinomas, particularly indicative of its spread beyond the prostate but also characteristic of hindgut NETs (10). In patients with hindgut NETs staining for chromogranin A often is only weakly positive or may even be negative (9, 11).

The presacral space lies between the rectum anteriorly, the sacrum posteriorly, and the endopelvic fascia laterally. It contains embryological remnants of different tissues. Tumors of this presacral space are rare, mostly benign, but several malignant tumors have also been reported (12), including NEN. Immunohistochemistry is important for the differentiation of NEN from other primary tumors or metastases of the presacral region (9, 13). Presacral NENs are extremely rare; to the best of our knowledge, about 70 cases have been reported so far mainly in single case reports (14–70) or small series (15, 17, 20, 26, 39, 42, 55, 71, 72). The majority of presacral NEN was diagnosed in female individuals of younger age compared to the median age of diagnosis in other gastroenteropancreatic NEN. According to the literature, presacral NENs are usually well-differentiated tumors with local involvement, but cases with distant metastases have also been reported (20, 72).

Therapeutic options of metastatic NEN include somatostatin analogs (SSA), chemotherapy, peptide receptor radionuclide therapy (PRRT), everolimus, and tyrosine kinase inhibitors (TKIs) (73). Even in more common NENs like pancreatic NEN, data of comparative treatment trials or on best sequence of treatments are not available at the moment. In rare subtypes like presacral NEN, data on treatment outcome are lacking. The aim of our study is to describe clinical, histopathological, therapeutic, and prognostic features of patients with presacral NEN who presented at one of five contributing NEN referral centers within the last 10 years. We were particularly interested in the number of patients we could collect in the participating NEN referral centers as a surrogate for the frequency of this disease, in the percentage of patients who were initially diagnosed as CUP-NEN, to analyze whether all had differentiated tumors and get a hint which therapeutic option may be of benefit in this extremely rare subgroup.

PATIENTS AND METHODS

All patients with neuroendocrine neoplasm and suspected primary tumor within the presacral space were included in this retrospective multicenter evaluation. In the case of initial presentation as NEN with unknown primary, investigations to detect the primary/exclude another primary tumor localization included gastroscopy, colonoscopy, CT or MRI, SSTR imaging [scintigraphy or specific positron emission tomography (PET)] and in some cases fluorodeoxyglucose (FDG)-PET-CT and endoscopic ultrasound. For the vast majority of patients (14 of 17), SSTR-based PET-CT was available during follow-up. In addition to the standard immunohistochemical stainings, such as synaptophysin, chromogranin A, and Ki67, further specific stainings were done according to local practice at the centers, e.g., for the transcriptional factors CDX-2 and TTF-1 in the majority of cases, and ISLET-1, prostate-specific acid phosphatase (PSAP), vimentin, CD56, and somatostatin receptor subtype 2 (SSTR2) in some cases. Several

patients received molecular diagnostics *via* "next generation sequencing" panels or whole exome/genome sequencing as part of the German Cancer Consortium (DKTK) Molecularly Aided Stratification for Tumor Eradication Research Trial (MASTER) (74–76).

The patients were identified *via* center-based databases or personal knowledge, and the available essential information was extracted and evaluated across centers. The following German centers have participated: Dresden, Essen, Halle (Saale), Hamburg, Heidelberg, and Marburg. Collected data included date of diagnosis, date of birth, sex, histology, stage, functionality, symptoms of tumor disease, localization of metastases, date of diagnosis of metastases, somatostatin receptor status, treatments with outcome, and date and cause of death or date of last contact. This study was conducted in accordance with the Declaration of Helsinki. All patients were included in the local disease databases conducted with approval of the local ethics committees at the respective sites. Written informed patient consent and approval for data collection and analysis were obtained upon admission to our institutions. For the use of the images, an additional consent was obtained in the selected cases.

Statistical analysis was performed IBM® SPSS® Statistics 27.0 (IBM, Armonk, NY, USA). Descriptive statistical analysis was performed for most parameters. Kaplan–Meier analyses of median duration of observation and overall survival were investigated.

RESULTS

Patient Characteristics and Clinical Presentation

We identified 17 patients (n = 17) in the databases of six German centers for neuroendocrine neoplasms [Dresden, Essen, Hamburg, Halle (Saale), Heidelberg and Marburg], who were diagnosed with a primary presacral NEN. Most patients were referred to one of our centers with the diagnosis of cancer of unknown primary (CUP) NET. Presacral NENs were diagnosed more frequently in women (n = 11; 64.7%) than in men (n = 6; 35.3%). In our study population, the initial diagnosis occurred at an age between 35 and 66 years. The patients had a median age of 50 years (mean, 50.3 years).

As mentioned above, an association with various anomalies such as tailgut cysts is frequently described in the literature (72). In our database, an association to an anomaly was detected in only one patient and suspected in another one. One patient showed a presacral localized histologically confirmed teratoma in addition to her primary presacral NEN G2. In another patient, a paraganglioma in the pterygopalatine fossa was suspected but could not be clearly distinguished from osseous metastasis due to a lack of histopathological confirmation. Even though there was no direct association with tailgut cysts, cystic portions of otherwise solid presacral NET could be detected on imaging in some cases (**Figure 1**).

According to our database analysis, presacral NENs are predominantly non-functioning. Only one patient had a functionally active presacral NET producing parathyroid hormone-related peptide (PTHrP). This patient developed a seizure due to paraneoplastic hypercalcemia. No patient suffered from carcinoid syndrome.

Most of the patients (14/17) presented clinically with locoregional symptoms caused by the space-occupying process of presacral NEN. Primary presacral NEN predominantly caused symptoms such as pain of the lower abdomen, pelvis, sacral region, perineum, or lower back (12/17); unilateral paresthesia of the lower limb (2/17); and defecation disorders, e.g., chronic constipation (4/11) or urination disorders (1/17) due to their mass effect. Systemic symptoms showed a minor role in presacral NEN. Only two patients presented with b-symptoms at initial diagnosis.

Patients with presacral NEN were often diagnosed at an advanced stage. At the time of diagnosis, most primary tumors showed a pronounced local extension with a size of 3–9 cm in diameter and frequently an infiltration of the sacrum and the

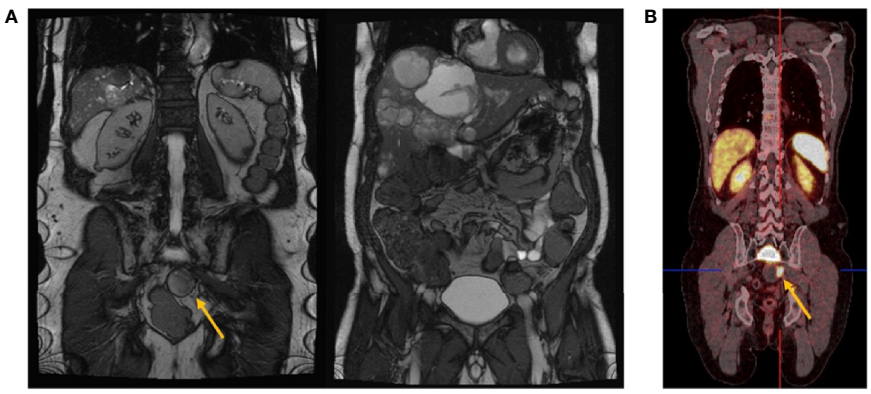

FIGURE 1 | **(A)** Magnetic resonance imaging. Coronal view demonstrating primary presacral neuroendocrine neoplasm (yellow arrow) and liver metastases. **(B)** Coronal view of a 68Ga-DOTATOC-PET/CT scan showing SSTR expression of the whole body. While the liver metastases showed a homogeneous SSTR expression, only a part of the presacral lesion showed a homogeneous SSTR expression (yellow arrow), suggesting a SSTR-negative/cystic portion besides the SSTR-positive solid presacral NEN. MRI and 68-DOTATOC-PET/CT are from the same patient (study-ID III) at different time points.

coccyx. In our cohort, most patients (12/17) had distant metastases at the time of diagnosis. Only five patients had a localized tumor stage. However, all patients except one with presacral NEN developed distant metastasis during the course of their disease in our series. Interestingly, one of the most frequent metastatic site in our cohort of patients with presacral NEN was the skeleton. Bone metastases were detected in 11 of 17 patients. Likewise, the liver was a common metastatic site (11/17). Furthermore, locoregional lymph node metastases occurred more frequently (10/17), whereas diffuse lymphatic metastasis to para-aortic and mesenteric lymph nodes and pulmonary metastases occurred less frequently (3/17). Metastases to the adrenal gland and peritoneum were diagnosed in two cases each (2/17). Brain metastases were not diagnosed in our cohort.

Patients characteristics are summarized in **Table 1**.

Histopathological Features

Predominantly, presacral NENs were histologically well differentiated. Most presacral NETs were classified as G2 tumors based on their Ki67 index. Only one presacral NET corresponded to a G1 NET with a Ki67 index of <2%, and three patients had a G3 presacral NET. Poorly differentiated presacral NEC turned out to be extremely rare. In our databases, there was only one presacral NEN that was histopathologically classified as large-cell neuroendocrine carcinoma (LCNEC) and had a Ki67 index of 80%.

Synaptophysin was strongly positive by immunohistochemistry in all samples of presacral NEN. In contrast, chromogranin A was only weakly positive in the majority of cases and even negative in two cases (**Table 2**).

Thyroid transcription factor 1 (TTF-1), a marker for metastases in NETs of pulmonary origin, and CDX2, a marker for metastases of gastrointestinal origin, were mostly negative in presacral neuroendocrine tumors. Only the presacral NEC was immunohistochemically positive for TTF-1, and one case of presacral NET was positive for CDX-2. Exclusion of a gastrointestinal primary tumor was performed by abdominal CT, gastroscopy, and colonoscopy in this case.

Vimentin—a marker for soft tissue tumors, but also expressed in various epithelial cancers—was examined immunohistochemically in only five presacral NETs, but it was detected in four of five cases.

Four presacral NETs were examined for PSAP by immunohistochemistry, and all resulted in positive detection of PSAP.

A representative example of microscopic tumor morphology and immunohistochemical stainings is shown in **Figures 2A–E**. **Table 2** summarizes the results of the histopathological reports.

Cytokeratin 7 (CK7) was not detected by immunohistochemistry (n = 9). Cytokeratin 18 (CK18) was positive (n = 3). CD56, a non-specific marker for neuroendocrine tumors, was positive in four tumors and negative in one.

Molecular Characterization

Molecular diagnostics was performed in three patients to identify molecular targets: in one NET G3 (case XIII), a colorectal panel was applied, whereas one NEC G3 (case IX) and one NET G2 (case XII) were enrolled in the MASTER Trial and underwent whole-exome and whole-genome sequencing, respectively (**Figure 3**). All patients were microsatellite stable. In case XIII, no targetable alterations were detected, and the absence of a pathogenic TP53 mutation confirmed the diagnosis of NET G3. In the NEC G3 case, the tumor mutational burden (TMB) was intermediate with 4.32 non-coding mutations per megabase; besides a TP53 mutation, several cyclin pathway alterations (CDKN2A mutation, CCND1 mutation, CDK6 amplification)

TABLE 1 | Patient characteristics.

Study ID	Sex	Age at diagnosis	Grading	Stage at diagnosis	Endocrine function	SSTR imaging	Symptoms related to presacral NEN	Associated anomalies
I	M	48	G2	IV	n.a.	Positive	Perineal pain	–
II	F	35	G2	IV	Non-functional	Positive	Defecation disorder	–
III	M	65	G2	IV	Non-functional	Positive	Asymptomatic	–
IV	F	46	G2	II	Non-functional	Positive	Abdominal and pelvic pain	–
V	F	66	G2	IV	Non-functional	Positive	Chronic obstipation	–
VI	M	53	G2	IV	Non-functional	Positive	Defecation disorder, perineal pain	–
VII	F	52	G3	IV	Non-functional	Positive	Abdominal pain	–
VIII	F	40	G3	III	Non-functional	Positive	Asymptomatic	–
IX	M	60	G3/ LCNEC	III	Non-functional	n.a.	Pain in the sacral region, paresthesia right lower limb, chronic obstipation	–
X	F	44	G2	IV	Non-functional	Positive	Pain in the sacral region	–
XI	F	65	G2	IV	Non-functional	Positive	Abdominal pain, diffuse backpain	–
XII	F	33	G2	IV	Non-functional	Positive	Asymptomatic	–
XIII	M	62	G3	III	Non-functional	Positive	Low backpain, paresthesia of the right lower limb, foot drop	–
XIV	F	41	G1	III or IV	Non-functional	n.a.	Pelvic pain	Paraganglioma, DD: bone metastasis
XV	M	50	G2	IV	Non-functional	Positive	Pelvic pain and swelling of the right hip	–
XVI	F	58	G2	IV	Parathyroid hormone-related peptide	n.a.	Pelvic pain, urinary tract obstruction, seizure due to paraneoplastic hypercalcemia	–
XVII	F	37	G2	IV	Non-functional	Positive	Pain in the sacral region	Teratoma

n.a., not assessed.

TABLE 2 | Immunohistochemical features of patients with primary presacral neuroendocrine neoplasms.

Study ID	Grading	Ki67	Chromogranin A	Synaptophysin	CD56	PSAP	Vimentin	TTF-1	CDX2	CK-7	CK-18
I	G2	5%	Negative	Positive	Positive	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
II	G2	12%	Positive	Positive	n.a.	Positive	Negative	Negative	Negative	Negative	n.a.
III	G2	7%	Negative	Positive	n.a.	n.a.	Negative	n.a.	Negative	n.a.	n.a.
IV	G2	–20%	Weak positive	Positive	n.a.	Positive	n.a.	Negative	Negative	n.a.	n.a.
V	G2	10%	Weak positive	Positive	n.a.	Positive	n.a.	Negative	Negative	Negative	Positive
VI	G2	5%	Weak positive	Positive	n.a.	Positive	Positive	Negative	Negative	n.a.	n.a.
VII	G3	30%	n.a.	Positive	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
VIII	G3	30%	Weak positive	Positive	n.a.	n.a.	n.a.	n.a.	n.a.	Negative	Positive
IX	G3/LCNEC	80%	n.a.	Positive	Negative	n.a.	Positive	Positive	Negative	Negative	n.a.
X	G2	–20%	Positive	Positive	n.a.	n.a.	Positive	n.a.	Negative	Negative	n.a.
XI	G2	–15%	Positive	Positive	Positive	n.a.	Positive	Negative	Negative	Negative	Positive
XII	G2	–15%	Weak positive	Positive	n.a.	n.a.	n.a.	Negative	Negative	n.a.	n.a.
XIII	G3	30%	Dot-like expression	Positive	Positive	n.a.	n.a.	Negative	Negative	Negative	n.a.
XIV	G1	<2%	Positive	Positive	Positive	n.a.	n.a.	n.a.	Positive	n.a.	n.a.
XV	G2	10%	Weak positive	Positive	n.a.	n.a.	n.a.	n.a.	n.a.	Negative	n.a.
XVI	G2	10%	Dot-like expression	Positive	n.a.	n.a.	n.a.	Negative	n.a.	n.a.	n.a.
XVII	G2	5%	n.a.	Positive	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a., not assessed.

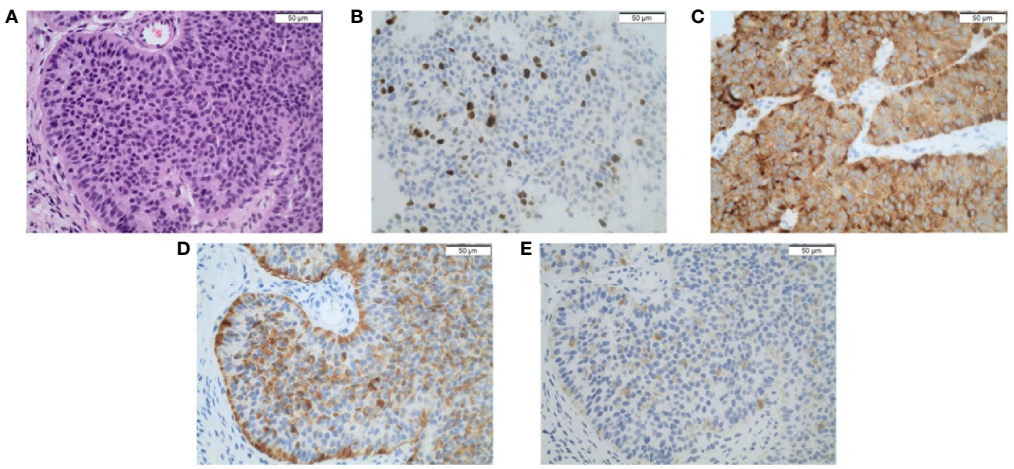

FIGURE 2 | **(A)** Primary presacral neuroendocrine neoplasm stained using H&E (100×). Immunohistochemical staining (100×) shows a well-differentiated neuroendocrine neoplasm with a **(B)** Ki67 index of 7% and positivity for **(C)** synaptophysin, **(D)** chromogranin a, and **(E)** PSAP. Scale bars represent 50 µm.

were detected. Case XII case showed a SETD2 frameshift insertion with presumably consecutive homologous DNA repair deficiency (HRD). TMB was low with 1.30 mutations per megabase.

Circulating Biomarkers

The general circulating neuroendocrine biomarkers chromogranin A (CgA) were determined in 15 of 17 patients. CgA was only slightly elevated (n = 7) or normal (n = 8) at initial diagnosis. Tumor progression during follow-up was not accompanied by increasing CgA levels.

Serotonin—the marker hormone of the carcinoid syndrome—was determined in seven of our patients with presacral NETs; there was no elevation of serotonin in serum.

Neuron-specific enolase (NSE) is a marker for neuronal tissue, neuroendocrine cells, and in particular a circulating marker for poorly differentiated NEN. NSE serum levels were elevated in 6 of 10 patients, with only two patients showing a pronounced elevation >100 µg/L.

Imaging

Imaging often reveals a solid tumor with sometimes cystic portions in the presacral space (see **Figure 1**). The morphological features of presacral NEN in CT and MR scans were unspecific.

SSTR imaging with specific PET-CTs like 68Ga-DOTATOC-PET/CT or SSTR scintigraphy was performed in 15/17 patients to exclude other potential primaries and for disease staging and treatment planning. Only one tumor showed no detectable SSTR expression. Most presacral NENs showed homogeneous SSTR expression (see for example **Figure 3D**), and only two tumors showed heterogeneous expression.

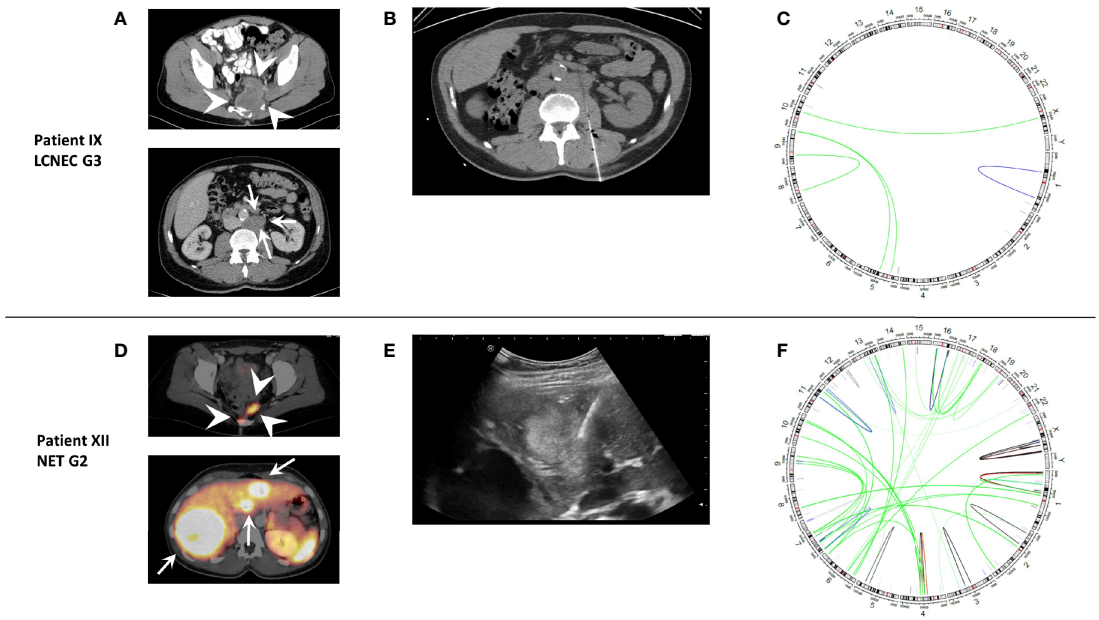

FIGURE 3 | Representative imaging and genomic rearrangements of molecularly characterized patients IX (LCNEC G3) and XII (NET G2). **(A)** CT and **(D)** DOTATOC-PET/CT of presacral primary (white arrowheads) and metastases (white arrows). **(B)** CT-guided biopsy of retroperitoneal lymph node metastasis and **(E)** ultrasound-guided biopsy of liver metastasis for fresh tissue for genomic analysis. **(C, F)** Circle plots of genomic rearrangements. Despite slightly lower tumor mutational burden, case XII shows a much higher number of rearrangements as a sign of homologous DNA repair deficiency possibly due to a pathogenic frameshift SETD2 mutation.

FDG-PET/CT was used in two patients with presacral NET and was not suitable for the detection of the primary tumor.

Treatment in Patients With Presacral NEN

Curative treatment of presacral NENs is only possible in a locally limited stage, when surgical resection of the primary tumor represents the only chance of cure. In our series, most patients with presacral NENs were already in an advanced metastatic stage of disease at the time of diagnosis. Therefore, a palliative systemic therapy was initiated in most cases.

However, even in an already metastatic stage, surgical resection of the primary tumor may be considered for the treatment of symptoms due to the mass effect of the primary tumor. In our cohort, the primary tumor was resected in nine cases, in four patients with localized disease, in two patients with metastatic disease in curative intent (combined with resection of metastases), and in three metastatic patients in palliative intention.

In total, 14 patients with presacral NENs received therapy with SSA. Half of the patients showed stable disease at least until the first follow-up. The other patients underwent therapy escalation due to progression (n = 6) at first follow-up or intolerance (n = 1). During the course of disease, patients frequently showed progression of presacral NEN under SSA; therefore, treatment with SSA was usually not sufficient for growth control in the long term.

In our study population, three patients received treatment with everolimus and did not benefit from this therapy due to progression in the first follow-up. One patient received the tyrosine kinase inhibitor cabozantinib as the seventh line of therapy and showed stable disease [progression-free survival (PFS) >7 months]. The dose of cabozantinib was reduced due to side effects, but treatment was continued at last follow-up.

With good SSTR expression in almost all presacral NEN, PRRT was initiated 15 times in a total of 12 patients. Two patients developed a complete remission, four patients a partial remission, and five patients a stable disease. Two patients developed a mixed response and one a progressive disease. The result of one performed PRRT could not be assessed due to pending staging. Overall, the 14 PRRT responses assessed led to a clinical benefit in 11 cases, giving a clinical benefit rate of 79%.

Liver-directed therapies also proved to be a useful therapeutic approach. One patient developed partial remission after SIRT. Two patients received a transcatheter arterial chemoembolization (TACE) and showed partial remission or stable disease.

Presacral NENs showed limited sensitivity to cytotoxic agents. Overall, presacral NENs responded poorly to chemotherapy. Seven patients received palliative chemotherapy (4× platinum-based chemotherapy with etoposide, 2× temozolomide/capecitabine, and 1× paclitaxel +/− carboplatin). Only the patient with LCNEC showed partial remission under chemotherapy (cisplatin/etoposide). All other patients did not benefit from chemotherapy.

Radiotherapy of the primary tumor was performed in four patients by external beam radiation therapy and in one patient as particle therapy. Three patients developed stable disease and two partial remission after radiotherapy.

Prognosis

Nine patients are currently still alive and in follow-up. Two patients were lost to follow-up. During a median follow-up time

of 44.5 months (mean, 56.0 months; range, 0.73–212.4 months), six patients died 0.7–56.2 months after diagnosis of presacral NEN. Three of these patients died of their presacral NEN. The other three had an unknown cause of death.

The Kaplan–Meier plot of duration of observation and overall survival is shown in **Figure 4**.

DISCUSSION

To the best of our knowledge, this is the largest study of presacral NEN so far. Besides clinical and pathological characteristics, we extensively analyzed the efficacy of different systemic therapeutics.

The pathological characteristics are in line with previous reports on presacral NENs. Expression of the neuroendocrine markers chromogranin A, synaptophysin, and CD56 is common, with CgA staining often being only weakly positive or even negative in some cases. When stained, Ck7, CDX2 (a marker of gastrointestinal origin), and TTF1 (a marker of pulmonary origin in NETs) were mostly negative, whereas PSAP, Ck18, and vimentin were mostly positive. This immunohistochemical profile resembles the profile of rectal NEN (11), which seems quite reasonable considering the hypothesis of a common ontogenetic origin from the embryonal hindgut (34). The similarity to rectal NEN is also supported by the only reported case of molecular profiling in presacral NEN we are aware of (19): here, an intestinal L cell was suggested as a putative cell of origin, with L-cell phenotype being reported in about 80% of rectal NETs (77). Most patients in our study showed well differentiated morphology with the vast majority being classified as NET G2. High-grade histology was detected in four patients, well-differentiated NET G3 in three, and poorly differentiated neuroendocrine carcinoma in one. This NEC was also the only TTF1-positive case in our cohort, demonstrating that TTF1 positivity is commonly observed in NEC of different origins and not a marker of pulmonary primary for NEC (in contrast to NET) (78).

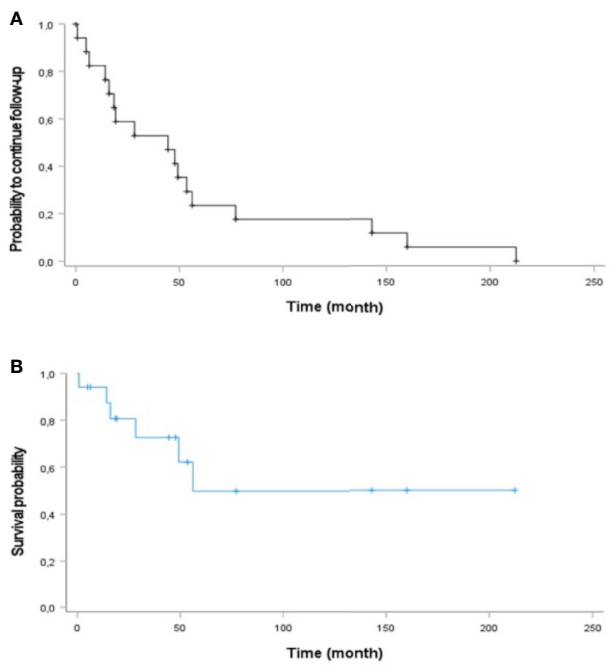

FIGURE 4 | Kaplan–Meier curve analysis of **(A)** duration of observation and **(B)** overall survival of patients with primary presacral NEN (n = 17).

Clinically, many patients showed local symptoms like pain and impairment of defecation as local symptoms of the tumor. However, in a remarkable proportion of patients, presacral NEN was identified as primary tumor of diagnosed NEN metastasis, and patients were referred to the centers as CUP NET cases.

Up to date, most cases of presacral NENs are published as single case reports or small series (14–72) (summarized in **Table 3**). In most of those cases, presacral NENs are treated locally with resection, and there is limited information on follow-up, metastasis, and systemic treatment. The largest case series that we could identify included 10 patients and reported on outcomes of different systemic therapeutic strategies for advanced disease (72). In our cohort, local resection was performed in 8 of 17 patients, half of them in palliative intention to treat local complaints. Several cases were treated with percutaneous radiotherapy, with encouraging results regarding local control and symptomatic improvement. In the literature, an association of presacral NENs with tailgut cysts and teratomas has been described. Additionally, an association with Currarino syndrome can be observed, an autosomal-dominant disorder caused by mutations in the motor neuron and pancreas homeobox 1 (MNX1) gene and characterized by presacral mass, sacral dysgenesis, anorectal anomalies (21, 49, 53). Most remarkably, whereas tailgut cysts or at least partially cystic primary tumors were observed in some of our patients, only one association with teratoma and none of other abnormalities like anorectal malformations or Currarino syndrome were present. Those abnormalities are quite often described in the case reports cited above; in the other larger case series of n = 10, only one patient presented with a teratoma. The observed difference between case reports and case series (including our analysis) could be attributed to a publication bias of more spectacular histological constellation for case reports and referral bias to centers where patients with advanced metastatic are more likely to be referred to. In our cohort, 12 of 17 patients presented with distant metastasis at first diagnosis, whereas more than 80% of the cases reported in the literature were localized or locally advanced.

When taking the systemic treatments applied for presacral NEN in our analysis into context, beside comparing them to NEN in general, a special focus should be laid on rectal NEN regarding their resemblance to presacral NEN as discussed above.

SSAs are among the first approved drugs for disease stabilizations for well-differentiated NETs, with octreotide for midgut NET in the PROMID trial (79) and lanreotide for enteropancreatic NET in the CLARINET trial (80). However,

TABLE 3 | Previously reported cases of presacral NEN.

Reference	Age	Sex	Histology	Anomalies	IHC +: positive (+): weak or focal positive –: negative	Metastases synchronous	Metastases metachronous	Treatment (best response, PFS in months)	Follow-up (months) †deceased
(42) Fiandaca 1988	35	F	NET	Teratoma	NR	LYM, HEP, OTH (ovary)		Surgery, PEB (NR)	NR (12 presurgery)
(70) Noshiro 1990	48	F	NET	–	NR	LYM		Surgery (CR, 24 og)	24
(60) Addis 1991	57	F	NET	–	CAM5.2+, S100(+), NSE(+), Vimentin-, GFAP-			Surgery (NR, 12 og)	12
(64) Lin 1992	18	F	NET	Tailgut cyst	NR			NR	NR
(55) Edelstein 1996	51	F	NET	–	NR			Surgery (CR, 18 og)	18
(71) Horenstein 1998	19	F	NET (Ki67 NR)	Tailgut cyst	CgA+, Syn+, NSE+, Cam5.2+, S100–, GFAP–			Surgery (CR, 48 og)	48
(71) Horenstein 1998	19	F	NET (Ki67 NR)	–	CgA+, Syn+, NSE+, Cam5.2+, GFAP–, NeuF–, serotonin–, Somatostatin–, VIP–, Gastrin–, Calcitonin–			2x Cis/Eto/Ifo, 3x Doxo/ DTIC/Cyclo, embolization (SD, 12 og) Surgery (CR, 36 og)	48
(71) Horenstein 1998	21	F	NET (Ki67 NR)	–	CgA+, Syn+, NSE+, Cam5.2+, Ck7–, Ck20–, GFAP –, serotonin–, somatostatin–, VIP–, Gastrin–, Calcitonin–		LR, OTH (Breast)	Surgery (CR, 12) Surgery Breast (CR, 1)	13
(68) Gorski 1999	42	F			NR			NR	
(56) Oyama 2000	52	M	NET G1 (Ki67 NR)	Tailgut cyst	NR			Surgery (CR, 6 og)	6
(43) Prasad 2000	69	F	NEC (Ki67 NR)	Tailgut cyst	Ck+, CgA+			Surgery(CR, 24og)	24
(58) Theunissen 2001	51	F	NET G2 ("LCNEC", Ki67 NR)	–	MNF116+, Vimentin(+), CEA–, CA125–, S100–, CgA +, Syn+			Cis/Eto (SD, 3)	3 †
(31) Mourra 2003	68	M	NET/NEC (Ki67 NR)	Tailgut cyst	NSE+, CgA+, Syn+, Ck+, EMA+, PSA–, CD45–			Surgery (CR, 12 og)	12
(26) Jacob 2004	42	F	NR	Tailgut cyst	NR			Surgery (NR)	NR
(16) Song 2004	41	F	NET (Ki67 NR)	Tailgut cyst	AE1/3+, Syn+, CgA+		HEP, BRA	Surgery (CR, 12) 5-FU (NR, 3) RT BRA (NR)	15
(48) Urioste 2004	22	M	NR	Teratoma, Currarino	NR			NR	
(18) Luong 2005	37	M	NET G1 (Ki67 2.9%)	Teratoma	Ck+, Syn+, NSE+, CgA–,	HEP, LYM, OSS		Lan (PD, 10) Lan (NR, 3)	18
(28) Mathieu 2005	49	F	NET (Ki67 NR)	Tailgut cyst	NR			Surgery (CR, 24 og)	24
(34) Kim 2007	58	F	NET (Ki67 NR	Imperforate anus	Syn+, CgA+, NSE+, Ck+, S100–			Surgery (CR, 10 og)	10
(24) Liang 2008	51	F	NET G2? (Ki67 > 1%)	Tailgut cyst	ER+, PR(+), Syn+, CgA+, PanCk+,			Surgery (NR)	NR (3 presurgery)
(20) La Rosa 2010	73	F	NET G1 (Ki67 < 2%)	Tailgut cyst	Syn+, CgB+, VMAT2+, SSTR2A+, PAP+, Ghrelin+, CgA+, Serotonin+, Somatostatin+, Ck20+, CDX2–,			Surgery (CR, 5)	5 (36 presurgery)

(Continued)

TABLE 3 | Continued

Reference	Age	Sex	Histology	Anomalies	IHC +: positive (+): weak or focal positive –: negative	Metastases synchronous	Metastases metachronous	Treatment (best response, PFS in months)	Follow-up (months) †deceased
(65) Pendlimari 2010 (32) Liu 2020	22	F	NET G2 (Ki67 5%)	Currarino, teratoma	VMAT1–, PP–, YY–, GRP–, Gastrin–, glicentin–, encephalin–, GFAP–, ER–, PR–, AR–, Ck7–, TTF1– CgA+, Syn+, CD56(+)	LYM		Surgery (CR, 24 og)	24
(59) Ciotti 2011	44	F	NET G1/G2 (Ki67 < 10%)	Currrarino, teratoma	CgA+, Syn+	LYM	LR, LYM, HEP	Surgery (CR, 16) Surgery LR, LYM (CR, 24) Octreotide, CT (PD)	40 †
(69) Harbeck 2011	39	F	NET G2 (Ki67 5%)/ NEC G3 (Ki67 30%)	–	CgA+, Syn+, Ck+, SSTR2+, serotonin–, glucagon–, somatostatin–		LYM, OSS	Surgery (CR, 30) PRRT (PR, 6 og)	36
(30) Spada 2011	63	F	NET G1 (Ki67 < 2%)	Tailgut cyst	AE1/3+, Syn+, PP+, AP+, CgA(+)	HEP		Surgery (CR, 25 og)	25
(30) Spada 2011	41	F	NET G2 (Ki67 18%)	Tailgut cyst	CgA+, Syn+, AE1/3+, SSTR2+	HEP	PLE, OSS, OTH (ovary)	Surgery (PR, NR) Carbo/Eto for HEP (NR, 7) PRRT (PR, NR) Surgery for PLE+Ovary (NR, NR) SSA (SD, NR) PRRT (SD, NR)	79
(62) Wöhlke 2011	55	F	NET G2 (Ki67 20%)	Tailgut cyst	AE1/3+, Syn+, CgA(+), somatostatin+, glucagon–, insulin–, gastrin–, CDX2–	LYM, HEP	OSS	Surgery, PRRT (CR, 22) PRRT (PR, 8)	30
(66) Zhong 2012	48	F	NET G1 (Ki67 1%)	–		OTH (muscle)		Surgery, cis/eto/doxo/cyclo RT (SD, 36 og)	36
(63) Zoccali 2012	64	M	NEN (Ki67 NR)	Tailgut cyst	AE1/3+, Syn+, CgA–, p63–			Surgery (NR)	NR
(57) Damato 2013	24	F	NET (Ki67 NR 5%)?	Tailgut cyst	Vimentin+, Ck+, S100–, Syn+, PSAP+			Surgery (CR, 3 og)	3
(41) Misawa 2013	53	F	NET/NEC (Ki67 20–60/70%)		AE1/3+, CAM5.1+/–, KL1+/–, S100 +/–, NSE+, Ubiquitin+, CD56+, CgA–, Syn+, LCA–, SMA–, Desmin–, CD10–/+, CD34–, HMB45–, GCDFP15–		LR, PER, PUL	Surgery (NR, 4) RTX for LR (NR, 4)	11 †
(67) Simpson 2014	64	F	NEN (Ki67 NR)	Teratoma	NR	NR		Surgery (NR)	NR
(39) Abukar 2014	61	M	NET G2 (Ki67 low)	Tailgut cyst	PAP+, CD56(+), Syn+, CgA+, MNF116+, AE1/3+			Surgery (NR)	NR
(17) Charalampakis 2014	35	M	NET G1 (Ki67 < 1%)?	Tailgut cyst	PanCk+, CgA+, Syn+			Surgery (CR, 36 og)	36
(37) Kim 2014	49	M	NET G2 (Ki67 5%)	Tailgut cyst	CgA+, Syn+, CD56+			Surgery, w&w for residual tumor (SD, 14 og)	24
(61) Menter 2014	69	M	NET G2 (Ki67 10%), PLE: Ki67 15%	–	PSAP+, TTF1–, PSA–, Ck20(+), CD56+, CgA+, Syn+, SSTR2+, Ck22+, Ck7–, EMA–, ERG–, S100–,	OSS, HEP, PUL, LYM, OTH, heart, duodenum, mesenterium		Surgery, RT (PR, 36)	72†
(22) Mitsuyama 2015	53	M	NET G2 (Ki67 12.5%)	Tailgut cyst	Vimentin+, panCk(+), EMA–, S100–, CD99–, CgA+, Syn+, SSTR2+			Surgery (CR, 10) Octreotide (NR)	28

(Continued)

TABLE 3 | Continued

Reference	Age	Sex	Histology	Anomalies	IHC +: positive (+): weak or focal positive –: negative	Metastases synchronous	Metastases metachronous	Treatment (best response, PFS in months)	Follow-up (months) †deceased
								Irradiation (NR, 10) Everolimus (NR, 8 og)	
(52) Sable 2014	35	F	NET G1 (Ki67 2%)	Teratoma	Ck+, Syn+			Surgery, CT	NR
(35) Falkmer 2015	57	M	NET G2 (Ki67 5–10%)	–	AE1/3+, Syn+, CgA+, CgB+, Ghrelin+, PYY(+), Motilin(+), VMAT2–, Serotonin–, Gastrin–, GIP–, CGRP–, CART–, Calcitonin–, ACTH–, Secretin–, VIP–, NRK–, Insulin–, IAPP–, glucagon–, GLP1–, GRP–, neurotensin–	LYM, OSS	LR, SKI, BRA, OTH (soft tissue, kidney, heart)	Watch&wait (NR, 27) Surgery (8) RTX (4) STZ/5-FU (PD, NR) PRRT (NR,19) Octreotide (NR, NR) Octreotide-HD (NR, NR) RTX (NR, 13) PRRT (NR, 25) PRRT (PR, 9)	135 †
(38) Jehangir 2016	74	M	NET (Ki67 NR)	Tailgut cyst	Syn+, NSE+, CgA–			Surgery (CR, 60 og)	60
(40) Ferrer-Márquez 2017	NR	NR	NET (Ki67 NR)	NR	NR			Surgery (CR, 24 og)	24
(40) Ferrer-Márquez 2017	NR	NR	NET (Ki67 NR)	NR				Surgery (CR, 24 og)	24
(40) Ferrer-Márquez 2017	NR	NR	NET (Ki67 NR)	NR				Surgery (CR, 6 og)	6
(45) Mora-Guzmán 2017	56	F	NET G1 (Ki67 < 2%)	Tailgut cyst	AE1/3+, CD56+, Syn+, CgA+			Surgery (CR)	7
(29) Al Khaldi 2018	53	F	NET G2 (Ki67 5-10%)	Tailgut cyst	CgA+, Syn+, Cam5.2+, AE1/3(+), CD56(+)		LYM, PUL, HEP	Surgery (CR, 24) SSA for LYM+PUL (NR, 6) SZT/5-FU (SD, 12) Everolimus (PD, 4)	46
(19) Erdrich 2018	77	M	NET G2 (Ki67 8.6%) HEP: NET G2 (Ki67 6.4%)	Tailgut cyst	CgA(+), Syn+	HEP		Surgery primary + HEP	NR
(14) Iwata 2019	25	F	NET G2, (Ki67 20%)	Tailgut cyst	Ck+, Syn+, CgA+, ER–, PR–			Surgery (NR)	NR (8 presurgery)
(51) Soyer 2018	14	M		Tailgut cyst					NR
(72) Yang 2018	39	F	NET G2 (Ki67 5–10%)	Tailgut cyst	CgA+, Syn+, Serotonin–, TTF1–, CDX2–, PAX8–, PP–		LR, HEP, PUL, PER, OTH (ovaries)	Surgery (CR, 12) Surgery + SSA for LR (CR,12) Surgery LR + PER, ablation (NR, 41) Surgery + RT for LR + PER + OTH (NR, 36) SIRT + SSA (NR, NR)	120

(Continued)

TABLE 3 | Continued

Reference	Age	Sex	Histology	Anomalies	IHC +: positive (+): weak or focal positive –: negative	Metastases synchronous	Metastases metachronous	Treatment (best response, PFS in months)	Follow-up (months) †deceased
(72) Yang 2018	41	F	NET (Ki67 NR)	Teratoma	Syn+, CgA+, Ck+, AE1/3+, S100+		OTH (ovaries, retroperitoneal)	Surgery, SSA (CR, 48) Surgery (ovaries, retroperitoneal) (NR, NR)	48
(72) Yang 2018	45	F	NET (Ki67 NR), BRA: NET G2, Ki67 18%	Anterior sacral meningocele, tailgut cyst	CAM5.2+, CgA+, Syn+, CDX2+		BRA, LYM, HEP, OSS	Surgery (CR, 9) Surgery brain (CR, 3) Octreotide, PRRT LR, LYM, HEP (SD, 36) PRRT for HEP, OSS (PR, NR)	46 (156 presurgery
(72) Yang 2018	46	M	NET G2 (Ki67 15%) BRA: NET G2 (Ki67 12%)	Tailgut cyst	Syn+, CgA+, AE1/3+, TTF1–, CDX2–	HEP, OSS		FOLFOX + Bev (SD?, 14 og) Everolimus + Oct, Irradiation (NR, 12) Surgery (BRA)	28
(72) Yang 2018	75	M	NET G1, Ki67 < 1%	–		LYM, OSS, PUL	HEP	Surgery, RTX, Oct (NR, 47) SIRT, Oct (PR, 27 og)	78
(72) Yang 2018	42	F	NET G2 (Ki67 6%)	–	Syn+, CD56+, NSE+, WT1+,		HEP	Surgery (CR, 12) Oct (NR, 25) Oct + CC-223 (NR, 9) SIRT (PR, 11) Surgery pancreas (NR, NR) Tem (PD, NR) Sunitinib (NR, NR) PRRT (SD, NR)	68
(72) Yang 2018	41	F	NET G2 (Ki67 13%)	–		PUL, HEP, OSS	BRA	Lan + IFN (NR, 9) Pazopanib (NR, 16) RT OSS BRA (SD, 6 og)	36
(72) Yang 2018	44	F	NEC G3 (Ki67 80–90%)	–	Ck+, Syn+, CD56+	LYM		Cis/Eto, RTX (PR, 5 og)	5
(72) Yang 2018	77	F	Large-cell NET/ NEC (Ki67 50%)	–	Syn+, CD56+, Villin+			Carbo/Eto (SD, 3 og) Oct (PD, 4)	7
(72) Yang 2018	50	F	NET (Ki67 NR)	–		LYM, HEP, OTH (pancreas)		Tem/Cap (NR), Oct (NR)	NR
(21) Chatani 2019	59	F	NET G2 (Ki67 NR), Adenocarcinoma	Teratoma, Currarino	Syn+, CgA+			Resection, RT (CR, 8 og)	8
(49) Coetzee 2019	60	F	NET G2 (Ki67 14%)	Teratoma, Currarino	CgA+, Syn+			Surgery (CR, 18 og)	18
(53) Colombo 2019	46	M	NET (Ki67 NR)	Currarino, dermoid cyst	NR				NR
(36) Kim 2019	78	M	NET G2 (Ki67 6.6%)	–	CgA+, Syn+, CD56+, Ck7-, TTF1-, CDX2-	HEP	PER	Surgery HEP (CR, 48) Octreotide + everolimus (SD, 14) Pazopanib (NR, 14) PRRT (SD, 2 og)	96
(15) Lee 2019	33	F	NET G1 (Ki67 1-2%)	Tailgut cyst	AE1/3+, Syn+, CgA+, CDX2(+), ER(+), Ck7(+), Ck20-			Surgery (NR, NR)	NR (96 presurgery)

(Continued)

TABLE 3 | Continued

Reference	Age	Sex	Histology	Anomalies	IHC +: positive (+): weak or focal positive –: negative	Metastases synchronous	Metastases metachronous	Treatment (best response, PFS in months)	Follow-up (months) †deceased
(46) Olczak 2019	49	M							
(50) Rod 2019	51	M	NET (Ki67 NR)	Currarino	NR			Surgery (NR, NR)	60
(27) Sakr 2019		M	NET (Ki67 NR)	Tailgut cyst	NR			Surgery (NR, NR), RT (NR, NR)	NR
(33) Singh 2019	63	M	NET G1 (Ki67 < 1%)	Tailgut cyst	Syn+, Ck+, CgA–, GFAP–, SMHC–, p63–, CD56–			Surgery (NR, NR)	NR
(25) Zhang 2019	36	F	NET G3 (Ki67 30%)	–	Syn+, CD56+, Ck+			Surgery (CR, 1 og)	1
(23) Kodera 2020	68	F	NET G1 (Ki67 < 2%)	Tailgut cyst	CD56+, SSTR2A+, PP+, PR(+), CgA–, p53–, ER–, gastrin–, serotonin–, somatostatin–, CDX2–, TTF1–			Surgery (CR, 12 og)	12
(32) Liu 2020	75	F	NET G2 (Ki67 3%), HEP: NET G2 (Ki67 6.8%)	Tailgut cyst, Currarino	NR	HEP		Octreotide (PD, 11) PRRT (NR, NR)	11
(32) Liu 2020 (54) Scott 2021	35	F	NET G2 (Ki67 4%)	Currarino, tailgut cyst	Syn+, SSTR2A+, Islet-1+, CgA–, Ck20–, TTF1–, CDX2–, PAX8–, GATA3–, Inhibin–Desmin–, S100–	LYM, OSS		Surgery, Octreotide (SD, 22 og) PRRT (NR, NR) Oct high dose (SD, NR)	36
(47) Rebelo 2020	48	M	NET G2 (Ki67 6%)	Tailgut cyst, Currarino	CD56+, Syn+, CgA–		HEP, OSS, OTH (spleen)	Surgery (CR, 18) CT	18
(54) Scott 2021	38	M	NET G2 (Ki67 7.5%), LYM: NET G2 (Ki67 9%)	Currarino, tailgut cyst	Syn+, CgA+, SSTR2A+, Islet–1+, PAX6+, CDX2–, TTF1–		LYM	Surgery (CR, 24) Surgery LYM (CR, NR)	24
(54) Scott 2021	62	F	NET G1 (Ki67 < 1%)	Currarino, teratoma	AE1/3+, CAM 5.2+, Syn+, CD56(+), Ck5/6–, p63–, S100–, desmin–, CD34–, CD45–, CgA–			Surgery (CR, 12 og)	12

Histopathology was adapted to the most current WHO 2019 classification according to the description in the report.

AP, acid phosphatase; AR, androgen receptor; BRA, brain; CAM, cell adhesion molecule; Cap, capecitabine; Cis, cisplatin; Ck, cytokeratin; CT, chemotherapy; CgA, chromogranin A; CgB, chromogranin B; CR, complete remission; cyclo, cyclophosphamide; doxo, doxorubicin; DTIC, dacarbazin; EMA, epithelial membrane antigen, ER, estrogen receptor; eto, etoposide; F, female, GCDFP, gross cystic disease fluid protein; GFAP, glial fibrillary acidic protein; GIP, gastric inhibitory peptide; GRP, gastrin releasing peptide; HEP, liver; Lan, lanreotide, LR, local recurrence; LYM, lymph nodes; M, male; NF, neurofilament; NR, not reported; NSE, neuron-specific enolase; Oct, octreotide; og, ongoing; OSS, bone; OTH, other, PD, progressive disease; PER, peritoneum; PgR, progesterone receptor; PLE, pleura; PP, pancreatic polypeptide; PR, partial remission; PRRT, peptide receptor radionuclide therapy; PSAP, prostatic specific acidic phosphatase; PUL, lung; PYY, peptide YY; RT, radiotherapy; SD, stable disease; SKI, skin; SSA, somatostatin analogue; SSTR, somatostatin receptor; syn, synaptophysin; Tem, temozolomide; VIP, vasoactive intestinal peptide; VMAT, vesicular monoamine transporter; w&w: watch & wait.

the use of SSAs in rectal NET is up for debate, since lanreotide failed to show PFS benefit *vs.* placebo in the CLARINET trial with very few rectal NET patients included. Nevertheless, SSAs are also commonly reported as effective treatment for presacral NET, with octreotide in 13 cases (22, 32, 36, 72), lanreotide in 1 case (72), and an unspecified SSA in 4 cases (29, 72). Fifteen patients in our analysis received SSAs, half of them lanreotide. Disease stabilization was observed in 50% (7/14).

PRRT is an effective treatment for NET of various locations since the mid-90s. With the conclusion of the NETTER trial, PRRT has shown its efficacy in a randomized phase III setting for intestinal NET (81), gaining approval in many countries. In rectal NET, PRRT has shown efficacy in case series (82). PRRT is reported in nine cases with presacral NET, all with encouraging long-term stabilizations and remission (32, 35, 36, 72). In accordance to this, PRRT was also one of the most effective treatments observed in our cohort, resulting in 43% responses (6/14) and 36% disease stabilizations (5/14).

The mTOR inhibitor everolimus has been approved for extrapancreatic NET after the positive phase III RADIANT-4 trial (83). The subgroup of rectal NETs in this trial showed significant PFS prolongation in this trial. Four cases of treatment of presacral NET are reported in the literature (22, 36, 72), with one showing long-term disease control, two short-term stabilizations, and one progressive disease. In our analysis, all three patients receiving everolimus showed disease progression.

The multi-tyrosine kinase inhibitor cabozantinib has shown promising antitumor activity in a preliminary report of a phase II trial for pancreatic and extrapancreatic NETs (72). We report the first patient receiving cabozantinib for metastatic presacral NET with an encouraging long-lasting disease stabilization in a heavily pretreated patient.

Cytotoxic chemotherapy is the main systemic treatment modality for NEN G3 and an effective option for pancreatic NET G1/G2 (73). In presacral NET, applications of the protocols FOLFOX (n = 1) (72), temozolomide + capecitabine (n = 2) (72), cisplatin + etoposide (n = 4) (58, 72), and carboplatin-etoposide (n = 2) (72) were reported, with heterogenous results. In our analysis, chemotherapy was applied seven times to six patients, mostly with unfavorable response. While well-differentiated presacral NET did not sufficiently respond to chemotherapy, treatment of the presacral neuroendocrine carcinoma with cisplatin + etoposide resulted in a partial remission.

Of three patients receiving molecular diagnostics, potential targetable alterations were detected in two patients: alterations in the cyclin pathway as potential target for a CDK inhibitor and HRD as a potential target for PARP inhibition and platinum-based chemotherapy most probable due to a SETD2 frameshift insertion (84–86). This is remarkable, since the only other case of molecularly profiled presacral NET reported earlier (19) showed a BRCA1 mutation, which also commonly leads to HRD.

Our study has several limitations, mainly due to its retrospective nature. On the other hand, considering the rarity of the disease, a prospective or even randomized trial is most likely not feasible. Furthermore, a central pathological or radiological review was not performed. However, all patients were included by experienced high volume NEN centers with well-established multidisciplinary diagnostic and therapeutic pathways.

In conclusion, we report the largest analysis of clinicopathological characteristics and treatment outcomes for presacral NEN so far. Presacral NENs are usually non-functioning and primarily cause locoregional symptoms. Plasma CgA levels are usually not elevated. Presacral NEN should be considered as possible primary in CUP-NET, especially when the immunohistochemical profile resembles a hindgut NET, and a rectal primary is excluded endoscopically. Functional imaging with SSR-based PET-CT is helpful for primary tumor identification and treatment planning. Local control could be achieved *via* radiotherapy. SSAs demonstrated limited efficacy, whereas PRRT showed promising activity for advanced disease. In our cohort, everolimus and chemotherapy were largely ineffective. Molecular diagnostics showed potential targetable alterations in selected cases. Further prospective evaluation and molecular characterization of this rare tumor entity are needed.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The dataset supporting the conclusions of this article is available on request by contacting the authors. **Tables 1**, **2** and **Figure 3** build the dataset. Requests to access these datasets should be directed to sprengea@uni-marburg.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki. All patients were included in the local disease databases conducted with approval of the local ethics committees at the respective sites. Written informed patient consent and approval for data collection and analysis were obtained upon admission to our institutions. For the use of the images, an additional consent was obtained in the selected cases.

AUTHOR CONTRIBUTIONS

ARi and DL contributed to conception and design of the study. SM, LA, JS, HL, SKru, ARi, ZK, and AK carried out the acquisition of data. SM, LA, and ARi analyzed and interpreted the data and drafted the manuscript. SM performed statistical analysis. ARa performed immunohistochemical stainings and pathological review. DL contributed PET-CT images. SKre provided molecular data and participated in data interpretation. TG was involved in data interpretation and critical review of the manuscript draft. All authors contributed to the article and approved the submitted version.

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Structured Reporting of Computed Tomography in the Staging of Neuroendocrine Neoplasms: A Delphi Consensus Proposal

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Background: Structured reporting (SR) in radiology is becoming increasingly necessary and has been recognized recently by major scientific societies. This study aims to build structured CT-based reports in Neuroendocrine Neoplasms during the staging phase in order to improve communication between the radiologist and members of multidisciplinary teams.

Materials and Methods: A panel of expert radiologists, members of the Italian Society of Medical and Interventional Radiology, was established. A Modified Delphi process was used to develop the SR and to assess a level of agreement for all report sections. Cronbach's alpha (Cα) correlation coefficient was used to assess internal consistency for each section and to measure quality analysis according to the average inter-item correlation.

Results: The final SR version was built by including n=16 items in the "Patient Clinical Data" section, n=13 items in the "Clinical Evaluation" section, n=8 items in the "Imaging Protocol" section, and n=17 items in the "Report" section. Overall, 54 items were included

in the final version of the SR. Both in the first and second round, all sections received more than a good rating: a mean value of 4.7 and range of 4.2-5.0 in the first round and a mean value 4.9 and range of 4.9-5 in the second round. In the first round, the Cα correlation coefficient was a poor 0.57: the overall mean score of the experts and the sum of scores for the structured report were 4.7 (range 1-5) and 728 (mean value 52.00 and standard deviation 2.83), respectively. In the second round, the Cα correlation coefficient was a good 0.82: the overall mean score of the experts and the sum of scores for the structured report were 4.9 (range 4-5) and 760 (mean value 54.29 and standard deviation 1.64), respectively.

Conclusions: The present SR, based on a multi-round consensus-building Delphi exercise following in-depth discussion between expert radiologists in gastro-enteric and oncological imaging, derived from a multidisciplinary agreement between a radiologist, medical oncologist and surgeon in order to obtain the most appropriate communication tool for referring physicians.

Keywords: radiology report, structured report, staging, neuroendocrine neoplasm, computed tomography

INTRODUCTION

Neuroendocrine neoplasms (NENs) are heterogeneous with respect to their site of origin and metastatic behaviour. About 25% of NENs secrete hormones leading to specific clinical signs and symptoms, while the remaining approximate 75% are so-called non-secreting NENs, which are often diagnosed incidentally and 40–50% are already in a metastatic tumour stage (1–3). Functioning NENs usually show up relatively early, so it might be difficult to detect lesions on radiological imaging, since these are often too small to be seen (4). While the detection and follow-up of NENs still pose a diagnostic challenge, radiological imaging is essential for the assessment of metastatic lesions (especially in the liver) and of tumour response to treatment, playing a key role in guiding treatment planning (5).

In such a complex scenario, an effective communication of imaging data to referring physicians is crucial for patient care. Radiology reports are the gold standard as to comprehensiveness and accuracy, and they are traditionally created as non-structured free text reporting (FTR) written in a narrative language. However, inconsistencies regarding content, style, and presentation format can reduce the clarity of FTR and hinder the communication of key information to the referring physician, potentially leading to incorrect diagnosis, delayed initiation of adequate treatment, or adverse patient outcomes (6–9).

Radiological structured reporting (SR) has several advantages over FTR, including a higher standardization of reporting style and lexicon with the adherence to established practice guidelines and recommendations, greater consistency and reproducibility (10–12), possibility of data mining and integration with artificial intelligence systems (13), shorter reporting time, lower error rate, and better communication with referring clinicians and other radiologists (8, 14, 15). To the latter regard, oncologists tend to prefer SR over FTR due to its ability to share information more clearly and in a more standardised way (16–18). Moreover, in 2018 the European Neuroendocrine Tumor Society (ENETS) launched an initiative to provide guidance for synoptic reporting of computed tomography (CT) and magnetic resonance imaging (MRI) examinations for staging and follow-up of bronchial, pancreatic and gastrointestinal NENs, where members of the imaging group stated a strong preference for a combination of limited and standardised options by way of drop-down menus, wherever possible (19).

Despite its established advantages, SR has not yet become commonplace in the radiological routine due to several reasons, including the current paucity of usable templates and of commercially available SR software solutions (6). In this context, the Italian Society of Medical and Interventional Radiology (SIRM) has created an Italian warehouse of SR templates that can be freely accessed by all SIRM members [including MRI for primary staging and restaging of rectal cancer (7), and chest CT in the management of COVID-19 pneumonia (9)], thus facilitating their routine use in a clinical setting (20).

Our purpose is to devise and evaluate an SR template for CT examinations performed for primary staging of NENs, with the goal to improve the standardization of reporting based on current best practice guidelines, as well as the communication between radiologists and clinicians, and among radiologists themselves.

MATERIALS AND METHODS

Expert Panel

Following extensive discussion between expert radiologists, a multi-round consensus-building Delphi exercise was performed to develop a comprehensive, focused SR template for CT at the staging phase of patients with NENs.

A SIRM radiologist with experience in abdominal imaging created the first draft of the SR with the collaboration of a surgeon and medical oncologist specialised in NENs.

A working team of 14 expert radiologists was set up, with members from the SIRM Chapters of Gastrointestinal and Abdominal Radiology and of Diagnostic Imaging in Oncology. Their aim was to revise the initial draft iteratively, with the objective of reaching a final consensus on the SR.

Selection of the Delphi Domains and Items

All panellists reviewed literature data on leading scientific databases (including PubMed, Scopus and Google Scholar), to assess papers on NENs, CT and structured radiology reports published from December 2000 to June 2021. The full text of the selected studies was reviewed by all members of the expert panel, and each of them developed and shared the list of Delphi items *via* email and/or teleconference.

The SR was divided into the following four sections: *(a)* Patient Clinical Data (including 16 items), *(b)* Clinical Evaluation (13 items), *(c)* Imaging Protocol (8 items) and *(d)* Report (17 items). A final section dedicated to key images was also included in the template.

Two Delphi rounds were performed. During the first round, each panellist independently contributed to refining the SR draft by means of online meetings or email exchanges. The level of panellists' agreement for each SR model was tested in the second Delphi through a Google Form questionnaire shared by email. Each expert expressed individual comments for each specific template section by using a five-point Likert scale (1 = strongly disagree, 2 = slightly disagree, 3 = slightly agree; 4 = generally agree, 5 = strongly agree).

After the second Delphi round, the last version of the SR was generated on the dedicated RSNA website (www.radreport.org) by using a T-Rex template format, in line with IHE (Integrating Healthcare Enterprise) and MRRT (Management Of Radiology Report Templates) profiles and accessible as open-source software, with technical support by Exprivia (Exprivia SpA, Bari, Italy). Such profiles determine both the format of radiology report templates [using version 5 of Hypertext Markup Language (HTML5)] and the transporting mechanism to request, retrieve, and stock these schedules. The radiology report was structured by using a series of "codified queries" integrated in the T-Rex editor's preselected sections (21).

Statistical Analysis

Answers from each panellist were exported into Microsoft Excel® format for ease of data collection and statistical analysis.

All panellists' ratings for each section were analysed, with descriptive statistics measuring the mean score, the standard deviation, and the sum of scores. An average mark of 3 was considered good, whereas 4 was considered excellent.

To measure the internal consistency of panellist ratings for each section of the report, a quality analysis based on the average inter-item correlation was performed with Cronbach's alpha (Cα) correlation coefficient (22, 23). The Cα test provides a measure of the internal consistency of a test or scale; it is expressed as a number between 0 and 1. Internal consistency describes the extent to which all the items in a test measure the same concept. Cα was determined after each round.

The nearer the Cα coefficient to 1.0, the more accurate the internal consistency of the categories in the scale. An alpha coefficient (α) ≥0.9 was considered excellent, α ≥0.8 good, α ≥0.7 acceptable, α ≥0.6 questionable, α ≥0.5 poor, and α <0.5 unacceptable. However, an α = 0.8 was seen as an acceptable parameter in internal reliability during iterations.

Data analysis was performed using Matlab Statistic Toolbox (The MathWorks, Inc., Natick, MA, USA).

RESULTS

Structured Report

The final SR (**Appendix 1**) template was built by including n=16 items in the "Patient Clinical Data" section, n=13 items in the "Clinical Evaluation" section, n=8 items in the "Imaging Protocol" section, and n=17 items in the "Report" section. Overall, 54 items were included in the final version of the SR.

The "Patient Clinical Data" section included patient clinical data, previous or family history of malignancies, risk factors, endocrine and or neuroendocrine neoplasms in young age, hereditary syndromes, and other genetic mutations. In this section, we included the item "Allergies" to the drug or no drug and contrast medium.

The "Clinical Evaluation" section collected previous examination results, a genetic panel, results of histopathological examination on biopsy specimen, Chromogranin A (CgA) level, Neuron-specific enolase (NSE) level, 5-hydroxyindoleacetic acid (5-HIAA) 24-h urine level, serum gastrin level, serum insulin level, serum glucagon level, serum VIP level, blood count, serum creatinine, liver function and clinical symptoms.

The "Imaging Protocol" section included data on the equipment used, the number of detectors and whether it was multidetector or dual energy, including data on the reconstruction algorithm and slice thickness. In addition, we collected data on contrast study protocol, including data on the contrast study phase, as well as data concerning the contrast medium, such as the active principle, commercial name, dosage, flow rate, concentration, and ongoing adverse events. In addition, in this section we included data about bowel preparation and contrast technique for gastro-enteric tract evaluation.

The "Report" section included data on lesion sites (primary tumour visible or not visible on CT imaging) and features such as number, site, size, and infiltration of neighbouring organs and/or structures for each site (e.g., lung, gastric, pancreatic, duodenal, small bowel, appendiceal and colorectal lesion). For small bowel lesions, we evaluated the desmoplastic reaction, while for pancreatic lesions assessment was made on the relationship with the pancreatic duct, with vessels and duodenum or ampulla, according to surgical planning. In addition, in this section we included tumour stage, node stage and metastases stage, as well as the presence of incidental radiological findings. To allow for maximum flexibility of SR use in different working

scenarios, only the Report section fields are mandatory, whereas all fields from the other SR sections can be filled in upon user discretion.

Consensus Agreement

Tables 1, **2** show single scores and the sum of scores of the 14 panellists for the SR in the first and second rounds, respectively.

Both in the first and second round, all sections received more than a good rating, with a mean value of 4.7 (range 4.2-5.0) in the first round, and a mean value of 4.9 (range 4.9-5) in the second round.

In the first round, the Cα correlation coefficient was 0.57, with an overall mean score of experts and sum of scores for the SR being 4.7 (range 1-5) and 728 (mean value 52.00, standard deviation 2.83), respectively.

In the second round, the Cα correlation coefficient was 0.82, with an overall mean score of experts and sum of scores for the SR being 4.9 (range 4-5) and 760 (mean value 54.29, standard deviation 1.64), respectively.

DISCUSSION

To the best of our knowledge, this is the first time that a panel of experts from a national radiological society has promoted the creation of a comprehensive SR template for the CT staging of NENs, systematically encompassing all steps of the radiological procedure (from registration of patient personal data to full details of CT examination and standardised reporting of relevant CT findings), along with the possibility of coupling together radiological and clinical data and in the perspective of integrating the SR template into the radiology workflow.

Our SR template was based on a multi-round consensus-building Delphi exercise following in-depth discussion between expert radiologists in gastrointestinal and oncological imaging. On a previous occasion, panellists had assessed and promoted a SR for MRI-based primary staging and restaging of rectal cancer (7). While such MRI templates were also based on a multi-round consensus-building Delphi exercise, in that earlier study the original draft derived from a single dedicated radiologist, without seeking any multidisciplinary agreement. On the other hand, in the present study the SR draft wa based on the multidisciplinary agreement of a radiologist, a medical oncologist and a surgeon, with the goal to achieve an optimal, all-around communication tool for referring physicians.

The approved SR template was divided into four sections (i.e., Patient Clinical Data, Clinical Evaluation, Imaging Protocol and Report), with a final section dedicated to key images. Although our template may appear somewhat long and complex (potentially slowing down the radiology workflow), it must be emphasised that only the Report section is mandatory, whereas other sections are optional. Furthermore, considering that not all data may be available to the radiologist, these open fields can also be filled in at a subsequent time. In addition, the possibility of

TABLE 1 | Single scores and sum of scores of 14 panellists for the structured report in the first round.

Panellist #	A1. Anthropometric data	A2. Personal assessments	A3. Allergies and adverse reactions	B1. Clinical information	C1. Exam data	C2. Contrast agent	C4. Adverse Events	D1. Lesion	D2. Lymphadenopathy	D3. Distantmetastasis	D8. Accessoryfindings	Total
1	5	4	5	5	5	5	5	5	5	5	5	54
2	5	4	5	5	5	5	5	5	5	5	5	54
3	3	3	5	5	5	5	5	5	5	5	5	51
4	4	4	4	5	5	5	5	5	5	5	4	51
5	5	5	5	5	5	5	5	5	5	5	5	55
6	5	5	5	5	4	5	5	5	5	5	5	54
7	5	5	5	5	5	5	5	5	5	5	5	55
8	4	4	5	4	5	5	5	4	5	5	5	51
9	5	5	5	5	5	5	5	5	5	5	1	51
10	4	4	5	5	5	5	5	5	5	5	5	53
11	5	5	5	5	5	5	5	5	5	5	4	54
12	3	4	5	5	5	5	5	5	5	3	3	48
13	5	3	4	5	3	5	4	3	5	3	5	45
14	4	4	5	5	5	5	5	5	5	5	4	52
Mean	4.43	4.21	4.86	4.93	4.79	5.00	4.93	4.79	5.00	4.71	4.36	52.00
Std	0.73	0.67	0.35	0.26	0.56	0.00	0.26	0.56	0.00	0.70	1.11	2.83

TABLE 2 | Single scores and sum of scores of 14 panellists for the structured report in the second round.

Panellist #	A1. Anthropometric data	A2. Personal assessments	A3. Allergies and adverse reactions	B1. Clinical information	C1. Exam data	C2. Contrast agent	C4. Adverse Events	D1. Lesion	D2. Lymphadenopathy	D3. Distant metastasis	D8. Accessory findings	Total
1	5	5	5	5	5	5	5	5	5	5	5	55
2	5	5	5	5	5	5	5	5	5	5	5	55
3	5	5	5	5	5	5	5	5	5	5	5	55
4	5	5	5	5	5	5	5	5	5	5	5	55
5	5	5	5	5	5	5	5	5	5	5	4	54
6	5	5	5	5	5	5	5	5	5	5	5	55
7	4	4	4	5	5	5	5	5	5	5	4	51
8	5	5	5	5	5	5	5	5	5	5	5	55
9	5	5	5	5	5	5	5	5	5	5	5	55
10	5	5	5	5	5	5	5	5	5	5	5	55
11	5	5	5	5	5	5	5	5	5	5	5	55
12	5	4	4	5	4	4	4	5	5	5	5	50
13	5	5	5	5	5	5	5	5	5	5	5	55
14	5	5	5	5	5	5	5	5	5	5	5	55
Mean	4.93	4.86	4.86	5.00	4.93	4.93	4.93	5.00	5.00	5.00	4.86	54.29
Std	0.27	0.36	0.36	0.00	0.27	0.27	0.27	0.00	0.00	0.00	0.36	1.64

connecting this template with the patient electronic health record allows for automatic import of available data.

All sections received more than a good rating in both Delphi rounds, with a mean value of 4.7 and 4.9 in the first and in the second round, respectively. In the first round, the Cα correlation coefficient was relatively poor (0.57), whereas in the second round, it was substantially improved (0.82). Moreover, the overall mean score of the experts in the second round was higher than that of the first round, with a lower standard deviation value being related to a greater agreement among experts for this SR. The sections with the lowest level of agreement were "Patient Clinical Data" and "Clinical Evaluation", reflecting the opinion that these sections were too long and may slow down daily practice. However, following a conference call, all panellists expressed their agreement once the optional nature of the sections had been clarified, and the importance of collecting patient clinical data and history for big data creation and connecting radiological with clinical data was demonstrated as well.

The "Patient Clinical Data" section included patient data, previous or family history of malignancies, risk factors, endocrine and or neuroendocrine neoplasms in young age, hereditary syndromes, and other genetic mutations. The "Clinical Evaluation" section collected previous examinations findings, a genetic panel, results of histopathological examination on biopsy specimen, chromogranin A (CgA) level, neuron-specific enolase (NSE) level, 5-hydroxyindoleacetic acid (5-HIAA) 24-h urine level, serum gastrin level, serum insulin level, serum glucagon level, serum VIP level, blood count, serum creatinine, liver function and clinical symptoms. This data could serve as a basis for creating potentially large databases, allowing not only for epidemiological statistical analysis, but also for building a radiomics model through the combination of radiological features and clinical data (24–29). To this end, genomic data could also be leveraged to build a radiogenomics model, which may be useful in the upper levels of personalised risk stratification and advanced precision medicine for early cancer diagnosis, cancer therapy selection, prognosis prediction, and assessment of treatment response and resistance to therapy (30–34).

A "strong agreement" was the result for the "Imaging Protocol" and "Report" sections. The "Imaging Protocol" section included data on the CT equipment used and related technical parameters (e.g., number of detector rows, multidetector single-or dual-energy, reconstruction algorithm and slice thickness). In addition, this section included data regarding the CT acquisition protocol (including post-contrast phases) and contrast medium administration (such as the active principle, commercial name, volume, flow rate, concentration, and ongoing adverse events), as well as data about bowel preparation and contrast technique for the evaluation of the gastrointestinal tract. Sharing examination techniques (not only within one's own department, but also with the radiology departments of other centres) allows for the standardisation and optimisation of study protocols. Indeed, during the follow-up phase, differences in acquisition parameters and segmentation algorithms are significant features that can lead to variability in volumetric assessment. Thus, slice

thickness and other protocol-related factors (such as the reconstruction kernel and field of view) should remain constant for reliable measurements to be performed. In the protocol optimization stage, enhanced communication between different centres can lead to an overall quality improvement through optimization of radiation dose and contrast administration, higher patient safety and overall better diagnostic quality (35, 36). Moreover, such enhanced communication can facilitate comparing the results obtained from different studies, thus reducing the variability due to different imaging protocols (37, 38).

The "Report" section included data on primary tumour being visible or not on CT imaging, as well as features such as lesion site, number, size, and infiltration of adjacent organs and/or structures for each site (e.g., lung, gastric, pancreatic, duodenal, small bowel, appendiceal, and colorectal lesions). Desmoplastic reaction was evaluated for small bowel lesions, whereas for pancreatic lesions the relationship with the pancreatic duct, vessels and duodenum or ampulla was taken into account, according to prospective surgical planning. In addition, in this section we included tumour stage, node stage and metastases stage, as well as the presence of incidental radiological findings. The possibility of using a SR template to guide the radiology workflow allows describing all the main radiological features that could be omitted with FTR, e.g., by mere distraction. For example, in pancreatic NENs, a correct evaluation of the stage of the neoplasm and an assessment of the relationship with the pancreatic duct, vessels, duodenum or the infiltration of neighbouring structures, allows for a correct stratification of patients and can avoid unnecessary major surgery compared to tumour enucleation (39–42). Using a checklist and a systematic search pattern may help to prevent such diagnostic errors. Both radiologists and referring clinicians are keen to reduce the rate of diagnostic errors, which, for radiologists, accounts for as much as 4% of reports (43–47). A retrospective overview of 3,000 MRI examinations was useful in determining clinically relevant extraspinal results in 28.5% of patients, which were not present in initial, unstructured reports (48). Similarly, the use of a checklist-style SR template has been shown to improve the rate of diagnosis of fracture-unrelated findings on cervical CT (49). SRs have also been shown to enhance the clinical impact on tumour staging and surgical planning for pancreatic and rectal carcinoma (50–52). Brook et al. compared the results of structured versus nonstructured reporting of CT findings for the staging and assessment of resectability for pancreatic cancer, and they concluded that surgeons were more confident about tumour resectability using SR compared to FTR (50). Sahni et al. showed that the use of an MRI-based SR improved rectal cancer staging when compared to FTR (52).

According to Weiss et al. [who described three levels of SR (53)], our SR is based on standardised terminology and structure, which are features needed for adhering to diagnostic-therapeutic recommendations and enrolment in clinical trials. This also reduces any ambiguity that may arise from non-conventional language and enables better communication between radiologists and clinicians. In addition, this SR template has the advantage of having been validated by a multidisciplinary group, potentially helping radiologists provide referring clinicians with all data required for correct patient management. However, this study has several limitations. Firstly, panellists were of the same nationality, and the contribution of experts from multiple countries could have allowed for a broader sharing, potentially increasing the consistency of the SR. Secondly, our study was not aimed at assessing the impact of the SR on the clinical management of NEN patients.

CONCLUSION

We developed a SR template for primary CT staging of NENs based on a multi-round consensus-building Delphi exercise, following in-depth discussion between expert radiologists in gastrointestinal and oncological imaging and derived from the multidisciplinary agreement of a radiologists, a medical oncologist and a surgeon specialised in NENs. A widespread adoption of SR could improve the quality, clarity, and reproducibility of reports across hospital departments and locations, improving patient health care and fostering research development.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Protocol number is: 13261–OSS, Azienda Ospedaliero - Universitaria Careggi, Florence, Italy. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.748944/full#supplementary-material

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Clinical Approach to Neuroendocrine Neoplasm Associated With Ovarian Teratoma

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Background: Neuroendocrine neoplasms are a heterogeneous group of cancers that develop from enterochromaffin cells of the diffuse endocrine system, with an increase in incidents over the last years. Ovarian neuroendocrine tumors (NET) are rare neoplasms, comprising 0.1% of all ovarian neoplasms and less than 5% of all neuroendocrine tumors. They may arise alone (as monodermal, specialized teratoma – ovarian carcinoid) or as a part of other ovarian lesion: cystic mature or immature teratomas. Due to the rarity and limited amount of such cases reported in the literature, there is no consensus on diagnostic and therapeutic procedures in this group of patients.

Materials and Methods: The group of 10 patients at the age of 19 to 77 years (mean 42.8 ± 17.9), diagnosed with unilateral NET within ovarian teratoma were analyzed. The histopathological type of tumor, progression free survival after surgical treatment and presence of hormonally active syndrome were assessed.

Results: 70% (n=7) of patients was diagnosed with mature cystic teratomas containing NET component and 30% (n=3) with monodermal teratoma (strumal carcinoid). All cases of monodermal teratomas were found in women at premenopausal age. Determined Ki67 ranged from 2% to 9%. Ninety percent of lesions (n=9) stained positive for synaptophysin and chromogranin, while markers: CK20, CK7, TTF-1 and CDX2 were negative in all cases, which ruled out their metastatic nature. None of the patients presented with carcinoid syndrome. All followed-up patients remain progression-free, which confirms surgical intervention being a crucial and sufficient method of treatment.

Conclusions: The prognosis and clinical behavior of NETs associated with ovarian teratomas are good with long progression-free survival.

Keywords: NET, teratoma, ovary tumor, neuroendocrine tumors, ovarian carcinoid

HIGHLIGHTS

- NET associated with ovarian teratomas are rare findings, possible in women of any age
- Surgical treatment is crucial and usually sufficient as a method of radical treatment of NETs associated with ovarian teratomas
- The prognosis of NETs associated with ovarian teratomas seem to be very good, with long progression-free survival, although there are no specific guidelines for follow-up

INTRODUCTION

Ovarian teratomas constitute frequent gynecological finding. They include mature cystic teratomas, immature teratomas and monodermal (specialized) teratomas. Mature cystic teratoma typically contains mature tissues of ecto-, meso- and endodermal origin. Immature (malignant) teratoma contains both mature and immature tissues e.g. tissue which resembles immature embryonal tissue (1).

On the other side neuroendocrine neoplasms (NENs) which are a heterogeneous group of neoplasm originating from enterochromaffin cells scattered throughout the body. They are mostly found in gastrointestinal and respiratory tracts with an estimated increasing incidence rate of 6.9/100,000 (2). Most of them occur sporadically, but there is known association with various genetic disorders, mainly MEN 1 and MEN 4 syndrome (3).

According to WHO classification of digestive system tumors, NENs might be highly or moderately differentiated called neuroendocrine tumors (NETs G1 or G2 with Ki 67 index below 20%) and poorly differentiated with Ki67 over 20% (NET G3 with still organoid histology or NEC (neuroendocrine carcinoma) without organoid histology (4). The degree of differentiation determines the prognosis and the method of treatment.

The new WHO classification of gynecologic NENs (2020) distinguish only well-differentiated tumors (NETs) and poorly differentiated carcinomas (NEC) and for neuroendocrine tumors of ovary WHO still recommends the use of "ovarian carcinoid" term instead of well-differentiated neuroendocrine tumor (1).

In general ovarian NETs are uncommon. They comprise only 0.1% of all ovarian neoplasms and less than 5% of all neuroendocrine tumors (5). Only part of them are primary ovarian tumors, while rest have metastatic origin usually from NETs of gastrointestinal or respiratory systems. Other gynecological organs like cervix, endometrium vagina or vulva are less frequently affected by NEN. In those localizations most NENs is diagnosed as NECs (6–8).

Most of ovarian NETs originate from monodermal teratomas. In general monodermal subtypes of teratomas are built only from one tissue type. In case of being built of a thyroid tissue they are called struma ovarii. If built from neuroendocrine tissue they are called ovarian carcinoid (insular, trabecular or mucinous type) (9). A presence of struma ovarii and carcinoid tissue in the same ovary is called a strumal carcinoid. The most common forms of ovarian carcinoid are insular and strumal type while trabecular and mucinous carcinoid are very rare (4). Most ovarian carcinoids of insular and trabecular type express chromogranin, synaptophysin and CD56, although trabecular one may be chromogranin negative. Insular and mucinous carcinoids may be positive with CDX2 (10). Both insular and trabecular carcinoids are typically CK7 positive and CK20 negative. Mucinous carcinoids are often CK20 positive and CK7 negative. Strumal carcinoids exhibit positive staining with neuroendocrine markers (carcinoid component) and thyroglobulin and thyroid transcription factor (TTF1) (thyroid component). The Ki67 proliferation index in primary ovarian carcinoid tumors of insular, trabecular and strumal types is usually less than 1% (5). The expression of somatostatin receptors on NEN cell surface (SSTR 2-5) (seen usually on well-differentiated NETs) enables diagnostic (imaging with 99mTc/68GaHYNIC/DOTA-peptides in SPECT/CT or PET/CT techniques respectively) and therapeutic procedures with the use of somatostatin analogues (peptide receptor radiation therapy (PRRT) with 177Lu or 90Y/177Lu-DOTA-TATE/TOC) (11).

MATERIAL AND METHODS

The aim of the study was to assess the clinical outcome of patients with postoperative diagnosis of neuroendocrine tumors (NET) in ovarian teratoma.

The study was designed as a retrospective analysis. Eligible patients were those who met main inclusion criteria: diagnose with NET in ovary teratoma which was histopathology confirmed according to WHO classification (2017 or 2019).

All patients meet the inclusion criteria were identified among the group of about 600 patients referred to our center during 2013-2020 (since the time of introduction of electronic documentation) due to suspicion of NET or confirmed NET diagnose. The identification was done by screening consecutive patients' records and collection of clinical/outcome data. Histopathological results were confirmed locally by pathologists with an expertise in the field of NETs blinded from clinical data.

Study variables included initial patients data, operation characteristics, postoperative complications, the course of disease and follow-up. Those data were extracted from the patient electronic records. The follow-up time was defined as the time from the patient's first visit to last visit.

Histological differentiation was assessed according to WHO classification for NET (2017, 2019), based on the morphological mitotic index (G1 <2 in 10 large visual fields (HPF), G2: 2-20/10HPF, and G3: > 20/10HPF) or immunohistochemically assessed tumor proliferative activity according to the Ki-67 index (G1 <3%, G2 3-20%, G3 > 20%). In cases where the mitotic index differed from the Ki-67 index, a higher index was used.

The surgical material was fixed in formalin, routinely processed and paraffin embedded. All cases were revaluated by a two pathologist experienced in a ovarian pathology. In each case, a single section including well-preserved part of tumor containing suspected neuroendocrine tissue was chosen. From this tissue block, 3 µm sections were prepared and immunohistochemistry with chromogranin [anti-chromogranin A antibody, Ventana (LK2H10)], synaptophysin [anti-synaptophysin antibody, Ventana (MRQ-40)] and MIB antibody was performed by a standard method. Briefly, the slides were dewaxed, rehydrated and incubated in 3% peroxide solution for 10 minutes to block endogenous peroxidase activity. Antigen retrieval was carried out by microwaving in citrate buffer (pH 6.0) for 5 minutes at 700 W, then for 5 minutes at 600 The LabVision (Thermo Fisher Scientific, USA) detection system was used. 3-amino-9-ethylcarbasole served as the chromogen. The slides were counterstained with Mayer's hematoxylin (DAKO, Denmark).

The follow-up examination included gynecological examinations with transvaginal ultrasound every 6-12 moths. Transabdominal ultrasound or abdominal and pelvic CT were performed 4-6 months after surgery, later every 12 month. Chromogranin A level was assessed on the first postoperative visit and in 6-12 months periods.

Statistical Analysis

Demographic and clinical characteristics were analyzed by producing tables of frequency for categorical variables and by calculation of the median and range for continuous variables. Length of follow-up is presented for the group as range and median value in months, from the time of curative surgery until the last follow-up appointment. IBM SPSS Statistics 26 was employed for the statistical analysis.

RESULTS

There were 10 patient eligible to include to the analysis. The mean age of patients at diagnosis was 42.8 ± 17.9 years (range 19–77). Seven of them were of reproductive age, and 3 were at menopause. In premenopausal patients, lesions were revealed in the majority of cases in a routine gynecological examination, in one case during pregnancy (ended later by caesarean section). In postmenopausal patients, the ovarian tumor was discovered while diagnosing specific complains either related to the disease itself (abdominal pain) or not (syncope). Two patients had a positive history of breast cancer treated surgically with subsequent radiotherapy, in oncological remission. None of the patients presented with carcinoid syndrome.

Each patient had a unilateral lesion and 70% of them (n=7) were located in the left ovary. The side did not correlate with an age of patients. All patients were operated not later than 6 months from diagnosis with laparoscopy or laparotomy. There were no complications in the course of surgeries or postoperative period. Postoperative imaging studies (CT scans and transvaginal ultrasounds) showed no abnormalities. In pathology the most common diagnosis was neuroendocrine tumor within ovarian teratoma present in 70% (n=7) of patients. Another 30% (n=3) were diagnosed with monodermal teratoma ovarian carcinoid. Tumors were usually large in their largest dimension. The mean teratoma diameter was 94.4mm [range 30-195)], however, neuroendocrine components in them were significantly smaller, 5-15 mm. No correlation was found between the size of tumor and a size of NET lesion or the affected side.

In premenopausal women there were 4 cases of NET within mature ovarian teratoma and 3 cases of monodermal teratoma with strumal carcinoid. In all cases of postmenopausal women (n=3) the NET within mature ovarian teratoma were diagnosed.

In 8 specimens, Ki-67 was determined and ranged from 2% to 9%. Forty percent (n=4) were classified as NET G1 and another 40% (n=4) as NET G2. In histological examination, synaptophysin and chromogranin were positive in 9 patients (**Figures 1**–**5**) In one patient, no histopathological staining was performed, but the morphology of the neuroendocrine component was typical. Serum chromogranin A (CgA) level was determined postoperatively in 7 patients after a neuroendocrine component was found in the histopathological examination; in 4 of them, CgA levels were elevated even several months after the surgery. Total time of observation ranges from 4 to 59 months, with a mean value of 29.7 ± 21.28 months. All patients who remain in observation are progression-free. Two patients at premenopausal age were lost to follow up after more than 3 years of observation.

All results are summarized in **Table 1**.

DISCUSSION

In recent years, the reported incidence of neuroendocrine tumors has been increasing, probably due to the better sensitivity of diagnostic tests and more frequent staining of chromogranin A and synaptophysin during the histopathological examination. Out of 10 cases, 50% (n=5) were detected over the last 2 years, while the remaining 50% (n=5) in the previous 5 years. The number of NETs primary to the gynecologic tract remains limited, with insufficient retrospective and prospective data (12), however the first case of a carcinoid ovarian teratoma was reported in 1939 (13).

Mature cystic teratomas typically occur in young women, while NET within ovarian teratoma is usually found in the postmenopausal age (14). However, in our study, only 30% (n=3) of patients were diagnosed after the menopause. Moreover, all patients diagnosed with ovarian carcinoid were in the premenopausal age (**Table 1**).

Carcinoid tumors of the ovary may be primary or metastatic. The morphological features of cells of metastatic carcinoids are similar to those which primarily arise in the ovary. Features that indicate the primary nature of the ovarian carcinoid are simultaneous presence of teratomatous elements in that tumor and unilaterality. Metastatic carcinoids are often bilateral, may be multinodular in each ovary, teratomatous elements are not present and extensive vascular invasion may be present.

Primary ovarian carcinoids are classified into four categories: insular, trabecular, strumal and mucinous, with insular being the

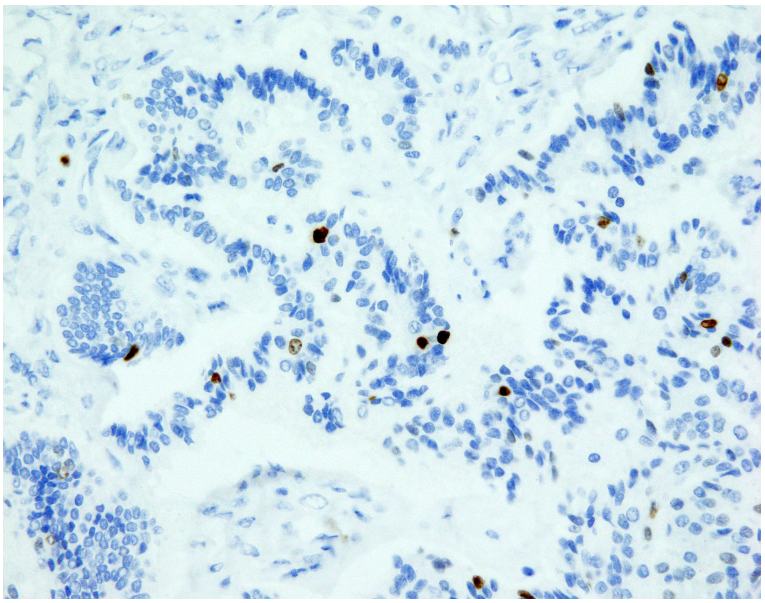

FIGURE 1 | NET focus in teratoma. Ki67 expression in NET foci of teratoma, zoom 40x. Immunohistochemical staining Ki67: proliferative activity around 1%.

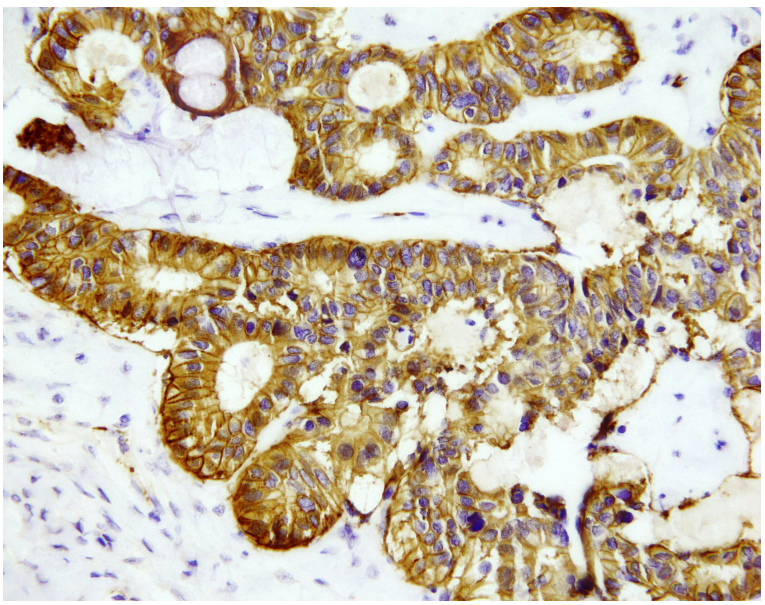

FIGURE 2 | NET focus in teratoma SSTR 2 expression, zoom 40x. Immunohistochemical staining with somatostatin receptor SSTR2: all cells show positive membrane reaction.

most common one (15). Out of them, insular type most commonly results in carcinoid syndrome and is the most commonly observed in Western countries, whereas trabecular and strumal NETs are primarily reported in Japan (16). All types are frequently associated with a mature cystic teratoma or mucinous tumor. The current study includes both insular and strumal lesions; neither trabecular nor mucinous NETs of the ovary were found among our patients.

Primary ovarian NET may occur on top of ovarian teratoma or in an otherwise normal ovary. The majority of ovarian teratomas, same as ovarian carcinoids within teratomas and their coexisting variants are found in routine abdominal

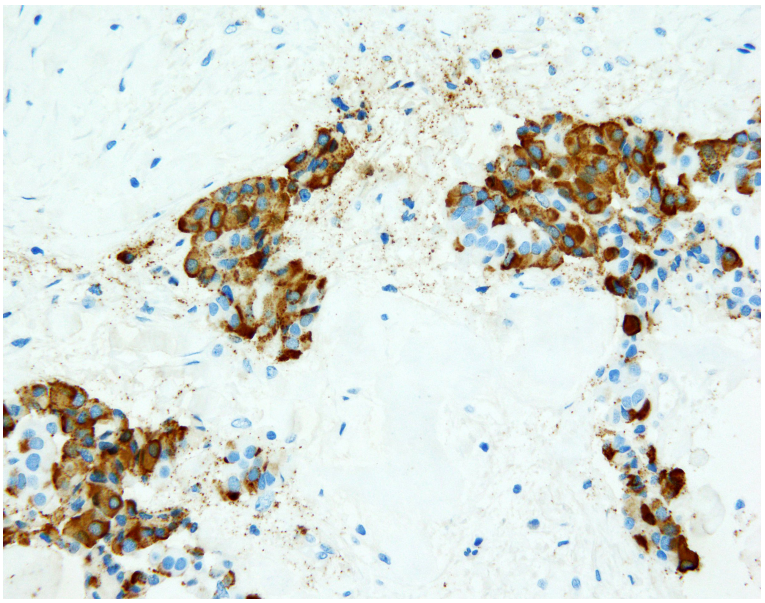

FIGURE 3 | NET focus in teratoma. Immunohistochemical staining (zoom 40x) with chromogranin A: around 85% of cells show positive granular, cytoplasmic reaction.

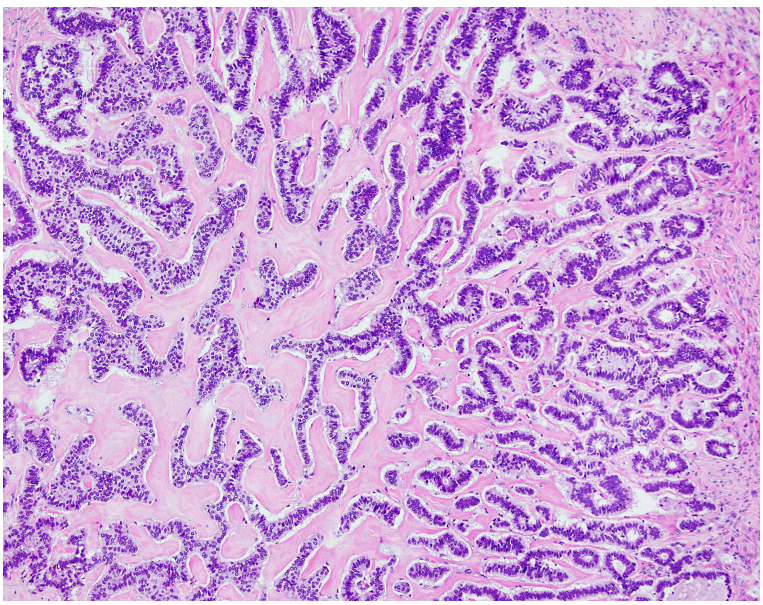

FIGURE 4 | NET foci in teratoma. Hematoxylin-eosin staining, zoom 10x. Long parallel ribbons and small islands and nests of cells with eosinophilic cytoplasm and oblong nuclei oriented perpendicular to main axis of ribbons.

ultrasound, which is in line with findings of our study but may be surprising considering the large size of those tumors.

If symptoms are present, they vary from non-specific complaints such as constipation or abdominal pain to emergency situations arising from tumor mass, as was seen in one of our patients. The biggest available study comes from Japan, where Soga et al. analyzed patients from 273 articles; 329 cases in total. Out of them, approximately half (43%) were pure ovarian NETs while 57% were associated with a teratoma. Tumors associated with mature teratomas were smaller, less frequently associated with metastases and had better 5-year survival [(17). Other authors also report excellent outcome after treating early-stage ovarian NETs arising in

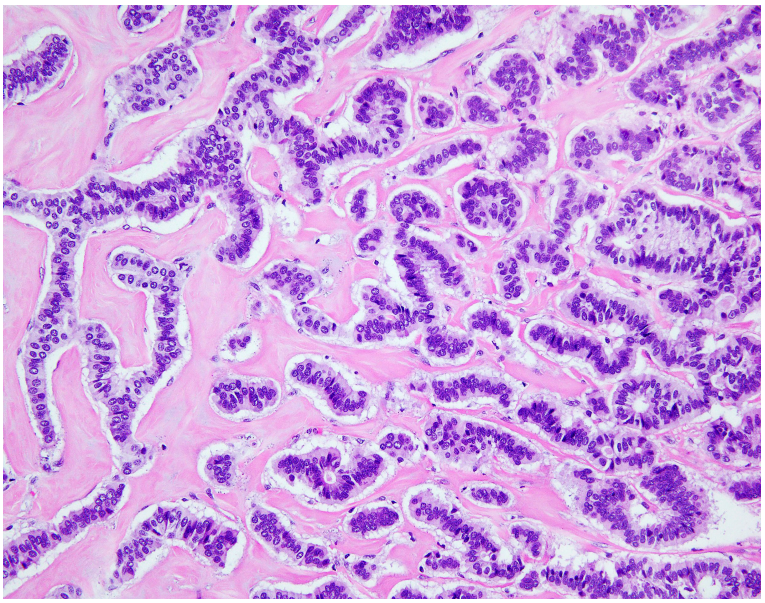

FIGURE 5 | NET foci in teratoma. Hematoxylin-eosin staining, zoom 20x. Long parallel ribbons and small islands and nests of cells with eosinophilic cytoplasm and oblong nuclei oriented perpendicular to main axis of ribbons.

TABLE 1 | Characteristics of patients and excised teratomas.

	Patients at premenopausal age (n = 7)	Patients at postmenopausal age (n = 3)	Total (n=10)
Age at diagnosis mean +/- SD, years (range)	33.14±9.06 (19-47)	65.33±10.4(57-77)	42.8±17.91 (19-77)
Months in observation mean +/- SD (range)	29.00±22.00 (4-55)	31.33±24.09 (15-59)	29.70±21.28 (4-59)
Histopatological types of NETs teratoma	• NET within mature ovarian teratoma (n=4) • Monodermal teratoma with strumal carcinoid (n=3)	NET within mature ovarian teratoma (n=3)	• NET within mature ovarian teratoma (n=7) • Monodermal teratoma with strumal carcinoid (n=7)
Side of ovary tumor	• Left (n=5) • Right (n=2)	• Left (n=1) • Right (n=2)	• Left (n=6) • Right (n=4)
Size of NEN lesion mean +/-SD [mm]	4.46±2.79 (n=6)	2.20 (n=1)	3.50±2.88 (range 2-9)
Chromogranin A level mean +/-SD [ng/ml]	69.25±27.58 (n=4)	195 (n=1)	94.4±61.10 (range 30-195)
Size of NEN lesion [mm]	7±2.83 (n=2)	12±4.24 (n=2)	4.46±2.79 (range 5-9)
Chromogranin A level [ng/ml]	5.34±2.71 (n=5)	10.0±10.04 (n=2)	6.67±5.18 (n=7)

n, numbers of cases; NET, neuroendocrine tumor; SD, standard deviation; mm, millimeters.

mature teratomas confined to one ovary with surgery alone (12, 18). Yamasaki et al. suggested that NETs arising in mature teratomas have low malignancy potential (19) and the aggressive course or metastasis was uncommon. In 2011, the Society of Gynecologic Oncology (SGO) issued a document on management of neuroendocrine tumors of the gynecologic tract. The surgery if radical can be considered as a curative without necessity of any adjuvant therapy (12). However, the prognosis and clinical behavior of NETs associated with ovarian teratomas have not been assessed yet, mainly due to its rarity.

According to the literature, approximately one third of all patients with neuroendocrine tumors within ovarian teratoma would present typical symptoms of carcinoid syndrome such as flushing and diarrhea due to bypassing the portal circulation by ovarian venous drainage (20). Some cases of hormonally active ovarian NETs were reported in Japan, however, they were not associated with ovarian teratoma (16, 21–24). Very few cases of primary ovarian NET associated with teratoma were complicated by severe course of the carcinoid syndrome (25, 26). There are also single reports on ACTH-secreting carcinoid components located in

an ovarian mature teratoma, manifesting with hypercortisolemia without circadian rhythm with a lack of cortisol suppression in dexamethasone tests (27). In presented study, none of the lesions had hormonal activity and thus, the patients did not experience symptoms related to the overproduction of hormones. Another rare but important issue is possibility of ovarian teratomas recurrence (28, 29). We had not observed relapse of tumors in our cohort.

Immunohistochemical staining of ovarian carcinoids include evaluation of neuroendocrine markers such as synaptophysin, chromogranin A and the proliferative activity of Ki-67/MIB1. In the case of clinical presentation of hormonal syndromes, the expression of hormones, particularly insulin, gastrin and serotonin, ACTH should be assessed. In case of all ovarian tumors, their metastatic nature should be ruled out. In few studies the utility of markers indicating the origin of neuroendocrine tumors, such as cytokeratin CK20, CK7, thyroid transcription factor (TTF-1) and CDX-2 in ovarian neuroendocrine tumors were determined. Rabban et al. examined the site of origin in 26 NETs (16 primary and 10 metastatic from midgut). Teratomatous elements were present in association with 10/16 primary ovarian NETs, whereas none were present in metastatic ones. Clinicopathologic features such as unilaterality, absence of multinodular growth, early stage, presence of teratomatous elements, and small size were the most helpful in suggesting a primary origin for an ovarian carcinoid tumor (10). Study of Gungor et al. states that metastatic carcinoid inside the ovary is always bilateral (30). However, to our knowledge, no case of primary bilateral NETs with ovarian teratoma has not been reported yet, as was not seen in our population. However, differential diagnosis should include metastatic neuroendocrine tumors (metastases from gastrointestinal tract are usually CK20+ and CDX2+ and metastases from lungs are usually CK7+ and TTF1 +), granulosa cell tumors (which is inhibin+ and calretinin+), poorly differentiated primary or metastatic adenocarcinomas (synaptophysin and chromogranin negative), Brenner tumors and androblastoma (9).

For follow-up the abdominal and pelvic imaging should be performed as well as chromogranin A (CgA) concentration assessed. It is important to remember that false-positive elevation of CgA is presented in many clinical settings like impaired renal function, chronic inflammations, chronic atrophic gastritis (type A), use of Proton Pump Inhibitors, glucocorticosteroids or others (11). For that reason, an isolated increase in CgA values require excluding other than NET causes of its elevation as observed in some of our patients in whom CgA increase was not accompanied by the disease relapse.

According to literature several other biochemical markers like circulating tumor cells, multiple transcript analysis, microRNA profile were tested as the tools for more accurate NET follow-up (31). Among them NETest (PCR based analysis of 51 different NET-related transcripts) seems to be the most promising in gastro-entero-pancreatic (GEP), and pulmonary NETs predicting radiologicalrelapse with 94% accuracy and 100% sensitivity (32). In the case of reproductive system NETs, the efficacy of NETest, has not been evaluated, yet.

In case of potentially inoperative or disseminated disease, there is a possibility, like in GEP-NETs, of SSTR assessment in the tumor tissue. It may be crucial for a further therapy planning (7, 11). The somatostatin analogues are recommended in treatment of well or moderately differentiated G1 and G2 GEP-NETs with good SSTR expression, while PRRT may be used after progression on somatostatin analogues in G1 and G2 GEP-NETs and additionally in G3 tumors either with good SSTR expression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The study protocol was approved by the Local Ethics Committee of the Jagiellonian University in Krakow. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MO, Data collection, data analysis, manuscript drafting, manuscript editing and approval, study coordination. AS-S, Data collection, data analysis, manuscript drafting, manuscript editing and approval. HO and MU-B, Data collection, manuscript drafting, manuscript editing and approval. AG-J, Data analysis, manuscript editing and approval. AH-D, Data analysis, manuscript drafting, manuscript editing and approval. All authors contributed to the article and approved the submitted version.

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Neuroendocrine Carcinoma as an Independent Prognostic Factor for Patients With Prostate Cancer: A Population-Based Study

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Background: Neuroendocrine carcinoma (NEC) is a rare and highly malignant variation of prostate adenocarcinoma. We aimed to investigate the prognostic value of NEC in prostate cancer.

Methods: A total of 530440 patients of prostate cancer, including neuroendocrine prostate cancer (NEPC) and adenocarcinoma from 2004 to 2018 were obtained from the national Surveillance, Epidemiology, and End Results (SEER) database. Propensity score matching (PSM), multivariable Cox proportional hazard model, Kaplan-Meier method and subgroup analysis were performed in our study.

Results: NEPC patients were inclined to be older at diagnosis (Median age, 69(61-77) vs. 65(59-72), P< 0.001) and had higher rates of muscle invasive disease (30.9% vs. 9.2%, P < 0.001), lymph node metastasis (32.2% vs. 2.2%, P < 0.001), and distal metastasis (45.7% vs. 3.6%, P < 0.001) compared with prostate adenocarcinoma patients. However, the proportion of NEPC patients with PSA levels higher than 4.0 ng/mL was significantly less than adenocarcinoma patients (47.3% vs. 72.9%, P<0.001). NEPC patients had a lower rate of receiving surgery treatment (28.8% vs. 43.9%, P<0.001), but they had an obviously higher rate of receiving chemotherapy (57.9% vs. 1.0%, P<0.001). A Cox regression analysis demonstrated that the NEPC patients faced a remarkably worse OS (HR = 2.78, 95% CI = 2.34–3.31, P < 0.001) and CSS (HR = 3.07, 95% CI = 2.55–3.71, P < 0.001) compared with adenocarcinoma patients after PSM. Subgroup analyses further suggested that NEPC patients obtained significantly poorer prognosis across nearly all subgroups.

Conclusion: The prognosis of NEPC was worse than that of adenocarcinoma among patients with prostate cancer. The histological subtype of NEC is an independent prognostic factor for patients with prostate cancer.

Keywords: neuroendocrine prostate cancer, prostate adenocarcinoma, clinicopathological characteristics, prognosis, SEER, survival

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INTRODUCTION

Prostate cancer, has the highest incidence of malignancy among men in the United States in 2021, which accounts for 26% of diagnoses (1, 2). Furthermore, it is also the second leading cause of cancer related deaths, only behind lung cancer (1). The predominant pathological type of prostate cancer is adenocarcinoma, and the assessment regarding incident rates, survival outcomes and therapeutic methods for prostate cancer are primarily according to this single histology (3). Neuroendocrine carcinoma (NEC) is a rare histological type, accounting for approximately 1% of newly diagnosed prostate cancer (4). Neuroendocrine prostate cancer (NEPC) possesses highly malignant characteristics such as poorly differentiated and high-grade (3, 5). In recent years, the incidence of NEPC has been rising and arouse wide concern (6, 7). Long-tern androgen-deprivation therapy (ADT) for prostate adenocarcinoma could contribute to castration resistant prostate cancer (CRPC), which may eventually develop to NEPC due to heterogeneity and evolution of prostate adenocarcinoma during therapy (8–11). Therefore, the extended application of ADT could partly explain the cause of the rising incidence of NEPC. Notably, the molecular mechanism by which NEPC transforms from prostate adenocarcinoma remains to be elucidated. Besides, as an increasingly recognized histologic subtype of prostate cancer, early diagnosis and effective treatment targeting specific biological characteristics for NEPC has not been developed.

Due to its rarity and a lack of associated published researches, NEPC is prone to be under-recognition and even neglected (12).However, given the upward incidence rates of NEPC in recent years as well as its refractory to medication, NEPC is attracting more attention worldwide increasingly. Currently, studies about NEPC were mainly case reports or retrospective researches based on small sample data. Therefore, our study compared NEPC with prostate adenocarcinoma comprehensively based on large population, aiming to overcome the remarkable challenges in the clinical treatment of patients with the rare subtype of prostate cancer. We utilized the national Surveillance, Epidemiology, and End Results (SEER) database (2004–2018) to compare the clinicopathological characteristics and survival outcomes between NEPC and prostate adenocarcinoma, the most common histological type of prostate cancer. Furthermore, we investigated the prognostic value of NEPC for patients with prostate cancer.

MATERIALS AND METHODS

Patients

This retrospective cohort study was conducted *via* the SEER database of the National Cancer Institute (http://seer.cancer.gov/). A total of 530440 patients of prostate cancer, including NEC and adenocarcinoma from 2004 to 2018 were obtained from the latest version of the SEER 18 database, as released in November 2020, using the SEER*Stat software (version 8.3.9). We identified prostate cancer according to the International Classification of Diseases for Oncology (Third Edition, ICD-O-3). NEPC, a generalized NEC of prostate, are classified by the American Joint Committee on Cancer (AJCC) as four histological subtypes, mainly including large cell neuroendocrine carcinoma (LCNE, ICD-0-3 codes 8013/3), small cell carcinoma (SCC, ICD-0-3 codes 8041/3), neuroendocrine carcinoma not otherwise specified (NEC NOS, ICD-0-3 codes 8246/3), and neuroendocrine differentiation (NED, ICD-0-3 codes 8574/3). And adenocarcinoma (ICD-0-3 code 8140/3) were included for comparison. All patients included were diagnosed by positive histology. Meanwhile, the exclusion criteria of patients were: (1) the information of age, race, marital status, survival time, surgery, radiotherapy, chemotherapy is unknown; (2) not the first tumor; (3) survival time < 1 month; (4) age at diagnosis < 18 years old; (5) with multiple primary tumor sites; (6) autopsy or death certificate only.

Clinical Variables

Variables covered demographic information (e.g., race, age at diagnosis, marital status and year of diagnosis), tumor characteristics [e.g., grade, tumor-node-metastasis (TNM) stage, lymph nodes and prostate-specific antigen (PSA)], treatment (e.g., surgery, radiation and chemotherapy), and survival information (survival months and vital status). In the SEER database, age is code as 18-59 years old, 60-74 years old and ≥75 years old. Race is coded as white, black, or other (e.g., American Indian/Alaskan native or Asian/Pacific Islander). Marital status is coded as married and not married. Between 2004 and 2018, patients were categorized according to 6th editions of the TNM classification. PSA was divided into four levels including 0-4.0 ng/ml, 4.1-10.0 ng/ml, 10.1-20 ng/ml, >20 ng/ml and unknown. We also enrolled treatment modality including surgery, chemotherapy, and radiation therapy information, which were divided with "Yes" and "No". The main outcome in this study were overall survival (OS) and cancer specific survival (CSS) according to data in the SEER database. OS was defined as the time interval from diagnosis to death for any cause or last follow-up. CSS refer to death from NEPC or prostate adenocarcinoma based on the recorded cause of death.

Statistical Analysis

Baseline demographic and clinicopathologic characteristics were performed to assess whether the distribution of the study population had significant differences between NEPC and prostate adenocarcinoma. Pearson's chi-square tests were adopted to calculate the differences in the distribution. We used Kaplan-Meier method and log-rank test to compare OS and CSS among patients with the two histological subtypes of prostate cancer. In order to overcome the effect of patient confounding bias, propensity score matching (PSM) method was adopted to remove the potential impact. Covariates of the two histological subtypes groups were matched with a ratio of 1:1 (R package "MatchIt"). The multivariable Cox proportional hazard model was performed to calculate hazard ratios (HR) and 95% confidence intervals (95% CI) according to histological types. We established two adjusted models in Cox regression analysis, in which covariates including age at diagnosis, marital status, lymph nodes examined, lymph nodes positive, PSA and

TNM stage were adjusted. We stratified the two histological subtypes groups based on the covariates into subgroups and applied stratified analyses to determine the subgroups that contribute to survival disadvantage of NEC. Interaction between the subgroups was calculated by R studio. The forest plot was applied to compare the impact of NEC and adenocarcinoma to survival outcomes of prostate cancer patients. Multivariate regression analysis was used to conduct subgroup analyses. Statistical analyses were performed using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Two-sided P values < 0.05 were considered as the threshold to define statistical significance.

RESULT

Patient Characteristics

This study enrolled 530440 eligible prostate cancer patients including 556 patients with NEPC and 529884 patients with prostate adenocarcinoma from SEER database between 2004 and 2018 (**Figure 1**). **Table 1** summarize the baseline demographic and clinicopathologic characteristics of these patients. The age at diagnosis of NEC patients were inclined to be older compared with adenocarcinoma patients, median age at diagnosis 69(61-77) vs. 65(59-72), age≥75 years (29.5% vs. 16.3%). The incidence of NEC in patients newly diagnosed were increasing roughly during our study period whereas the incidence of adenocarcinoma remained stable. Significantly, the NEC patients formed a higher proportion with a more advanced stage than the adenocarcinoma patients (59.6% vs. 12.2%, P<0.001), as displayed by a higher proportion of muscle invasive disease (30.9% vs. 9.2%, P<0.001), lymph node metastasis (32.2% vs. 2.2%, P<0.001), and distal metastasis (45.7% vs. 3.6%, P < 0.001). Lymph nodes were more likely to be examined in adenocarcinoma patients (11.2% vs. 24.7%, P<0.001) whereas positive lymph nodes were more common in the NEC patients (9.2% vs. 1.6%, P <0.001). Additionally, NEC patients with PSA levels higher than 4.0 ng/mL accounted for 47.3%, compared with 72.9% of adenocarcinoma patients. Furthermore, NEC patients had a lower rate of receiving surgery treatment compared with adenocarcinoma patients (28.8% vs. 43.9%, P<0.001). However, NEC patients were prone to receiving chemotherapy treatment, which accounted for 57.9% compared with 1.0% of adenocarcinoma patients. There was no significant difference of radiation between NEC and adenocarcinoma patients.

NEPC are defined by AJCC as different histological subtypes, including LCNE, SCC, NEC NOS, and NED. The first three are *de novo* NEPC while NED originated from the trans-differentiation of adenocarcinoma during the process of resistance to ATD or androgen receptor pathway inhibitors (ARPIs) treatment. The results of comparison among the four histological subtypes of NEPC and prostate adenocarcinoma are summarized in **Table 2**. The four histological subtypes patients all had higher proportions of muscle invasive disease (LCNE 50.0% vs. SCC 30.7% vs. NEC NOS 31.0% vs. NED 30.4%), lymph node metastasis (LCNE 50.0% vs. SCC 31.4% vs. NEC NOS 34.1% vs. NED 31.2%), and distal metastasis (LCNE 66.7% vs. SCC 46.6% vs. NEC NOS 45.7% vs. NED 42.4%), as compared to prostate adenocarcinoma patients (9.2%, 2.2%, 3.6%) respectively. Three histological subtypes of NEPC patients had low rates to receiving surgery treatment (SCC 25.0% vs. NEC NOS 31.0% vs. NED 32.8%) than that of adenocarcinoma (43.9%) except for LCNE (83.3%). However, the proportions of receiving radiation treatment of SCC (38.5%), NEC NOS (36.4%), and NED 33.6%) had no significant difference as compared to adenocarcinoma (35.2%) except for LCNE (66.7%). Additionally, the proportions of receiving chemotherapy treatment of LCNE (50.0%), SCC (67.9%) and NEC NOS (55.0%) patients were obviously higher than that of adenocarcinoma patients (1.0%) while NED patients (37.6%) were between *de novo* NEPC and prostate adenocarcinoma patients. Notably, NED patients with PSA levels higher than 4.0 ng/mL accounted for 72.0%, which was significantly higher than that of the other three histological subtypes of NEPC patients (LCNE 50.0%, SCC 36.9%, NEC NOS 47.3%). We speculated that it may attributed to the mixed adenocarcinoma and NEC components of NED.

Survival Analyses

We performed Kaplan-Meier curves to compare the OS and CSS between the four histological subtypes of NEC and adenocarcinoma patients (**Figure 2**). The LCNE patients had the worst OS and CSS among all histological subtypes, followed by SCC, NEC NOS, NED, and adenocarcinoma patients. Intriguingly, these results suggested that the OS and CSS of NED patients were better than that of *de novo* NEC patients but worse than that of adenocarcinoma patients. Furthermore, we performed the survival analysis of 1-, 2-, 3-, 4- and 5-year OS and CSS rates of patents with the four histological subtypes of NEPC and prostate adenocarcinoma (**Table 3**). The LCNE patients had the worst 5-year OS rate among all histological subtypes, followed by SCC, NEC NOS, NED, and adenocarcinoma patients. Compared with the four histological subtypes of NEPC, the 1-, 3- and 5-year OS rate of adenocarcinoma (97.7%, 92.7%, 88.0%) roughly remained stable. The 1-, 2-, 3-, 4- and 5-year CSS revealed the similar outcomes.

Due to the imbalanced basic demographic and clinicopathologic characteristics, we conducted PSM *via* R software to minimize confounding factors. All the covariates in the present study were matched between the two groups. The baseline after PSM was shown in **Table 4**. We matched 401 NEPC patients with 401 prostate adenocarcinoma patients with a ratio of 1:1. After eliminating the selection bias, all variables were matched as defined by the P value >0.05. We performed multivariable Cox proportional hazard regression based on a non-adjusted model and three adjusted models (**Table 5**). Adjusted I model adjusts for age, marital status, lymph nodes examined and lymph nodes positive and adjusted II model adjusted for age, marital status, lymph nodes examined and lymph nodes positive, T stage, N stage, M stage, PSA level. NEPC patients faced a remarkably worse OS (HR = 2.78, 95% CI = 2.34–3.31, P < 0.001) and CSS (HR = 3.07, 95% CI = 2.55–3.71,

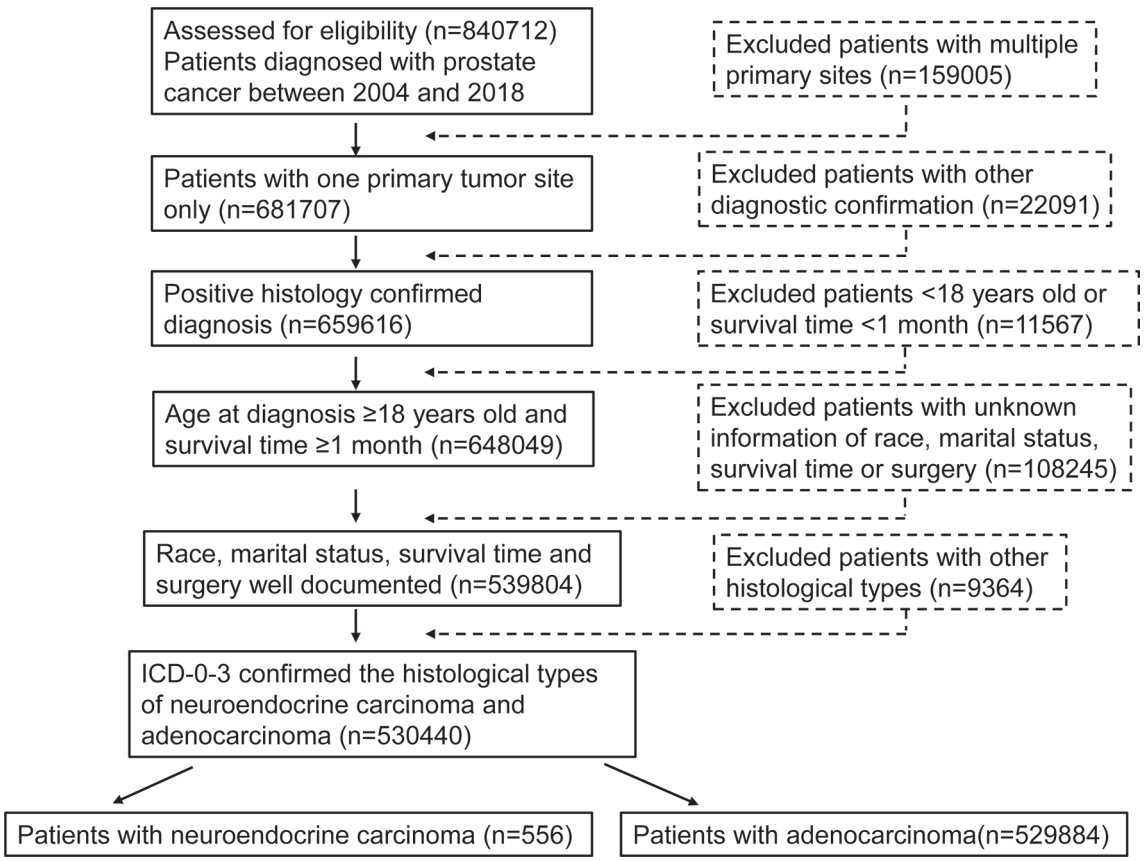

FIGURE 1 | Flowchart of patient selection steps.

P < 0.001) compared with prostate adenocarcinoma patients. These findings emphasized the worse survival outcomes for the histological subtype of NEC.

Subgroup Analyses

After discovering the shortened survival of NEPC patients, we next aimed to evaluate the prognostic consistency and difference in diverse subgroups of prostate cancer patients between NEC and adenocarcinoma patients (**Figure 3**). According to the baseline demographic and clinicopathologic characteristics, NEC and adenocarcinoma patients were divided into subgroups, respectively. The results demonstrated that NEC patients obtained significantly poorer prognosis than adenocarcinoma patients across all subgroups except for G2 (HR = 3.25, 95%CI=0.68–15.4, P=0.1371), stage II (HR = 2.56, 95%CI=0.64–10.2, P=0.1831) and lymph nodes negative (HR = 3.57, 95%CI=0.94–13.4, P=0.0602) subgroups. We suspected that the insufficient sample size may contribute to no statistic difference of the three subgroups above. Nonetheless, the general tendency for the worse survival outcomes were existing in NEPC patients. Similar results were shown in subgroup analysis for CSS (**Figure 4**).

Furthermore, we performed subgroup analysis to test the interaction after adjusting for the potential covariates (**Figure 5**). No significant difference was found for age at diagnosis, race, marital status, grade, T stage, N stage, lymph nodes examined, lymph nodes positive, radiation in both OS and CSS. The results uncovered that NEPC patients had a poorer survival outcome out of all subgroups. These results indicated that among patients with prostate cancer, the histological subtype of NEC had poorer prognosis than adenocarcinoma, which was not affected by other potential variates. Especially, it was reasonable to speculate that the histological subtype of NEC was an independent prognostic factor for patients with prostate cancer.

DISCUSSION

Our study is the most representative and comprehensive of the latest primary survival information of NEC compared with the most common histological type of prostate cancer. Given that NEPC is a rare and highly aggressive malignancy, majority of investigations are based on case reports or retrospective studies limited by small sample sizes (13–17). Consequently, the present study performed an investigation of a prostate cancer patient cohort based on large population from SEER registries between 2004 and 2018. We aimed to compare the survival outcomes of NEC with adenocarcinoma among prostate cancer patients

TABLE 1 | Baseline demographic and clinicopathologic characteristics of patients with prostate adenocarcinoma compare to NEPC.

Characteristics	NEPC (n = 556)	Prostate Adenocarcinoma (n = 529884)	P value
Median age, y (IQR)	69(61-77)	65(59-72)	<0.001
Age at diagnosis, n (%)			<0.001
18-59	115(20.7)	138473(26.1)	
60-74	281(50.5)	304879(57.5)	
≥75	160(28.8)	86532(16.3)	
Race, n (%)			0.001
White	464(83.5)	414093(78.1)	
Black	57(10.3)	86273(16.3)	
Other	35(6.2)	29518(5.6)	
Marital status, n (%)			<0.001
Married	377(67.8)	395936(74.7)	
Not married	179(32.2)	133948(25.3)	
Year of diagnosis, n (%)			<0.001
2004-2008	117(21.0)	181794(34.3)	
2009-2013	203(36.5)	178887(33.8)	
2014-2018	236(42.5)	169203(31.9)	
Grade, n (%)			<0.001
G1	1(0.2)	40933(7.7)	
G2	12(2.2)	213722(40.3)	
G3	240(43.2)	226212(42.7)	
G4	41(7.4)	876(0.2)	
Unknown	262(47.1)	48141(9.1)	
Stage, n (%)			<0.001
I	0(0.0)	1478(0.3)	
II	45(8.1)	340943(64.3)	
III	7(1.3)	35235(6.6)	
IV	324(58.3)	29774(5.6)	
Unknown	180(32.4)	122454(23.1)	
T stage, n (%)			<0.001
T1	52(9.4)	161210(30.4)	
T2	92(16.5)	203640(38.4)	
T3	50(9.0)	41566(7.8)	
T4	122(21.9)	7284(1.4)	
Unknown	207(43.2)	116184(21.9)	
N stage, n (%)			<0.001
N0	152(27.3)	393696(74.3)	
N1	179(32.2)	11647(2.2)	
Unknown	225(40.5)	124541(23.5)	
M stage, n (%)			<0.001
M0	122(21.9)	394657(74.5)	
M1	254(45.7)	18815(3.6)	
Unknown	180(32.4)	116412(22.0)	
Lymph nodes examined, n (%)			<0.001
None	471(84.7)	393040(74.2)	
More than one	62(11.2)	130720(24.7)	
Unknown	23(4.1)	6124(1.2)	
Lymph nodes positive, n (%)			<0.001
None	16(2.9)	122138(23.0)	
More than one	51(9.2)	8448(1.6)	
Unknown	489(87.9)	399298(75.4)	
PSA, ng/mL, n (%)			<0.001
0-4.0	127(22.8)	57511(10.9)	
4.1-10.0	92(16.5)	265323(50.1)	
10.1-20.0	42(7.6)	66929(12.6)	
>20.0	129(23.2)	53942(10.2)	
Unknown	166(29.9)	86179(16.3)	
Surgery			<0.001
No	396(71.2)	297267(56.1)	
Cryoprostatectomy	0(0)	4875(0.9)	
Laser ablation	3(0.5)	1202(0.2)	
TURP	116(20.9)	25297(4.8)	
Partial prostatectomy	2(0.4)	1123(0.2)	
Radical prostatectomy	39(7.0)	200120(37.8)	

(Continued)

TABLE 1 | Continued

Characteristics	NEPC (n = 556)	Prostate Adenocarcinoma (n = 529884)	P value
Radiation			<0.001
No	349(62.8)	343593(64.8)	
Beam radiation	199(35.8)	128148(24.2)	
Radioactive implants	3(0.5)	34974(6.6)	
Combination of beam with implants or isotopes	1(0.2)	20895(3.9)	
Radioisotopes	0(0)	871(0.2)	
Radiation method unknown	4(0.7)	1404(0.3)	
Chemotherapy			<0.001
No	234(42.1)	524571(99.0)	
Yes	322(57.9)	5313(1.0)	
Overall mortality			<0.001
Alive	113(20.3)	431549(81.4)	
Dead	443(79.7)	98335(18.6)	
Cause special mortality			<0.001
Alive	150(27.0)	495892(93.6)	
Dead	406(73.0)	33992(6.4)	

PSA, prostate-specific antigen; NEPC, neuroendocrine prostate cancer; IQR, interquartile range; TURP, Transurethral resection of prostate.

TABLE 2 | Baseline demographic and clinicopathologic characteristics of patients with prostate adenocarcinoma compare to four histological subtypes of NEPC.

Characteristics	Prostate adenocarcinoma (n = 529884)	NEPC (n = 556)				P value
		LCNE (n = 6)	SCC (n = 296)	NEC NOS (n = 129)	NED (n = 125)	
Age at diagnosis, y, n (%)						<0.001
18-59	138473(26.1)	3(50.0)	56(18.9)	32(24.8)	24(19.2)	
60-74	304879(57.5)	1(16.7)	151(51.0)	58(45.0)	71(56.8)	
≥75	86532(16.3)	2(33.3)	89(30.1)	39(30.2)	30(24.0)	
Race, n (%)						0.020
White	414093(78.1)	5(83.3)	244(82.4)	107(82.9)	108(86.4)	
Black	86273(16.3)	0(0)	32(10.8)	13(10.1)	12(9.6)	
Other	29518(5.6)	1916.7)	20(6.8)	9(7.0)	5(4.0)	
Marital status, n (%)						0.002
Married	395936(74.7)	3(50.0)	208(70.3)	86(66.7)	80(64.0)	
Not married	133948(25.3)	3(50.0)	88(29.7)	43(33.3)	45(36.0)	
Year of diagnosis, n (%)						<0.001
2004-2008	181794(34.3)	1(16.7)	57(19.3)	37(28.7)	22(17.6)	
2009-2013	178887(33.8)	3(50.0)	109(36.8)	48(37.2)	43(34.4)	
2014-2018	169203(31.9)	2(33.3)	130(43.9)	44(34.1)	60(48.0)	
Grade, n (%)						<0.001
G1	40933(7.7)	0(0)	0(0)	0(0)	1(0.8)	
G2	213722(40.3)	0(0)	6(2.0)	2(1.6)	4(3.2)	
G3	226212(42.7)	1(16.7)	79(26.7)	72(55.8)	88(70.4)	
G4	876(0.2)	1(16.7)	27(9.1)	11(8.5)	2(1.6)	
Unknown	48141(9.1)	4(66.7)	184(62.2)	44(34.1)	30(24.0)	
Stage, n (%)						<0.001
I	1478(0.3)	0(0)	0(0)	0(0)	0(0)	
II	340943(64.3)	0(0)	22(7.4)	13(10.1)	10(8.0)	
III	35235(6.6)	0(0)	1(0.3)	2(1.6)	4(3.2)	
IV	29774(5.6)	5(83.3)	176(59.5)	74(57.4)	69(55.2)	
Unknown	122454(23.1)	1(16.7)	97(32.8)	40(31.0)	42(33.6)	
T stage, n (%)						<0.001
T1	161210(30.4)	0(0)	27(9.1)	13(10.1)	12(9.6)	
T2	203640(38.4)	1(16.7)	55(18.6)	19(14.7)	17(13.6)	
T3	41566(7.8)	0(0)	27(9.1)	9(7.0)	14(11.2)	
T4	7284(1.4)	3(50.0)	64(21.6)	31(24.0)	24(19.2)	
Unknown	116184(21.9)	2(33.3)	123(41.6)	57(44.2)	58(46.4)	
N stage, n (%)						<0.001
N0	393696(74.3)	0(0)	84(28.4)	34(26.4)	34(27.2)	
N1	11647(2.2)	3(50.0)	93(31.4)	44(34.1)	39(31.2)	
Unknown	124541(23.5)	3(50.0)	119(40.2)	51(39.5)	52(41.6)	

(Continued)

TABLE 2 | Continued

Characteristics	Prostate adenocarcinoma (n = 529884)	NEPC (n = 556)				P value
		LCNE (n = 6)	SCC (n = 296)	NEC NOS (n = 129)	NED (n = 125)	
M stage, n (%)						<0.001
M0	394657(74.5)	1(16.7)	60(20.3)	32(24.8)	29(23.2)	
M1	18815(3.6)	4(66.7)	138(46.6)	59(45.7)	53(42.4)	
Unknown	116412(22.0)	1(16.7)	98(33.1)	38(29.5)	43(34.4)	
Lymph nodes examined, n (%)						<0.001
None	393040(74.2)	6(100.0)	258(87.2)	102(79.1)	105(84.0)	
More than one	130720(24.7)	0(0)	25(8.4)	19(14.7)	18(14.4)	
Unknown	6124(1.2)	0(0)	13(4.4)	8(6.2)	2(1.6)	
Lymph nodes positive, n (%)						<0.001
None	122138(23.0)	0(0)	7(2.4)	3(2.3)	6(4.8)	
More than one	8448(1.6)	0(0)	21(7.1)	16(12.4)	14(11.2)	
Unknown	399298(75.4)	6(100.0)	268(90.5)	110(85.3)	105(84.0)	
PSA, ng/mL, n (%)						<0.001
0-4.0	57511(10.9)	1(16.7)	81(27.4)	32(24.8)	13(10.4)	
4.1-10.0	265323(50.1)	1(16.7)	47(15.9)	17(13.2)	27(21.6)	
10.1-20.0	66929(12.6)	0(0)	18(6.1)	13(10.1)	11(8.8)	
>20.0	53942(10.2)	2(33.3)	44(14.9)	31(24.0)	52(41.6)	
Unknown	86179(16.3)	2(33.3)	106(35.8)	36(27.9)	22(17.6)	
Surgery						<0.001
No	297267(56.1)	1(16.7)	222(75.0)	89(69.0)	84(67.2)	
Cryoprostatectomy	4875(0.9)	0(0)	0(0)	0(0)	0(0)	
Laser ablation	1202(0.2)	0(0)	1(0.3)	0(0)	2(1.6)	
TURP	25297(4.8)	5(83.3)	58(19.6)	29(22.5)	24(19.2)	
Partial prostatectomy	1123(0.2)	0(0)	2(0.7)	0(0)	0(0)	
Radical prostatectomy	200120(37.8)	0(0)	13(4.4)	11(8.5)	15(12.0)	
Radiation						<0.001
No	343592(64.8)	2(33.3)	182(61.5)	82(63.6)	83(66.4)	
Beam radiation	128148(24.2)	4(66.7)	109(36.8)	46(35.7)	40(32.0)	
Radioactive implants	34974(6.6)	0(0)	3(1.0)	0(0)	0(0)	
Combination of beam with implants or isotopes	20895(3.9)	0(0)	0(0)	0(0)	1(0.8)	
Radioisotopes	871(0.2)	0(0)	0(0)	0(0)	0(0)	
Radiation method unknown	1404(0.3)	0(0)	2(0.7)	1(0.8)	1(0.8)	
Chemotherapy						<0.001
No	524571(99.0)	3(50.0)	95(32.1)	58(45.0)	78(62.4)	
Yes	5313(1.0)	3(50.0)	201(67.9)	71(55.0)	47(37.6)	
Overall mortality						<0.001
Alive	431549(81.4)	1(16.7)	48(16.2)	21(16.3)	43(34.4)	
Dead	98335(18.6)	5(83.3)	248(83.8)	108(83.7)	82(65.6)	
Cause special mortality						<0.001
Alive	495892(93.6)	1(16.7)	65(22.0)	31(24.0)	53(42.4)	
Dead	33992(6.4)	5(83.3)	231(78.0)	98(76.0)	72(57.6)	

PSA, prostate-specific antigen; NEPC, neuroendocrine prostate cancer; TURP, Transurethral resection of prostate; LCNE, large cell neuroendocrine carcinoma; SCC, small cell carcinoma; NEC NOS, neuroendocrine carcinoma not otherwise specified; NED, neuroendocrine differentiation.

according to clinicopathologic characteristics and explore the prognostic values in NEPC. Several meaningful conclusions could be obtained from our study. Among patients with prostate cancer, NEC had a worse prognosis than adenocarcinoma, even after adjustment for potential covariates. Moreover, subgroup analysis suggested that NEC patients obtained significantly poorer survival outcomes than adenocarcinoma patients across almost all subgroups. Last but not the least, there was no interaction among age at diagnosis, race, marital status, year of diagnosis, grade, T stage, N stage, lymph nodes examined, lymph nodes positive, radiation and the histological subtype of NEC was an independent prognostic factor for prostate cancer.

Although NEPC is a rare entity, the incidence rates of it maintained an upward trend in recent years (18). It had risen by approaching 6.8% per year, which could be mainly attributed to advanced medical technology and improved diagnostic methods (10, 18). Specially, the incident rates of small cell carcinoma (SCC) had a similar increasing trend of nearly 7.0% per year (18). Previous studies revealed that it was quite possible that the rise in incidence of NEPC was driven by SCC (19–21). On the other hand, several studies hold the view that the utilization of ADT was related to the incidence of NEPC (22–24). ADT was a primary therapy for prostate cancer targeting the androgen axis, which was first put forward by Huggins and Hodges in 1941 (11). Recently, the incidence rates of NEPC rose accompanied by the utilization of highly potent ADT, such as abiraterone and enzalutamide before or after chemotherapy for CRPC (25, 26). Long-term androgen deprivation could promote

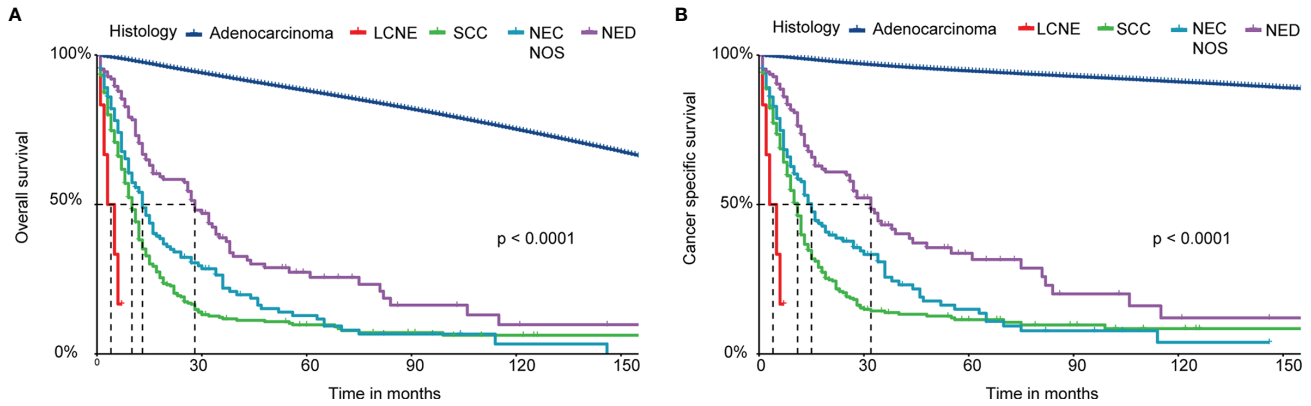

FIGURE 2 | Survival analysis of OS and CSS for patients with four histological subtypes of NEPC and prostate adenocarcinoma. **(A)** Kaplan-Meier analysis of OS; **(B)** Kaplan-Meier analysis of CSS. NEPC, neuroendocrine prostate cancer; LCNE, large cell neuroendocrine carcinoma; SCC, small cell carcinoma; NEC NOS, neuroendocrine carcinoma not otherwise specified; NED, neuroendocrine differentiation; OS, overall survival; CSS, cancer-specific survival.

adenocarcinoma cells lose androgen receptor (AR) expression and eventually developed to NEC cells, which was called treatment-related NEPC (t-NEPC) (10, 27). However, it was reported that the utilization of ADT obviously decreased in 2004 and 2005 while the incident rates of NEPC, by contrast, displayed an increasing trend (19). Hence, such hypothesis is still not exactly elucidated. The upward incident trend of NEPC were supposed to be highlighted and the issue of long-term exposure to ADT in the clinic was warranted to be resolved in the coming years.

In the present study, NEC patients with PSA levels higher than 4.0 ng/mL accounted for 43.7%, compared with 72.9% of adenocarcinoma patients. This result suggested that except for loss of AR, NEPC patients are typically manifested by the downregulation of PSA (28). Our investigation was consistent with previous studies, which demonstrated that the PSA marker was usually expressed in adenocarcinoma while SCC, large cell carcinoma, or mixed adenocarcinoma neuroendocrine histology were scarcely expressed PSA (29). Hence, the low or non-rising serum PSA levels in tumor cells may indicate a relatively poorer prognosis (30). It also implied that serum PSA screening may not be effective for detection of NEPC in the clinic (7). The US Preventive Services Task Force (USPSTF) has recommended against PSA screening first in 2008 for men aged 75 years and older and then in 2012 for all men. However, since USPSTF's 2012 recommendation, the incidence of advanced-stage prostate cancer has continued to rise though rates of localized disease have declined (6). Currently, the diagnosis of NEPC is mainly according to metastatic tumor biopsy confirming tumor morphology. Although there were no standard criteria for the best opportunity to conduct tumor biopsy, the NCCN guidelines recommended performing metastatic biopsy in suspected patients with particularly atypical spread, aggressive characteristics, and/or development with low serum PSA levels (30). Serum NE markers like CgA and NSE levels as well as synaptophysin (SYP), chromogranin and CD56 were classic biomarkers of NE cell, which were frequently upregulated in NEPC by immunohistochemistry (IHC), but neither of them was

TABLE 3 | Overall survival and cancer specific survival of patients with NEPC and prostate adenocarcinoma.

Year	Overall survival					Cancer specific survival				
	Prostate	NEPC				Prostate	NEPC			
	Adenocarcinoma	LCNE	SCC	NEC NOS	NED	Adenocarcinoma	LCNE	SCC	NEC NOS	NED
1	97.7 (97.7-97.8)	0	38.1 (32.8-44.3)	54.0 (46.0-63.5)	70.4 (62.6-79.2)	98.8 (98.8-98.9)	0	39.9 (34.4-46.2)	57.6 (49.6-67.0)	73.5 (65.8-82.0)
2	95.2 (95.1-95.2)	0	19.1 (14.9-24.5)	32.3 (24.9-41.9)	57.4 (48.9-67.3)	97.5 (97.5-97.6)	0	20.5 (16.0-26.2)	37.7 (29.8-47.9)	59.9 (51.4-69.8)
3	92.7 (92.7-92.8)	0	11.7 (8.3-16.5)	22.0 (15.4-31.4)	38.9 (30.4-49.7)	96.4 (96.4-96.5)	0	13.8 (10.0-19.1)	25.7 (18.3-36.1)	43.2 (34.2-54.5)
4	90.3 (90.2-90.4)	0	10.8 (7.5-15.5)	15.1 (9.5-24.1)	28.8 (20.9-39.8)	95.5 (95.4-95.6)	0	12.7 (9.0-17.9)	17.7 (11.2-27.8)	35.6 (26.7-47.4)
5	88.0 (87.9-88.1)	0	9.7 (6.6-14.4)	11.6 (6.7-20.2)	25.6 (17.8-36.8)	94.7 (94.6-94.8)	0	11.5 (7.9-16.6)	13.6 (7.9-23.4)	31.6 (22.7-44.0)

NEPC, neuroendocrine prostate cancer; LCNE, large cell neuroendocrine carcinoma; SCC, small cell carcinoma; NEC NOS, neuroendocrine carcinoma not otherwise specified; NED, neuroendocrine differentiation.

TABLE 4 | Propensity score matching for baseline factors.

Characteristics	Prostate Adenocarcinoma (n = 484)	NEPC (n = 484)	P value
Age at diagnosis, y, n (%)			0.722
18-59	92 (19.0)	101 (20.9)	
60-74	249 (51.4)	248 (51.2)	
≥75	143 (29.5)	135 (27.9)	
Race, n (%)			0.222
White	382 (78.9)	396 (81.8)	
Black	74 (15.3)	56 (11.6)	
Other	28 (5.8)	32 (6.6)	
Marital status, n (%)			0.500
Married	310 (64.0)	321 (66.3)	
Not married	174 (36.0)	163 (33.7)	
Year of diagnosis, n (%)			0.006
2004-2006	127 (26.2)	97 (20.0)	
2007-2009	137 (28.3)	180 (37.2)	
2010-2012	220 (45.5)	207 (42.8)	
Grade, n (%)			0.004
G1	2 (0.4)	1 (0.2)	
G2	23 (4.8)	12 (2.5)	
G3	234 (48.3)	237 (49.0)	
G4	11 (2.3)	33 (6.8)	
Unknown	214 (44.2)	201 (41.5)	
Stage, n (%)			NaN
I	0 (0.0)	0 (0.0)	
II	50 (10.3)	45 (9.3)	
III	20 (4.1)	7 (1.4)	
IV	210 (43.4)	270 (55.8)	
Unknown	204 (42.1)	162 (33.5)	
T stage, n (%)			0.011
T1	56 (11.6)	48 (9.9)	
T2	83 (17.1)	76 (15.7)	
T3	50 (10.3)	42 (8.7)	
T4	58 (12.0)	99 (20.5)	
Unknown	237 (49.0)	219 (45.2)	
N stage, n (%)			<0.001
N0	172 (35.5)	127 (26.2)	
N1	88 (18.2)	149 (30.8)	
Unknown	224 (43.9)	208 (49.1)	
M stage, n (%)			0.006
M0	137 (28.3)	106 (21.9)	
M1	168 (34.7)	214 (44.2)	
Unknown	179 (37.0)	164 (33.9)	
Lymph nodes examined, n (%)			0.245
None	405 (83.5)	405 (83.5)	
More than one	47 (9.7)	57 (11.8)	
Unknown	32 (6.6)	22 (4.5)	
Lymph nodes positive, n (%)			0.188
None	19 (3.9)	16 (3.3)	
More than one	31 (6.4)	46 (9.5)	
Unknown	434 (89.7)	422 (87.2)	
PSA, ng/mL, n (%)			0.012
0-4.0	65 (13.4)	83 (17.1)	
4.1-10.0	90 (18.6)	76 (15.7)	
10.1-20.0	43 (8.9)	37 (7.6)	
>20.0	160(33.1)	125 (25.8)	
Unknown	126 (26.0)	163 (33.7)	
Surgery			0.003
No	367 (75.8)	339 (70.0)	
Cryoprostatectomy	2 (0.4)	0 (0)	
Laser ablation	0(0)	2(0.4)	
TURP	64(13.2)	106(21.9)	
Partial prostatectomy	2(0.4)	1(0.2)	
Radical prostatectomy	49(10.1)	36(7.4)	

(Continued)

TABLE 4 | Continued

Characteristics	Prostate Adenocarcinoma (n = 484)	NEPC (n = 484)	P value
Radiation			0.046
No	327 (67.6)	310 (64.0)	
Beam radiation	139 (28.7)	166 (34.3)	
Radioactive implants	8(1.7)	3(0.6)	
Combination of beam with implants or isotopes	7(1.4)	1(0.2)	
Radioisotopes	1(0.2)	0(0)	
Radiation method unknown	2(0.4)	4(0.8)	
Chemotherapy			0.479
No	246 (50.8)	234 (48.3)	
Yes	238 (49.2)	250 (51.7)	
Overall mortality			<0.001
Alive	241 (49.8)	105 (21.7)	
Dead	243 (50.2)	379 (78.3)	
Cause special mortality			<0.001
Alive	294 (60.7)	141 (29.1)	
Dead	190 (39.3)	343 (70.9)	

PSA, prostate-specific antigen; TURP, Transurethral resection of prostate; NEPC, neuroendocrine prostate cancer.

necessary for the diagnosis of NEPC in the clinic (31, 32). In order to achieve early diagnosis and effective treatment, it will be crucial to confirm feasible biomarkers that can detect the emergence of NEPC transformation during sequential therapies. A further investigation of biological characteristics of NEPC is indispensable to overcome the obstacle of this highly malignant prostate cancer.

The prevalent therapeutic modalities for prostate adenocarcinoma patients mainly include surgical removal of the prostate (radical prostatectomy), or radiation therapy with or without ADT. For early-stage or localized tumors, radical prostatectomy or radiation therapy is potentially effective and safe treatment option (33). ADTs is still first-line treatment for metastatic prostate cancer. However, after initial response to ADT, the tumor develops an androgen-insensitive form known as CRPC (34). ARPIs including abiraterone, enzalutamide, apalutamide and darolutamide have been developed for CRPC treatment. Nevertheless, partial ARPI- resistant CRPC may eventually develop NEPC due to AR- independent mechanisms in prostate cancer.

Our study suggested that the median OS of NEPC patients was only 12 months compared with 42 months of prostate adenocarcinoma patients. The severe invasiveness and the delayed diagnosis contributed to the final poor survival outcomes of NEPC. For example, we found that NEPC patients had an extremely high rate of metastasis, accounted for 45.4% of the group. In addition, the proportion of receiving surgery treatment for NEPC patients was significantly lower than prostate adenocarcinoma patients due to patients in advanced stage missing optimal opportunity for surgery. Until now, radical resection and palliative resection are the primary treatment for early NEPC without distal metastasis (35). Currently, the first-line treatment for NEPC is platinum- based chemotherapy, such as a combination of cisplatin and etoposide (36, 37). Cisplatin-or etoposide-based systemic chemotherapies, combined with surgery or radiation is the main therapy for NEPC with metastasis currently (38). The initial response of NEPC to chemotherapy is considerable. Unfortunately, its limitations are obvious: high and short response duration owing to acquired drug resistance (36). However, the effect of systemic treatment is not so satisfactory. Accurate assessment, early diagnosis and timely treatment of NEPC is critical for enhancing the clinical effect and thereby improving the prognosis.

Considering that the poor prognosis of NEPC is overwhelming, the novel effective therapeutic methods aiming at specific targets is warranted to be explored. Currently, emerging molecular targets with in the landscape of NEC differentiation put insight into individual therapy for NEPC. Rearrangement of TMPRSS2–ERG in NEPC was a crucial finding to prove that NEPC is evolved from conventional prostate adenocarcinoma (39). In the progression of evolution, several underlying molecular mechanism function, including loss of AR and tumor suppressors (TP53, PTEN, RB1) and induction of neural programs (39, 40). Especially, activation of mitotic programs such as Aurora kinase A (AURKA) upregulation and MYCN amplification are involved. AURKA, associated with MYCN amplification could regulates the assembly of mitotic spindle apparatus and eventually influences chromosome separation (41, 42). In addition, epigenetics regulation changes play an important role as well. Transcription factor RE1-silencing transcription factor (REST), suppressing neuronal differentiation, was found to be downregulated in 50% NEPC (43). Furthermore,

TABLE 5 | Multivariable Cox proportional hazard model.

Outcomes	NEPC HR (95% CI)	P-value
Overall survival		
Non-adjusted	23.20 (21.02-25.60)	<0.001
Adjust I	19.24 (17.43-21.23)	<0.001
Adjust II	6.35 (5.75-7.02)	<0.001
PSM	2.78 (2.34-3.31)	<0.001
Cancer specific survival		
Non-adjusted	48.08 (43.38-52.29)	<0.001
Adjust I	34.65 (31.24-38.43)	<0.001
Adjust II	7.70 (6.94-8.56)	<0.001
PSM	3.07 (2.55-3.71)	<0.001

PSM, propensity score matching; NEPC, neuroendocrine prostate cancer. HR, hazard ratios.

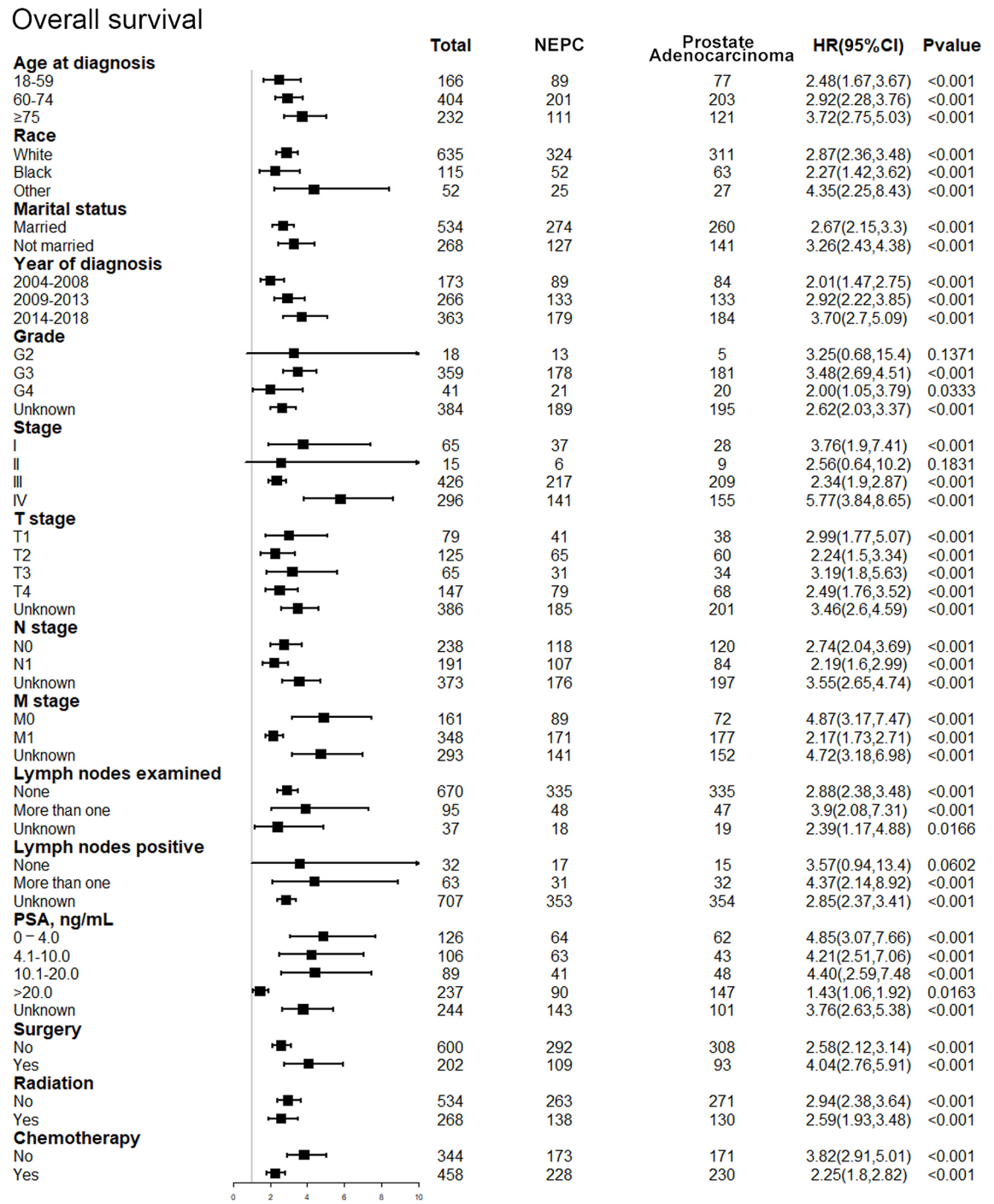

FIGURE 3 | Forest plot of the subgroup analysis for NEPC and prostate adenocarcinoma in OS. NEPC, neuroendocrine prostate cancer; OS, overall survival.

microenvironment changes including endogenous IL-6 expression (44), MMP-9 production and other pro-inflammation cytokines upregulation fulfil complicated and comprehensive function in the process of adenocarcinoma transdifferentiating into NEC (45). Correspondingly, AURKA inhibitor PHA-739358 (danusertib) was confirmed to be effective on the growth of NE tumor cells and mouse xenograft models (46). This kinase inhibitor is being evaluated in phase II clinical trials and is expected to be applied for individual therapy prospectively in the clinic (46). Besides, other promising therapeutic targets for NEPC are also currently

Cancer specific survival

	Total	NEPC	Prostate Adenocarcinoma	HR(95%CI)	Pvalue
Age at diagnosis					
18-59	166	89	77	2.44(1.62,3.67)	<0.001
60-74	404	201	203	3.1(2.37,4.04)	<0.001
≥75	232	111	121	4.54(3.22,6.4)	<0.001
Race					
White	635	324	311	3.16(2.56,3.9)	<0.001
Black	115	52	63	2.2(1.36,3.58)	0.0013
Other	52	25	27	6.88(3.08,15.3)	<0.001
Marital status					
Married	534	274	260	2.99(2.37,3.76)	<0.001
Not married	268	127	141	3.49(2.53,4.82)	<0.001
Year of diagnosis					
2004-2008	173	89	84	2.21(1.57,3.11)	<0.001
2009-2013	266	133	133	3.34(2.48,4.5)	<0.001
2014-2018	363	179	184	3.91(2.78,5.5)	<0.001
Grade					
G2	18	13	5		0.9891
G3	359	178	181	3.76(2.85,4.97)	<0.001
G4	41	21	20	2.04(1.04,3.97)	0.0359
Unknown	384	189	195	2.92(2.22,3.86)	<0.001
Stage					
I	65	37	28	13.6(4.13,45.3)	<0.001
II	15	6	9	5.08(0.93,27.7)	0.0605
III	426	217	209	2.41(1.94,3)	<0.001
IV	296	141	155	6.85(4.35,10.7)	<0.001
T stage					
T1	79	41	38	3.44(1.94,6.08)	<0.001
T2	125	65	60	2.62(1.68,4.09)	<0.001
T3	65	31	34	3.42(1.87,6.26)	<0.001
T4	147	79	68	2.52(1.75,3.62)	<0.001
Unknown	386	185	201	3.91(2.86,5.33)	<0.001
N stage					
N0	238	118	120	3.11(2.26,4.29)	<0.001
N1	191	107	84	2.28(1.64,3.17)	<0.001
Unknown	373	176	197	4(2.91,5.49)	<0.001
M stage					
M0	161	89	72	7.45(4.35,12.7)	<0.001
M1	348	171	177	2.27(1.79,2.86)	<0.001
Unknown	293	141	152	5.67(3.65,8.8)	<0.001
Lymph nodes examined					
None	670	335	335	3.18(2.59,3.89)	<0.001
More than one	95	48	47	4.09(2.08,8.01)	<0.001
Unknown	37	18	19	2.74(1.24,6.05)	0.0122
Lymph nodes positive					
None	32	17	15	5.19(1.1,24.5)	0.0371
More than one	63	31	32	4.23(1.99,8.97)	<0.001
Unknown	707	353	354	3.15(2.59,3.83)	<0.001
PSA, ng/mL					
0 − 4.0	126	64	62	4.96(3.06,8.03)	<0.001
4.1-10.0	106	63	43	5.25(2.90,9.51)	<0.001
10.1-20.0	89	41	48	5.06(2.87,8.91)	<0.001
>20.0	237	90	147	1.42(1.03,1.95)	0.0293
Unknown	244	143	101	4.95(3.28,7.46	<0.001
Surgery					
No	600	292	308	2.85(2.31,3.51)	<0.001
Yes	202	109	93	4.7(3.04,7.27)	<0.001
Radiation					
No	534	263	271	3.41(2.7,4.31)	<0.001
Yes	268	138	130	2.63(1.92,3.59)	<0.001
Chemotherapy					
No	344	173	171	4.85(3.53,6.65)	<0.001
Yes	458	228	230	2.36(1.87,2.98)	<0.001

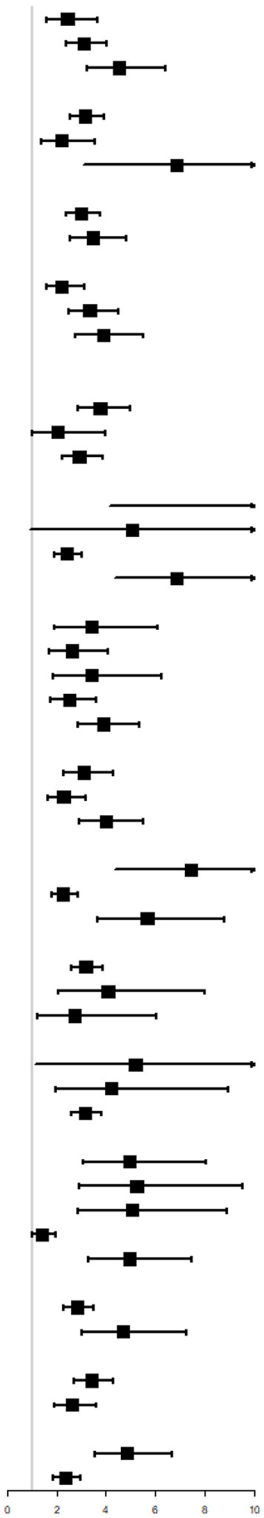

FIGURE 4 | Forest plot of the subgroup analysis for NEPC and prostate adenocarcinoma in CSS. NEPC, neuroendocrine prostate cancer; CSS, cancer-specific survival.

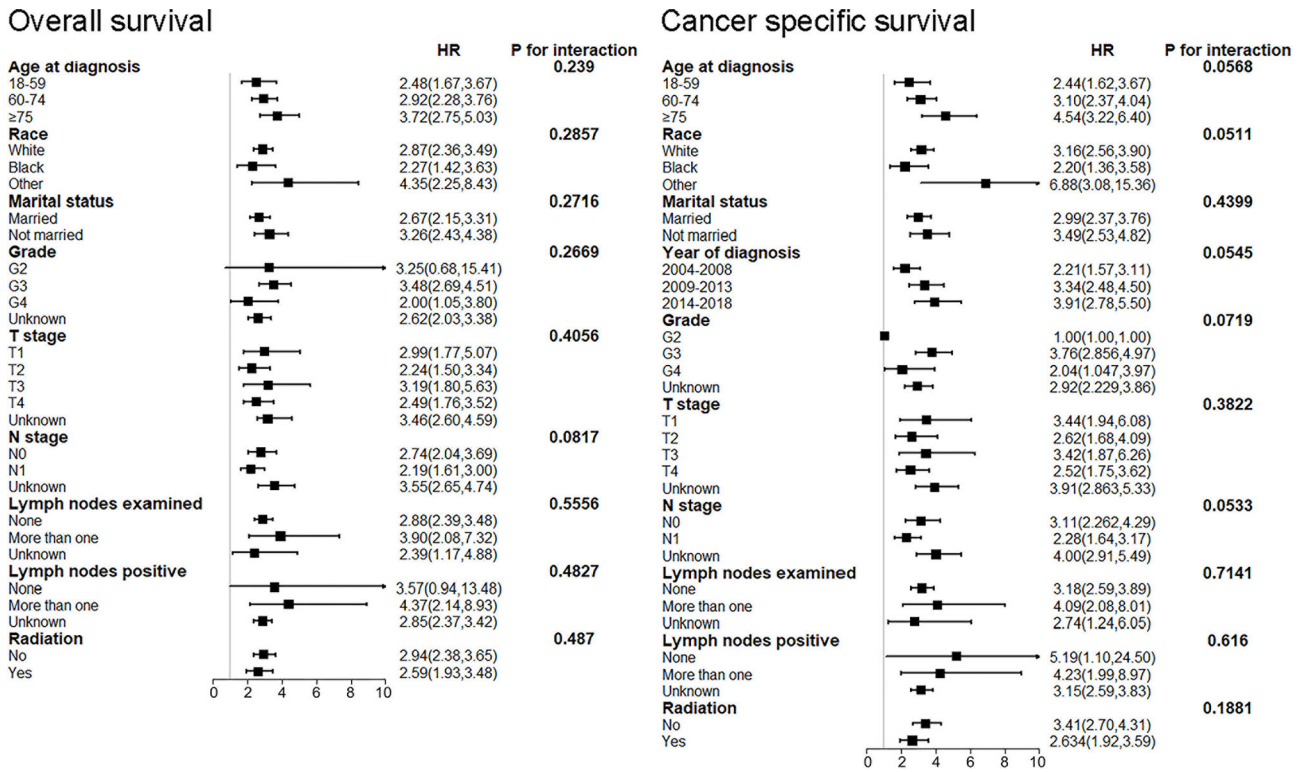

FIGURE 5 | Subgroup analysis for interaction between NEPC and potential covariates in both OS and CSS. NEPC, neuroendocrine prostate cancer; OS, overall survival; CSS, cancer-specific survival.

undergoing investigation in clinical trials, such as rocalpituzumab tesirine (DLL3 inhibitor) (47), GSK126 (EZH2 inhibitor) (48), and avelumab (immune-checkpoint PDL1 inhibitor) (49). Therefore, the remarkable progress in the molecular mechanism of NEPC established the foundation for the new effective treatment.

Peptide receptor radionuclide therapy (PRRT) is considered a curative and safe treatment option for NEPC (50). NEC cells have a higher expression of somatostatin receptors (SSTRs) than normal cells, which renders SSTR2 a potential target for NEPC treatment. The radiolabelled (Lutetium-177 or Yttrium-90) somatostatin analogues (SSAs) can target SSTR subtypes on the tumor cell surface and cause DNA damage in the cell nucleus which subsequently leads to cell death (51). Currently, 177Lu-DOTATATE or 177Lu-oxodotreotide is registered for the treatment of progressive and advanced grade 1–2 NEPC (50). On the other hand, 177Lu-PSMA-617 targets prostate-specific membrane antigen (PSMA), a cell-surface protein enriched in prostate cancer, which is used to treat metastatic prostate cancer (52). Besides, Radium-223 (223Ra) is another radiopharmaceutical treatment for patients with metastatic castration resistant prostate cancer patients (mCRPC) with symptomatic bone metastases and no known visceral metastatic disease (53). However, no research has showed that 223Ra could be performed in the treatment of NEPC.

De novo NEPC is a rare clinical entity, accounting for approximately 1% of all prostate cancers. Correspondingly, t-NEPC occurs in 10–17% of patients with CRPC by developing resistance to ADT and/or APRI treatment (54). The managements for the two types of NEPC are not identical and the difference in the details should attract enough attention (55). For locally advanced *de novo* NEPC, radiation therapy and radical resection are usually recommended. Given that majority of *de novo* NEPC patients present with distal metastatic disease at diagnosis, platinum-based chemotherapy should be adopted rather than ADT or APRI treatment (56). Previous researched suggested that t-NEPC occur in approximately 30% of metastatic CRPC, which suggests a strong possibility of distal metastasis at diagnosis. Thus, radiation therapy or radical resection is not recommended generally for t-NEPC. Considering prostate adenocarcinoma admixed with extensive neuroendocrine differentiation in t-NEPC, a trial of ADT in combination with cytotoxic chemotherapy is recommended. The chemotherapy regimens for *de novo* NEPC are usually platinum plus etoposide combinations. However, t-NEPC is frequently treated with docetaxel or a combination of carboplatin plus docetaxel rather than etoposide. Because docetaxel is an effective chemotherapeutic agent both for neuroendocrine and the adenocarcinoma components (56).

Due to the rarity of NEPC, our study conducted a retrospective study enrolling 482 patients with NEPC from the SEER. Thus, based on a large population, we had sufficient cases to make more credible and valuable analyses. Moreover, we provided the latest and

comprehensive clinicopathological information of NEPC according to the recent released database. Nevertheless, our study had several limitations. Firstly, the detailed information such as chemotherapy regimens and operational styles were not available from the SEER, which was a severe obstacle for us to estimate the effect of treatment and assess the survival outcomes. Secondly, the retrospective nature of the study caused unavoidable selection biases, although PSM was performed. Thirdly, the ADT exposure history can't be provided by the SEER. This factor is a critical variable for investigating the issue about adenocarcinoma transdifferentiates into NEC.

CONCLUSION

The results of our study suggested that the prognosis of NEC was worse than that of adenocarcinoma among prostate cancer patients, even after adjustment for demographic and clinicopathological characteristics by PSM. Subgroup analysis further demonstrated that NEPC patients obtained significantly poorer prognosis than prostate adenocarcinoma patients across nearly all subgroups. Besides, the histological subtype of NEC was an independent prognostic factor for prostate cancer.

DATA AVAILABILITY STATEMENT

The dataset from SEER database generated and/or analyzed during the current study are available in the SEER dataset repository (https://seer.cancer.gov/).

ETHICS STATEMENT

The data from SEER are publicly available and de-identified.

AUTHOR CONTRIBUTIONS

JPY: designed this research, completed the data analysis statistics work, and wrote the manuscript. YL: conception and design, collection and assembly of data, and data analysis and interpretation. XL, JS, YZ, and JY: worked in data collection and analysis. MZ: guided all research work, reviewed the manuscript, and provided financial support. All authors contributed to the article and approved the submitted version.

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Tumor Macroscopic Morphology Is an Important Prognostic Factor in Predicting Chemotherapeutic Efficacy and Clinical Outcomes of Patients With Colorectal Neuroendocrine Neoplasms, One Multicenter Retrospective Cohort Study

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Background and Aims: Locally advanced and metastatic colorectal neuroendocrine neoplasm (NEN) is a rare disease with a dismal prognosis. We aimed to explore the value of the macroscopic morphology of NENs in the management of TNM stage II-IV colorectal NENs, which has not been fully elucidated in previous reports.

Methods: We retrospectively enrolled 125 eligible patients with TNM stage II-IV colorectal NENs who were diagnosed between 2000 and 2020 from three Chinese hospitals. All were categorized into either protruding or ulcerative NEN groups through endoscopic evaluation of their macroscopic morphology. Clinicopathological data were collected and compared between the two groups. Survival analysis was performed to assess the survival outcomes between the two groups.

Results: A total of 77 and 48 patients had protruding and ulcerative NENs, respectively. Patients with ulcerative NENs had a larger median tumor size (P<0.001) and higher median Ki-67 index (P<0.001), and a larger proportion of these patients had grade G3 disease (P=0.001) and poorly differentiated neoplasms (P=0.001), as well as higher frequencies of T3 and T4 tumors (P=0.006) than patients with protruding NENs. In addition, patients with ulcerative NENs showed a much lower response to first-line chemotherapy [50% (95% CI: 27.3% - 72.7%) versus 20% (95% CI: 3.1% - 36.9%), P=0.03] and a worse 3-year progression-free survival (PFS) rate [19.7% (95% CI: 7.2% - 32.2%) versus 49.5% (95% CI: 37.5% - 61.5%), P=0.001] and 3-year overall survival (OS) rate [30.7% (95% CI: 15.6% - 45.8%) versus 76.9% (95% CI: 66.5% - 87.3%), P<0.001] than those with protruding NENs. The multivariate analysis results indicated that the macroscopic shape of NENs was an independent prognostic factor affecting both PFS

(HR = 1.760, 95% CI: 1.024 – 3.026, P = 0.04) and OS (HR = 2.280, 95% CI: 1.123 – 4.628, P = 0.02).

Conclusions: Ulcerative NENs were more malignant and chemotherapy resistant than protruding NENs. Tumor macroscopic morphology is a valuable prognostic factor for stage II-IV colorectal NENs.

Keywords: colon, rectum, neuroendocrine tumors, morphology, endoscopy

INTRODUCTION

Colorectal neuroendocrine neoplasms (NENs) are derived from diffuse neuroendocrine cells throughout the colon and rectum (1). Although it is a rare disease, it has presented an increasing incidence in recent decades, owing to the popularization of colonoscopy screening (2–4). One study from the Netherlands indicated that the incidence of colorectal NENs doubled from 2006 to 2011, with incidence rates increasing from 0.36 per 100000 inhabitants to 0.75 per 100000 inhabitants (5).

Colorectal NENs are a group of heterogeneous diseases ranging from indolent tumors to highly aggressive carcinomas. In the World Health Organization (WHO) 2010 classification and nomenclature system for digestive NENs, colorectal NENs were classified into G1, G2 and G3 based on the mitotic count and/or Ki-67 index. G1 and G2 NENs were regarded as well-differentiated NENs, while G3 NENs were regarded as poorly differentiated neuroendocrine carcinomas (NECs) and included small cell carcinomas (SCCs) and large cell carcinomas (LCCs) (2). In the recent 2019 edition of the WHO classification system, well-differentiated G3 NENs are separated from NECs and termed G3 neuroendocrine tumors (NETs), which are less aggressive and present better clinical outcomes than NECs. G1 and G2 NENs and well-differentiated G3 NENs are collectively referred to as NETs (3, 6).

Most diagnosed colorectal NENs are small, indolent and localized lesions confined within the submucosal layer. One report based on the Surveillance, Epidemiology and End Results (SEER) database included 9602 cases with colorectal NENs, and localized NENs (Tis/T1N0M0) based on the European Neuroendocrine Tumor Society (ENETS) and Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) guidelines for TNM assessment of colorectal NENs accounted for 75.2% of all colorectal NENs (7). Therefore, most previous studies have focused on the management strategies for localized NENs and have indicated that endoscopic therapy is a reliable choice and could guarantee a favorable prognosis (8, 9). However, the optimal management scheme for locally advanced (T2-4N0M0 and T0-4N1M0) and metastatic (T0-4N0-1M1) NENs has not been well established due to its rarity and heterogeneity (10). Locally advanced and metastatic NENs refer to neoplasms invading into or through the muscularis propria or neoplasms with involvement of lymph nodes or distant metastasis, which are categorized as stage II-IV NENs based on the ENETS and UICC/AJCC guidelines (11). Although they constitute only a small proportion of diagnosed colorectal NENs, they present high malignancy and strong aggressiveness, which negatively affects the survival of patients.

Currently, the recognized prognostic factors include tumor size, grade, histological differentiation, depth of tumor invasion, status of regional lymph nodes and distant organ metastasis. The therapeutic scheme has been established based on comprehensive evaluation of these factors (2, 12). However, the tumor morphology has long been ignored in previous studies, even though it can be easily obtained through endoscopic examination. Although numerous prior reports have demonstrated the association between morphology and tumor characteristics for colorectal adenocarcinomas, there remain few studies on the value of morphology in the evaluation, treatment and surveillance of colorectal NENs (13, 14). NENs are typically divided into protruding and ulcerative lesions in patient medical records based on gross observation from colonoscopy examination. In this study, we aimed to determine whether the macroscopic morphological features of tumors had an impact on the clinical manifestations and outcomes of stage II-IV colorectal NENs.

MATERIALS AND METHODS

Study Design

Our study received approval from the Ethics Committee of the National Cancer Center and complied with the Declaration of Helsinki of the World Medical Association. We performed a multicenter retrospective cohort study, and included 92 patients from Cancer Hospital Chinese Academy of Medical Sciences, 25 from China-Japan Friendship Hospital, and 8 from Beijing Hospital. Patients were categorized into a protruding subgroup and an ulcerative subgroup based on the endoscopic evaluation of tumor shape. Our primary outcomes of interest included tumor grade, depth of invasion, involvement of regional lymph nodes, distant organ metastasis and chemotherapeutic efficacy of first-line treatment. Secondary outcomes included cancer progression and disease-specific mortality.

Tumor Morphology

Tumor shape was characterized based on endoscopic findings and was classified into protruding and ulcerative neoplasms. Lesions with obvious elevation over the surrounding normal mucosa were regarded as protruding tumors. Lesions with part of the mucosal surface that was lower than the surrounding normal mucosa were categorized into ulcerative tumors (**Figure 1**).

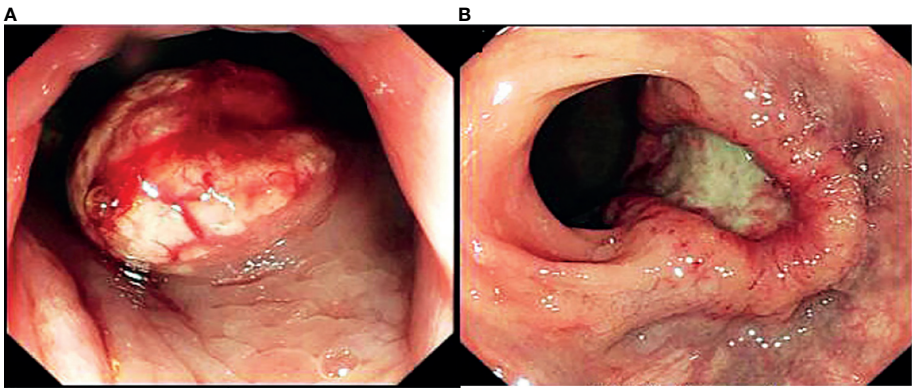

FIGURE 1 | Endoscopic findings of macroscopic morphology of stage II-IV NENs. **(A)** protruding NENs, **(B)** ulcerative NENs.

Patients and Data

We retrospectively collected data from patients who received treatment at our institutions between 2000 and 2020. The inclusion criteria were as follows: 1) tumors located in the colon or rectum; 2) tumors pathologically confirmed as NENs; and 3) tumors that invaded into or through the muscularis propria, involved regional lymph nodes or showed distant metastasis. The exclusion criteria were as follows: 1) tumors located in the appendix; 2) tumors confined within the submucosa; 3) accompanying malignancies of other origin; and 4) a lack of complete data. From 2000 to 2020, 315 cases of colorectal NENs were diagnosed and treated at the National Cancer Center, Cancer Hospital Chinese Academy of Medical Sciences, China-Japan Friendship Hospital and Beijing Hospital. After excluding 145 patients with neoplasms confined to the submucosa, 33 patients with indeterminate tumor morphology, 7 patients with malignancies of other origins and 5 patients with NENs located in the appendix, 125 qualified patients with complete clinicopathologic and survival data were enrolled in our study. The data needed in our report were collected from either the hospital database or *via* telephone call. The last follow-up visit was July 1, 2021. Overall survival (OS) was calculated between the date of initial treatment and cancer-specific death. Progression-free survival (PFS) was obtained between the date of initial therapy and cancer progression based on imaging evaluations.

Statistical Analysis

Continuous data that followed a normal distribution are presented as the mean ± standard deviations (SD) and were compared using a *t*-test. Continuous variables that did not follow the normal distribution are reported as median with interquartile range (IQR) and were analyzed using the Mann-Whitney *U* test. Categorical and ordinal factors are presented as frequency with percentage and were subsequently compared by $\chi 2$ test for categorical data and Mann-Whitney *U* test for ordinal data. Cumulative incidence of cancer specific mortality (CSM) was calculated by a competing risk model, death from other causes was recognized as a competitive event of cancer-specific death. Gray's test was used to determine the intergroup difference in the CSM. OS and PFS rates were determined using the Kaplan–Meier method. Univariable and multivariable logistic regression analyses were utilized to determine the relationship between macroscopic morphological patterns and prognosis. All data were calculated and analyzed using the Statistical Package for the Social Sciences (SPSS version 24.0; IBM Corp., Armonk, NY). Statistical significance was set at a two-sided P-value<0.05.

RESULTS

Patient Characteristics

Patient demographics and clinicopathological manifestations are summarized in **Table 1**. A total of 125 patients with a mean age of 56.1 ± 11.8 years old and mean body mass index (BMI) of 24.3 ± 3.0 kg/m^2 were enrolled in our study, consisting of 81 (64.8%) male and 44 (35.2%) female patients. Most patients (75.2%) had NENs in the rectum, followed by the cecum and ascending colon (9.6%), sigmoid colon (8.8%), descending colon (4.0%) and transverse colon (2.4%). All NENs had a median size of 3.0 (IQR 2.0–5.0) cm, with 16.8%, 22.4% and 60.8% having G1, G2 and G3 grades, respectively. Sixty-four patients (51.2%) were pathologically confirmed to have poorly differentiated disease and were categorized into the NEC group, and the remaining 61 patients (48.8%) were found to have well-differentiated disease and categorized into the NET group. In terms of the immunohistochemical markers, the expression of synaptophysin, chromogranin and CD 56 was detected in 94.4%, 67.8% and 87.5% of evaluable patients, respectively. The median Ki-67 index in the whole cohort was 40.0% (IQR 5.0%-70.0%). Most patients with NENs had tumors invading through the muscularis propria (68.8%) and involving regional lymph nodes (84.0%). Forty-eight (38.4%) patients had distant metastasis at the initial date of diagnosis. Based on the ENETS and UICC/AJCC TNM assessment of colorectal NENs, 15 (12.0%), 62 (49.6%) and 48 (38.4%) patients were classified as having stage II, III and IV disease, respectively. Extramural vascular invasion (EMVI) and perineural invasion (PNI) were found in 54.3% and 43.6% of the evaluable patients, respectively. With regard to the treatment regimens, surgery, chemotherapy

TABLE 1 | Demographic and clinicopathological manifestations.

Variables	All (n = 125)	Protruding (n = 77)	Ulcerative (n = 48)	P-value
Gender, n (%)				0.97
Male	81 (64.8%)	50 (64.9%)	31 (64.6%)	
Female	44 (35.2%)	27 (35.1%)	17 (35.4%)	
Age (yr, mean ± SD)	56.1 ± 11.8	54.9 ± 11.0	58.0 ± 12.7	0.15
BMI (kg/m^2, mean ± SD)	24.3 ± 3.0	24.6 ± 3.4	23.9 ± 3.0	0.29
Family history of cancer, n (%)	28 (22.4%)	19 (25.0%)	9 (18.8%)	0.45
Smoking	44 (35.2%)	24 (31.2%)	20 (41.7%)	0.22
Alcohol consumption	43 (34.4%)	27 (35.1%)	16 (33.3%)	0.93
Location, n (%)				0.67
Rectum	94 (75.2%)	61 (79.2%)	33 (68.8%)	
Sigmoid colon	11 (8.8%)	6 (7.8%)	5 (10.4%)	
Descending colon	5 (4.0%)	2 (2.6%)	3 (6.3%)	
Transverse colon	3 (2.4%)	2 (2.6%)	1 (2.1%)	
Cecum and ascending colon	12 (9.6%)	6 (7.8%)	6 (12.5%)	
Size (cm), median (IQR)	3.0 (2.0, 5.0)	2.5 (1.7, 4.0)	5.0 (3.0, 7.0)	<0.001
Grade				0.001
G1	21 (16.8%)	18 (23.4%)	3 (6.25%)	
G2	28 (22.4%)	22 (28.6%)	6 (12.5%)	
G3	76 (60.8%)	37 (48.1%)	39 (81.3%)	
Differentiation				0.001
NET	61 (48.8%)	47 (61.0%)	14 (29.2%)	
NEC	64 (51.2%)	30 (39.0%)	34 (70.8%)	
Synaptophysin, n (%)	118 (94.4%)	72 (93.5%)	46 (95.8%)	>0.99
Chromogranin, n (%)	78 (67.8%)	46 (59.7%)	32 (66.7%)	0.53
CD56, n (%)				0.27
Positive	91 (72.8%)	63 (90.0%)	28 (82.4%)	
Negative	13 (10.4%)	7 (10.0%)	6 (17.6%)	
Unknown	21 (16.8%)	7 (9.1%)	14 (29.2%)	
Ki-67 (%), median (IQR)	40.0% (5.0%, 70.0%)	10.0% (3.0%, 60.0%)	60.0% (27.5%, 80.0%)	<0.001
T stage, n (%)				0.006
T1, T2	39 (31.2%)	31 (40.3%)	8 (16.7%)	
T3, T4	86 (68.8%)	46 (59.7%)	40 (83.3%)	
N stage, n (%)				0.73
N0	20 (16.0%)	13 (16.9%)	7 (14.6%)	
N1	105 (84.0%)	64 (83.1%)	41 (85.4%)	
M stage, n (%)				0.18
M0	77 (61.6%)	51 (66.2%)	26 (54.2%)	
M1	48 (38.4%)	26 (33.8%)	22 (45.8%)	
TNM stage, n (%)				0.40
II	15 (12.0%)	10 (13.0%)	5 (10.4%)	
III	62 (49.6%)	41 (53.2%)	21 (43.8%)	
IV	48 (38.4%)	26 (33.8%)	22 (45.8%)	
EMVI, n (%)				0.11
Positive	51 (40.8%)	30 (48.4%)	21 (65.6%)	
Negative	43 (34.4%)	32 (51.6%)	11 (34.4%)	
Unknown	31 (24.8%)	15 (19.5%)	16 (33.3%)	
PNI, n (%)				0.67
Positive	41 (32.8%)	28 (45.2%)	13 (40.6%)	
Negative	53 (42.4%)	34 (54.8%)	19 (59.4%)	
Unknown	31 (24.8%)	15 (19.5%)	16 (33.3%)	
Surgery	101 (80.8%)	66 (85.7%)	35 (72.9%)	0.08
Chemotherapy	94 (75.2%)	56 (72.7%)	38 (79.2%)	0.42
Radiotherapy	21 (16.8%)	10 (13.0%)	11 (22.9%)	0.15

Patients with unknown information were not included in the χ^2 -test.

SD, standard deviation; BMI, body mass index; IQR, interquartile range; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; EMVI, extramural vascular invasion; PNI, perineural invasion.

and radiotherapy were performed in 80.8%, 75.2% and 16.8% of the patients, respectively.

In the whole cohort, 77 (61.6%) and 48 (38.4%) patients were characterized as having protruding and ulcerative lesions, respectively. Statistical analysis showed that there were no significant discrepancies between the groups in terms of the distribution of sex (P= 0.97), age (P=0.15), BMI (P=0.29), family history of cancer (P=0.45), smoking (P=0.22), alcohol consumption (P=0.93), location (P=0.67), positive rates of synaptophysin (P>0.99), chromogranin (P=0.53) and CD 56 (P=0.27), regional lymph node status (P=0.73), distant metastasis (P=0.18), TNM stage (P=0.40), EMVI (P=0.11), PNI

(P=0.67), and intervention by surgery (P=0.08), chemotherapy (P=0.42), or radiotherapy (P=0.15). The Mann-Whitney *U* test demonstrated that the patients with ulcerative NENs presented a larger median size (5.0 cm in the ulcerative group versus 2.5 cm in the protruding group, P<0.001) and higher median Ki-67 index (60.0% in the ulcerative group versus 10.0% in the protruding group, P<0.001) than patients with protruding NENs. With regard to the grade and differentiation of NENs, a higher proportion of patients with ulcerative NENs had grade G3 disease (81.3% in the ulcerative group versus 48.1% in the protruding group, P=0.001) and poorly differentiated NEC neoplasms (70.8% in the ulcerative group versus 30.9% in the protruding group, P=0.001). In terms of the depth of cancer invasion, the patients with ulcerative NENs were more prone to experiencing invasion through the muscularis propria; 40 (83.3%) and 46 (59.7%) in the ulcerative group and protruding group presented T3 and T4 stage tumors, respectively (P=0.006).

The Predictive Value of Morphology for NEN Patients Receiving First-Line Chemotherapy

Detailed information regarding the first-line chemotherapy schedule and treatment efficacy was available for 47 patients, including 35 patients who had distant metastasis at the initial diagnosis and 12 patients who had local NENs but experienced progression after radical surgical treatment. Of the 47 patients, 16 responded to first-line chemotherapy, with an overall response rate of 34%. Eleven of the 22 patients with protruding NENs and 5 of the 25 patients with ulcerative NENs responded to first-line chemotherapy, with response rates of 50.0% and 25.0%, respectively (**Table 2**). Patients with ulcerative NENs were significantly less sensitive to chemotherapy (P=0.03).

Oncological Outcomes

A median follow-up period of 26 months (range 1–183 months) was reached in our research. Eight patients were lost to follow-up due to loss of communication or unexpected death from other accidents, resulting in a follow-up completion rate of 93.6%. Kaplan-Meier survival curves were used to determine the PFS and OS rates of the whole cohort and for subgroup analyses by NEN morphology (**Figure 2**).

In the whole cohort, the 3-year PFS and OS rates were 38.4% (95% CI: 29.2% - 47.6%) and 57.2% (95% CI: 47.2% - 67.2%), respectively, with the median PFS and OS being 12 and 62 months, respectively. We subsequently evaluated the difference in survival outcomes between protruding and ulcerative NENs. In the protruding group, the 3-year PFS rate was 49.5% (95% CI: 37.5% - 61.5%), and the median PFS was 30 months. In the ulcerative group, the 3-year PFS rate was 19.7% (95% CI: 7.2% - 32.2%), and the median PFS was only 4 months. In terms of OS, the 3-year OS rates were 76.9% (95% CI: 66.5% - 87.3%) and 30.7% (95% CI: 15.6% - 45.8%) for the protruding and ulcerative groups, respectively. The median OS in the ulcerative group was 25 months, while the median OS in the protruding group could not be calculated, as more than half of the patients were still alive by the end of our follow-up. Patients with ulcerative NENs had significantly worse PFS (log-rank P=0.001) and OS (log-rank P<0.001) rates than those with protruding NENs.

Stratification Analysis Based on the Presence or Absence of Distant Metastasis

Tumor features stratified by TNM stage and morphology are shown in **Table 3**. Given the limited sample size of our study, patients with TNM stage II and III disease were analyzed together as regional disease. For individuals with regional NENs, we still found that patients with ulcerative lesions were prone to neoplasms of a larger size (P=0.002), higher grade (P=0.003), and poorer histological differentiation (P=0.008) with a higher Ki-67 index (P=0.02) and deeper layers of intestinal wall

TABLE 2 | Data regarding NENs received first-line chemotherapy.

	Chemotherapy regimens	Patients received first-line chemotherapy	Patients responded to first-line chemotherapy	In total	P-value
Protruding (n=22)	cisplatin/carboplatin+etoposide	10	7	11 (50.0%)	0.03*
	oxaliplatin+capecitabine/5-Fu	5	1		
	temozolomide + capecitabine	1	0		
	temozolomide + S-1	3	2		
	irinotecan + S-1	1	1		
	etoposide + thalidomide	1	0		
	AK105 + anlotinib	1	0		
Ulcerative (n=25)	cisplatin/carboplatin/oxaliplatin+etoposide	11	3	5 (20.0%)	
	oxaliplatin+capecitabine/5-Fu	7	1		
	temozolomide + capecitabine	3	0		
	irinotecan + capecitabine	1	1		
	cisplatin + irinotecan	1	0		
	oxaliplatin +fruquintinib	1	0		
	AK105 + anlotinib	1	0		

**The P-value refers to the comparison between response rates in protruding and ulcerative groups.*

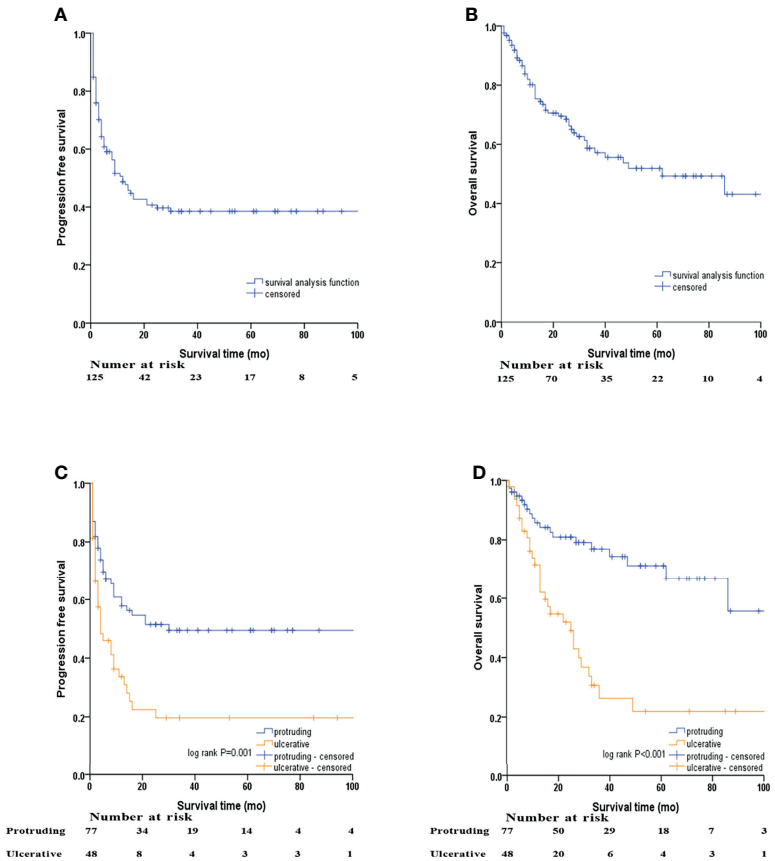

FIGURE 2 | Kaplan–Meier survival analyses by macroscopic morphology of NENs. **(A)** PFS of the whole cohort, **(B)** OS of the whole cohort, **(C)** PFS of protruding and ulcerative NENs in the whole cohort, **(D)** OS of the protruding and ulcerative NENs in the whole cohort.

TABLE 3 | Stratification analysis by presence or absence of distant metastasis.

Variables	Regional NENs			Metastatic NENs		
	Protruding (n = 51)	Ulcerative (n = 26)	P-value	Protruding (n = 26)	Ulcerative (n = 22)	P-value
Size (cm), median (IQR)	2.1 (1.5, 3.6)	4.0 (2.0, 7.5)	0.002	3.0 (2.1, 4.0)	5.0 (4.0, 6.0)	0.001
Grade, n (%)			0.003			0.04
G1 and G2	28 (54.9%)	5 (19.2%)		12 (46.2%)	4 (18.2%)	
G3	23 (45.1%)	21 (80.8%)		14 (53.8%)	18 (81.8%)	
Differentiation, n (%)			0.008			0.03
NET	32 (62.7%)	8 (30.8%)		15 (57.7%)	6 (27.3%)	
NEC	19 (37.3%)	18 (69.2%)		11 (42.3%)	16 (72.7%)	
Ki-67 (%), median (IQR)	5.0% (2.0%, 50.0%)	65.0% (20.0%, 80.0%)	0.02	25.0% (5.0%, 65.0%)	60.0% (29.0%, 80.0%)	0.13
T stage, n (%)			0.03			0.45
T1, T2	27 (52.9%)	7 (26.9%)		4 (15.4%)	1 (4.5%)	
T3, T4	24 (47.1%)	19 (73.1%)		22 (84.6%)	21 (95.5%)	

IQR, interquartile range; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; NEN, neuroendocrine neoplasm.

invasion (P=0.03) than patients with protruding lesions. In terms of patients with metastatic disease, only size (P=0.001), tumor grade (P=0.04) and histological differentiation (P=0.03) demonstrated a significant difference between patients with protruding and ulcerative NENs. No obvious discrepancies in the Ki-67 index (P=0.13) or T stage (P=0.45) were observed between the two groups.

Kaplan–Meier survival curves based on stratification analysis of TNM stages and tumor morphology were also performed (**Figure 3**). For patients with regional NENs, ulcerative NENs

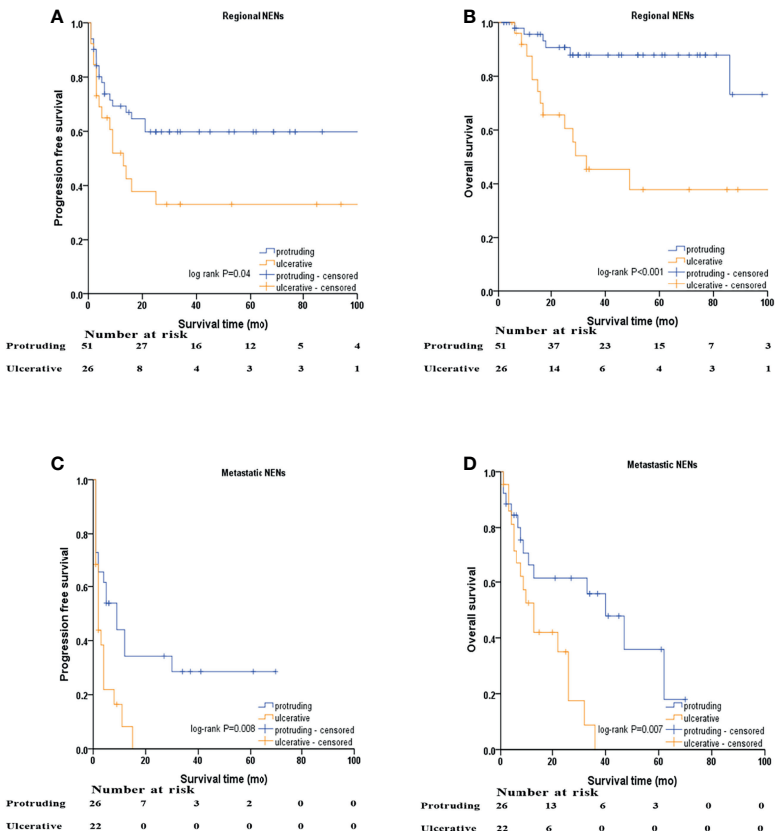

FIGURE 3 | Kaplan–Meier survival analyses after stratified by M stage and macroscopic morphology of NENs. **(A)** PFS of protruding and ulcerative NENs in the patients with regional NENs, **(B)** OS of protruding and ulcerative NENs in the patients with regional NENs, **(C)** PFS of protruding and ulcerative NENs in the patients with metastatic NENs, **(D)** OS of protruding and ulcerative NENs in the patients with metastatic NENs.

were significantly associated with decreased 3-year PFS [33.1% (95% CI: 13.7% - 52.5%) in ulcerative NENs versus 59.8% (95% CI: 45.7% - 73.9%) in protruding cases, log-rank P=0.04] and OS [45.4% (95% CI: 24.2% - 66.6%) in ulcerative NENs versus 87.9% (95% CI: 77.9% - 97.9%) in protruding cases, log-rank P<0.001].

For patients with metastatic NENs, ulcerative NENs were also associated with a worse 3-year PFS [0 in ulcerative NENs versus 28.6% (95% CI: 9.4% - 47.8%) in protruding cases, log-rank P=0.008] and OS [0 in ulcerative NENs versus 55.8% (95% CI: 34.8% - 76.8%) in protruding cases, log-rank P=0.007].

TABLE 4 | Stratification analysis by tumor size.

Variables	Size ≤ 2cm			Size > 2cm		
	Protruding (n = 31)	Ulcerative (n = 10)	P-value	Protruding (n = 46)	Ulcerative (n = 38)	P-value
Grade, n (%)			0.007			0.06
G1 and G2	23 (74.2%)	2 (20.0%)		17 (37.0%)	7 (18.4%)	
G3	8 (25.8%)	8 (80.0%)		29 (63.0%)	31 (81.6%)	
Differentiation, n (%)			0.001			0.19
NET	26 (83.9%)	2 (20.0%)		21 (45.7%)	12 (31.6%)	
NEC	5 (16.1%)	8 (80.0%)		25 (54.3%)	26 (68.4%)	
Ki-67 [median (IQR)]	4.0% (2.0%, 10.0%)	70.0% (35.0%, 80.0%)	0.001	40.0% (5.0%, 62.5%)	60.0% (23.8%, 80.0%)	0.09
T stage, n (%)			0.02			0.79
T1, T2	24 (52.9%)	3 (30.0%)		7 (15.2%)	5 (13.2%)	
T3, T4	7 (47.1%)	7 (70.0%)		39 (84.8%)	33 (86.8%)	

NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma.

Stratification Analysis Based on Tumor Size

Tumor features stratified by tumor size and morphology are shown in **Table 4**. For NENs ≤ 2.0 cm, ulcerative lesions were characterized with higher grade (P=0.007), and poorer histological differentiation (P=0.001) with a higher Ki-67 index (P=0.001) and deeper layers of intestinal wall invasion (P=0.02) than protruding lesions. Regarding NENs > 2.0 cm, ulcerative group showed a higher proportion of NENs with G3 grade, poor histological differentiation and higher Ki-67 index than protruding group. However, this tendency did not reach statistical significance, which might be due to the limited sample size of our study.

Kaplan–Meier survival curves based on stratification analysis of tumor size and morphology are presented (**Figure 4**). For patients with NENs ≤ 2.0 cm, ulcerative NENs were significantly associated with decreased 3-year PFS [10.0% (95% CI: 0 - 28.6%) in ulcerative NENs versus 68.1% (95% CI: 50.7% - 85.5%) in protruding cases, log-rank P<0.001] and OS [28.1% (95% CI: 0 – 60.0%) in ulcerative NENs versus 90.9% (95% CI: 78.7% - 100%) in protruding cases, log-rank P<0.001]. For patients with NENs > 2.0 cm, the 3-year PFS rates were 22.2% (95% CI: 7.3% - 37.1%) and 37.2% (95% CI: 22.3% - 52.1%) in the ulcerative and protruding NENs, respectively, which was not statistically different (log-rank P=0.21). However, we still observed significant decreased 3-year OS rate in ulcerative patients compared to protruding patients [26.2% (95% CI: 9.1% - 43.3%) in ulcerative NENs versus 67.2% (95% CI: 52.3% - 82.1%) in protruding cases, log-rank P=0.02].

Cumulative Incidence of Death and Competing Risk Analysis

A total of 51 (40.8%) patients died by the end of our follow-up, of which 49 (96.1%) died from colorectal NENs and 2 (3.9%) died from other diseases. The 3-year cumulative incidence of NENs-specific death were 60.4% and 19.5% in ulcerative group and protruding group, respectively (**Figure 5**). Patients with ulcerative NENs had significantly increased CSM (P<0.001).

Univariate and Multivariate Cox Regression Analyses

Univariable Cox proportional hazards regression analysis was performed to identify variables showing a significant association with DFS and OS, and the identified factors were enrolled in

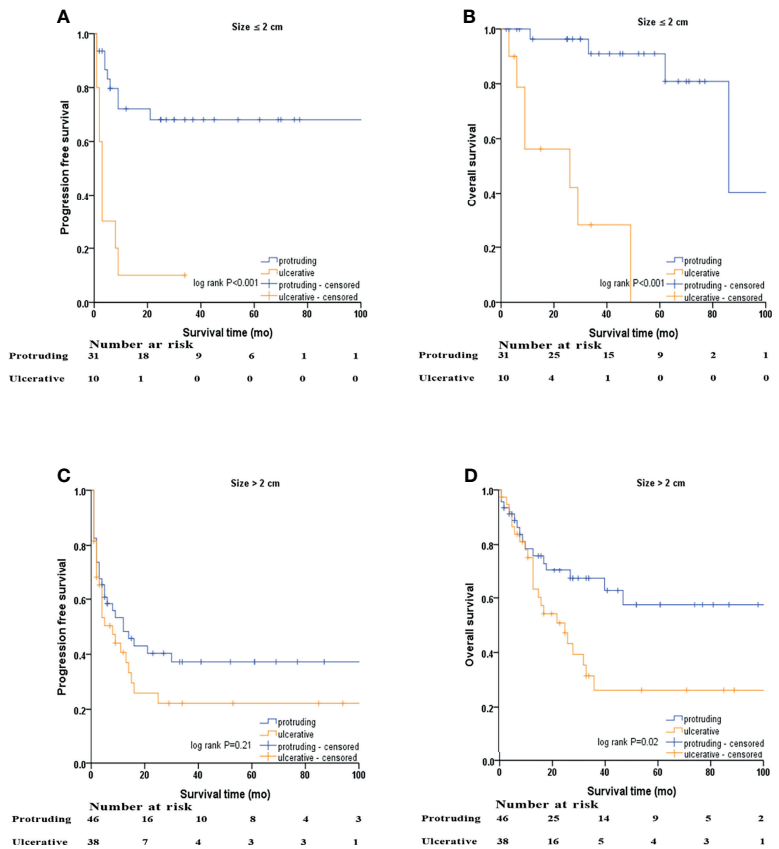

FIGURE 4 | Kaplan–Meier survival analyses after stratified by tumor size and macroscopic morphology of NENs. **(A)** PFS of protruding and ulcerative NENs in the patients with NENs below 2 cm, **(B)** OS of protruding and ulcerative NENs in the patients with NENs below 2 cm, **(C)** PFS of protruding and ulcerative NENs in the patients with NENs above 2 cm, **(D)** OS of protruding and ulcerative NENs in the patients with NENs above 2 cm.

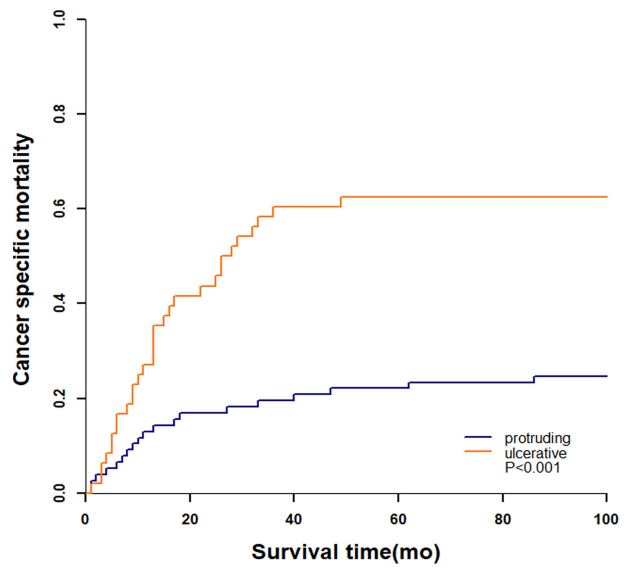

FIGURE 5 | Cancer specific mortality of patients from competing risk model.

subsequent multivariable Cox proportional hazards regression analysis to evaluate the value of tumor morphology in predicting prognosis (**Tables 5**, **6**). After controlling for confounding factors, an ulcerative growth pattern (HR=1.760; 95% CI=1.024–3.026; P=0.04) and M1 stage (HR=2.006; 95% CI=1.067–3.774; P=0.03) were confirmed to be an independent risk factor associated with cancer progression. After controlling for confounding factors, ulcerative NENs (HR=2.280; 95% CI=1.123–4.628; P=0.02), age > 60 (HR=2.055; 95% CI=1.025–4.120; P=0.04), poor histological differentiation (HR=4.713; 95% CI=1.345–16.516; P=0.02) and M1 stage (HR=3.651; 95% CI=1.601–8.327; P=0.002) were significantly associated with increased mortality.

Subgroup Analyses

Multivariate Cox proportional hazard regression analyses for PFS and OS were performed for subgroups based on sex, age, location, size, grade, differentiation, T stage, N stage, M stage, surgery, chemotherapy, or radiotherapy. Ulcerative NENs were associated with worse PFS in subgroups of patients with rectal NENs, patients with G1 and G2 NENs, patients with T3 and T4 stage NENs, patients with N1 stage NENs, and patients who

TABLE 5 | Univariable and multivariable Cox proportional hazards regression analysis for PFS.

Variables	Univariable			Multivariable		
	HR	95% CI	P-value	HR	95% CI	P-value
Morphology (ulcerative vs protruding)	2.121	1.325-3.393	0.002	1.760	1.024-3.026	0.04
Size (>2 cm vs ≤ 2cm)	2.027	1.120-3.667	0.02	.880	0.385-2.012	0.76
Differentiation (NEC vs NET)	2.308	1.409-3.780	0.001	1.724	0.957-3.105	0.07
T stage (T3 T4 vs T1, T2)	2.599	1.422-4.749	0.002	1.421	0.577-3.498	0.45
M stage (M1 vs M0)	2.632	1.639-4.225	<0.001	2.006	1.067-3.774	0.03
Surgery (yes vs no)	0.398	0.233-0.680	0.001	0.693	0.348-1.381	0.30

PFS, progression free survival; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; HR, hazards ratio; CI, confidence interval.

TABLE 6 | Univariable and multivariable Cox proportional hazards regression analysis for OS.

Variables	Univariable			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (> 60 vs ≤ 60)	2.515	1.424-4.443	0.001	2.055	1.025-4.120	0.04
Morphology (ulcerative vs protruding)	3.428	1.915-6.137	<0.001	2.280	1.123-4.628	0.02
Location (colon vs rectum)	1.914	1.051-3.486	0.03	0.922	0.453-1.877	0.82
Size (> 2cm vs ≤2cm)	2.673	1.242-5.755	0.01	1.041	0.322-3.368	0.95
Grade (G3 vs G1, G2)	3.576	1.674-7.639	0.001	0.904	0.222-3.686	0.89
Differentiation (NEC vs NET)	4.401	2.240-8.645	<0.001	4.713	1.345-16.516	0.02
T stage (T3, T4 vs T1, T2)	3.146	1.412-7.008	0.005	0.658	0.184-2.347	0.52
N stage (N1 vs N0)	3.634	1.125-11.742	0.03	2.458	0.699-8.644	0.16
M stage (M1 vs M0)	4.313	2.382-7.808	<0.001	3.651	1.601-8.327	0.002
Surgery (yes vs no)	0.313	0.166-0.591	<0.001	0.582	0.240-1.414	0.23

OS ,overall survival; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; HR, hazards ratio; CI, confidence interval.

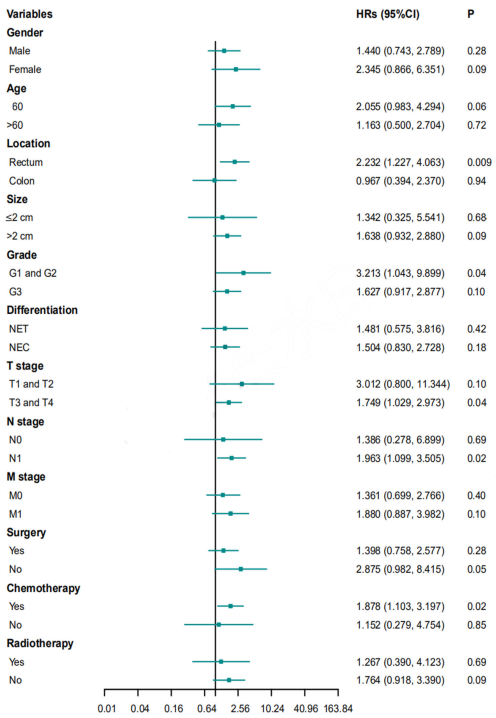

FIGURE 6 | HRs with 95% CIs for PFS comparing protruding NENs and ulcerative NENs in different subgroups. HRs, hazard ratios; CI, confidence interval; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; PFS, progression free survival.

received chemotherapy (**Figure 6**). In terms of OS, patients with ulcerative NENs had poorer OS than those with protruding NENs in subgroups with a younger age, rectal NENs, smaller lesions, G1 and G2 grade NENs, NETs, T1 and T2 stage, N1 stage, and M0 stage as well as patients who received chemotherapy and those who did not (**Figure 7**).

DISCUSSION

Colorectal NENs are highly heterogeneous tumors with significantly different clinicopathological features and clinical outcomes. Endoscopic examination is essential, as the endoscopic appearance of NENs can provide an indispensable reference for subsequent diagnosis and treatment regimens. In prior reports, NEN size measured by endoscopy has been widely acknowledged as an important prognostic factor, and a NEN size of 2 cm was set as the cutoff point for endoscopic or radical surgical treatment of Tis/T1N0M0 NENs (11, 15, 16). However, even for diminutive NENs below 10 mm, lymph node metastasis can be observed and lead to a dismal prognosis (17, 18). Therefore, size alone is not a reliable enough endoscopic indicator to determine the management strategy of colorectal NENs. Our reports explored the value of the growth patterns of NENs in predicting pathological manifestations, chemotherapy sensitivity to first-line schemes and prognosis.

Numerous studies have focused on the macroscopic morphology of colorectal adenocarcinomas. Some reports classified tumors into polypoid and nonpolypoid types based on the presence or absence of elevated lesions compared with adjacent mucosa and concluded that nonpolypoid tumors exhibit more malignant characteristics and a poorer prognosis than polypoid tumors (19, 20). Others divided colorectal cancers into expansive, infiltrative and ulcerative subtypes or depressed, laterally spreading, protruding and ulcerative subtypes (13, 14). Unlike epithelial tumors, colorectal NEN is a rare subepithelial disease, and the classification system from adenocarcinomas may not be suited for NENs.

Several previous reports have explored the predictive value of the endoscopic features of NENs, but most of them focused on early, localized lesions less than 2 cm in size (21, 22). Normally, endoscopic evaluation for NENs includes an analysis of shape, color, and surface changes (depression, erosion, hemorrhage, ulceration and hyperemia) (23). Some studies have divided NENs into lesions with typical endoscopic features and lesions with atypical endoscopic features (24, 25). NENs with typical endoscopic findings appear as yellowish, sessile, smooth and submucosal tumors. They present favorable clinicopathological manifestations and clinical outcomes (26, 27). NENs with an atypical endoscopic appearance are morphologically unusual, showing irregular surfaces with depressions, ulceration, erosion, hemorrhage and hyperemia or being pedunculated. They are associated with high frequencies of lymph node metastasis, distant metastasis and a poor prognosis (23, 25). Moreover, some reports have indicated that NENs with a central depression on the surface had a higher tendency for incomplete endoscopic excision (28). Subsequently, Xiang-Yao Wang et al. classified NENs into type I (protruded), II (flat and slightly elevated) and III (depression and ulcer on the surface) and demonstrated that patients with type II and III NENs had a higher risk of incomplete endoscopic resection (21). In 2020, Luohai Chen et al. proposed a novel scoring system based on the endoscopic assessment of the size, shape and mucosal surface of primary rectal tumors, and it showed great value in identifying patients with endoscopically advanced disease and for monitoring tumor recurrence (22). However, most of the patients included in these reports had small, early and localized disease, and most NENs were indolent, diminutive, of a low grade and well differentiated. In addition, most of these previous studies focused only on the value of the endoscopic appearance of NENs in endoscopic therapy, and few have explored their value in predicting clinicopathologic features, chemotherapy sensitivity and clinical outcomes. Although locally advanced and metastatic NENs constitute only a small portion of colorectal NENs, they are usually characterized by greater malignancy, increased aggressiveness and a worse prognosis than localized NENs (29). Locally advanced and metastatic NENs therefore deserve more clinical attention, and our study focused on the value of cancer morphology in the management of these colorectal NENs.

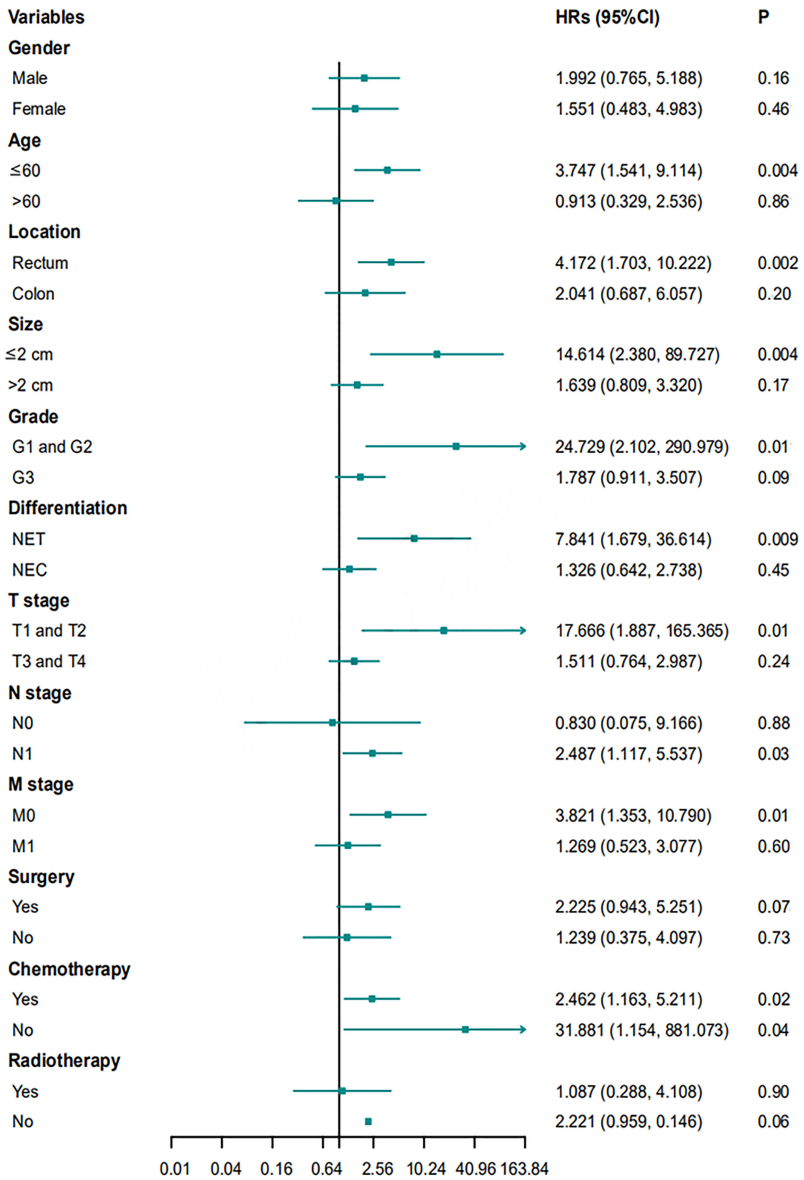

FIGURE 7 | HRs with 95% CIs for OS comparing protruding NENs and ulcerative NENs in different subgroups. HRs, hazard ratios; CI, confidence interval; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; OS, overall survival.

We categorized stage II-IV colorectal NENs into protruding and ulcerative subtypes based on endoscopic evaluation of the presence or absence of elevated lesions compared with adjacent mucosa. The ulcerative group was characterized by significantly more malignant features than the protruding groups, including larger tumor sizes, higher frequencies of G3 NENs and poorly differentiated NECs, an increased Ki-67 index, and a higher proportion of T3 and T4 NENs. In the stratification analysis based on the presence or absence of distant metastasis, all these increased malignant characteristics were further verified for ulcerative NENs with regional disease. Regarding patients with metastatic diseases, size, grade and differentiation followed a similar path on ulcerative NENs group. In the stratification analysis based on tumor size, the increased Ki-67 index, higher frequencies of G3 NENs, NECs and T3 and T4 NENs of ulcerative group compared to protruding group were only statistically confirmed in patients with NENs size ≤ 2cm. For patients with NENs size > 2cm, no significant difference was observed, which may be due to the small sample size of our study. Overall, it may suggest that ulcerative NENs might be more aggressive than protruding NENs. For NENs ≤ 2cm, tumor macroscopic morphology may serve as an important reference index, NENs with ulcerative shape might not be suitable for endoscopic resection.

Given the rarity of colorectal NENs, there are no widely acknowledged optimal systematic chemotherapy regimens for the treatment of metastatic or recurrent disease. Most physicians adopt chemotherapy recommendations for colorectal adenocarcinomas and pulmonary NENs (30). In summary, temozolomide regimens (temozolomide plus capecitabine) and platinum regimens (cisplatin or carboplatin plus etoposide) are the cornerstones of first-line chemotherapy strategies for colorectal NETs and NECs, respectively. Moreover, the response rates vary widely between 14% and 75% according to the literature reports, and few markers have been found to predict the efficacy of systematic chemotherapy (10, 11). A total of 47 patients had evaluable data for first-line chemotherapy in our study, including 22 patients with protruding NENs and 25 with ulcerative NENs. Patients with protruding NENs had a significantly higher response rate than those with ulcerative NENs [50% (95% CI: 27.3% - 72.7%) versus 20% (95% CI: 3.1% - 36.9%), P=0.03], which implied that macroscopic morphology might be a valuable tool in predicting the chemotherapeutic sensitivity of colorectal NENs.

The 3-year PFS rates were 38.4% (95% CI: 29.2% - 47.6%), 49.5% (95% CI: 37.5% - 61.5%), and 19.7% (95% CI: 7.2% - 32.2%), and the 3-year OS rates were 57.2% (95% CI: 45.6% - 65.6%), 76.9% (95% CI: 66.5% - 87.3%), and 30.7% (95% CI: 15.6% - 45.8%) for the entire cohort, protruding NENs and ulcerative NENs, respectively. Patients with ulcerative NENs had significantly worse PFS and OS rates than protruding NENs, which was further confirmed in further univariable and multivariable Cox proportional hazards regression analyses after controlling for confounding factors. The 3-year cumulative incidence of CSM were 60.4% and 19.5% in ulcerative group and protruding group, respectively. This difference between the two groups were statistically confirmed through competitive risk analysis model. This result indicated that tumor shape might be a strong candidate for predicting the clinical outcomes of colorectal NENs. Patients with ulcerative NENs have a higher risk of tumor progression and cancer-specific death and require more intensive treatment and surveillance strategies than those with protruding NENs.

To our knowledge, macroscopic morphology has long been ignored in the current management of stage II-IV colorectal NENs, and few studies have explored its significance in predicting chemotherapy sensitivity, tumor recurrence and progression and survival outcomes. Our report demonstrated that gross morphology should be taken into account as an important parameter in the diagnosis, treatment and surveillance of these colorectal NENs. However, our study had the following limitations. First, due to the retrospective nature of our report, we enrolled patients over a period of 20 years, and bias from patient selection and data collection could not be completely avoided. Second, the sample size of our study cohort was relatively small; we included only 125 cases so our conclusions need to be confirmed in multicenter studies with larger sample sizes.

In conclusion, endoscopic evaluation of the macroscopic morphology of NENs may have a role in the management of stage II-IV colorectal NENs. In our cohort, ulcerative NENs present more malignant and aggressive potential, poor response to first-line chemotherapy regimens and decreased rates of PFS and OS when compared to protruding NENs. Tumor shape should be evaluated as an independent factor in the management of advanced colorectal NENs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZW anlyszed the data and wrote this article. KA and RL collected the data. QL designed this study and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: An Unusual Case of Ectopic ACTH Syndrome Caused by Mediastinal Paraganglioma

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Ectopic adrenocorticotrophic hormone (ACTH) syndrome is not common, which is more unusual when caused by paraganglioma. We herein present a 40-year-old Chinese male who was diagnosed with ACTH-dependent Cushing's syndrome. However, the localization of the ACTH source was troublesome due to the inconsistent results of the high-dose dexamethasone suppression test and the desmopressin stimulation test. Bilateral inferior petrosal sinus sampling was performed, and ectopic ACTH syndrome was diagnosed. After ^{68}Ga-DOTATATE-PET/CT and ^{18}F-FDG-PET/CT were performed, it was localized in the anterior mediastinum. Post-operation histopathology demonstrated an ACTH-secreting mediastinal paraganglioma. The patient obtained complete clinical remission after a mediastinal tumorectomy.

Keywords: ectopic ACTH syndrome, Cushing's syndrome, paraganglioma, mediastinal, PET/CT

INTRODUCTION

Cushing's syndrome (CS) is a general term for diseases caused by excessive cortisol secretion in the adrenal cortex due to various reasons. The accompanying clinical presentations are all attributed to hypercortisolism. Ectopic adrenocorticotrophic hormone (ACTH) syndrome, a rare type of ACTH-dependent CS, is a disease referable to the abnormal secretion of ACTH from tumors outside of the pituitary, which stimulates bilateral adrenal gland hyperplasia and excessive secretion of cortisol. According to the literature, the most common cause of ectopic ACTH syndrome (EAS) is lung cancer (1). Paraganglioma-associated ACTH-secreting tumor was rarely reported in clinics to date (2).

Here we report a Chinese patient diagnosed with ACTH-dependent CS, which was ultimately attributed to an ACTH-secreting mediastinal paraganglioma.

CASE PRESENTATION

A 40-year-old male complaining of pitting edema in the whole body with limb weakness, polydipsia, and polyuria was admitted to our Endocrinology Unit. No one in his family suffers from a similar disease.

The physical examinations revealed vital signs of heart rate of 91 beats/min and blood pressure of 148/84 mmHg. He has abdominal obesity with a BMI of 29.5 kg/m^2 and a waist–hip ratio of 0.98. The patient had a classic Cushing's syndrome appearance, presented as central obesity, moon face, buffalo back, scattered bruises in the upper limbs, and pitting edema of the whole body.

The laboratory examination showed an increased level of 24-h urine-free cortisol (24-h UFC) and the disappearance of circadian rhythm in plasma total cortisol (PTC), accompanied by an elevated ACTH level, which confirmed the ACTH-dependent Cushing's syndrome diagnosis for this patient. These were also presented with severe hypokalemia, hyperglycemia, and alkalosis, which were relevant to CS. The high-dose dexamethasone suppression test (HDDST) showed a low suppressing rate of plasma ACTH and PTC. However, the 24-h UFC was suppressed by more than 50%. Desmopressin stimulation test was performed, and the results showed an intensively stimulated rate of ACTH level, with a peak rate 836% compared to baseline (**Table 1**). The thoracic computed tomography (CT) result suggested a nodule in the left anterior mediastinum, about 1.6 cm in diameter (**Figure 1A**). The enhanced magnetic resonance imaging (MRI) of the pituitary revealed a 3 × 2-mm nodule in the right side of the pituitary after enhancement, which was suspected as pituitary microadenoma (**Figure 1B**). All the above-mentioned tests made the identification of Cushing's disease (CD) and EAS troublesome. A subsequent bilateral inferior petrosal sinus sampling (BIPSS) combined with desmopressin (DDAVP) stimulation test was performed, and the results showed that there was neither a lateralization ACTH rate of the bilateral inferior petrosal sinus (IPS) nor a higher ACTH level in IPS relative to peripheral blood before and after the DDAVP stimulation.

In order to identify the localization of the ACTH-secreting tumor, we recommended that the patient finish the positron emission tomography CT (PET/CT) scan. After the ^{68}Ga-DOTATATE-PET/CT and ^{18}F-fluorodeoxyglucose (FDG)-PET/CT were finished, the results showed both ^{68}Ga (**Figures 1C, D**) and glucose (**Figure 1E**) uptake in the anterior mediastinal nodule, which suggested a neuroendocrine tumor. Therefore, EAS caused by the anterior mediastinal nodule was considered, and anterior mediastinal tumorectomy was performed. After the anterior mediastinal tumorectomy was performed, a 2 × 1.7 × 1-cm anterior mediastinal mass was removed. Immunohistochemical staining showed positive staining for chromogranin A and synaptophysin, which could happen in either paraganglioma or carcinoid tumors. However, S100 protein, which is a characteristic of paraganglioma, showed positive staining in the sustentacular cells. So, considering the positive staining of ACTH and PCK and the negative staining of EMA, we took the diagnosis of a paraganglioma with ACTH-secreting function (**Figure 2**).

After the operation, the ACTH and cortisol concentrations declined to low levels, with 13.03 ng/L and 224 nmol/L, respectively. The clinical symptoms were improved significantly, and the edema of both lower limbs subsided. Then, 10 mg prednisone per day was prescribed for the patient post-operation, and this dose was gradually tapered. At 6 months after the operation, the hypothalamus–pituitary–adrenal axis of the patient was recovered, and prednisone replacement therapy was stopped. He is now still presenting with total clinical remission at 16 months post-surgery. Follow-up of this patient is still ongoing (**Table 2**).

TABLE 1 | Laboratory and hormone values of the patient.

Parameters	Values	Reference range
Blood potassium (mmol/L)	2.98	3.50–5.30
FBG (mmol/L)	11.22	3.90–5.90
ACTH (ng/L)	162.10	5.00–78.00
8:00 PTC (nmol/L)	2,247.00	147.30–609.30
24:00 PTC (nmol/L)	2,104.00	147.30–609.30
24-h UFC (ug/24 h)	9,174.30	20.30–127.60
ACTH after HDDST (ng/L)	222.30	
PTC after HDDST (nmol/L)	1,746.00	
24-h UFC after HDDST (ug/24 h)	3,421.20	
ACTH before DDAVP stimulation test (ng/L)	212.50	
ACTH after DDAVP stimulation test (ng/L)	1,834.00	
ACTH after the surgery (ng/L)	13.03	
PTC after the surgery (nmol/L)	224.00	

FBG, fasting blood glucose; ACTH, adrenocorticotrophic hormone; PTC, plasma total cortisol; UFC, urine-free cortisol; HDDST, high-dose dexamethasone suppression test; DDAVP, desmopressin.

PERSPECTIVE OF THE PATIENT

"I was a policeman. My body was very strong before I got sick. But I became very weak since more than 1 year ago. I felt tired every day. There was no strength in my limbs, especially in the lower limbs. My mood got worse gradually, lazy words, and depression. All the symptoms progressed rapidly within 3 months, and a lumbar fracture occurred, which made me feel a terrible backache. I remember that the first time I went to the endocrinology department, I could not walk by myself and had to take a wheelchair and accompanied by my wife. After the operation, my muscle strength became better, and the mood significantly improved. At 1 to 2 months later, my symptoms gradually disappeared. Now I can come to the clinic for follow-up visits by myself, and I have resumed my routine work".

DISCUSSION

Cushing's syndrome is characterized by hypercortisolism, and its symptoms and signs are caused by long-term exposure to excessive glucocorticoids. Patients with CS clinically present with central obesity, hypertension, peripheral edema, glucose intolerance, and hypokalemia.

Cushing's syndrome can be classified into two types: ACTH-independent and ACTH-dependent CS. In this case, the patient presented with pitting edema, full moon face, buffalo back, and elevated cortisol and ACTH concentrations. He was diagnosed with ACTH-dependent CS after initial tests. The most common

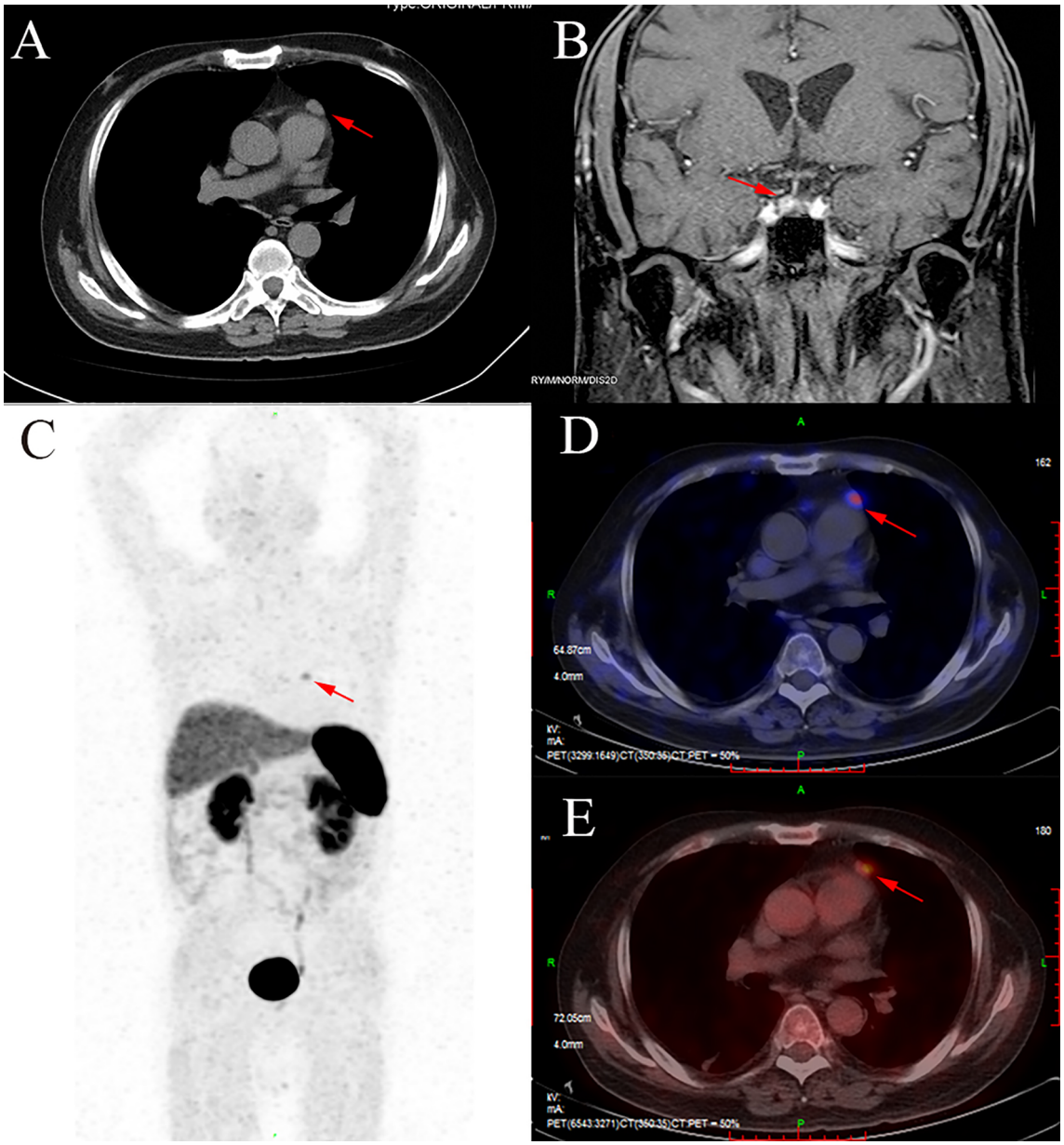

FIGURE 1 | The CT, MRI, and PET/CT images of this patient. **(A)** A soft tissue density nodule in the mediastinum (red arrow). **(B)** A nodule in the right side of the pituitary (red arrow). **(C, D)** The anterior mediastinal nodule with increased ^{68}Ga uptake (red arrow). **(E)** The anterior mediastinal nodule with increased fluorodeoxyglucose uptake (red arrow).

cause of ACTH-dependent CS is CD, which means the endogenous secretion of ACTH from a pituitary adenoma. Only 10% of cases of ACTH-dependent CS were caused by ectopic ACTH syndrome, which stands for endogenous secretion of ACTH from the ectopic tumor (3).

The critical point and challenge of differential diagnosis for ACTH-dependent CS is to localize the ACTH-secreting tumor. In this case, the process of tumor localization was troublesome. Initially, the HDDST showed an inconsistent result in plasma and urinary cortisol. The blood ACTH and PTC were insufficiently suppressed by HDDST, whereas the 24-h urine-free cortisol was inhibited by more than 50% compared to baseline. The DDAVP stimulation test showed a positive response of ACTH. Additionally, pituitary MRI suggested an existing pituitary microadenoma with a diameter of less than 6 mm. These results made the differential diagnosis of CD or EAS controversial. Considering that the 24-h UFC concentration could be affected by urine volume and the inspection methodology, nearly 20% of ACTH-secreting pituitary microadenoma cannot be suppressed by HDDST according to

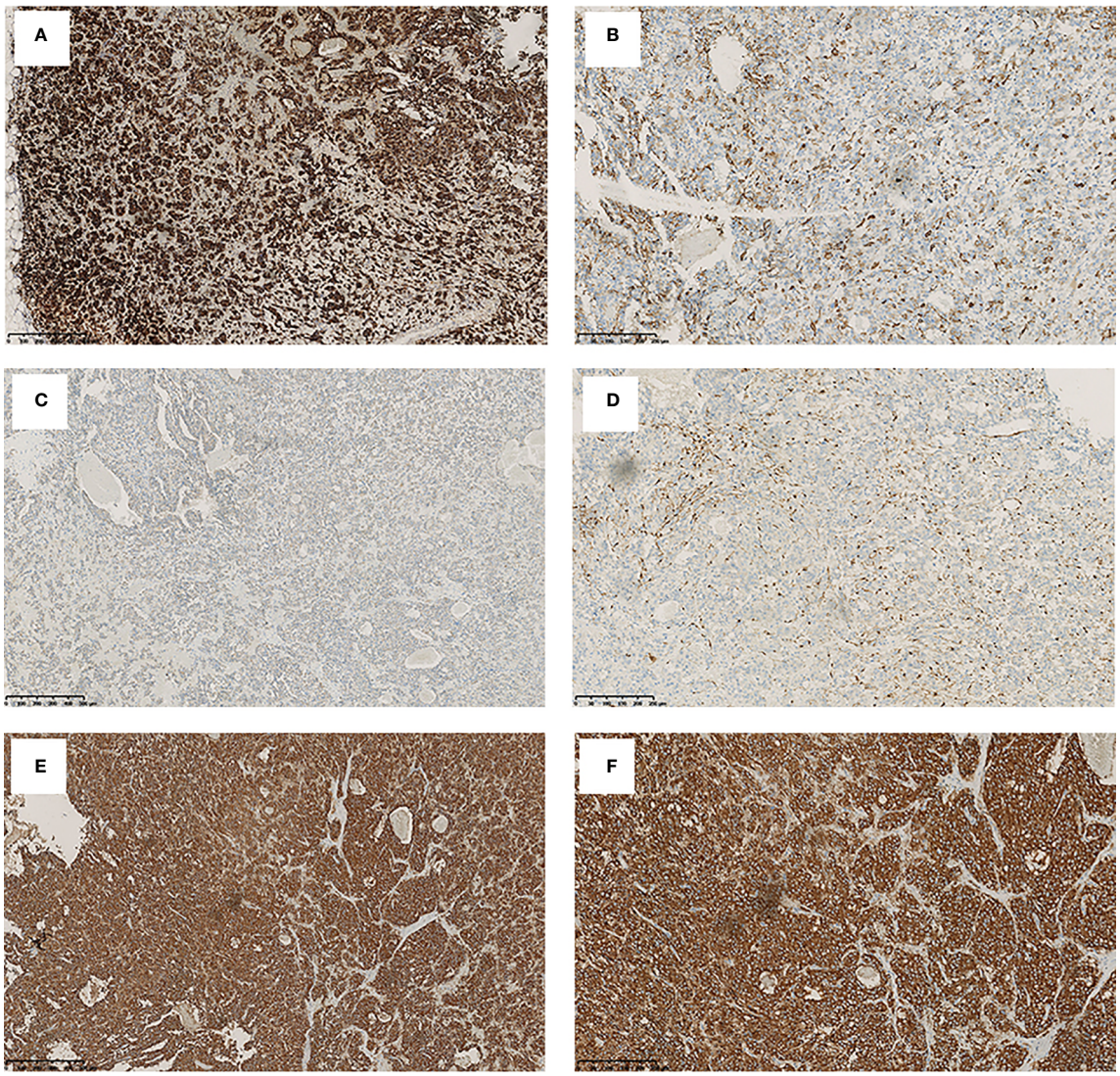

FIGURE 2 | The immunohistochemical staining pattern of mediastinal mass. Tumor cells show a positivity for adrenocorticotrophic hormone **(A)** (original magnification, ×100), chromogranin A **(B)** (original magnification, ×200), PCK **(C)** (original magnification, ×100), and S100 **(D)** (original magnification, ×200) and synaptophysin **(E, F)** (original magnification, ×100 and ×200).

previous literature (4). Previous literature had reported that some patients with ectopic ACTH syndrome could also respond to DDAVP (5). BIPSS was performed to differentiate the source of ACTH. After sampling, there was no advantage gradient of ACTH in IPS relative to peripheral blood. EAS should be highly suspected. So, ^{68}Ga-DOTATATE-PET/CT and ^{18}F-FDG-PET/CT were recommended to search for the ACTH source, and it was found that the anterior mediastinal nodule had increased glucose uptake and an increased expression of somatostatin receptors, which is consistent with

TABLE 2 | Biochemical and hormone values before and after the surgery.

	Before	After	2 months later	11 months later	Reference value
PTC (nmol/L)	2,060.00	224.00	183.00	331.00	147.30–609.30
ACTH (ng/L)	330.80	13.03	33.47	31.72	5.00–78.00
Blood potassium (mmol/L)	3.04	4.79	4.67	4.50	3.50–5.30
Blood calcium (mmol/L)	2.04	2.07	2.41	2.34	2.11–2.52
FBG (mmol/L)	11.71	5.25	4.91	5.06	3.90–5.90

PTC, plasma total cortisol; ACTH, adrenocorticotrophic hormone; FBG, fasting blood glucose.

neuroendocrine tumors that might be of ectopic ACTH source. After the anterior mediastinal mass was removed, the level of ACTH and cortisol decreased to normal range immediately. It confirmed our speculation.

This case report demonstrates that hybrid imaging modalities are of great help to improve the detection of the ectopic ACTH source, such as PET/CT, and for determination of the location and function of the tumor. The combination of ^{68}Ga-DOTATATE-PET/CT and ^{18}F-FDG-PET/CT could improve the detection rate of tumors, especially neuroendocrine tumors (6).

Paraganglioma originates from widely distributed specific neural crest chromaffin cells and is relatively rare in clinical practice. Paraganglioma could occur in the kidney (7), paranasal sinuses (8), and pulmonary (9, 10) and cervical (11) sites, while the mediastinum is an uncommon site of occurrence. According to the reference, the total incidence of pheochromocytomas and paragangliomas is only about 3 per million (12). Moreover, the morbidity of pheochromocytomas associated with EAS accounted for only 3% of the total EAS cases (2). Paragangliomas are classified into functional and non-functional, and functional paragangliomas mainly secrete catecholamines. ACTH-secreting paragangliomas are very rare. The postoperative pathological result reported as paraganglioma is beyond our expectation. Based on literature reviews, less than 20 cases were reported about ACTH-secreting paraganglioma (13), and only 4 cases were described to originate from the mediastinum (14). Since the patient had a clinical manifestation of neither hyper-catecholamine nor elevated serum or urinary catecholamine, a non-functional mediastinal paraganglioma secreting ACTH and leading to EAS was diagnosed eventually in this case.

Tumorectomy is the first choice for the treatment of EAS. After removal of the ectopic ACTH source, the clinical disorders of the patient can be gradually relieved. Like this patient, the edema disappeared, and the blood potassium, cortisol, and ACTH levels returned to normal after the surgery. What should be noted is that ectopic ACTH syndrome has an undesirable prognosis. The prognosis is related to the nature of the tumor. According to literature reports, only 47% of EAS patients can be cured (1). For the recrudescence of neuroendocrine tumors, even if being removed by operation, follow-up is recommended for at least 10 years (15).

CONCLUSIONS

In summary, ectopic ACTH syndrome is challenging to locate and is easy to be missed and misdiagnosed. The dexamethasone suppression test and BIPSS lead to the correct diagnosis. Functional imaging methods with radioisotopes help localize these tumors, such as ^{68}Ga-DOTATATE-PET/CT and ^{18}F-FDG-PET/CT. The paraganglioma-related EAS is very rare. The optimal choice for these patients is tumorectomy. After removal of the tumor, the patient can obtain complete clinical remission. However, the evaluation of prognosis requires further long-term follow-up studies with more samples.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

BL took charge of original draft writing. ZY performed follow-up and management of the patient. HH contributed to manuscript review and editing. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Intracranial Germinoma Misdiagnosed as Hyperthyroidism: A Case Report and Review of the Literature

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Intracranial germ cell tumors (GCTs) are relatively rare, which account for 0.5% of all primary intracranial neoplasms. Intracranial germinomas most commonly occur in the pineal and suprasellar region, making up the majority of all intracranial GCTs. For its diversified clinical manifestations, the diagnosis is easily confused with other diseases. Here, we present a case of a 19-year-old boy with intracranial germinoma who was preliminarily misdiagnosed as hyperthyroidism for the symptoms of weight loss and thyroid dysfunction.

Keywords: intracranial germinoma, hyperthyroidism, hypopituitarism, central diabetes insipidus, radiotherapy

BACKGROUND

Primary intracranial germ cell tumors (GCTs) originate from primordial germ cells. Germinomas comprise the majority of GCTs and usually develop in the midline structures, especially in the pineal followed by the suprasellar region (1). They mainly affect children and young adults, and have a male predominance (2). Depending on the size and location of the GCTs, there are different clinical manifestations: hypopituitarism, diabetes insipidus, intracranial hypertension, etc. The diagnosis of intracranial germinoma is easily confused with other diseases due to its diverse clinical manifestations. Here, we report a case of intracranial germinoma in a 19-year-old boy who was misdiagnosed as hyperthyroidism.

CASE PRESENTATION

A 19-year-old boy was admitted to the hospital for complaints of fatigue, poor appetite, and weight loss without headache, nausea, vomiting, polydipsia, and polyuria. Pre-admission thyroid hormones determination: thyroid stimulating hormone(TSH) <0.005 mU/L (Reference range 0.27–4.2 mU/L), free triiodothyronine (FT3) 7.66 pmol/L (Reference range 3.60–7.50 pmol/L), free thyroxine (FT4) 19.52 pmol/L (Reference range 12.0–22.0pmol/L). The preliminary diagnosis was hyperthyroidism.

Physical examination: T: 36.5°C, R: 18 bpm, BP: 85/52 mmHg, HR: 87 bpm, Height: 173 cm, Weight: 44 kg, BMI: 14.7kg/m^2. Clear consciousness, dry skin, and normal development. Neurological examination was negative.

Laboratory examination: blood glucose, hepatic function, renal function, routine blood count, and stool routine were normal. Redetermination of thyroid hormone on admission: TSH <0.005 mU/L, FT3 6.93 pmol/L, FT4 18.03 pmol/L. TSH receptor antibody (TRAb), thyroglobulin antibody (TGAb), and thyroid peroxidase antibody (TPOAb) were negative. The thyroid function of the patient was changing without any drug intervention over time (**Table 1**). Single-photon emission computed tomography (SPECT) thyroid imaging revealed decreased thyroid uptake of technetium. The results of additional hormone test showed secondary hypoadrenocorticism, secondary hypogonadism, and hyperprolactinemia (**Table 2**). Then, the patient was treated with hydrocortisone 50mg per day. A few days later, the patient began to complain about polydipsia and polyuria. The serum sodium concentration increased from 142 to 158 mmol/L and there was no change in urine specific gravity (1.004) during the water deprivation test, while a great increase in urine specific gravity (increased from 1.004 to 1.018) was observed after administration of desmopressin, this confirmed central diabetes insipidus (CDI). His polydipsia and polyuria were relieved by desmopressin. Contrast-enhanced MRI revealed nodular signals in the pineal, suprasellar region, and fourth ventricle (1.0, 2.7, and 1.1 cm in diameter, respectively) (**Figure 1A**). A spine MRI excluded metastatic lesions. Serum β-human chorionic gonadotropin (β-HCG) was 8.56 IU/L (Reference range <3.81 IU/L)and serum Alpha-fetoprotein (α-FP) was within the normal reference range. Consequently, the diagnosis of intracranial germinoma was considered. After the completion of diagnostic radiation therapy of 20 Gy and subsequent radiotherapy(the patient received three-dimensional conformal radiotherapy and the total dose was 40 Gy in fractions of 1.8–2.0 Gy per day, 5 d/wk), the lesions of the suprasellar, the pineal, and fourth ventricle almost disappeared (**Figure 1B**). Redetermination of the thyroid axis revealed that all the indices gradually returned to the normal range, with ACTH fluctuating around the lower limit of the reference range and low levels of cortisol. The patient was treated with hydrocortisone 20 mg/day and desmopressin 0.05 mg per day for cortisol replacement and CDI, respectively. His symptoms improved significantly, the 24-hour intake and output were maintained at about 2,000 ml, blood pressure returned to normal, and his weight increased. No recurrence occurred after a follow-up period of one year.

TABLE 2 | Hormone test results.

Hormones	Lab value	Reference range
ACTH (8:00 A.M.)	10.87	5.00–78.00 ng/L
Cortisol (8:00 A.M.)	50.79	147.30–609.30 nmol/L
GH	2.10	0.03–2.47 ng/ml
LH	<0.10	1.70–8.60 mIU/L
FSH	<0.10	1.50–12.40 mIU/L
Estradiol	<5.00	25.80–60.70 pg/ml
Testosterone	1.99	0.28–11.10 ng/ml
PRL	78.22	4.60–21.40 ng/mL

ACTH, adrenocorticotropic hormone; GH, growth hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin.

DISCUSSION

Primary central nervous system (CNS) germ cell tumors (GCTs) are rare, which represent approximately 0.5% of all primary intracranial neoplasms (3). These rare tumors primarily affect children (especially 10–14 years old) and young adults with a male preponderance (2, 3). The majority of intracranial GCTs appear in the midline structures of the brain, such as the pineal and suprasellar region (1). CNS GCTs include a heterogeneous group of neoplasms, which are commonly classified into germinomas and non-germinomatous germ cell tumors (NGGCTs) (4, 5). Germinomas comprise the largest proportion of CNS GCTs and show high radiosensitivity and also excellent prognosis. The optimal treatment is either radiotherapy alone or chemotherapy followed by radiotherapy (3, 6).

The diversity of clinical manifestations is related to the size and location of tumors. Tumors occurring in the pineal region can easily block the midbrain aqueduct, causing obstructive hydrocephalus, high intracranial pressure, Parinaud's syndrome, etc. (7), while suprasellar tumors mostly lead to hypothalamo-hypophyseal insufficiency with corresponding clinical manifestations, namely, delayed growth, delayed or precocious puberty, central diabetes insipidus, fatigue, weight loss, etc. (8). This patient showed symptoms of fatigue and weight loss, had low levels of TSH and normal levels of FT4

TABLE 1 | Serum thyroid hormones with reference range.

Serum Thyroid Hormones	TSH	T3	FT3	T4	FT4
Reference range	0.27–4.2mU/L	1.3–3.1nmol/L	3.60–7.50pmol/L	62–164nmol/L	12.0–22.0pmol/L
On admission	<0.005		6.93		18.03
1 week later	0.006		3.84		14.88
2 weeks later	0.012		3.21		14.19
3 weeks later	0.018	1.19	3.66	86.22	12.48
2 weeks after RT	0.058	0.86	2.72	73.63	10.67
1 month after RT	0.765		2.76		11.33
2 months after RT	0.883	1.20	3.22	88.63	12.64
4 months after RT	1.630		3.59		14.67

TSH, thyroid stimulating hormone; T3, triiodothyronine; FT3, free triiodothyronine; T4, thyroxine; FT4, free thyroxine; RT, radiotherapy.

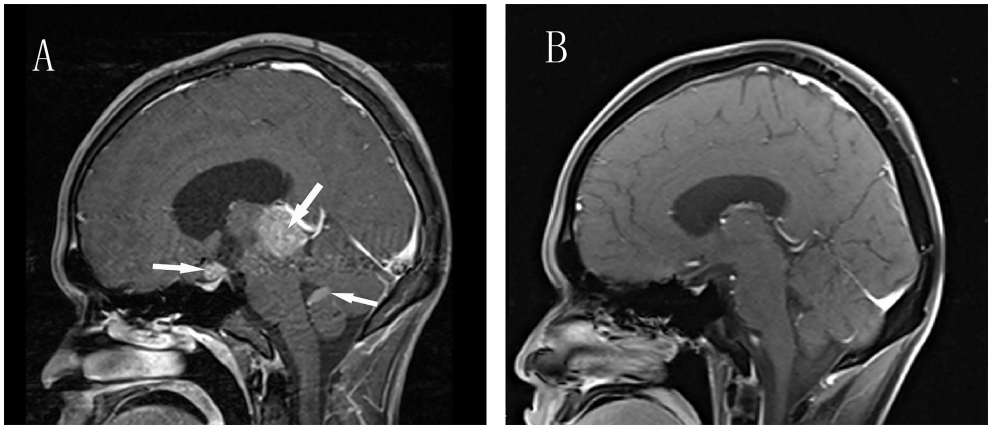

FIGURE 1 | Enhanced Brain MRI before and after Radiotherapy. **(A)** Sagittal contrast-enhanced T1-weighted MR image revealing three masses in the pineal, suprasellar region and fourth ventricle(arrows). **(B)** After radiotherapy, T1-weighted MR image show total disappearance of the lesions.

and FT3, so subclinical hyperthyroidism was considered. However, we noticed the patient paradoxically had poor appetite and low blood pressure, instead of hypermetabolic symptoms such as heat intolerance, sweating, and increased appetite. Additionally, during serial follow-up of the thyroid hormone after admission, the FT3 and FT4 levels were on a downward trend. The results of the pituitary hormone test showed he had secondary hypoadrenocorticism, secondary hypogonadism, and hyperprolactinemia. In the absence of cortisol, the symptoms of polydipsia and polyuria of the patients with diabetes insipidus cannot be obvious. After being given hydrocortisone, the symptoms of polydipsia and polyuria become apparent for an increase in blood volume (9). The subsequent water deprivation vasopressin test confirmed central diabetes insipidus. The patient had normal physical development, which might suggest the tumor had been present and developed after his puberty.

Although histopathology is the golden standard for the diagnoses of CNS GCTs, it is difficult to obtain the pathological specimen clinically. Imaging examination is helpful in diagnosis, typical MRI abnormalities in typical locations are strongly suggestive of intracranial GCTs. Intracranial GCTs lesions that involve both the pineal gland and suprasellar region are frequently termed as bifocal GCTs (10), of which the majority are germinomas (11). Synchronous neoplasms in any other location such as the fourth ventricle are considered to represent disseminated foci (12). MRI examinations of the patient revealed localized lesions were distributed in the pineal, suprasellar region, and fourth ventricle and presented significantly heterogeneous enhancement. Alpha-fetoprotein (α-FP) and Beta-Human chorionic gonadotropin (β-hCG) are two markers of GCTs, which are not produced by any other primary intracranial neoplasms (13). β-hCG is more valuable in diagnosing germinomas, because germinomas may secrete low levels of β-hCG instead of α-FP (14). Hu et al. suggest β-hCG ≥8.2 IU/L in CSF or serum β-hCG ≥2.5 IU/L as cutoff values for the clinical diagnosis of intracranial GCTs (15). In our patient, the high serum β-hCG (8.56 IU/L) and normal α-FP further support the diagnosis.

Because of the high radiosensitivity of germinomas compared with other intracranial tumors, diagnostic radiotherapy with a dose of 20 Gy was once used without histological verification (16–18). Prompt response to low-dose radiation (tumor can be reduced in mean diameter by more than 80% at 15-20 Gy) was deemed as one of the criteria for the clinical diagnosis of intracranial germinoma (19, 20).

For CNS germinomas, there has been consensus that radiotherapy should be the first line treatment and adjuvant chemotherapy could be conducive to the reduction of radiation dosage, while surgical resection plays a limited role (21, 22). The favored management for patients with symptomatic obstructive hydrocephalus is endoscopic third ventriculostomy (ETV) (22). Craniospinal irradiation (CSI) with focal boosts to tumor sites remains the standard of care for metastatic germinoma. The SIOP CNS GCT 96 study demonstrated 98% overall survival at 5 years with a CSI dose of 24 Gy followed by a 16 Gy boost to the tumor sites, no case of relapse was reported during a median follow-up of 6 years, and there was no additional benefit of chemotherapy (23). A retrospective review presented 10 patients with histologically proven primary intracranial germinoma who were treated by low-dose CSI with local boosts to a total dose of 40 Gy, all patients were alive with a median follow up time of 10.9 years, none with relapsed disease (24).

After trial therapy with a dose of 20 Gy and subsequent radiation, significant shrinkage of the lesions of the patient was observed. As the tumor volume shrunk obviously, his thyroid function gradually returned to normal. It suggested that the changes in his thyroid function were related to transient hyposecretion of TSH caused by tumor compression, which was misdiagnosed as subclinical hyperthyroidism.

CONCLUSION

Because germinomas occur at different ages with different course and lesion sites, the clinical manifestations and lab results are diverse and deceptive. This patient was misdiagnosed as subclinical hyperthyroidism for TSH reduction at first. Therefore, it is of great help to the diagnosis by dynamic observation of the changes in symptoms and lab results. By the way, imaging examination, β-hCG test and diagnostic radiotherapy are valuable for the diagnosis of germinoma.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JT wrote the manuscript. JW and ZY were responsible for the collection of the clinical data and the follow-up of the patient. HH designed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Ischemic Infarction of Pituitary Apoplexy: A Retrospective Study of 46 Cases From a Single Tertiary Center

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Objective: Ischemic infarction of pituitary apoplexy (PA) is a rare type of pituitary apoplexy. This study aims to characterize ischemic PA via clinical presentations, imaging data, histopathological manifestations, and focus on the management and prognosis of the disease.

Methods: This study retrospectively identified 46 patients with ischemic PA confirmed using histopathology at a single institution from January 2013 to December 2020. The clinical presentations, imaging data, laboratory examination, management, and outcomes were collected. We then summarized the clinical presentations, imaging features, intraoperative findings, and histopathological manifestations, and compared the outcomes based on the timing of surgical intervention.

Results: Headache was the most common initial symptom (95.65%, 44/46), followed by visual disturbance (89.13%, 41/46), and nausea and vomiting (58.70%, 27/46). 91.3% of the patients had at least one pituitary dysfunction, with hypogonadism being the most common endocrine dysfunction (84.78%, 39/46). Cortisol dysfunction occurred in 24 (52.17%) patients and thyroid dysfunction occurred in 17 (36.96%). Typical rim enhancement and thickening of the sphenoid sinus on MRI were seen in 35 (85.37%) and 26 (56.52%) patients, respectively. Except for one patient with asymptomatic apoplexy, the remaining patients underwent early ($\leq$ 1 week, 12 patients) and delayed ($>$ 1 week, 33 patients) transsphenoidal surgery. Total tumor resection was achieved in 27 patients and subtotal tumor resection in 19 patients. At surgery, cottage cheese–like necrosis was observed in 50% (23/46) of the patients. At the last follow-up of 5.5 $\pm$ 2.7 years, 92.68% (38/41) of the patients had gained a significant improvement in visual disturbance regardless of surgical timing, and 65% of the patients were still receiving long-term hormone replacement therapy.

Conclusion: Patients with ischemic PA can be accurately diagnosed by typical imaging characteristics preoperatively. The timing of surgical intervention does not significantly affect the resolution of neurological and endocrinological dysfunctions. Preoperative endocrine dysfunctions are common and usually appear to be poor after surgical intervention.

Keywords: pituitary apoplexy, ischemic infarction, coagulative necrosis, pituitary ring sign, ghost cells

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INTRODUCTION

Pituitary apoplexy (PA) is a rare, life-threatening emergency caused by hemorrhage and/or ischemia of a preexisting pituitary adenoma, and is most often a clinically non-functioning macroadenoma (Briet et al., 2015). According to epidemiological studies, the prevalence of pituitary apoplexy is about 6.2 cases per 100,000 people. Approximately 2–12% of patients with various types of pituitary adenoma experience apoplexy (Wakai et al., 1981; McFadzean et al., 1991; Bonicki et al., 1993; da Motta et al., 1999; Randeva et al., 1999; Ayuk et al., 2004; Verrees et al., 2004; Liu and Couldwell, 2006; Dubuisson et al., 2007; Murad-Kejbou and Eggenberger, 2009; Turgut et al., 2010; Möller-Goede et al., 2011), and more than 75% of apoplexy occurs in patients with an undiagnosed pituitary adenoma (Randeva et al., 1999; Biousse et al., 2001; Chacko et al., 2002; Ayuk et al., 2004; Sibal et al., 2004; Gruber et al., 2006; Dubuisson et al., 2007; Liu et al., 2010; Leyer et al., 2011; Möller-Goede et al., 2011; Pal et al., 2011; Sarwar et al., 2013; Bujawansa et al., 2014; Kinoshita et al., 2014). The clinical presentations of pituitary apoplexy vary, including sudden headache, nausea and vomiting, ophthalmic dysfunction, fever, altered mental status, and even death. The pathophysiological mechanism of PA remains unclear. There have been numerous reports on the diverse predisposing factors of pituitary apoplexy, such as head trauma, pregnancy, diabetes mellitus, hypertension, anticoagulant medications, dynamic study of the pituitary gland, and surgeries (Möller-Goede et al., 2011). Compared with symptomatic pituitary apoplexy, approximately 25% of patients experienced asymptomatic pituitary apoplexy (Wakai et al., 1981; Fraioli et al., 1990; Onesti et al., 1990; Bonicki et al., 1993; Kinoshita et al., 2014).

Hemorrhage has been known as the primary cause of pituitary apoplexy. A pure ischemic infarction of pituitary apoplexy is rarely reported compared with hemorrhage. With the limited numbers of ischemic PAs reported in the literature, there is a poor understanding of the pathophysiology of this entity. This study reported the largest sample size of the patients presenting with a pure ischemic PA. Herein, we summarized the clinical presentations, endocrine function, and imaging data of patients with ischemic PA. Moreover, we further explored the effect of surgical timing on neuro-ophthalmic and endocrine outcomes. We hope that this study can provide a more comprehensive understanding and optimize the management of ischemic PA.

MATERIALS AND METHODS

We retrospectively analyzed patients with ischemic PA confirmed by pathology findings within the Beijing Tiantan Hospital from January 2012 to December 2020. The inclusion criteria are as follows: (1) The patient was diagnosed with a pituitary adenoma by cranial CT or/and MRI; (2) postoperative pathological examinations confirmed patients with pituitary apoplexy and only exhibited an ischemic infarction; (3) patients had complete clinical data. The medical records of index hospitalization and the last clinical visit were reviewed to obtain information on demographic data, clinical presentation, laboratory examination, neuro-ophthalmic evaluation, imaging data, management profile, and pathological manifestations. The baseline characteristics of the included patients are described in **Table 1**.

Endocrinological assessments primarily included thyroid-stimulating hormone (TSH) and thyroid hormone levels, growth hormone (GH), insulin-like growth factor (IGF), luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and random cortisol. Diabetes insipidus (DI) was diagnosed when serum osmolality was > 295 mOsm/kg, whereas a corresponding urine osmolality was < 300 mOsm/kg in fluid deprivation tests in the case of polyuria and polydipsia, and a subsequent response to arginine vasopressin was observed.

TABLE 1 | Baseline characteristics of among 46 patients with ischemic PA.

Clinical factors	Values
Age (yr)	46.78 ± 12.32
Sex (M/F)	35M/11F
Symptoms and sign	
Headache	95.65% (44/46)
Nausea and vomiting	58.70% (27/46)
Hyponatremia	39.13% (18/46)
Diabetes insipidus	10.87% (5/46)
Fever	10.87% (5/46)
Neuro-ophthalmic examination	
Decrease visual acuity	89.13% (41/46)
Visual field deficit	82.61% (38/46)
Ocular palsy	50.00% (23/46)
Endocrine dysfunction	
Hypocorticolism	52.17% (24/46)
Hypothyroidism	36.96% (17/46)
Hypogonadism	84.78% (39/46)
Corticotropic deficiency or secondary hypothyroidism	58.70% (27/46)
Corticotropic deficiency and secondary hypothyroidism	30.43% (14/46)
Imaging characteristics	
Maximal tumor diameter (mm)	26.54 ± 6.03
Knosp classification (1–2 vs. 3–4)	28 vs. 14
Pituitary ring sign	85.37% (35/41)
Thickening of the sphenoid sinus mucosa	56.52% (26/46)
Ischemic imaging	23.91% (11/46)
Treatment	
Surgery (early vs. delayed)	12 vs. 33
EOR (Total resection vs. Subtotal resection)	27 vs. 19
Histology	
NFPA vs. FPA	36 vs. 6
Follow-up	
Duration (m)	66.29 ± 32.85
Recurrence	2

NFPA, non-functioning pituitary apoplexy; FPA, functioning pituitary apoplexy; yr, year; M, male; F, female; mm, millimeter; m, month; EOR, extent of resection.

Abbreviations: PA, pituitary apoplexy; TSS, transsphenoidal surgery; TSH, thyroid-stimulating hormone; GH, growth hormone; IGF, insulin-like growth factor; LH, luteinizing hormone; FSH, follicle-stimulating hormone; DI, diabetes insipidus; ACTH, adrenocorticotropic hormone; FPA, functioning pituitary adenoma; NFPA, non-functioning pituitary adenoma; DWI, diffusion-weighted imaging.

Prolactin deficiency or excess was determined according to a clinical reference range. At admission, random serum cortisol levels of < 50 nmol/L (range 50–250 mmol/L) indicated hypocortisolism. Low free T4 together with low or inappropriate normal TSH was considered secondary hypothyroidism. Gonadotropic deficiency in men and premenopausal women was defined as low testosterone levels with having low or inappropriately normal gonadotrophin levels, and in postmenopausal women, it was defined as inappropriately low gonadotrophin levels for menopausal age. Ophthalmologic evaluations included assessments of binocular visual acuity, visual field, and cranial nerve functions and were performed by an experienced ophthalmologist.

Two experienced radiologists evaluated all imaging data. The tumor size was classified as microadenoma (<1 cm), macroadenoma (1–4 cm), and giant pituitary adenoma (>4 cm). The relationship between the tumor and the cavernous sinus was evaluated according to knosp criteria, and patients with knosp grades 3 and 4 (tumor invasion beyond a line tangential to the lateral margins of the cavernous internal carotid artery, and total internal carotid artery encasement, respectively) were defined as positive cavernous sinus invasion. Based on the interval from initial onset to severe symptoms such as visual disturbance, the patients with ischemic PA were classified as acute onset (<3 days), subacute onset (3–14 days), and chronic onset (>14 days) (Xiao et al., 2015). All patients finally underwent transsphenoidal surgery (TSS) by experienced neurosurgeons. Early surgery was defined as an operation performed within 7 days of symptom onset, whereas delayed surgery was defined as an operation beyond 7 days of symptom onset. All patients were followed up for more than 6 months. On the last follow-up, the requirement of hormone replacement was considered pituitary dysfunction. The comparison of clinical presentations and outcomes of ischemic PA between early and delayed surgery groups is summarized in **Table 2**.

Continuous variables with normal distribution were described as means and SD, and categorical variables as medians and range. A *t*-test (normal distribution) or a Mann–Whitney rank-sum test was used when two sets of continuous variables were compared. Categorical variables were tested using a χ^2-test. A two-sided $p < 0.05$ was considered statistically significant. The results were graphically represented when deemed necessary. SPSS version 22 (IBM Corp, Armonk, New York, United States) was used for statistical analysis.

RESULTS

Patient Demographics

Table 1 summarizes the baseline characteristics of the patients in detail. A total of 46 patients were diagnosed with ischemic PA, including 35 males and 11 females, with a male-to-female ratio of more than 3:1. At diagnosis, the mean age was 46.78 years (*SD* = 12.32 years) ranging from 25 to 76 years. Compared with functioning pituitary adenoma, patients with non-functioning pituitary adenoma were significantly older ($p = 0.038$, *t*-test) (**Table 3**). The patients were mainly in their 40–50 s. Twenty patients had acute onset, 13 patients had subacute onset, and 12 had chronic onset. The median duration from symptom onset to the diagnosis of ischemic PA was 20 days (range, 3–90 days). Eleven patients had at least one or more possible predisposing factors, including diabetes mellitus, hypertension, and antiplatelet medications. Six patients suffered hypertension, six patients suffered diabetes mellitus, two patients suffered diabetes mellitus and hypertension, and one patient was using antiplatelet therapy due to a history of cerebral infarction. Dopamine receptor agonists like bromocriptine, cabergoline, and somatostatin analogs were not seen in our study.

Headache was the most common symptom. All but one patient presented with a headache, including 21 patients who suffered sudden-onset headache. Among the patients with headaches, 27 patients were accompanied by nausea and vomiting. There were no patients with alerted mental status. Decreased vision was observed in 41 patients, and 4 patients were nearly blind. Thirty-eight patients presented with temporal hemianopia, and 23 patients experienced ophthalmoplegia. Fifteen patients had temporal hemianopia and oculomotor nerve paralysis. Five patients had a fever and 5 patients suffered from polydipsia and polyuria preoperatively.

Laboratory Examination

Pituitary dysfunctions were common in this cohort. Forty-two (42/46, 91.30%) patients had one or more pituitary dysfunctions. Hypogonadism is the most common pituitary dysfunction in this study (84.78%, 39/46). At admission, low random cortisol levels and secondary hypothyroidism were seen in 24/46 (52.17%) and 17/46 (36.96%), respectively. Fourteen patients suffered hypocortisolism combined with secondary hypothyroidism. There was no significant difference in endocrine dysfunctions between the early and delayed surgery groups (**Table 2**), as well as in the functioning pituitary adenoma (FPA) and non-functioning pituitary adenoma (NFPA) groups (**Table 3**). Six patients had mild hyperprolactinemia, and the levels of prolactin did not exceed 2 times the upper limit. Nineteen patients had low serum prolactin. Hyponatremia occurred in 17 patients, including 10 cases (135–130 mmol/L), 5 cases (125–130 mmol/L), and 2 cases (< 125 mmol/L) of serum sodium.

Neuroimaging Findings

In this study, there were 45 macroadenomas and 1 giant adenoma. The mean diameter of the tumor was 26.54 mm (*SD* = 6.03 mm) ranging from 14 to 48 mm. In terms of tumor invasiveness, 29 and 14 patients were identified as knosp 1–2 and knosp 3–4, respectively (**Table 1**). There was a significant difference in tumor invasiveness between the early and delayed surgery groups ($p = 0.049$, Pearson χ^2) (**Table 2**). In addition, 83.33% (35/42) of the patients showed iso-intensity in T1-weighted and hyperintensity in T2-weighted imaging (**Figures 1A,B**). After gadolinium administration, 87.5% (35/40) of the patients showed rim enhancement, also known as the pituitary ring sign (**Figure 1C**). Sixty-five percent (26/40) of the patients showed thickening of the sphenoid sinus on MRI. Eleven patients had cerebral ischemia lesions on MRI. Among these patients, two patients showed bilateral frontal subcortical

TABLE 2 | Comparison of clinical presentation and outcomes of ischemic PA between early and delayed surgery groups.

	Early surgery (n = 12)	Delayed surgery (n = 33)	P-value
Age* (yr)	46.58 ± 14.34	46.30 ± 11.65	0.864
Sex^ (M/F)	11M/1F	24M/9F	0.344
Symptoms and sign			
Headache^	100% (12/12)	96.97% (32/33)	1
Nausea and vomiting^	66.67% (8/12)	57.58% (19/33)	0.836
Hyponatremia#	50.00% (6/12)	63.64% (21/33)	0.409
Ophthalmic examination			
Decrease visual acuity&	91.67%% (11/12)	90.91% (30/33)	1
Visual field deficit^	83.33% (10/12)	51.52% (17/33)	0.114
Ocular palsy#	41.67% (5/12)	54.55% (18/33)	0.445
Endocrine dysfunction			
Hypocorticolism#	50.00% (6/12)	54.55% (18/33)	0.787
Hypothyroidism^	16.67% (2/12)	45.45% (15/33)	0.157
Hypogonadism^	83.33% (10/12)	84.85% (28/33)	1
Corticotropic deficiency or secondary hypothyroidism#	50.00% (6/12)	63.64% (21/33)	0.409
Corticotropic deficiency and secondary hypothyroidism^	16.67% (2/12)	36.36% (12/33)	0.369
Imaging characteristics			
Maximal tumor diameter* (mm)	27.08 ± 5.089	25.70 ± 5.193	0.430
Knosp classification# (1–2 vs. 3–4)	(5 vs. 6)	(7 vs. 24)	0.049
Pituitary ring sign&	100% (11/11)	79.31% (23/29)	0.162
Thickening of sphenoid sinus mucosa^	83.33% (10/12)	53.33% (16/33)	0.080
Clinical outcomes			
Total improved vision^	90.91% (10/11)	82.14% (23/28)	0.850
Total improved ocular palsy	100% (5/5)	100% (16/16)	–
Improved hypocortisolism	20% (1/5)	13.33% (2/15)	–
Improved hypothyroidism	0% (0/2)	35.71% (5/14)	–
EOR^ (Total resection vs. Subtotal resection)	1 vs. 11	19 vs. 14	0.009
Follow-up (m)	72.11 ± 28.52	64.51 ± 34.90	0.504

**P-value calculated by t-test.*
#P-value calculated by Pearson Chi-Square.
^P-value calculated by Continuity Correction.
&P-value calculated by Fisher's Exact Test.

ischemia on MRI, two patients had a lacunar infarction in the basal ganglia, three patients showed multiple lacunar infarct lesions, two patients had white matter ischemic lesions on MRI, and two patients showed ischemic demyelinating lesions.

Intraoperative Findings and Pathology

At surgery, 96.52% (42/46) of the patients had solid tumor masses with poor or medium blood supply. The tumors were characterized by a grayish-yellow or a white-yellow mass with a soft texture or/and fibrous tissue, which is different from a classic pituitary adenoma. H&E staining of the tumor specimens showed massive acellular coagulative necrosis without any hemorrhagic changes under the microscope (**Figure 1D**). "Ghost cells" are a typical manifestation characterized by ghost outlines and no cellular structure. Moreover, white-yellow cottage cheese–like tissue was often seen during the operation (50%, 23/46). Four tumor samples lacked enough viable tumor tissue to make a biochemical diagnosis. Of the remaining 42 patients, there were 36 non-functional adenomas, 4 prolactinomas, 1 mixed prolactinoma/somatotrophinoma, and 1 TSH adenoma.

Treatment and Prognosis

Four patients initially presented with mild headaches and transient double vision. They received watchful observation and supportive management. The symptoms were temporarily relieved; however, they progressed into a visual disturbance in later observations. These patients finally underwent TSS. The other 42 patients received TSS after diagnosis of their initial symptoms. The remaining 12 patients received early surgical intervention ($\leq$ 7 days) and 33 patients received delayed surgical intervention ($>$ 7 days), except for one patient with asymptomatic apoplexy. Total tumor resection was performed in 27 patients and subtotal tumor resection in 19 patients. Patients who received delayed surgical intervention significantly obtained a higher rate of total tumor resection compared with early surgery group (p = 0.009, continuity correction). There were no obvious postoperative complications. The mean follow-up duration was 66.29 months (SD = 32.85 months) ranging from 7.83 to 115 months (**Table 1**). Complete remission of decreased vision was seen in 10 out of 11 patients undergoing early surgery vs. 23 of 28 undergoing delayed surgery (p = 0.850, continuity correction) (**Table 2**). The duration of recovery ranged from 1

TABLE 3 | Comparison of clinical presentation and outcomes of ischemic PA between NFPA and FPA groups.

Clinical factors	NFPA (*n* = 36)	FPA (*n* = 36)	*P*-value
Age* (yr)	47.56 ± 12.25	36.33 ± 8.45	0.038
Sex (M/F)[&]	29M/7F	4M/2F	0.593
Symptoms and sign			
Headache[&]	97.22% (35/36)	83.33% (5/6)	0.268
Nausea and vomiting[&]	58.33% (21/36)	28.57% (2/6)	0.384
Ophthalmic examination			
Decrease visual acuity [&]	97.22% (35/36)	66.67% (4/6)	0.049
Visual field deficit[&]	66.67% (24/36)	50.00% (3/6)	0.649
Ocular palsy^	50.00% (18/36)	16.67% (1/6)	0.282
Endocrine dysfunction			
Hypocortisolism[&]	52.78% (19/36)	33.33 (2/6)	0.663
Hypothyroidism^	41.67% (15/36)	16.67% (1/6)	0.476
Hypogonadism[&]	80.56% (29/36)	100% (6/6)	0.567
Corticotropic deficiency or secondary hypothyroidism[&]	58.33% (21/36)	33.33% (2/6)	0.384
Corticotropic deficiency and secondary hypothyroidism^	33.33% (12/36)	16.67% (1/6)	0.733
Imaging characteristics			
Maximal tumor diameter*(mm)	26.25 ± 5.26	27.67 ± 10.33	0.603
Knosp classification[&]	81.25% (26/32)	100% (6/6)	0.562
Pituitary ring sign[&]	63.33% (19/30)	85.71% (6/7)	0.389
Thickening of sphenoid sinus mucosa[&]	57.58% (20/36)	71.43% (4/6)	0.685
Ischemic imaging^	34.62% (9/36)	14.29% (1/6)	1
Treatment			
Surgery (early vs. delayed)^	32.35% (11/36)	16.67% (1/5)	1
Clinical outcomes			
Total improved vision[&]	84.85% (28/33)	100% (4/4)	1
Total improved ocular palsy	100% (18/18)	100% (1/1)	–
Improved hypocortisolism	6.25% (1/16)	0% (0/1)	–
Improved hypothyroidism	23.08% (3/13)	0% (0/1)	
EOR[&] (Total resection: Subtotal resection)	14:22	4:2	0.375
Follow-up* (m)	68.43 ± 31.57	79.32 ± 33.36	0.442

**P-value calculated by t-test.*
[#]P-value calculated by Pearson Chi-Square.
^P-value calculated by Continuity Correction.
[&]P-value calculated by Fisher's Exact Test.

week to 6 months, with the majority of recovery at 3 months (78.95%, 30/38). The patients with ocular palsy recovered first among the ophthalmological dysfunctions, with 50% of the patients having significantly recovered before discharge. The rest of the patients had recovered completely during the 3-month follow-up (**Table 4**).

After TSS, preoperative cortisol dysfunction was restored in three patients and secondary hypothyroidism in five patients. However, seven patients had postoperative new-onset cortisol dysfunction and five had postoperative secondary hypothyroidism during the last follow-up. Fifteen patients suffered postoperative new-onset polydipsia and polyuria and quickly recovered. The five patients with preoperative diabetes insipidus were completely cured after the operation, with the recovery duration ranging from 2 to 12 months. There was no significant difference in the prognosis of pituitary function between the early and delayed surgery groups (**Table 2**). Hormone replacement therapy was performed in the patients with long-term pituitary dysfunction, with testosterone only being administered to the men. As shown in **Table 1**, total tumor resection was achieved in 27 patients and subtotal tumor resection in 19 patients. Moreover, patients who received delayed surgery achieved a significantly higher rate of total tumor resection (p = 0.009, continuity correction) (**Table 2**). At the last follow-up, two patients revealed tumor recurrence through MRI examination and underwent TSS again. The prognosis was satisfactory without any severe complications.

DISCUSSION

Ischemic pituitary apoplexy, a rare type of pituitary apoplexy, shows unique intraoperative findings, imaging characteristics, and histological manifestations, different from hemorrhagic PA. There is a limited understanding of ischemic PA with a small number of reported cases. This study reported the largest number of ischemic PA patients confirmed by histopathology. The summarized clinical manifestations, imaging data, and

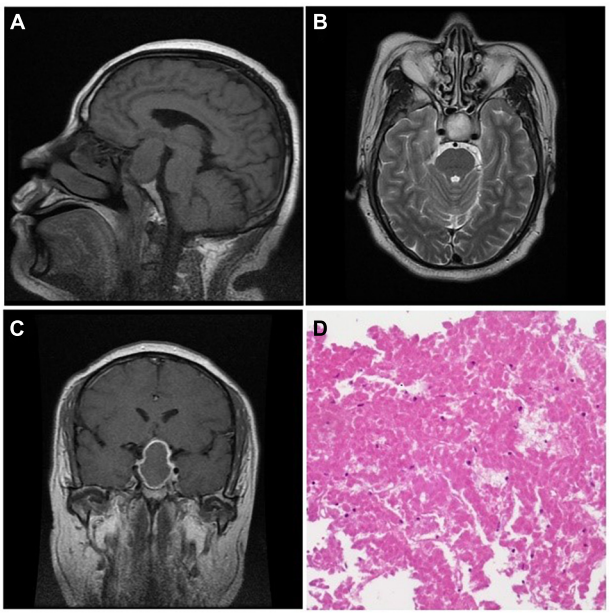

FIGURE 1 | The MRI showed iso-intensity in T1-weighted imaging (**A**, sagittal) and hyperintensity in T2-weighted imaging (**B**, axial). After gadolinium administration, the imaging showed central iso-intensity with an enhanced rim (**C**, coronal). Histological manifestation **(D)** showed a large area of red massive coagulative necrosis without cellular structure (H&E ×100).

TABLE 4 | Baseline characteristics of patients with ischemic PA between preoperative and postoperative.

	Preoperative	Postoperative
Symptoms and sign		
Headache	95.65% (44/46)	0
Hyponatremia	36.96% (17/46)	0
Diabetes insipidus	10.87% (5/46)	0
Neuro-ophthalmic examination		
Decrease visual acuity	89.13% (41/46)	15.91% (7/44)
Visual field deficit	60.87% (28/46)	11.11% (3/27)
Ocular palsy	50.00% (23/46)	0
Endocrine dysfunction		
Hypocortisolism	52.17% (24/46)	65.00% (26/40)
Hypothyroidism	36.96% (17/46)	46.34% (19/41)
Hypogonadism	82.61% (38/46)	63.16% (24/38)

intraoperative findings are further discussed as well as the effect of surgical timing on the prognosis of ischemic PA.

The average age of the patients in this study was 46.78 years, and the patients were mainly in the 40–50 s, which is similar to the pituitary apoplexy findings within previous reports showing that patients were primarily in their 50–60 s (Wakai et al., 1981; Fraioli et al., 1990; Onesti et al., 1990; McFadzean et al., 1991; Jugenburg et al., 1995; Biousse et al., 2001; Dubuisson et al., 2007; Möller-Goede et al., 2011). The male/female ratio was more than 3:1 showing a similar gender preponderance with previously reported studies (Wakai et al., 1981; McFadzean et al., 1991; Bills et al., 1993; Chacko et al., 2002; Sibal et al., 2004; Dubuisson et al., 2007; Zhang et al., 2009; Möller-Goede et al., 2011; Sarwar et al., 2013; Bujawansa et al., 2014; Vargas et al., 2014).

The patients in our study presented with classic symptoms, including headache, visual disturbance, and nausea and vomiting, without any life-threatening situations. Similar to previous studies, patients with ischemic PA are more likely to experience a long course of progression and relatively mild symptoms than classic pituitary apoplexy (Semple et al., 2008; Wang et al., 2019). More than 50% (25/45) of the patients in our study experienced subacute or chronic progression of symptoms from the initial onset, including headache, visual disturbance, and limited ocular movement, which partly explains why 33 patients received delayed surgical intervention. Moreover, asymptomatic pituitary apoplexy was reported to be common in the other subtypes of pituitary apoplexy (Briet et al., 2015). Only one patient in our study presented with asymptomatic pituitary apoplexy, which proposes the hypothesis that ischemic PA may lead to a large tumor infarct area, thus significantly increasing intrasellar pressure.

Until now, the etiology of pituitary apoplexy is still not well understood. According to previous reports, the precipitating factors of pituitary apoplexy include pituitary irradiation, intracranial pressure change, head trauma, hormone therapy, pregnancy, diabetes mellitus, diabetic ketoacidosis, cerebral angiography, anticoagulant drugs, pituitary function stimulation test, blood dialysis, and surgery (Briet et al., 2015). In addition, 31.43% (11/46) of patients in this study had at least one of the predisposing factors, including diabetes mellitus, hypertension, and anticoagulant therapy. These precipitating factors were similar to classic pituitary apoplexy. Moreover, 23.91% (11/46) of patients had focal ischemia or infarcted lesions on MRI. The manifestations of cerebrovascular fragility suggest that vascularization properties may play an important role in ischemic PA. Generally, due to the limited reported case of ischemic PA, it is challenging to determine the specific predisposing factors of ischemic PA.

The pathophysiology of ischemic PA remains unclear, but unique vascularization properties may contribute to its etiology. Based on the previous research, the possible mechanisms are as follows: (1) The rapid growth of tumors exceeds the angiogenesis supply (Oldfield and Merrill, 2015); (2) pituitary tumors have less vascular supply (Jugenburg et al., 1995; Turner, 2001); (3) tumor compression leads to increased intrasellar pressure (Rovit and Fein, 1972); (4) the decrease of systemic blood pressure leads to the decrease of blood supply (Biousse et al., 2001; Elsässer Imboden et al., 2005; Möller-Goede et al., 2011); (5) the fragility of blood vessels and vascular embolisms (Biousse et al., 2001). Of note, these factors may act together instead of singularly. As mentioned previously, patients in this cohort have a high rate of ischemia or infarcted lesions on MRI, which convinced us that the fragility of the blood vessels may be the primary cause of ischemic infarction. Coagulative necrosis of PA was generally considered the result of ischemic infarction (Xiao et al., 2015; Wang et al., 2019). However, Chacko et al. (2002) considered coagulative necrosis to be a late pathological manifestation of hemorrhagic infarction after an extended time interval between

the acute onset and surgery (>8 weeks), which is obviously inconsistent with the findings of our study and other research (Nishioka et al., 2005; Kim et al., 2008; Xiao et al., 2015; Ogawa et al., 2016; Wang et al., 2019).

Imaging manifestations correspond to the underlying pathophysiology of ischemic PA. MRI is an effective imaging method for the diagnosis of pituitary apoplexy. The diffusion-weighted imaging (DWI) sequence of MRI can detect the infarcted area in a short time after tumor infarction (Rogg et al., 2002). Unfortunately, DWI is not routinely used in our patients. The MRI appearance of hemorrhagic PA was different at each stage (Dubuisson et al., 2007; Vaphiades, 2017; Waqar et al., 2017; Goyal et al., 2018). At the acute phase (7 days), the tumor showed iso- to hypointensity on T1WI and hypointensity on T2WI. During the subacute phase (7–14 days), hyperintensity on T1WI and T2WI can be observed. During the chronic phase (> 14 days), hypointensity on T1WI and T2WI can be observed. The MRI manifestations in our study are similar to previous research (Xiao et al., 2015; Ogawa et al., 2016; Wang et al., 2019). The tumor presented with iso- or hyperintensity on T1W and T2WI without enhancement after gadolinium injection regardless of what phase they were in. Rim enhancement of the tumor on MRI after gadolinium administration is a unique appearance for ischemic PA and was seen in 87.5% (35/40) of the patients in our study. It has been reported that rim enhancement, the outermost portion of the infarcted pituitary adenoma, was found to be the presence of granulation tissue and lymphocytosis based upon histological examination (Kleinschmidt-DeMasters and Lillehei, 1998). To our knowledge, rim enhancement, known as the pituitary ring sign, can also be seen in several pituitary diseases, such as craniopharyngioma, lymphocytic hypophysitis, and pituitary abscesses (Rogg et al., 2002; Arita et al., 2006; Zhu et al., 2019). Considering the presentation and imaging characteristic of pituitary apoplexy, the diagnosis of pituitary adenoma can be accurately made in sellar region diseases. Similar to typical pituitary apoplexy, thickening of the sphenoid sinus mucosa can also be seen in the majority of patients in this study and is likely attributed to increased pressure in the venous drainage system within the sinus area; thus, it is an indirect result of the increased intrasellar pressure (Liu and Couldwell, 2006; Briet et al., 2015; Vaphiades, 2017). Generally, MRI manifestations of ischemic PA have several unique characteristics corresponding to the histopathological findings, which are conducive to an accurate diagnosis before surgery (Semple et al., 2006, 2008; Wang et al., 2019).

Imaging features and intraoperative findings were the primary manifestations of the underlying pathophysiology of ischemic PA. During surgery, a yellow cottage cheese–like tissue was often observed (52.17%, 24/46). Similar to previous studies, the tumor in this study presented as yellow-white or yellow-grayish, with soft or uneven texture and poor-medium blood supply (Ogawa et al., 2016; Wang et al., 2019). Histological examination revealed massive coagulative necrosis with no intact adenoma cells. Ghost cells, with only ghost outlines and an acellular structure, were considered a unique manifestation of the pathological diagnosis of coagulative necrosis (Semple et al., 2008; Wang et al., 2019). It has been reported that the cottage cheese–like tissue, which may appear as iso- to hyperintensity on T1WI and non-enhancement on MRI after gadolinium injection, is in accordance with massive coagulative necrosis that has no intact adenoma cells under the light microscope (Wang et al., 2019).

Despite the advances in neurosurgical techniques and neurointensive care, there is still a lack of agreement regarding the best management of pituitary apoplexy. Supportive treatment and hormone replacement are essential for preoperative care and endocrine dysfunction. In this study, patients with hyponatremia, low serum cortisol, and secondary hypothyroidism received supportive therapy.

Four patients presented with headache and transient double vision; all patients received medical therapy and watchful observation. Surgical intervention was finally performed due to visual disturbance onset. Some studies have revealed an increasing role for conservative therapy in select cases, which often did not experience visual disturbance (Maccagnan et al., 1995; Ayuk et al., 2004; Gruber et al., 2006; Bujawansa et al., 2014; Singh et al., 2015). Five large retrospective studies compared the outcomes of conservative therapy and surgical treatment of patients with PA, and found that conservative treatment was able to achieve acceptable outcomes compared with surgical treatment (Ayuk et al., 2004; Sibal et al., 2004; Gruber et al., 2006; Leyer et al., 2011; Bujawansa et al., 2014). Although selection bias cannot be ignored that patients who received conservative treatment may have less severe visual dysfunction, therefore, pituitary apoplexy is increasingly considered to be a uniform diagnosis. For mild symptoms and subacute onset, conservative therapy should be more considered.

The surgical practice reflected the high rate of visual disturbance in our study. Moreover, 93.48% (43/46) of patients presented with visual loss and/or ocular palsy, which prompted the patients to receive surgical treatment. Four patients had almost no light perception. Neuro-ophthalmic outcomes were satisfactory that all patients obtained partial or complete remission, which was consistent with previous reports (Semple et al., 2006, 2008; Wang et al., 2019). However, there was no significant difference in ophthalmological outcomes between the early and the delayed surgery groups (**Table 2**). Furthermore, except for one patient who had slowly progressing visual loss and received early surgery due to sudden onset of headache, the rest of the patients obtained a partial visual recovery in the delayed surgery group. In addition, although **Table 2** showed that there were no significant differences in preoperative baseline characteristics between the early and the delayed surgery groups, the incidence of visual loss was higher in the early surgery group. There is a possibility that patients in the early surgery group may have more severe ophthalmological dysfunction and therefore achieves a more favorable prognosis similar to that within the delayed surgery group. Moreover, there is a lack of a good evaluation system for preoperative ophthalmological dysfunction. Using Pituitary Apoplexy Score system (Rajasekaran et al., 2011), mild bilateral visual acuity impairment would obtain a higher score than the patients with unilateral blindness. The patients with unilateral blindness were

more severe and more likely to receive early surgical intervention. Several studies reported that early surgical intervention did not show any statistically significant differences in the visual outcomes compared with the delayed surgery group (Bujawansa et al., 2014; Giritharan et al., 2016; Rutkowski et al., 2018). However, the selection bias in these studies cannot be ignored. Patients with severe situations would be more likely to undergo early surgical intervention. In contrast, several studies revealed that early surgical intervention could significantly improve visual outcomes (Bills et al., 1993; Randeva et al., 1999; Woo et al., 2010; Seuk et al., 2011). Generally, surgical intervention can achieve marked decompression and obtain satisfactory visual outcomes. Although the effect of surgical timing is still in the debate, early surgery should be advocated and seems to have a better visual outcome in this study.

Acute endocrine dysfunctions are prevalent in PA at the onset. It is reported that up to 80% of patients developed partial or panhypopituitarism (Rajasekaran et al., 2011). Our study reported a higher incidence of partial pituitary dysfunction, up to 91.30% (42/46). Similar to classic pituitary apoplexy, pituitary dysfunction showed no significant improvement regardless of the timing of surgical intervention (**Table 2**; Sibal et al., 2004; Gruber et al., 2006; Bujawansa et al., 2014; Singh et al., 2015; Giritharan et al., 2016; Rutkowski et al., 2018). Sixty-five percent of patients still received hormone replacement therapy at the last follow-up. Arafah et al. (1990) reported that the cases with pituitary dysfunction of classic PA recovered after surgical treatment. However, this study only included eight patients, a small sample size that does not provide strong recommendations. Surgery rarely leads to new pituitary dysfunction for patients without preoperative pituitary dysfunction. In general, endocrine dysfunction has shown no improvement following surgical intervention, and most patients still need long-term hormone replacement therapy postoperatively.

Patients in this study all obtained satisfactory curative effects after TSS without any complications. The total tumor resection rate was significantly higher in the early surgery group than in the delayed surgery group ($p = 0.009$, continuity correction) (**Table 2**). This result may be attributed to the high rate of invasiveness among the patients who underwent early surgery ($p = 0.049$, Pearson χ^2) (**Table 2**). As mentioned previously, a tumor with more invasiveness leads to an increase of intrasellar pressure; thus, it is prone to experiencing pituitary apoplexy (Rovit and Fein, 1972). Therefore, periodic follow-up is necessary for the management of the disease. In summary, conservative therapy is suitable for selective patients; however, watchful observation is still necessary. Even during the long course of symptom onset, surgical intervention can achieve satisfactory outcomes regardless of surgical timing. The main strengths of our study are the large sample size of patients with ischemic PA, detailed clinical data, prognostic information, and prolonged follow-up. However, this study has limitations like all retrospective studies including selection bias and missing data. Two patients were lost during the follow-up. MRI examination was not implanted for four patients and partial endocrine information was missed in several patients. Besides, some important clinical factors are not available due to the limit of retrospective nature, like Ki-67. Furthermore, quite a few patients did not receive timely surgical decompression. Possible reasons are as follows: (1) Patients with chronic symptom onset are more likely to receive delayed surgery due to relatively mild symptoms and long onset duration; (2) many patients are transferred from other tertiary hospitals, which is a time-consuming process. A larger prospective multicenter controlled study and experimental investigation should be conducted to elucidate the natural history and pathogenesis of ischemic PA as well as formulate guidelines for the management of ischemic PA.

CONCLUSION

Patients with ischemic PA can be accurately diagnosed by typical imaging characteristics preoperatively. The timing of surgical intervention does not significantly affect the resolution of neurological and endocrinological dysfunctions. Preoperative endocrine dysfunctions are common and usually appear to be poor after surgical intervention.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Beijing Tiantan Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

QZ and LW: study concept and design. QZ, YcL, ZF, and YkL: data acquisition and analysis. QZ and CZ: formal analysis and investigation. QZ: writing—original draft preparation. YcL, ZF, and YW: writing—review and editing. HZ, TL, JY, YZ, and YW: resources. LW: supervision and funding acquisition. All authors contributed to the article and approved the submitted version.

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Prognostic Analysis for Patients With Parathyroid Carcinoma: A Population-Based Study

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Background: Parathyroid carcinoma (PC) is a rare but often lethal malignancy for which staging system, prognostic indicators, and treatment guidelines are still not established. We aimed to explore the prognostic parameters and construct a nomogram for cancer-specific survival (CSS) of PC.

Methods: A retrospective analysis of 604 PC patients in the SEER database from 2001 through 2018 was performed. All the cases were randomly assigned to the training cohort ($n = 424$) or the validation cohort ($n = 180$) at a ratio of 7:3. The Kaplan–Meier method and Cox regression model were applied to estimate the CSS and risk factors, and a nomogram was constructed. The predictive accuracy and discriminative ability of the nomogram in CSS were assessed by concordance index (C-index), the area under the curve (AUC) of receiver operating characteristics (ROC), and the calibration curve.

Results: Age at diagnosis > 70 years [hazard ratio (HR): 3.55, 95% CI: 1.07–11.78, $p = 0.039$] and tumor size > 35 mm (HR 4.22, 95% CI: 1.67–10.68, $p = 0.002$) were associated with worse CSS. Compared with distant metastasis, localized (HR 0.17, 95% CI: 0.06–0.47, $p = 0.001$) and regional lesions (HR 0.22, 95% CI: 0.07–0.66, $p = 0.007$) showed an improved CSS rate. Parathyroidectomy was the recommended treatment ($p = 0.02$). The C-index of the nomogram was 0.826, and the AUC for 5-, 10-, and 15-year CSS was 83.7%, 79.7%, and 80.7%, respectively. The calibration curve presented good agreement between prediction by nomogram and actual observation.

Conclusion: Age at diagnosis > 70 years, tumor size > 35 mm, and distant metastasis were independent risk factors for PC-specific mortality. Parathyroidectomy was currently the most recommended treatment for PC. This nomogram provided individualized assessment and reliable prognostic prediction for patients with PC.

Keywords: parathyroid carcinoma, prognostic factor, cancer-specific survival (CSS), nomogram, validation

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BACKGROUND

Parathyroid carcinoma (PC) is a rare endocrine malignancy, accounting for 0.005% of all malignancies (Salcuni et al., 2018) and 0.5–5% of all patients with primary hyperparathyroidism (PHPT) (Rawat et al., 2005). Patients with PC are often characterized by markedly elevated serum calcium and parathyroid hormone (PTH) (Cetani et al., 2016) who typically present with

metabolic complications, including renal failure, bone disease, pancreatitis, cardiac arrhythmia, and occasionally a neck mass (Schulte and Talat, 2012). Most patients succumb to target organ damage caused by uncontrollable hypercalcemia rather than tumor burden. As PC is difficult to distinguish from parathyroid adenoma (PA), its preoperative and even intraoperative diagnoses are challenging. Preoperative ultrasound, although not suggestive of malignancy, is usually helpful in locating the abnormal parathyroid glands. As a supplement, CT may reveal an invasive parathyroid tumor and suggest a possible malignancy (Harari et al., 2011). However, it was reported that about 25% of cases are not being recognized by the surgeon at the time of initial parathyroidectomy (Kebebew, 2001). In terms of molecular diagnostics, if the PHPT patients were found to have the CDC73 (alternatively known as HRPT2) and/or MEN1 gene mutation, PC should be highly suspected (Schulte and Talat, 2012). The histological characteristics of PC reported in the literature include capsule infiltration, angioinvasion, tumor necrosis, fibrosis, numerous mitotic figures, and nuclear atypia (Schantz and Castleman, 1973; Bondeson et al., 1993). The use of adjuvant therapy is currently controversial owing to the lack of evidence-based clinical practice. Radical surgery with sufficient margins has been recommended as the only potential cure for PC (Schulte et al., 2014), although there is no consensus on a systematic oncological surgical approach or a dedicated terminology to describe it. Postoperatively, the clinical course of PC patients varies greatly. Recurrence was most common within the neck, with an r rate of 40–60% (Lee et al., 2007; Talat and Schulte, 2010). Distant metastasis occurs in about 30% of cases, usually to the lung and bone, and less frequently to the liver and visceral organs (Schulte and Talat, 2012; Lenschow et al., 2020).

Nevertheless, due to the rarity of PC and the paucity of large cohort studies or prospective research, it is still difficult to counsel patients on their natural course and prognosis. A population-based database allows us to have a large enough sample size to answer this question. In this study, we sought to conclude the clinicopathological features and explore the prognostic factors associated with cancer-specific survival (CSS) utilizing the national Surveillance, Epidemiology, and End Results (SEER) database. Meanwhile, we developed and internally validated a clinical nomogram incorporating independent prognostic factors, which can predict the CSS in patients with PC. It may provide individualized assessment and reliable prognostic prediction for patients with PC, which may build a foundation for a staging system.

MATERIALS AND METHODS

Data Source and Patient Selection

The data of patients with PC from 2001 through 2018 were extracted from 18 population-based cancer registries of the SEER database[1] using the SEER*Stat program (version 8.3.9), which is a cancer incidence registry that includes about 30% of the United States population. The extraction conditions were as follows: "Primary Site = C75.0-Parathyroid gland" and "Behavior code ICD-0-3 = Malignant." The following variables were extracted: patient ID, Age at diagnosis, Sex, Year of diagnosis, Race/ethnicity, Laterality, Histology, Combined Summary Stage (extent of disease), Chemotherapy recode, Radiotherapy recode, Surgery of primary site, Tumor Size, Regional nodes examined, Regional nodes positive, Survival months, and SEER cause-specific death classification. The exclusion criteria in the study were as follows: a) unknown vital status (study cutoff used) and b) metastatic disease originating from other organ sites. The demographic and clinicopathological data of all eligible cases were collected and retrospectively analyzed.

Cohort Definition and Variable Recode

The patients with PC were divided into the training and validation cohorts with a ratio of 7:3 using the R studio (version 4.0.3[2]) function "createDataPartition" to ensure that outcome events were distributed randomly between the two cohorts. The training cohort was used to screen variables and construct the nomogram predictive model, while the validation cohort was applied to validate the model based on the training cohort.

The variables from the selected cohorts included the following: age at diagnosis ($\leq$70 and $>$ 70), gender (male and female), race (white, black and other), extent of disease (distant, regional, localized, and unknown), surgery of primary site, radiotherapy (yes and no), lymph node (LN) metastasis (yes and no), and tumor size ($\leq$35 and $>$ 35 mm). The cutoff point of continuous variables such as age and tumor size for risk stratifications was generated by the "surv_cutpoint" function of the "Survminer" R package. Surgery of primary site was divided into four subgroups: no surgery, en bloc radical resection, parathyroidectomy (simple/partial surgical removal of primary site, total surgical removal of primary site and local tumor excision), and other.

The main endpoint was CSS according to data in the SEER database. CSS was defined as the proportion of patients with a type of cancer who did not die of cancer after a specific time period. These patients may still be alive, or they may have died of some other cause.

Construction of the Nomogram

Cancer-specific survival of patients in the different risk groups was assessed using the Kaplan–Meier method. Univariate Cox proportional hazards model was used to check each factor's power in predicting CSS. Subsequently, factors with a p-value < 0.05 in univariate analysis were further analyzed in a multivariate Cox proportional hazards model using a backward model selection procedure (elimination criterion: $p > 0.10$). The hazard ratios (HRs) and corresponding 95% CIs were calculated. Finally, according to the regression coefficients of each independent risk factor in the multivariate analysis, the nomogram was visualized to predict the probability of 5-, 10-, and 15-year CSS rates in patients with PC.

[1] https://seer.cancer.gov/

[2] http://www.r-project.org

Discrimination and Calibration of the Nomogram

The performance of the nomogram was evaluated by discrimination and calibration. The C-index was calculated to reflect the discrimination ability. The value of the C-index varies from 0.5 to 1.0, where 0.5 represents random chance, and 1.0 indicates a perfect ability to stratify patients into different prognosis groups. Meanwhile, time-dependent receiver operating characteristic (tROC) curves and the corresponding area under the curve (AUC) values at 5, 10, and 15 years were utilized to estimate the predictive accuracy. Typically, C-index and AUC values greater than 0.7 suggest a reasonable estimation.

Calibration curves with 1,000 bootstrap resamples were generated to test the calibration of the nomogram, which showed the correlation between the predicted probability and the frequency of the observed outcome. The standard curve was a straight line with slope 1 through the origin of the axes. The closer the calibration curve was to the standard curve, the better the prediction ability of the nomogram was.

Statistical Analysis

Continuous variables conforming to the normal distribution were represented as the mean ± SD; otherwise, the median and interquartile range (IQR) were used. Categorical variables were shown as frequencies and their proportions. Statistical differences of distribution in variables between the training cohort and the validation cohort were analyzed by using the chi-square test. Statistical significance was cohort at two-sided $p < 0.05$. All statistical analyses and visualization are performed using the R studio version 4.0.3 software (see text footnote 2).

Ethics Statement

This study was exempt from the approval processes of the Institutional Review Boards because the SEER database patient information was de-identified.

RESULTS

Demographics and Clinicopathological Characteristics of Patients

A total of 604 patients with PC were extracted from the SEER database from 2001 to 2018. The baseline clinicopathological characteristics and treatment information of all patients were summarized in **Table 1**. The median age of all patients was 59 years (IQR: 48–68). The incidence of PC was roughly the same in men (51.5%) and women (48.5%). Among all patients, 455 (75.3%) patients were white, 601 (99.5%) patients had a unilateral lesion, 543 (89.9%) patients were not recorded to have a specific pathological type, and 577 (95.5%) patients accepted the treatment. A minority of patients who had distant metastasis (5.0%) and LN metastasis (6.1%) experienced radiotherapy (10.6%) and chemotherapy (0.2%). The median follow-up time was 76 months (IQR: 33–135),

TABLE 1 | Demographics and clinicopathological characteristics of all patients with parathyroid carcinoma.

Characteristic	Level	Number/ Median	%/IQR
N		604	100%
Age (year)		59	[48.0, 68.0]
Age group			
	≤20	4	0.7%
	>20, ≤40	77	12.7%
	>40, ≤60	255	42.2%
	>60, ≤80	232	38.4%
	>80	36	6.0%
Gender			
	Male	311	51.5%
	Female	293	48.5%
Race			
	White	455	75.3%
	Black	98	16.2%
	Other	51	8.4%
Follow-up time (months)		76	[33.0,135.0]
Laterality			
	Unilateral	601	99.5%
	Bilateral	3	0.5%
Histology type			
	Carcinoma, NOS	543	89.9%
	Papillary carcinoma	24	4.0%
	Adenocarcinoma	20	3.3%
	Other	17	2.8%
Extent of disease			
	regional	178	29.5
	localized	347	57.5%
	distant	30	5.0%
	unknown	49	8.1%
Treatment			
	Yes	577	95.5%
	No	27	4.5%
Surgery			
	Parathyroidectomy	488	80.8%
	En bloc radical resection	51	8.4%
	Other	38	6.3%
	No surgery	27	4.5%
Radiotherapy			
	Yes	64	10.6%
	No	540	89.4%
Chemotherapy			
	Yes	1	0.2%
	No	603	99.8%
Lymph node metastasis			
	Yes	37	6.1%
	No	135	22.4%
	NA	432	71.5%
Tumor size (mm)			
	≤20	125	20.7%
	>20, ≤40	189	31.3%
	>40, ≤60	43	7.1%
	>60	20	3.3%
	NA	227	37.6%
Tumor-specific death			
	Yes	62	10.3%
	No	542	89.7%

IQR, interquartile range; NOS, not otherwise specified; mm, millimeter.

and the proportion of tumor-specific deaths was about 10.3%. The number of cases diagnosed each year was visualized as **Figure 1**, which showed that the incidence rate of PC presented a steady trend over the past two decades. The patient characteristics of the training ($n = 424$) and validation cohorts ($n = 180$) were concluded in **Table 2**. Since all cases were randomly assigned to the two cohorts, there was no statistical difference in the distribution of variables between them (all the p-values > 0.05).

Independent Prognostic Factors in the Training Cohort

To evaluate the impact of different factor on CSS of PC patients, the Kaplan–Meier survival analysis was performed in the training cohort. As shown in **Figure 2**, there were significant differences of CSS among age ($p = 0.023$), extent of disease ($p < 0.001$), surgery ($p < 0.001$), and tumor size ($p = 0.008$). Further, based on the univariate (**Figure 3**) and multivariate Cox (**Figure 4**) proportional hazards regression analyses, four independent prognostic factors were identified in the training cohort: age (>70: HR 3.55, 95% CI: 1.07–11.78, $p = 0.039$), tumor size (>35 mm: HR 4.22, 95% CI: 1.67–10.68, $p = 0.002$), extent of disease (localized: HR 0.17, 95% CI: 0.06–0.47, $p = 0.001$; and regional: HR 0.22, 95% CI: 0.07–0.66; $p = 0.007$), and surgery (parathyroidectomy: HR 0.29, 95% CI: 0.10–0.83; $p = 0.021$) were all significantly associated with CSS in patients with PC.

Prognostic Nomogram for Cancer-Specific Survival

As shown in **Figure 5**, the nomogram based on the above four independent prognostic factors was developed for the prediction of the 5-, 10-, and 15-year CSS rates in patients with PC. It demonstrated that the extent of disease contributed the most to the prognosis. Each level of each variable was assigned a score on the points scale. The total score was obtained by adding the scores of each selected variable. Then, the prediction corresponding to that total score helped to predict the CSS rate for each patient.

Validation and Calibration of the Nomogram

The C-index value was 0.826 in the training cohort and 0.872 in the validation cohort, which was greater than 0.7, reflecting the good discrimination ability of the model. The AUC values in the ROC curve were 83.7 (95% CI: 75.3–92.0), 79.7 (95% CI: 70.6–89.2), and 80.7 (95% CI: 71.0–90.3) in the training cohort and 89.5 (95% CI: 79.9–99.1), 81.1 (95% CI: 67.6–94.6), and 79.8 (95% CI: 65.1–94.5) at 5, 10, and 15 years in the validation cohort, respectively (**Figure 6**). The calibration curves also presented a favorable consistency between the actual observation and the nomogram prediction of the 5-, 10-, and 15-year CSS rates in both the training and validation cohorts (**Figure 7**).

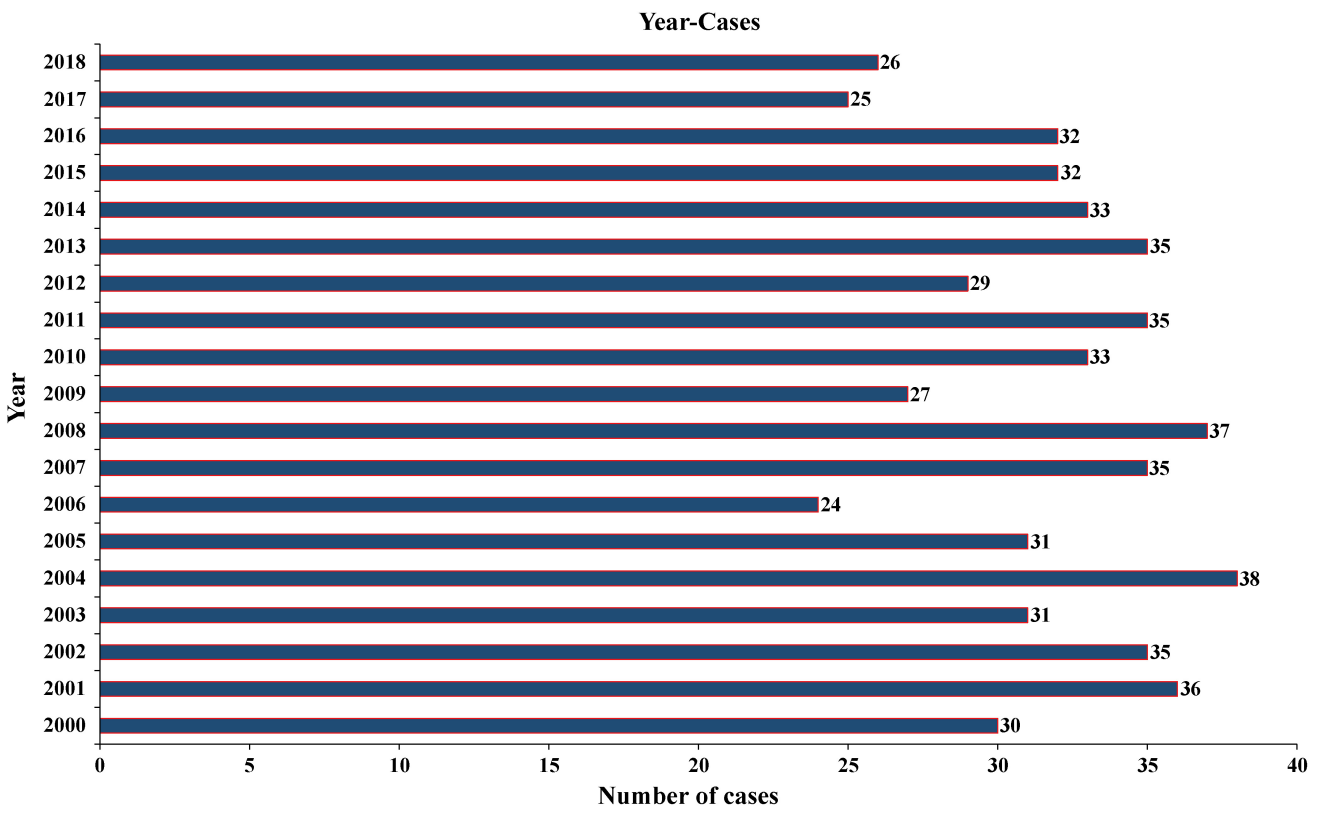

FIGURE 1 | Annual number of PC cases recorded in 18 registries of the United States from 2001 to 2018. PC, parathyroid carcinoma.

TABLE 2 | Baseline clinicopathological characteristics and treatment experience of patients in the training and validation cohort (the subdivision of the continuous variables age and diameter of the tumor generated by the "surv_cutpoint" function of the "Survminer" R package).

Characteristic	Level	Training cohort	Validationcohort	*P* value
N	604	424	180	
Age (%)				0.853
	≤70	335 (79.0)	141 (78.3)	
	>70	89 (21.0)	39 (21.7)	
Gender (%)				0.903
	Female	205 (48.3)	88 (48.9)	
	Male	219 (51.7)	92 (51.1)	
Race (%)				0.242
	White	314 (74.1)	141 (78.3)	
	Black	69 (16.3)	29 (16.1)	
	Other	41 (9.7)	10 (5.6)	
Region (%)				0.807
	unknown	36 (8.5)	13 (7.2)	
	regional	124 (29.2)	54 (30.0)	
	localized	245 (57.8)	102 (56.7)	
	distant	19 (4.5)	11 (6.1)	
Surgery (%)				0.780
	No surgery	21 (5.0)	6 (3.3)	
	En bloc radical resection	35 (8.3)	16 (8.9)	
	Other	28 (6.6)	10 (5.6)	
	Parathyroidectomy	340 (80.2)	148 (82.2)	
Radiotherapy (%)				0.549
	No	377 (88.9)	163 (90.6)	
	Yes	47 (11.1)	17 (9.4)	
Tumor size (%)				0.473
	≤35	192 (45.3)	89 (49.4)	
	>35	66 (15.6)	30 (16.7)	
	NA	166 (39.2)	61 (33.9)	
Lymph node metastasis (%)				0.458
	No	89 (21.0)	46 (5.6)	
	Yes	27 (6.4)	10 (5.6)	
	NA	308 (72.6)	124 (68.9)	
Tumor-specific death (%)				
	No	383 (90.3)	159 (88.3)	0.460
	Yes	41 (9.7)	21 (11.7)	

DISCUSSION

The accurate and effective prognostic evaluation was of great clinical significance for individualized treatment and follow-up. However, due to the rarity of PC, there was little clinical evidence about its prognosis, and no predictive model was available for predicting the prognosis of patients with PC. In this population-based study, we identified four independent prognostic factors of the patients with PC based on the SEER database: age, extent of disease, surgical approach, and tumor size. At the same time, the nomogram prediction model was used to visualize the overall impact of these factors on the CSS rate of each patient. Validation of the nomogram showed it had good discriminative and calibration ability.

The incidence of PC was reported differently in different countries and regions. It was considered to account for approximately 1% of all cases of PHPT (Wilhelm et al., 2016) and 0.005% of all cancers in the United States (Ferraro et al., 2019). However, in China, the proportion of PC in the PHPT rose to 5–7% (Zhao et al., 2013), and its annual incidence in the European Union was about 2 cases per 10 million people in 2008 (van der Zwan et al., 2012). According to an analysis from the SEER database, the incidence of PC increased significantly from 3.58 to 5.73 per 10 million people from 1988 to 2003 (Lee et al., 2007). This may be due to the change in the National Institutes of Health (NIH) guidelines for parathyroidectomy for asymptomatic hyperparathyroidism (2002) leading to more PC patients being diagnosed early *via* increased serum calcium screening (Asare et al., 2015). Lo et al. (2018) reported that the increasing incidence was primarily due to smaller tumors (<3 cm) and regional disease (locally invasive and LN-positive disease). They also demonstrated that

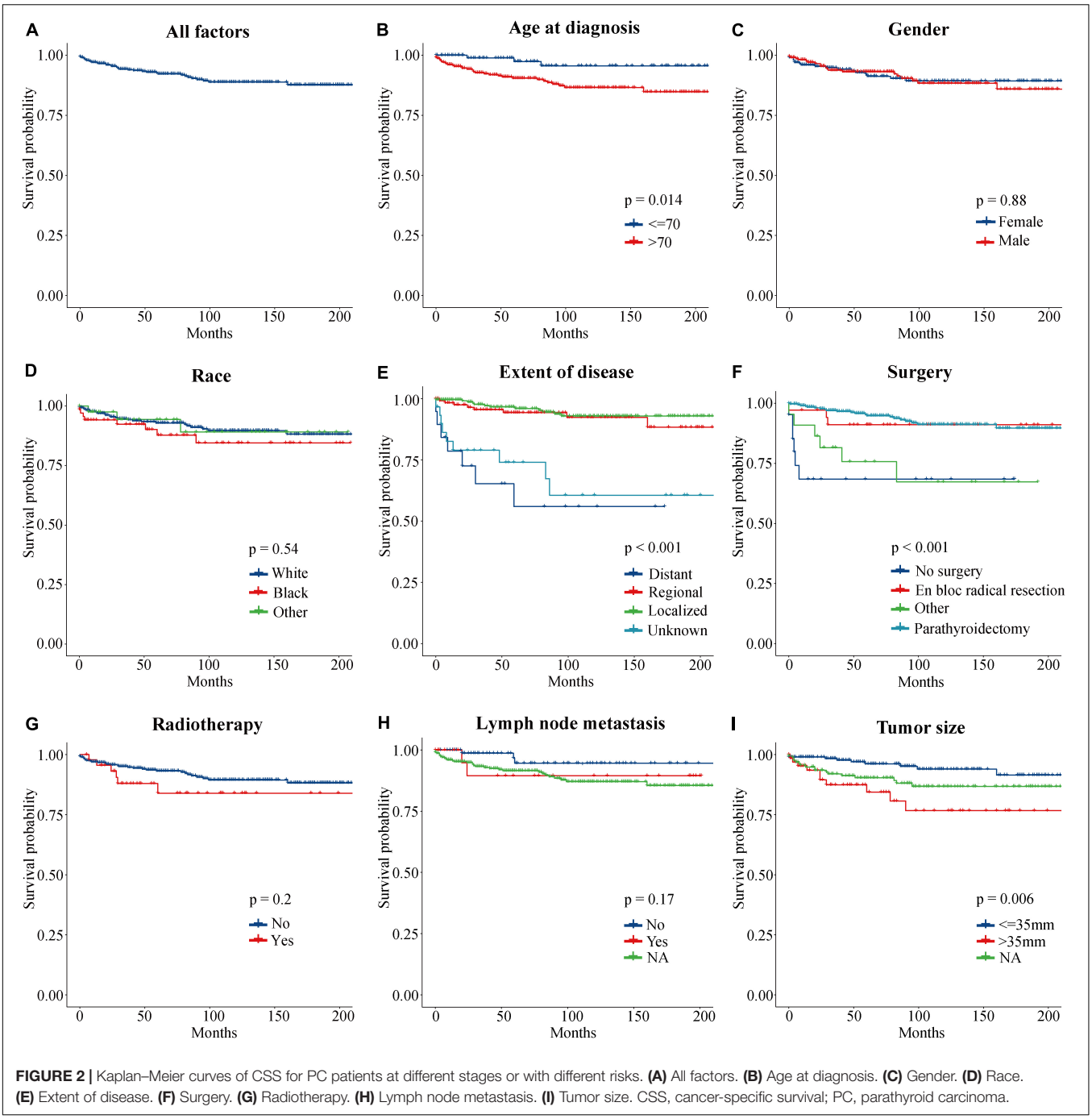

FIGURE 2 | Kaplan–Meier curves of CSS for PC patients at different stages or with different risks. **(A)** All factors. **(B)** Age at diagnosis. **(C)** Gender. **(D)** Race. **(E)** Extent of disease. **(F)** Surgery. **(G)** Radiotherapy. **(H)** Lymph node metastasis. **(I)** Tumor size. CSS, cancer-specific survival; PC, parathyroid carcinoma.

the incidence of PC has not increased since 2001 and instead remained stable, which was consistent with our findings. As shown in **Figure 1**, from 2001 to 2018, the number of cases recorded in 18 registered areas in the United States had remained at about 30 each year.

Regarding the effect of tumor size on the PC patient's prognosis, previous studies have drawn different conclusions. Hundahl et al. (1999) analyzed 286 PC patients in the National Cancer Database (NCDB) from 1985 to 1995 and found that tumor size was not an important prognostic marker, which was supported by the research of Lee et al. (2007) and Talat and Schulte (2010). However, Hsu concluded that tumor size $\geq$ 3 cm was associated with LN metastasis (Hsu et al., 2014), and Asare et al. (2015) found that tumor size $>$ 4 cm was correlated with an increased risk of death from PC. Among 520 patients with PC in the SEER database, tumor size $>$ 3 cm was associated with worse CSS in patients with PC (HR 5.60, 95% CI: 1.50–21.20, $p = 0.012$) (Lo et al., 2018). A retrospective review from a tertiary-referral cancer hospital suggested that tumor size $>$ 3.2 cm may increase the risk of distant metastasis by more

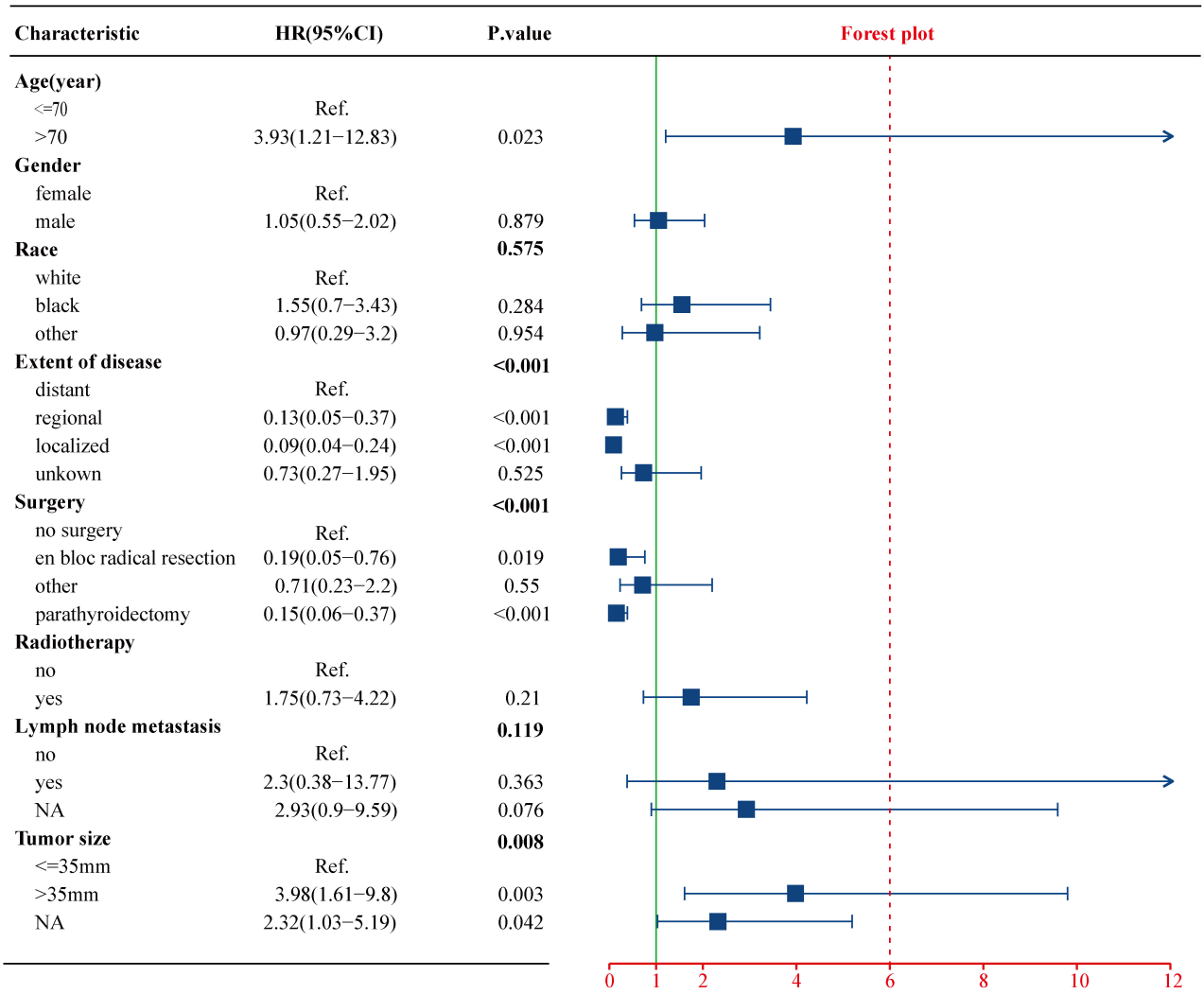

FIGURE 3 | Univariate Cox regression analysis of the clinicopathological parameters for CSS using the training cohort. CSS, cancer-specific survival.

than three times (Asare et al., 2019). In this study, Kaplan–Meier survival (**Figure 2I**) and Cox regression (**Tables 1**, **2**) analysis jointly confirmed that tumor size > 35 mm was an independent adverse prognostic factor for PC patients. Patients with tumors size > 35 mm had an increased risk of cancer-specific death as compared to < 35 mm (HR 4.22, CI: 1.67–10.68, $p = 0.002$).

Distant metastasis was uniformly considered a poor prognostic factor for PC patients. In the present study, localized (HR 0.17, CI: 0.06–0.47, $p = 0.001$) or regional (HR 0.22, CI: 0.07–0.66, $p = 0.007$) PC showed significantly higher CCS as compared with distant metastases (in terms of the extent of disease, the SEER database defined "distant" as a neoplasm that had spread to parts of the body remote from the primary tumor either by direct extension or by discontinuous metastasis to distant organs or tissues or *via* the lymphatic system to distant LNs). Moreover, the nomogram exhibited that the degree of disease contributed the most to the prognosis (**Figure 5**). The primary data recorded for distant metastases in this study included the lung (4 cases), brain (1 case), bone (1 case), and other organs (2 cases). Similarly, Asare et al. (2019) found that distant metastasis reflected the highest risk factor for survival in patients with PC by compiling 37 years' worth of patient management data from a single institution. At the same time, they noted that the lung was the most common site of metastasis and that patients with bone metastases may have a shorter survival period. Lee et al. (2007) and Harari et al. (2011) also clarified a definite association between metastatic disease at diagnosis and poorer overall survival (OS). Lo et al. (2018) confirmed that the presence of metastatic disease was prognostic and associated with CSS of PC patients.

Unexpectedly, no clear relationship was found between regional LN metastasis and prognosis of patients with PC (**Figures 2H**, **3**). This may be due to the absence of complete information about LN status in the vast majority of patients (71.5%); no strong conclusion could be drawn about the prognostic value of LN status. The same phenomenon had

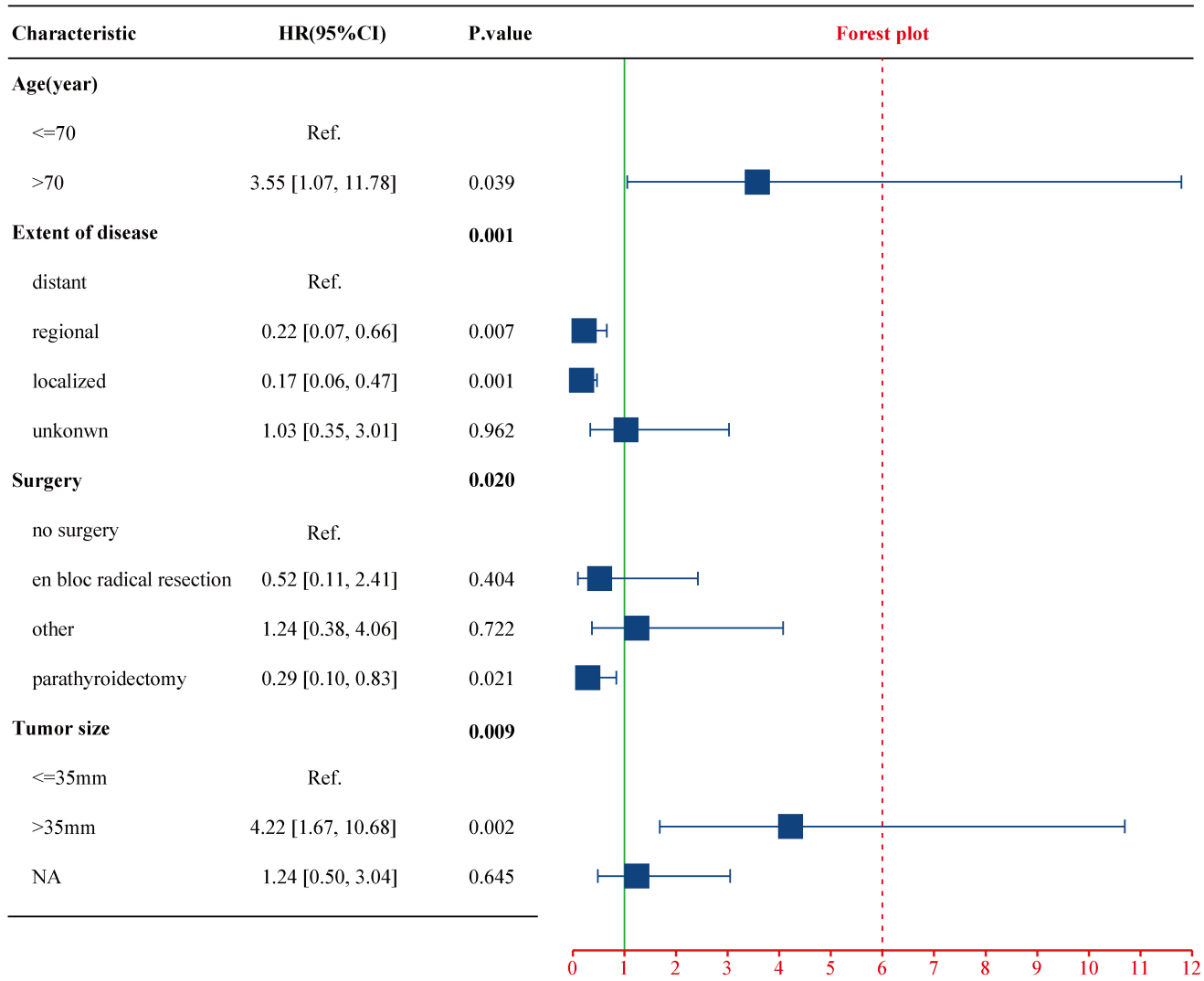

FIGURE 4 | Multivariate Cox regression analysis of the select factors for CSS using the training cohort. CSS, cancer-specific survival.

been seen in similar studies of SEER databases (Hsu et al., 2014; Lo et al., 2018). However, some studies suggested that positive LNs were associated with poor prognosis. A study from the NCDB database confirmed that positive LNs predict lower OS and an increased risk of death (Sadler et al., 2014). The researchers suggested to remove ipsilateral central compartment LN dissection for patients with clinically suspicious LNs, but not prophylactic ipsilateral central LN dissection for all patients. And Schulte et al. (2012) proposed to place all PC patients with positive LNs, distant metastases, and vascular or tissue invasion into a high-risk category. Therefore, this study believed that the effect of LN status on the prognosis of PC patients still needed more prospective large-sample studies to confirm.

Age at diagnosis > 70 years proved to be an independent risk prognostic factor for PC, associated with poorer CSS (HR 3.55, 95% CI: 1.07–11.78, $p = 0.039$). Some previous studies had confirmed similar findings. Sadler et al. (2014) demonstrated that age at diagnosis > 57 years increased the risk of death 5 years after surgery and portended a poor OS. Lee et al. (2007) observed that young age was associated with an improved OS rate. However, since PC was a relatively indolent disease and the median age at diagnosis was 59 years (**Table 1**), OS as the primary endpoint may be confounded by lifespan and current comorbidity of the patients. Lo et al. had analyzed age at diagnosis as categorical variables, using thresholds of < 45, 45–59, 60–69, 70–79, and 80 + years, but did not find age at diagnosis to be associated with an increased risk of cancer-specific death. This may be due to the improper cutoff values concealing the difference between the age group. Silva-Figueroa et al. (2017) took the recurrence-free survival (RFS) as the endpoint of the study, confirming that age at diagnosis older than 65 years was negatively correlated with RFS rate (Zhou et al., 2021).

The only curative treatment of PC was surgery and the best chance of cure could be achieved by complete

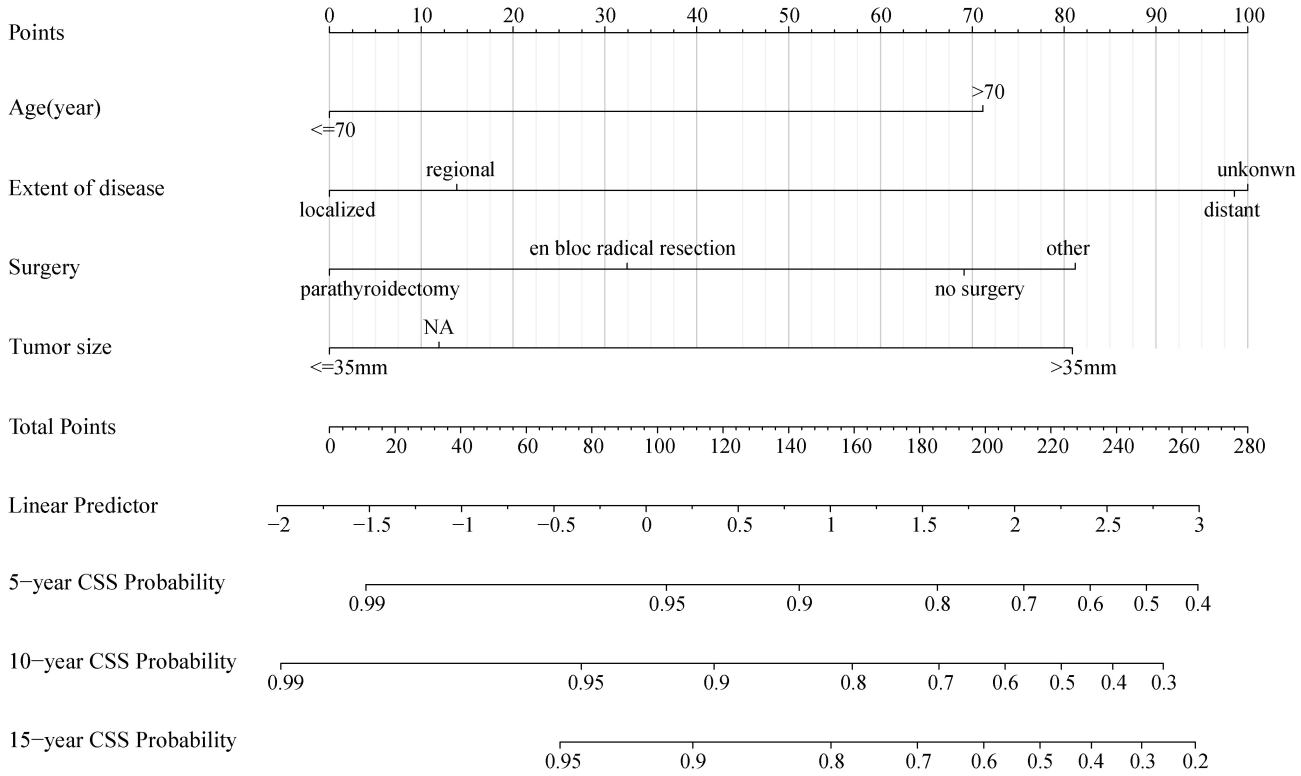

FIGURE 5 | Nomogram for predicting probabilities of cancer-specific survival in patients with parathyroid carcinoma. NA, not availability.

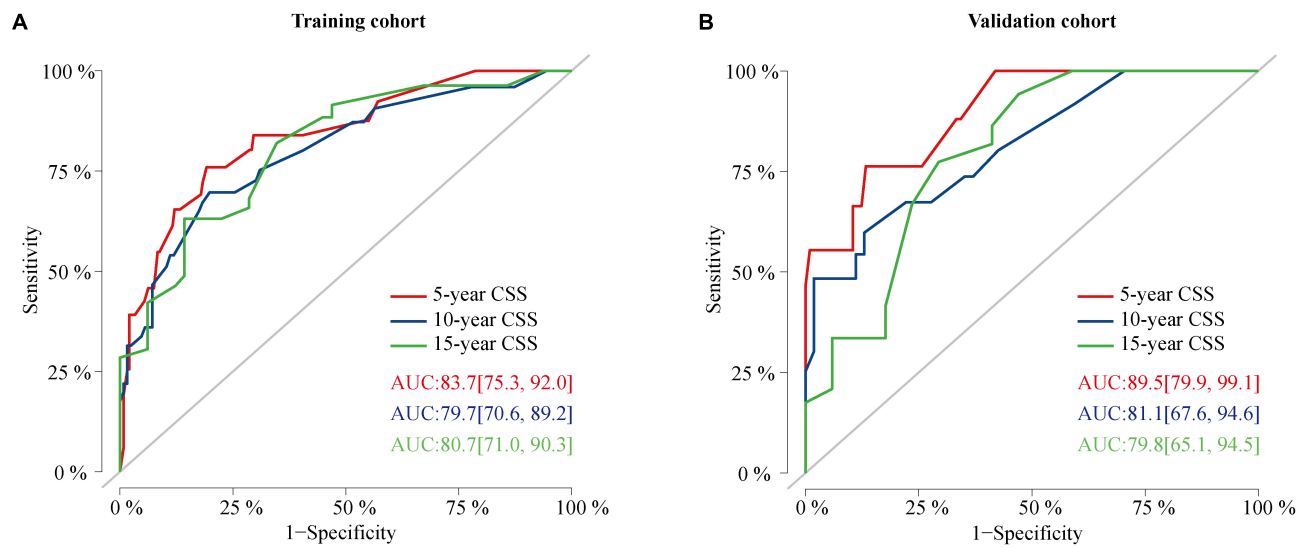

FIGURE 6 | ROC curves of the nomogram. **(A)** ROC curves of the nomogram for predicting CSS probability at 5, 10, and 15 years in the training cohort. **(B)** ROC curves of the nomogram for predicting CSS probability at 5, 10, and 15 years in the validation cohort. ROC, receiver operating characteristic curve; CSS, cancer-specific survival.

excision, avoiding capsular disruption at the first operation (Schulte and Talat, 2012; Wilhelm et al., 2016; Rodrigo et al., 2020). Survival analysis (**Figure 2F**) showed that the CSS rates of parathyroidectomy and en bloc radical resection were significantly higher than those of non-surgical patients ($p < 0.001$). Univariate Cox analysis showed that compared with non-surgical patients, parathyroidectomy (HR 0.15, CI: 0.06–0.37, $p < 0.001$) and en bloc radical resection (HR

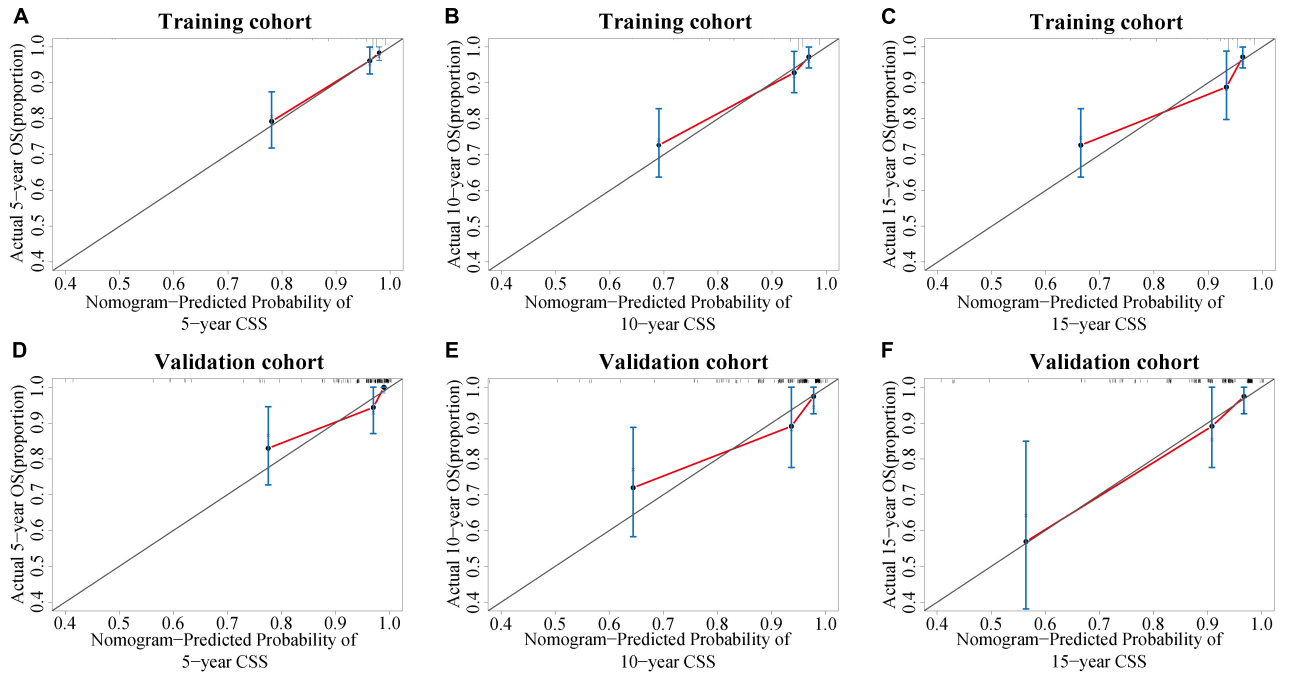

FIGURE 7 | Calibration curves of the nomogram. **(A–C)** Calibration curves of the nomogram for predicting the 5-, 10-, and 15-year CSS of PC patients in the training cohort. **(D–F)** Calibration curves of the nomogram for predicting the 5-, 10-, and 15-year CSS of PC patients in the internal validation cohort. The x-axis indicates the predicted survival probability, and the y-axis indicates the actual survival probability. The diagonal 45° line (gray line) indicates that the prediction agreed with actuality. CSS, cancer-specific survival; PC, parathyroid carcinoma.

0.19, CI: 0.05–0.76, $p < 0.019$) could improve the prognosis of PC patients, which was consistent with previous literature reports (Zhou et al., 2021). However, multivariate Cox analysis presented that only parathyroidectomy (HR 0.29, CI: 0.10–0.83, $p = 0.021$) was an independent prognostic factor for higher CSS rates. This finding could be interpreted as that the surgical treatment code in SEER was not specific enough to describe the exact scope of resection, which led to the possibility that the frequency of en bloc resection was underestimated. Regarding the management of the LNs, clinically suspected LNs should be removed, while prophylactic central or lateral neck dissection was not recommended (Christakis et al., 2016; Rodrigo et al., 2020).

For radiotherapy, neither survival analysis ($p = 0.2$) nor Cox regression analysis ($p = 0.239$) suggested that it could not improve the CSS rate in PC patients, which was consistent with the mainstream viewpoint (Asare et al., 2015; Christakis et al., 2016; Limberg et al., 2021). As PC was considered to be a radio-resistant tumor (Salcuni et al., 2018), a study by the Anderson Cancer Center confirmed that in high-risk cases, postoperative adjuvant radiotherapy achieves long-term disease control (Christakis et al., 2017). Therefore, the American Association of Endocrine Surgeons considered that adjuvant external radiation therapy should not be performed routinely after surgery but as a palliative option (Wilhelm et al., 2016). Chemotherapy was performed in only one patient in the study, and further analysis could not be explored. Nevertheless, literatures had certified that except for the partial response in a few case reports, cytotoxic chemotherapy has not shown effectiveness in the treatment of PC, and there were no standardized protocols to use it (Cetani et al., 2016; Tsoli et al., 2017). Additionally, this study also concluded that although there were multiple parathyroid glands, PC was mostly unilateral (99.5%), and the gender distribution for the 604 cases was nearly equal: 51.5% were male and 48.5% were female. There was no significant difference in the prognosis of PC among different races ($p = 0.575$).

As far as we know, this may be the first nomogram model to predict the CSS rate of patients with PC. Although the nomogram presented a good performance of discrimination and calibration, it has some limitations that should be acknowledged. Firstly, this study was a retrospective analysis with inherent biases. Secondly, the registry lacks preoperative calcium and PTH levels to identify biochemical prognostic. And recurrence was an important prognostic parameter for PC, but we were unable to evaluate it due to the lack of relevant records in the SEER database. Finally, although external validation was difficult to achieve due to the limited number of cases in a single institution, it was necessary.

CONCLUSION

In conclusion, this study demonstrated a relatively stable incidence trend of PC over the past two decades. Age at diagnosis > 70 years, tumor size > 35 mm, and distant metastasis

were independent risk factors for CSS in patients with PC. Gender, race, radiotherapy, and regional LN metastasis were not associated with CSS. Parathyroidectomy was currently the most recommended for PC. This nomogram provided individualized assessment and reliable prognostic prediction for patients with PC, which may build a foundation for a staging system. More future prospective studies are needed to confirm and improve this model.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov/data.

AUTHOR CONTRIBUTIONS

XQ: administrative support. BQ and YQ: collection and assembly of data. BQ, YQ, and LH: data analysis and interpretation. BQ: manuscript writing. SZ, LM, and XQ: critical revision of the manuscript. All authors contributed to the conception and design and approved the final manuscript.

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frontiers in Endocrinology

CASE REPORT
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Case Report: New CDKN1B Mutation in Multiple Endocrine Neoplasia Type 4 and Brief Literature Review on Clinical Management

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Background: The fourth type of multiple endocrine neoplasia (MEN) is known as a rare variant of MEN presenting a MEN1-like phenotype and originating from a germline mutation in CDKN1B. However, due to the small number of cases documented in the literature, the peculiar clinical features of MEN4 are still largely unknown, and clear indications about the clinical management of these patients are currently lacking. In order to widen our knowledge on MEN4 and to better typify the clinical features of this syndrome, we present two more cases of subjects with MEN4, and through a review of the current literature, we provide some possible indications on these patients' management.

Case Presentation: The first report is about a man who was diagnosed with a metastatic ileal G2-NET at the age of 34. Genetic analysis revealed the mutation p.I119T (c.356T>C) of exon 1 of CDKN1B, a mutation already reported in the literature in association with early-onset pituitary adenomas. The second report is about a 76-year-old woman with a multifocal pancreatic G1-NET. Genetic analysis identified the CDKN1B mutation c.482C>G (p.S161C), described here for the first time in association with MEN4 and currently classified as a variant of uncertain significance. Both patients underwent biochemical and imaging screening for MEN1-related diseases without any pathological findings.

Conclusions: According to the cases reported in the literature, hyperparathyroidism is the most common clinical feature of MEN4, followed by pituitary adenoma and neuroendocrine tumors. However, MEN4 appears to be a variant of MEN with milder clinical features and later onset. Therefore, these patients might need a different and personalized approach in clinical management and a peculiar screening and follow-up strategy.

Keywords: men, multiple endocrine neoplasia, CDKN1B, familiar, hyperparathyroidism, neuroendocrine tumor, pituitary adenoma

INTRODUCTION

Multiple endocrine neoplasia (MEN) is a rare genetic syndrome that predisposes patients to develop tumors in one or more endocrine organs. Depending on the endocrine glands most frequently involved, MEN is classified into different categories, numbered from 1 to 4. MEN1 is mainly characterized by primary hyperparathyroidism, pituitary adenomas, and neuroendocrine tumors (NETs) and is caused by a mutation in menin. MEN2A is characterized by medullary thyroid carcinoma, pheochromocytoma, and hyperparathyroidism, and MEN2B by medullary thyroid carcinoma, pheochromocytoma, ganglioneuromas, and musculoskeletal anomalies. Both MEN2A and MEN2B originate from a mutation in the RET oncogene. The fourth type of multiple endocrine neoplasia is the most recently introduced (1) and includes subjects phenotypically similar to MEN1 but not carrying any germline mutation in the the menin gene. This condition, previously known as MENX, was first reported in humans by Pellegata et al. (2), who described it as a potential cause of mutation of the CDKN1B gene.

CDKN1B encodes for the p27 protein, an important regulator of the cell cycle, which plays a pivotal role in a wide range of cellular activities such as inhibition of cyclin-dependent kinase, regulation of apoptosis, and interaction with the cytoskeleton (3). Heterozygous mutations in CDKN1B have been proven to encode for a p27 protein with either a truncated structure or reduced binding activity, leading to loss of major tumor-suppressor functions (4). As such, CKDN1B is thought to act as a tumor suppressor through a haploinsufficiency mechanism.

CDKN1B mutations have been associated with a wide variety of endocrine and non-endocrine neoplasms such as luminal breast cancer (5), prostate cancer (6), and hairy cell leukemia (7). However, the specific mechanism promoting the development of a MEN1-like phenotype is still unclear. Menin physiologically regulates both CDKN1B transcription and p27 expression through epigenetic factors, and its inactivation was found to induce a p27 downregulation (8). Therefore, tumor development in MEN1 and MEN4 could share a common pathway (9).

To date, 23 different mutations of CDKN1B have been described in the literature in association with MEN4, including a total of 57 carriers. Forty-two of these subjects developed at least one endocrine neoplasm, while the involvement of multiple endocrine organs was detected in 17 of them (**Table 1**). Phenotypic and haplotypic analyses of the carriers' families suggest that MEN4 follows an autosomal dominant pattern of transmission, although disease penetrance is not yet completely known. Primary hyperparathyroidism is reported as the most common clinical feature, followed by pituitary adenomas and neuroendocrine tumors. In addition, several non-endocrine neoplasms have also been described, such as breast cancer, prostate cancer, colon cancer, papillary thyroid carcinoma, angiomyolipoma, meningioma, and adrenal adenoma.

Due to the small number of cases reported so far, it is still unclear whether MEN4 should be considered only as a rare variant of MEN1 or whether it presents some distinctive clinical features. Furthermore, clear guidelines on the clinical management of these patients are lacking. In a recent review, Frederiksen et al. have suggested screening these patients for hyperparathyroidism and pituitary tumors in adolescence and performing screening for NETs according to the guidelines provided for MEN1 (25).

In this report, we describe two patients developing a neuroendocrine tumor and carrying a germline mutation in CDKN1B. Moreover, through a new review of the most recent cases in the literature, we try to start outlining the first differences between MEN1 and MEN4 and to provide new indications on the clinical management of these patients.

CASE DESCRIPTION

Case 1

Clinical History

The first case is a 43-year-old man with no significant previous medical history, who was diagnosed with a metastatic ileal G2-NET at the age of 34. The tumor presented at diagnosis as an ≈3-cm ileal mass with multiple metastasis at the lymph nodes, mesentery, and liver. Histological examination of the ileal mass, removed by segmental ileal resection as conditioning a strict stenosis, documented the following: infiltration of the entire wall from the mucous membrane to the subserous adipose tissue; mitosis number: 1 × 10 HPF; Ki67: about 3%; and presence of diffuse neoplastic permeation of lymphatic vessels, both peritumoral and distant. Due to the widespread localization of the disease, treatment with first-generation somatostatin receptor ligands (SRLs) was started. After almost 10 years of substantial stability of disease in SRL therapy, recent 68-Ga-DOTATOC PET and abdomen MRI scans documented a progression. As such, the patient is currently being evaluated for peptide receptor radionuclide therapy.

Genetic and Clinical Testing

In consideration of the multiple localizations of the disease at a relatively young age, the patient was also advised to undergo a genetic evaluation for MEN. The analysis was performed through next-generation sequencing (NGS) (Illumina, Inc.Hayward, CA, USA: Illumina-Nextera Rapid Capture Custom Enrichment kit) of coding regions and intron/exon junctions of AIP, CDC73, CDKN1B, MEN1, and PRKAR1a.

The analysis identified the mutation p.I119T (c.356T>C) in heterozygosity of exon 1 of CDKN1B. This mutation was identified in control databases in 152 of 279,092 alleles at a frequency of 0.0005446 (gnomAD). *In-silico* analysis does not predict a difference in splicing. This variant was determined to be of uncertain significance according to ACMG guidelines, 2015, and had already been reported in the literature in association with early-onset pituitary adenoma (20, 22).

The patient was then offered screening for MEN1-related diseases, including assessment of parathyroid function (blood samples for PTH, calcium, phosphorus, albumin, 25OH vitamin D) and pituitary function (blood samples for TSH, FT4, IGF-1, ACTH, prolactin, FSH, LH, testosterone, morning cortisol at h 8 a.m. and after stimulation with 1 mcg ACTH test), and MRI of sella turcica. All the examinations reported no significant findings.

TABLE 1 | Studies describing families with MEN4 syndrome associated with CDKN1B mutations.

Mutation	Type of mutation	Carriers with MEN4-associated features			Asymptomatic family carriers (age at assessment)	Total carriers	Reference
		Sex	Main MEN features (age at diagnosis)	Other significant history of neoplasia			
c.G692A (p.W76X)	Nonsense	F	GH-producing pituitary macroadenoma (30 years old) Primary hyperparathyroidism (PHPT) (46 years old)	NO	One sister without MEN main features but with a history of angiomyolipoma diagnosed at 52 years old. Another sister (44 years old) and her daughter (16 years old) completely asymptomatic.	4	Pellegata et al. (2)
c.59_77dup19 (pK25fs)	Frameshift	F	Small-cell neuroendocrine cervical carcinoma (45 years old) ACTH-secreting pituitary adenoma (46 years old) PHPT (47 years old)	NO	None	1	Georgitisi et al. (10)
ATG-7G>C	Mutation in 5′UTR	F	PHPT (61 years old)	Bilateral NF adrenal masses (63 years old)	2 daughters (46 and 48 years old)	3	Agarwal et al. (11)
c283C>T (p.P95S)	Missense	F	PHPT with multiple adenoma (46 years old), metastatic gastrinoma (50 years old)	NO	None	1	Agarwal et al. (11)
c.595T>C (p.*199Qext60)	Nonsense	F	PHPT with multiple adenoma (50 years old)	NO	None	2	Agarwal et al. (11)
		F	PHPT (66 years old)	NO			Agarwal et al. (11)
c.163G>A (p.Ala55Thr)	Missense	F	Metastatic gastrinoma (42 years old) PHPT (51 years old)	NO	None	1	Belar et al. (12)
c.678C>T (p.P69L)	Missense	F	PHPT (67 years old) Metastatic bronchial carcinoid (67 years old) Non-functioning pituitary microadenoma (79 years old)	Papillary thyroid carcinoma (64 years old)	None	1	Molatore et al. (13)
c.-456_-453delCCTT	Deletion in 5′UTR	F	GH-secreting adenoma (62 years old) Non-functioning pancreatic NET (62 years old)	NO	None	1	Occhi et al. (14)
c.374_375delCT	Frameshift	F	PHPT from multiple adenoma (41 years old) Metastatic gastrinoma (50 years old)	NO	1 son (34 years old)	2	Tonelli et al. (15)
c.25G>A (Gly9Arg)	Missense	M	PHPT (68 years old)	NO	None	1	Costa-Guda et al. (16)
c.397CA (p.Pro133Thr)	Missense	F	PHPT (53 years old)	NO	None	3	Costa-Guda et al. (16)
		F	PHPT (56 years old)	Papillary thyroid carcinoma (56 years old) Uterine leiomyoma (NA) Grade II meningioma (NA)	None		Bugalho and Domingues (17)
		F	PHPT from multiple adenoma(49 years old) Mutation also in MEN1	Fibrocystic breast disease (NA)	None		Borsari et al. (8)
c.-80C>T	Missense	M	PHPT (38 years old)	NO	None	1	Borsari et al. (8)
c.-29_-26delAGAG	Deletion in 5′UTR	F	PHPT (61 years old)	NO	None	5	Borsari et al. (8)
		F	GH-secreting pituitary adenoma (5 years old)	NO	Mother (age not reported)		Sambugaro et al. (18)
		F	Gastric carcinoid tumor (69 years old) PHPT (74 years old)	NO	None		Malanga et al. (19)
		M	ACTH-secreting pituitary microadenoma (13 years old)	NO	None		Chasseloup et al. (20)
c378G>C (pE126D)	Missense	F	PHPT (15 years old)	NO	Mother (46 years old) Grandfather (74 years old)	3	Elston et al. (21)

(Continued)

TABLE 1 | Continued

Mutation	Type of mutation	Carriers with MEN4-associated features			Asymptomatic family carriers (age at assessment)	Total carriers	Reference
		Sex	Main MEN features (age at diagnosis)	Other significant history of neoplasia			
c.320delA (p.Q107Rfs*12)	Frameshift	F	ACTH-secreting pituitary microadenoma (16 years old)	NO	4 (mother, brother, and 2 children)	5	Chasseloup et al. (20)
c.376G>C (p.E126Q)	Missense	F	ACTH-secreting pituitary microadenoma (12 years old)	NO	None	1	Chasseloup et al. (20)
c.407A>G (p.D136G)	Missense	M	ACTH-secreting pituitary microadenoma (15 years old)	NO	None	1	Chasseloup et al. (20)
c.356T>C (p.I119T)	Missense	F	ACTH-secreting pituitary microadenoma (15 years old)	NO	None	2	Chasseloup et al. (20)
		F	GH-secreting adenoma (NA)	NO	(aunt with GH-secreting adenoma, not tested for CDKN1B mutation)		Tichomirowa et al. (22)
c.286AOC (p.K96Q)	Missense	F	PRL-secreting adenoma (NA)	Breast cancer (41 years old)	1 sister (age not reported)	2	Tichomirowa et al. (22)
c.285dupC (Lys96Glnfs*29)	Duplication	M	PHPT (60 years old)	Positive for NF1. Prostate cancer with transdifferentiation in metastatic small cell carcinoma (60 years old) Multiple spinal neurofibromas	None	1	Brock et al. (23)
c.281C>T, (Pro94Leu)	Missense	F	Hyperprolactinemia (adenoma non-detected at MRI) (35 years old) PHPT (51 years old)	Breast cancer (37 years old) Ovarian serous cystadenoma (50 years old) Multifocal papillary thyroid carcinoma (55 years old)	None	1	Chevalier et al. (24)
c.206C>T, p. (Pro69Leu9)	Missense	F	Non-functioning macroadenoma (66 years old) PHPT (66 years old)	Subclinical cortisol autonomous secretion by adrenal adenoma (NA)	None	1	Chevalier et al. (24)
c.121_122 delTT (pLeu41Asnfs*83	Frameshift	F	PHPT and ACTH-secreting pituitary microadenoma (37 years old)	NO	All the patients belong to the same family, no other healthy carrier reported	13	Frederkisen et al. (25)
		F	PHPT and non-functioning pancreatic NET (67 years old)	NO			
		F	PHPT and non-functioning pituitary microadenoma (66 years old)	NO			
		M	PHPT and non-functioning pituitary microadenoma (64 years old)	NO			
		M	PHPT and non-functioning pituitary macroadenoma (46 years old)	NO			
		F	PHPT- and ACTH-secreting pituitary microadenoma (NA)	NO			
		5F + 2M	PHPT (NA)	One F developed had a recurrency of PHPT after first surgery and had breast cancer (77 years old)			

M, male; F, female; PHPT, primary hyperparathyroidism; NA, not assessed.

Family History

Genetic evaluation was then extended to the patient's relatives, with positive findings in the father and younger brother. The same screening for MEN1 was then offered to all family carriers, with the addition of an abdominal ultrasound (not performed in the index patient because of periodic CT follow-up).

The patient's brother, aged 31, had no significant medical history and no symptoms. Sellar MRI and abdominal ultrasound documented no pathological findings. Parathyroid and pituitary function resulted normal as well.

The patient's father, aged 70, had a history of clear-cell renal carcinoma diagnosed at the age of 57, in complete remission after unilateral nephrectomy and adrenalectomy. Screening for MEN1-related diseases revealed also, in this case, no significant findings at MRI, abdomen ultrasound, and pituitary and parathyroid function assessments.

Case 2

Clinical History

We also present the case of a 76-year-old woman, reporting in medical history only an ovarian dermoid cyst removed when she was 39. At the age of 73, during a routine abdominal ultrasound, an ≈3-cm pancreatic mass was detected. A subsequent CT scan confirmed the presence of a 3.2-cm solid and partially hypervascular mass in the pancreatic uncinate process, suggestive of NET. The diagnosis of NET was supported by enhanced uptake at 68-Ga-DOTATOC PET and confirmed through endoscopic ultrasound biopsy. No further localization of the disease was observed, so the patient underwent the Whipple procedure with complete remission of the disease. At the last 3-year follow-up, there was no evidence of disease recurrence.

Histological examination of the mass documented "three different foci of G1-NET of the pancreatic head (according to WHO 2010), respectively, 2.9, 0.6, and 0.5 cm; Ki67: 1%–2%; mitotic index: <1 mitosis/10 HPF; angioinvasion present, no necrosis or neural invasion detected; and no lymph nodes involved".

Genetic and Clinical Testing

Due to the multifocality of the disease, the patient was advised for genetic testing, performed as described in case 1. The analysis showed a missense heterozygous variant in exon 2 of CDKN1B (c.482C>G, p.S161C). This mutation has never been reported so far in any patient with MEN4 phenotype. It is present in population databases (rs373917399, ExAC 0.04%) and has an allele count higher than expected for a pathogenic variant (PMID: 28166811). The Genome Aggregation Database (gnomAD) reported an allele frequency of 0.00014. The variant is located into the C-terminal RhoA binding domain (26). Since p27 modulates actin dynamics by direct regulation of the RhoA pathway (27), this variant could affect the interaction between p27 and RhoA and the consequent regulation of actin dynamics and cell motility. However, *in-silico* analysis supports that this missense variant does not alter protein structure/function, and thus, it has been currently classified as a variant of uncertain significance.

The patient was then offered screening for MEN-related diseases, as described in case 1. Sellar MRI documented partial empty sella, but no pituitary function impairment was detected at biochemical exams. Parathyroid function resulted normal as well.

Family History

Genetic evaluation was extended to the patient's family, with a positive finding in the patient's 76-year-old sister. She was also offered a screening test for MEN-related diseases, but she refused to undergo any examination because she was not reporting any symptoms.

DISCUSSION

MEN4, although basically recognized as a variant of MEN1, is still a condition whose clinical features are relatively unknown, mainly because of the small number of cases in the literature. However, with the increase of MEN4 reports, we have the opportunity to better characterize its similarities with MEN1, but also to begin to delineate the differences.

Both MEN1 and MEN4, despite an autosomal dominant transmission which theoretically predicts an equal distribution in both sexes, are more common in women (57% MEN1, 75% MEN4). The significantdifference in gender prevalence reported in MEN4 could still be due to the small population examined. However, it confirms a trend also present in MEN1 of greater penetrance in the female sex, whose causes are still not clear (28).

Primary hyperparathyroidism results in MEN4, as in MEN1, the most frequent endocrine neoplasm. However, in MEN4, the prevalence seems to be lower (75% vs. >93%) and with a more advanced mean age at diagnosis (53 vs. 40 years) (28). According to case reports in the literature, the youngest age of onset is 15 years in MEN4, while in MEN1, primary hyperparathyroidism might occur at a much earlier age (as young as 5 years) (29). Thus, as suggested by Frederiksen et al. (25), screening for hyperparathyroidism in subjects with MEN4 might start in adolescence and not in childhood as MEN1. Moreover, in MEN4, hyperparathyroidism is more frequently caused by one single parathyroid adenoma, while the involvement of multiple parathyroids and/or recurrence after surgery is quite rare (only five cases documented). Therefore, in patients with MEN4, we suggest that the surgical approach could be limited to the removal of the single hyperfunctioning parathyroid without the necessity of subtotal parathyroidectomy.

The prevalence of pituitary adenoma in MEN4 is around 40%, substantially overlapping with MEN1 (28). Pituitary adenomas may affect subjects of all ages; although the mean age is 33–35 years, the youngest case report is a 5-year-old girl. Therefore, we suggest assessing basal pituitary function in any MEN4 patient and eventually performing an MRI of sella turcica if the hormonal profile should prove abnormal. Interestingly, although prolactinomas are the most common form of functioning adenoma in MEN1, in MEN4, they seem to be the rarest type, with only one case reported. In contrast, corticotropinomas, which represent only 5% of the cases in MEN1 (28), appear to account for almost 40% of all pituitary adenomas in MEN4.

Currently, it is not possible to determine whether this difference is due to a statistical issue related to the small number of cases or to a specific effect of CDKN1B on pituitary tumor development.

Neuroendocrine tumors occur in ≈20% of MEN4 subjects, a significantly lower percentage than MEN1 (≈50%). The mean age is around 55 years, and the youngest so far is our patient at the age of 34. Localization of the primary tumor includes the pancreas, small intestine, and lung. A small cell neuroendocrine tumor of the cervix was also described in one woman (10). The most common form of functioning NET is gastrinoma, as in MEN1, while no cases of VIPomas, glucagonomas, insulinomas, or somatostatinomas have been reported so far.

The significant difference in NET prevalence between MEN1 and MEN4 could be due to discrepancies in the clinical

assessment of patients. In MEN1, it is indeed recommended to investigate the presence of neuroendocrine tumors even in asymptomatic subjects, performing periodic biochemical and imaging investigations (30). However, there are no such clear guidelines for MEN4 patients, so the cases reported so far may not have been screened for NETs as accurately as in MEN1, especially if the patient did not show any symptoms.

In agreement with Frederiksen et al., considering the severe comorbidity associated with NETs and the lack of conclusive data on the real prevalence in MEN4, we suggest that all MEN4 patients should be screened for NETs according to the same guidelines as for MEN1.

Moreover, in MEN4, also non-endocrine neoplasms have been reported such as breast cancer, prostate cancer, colon cancer, angiomyolipoma, meningioma, and adrenal adenoma. Many of these conditions have also been reported in MEN1 (28). In addition, CDKN1B has been recognized as a potential driver mutation in the development of breast cancer and prostate cancer (31). Recent studies have also highlighted the predisposition of Cdkn1b-mutated rats in developing pheochromocytoma (32), although no cases have been reported in humans yet.

Finally, we must consider that, despite the wide variety of neoplasms associated with CDKN1B, the true oncogenic risk in CDKN1B-mutated carriers is still unknown. Most of the patients developed isolated neoplasms, difficult to distinguish from sporadic forms, and only rarely manifested a true MEN. Moreover, a significant number of CDKN1B mutation carriers were totally asymptomatic. As such, we cannot exclude that CDKN1B mutation alone may not be sufficient to determine a clinically overt MEN, but other factors might be involved in triggering tumorigenesis in these patients.

CONCLUSIONS

Thanks to the increased number of cases reported on MEN4, it is possible to more accurately define both similarities and differences in comparison with MEN1. MEN4 appears to be a variant with a later onset, less penetrance, and milder clinical features. Hyperparathyroidism is the most common clinical feature, although in our cases this condition was not found; since recurrence and/or multiple parathyroid involvement appears to be rare, a less aggressive surgical approach than in MEN1 could be justified. We also suggest that all MEN4 carriers, even asymptomatic, should be screened for neuroendocrine tumors, considering that this could also represent the only pathological manifestation, as we report in our cases. Even if not reported in our patients, according to the literature, MEN4 patients should also be screened for pituitary adenomas. In conclusion, larger case series are needed to clarify the peculiar features of MEN4, to establish a specific diagnostic and therapeutic standard, and to set up an appropriate follow-up strategy. Moreover, specific studies performed on CDKN1B carriers are needed to assess the real oncological risk in these subjects and to elaborate a standardized screening protocol.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Board IRCCS Humanitas Research Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

EL and AB conceived and designed the study. Data collection, analysis, and interpretation were done by AB. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Unique Finding of a Primary Central Nervous System Neuroendocrine Carcinoma in a 5-Year-Old Child: A Case Report

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Neuroendocrine tumors (NETs) are rare neoplasms predominantly arising in the gastrointestinal-tract or the lungs of adults. To date, only ten cases of primary central nervous system (CNS) NETs have been reported, with just three of them describing a neuroendocrine carcinoma (NECA) and none occurring in a child. We report on a previously healthy 5-year-old boy, who presented with headaches, nausea and vomiting, and was diagnosed with a left cerebellar solid mass with a cystic component. After gross-total resection, histology revealed a neuroendocrine carcinoma. Molecular analysis of the tumor tissue showed a *KRAS*-splice-site mutation (c451-3C > T). The *KRAS*-mutation was discovered to be a maternal germline mutation, previously described as likely benign. After extensive search for an extracranial primary tumor, including Ga-68 DOTANOC-PET-CT, the diagnosis of a primary CNS NECA was established, and proton irradiation was performed. Unfortunately, the patient developed an in-field recurrence just 5 weeks after the end of radiotherapy. The tumor was re-resected with vital tumor tissue. Six cycles of chemotherapy were initiated, consisting of cisplatin, carboplatin, etoposide and ifosfamide. The patient remains disease free 22 months after the end of treatment, supporting the beneficial effect of platinum- and etoposide-based chemotherapy for this tumor entity.

Keywords: pediatric brain tumor, neuroendocrine carcinoma (NEC), primary CNS tumor, rare entities, neuroendocrine tumors

INTRODUCTION

Tumors of the central nervous system (CNS) are the most frequent type of solid neoplasms in children (Ostrom et al., 2020). However, they are comprised of more than hundred different entities, and while the more frequent ones are considered to be a rare disease (i.e., with an incidence of <1:2,000), some entities are only described anecdotally (Richter et al., 2015). We present the case of a primary CNS neuroendocrine carcinoma, a tumor entity that has been recently described

in a few case reports on adult patients (Tamura et al., 2014; Liu et al., 2016; Reed et al., 2019), but not yet in the pediatric population.

CASE REPORT

We report on a previously healthy 5-year-old boy, who had been suffering from headaches, accompanied by nausea and intermittent vomiting for 4 weeks prior to diagnosis. Magnet resonance imaging (MRI) of the head showed a left cerebellar cystic mass and hydrocephalus with signs of transependymal cerebrospinal fluid (CSF) diapedesis. The appearance on MRI (**Figures 1A,B**) with comparatively low apparent diffusion coefficient (ADC) values within the solid component directed toward higher cellularity (**Figure 1C**). Surgical resection of the tumor mass was performed. Postoperative MRI did not show any signs of residual tumor nor metastasis.

Histologic analysis of the tumor material revealed an epithelial neoplasm composed of predominantly small uniform cells with focally moderately anaplastic elements (**Figure 2A**). Immunohistochemically, expression of pancytokeratin (**Figure 2B**), CK8, CK18 CK19, and EMA was detectable. No immunopositivity was observed for CK7, CK20, and p63. Chromogranin A was expressed in the majority of tumor cells (**Figure 2C**) and few cells displayed synaptophysin, (**Figure 2D**) pointing toward a neuroendocrine differentiation, yet CD56 was negative within the tumor cells (**Figure 2E**). No serotonin and CD117 immunoreactivity could be detected, whereas SSTR2 (**Figure 2F**) and SSTR5 expression was present. To exclude other CNS and non-CNS tumors a broad panel of immunohistochemical stainings was performed (**Table 1**). A smaller fraction of cells showed a moderately intense expression of NeuN. OTX2 was moderately intense expressed in the majority of tumor cells (**Figure 2H**). The anti-pHH3 staining (**Figure 2I**) revealed up to 22 mitoses per mm^2 and the Ki-67 proliferation index was 35.4% (**Figure 2J**). Due to the combined expression of epithelial and neuroendocrine markers the tumor was classified as neuroendocrine carcinoma.

Further molecular analysis was performed using the Ion AmpliSeq Cancer HotSpot Panel v2 and Oncomine Comprehensive Assay v3 (both: Thermo Fisher Scientific, Waltham, MA, United States), showing a splice site mutation in *KRAS* (c451-3C > T) with an allele frequency of 50%, which was later confirmed as a germ-line mutation, inherited from the patient's asymptomatic mother. No alterations were detected in *MEN1* and *RET*. An extensive search for a primary tumor outside the CNS was initiated. F-18-FDG PET-CT of the cervical, thoracal, and abdominal area did not show signs of increased uptake. Ga-68-DOTANOC PET-CT from the head to the symphysis demonstrated a slightly elevated uptake in the area of tumor resection without any other areas of increased uptake. In addition, ultrasound of the thyroid and abdomen as well as a capsule endoscopy were performed. None of these examinations showed signs of an extracranial primary tumor. Neuron-specific enolase was slightly elevated (25.6 μg/L), other markers of neuroendocrine tumors (NETs), such as insulin, glucagon, vasoactive intestinal peptide (VIP) were within the normal range. Therefore, the diagnosis of a primary neuroendocrine carcinoma (NECA) of the CNS was established and focal proton therapy initiated 5 weeks after diagnosis (54 Gy/60 Gy (PTV1/PTV2) relative biologic effectiveness in 30 fractions).

The MRI 5 weeks after completion of radiotherapy showed local recurrence of the primary tumor within the field of irradiation (**Figure 3A**). En-bloc re-resection was performed and biopsies from the surrounding tissue were taken (**Figure 3B**). Vital tumor cells of the previously diagnosed NECA were found within the resected tissue, while the biopsies from the surrounding areas were negative for tumor cells. Systemic cytotoxic therapy with a total of six cycles was given (cumulative doses: cisplatin 200 mg/m^2, carboplatin 2,400 mg/m^2, etoposide 2,400 mg/m^2, ifosfamide 12,000 mg/m^2). At the end of chemotherapy, no sign of recurrence or metastases was detected in MRI. Even 22 months after the end of chemotherapy there is

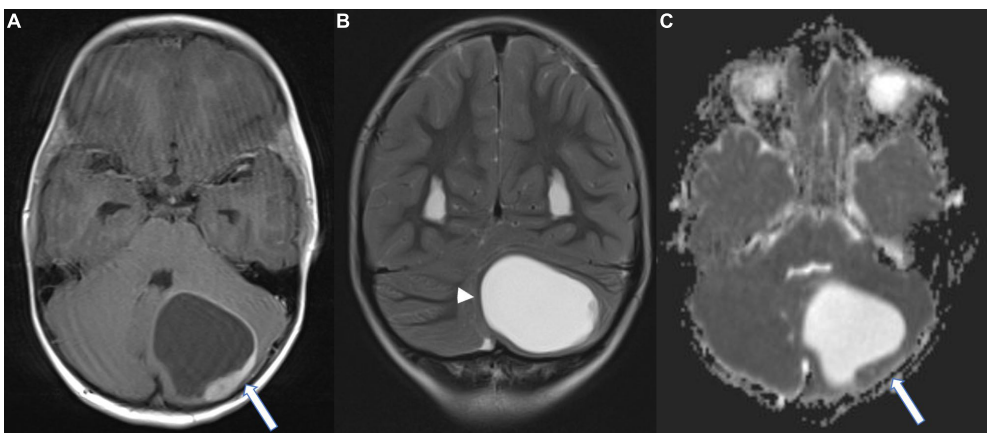

FIGURE 1 | Magnet resonance (MR) images at the time of diagnosis; **(A)** Axial T1-weighted contrast-enhanced image showing a left cerebellar cystic mass with a peripheral contrast enhancing component (arrow); **(B)** Coronal T2-weighted image. Note the infratentorial midline shift due to the mass effect of the cystic component (arrowhead). **(C)** Axial diffusion-weighted image (ADC-Map) demonstrating low ADC values of the solid component (arrow), suggestive of high cellular density.

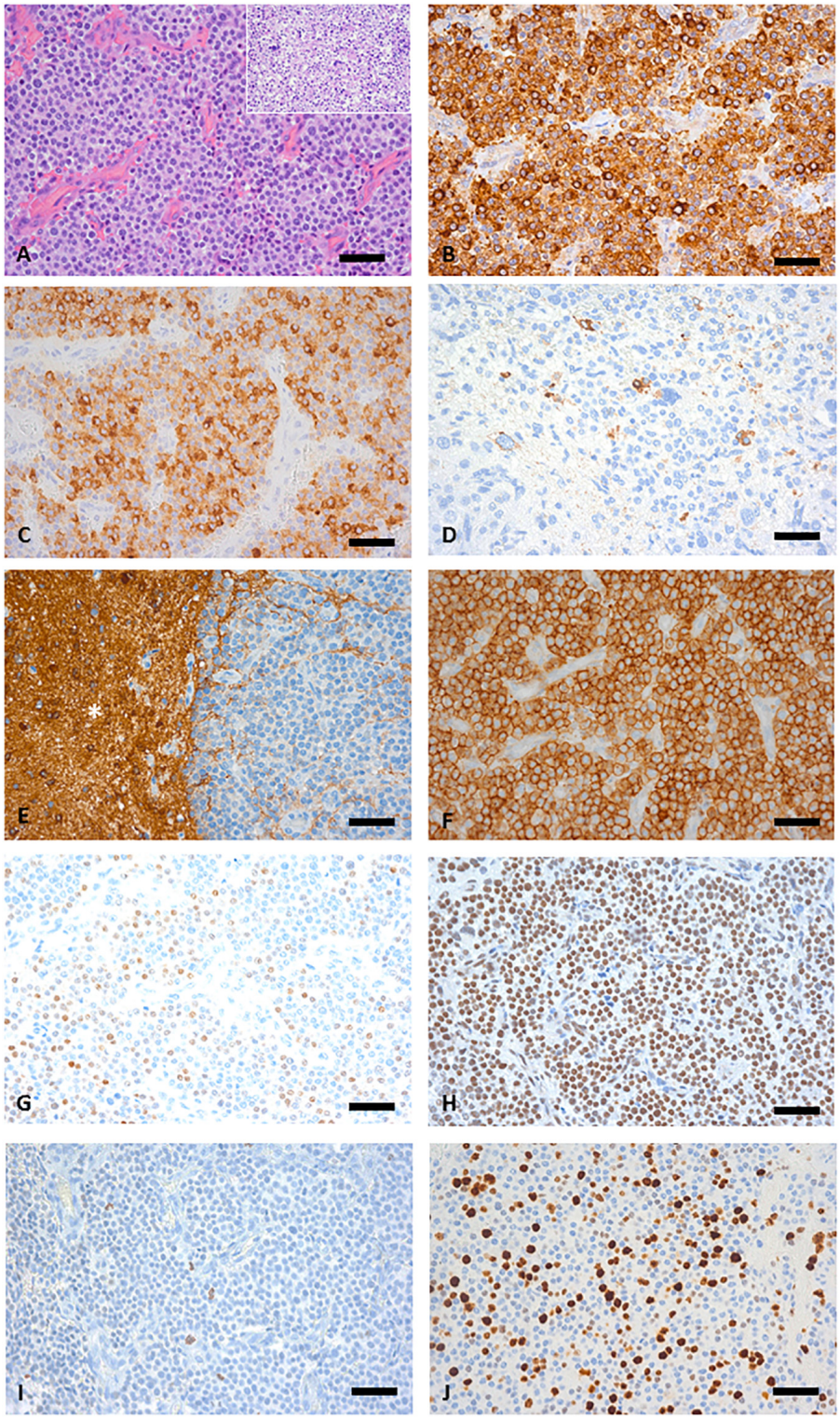

FIGURE 2 | (A) Hematoxylin and eosin (HE) sections of the biopsy specimen showing a highly cellular tumor growing in a sheet-like pattern separated by fibrovascular septa, inset depicts an area with pleomorphic tumor cells. **(B)** Staining with pan-cytokeratin [Lu-5] antibody confirms an epithelial origin. **(C)** Anti-Chromogranin A staining is positive in most tumors cells. **(D)** Only few scattered synaptophysin positive tumor cells were detectable. **(E)** NCAM staining was negative in the tumor tissue but the asterisk (*) indicates positive NCAM staining in the adjacent white matter. **(F)** Widespread SSTR2 expression. **(G)** NeuN showing a moderate intensity in a fraction of tumor cell nuclei. **(H)** Widespread expression of OTX2. **(I)** pHH3 reveals frequent mitotic figures. **(J)** Ki67 proliferation; 35.4%. **(A–J)** Original magnification x400, scale bar represents 50 μm.

TABLE 1 | All antibodies/company used and the respective results in the tumor cells.

Antibody	Company, clone	Tumor cells
Pancytokeratin	Dako/Agilent; Lu-5	Pos
CK5/6	Dako/Agilent; D5/16 B4	Neg
CK7	Dako/Agilent; DOV-TL 12/30	Neg
CK8	BD Biosciences; CAM 5.2	Pos
CK18	Dako/Agilent; DC 10	Pos
CK19	Dako/Agilent; RCK108	Pos
CK20	Dako/Agilent; Ks 20.8	Neg
p63	Ventana/Roche; 4A4	Neg
EMA	Dako/Agilent; E29	Pos
Chromogranin A	Dako/Agilent; DAK-A3	Pos
CD56	Monosan; 123C3	Neg
Synaptophysin	Dako/Agilent; DAK-SYNAP	Scattered cells pos
Serotonin	Dako/Agilent; 5HT-H209	Neg
SSTR2	Abcam; UUMB1	Pos
SSTR5	Abcam; UMB4	Pos
CD117	Dako/Agilent; polyclonal	Neg
Pit-1	Santa Cruz; D-7	Neg
SF1	R&D Systems; N1665	Neg
ACTH	Dako/Agilent; 02A3	Neg
TTF1α	Ventana/Roche; 8G7G3/1	Neg
GFAP	Dako/Agilent; polyclonal	Neg
Olig2	IBL; polyclonal	Neg
S100	Dako/Agilent; polyclonal	Neg
Smooth muscle actin	Dako/Agilent; 1A4	Neg
Vimentin	Dako/Agilent; V9	Neg
MAP2	Merck/Millipore; AP20	Neg
Neurofilament H phosphorylated	Covance; SMI-31	Neg
Neurofilament H non-phosphorylated	Covance; SMI-32	Neg
NeuN	Merck/Millipore; A60	Some cells positive
Lin28A (A177)	Cell Signaling Technology; polyclonal	Neg
CRX (A-9)	Santa Cruz Biotechnology	Neg
CD99	BioGenex; EP8	Neg
BCoR (C-10)	Santa Cruz Biotechnology	Neg
NUT	Cell Signaling Technology; C52B1	Neg
OCT-3/4 (C-10)	Santa Cruz Biotechnology	Neg
H3 p.K28me3	Invitrogen; polyclonal	Pos
H3 p.K28M	Abcam; EPR18340	Neg
SMARCB1	BD Biosciences; 25/BAF47	Pos
SMARCA4	Abcam; EPNCIR111A	Pos
SDHB	Abcam; (21A11AE7)	Pos
OTX2	ThermoFisher; 1H12C4B5	Pos
MelanA	Biocare Medical; HMB45/MART-1/Tyrosinase	Neg
Ki67	Dako/Agilent; MIB-1	35% proliferation
pHH3	Merck/Sigma-Aldrich; polyclonal	≥2 mitoses/mm^2
MSH2	Cell Marque; G219-1129	Pos
MSH6	Cell Marque; 44	Pos
MLH1	Ventana/Roche; M1	Pos
PMS2	Cell Marque; EPR3947	Pos
ALK	Zytomed; 1A4;	Neg
NTRK	Abcam; EPR17341	Neg

still no sign of recurrence, the patient is in good clinical condition, attending school and participating in daily life activities without any limitations.

DISCUSSION

Neuroendocrine carcinomas (NECAs) are a subgroup of neuroendocrine tumors (NETs) originating from neuroendocrine cells, defined by increased proliferation markers (Ki-67 index > 20%) and loss of differentiated histomorphology (DeLellis, 2001; Oronsky et al., 2017). These widely dispersed cells are characterized by the presence of endocrine and neuronal features and can give rise to NETs in virtually all organs. However, in adults the majority of primaries arise in the gastrointestinal tract (62–67%) and the lungs (22–27%) (DeLellis, 2001; Oronsky et al., 2017).

Neuroendocrine tumors (NETs) are extremely rare in the pediatric population, so that the incidence can only be estimated, with a range from 1 to 5 per 1,000,000 people with hardly any case reported in children below the age of 10 (Navalkele et al., 2011; Diets et al., 2017; Stawarski and Maleika, 2020). The most common site of occurrence in the pediatric population is the appendix (Diets et al., 2017). However, so far, no case of primary CNS NET has been described in the pediatric population.

While most NETs occur sporadically, certain genetic syndromes predispose the development, including multiple endocrine neoplasia type 1 and 2 (MEN-1 and MEN-2) (Walls, 2014), neurofibromatosis (Gut et al., 2015), tuberous sclerosis (Gut et al., 2015) and von Hippel-Lindau disease (Rednam et al., 2017). Occurrence at young age and/or positive family history should prompt for genetic counseling and testing. Our patient showed neither clinical characteristics of these syndromes, nor was MEN 1/2 detected in the genetic analysis.

Irrespective of the primary site, NETs share some histologic characteristics and can be divided into differentiated NETs and NECAs. Well differentiated NETs are characterized by "organoid" or neuroendocrine shaped arrangement of tumor cells producing neurosecretory granules, intensely reacting to neuroendocrine markers, including synaptophysin and chromogranin A. Staining intensity with neuroendocrine markers in NECA can be less, but is by definition present. In contrast to NETs, proliferation index with Ki-67 is >20% in NECAs. Histomorphology in NECA is undifferentiated with solid or diffuse growth and nuclear atypia (Klimstra et al., 2010; Oronsky et al., 2017). Previous case reports of primary CNS NETs include seven cases of differentiated NETs (Porter et al., 2000; Deshaies et al., 2004; Ibrahim et al., 2010; Hood et al., 2014; Liu et al., 2016; Vernieri et al., 2016; Hakar et al., 2017) and three cases of NECAs (Tamura et al., 2014; Liu et al., 2016; Reed et al., 2019). Primary CNS NECAs were described to stain positive for neuroendocrine markers, including chromogranin and synaptophysin, and negative for the glial marker GFAP. This also was the case in our patient, who was positive for CK8, CK18, and CK19, additionally, while being negative for CK7 and CK20, vimentin, S100, Olig2, and MAP2. In contrast to a previous case report of a neuroendocrine tumor in the brain, which was slightly CD56 positive (Tamura et al., 2014),

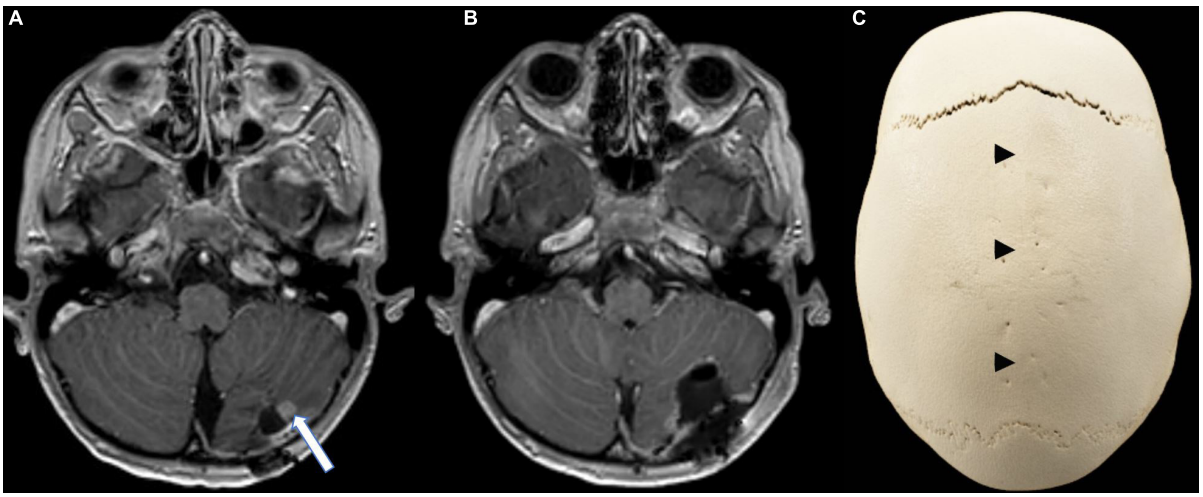

FIGURE 3 | Follow-up imaging. **(A)** Axial contrast-enhanced T1-weighted MR image showing a contrast enhancing nodule (arrow) at the infero-lateral border of the resection cavity, strongly suggestive of local recurrence. **(B)** Axial contrast-enhanced T1-weighted MR image after en-bloc resection of the recurrent tumor. **(C)** CT reconstruction (Volume rendering) of the skull highlighting the dolichocephaly and the premature synostosis of the sagittal suture (arrow heads) when compared to the open sutures.

we could not detect CD56 expression. Somatostatin receptor (SSTR) status was not reported in other cases of CNS NECA, however, 100% of cells in our specimen were positive for SSTR-2, and 80% were positive for SSTR-5. The unusual pathological and immunohistochemical features of this tumor require careful delineation from other pediatric CNS tumor types. The expression of chromogranin A and synpatophysin raises the suspicion of a CNS embryonal tumor, particularly medulloblastoma, atypical teratoid/rhabdoid tumor, CNS neuroblastoma, FOXR2-activated or CNS embryonal tumor NEC/NOS. However, none of these tumors displays expression of cytokeratin throughout the whole tumor tissue. On the other hand, tumors of the choroid plexus are characterized by widespread cytokeratin expression. Yet, these tumors show a papillary architecture, which was not present in our case and no neuroendocrine differentiation. Furthermore, an ectopic pituitary adenoma was excluded due to lack of Pit1, SF1, and ACTH expression. Thus, the morphological and immunohistochemical features do not support the diagnosis of any other CNS tumor entity. Interestingly, the tumor showed expression of OTX2, which plays an important role in the development of the midbrain-hindbrain region (Di Giovannantonio et al., 2014) and is expressed in medulloblastomas (de Haas et al., 2006).

Because of the rarity of primary intracranial neuroendocrine carcinomas, it is obligatory to perform a thorough screening for an extracranial primary before establishing the diagnosis. In addition to the standard screening methods, including MRI/CT of the chest and abdomen, thyroid ultrasound, gastro- and colonoscopy, chromogranin and further symptom based biochemical testing, the recently developed method of receptor-based PET-CT/MRI adds more sensitivity to the already available functional imaging (Ambrosini et al., 2008; Naswa et al., 2012; Raphael et al., 2017).

While for some low-grade NETs surgical resection is sufficient, NECAs necessitate further chemotherapy, which in most cases is platinum-based (Oronsky et al., 2017; Rinke and Gress, 2017). However, all case reports on primary CNS NECAs reported radiation therapy as their first choice (Tamura et al., 2014; Reed et al., 2019). Considering the beneficial effect of radiotherapy in most pediatric CNS high-grade malignancies, proton therapy was the first-line treatment. Unfortunately, the tumor recurred very rapidly 5 weeks after the end of radiation within the irradiation field, necessitating re-resection and systemic cytotoxic therapy.

Besides the previously mentioned genetic syndromes, loss of RB1 and p53 function is one of the molecular characteristics described in various NECA locations (Kawasaki et al., 2020; McNamara et al., 2020). Further mutations differ depending on the localization and grade, with frequent alterations in *PIK3CA/PTEN*, *BRAF*, and *KRAS* (Sahnane et al., 2015; Olevian et al., 2016; Vijayvergia et al., 2016; Oronsky et al., 2017; Von Arx et al., 2019). No genetic information was available on the previously published primary CNS NECAs (Tamura et al., 2014; Liu et al., 2016; Reed et al., 2019), however, the tumor material of our patient was extensively analyzed, resulting in the detection of a germ-line splice site mutation in *KRAS* (exon5: c.451-3C > T), an alteration previously not described in NECAs. This variant is located in an alternate transcript (*KRAS-A*) of *KRAS* and likely benign, since it was only identified in an unaffected parent of a patient with Noonan syndrome. This is further supported by the finding of the same variant in our patient's mother, who did not suffer from any malignancy and the unremarkable family history regarding oncologic diseases. Interestingly, craniosynostosis and scaphocephaly was found in our patient (**Figure 3C**). Most cases of craniosynostosis develop sporadically, especially sagittal synostosis, where genetic alterations can be found in less than 1% of cases (Wilkie et al., 2017). Several molecular alterations have been

identified as important in the development of craniosynostosis, including ERF, a regulator in the RAS-MAP-kinase pathway, as well as KRAS itself, mostly within syndromic cases (Addissie et al., 2015; Wilkie et al., 2017). Again, our patient did not show any clinical signs of Noonan or similar RASopathies, and it remains unclear whether the *KRAS* mutation detected in our young patient is to be considered as a polymorphism, not involved in the development of neither the craniosynostosis nor the neuroendocrine carcinoma, or if it played a role in the disease development.

CONCLUSION

To the best of our knowledge, primary CNS neuroendocrine tumors have not yet been described previously in children. Reports about primary CNS NECAs appeared only recently and covered just the adult population. Extensive screening is necessary to exclude any extracranial primary tumor before establishing this diagnosis. The aggressiveness of this tumor has been demonstrated by its rapid in-field recurrence after irradiation. Similar to extracranial NECAs, platinum-based chemotherapy seems to be the therapy of choice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

NS, JG, IS, and AP: study concept and design. NS, CH, ST, M-TS, CL-C, LM, JG, AA, TC, IS, and AP: acquisition of data. NS, CH, ST, M-TS, LM, JG, AA, TC, IS, and AP: analysis and interpretation of data and critical revision of the manuscript for important intellectual content. NS and AP: drafting of the manuscript. JG, IS, and AP: study supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.810645/full#supplementary-material

Supplementary Figure 1 | Pre-existing cerebellar tissue shows synaptophysin (arrow) and NCAM expression (asterisk).

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A Nomogram Predicting the Overall Survival and Cancer-Specific Survival in Patients with Parathyroid Cancer: A Retrospective Study

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Purpose: This study aimed to explore a visual model for predicting the prognosis of patients with parathyroid carcinoma (PC) and analyze related biochemistries in different groups of stage.

Methods: The training dataset of 342 patients with PC was obtained from the Surveillance, Epidemiology, and End Results (SEER) database, and the validation dataset included 59 patients from The First Affiliated Hospital of Zhengzhou University. Univariate and multivariate Cox regression analyses were performed to evaluate significant independent prognostic factors. Based on those factors, nomograms and Web-based probability calculators were constructed to evaluate the overall survival (OS) and the cancer-specific survival (CSS) at 3, 5, and 8 years. The concordance index (C-index), receiver operating characteristic (ROC) curve, calibration curve, and decision curve analysis (DCA) were used to evaluate the nomogram in the training set and validation set. Moreover, biochemistries from the validation set were retrospectively analyzed in different groups of stage by Kruskal–Wallis test.

Results: Age, marital status, tumor size, stage, lymph node status, and radiation were identified as prognostic factors of OS. In contrast, only tumor size and stage were predictive for CSS. The nomogram was developed based on these independent factors. The C-index, ROC curve, calibration curve, and DCA of the nomogram in both training and validation sets showed that the nomogram had good predictive value, stability, and clinical benefit in predicting 3-, 5-, and 8-year OS and CSS in PC patients. Among the 59 PC patients from our hospital, lower albumin (ALB) levels and higher postoperative parathyroid hormone (PTH) levels were found in patients with distant metastasis (Distant vs. Regional ALB levels: $p = 0.037$; Distant vs. Local ALB levels: $p = 0.046$; Distant vs. Regional postoperative PTH levels: $p = 0.002$; Distant vs. Local postoperative PTH: $p = 0.002$).

Conclusion: The established nomogram application can provide accurate prognostics for patients with PC in the Chinese population, but it must be validated on prospectively collected real-world data.

Keywords: parathyroid carcinoma, prognosis, nomogram, SEER, distant metastasis

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INTRODUCTION

Parathyroid carcinoma (PC) is one of the rarest causes of primary hyperparathyroidism (PHPT), accounting for 0.5%–5% of the total PHPT cases (1). The incidence rate of PC in the population is increasing (2, 3). A meta-analysis reported 234 cases of PC patients in China, and the number of cases increases yearly (4).

Surgery leads to the best chance of survival with a 5-year and 10-year overall survival (OS) rate of 78%–91% and 60%–72%, respectively (1). The cancer-specific survival (CSS) rate was recorded as 89.4%, with a median number of 75 months (3). The indolent course of PC, compared with other invasive tumors, had better survival, but it shows frequent recurrence (1). In a center where the management and patient outcomes of PC have not changed significantly over the past 35 years, the 5-year disease-free survival rate was about 62.3% (5). In addition, almost all patients died from this cancer due to complications of hypercalcemia rather than tumor burden (1, 6). Currently, surgical resection with negative margin therapy is the only effective treatment strategy, as there is no evidence that radiotherapy and chemotherapy are effective for PC (7, 8).

Recently, the 8th American Joint Committee on Cancer (AJCC) staging system adopted Schule's staging system for tumor patients (9). Another mainstream staging method is proposed by Shaha and Shah (10), including both tumor size and disease progress. Nevertheless, some elements that have been reported to be related to PC, including age, serum calcium levels, intact parathyroid hormone (PTH) levels, vascular invasion, local excision, and absence of parafibromin staining, are not considered in the TNM staging system (2, 11–14). Therefore, to a certain extent, the TNM staging is incomplete and does not provide an individual prediction of PC patients.

Considering the various clinicopathological characteristics that could influence the prognosis of PC, an instrument integrating the relevant predictors is urgently needed for facilitating advanced therapy and improving the quality of life of patients. A nomogram is a simplified visual model for statistical predictions combining independent factors. Many researchers reported possible prognostic factors for PC, but there is no corresponding nomogram.

In this study, prognostic nomograms regarding OS and CSS were developed from PC patient data registered between 2000 and 2018 in the Surveillance, Epidemiology, and End Results (SEER) database. External data from a single Chinese center were utilized to validate these nomograms, and the patient characteristics reported by our hospital were analyzed.

METHODS

Ethics Statement

This research was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Number: 2021-KY-1062-001). Patients from the SEER database had consented to be studied publicly in any scientific research worldwide.

Population

The primary training dataset was downloaded with the SEER*Stat Software (Version 8.3.9.2; National Cancer Institute, Bethesda, MD, USA). The primary site code C75.0 for PC was applied in the SEER research Plus Data,18 Registries, Nov 2020 Sub (2000–2018) database. The exclusion criteria were listed as follows: 1) International Classification of Childhood Cancer (ICCC) site recode International Classification of Disease for Oncology-3 (ICD-O-3)/WHO 2008 was not parathyroid; 2) patients diagnosed at autopsy or *via* death certificates; 3) survival months were 0; 4) unknown race record, stage, surgery, marital status, tumor size, or cause-specific death classification (**Figure 1**).

The validation dataset was obtained from The First Affiliated Hospital of Zhengzhou University, a single Chinese center. Patients diagnosed with PC and hospitalized for the first resection between 2012 and 2020 were included in this study. Patients with inaccessible follow-up information were excluded (**Figure 1**).

Variables

The variables utilized for analysis were age at diagnosis, sex, race record, marital status at diagnosis, tumor size, surgery information, stage, laterality, lymph node (LN) status, and radiotherapy data. Moreover, laboratory and clinicopathologic variables were obtained from our hospital, such as blood calcium, PTH levels, tumor volume, and Ki-67. The TNM staging of PC was not included in this analysis because they were not proposed until 2017 at the eighth edition guideline of AJCC (15).

1) The tumor size included "Extent of Disease (EOD) 10-size (1988–2003)," "Collaborative Stage (CS) tumor size (2004–2015)," and "Tumor Size Summary (2016+)" in the SEER*Stat Software.

2) Age and tumor size were converted into two groups in the training set, and the optimal cutoff was calculated by X-tile software (Version 3.6.1; Robert, MD) (16). As shown in **Figure 2**, the optimal cutoff for age was 66 years, and the optimal cutoff for tumor size was 41 mm. Age was classified as greater than 66 years and less than or equal to 66 years, and tumor size was classified as greater than 41 mm and less than or equal to 41 mm.

3) "Marital status" contained "Married," "Single," and "Others" groups. Furthermore, patients in the "Others" group were "Divorced," "Separated," Unmarried or Domestic Partner," or "Widowed,".

4) The stage was composed of "COMBINED SUMMARY STAGE (2004+)" and "historic stage A (1973–2015)." Furthermore, the staging was aligned to the Summary Stage 2018 Coding Manual v2.0 from the SEER website (**Supplementary Material**) (17). Tumors confined to the parathyroid glands without distant metastasis are called localized group; tumors infiltrating the thyroid, surrounding muscles, recurrent laryngeal nerve, trachea, esophagus, or other tissues and organs without distant metastasis are called regional group; tumors transferring to other organs or distant lymph node were defined as distant metastasis group.

5) Radical surgery referred to the resection extension of the lesion parathyroid, ipsilateral thyroid, and central neck dissection. "Excisional biopsy", "Local tumor excision, nos (with pathology

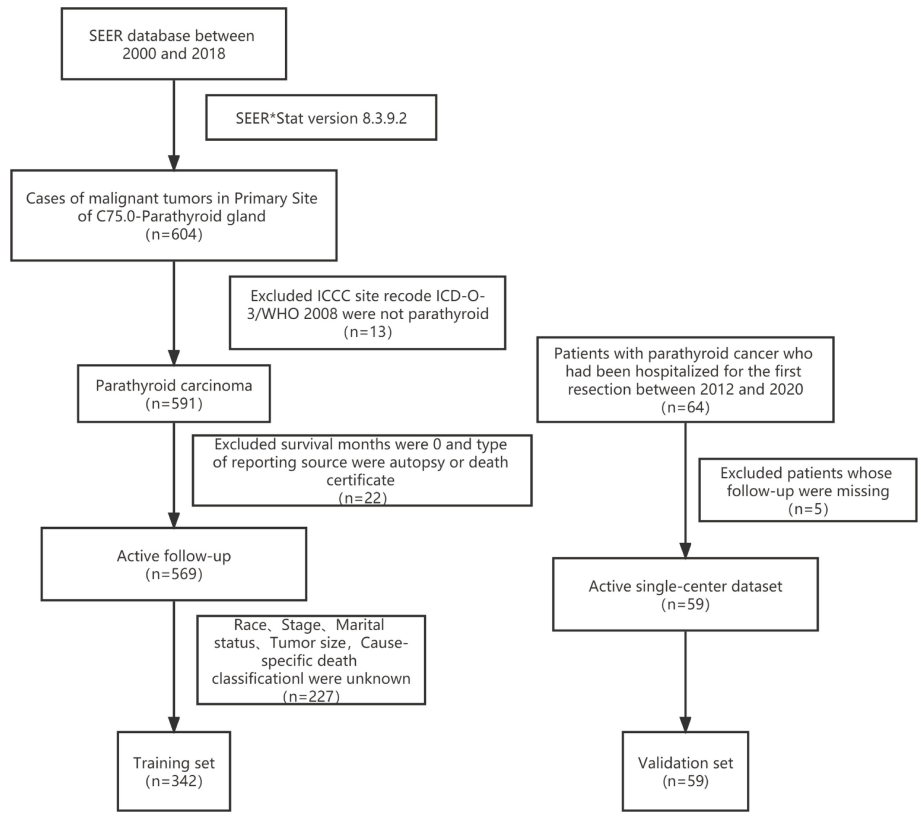

FIGURE 1 | The data process flowchart.

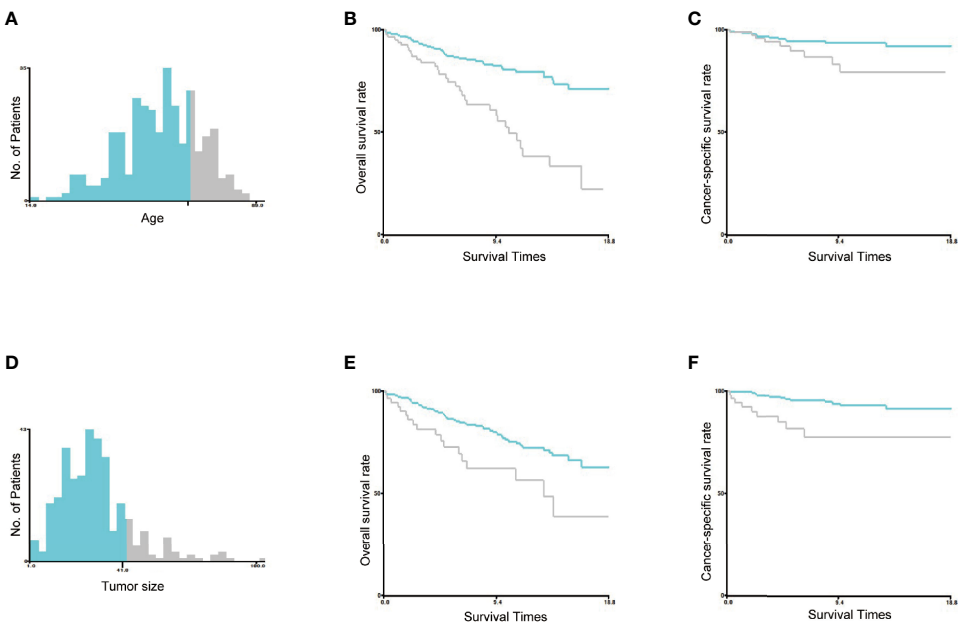

FIGURE 2 | Identification of the optimal cutoff values of age and tumor size. The optimal cutoff values of age were identified as 66 years based on both overall survival and cancer-specific survival **(A–C)**. The optimal cutoff value of tumor size was identified as 41 mm based on both overall survival and cancer-specific survival **(D–F)**.

specimen)," "Photodynamic therapy (PDT)," "Simple/partial surgical removal of primary site," "debulking," "Surgery, NOS," and "Total surgical removal of primary site" were defined as "others." "None; no cancer-directed surgery of primary site" was defined as "No surgery."

6) OS and CSS were selected as the outcomes. OS refers to death due to any cause, while CSS represents PC-specific death. The survival months were defined as from the surgery date to November 2020 and June 30, 2021, respectively, for the SEER and Chinese single-center datasets.

Statistical Analysis

The number and the percentage [N (%)] were used to describe the categorical data, and these data were compared by the chi-square test and Kruskal-Wallis test. The mean ± standard deviation (SD) and median with range were used to describe the normally and non-normally distributed quantitative variable, respectively. Kruskal–Wallis test was used to compare the levels of quantitative variables in different groups. The Kaplan–Meier method was used to estimate the survival and was compared by a log-rank test. The prognostic factors significantly affecting OS and CSS were explored by univariate and multivariate Cox regression analysis. Additionally, the hazard ratio (HR) and 95% confidence interval were reported for prognostic factors, and the receiver operating characteristic (ROC) curves and calibration curves were calculated to validate the nomogram. The bilateral $p < 0.05$ was regarded as significant. All data were analyzed by R software (Version 4.0.3) and IBM SPSS software (Version 22.0).

RESULTS

Demographic and Clinical Characteristics

A total of 342 patients from the SEER database and 59 patients from our hospital were identified in this study. The baseline demographics and clinical characteristics are listed in **Table 1**.

TABLE 1 | Demographic and clinical characteristics in the SEER database and a single Chinese center*.

	SEER data (N = 342)	Single-center data (N = 59)	*p* value
Sex			
Women	164 (48.0%)	32 (54.2%)	0.400
Men	178 (52.0%)	27 (45.8%)	
Age (years)			
≤66	258 (75.4%)	50 (84.7%)	0.118
>66	84 (24.6%)	9 (15.3%)	
Race			
White	255 (74.6%)	NA	NA
Black	53 (15.5%)	NA	
Others[a]	34 (9.9%)	59 (100%)	
Marital Status			
Married	219 (64.0%)	56 (94.9%)	<0.001
Single	64 (18.7%)	3 (5.1%)	
Others[b]	59 (17.3%)	NA	
Tumor Size (mm)			
1–41	288 (84.2%)	45 (76.3%)	0.136
>41	54 (15.8%)	14 (23.7%)	
Surgery			
No surgery	5 (1.5%)	NA	<0.001
Radical surgery	30 (8.8%)	31 (52.5%)	
Others[c]	307 (89.8%)	28 (47.5%)	
Stage			
Localized	210 (61.4%)	35 (59.3%)	0.069
Regional	114 (33.3%)	16 (27.1%)	
Distant	18 (5.3%)	8 (13.6%)	
Laterality			
Left	23 (6.7%)	29 (49.2%)	<0.001
Right	29 (8.5%)	28 (47.5%)	
Unknown[d]	290 (84.8%)	2 (3.4%)	
LN Status			
Negative	102 (29.8%)	31 (52.5%)	0.002
Positive	10 (2.9%)	2 (3.4%)	
Unknown[e]	230 (67.3%)	26 (44.1%)	
Radiation			
No	294 (86.0%)	56 (94.9%)	0.058
Yes	48 (14.0%)	3 (5.1%)	
CSS			
Alive	319(93.3%)	55 (93.2%)	1

(Continued)

TABLE 1 | Continued

	SEER data (N = 342)	Single-center data (N = 59)	*p* value
Death	23 (6.7%)	4 (6.8%)	
OS			
Alive	267 (78.1%)	49 (83.1%)	0.491
Death	75 (21.9%)	10 (16.9%)	
Survival months			
Median [Min, Max]	81.5 [1.0, 226.0]	44.0 [5.0, 117.0]	<0.001

[a] *Including Asian and American Indian*
[b] *Including divorced, separated, unmarried, domestic partner, and widowed.*
[c] *Including local tumor excision, simple/partial surgical removal of primary site, debulking, etc.*
[d] *Without record.*
[e] *Regional neck lymphadenectomy not performed.*
**SEER, Surveillance, Epidemiology, and End Results; LN, lymph node; CSS, cancer-specific survival; OS, overall survival.*

The mean age of the patients was 55.7 ± 13.8 years, and the mean tumor size was 29.6 ± 15.4 mm. In the training set (n = 342), there were 178 (52%) female patients and 255 (74.6%) white patients; 5 (1.5%) patients did not undergo surgery, and 30 (8.8%) underwent radical surgery treatment. In terms of stage, 18 patients (5.3%) developed distant metastasis. In addition, 112 patients (32.7%) underwent cervical lymph node resection, of whom 10 patients had lymph node metastasis. Moreover, 48 patients (14.0%) received radiation therapy. In the validation set (n = 59), there were 27 men and 32 women, the mean age was 49.0 ± 15.4 years, and the mean tumor size was 32.1 ± 12.7 mm; all populations were Asian; 2 patients' tumors were "intraglandular parathyroid cancer." There were no statistically significant differences in gender, age, clinical stage, radiotherapy, overall deaths, and cancer-specific deaths between the training and validation sets ($p > 0.05$). There were differences in marital status ($p < 0.001$), surgery ($p < 0.001$), and lymph node status ($p = 0.002$).

Prognostic Factors of Overall Survival and Cancer-Specific Survival

As shown in **Table 2**, 10 variables were enrolled for univariate Cox regression analysis. The significant variables were analyzed

TABLE 2 | Univariate Cox regression analysis of OS and CSS in patients with parathyroid cancer in the training set.

	OS			CSS		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***p***
Sex						
Men	**Reference**			**Reference**		
Women	1.580	0.992–2.515	0.054	1.280	0.561–2.922	0.555
Age						
≤66	**Reference**			**Reference**		
>66	3.067	1.935–4.863	<0.001	2.414	1.042–5.596	0.040
Race						
White	**Reference**			**Reference**		
Black	1.263	0.687–2.324	0.452	1.579	0.574–4.346	0.377
Others[a]	1.743	0.907–3.346	0.095	1.623	0.470–5.608	0.444
Marital status						
Married	**Reference**			**Reference**		
Single	1.342	0.713–2.526	0.363	1.540	0.542–4.378	0.418
Others[b]	2.599	1.563–4.323	<0.001	1.904	0.714–5.074	0.198
Tumor size						
1–41 mm	**Reference**			**Reference**		
>41 mm	2.177	1.280–3.703	0.004	4.230	1.827–9.790	<0.001
Surgery						
No surgery	**Reference**			**Reference**		
Radical surgery	0.169	0.042–0.679	0.012	0.185	0.017–2.057	0.170
Others[c]	0.191	0.060–0.613	0.005	0.187	0.025–1.404	0.103
Stage						
Localized	**Reference**			**Reference**		
Regional	1.599	0.973–2.626	0.064	2.173	0.837–5.639	0.111
Distant	5.737	2.889–11.393	<0.001	11.835	4.066–34.455	<0.001
Laterality						
Left	**Reference**			**Reference**		
Right	1.211	0.289–5.070	0.794	1.172	0.196–7.021	0.862

(Continued)

TABLE 2 | Continued

	OS			CSS		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***p***
Unknown[d]	1.293	0.405–4.126	0.664	0.580	0.134–2.516	0.467
LN Status						
Negative	**Reference**			**Reference**		
Positive	6.681	2.465–18.108	<0.001	7.117	1.423–35.785	0.017
Unknown[e]	1.095	0.646–1.855	0.736	1.049	0.407–2.707	0.920
Radiation				7.117	1.423–35.785	0.017
No	**Reference**			**Reference**		
Yes	1.814	1.026–3.202	0.041	1.836	0.680–4.958	0.230

[a] *Including Asian and American Indian.*
[b] *Including divorced, separated, unmarried, domestic partner, and widowed.*
[c] *Including local tumor excision, simple/partial surgical removal of primary site, debulking, etc.*
[d] *Without record.*
[e] *Regional neck lymphadenectomy not performed.*
**OS, overall survival ; CSS, cancer-specific survival; HR, hazard ratio; CI, confidence interval; LN, lymph node.*

by multivariate Cox regression (backward methods) later. As shown in **Table 3**, age, marital status, tumor size, stage, LN status, and radiation were identified as independent variables for OS. However, only tumor size and stage were independent factors for CSS.

Construction of Nomograms

Based on the above variables, we constructed a visualized model to predict the OS and CSS of PC patients at 3, 5, and 8 years. The total score predicts the OS/CSS probabilities through the nomograms and is obtained by adding the score of each variable (**Figure 3**).

Evaluation and External Validation of Nomograms

The nomograms from the SEER set (training set) and Chinese single-center set (validation set) were evaluated and validated by the concordance index (C-index), the area under the curve (AUC), calibration curves, and decision curve analysis (DCA). In the training set, the C-index of the nomogram for OS was 0.736 (95% CI, 0.674–0.798; $p < 0.001$) and 0.820 (95% CI, 0.668–0.972; $p < 0.001$) in the validation set. The AUC of ROC curves in predicting OS at 3, 5, and 8 years in the training set was 0.749, 0.744, and 0.794 **(Figures 4A-C)**, respectively, while the AUC of ROC curves for the validation set was 0.846, 0.896, and 0.702,

TABLE 3 | Multivariate Cox regression analysis of OS and CSS in patients with parathyroid cancer in the training set.

	OS			CSS		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age						
≤66	**Reference**					
>66	3.262	1.870–5.689	<0.001			
Marital status						
Married	**Reference**					
Single	2.134	1.081–4.212	0.029			
Others[a]	1.745	0.991–3.073	0.054			
Tumor size						
1–41 mm	**Reference**			**Reference**		
>41 mm	2.180	1.248–3.810	0.006	3.657	1.566–8.543	0.003
Marital status						
Married	**Reference**			**Reference**		
Single	1.160	0.683–1.971	0.583	2.114	0.813–5.494	0.124
Others[b]	3.262	1.870–5.689	<0.001	10.090	3.438–29.614	<0.001
LN Status						
Negative	**Reference**					
Positive	6.678	2.318–19.240	<0.001			
Unknown[c]	0.995	0.580–1.706	0.985			
Radiation						
No	**Reference**					
Yes	2.075	1.108–3.886	0.023			

[a] *Including divorced, separated, unmarried, domestic partner, and widowed.*
[b] *Including local tumor excision, simple/partial surgical removal of primary site, debulking, etc.*
[c] *Regional neck lymphadenectomy not performed.*
**OS, overall survival ; CSS, cancer-specific survival; HR, hazard ratio; CI, confidence interval; LN, lymph node.*

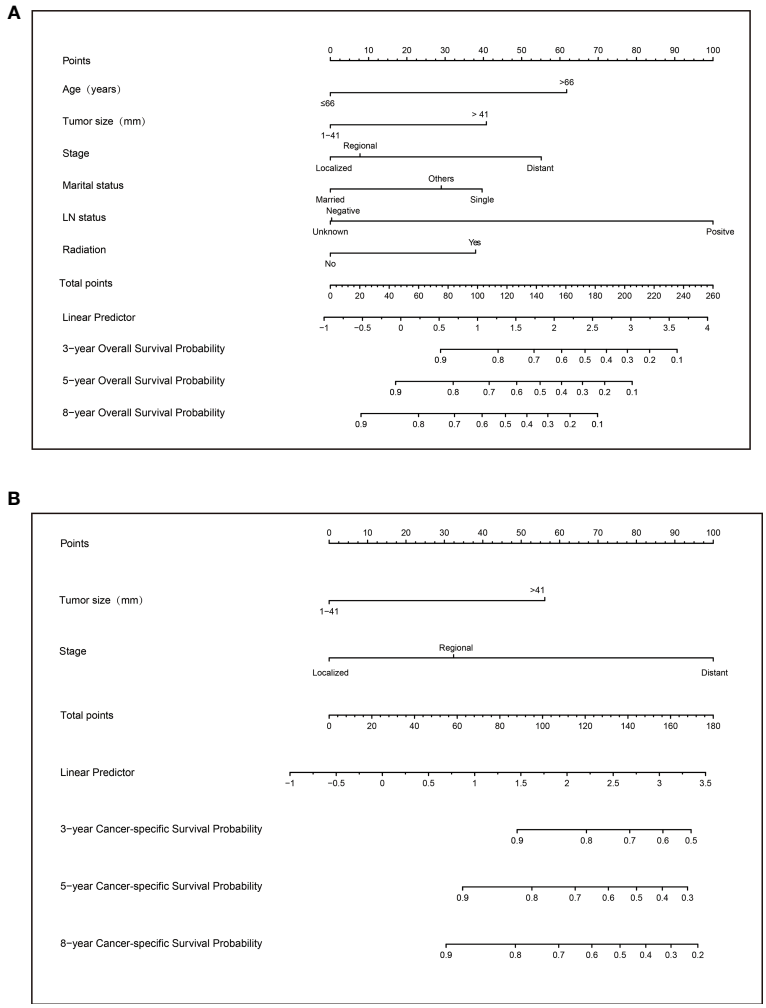

FIGURE 3 | Nomograms to predict 3-, 5-, and 8-year overall survival **(A)** and cancer-specific survival **(B)** for patients with parathyroid carcinoma.

respectively (**Figures 4D-F**). The 3-, 5-, and 8-year calibration curves in the training set and validation set for OS prediction proved to have a satisfying fit (**Figure 5**). The ordinate represents the net benefit, and the abscissa is the threshold probability in DCA curves. "All" refers to all interventions, "None" refers to no intervention, and "Nomogram" refers to the intervention under a certain threshold probability. As shown in **Figure 6**, the DCA curves show a better net clinical benefit.

The C-index of the nomogram for CSS was 0.772 (95% CI, 0.661–0.883; $p < 0.001$) in the training set and 0.943 (95% CI, 0.881–1; $p = 0.003$) in the validation set. The AUC of ROC curves in predicting CSS at 3, 5, and 8 years in the training set was 0.750, 0.744, and 0.779, respectively **(Figures 7A-C)**. The AUC of ROC curves in predicting CSS at 3, 5, and 8 years in the validation set was 0.926, 0.935, and 0.935 (**Figures 7D-F**). Furthermore, the 3-, 5-, and 8-year calibration curves and DCA curves in the training set for CSS prediction proved to have a satisfying fit and net benefit (**Figures 8A-F**). The 3-, 5-, and 8-year CSS calibration curves and DCA curves in the validation set could not be obtained because there were few cancer-specific deaths.

Web-Based Probability Calculator

Applying the above results, dynamic Web-based probability calculators for predicting OS (https://parathyroidcancer.shinyapps.io/dynnomapp/) and CSS (https://parathyroidcancercss.shinyapps.io/dynnomapp/) were constructed (**Figure 3**). The calculators provide a convenient way to predict the individual OS and CSS rates at 3, 5, and 8 years based on PC patient clinical characteristics (**Figure 9**).

Laboratory and Pathological Indices of Parathyroid Carcinoma Patients

Considering the effects of the stage on OS and CSS of PC, further exploration of the validation set would provide valuable insight. The laboratory and pathologic characteristics of PC patients in our hospital were collected, as shown in **Table 4**. Some variables had

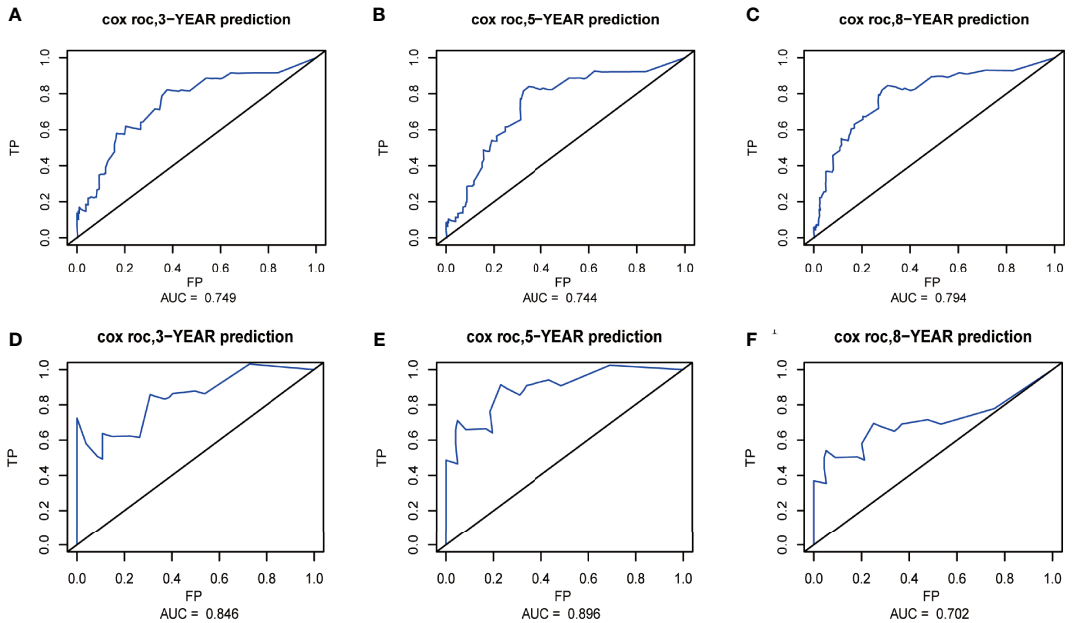

FIGURE 4 | Receiver operating characteristic (ROC) curves at 3, 5, and 8 years in the training set **(A–C)** and validation set **(D–F)** for validating the overall survival (OS) prediction nomogram.

missing values, such as correction serum calcium (crCa), albumin (ALB), creatinine (Cr), alkaline phosphatase (ALP), and Ki-67. The postoperative serum calcium and PTH levels were continuously monitored. Patients with distant metastasis was identified in eight cases during the follow-up interval. The detailed information is described in the **Supplementary Material**.

The differences between laboratory indicators and Ki-67 between different stages were evaluated (**Figure 10**). The levels of ALB, crCa, tumor size, tumor volume, and postoperative PTH demonstrated statistically significant differences between stages. Lower ALB levels and higher postoperative PTH levels were found in patients with distant metastasis. The tumor size and tumor volume in the local disease group were smaller than those in the regional and distant metastasis groups. The level of crCa in the distant metastasis group was statistically higher than that in the local disease group.

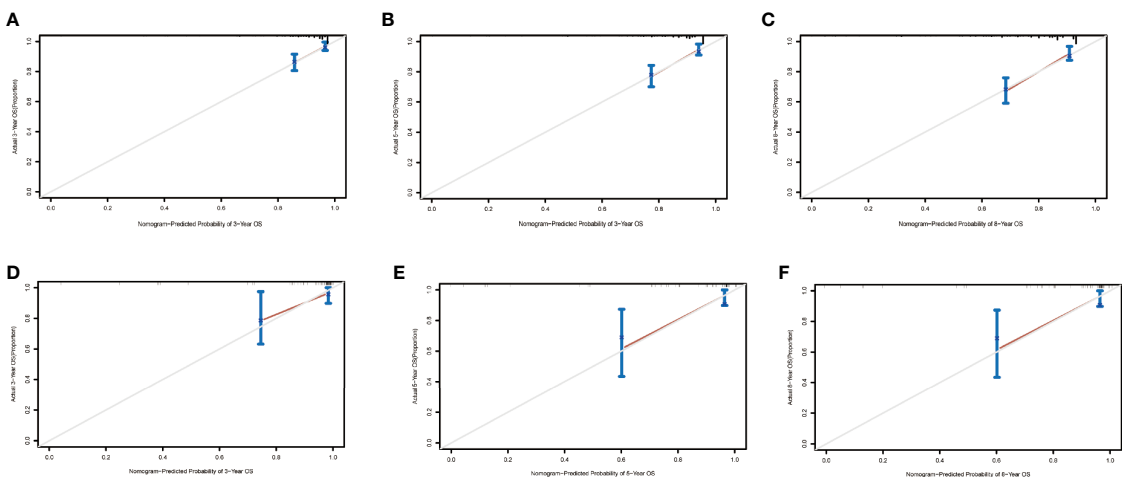

FIGURE 5 | Calibration curves at 3, 5, and 8 years in the training set for validating the overall survival (OS) prediction nomogram **(A–C)**. Calibration curves at 3 and 5 years in the validation set for the OS prediction nomogram **(D–F)**.

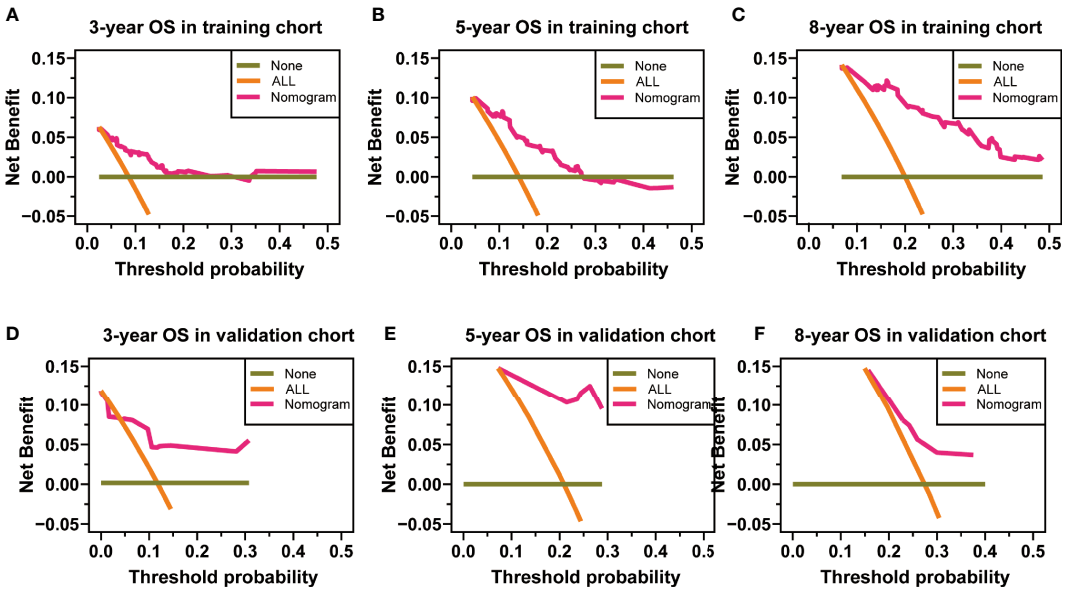

FIGURE 6 | Decision curve analysis at 3, 5, and 8 years in the training set for the overall survival (OS) prediction nomogram **(A–C)**. Decision curve analysis at 3, 5, and 8 years in the validation set for the OS prediction nomogram **(D–F)**.

DISCUSSION

To our knowledge, few studies investigated prognostic factors for patients with PCs, but there was no research that developed a predictive nomogram (11–13, 18–20). This study includes the largest number of patients with a confirmed diagnosis of PC who underwent their first surgery in a single Chinese center. We constructed the nomograms to predict the OS and CSS according to the SEER database, and validation was performed with the Chinese single-center dataset. Extra analysis of the laboratory

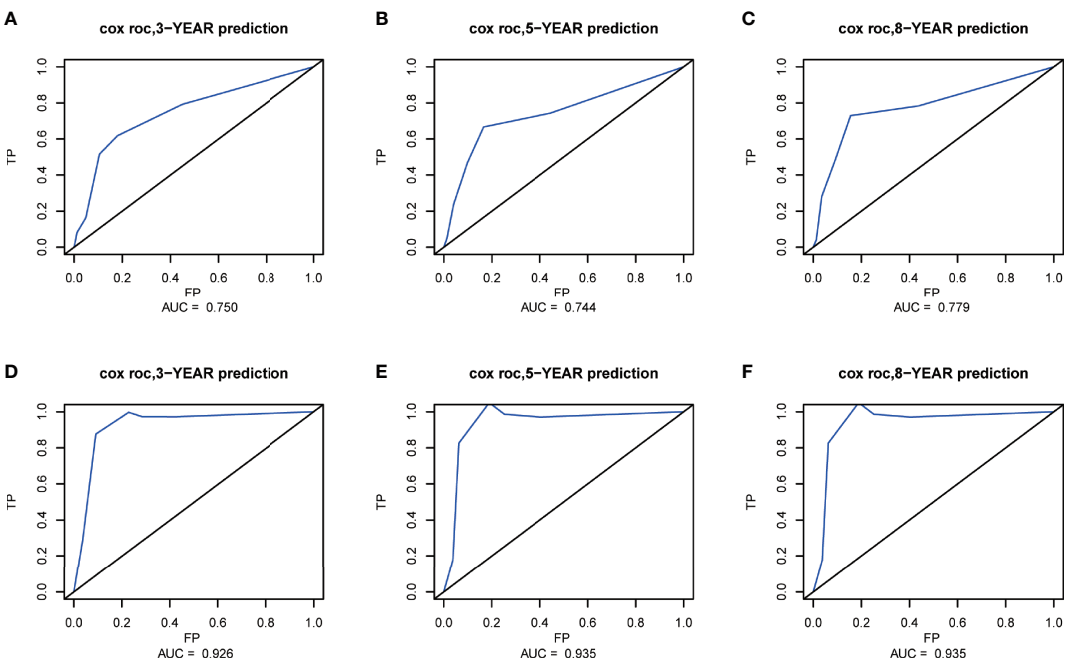

FIGURE 7 | Receiver operating characteristic (ROC) curves at 3, 5, and 8 years in the training set **(A–C)** and validation set **(D–F)** for validating the cancer-specific survival (CSS) prediction nomogram.

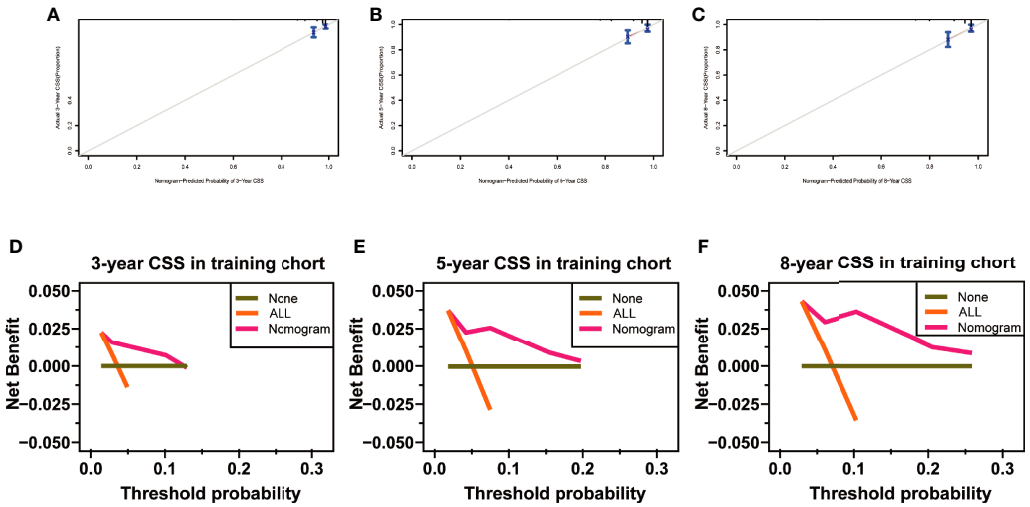

FIGURE 8 | Calibration curves at 3, 5, and 8 years in the training set for validating the cancer-specific survival (CSS) prediction nomogram **(A–C)**. Decision curve analysis at 3, 5, and 8 years in the training set for the CSS prediction nomogram **(D–F)**.

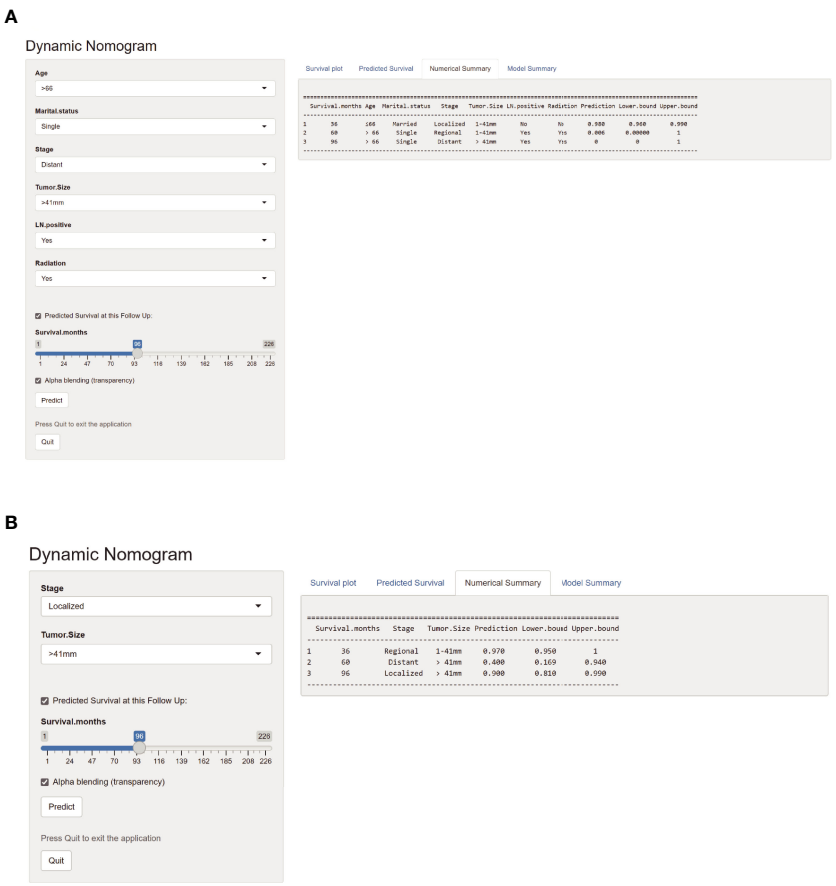

FIGURE 9 | Web-based probability calculators. The 3-, 5-, and 8-year overall survival rate probability was calculated **(A)**. The 3-, 5-, and 8-year cancer-specific survival rate probability was calculated **(B)**.

TABLE 4 | Laboratory and clinicopathologic indices of parathyroid carcinoma patients in the single Chinese centera.

	Overall (N = 59)	Distant (N = 8)	Regional (N = 16)	Localized (N = 35)
Ca (mmol/L)				
Mean (SD)	3.36 (0.69)	3.70 (0.77)	3.31 (0.47)	3.31 (0.76)
crCa (mmol/L)				
Mean (SD)	3.34 (0.74)	3.85 (0.75)	3.30 (0.46)	3.25 (0.81)
Missing	5 (8.5%)	1 (12.5%)	2 (12.5%)	2 (5.7%)
P (mmol/L)				
Median [Min, Max]	0.80 [0.27, 1.91]	1.00 [0.56, 1.48]	0.82 [0.27, 1.04]	0.77 [0.40, 1.91]
PTH (ng/L)				
Median [Min, Max]	684 [58.3, 2,690]	1,590 [177, 2,420]	831 [58.3, 2,620]	463 [88.3, 2,690]
ALB (g/L)				
Median [Min, Max]	41.2 [34.0, 54.3]	37.4 [34.9, 41.9]	44.8 [34.3, 48.6]	41.3 [34.0, 54.3]
Missing	5 (8.5%)	1 (12.5%)	2 (12.5%)	2 (5.7%)
Cr (IU/L)				
Median [Min, Max]	71.4 [6.00, 342]	100 [6.00, 255]	76.5 [28.0, 153]	68.5 [19.0, 342]
Missing	1 (1.7%)	0 (0%)	0 (0%)	1 (2.9%)
ALP (IU/L)				
Median [Min, Max]	189 [42.0, 3,590]	233 [64.0, 1,080]	291 [64.0, 3,590]	185 [42.0, 3,320]
Missing	3 (5.1%)	0 (0%)	1 (6.3%)	2 (5.7%)
Postoperative PTH (ng/L)				
Median [Min, Max]	18.1 [1.83, 736]	69.1 [9.81, 736]	12.4 [3.62, 58.7]	16.2 [1.83, 199]
Postoperative Ca (mmol/L)				
Mean (SD)	2.22 (0.22)	2.18 (0.16)	2.25 (0.22)	2.21 (0.24)
Tumor Size (mm)				
Mean (SD)	32.1 (12.7)	42.3 (12.6)	38.2 (12.3)	26.9 (10.4)
Tumor Volume (mm3)				
Median [Min, Max]	7,500 [240, 60,000]	12,900 [7,500, 60,000]	16,800 [1,950, 60,000]	4,880 [240, 39,000]
Ki-67				
Median [Min, Max]	5 [1, 50]	5 [2, 20]	5 [1, 50]	5 [1, 40]
Missing	11 (18.6%)	1 (12.5%)	2 (12.5%)	8 (22.9%)

[a] *Ca, calcium; crCa, correction serum calcium; P, phosphorus; PTH, parathyroid hormone; ALB, albumin; Cr, creatinine; ALP, alkaline phosphatase.*

investigations at different stages was performed. Notably, the prognostic factors for disease-free survival of PC patients in our hospital were reported (21).

There were some differences between the SEER dataset and the Chinese single-center dataset. Some potential causal factors include religious beliefs, economic conditions, incidence, treatment strategy, and so on. Univariate and multivariate Cox regression proportional analysis showed that the independent prognostic factors for OS in patients with PC were age, marital status, tumor size, stage, positive lymph nodes, and radiation therapy. Larger tumor size and distant metastasis were identified as significantly poor prognostic factors of poor CSS.

Age was expected to have an essential role in predicting OS. Additionally, the view that the prognosis of cancer patients is worse with aging is acceptable. Sadler et al. (20) and Silva-Figueroa et al. (12) showed the same effect of age on patients with PC as we did. Older patients tend to have worse comprehensive physical conditions, which results in a higher incidence of complications after surgery and a longer recovery time (22, 23). Therefore, better general geriatric medical management could lead to improved OS of PC patients.

Our finding indicates that marital status was associated with the OS of PC patients, and married patients had a better prognosis than that of single or other patients. Marital status is not only an independent prognostic indicator of many cancers (24–27) but also a risk factor for developing cancers (28, 29). A variety of reasons may lead to this result. With family support or spouse support, married patients were better equipped to overcome cancer better than patients with other marital statuses. Having relatively strong financial resources makes it easier to obtain better treatment for married patients, which is associated with a better prognosis. Our findings suggest that married PC patients have a better prognosis than other patients. However, we have no information on socioeconomic status available for prediction. It highlights the potentially significant impact that social support can have on survival. Nevertheless, these results still needed to be verified.

PC is a radioresistant tumor, and there is no indication that radiotherapy is performed as a regular treatment (30). The PC analysis in the National Cancer Data Base (NCDB) showed that radiotherapy did not improve the OS rate of patients (31). Among the largest series of patients published, the 5-year OS rate of patients receiving radiotherapy is lower. In contrast, this may reflect those patients with the more advanced disease who are referred for radiotherapy (20). The same result was observed in this study. Christakis et al. (32) showed that long-term disease control for high-risk patients could be achieved through reoperation and postoperative radiotherapy at Anderson Cancer Center. The effect of radiotherapy should be investigated in a long-term study including more patients, as there are only 8 cases in the above study (32).

It is controversial that a positive lymph node status is associated with a poor prognosis. However, this study and others revealed that lymph nodes status is significantly

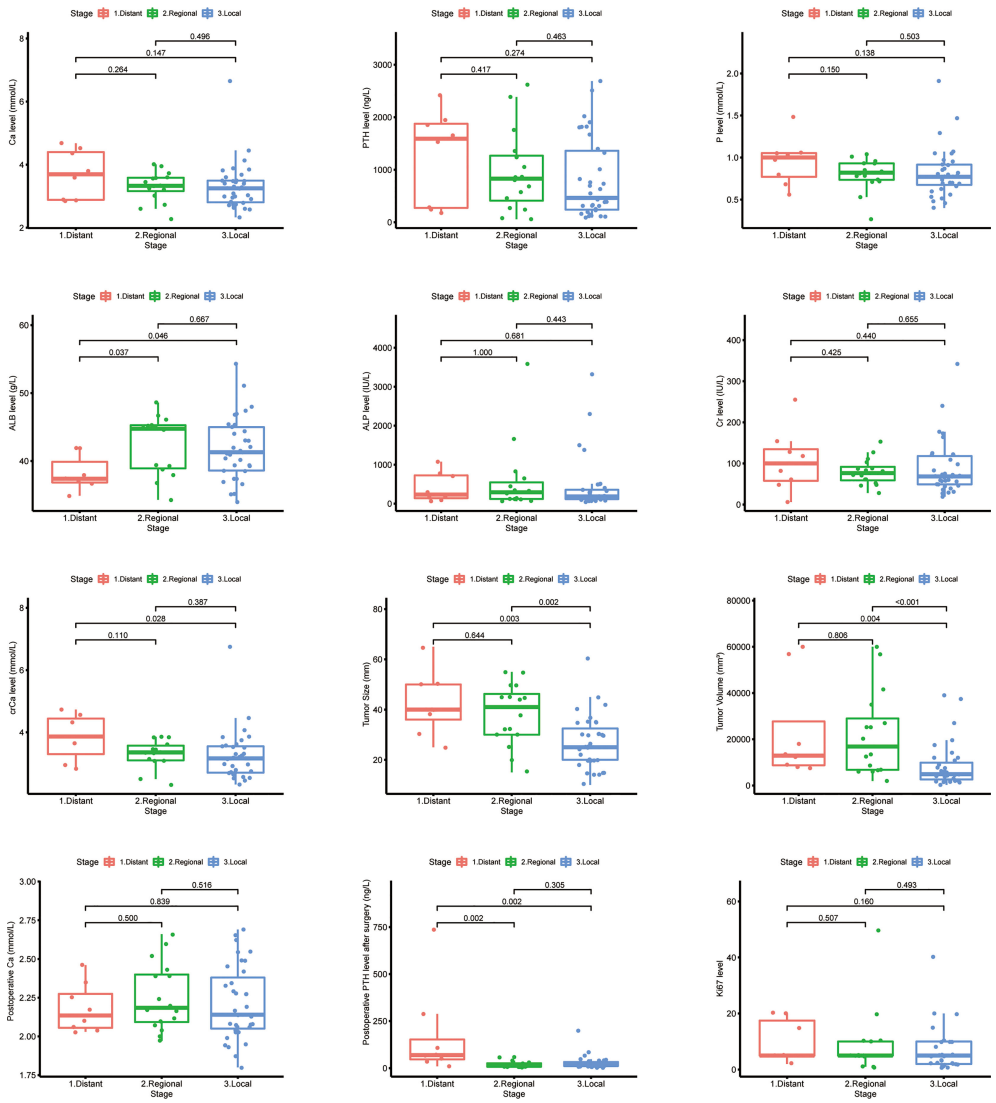

FIGURE 10 | Laboratory and pathologic indicator levels among different stages in Chinese single-center parathyroid carcinoma patients.

associated with poor prognosis (20, 33–35). Less than a third of the patients (**Table 1**) undergoing neck dissection have had the lymph nodes investigated. Therefore, a considerable proportion of patients likely have insufficient staging of the lymphatic involvement of the disease.

Tumor size and stage have always been considered the key factors affecting the prognosis of PC (3, 13, 18). The most common cutoff value of tumor size was recognized as 3 cm, although it was 4.1 cm in this study (10). This may be related to the number of patients and outcome indicators in different studies. The involvement of distant organs or lymph nodes in parathyroid cancer indicates poor outcomes. The OS and CSS Kaplan–Meier curves of the distant metastasis group showed a significant difference from the other in the validation set. Mostly, distant metastasis patients without reoperation suffered from hypercalcemia (36).

Therefore, the early identification of distant metastasis is valuable in parathyroid cancer management. Patients with distant metastases were likely to have lower ALB levels and higher postoperative PTH levels compared to other patients. The correlation between serum ALB and tumor prognosis was found in various tumors (37). A meta-analysis showed that lower ALB levels before treatment were significantly associated with poorer metastasis-free survival in nasopharyngeal carcinoma (38). In addition, lower ALB levels in gastric, rectal, and cervical cancers have also been shown to be prognostic predictors of tumors (39–41). ALB is closely associated with inflammatory and dystrophic status in cancer patients. ALB is mainly synthesized by the liver and is the most and smallest molecular in plasma (42). They also activate DNA replication in cancer cells, thereby promoting tumor proliferation and immune escape (43, 44). Thus, PC can lead to lower ALB levels by inducing malnutrition

and anorexia, further leading to disease progression and forming a vicious circle. When evaluating PC patients, it is recommended that serum ALB levels be routinely checked and adequately evaluated accordingly. And for metastatic parathyroid cancer patients, early nutritional intervention to correct ALB levels may be a new idea.

Several limitations of this study should be addressed. Firstly, since this is a retrospective study, information and selection bias may be present. Secondly, the eighth edition of the AJCC staging system and much clinicopathological information, such as recurrence and calcium and PTH levels, and some individual molecular factors were not found in the SEER database. Lastly, the classification of age and tumor size based on this study may not apply to other studies. Larger cohorts are necessary for further validation of the present nomograms.

In conclusion, predictive nomograms integrating independent prognostic factors and Web-based probability calculators based on the SEER database were constructed to predict the OS and CSS rates of PC patients. The established nomogram application can provide accurate prognostics for patients with PC in the Chinese population, but it must be validated on prospectively collected real-world data.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This research was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Number: 2021-KY-1062-001). Patients from the SEER database had consented to be studied publicly in any scientific research worldwide.

AUTHOR CONTRIBUTIONS

MT had full access to all of the data from both SEER and our hospital, performed the statistical analysis, and drafted the article. SL took responsibility for the data integrity and interpretation. XW and MJ verified the accuracy of the data and performed proofreading. XL supervised and proofread. All authors participated in the concept and design. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.850457/full#supplementary-material

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CASE REPORT
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Case Report: Hypoglycemia Due to Metastatic Insulinoma in Insulin-Dependent Type 2 Diabetes Successfully Treated With 177 Lu-DOTATATE

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We describe a 96-year-old man with insulin-dependent type 2 diabetes mellitus who, despite insulin cessation, presented with recurrent hypoglycemia associated with confirmed inappropriate endogenous hyperinsulinemia. ^{68}Ga-DOTATATE-PET/CT scans demonstrated increased uptake in the pancreatic tail with multiple large intensely active liver metastases. Liver biopsy confirmed the diagnosis of well-differentiated metastatic neuroendocrine tumor. He was unsuitable for surgical resection and long-acting somatostatin analog therapy was ineffective. Subsequent management with four cycles of Lutate [177-Lutetium-DOTA^{0}-Tyr^{3}-octreotate (^{177}Lu-DOTATATE)] resulted in resolution of hypoglycemia and ongoing clinical, biochemical, and radiological response 6 years after. This case is unique due to not only the paradoxical entity of insulinoma in insulin-dependent diabetes but also the positive sustained outcome after ^{177}Lu-DOTATATE, given that unresectable metastatic insulinoma carries a poor prognosis. We review published cases of metastatic insulinoma in patients with diabetes mellitus as well as the literature to-date investigating efficacy and safety of Lutate therapy in metastatic insulinoma.

Keywords: hypoglycemia, insulinoma, diabetes, type 2 diabetes, lutate, lutetium, neuroendocrine tumor

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INTRODUCTION

Although insulinomas are the most common functioning pancreatic neuroendocrine tumors (panNETs) and most common cause of endogenous hyperinsulinemic hypoglycemia, they are rare, occurring in approximately one to four per million people annually (1, 2). Insulinoma is exceptionally rare in a patient with pre-existing diabetes mellitus but important not to miss as a cause of recurrent hypoglycemia when iatrogenic causes have been excluded. Insulinomas are predominantly non-metastatic (90%–95%), sporadic, solitary, small (<2cm), and intrapancreatic NETs and most commonly occur in the fifth to sixth decades of life with equal sex distribution (3, 4). Surgical resection is the only cure, with both cure rates and 10-year survival rate >90% in patients with non-metastatic insulinoma following resection (5, 6).

Metastatic insulinoma, however, carries a poor prognosis. A large European registry including 81 patients with metastatic insulinoma reported a 5-year survival rate of 55.6% (7). Data from an

American registry of patients (n = 121) revealed a much lower 5-year survival rate in unresectable metastatic insulinoma compared to those who underwent surgery (14% vs. 84%, $p < 0.001$) (8). Management of recurrent hypoglycemia in these patients is extremely challenging given lack of definitive surgical cure and limitations of available medical options including paucity of data in insulinoma specifically, modest efficacy, and treatment-related side effects and toxicity (9). ^{177}Lu-DOTATATE (Lutate) has an emerging evidence basis in patients with gastroenteropancreatic (GEP) NETs and shows promise in managing hypoglycemia secondary to metastatic unresectable insulinoma; however, further studies are required (10, 11).

We present a 96-year-old man with insulin-dependent type 2 diabetes mellitus (T2DM) and recurrent refractory life-threatening hypoglycemia secondary to metastatic insulinoma. ^{177}Lu-DOTATATE resulted in resolution of hypoglycemia and reduction in metastatic disease burden, with ongoing clinical, biochemical, and radiological response at 6-year follow-up. This case is unique due to not only the paradoxical entity of insulinoma in insulin-dependent diabetes but also the positive sustained well-documented outcome after ^{177}Lu-DOTATATE, given that unresectable metastatic insulinoma carries a poor prognosis. We review the few published cases of metastatic insulinoma in patients with diabetes and detail the limited existing data investigating ^{177}Lu-DOTATATE therapy in patients with metastatic insulinoma.

CASE DESCRIPTION

A 96-year-old man was referred to our Endocrinology institution by his general practitioner for difficult management of longstanding T2DM. He had been experiencing recurrent "funny turns" (six episodes in the preceding 12 months) associated with hypoglycemia culminating in an episode of loss of consciousness necessitating hospitalization. During this period, there was significant reduction in diabetic regimen intensity including insulin cessation; however, weight had remained stable. His hypoglycemic episodes were predominantly fasting and relieved with carbohydrate consumption.

DIAGNOSIS, TREATMENT, AND OUTCOMES

Common differentials such as renal/liver failure (eGFR, 55 ml/min/1.73 m^2), hypocortisolemia, growth hormone deficiency, and malabsorption were excluded. He subsequently underwent a 75-g oral glucose tolerance test (OGTT), which indicated abnormal insulin physiology. The OGTT results (**Table 1A**) revealed inappropriate fasting hyperinsulinemia (23 μIU/ml) in the setting of fasting hypoglycemia (2.6 mmol/L). This was followed by significant hyperglycemia 2 hours after glucose load (15.8 mmol/L) and insufficient insulin response (61 μIU/ml). After 5 hours, he again demonstrated inappropriate hyperinsulinemia (24 μIU/ml) with hypoglycemia (2.5 mmol/L). Thus, the 75-g OGTT indicated a dysregulated relationship between glucose and insulin concentrations, with inappropriate hyperinsulinemia in the setting of hypoglycemia at fasting and 5 hour after glucose load and with significant hyperglycemia and insufficient insulin response at 2 hours after glucose load. During prolonged inpatient fast, several episodes of hypoglycemia occurred during which inappropriate endogenous hyperinsulinemia was confirmed with elevated insulin and C-peptide concentrations during hypoglycemia (**Table 1B**). Sulfonylurea use and insulin antibodies were excluded, raising suspicion for an insulinoma.

TABLE 1A | Paired serum glucose and insulin concentrations during 5-hour 75-g oral glucose tolerance test (OGTT).

	Glucose (mmol/L)	Insulin (mU/L)
Fasting	2.6	23
0.5 hours	6.3	30
1 hour	11.3	47
1.5 hours	14.1	57
2 hours	15.8	59
2.5 hours	14.4	61
3 hours	11.2	49
3.5 hours	7.4	40
4 hours	5.0	33
4.5 hours	3.6	29
5 hours	2.6	24

Normal range for glucose fasting, 3.6–6.0 mmol/L; normal range for insulin fasting, <10 mU/L; normal range for glucose 2 hours after glucose load, 3.6–7.7 mmol/L.

Computed tomography (CT) scan of the abdomen could not identify focal or diffuse pancreatic enlargement although detected three arterially enhancing liver lesions, measuring 8.0, 6.0, and 2.0 cm. ^{68}Gallium-DOTATATE–positron emission tomography (PET)/CT scan, however, showed a small focus of moderately intense tracer accumulation in the pancreatic tail (**Figure 1**) and extensive active metastatic disease in both lobes of the liver (**Figures 2**, **3**). Serum tumor markers α-fetoprotein (AFP), carcinoembryonic antigen (CEA), and cancer antigen (CA)-19.9 were negative. Subsequent liver biopsy revealed a well-differentiated, metastatic NET. Tumor cells were characterized by eccentric round-oval nuclei with mild nuclear pleomorphism

TABLE 1B | Paired glucose, C-peptide, insulin, and proinsulin concentrations during inpatient fasting episodes of hypoglycemia.

Study	Result	Sample 1	Sample 2	Normal Range (Units)
Glucose	Low	2.4	1.0	3.0–5.5 (mmol/L)
C-peptide	High	11.02	8.9	0.4–4.5 (ng/mL)
Insulin	High	34.2	29.1	2.6–24.9 (mU/L)
Proinsulin	High	>99.9	>99.9	<13.3 (nmol/L)

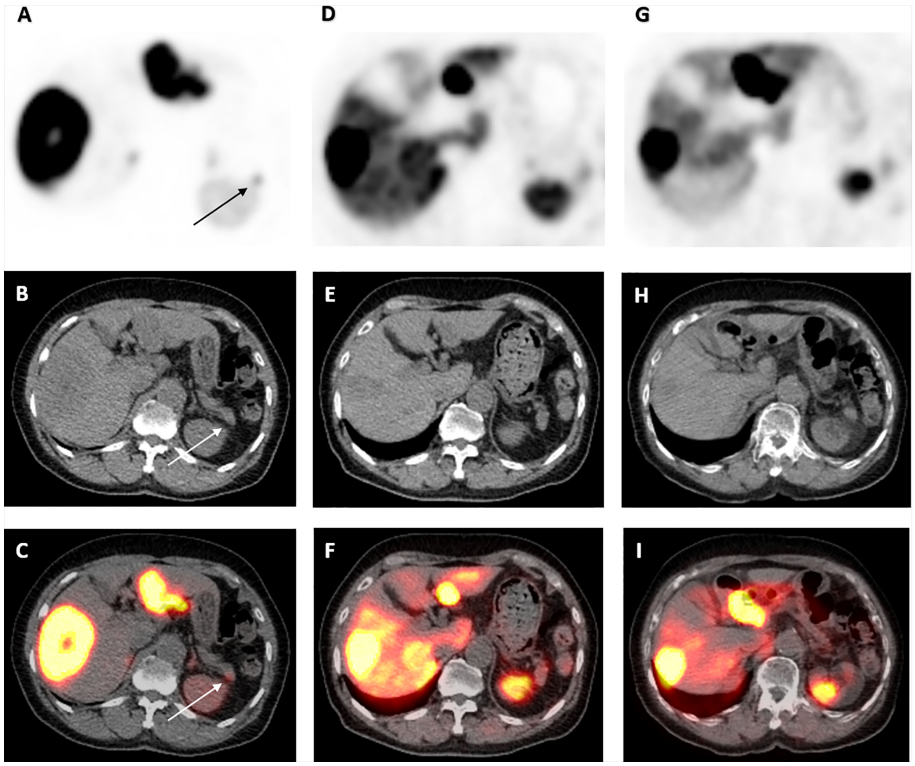

FIGURE 1 | 68Ga-DOTATATE PET/CT scan images of pancreatic tail lesion pre- and post-177 Lu-DOTATATE. Axial view images of ^{68}Ga-DOTATATE PET/CT scan demonstrating interval reduction in avidity in moderately intense focus of activity at the tip of the pancreatic tail (arrow) on PET, low-dose CT and PET/CT fusion (top to bottom) from baseline, to 1 and 4 years after ^{177}Lu-DOTATATE therapy (left to right). Physiological uptake in the spleen and remainder of the liver is also visualized.

and moderate eosinophilic granular cytoplasm. One mitosis per 10 hpf was noted. Tumor cells exhibited strongly positive staining for neuroendocrine markers chromogranin A, synaptophysin, and cluster differentiation (CD)-56, whereas staining was negative for insulin, TTF1, and CDX2. The Ki67 proliferative index was estimated at 2%–3%.

Given advanced age and extensive liver metastases, the patient was deemed unsuitable for surgical resection. He failed initial medical treatment consisting of four weekly intramuscular injections of long-acting Octreotide (30 mg) with another hypoglycemic syncopal event requiring hospital admission. He was then trialed on four cycles of intravenous ^{177}Lu-DOTATATE with cumulative dose of 32 GBq over 5 months. He did not experience any dose-limiting side effects such as acute kidney injury or cytopaenia. Hypoglycemic episodes abated, and after a few months, he developed symptomatic hyperglycemia up to 24 mmol/L, which was insufficiently controlled with re-commencement of oral hypoglycemic therapy.

Paradoxically, this elderly man with endogenous hyperinsulinemia from metastatic insulinoma, having had his metastatic disease controlled on ^{177}Lu-DOTATATE, began to require exogenous insulin administration for glycemic control.

Repeat ^{68}Ga-DOTATATE-PET/CT scans 1 and 4 years after ^{177}Lu-DOTATATE completion demonstrated significant durable response with reduction in uptake in the pancreatic tail (**Figure 1**) and throughout the liver (**Figures 2**, **3**). Serial chromogranin A levels also declined from 3,992 to 119 ng/ml (normal range ≤104 ng/ml). He is currently well 6 years after completion of ^{177}Lu-DOTATATE for his insulinoma at the age of 96 years with sustained clinical, biochemical, and radiological response.

TIMELINE

April, 2015	Onset of "funny turns"
October, 2015	Endocrinology institution referral
November, 2015	Histopathological diagnosis of metastatic insulinoma
December, 2015	Octreotide commencement
January, 2016	Last serious hypoglycaemic episode
January, 2016	First cycle of ^{177}Lu-DOTATATE (8,009 MBq) Octreotide cessation
February, 2016	Second cycle of ^{177}Lu-DOTATATE (8,010 MBq)
March, 2016	Resolution of hypoglycemia
April, 2016	Third cycle of ^{177}Lu-DOTATATE (7,928 MBq)
June, 2016	Fourth cycle of ^{177}Lu-DOTATATE (7,976 MBq)
July, 2016	Onset of symptomatic hyperglycemia
August, 2016	Oral hypoglycemic agent re-commencement
June, 2017	Exogenous insulin re-commencement
January, 2021	Last ^{68}Ga-DOTATATE-PET/CT scan
February, 2022	Last clinical assessment

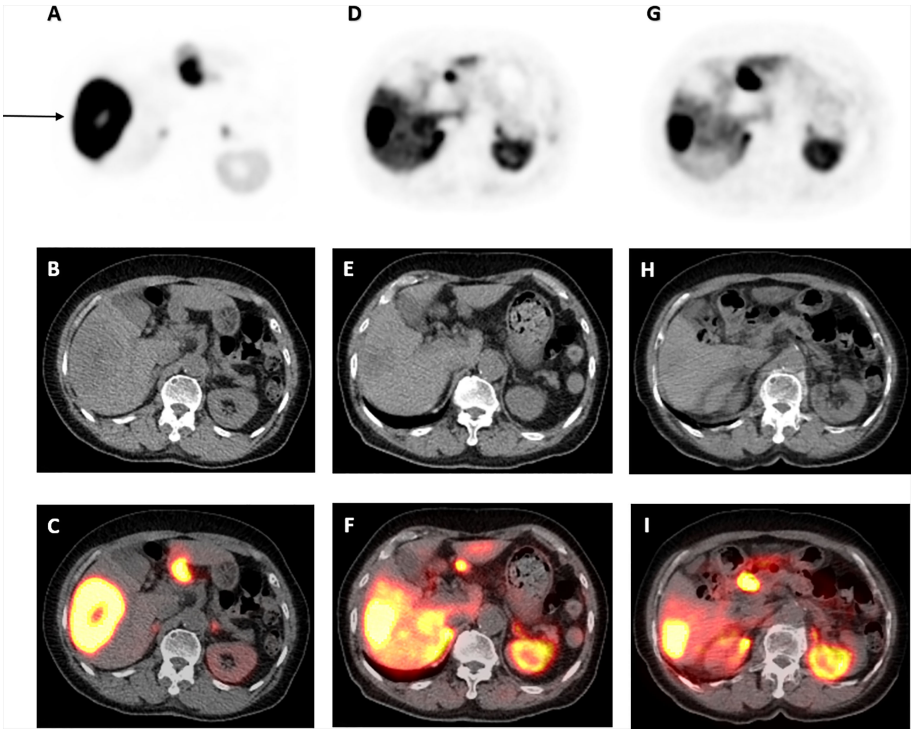

FIGURE 2 | 68Ga-DOTATATE PET/CT scan images of dominant right liver lobe lesion pre- and post-177 Lu-DOTATATE. Axial view images of ^{68}Ga-DOTATATE PET/CT scan demonstrating interval reduction in size in dominant right liver lobe lesion (arrow) on PET, low-dose CT and PET/CT fusion (top to bottom) from baseline, to 1 and 4 years after ^{177}Lu-DOTATATE therapy (left to right). Physiological uptake in the spleen and remainder of the liver is also visualized.

DISCUSSION

Insulinomas are considered functional panNETs as they result in endogenous hyperinsulinemia, producing the clinical syndrome of recurrent hypoglycemia (most commonly fasting) (1). Whipple's triad, which is well-documented, serves as the trigger for further investigation (12, 13). Biochemical diagnosis is confirmed with evidence of inappropriate endogenous hyperinsulinemia (elevated/non-suppressed insulin and C-peptide concentrations) during either spontaneous or provoked hypoglycemia (during prolonged inpatient 72-hour fast), after excluding sulfonylurea use (12, 13). Given that surgical resection is the only cure, the next step after confirmed biochemical diagnosis is meticulous localization of the culprit tumor. Localization begins with structural imaging including ultrasound, CT, or magnetic resonance imaging (MRI), followed by functional imaging such as ^{68}Ga-DOTATATE-PET/CT scans, which facilitate detection of occult metastases (12, 13). Endoscopic ultrasound (EUS) in combination with fine needle aspiration is the most accurate diagnostic tool for insulinoma with sensitivity and specificity up to 95% and allows histopathological examination of the primary pancreatic tumor and adjacent lymph nodes. EUS, however, is not as sensitive in detecting pancreatic tail tumors (3, 4). Positive tumor cell staining for insulin is supportive but not mandatory for insulinoma diagnosis as up to 20% of patients with pre-operative hyperinsulinemic hypoglycemia and resolution post-tumor resection have negative insulin staining (14). In a retrospective analysis of 80 patients with insulinoma, malignant insulinoma was less likely to stain positive for insulin compared to benign insulinoma (3/7 vs. 66/73, $p = 0.015$) (15). Hypotheses for lack of insulin staining include defects in insulin storage capacity and sampling errors.

The OGTT is not indicated in the diagnostic algorithm for suspected cases of insulinoma (12), partly due to the occurrence of hypoglycemia during OGTT in some healthy individuals (16). Regardless, the results of a 5-hour OGTT provided the first indication of abnormal insulin physiology in our patient. The OGTT demonstrated inappropriately high fasting insulin during hypoglycemia and insufficient insulin response to hyperglycemia post-glucose ingestion, suggesting that insulin release was occurring largely independent of changes in serum glucose. Under normal physiological conditions, glucose metabolism is intimately coupled with β-cell insulin secretion such that blood glucose concentrations are maintained between 3.5 and 5.5 mmol/L (17), with a sigmoidal pattern of insulin release to higher glucose concentrations in isolated normal human islet cells (18). However, in the presence of an insulinoma, this glucose-insulin coupling becomes dysregulated. Small-scale *in vitro* studies have shown that cultured human insulinoma cells can have near maximal glucose responsiveness at blood glucose levels of 1.0–3.0 mmol/L (19) with no significant increase in

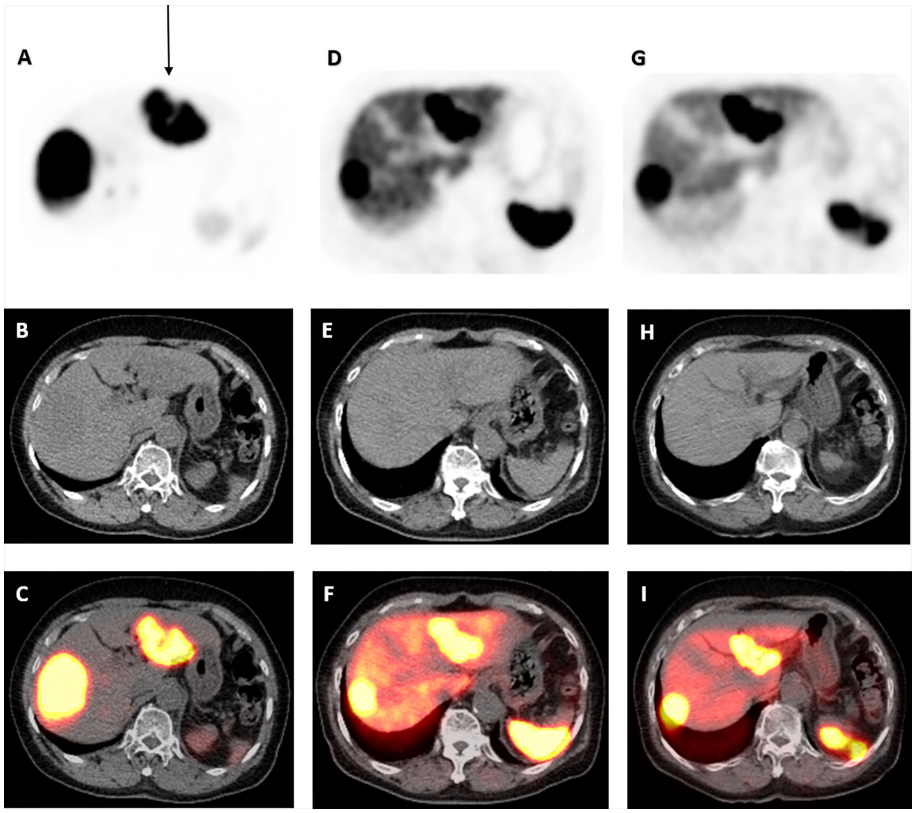

FIGURE 3 | 68Ga-DOTATATE PET/CT scan images of dominant left liver lobe lesion pre- and post-177 Lu-DOTATATE. Axial view images of ^{68}Ga-DOTATATE PET/CT scan demonstrating interval reduction in size in dominant left liver lobe lesion (arrow) on PET, low-dose CT, and PET/CT fusion (top to bottom) from baseline, to 1 and 4 years after ^{177}Lu-DOTATATE therapy (left to right). Physiological uptake in the spleen and remainder of the liver is also visualized.

insulin release with rise in glucose concentration from 2.8 to 8.3 mmol/L in one study (20) and plateau at 10.0–15.0 mmol/L in two other studies (19, 21). The autonomous insulin secretion despite low glucose concentrations and the blunted insulin response to higher glucose concentrations can give rise to fasting hypoglycemia and postprandial hyperglycemia, respectively. This abnormal insulin secretion pattern was not only evident in our patient but also the most common pattern in a retrospective analysis published in 2008 of 64 patients who underwent 100-g OGTT prior to insulinoma resection (22).

Insulinoma is an exceptionally rare occurrence in patients with diabetes. Among 313 confirmed insulinoma cases at the Mayo Clinic between 1927 and 1992, there was only one patient with pre-existing diabetes (23), whereas a cohort from Japan of 443 cases of insulinoma included one diabetic patient (24). A single institution in Taiwan reported one patient with diabetes out of 23 insulinoma cases seen between 1984 and 2006 (25). Potential explanations for the low reported incidence of diabetes in insulinoma cases include a) lack of reporting in the literature, b) missed or delayed diagnosis due to difficulty differentiating iatrogenic from insulinoma-induced hypoglycemia, c) insulin resistance or pre-existing hypoglycemia unawareness masking the clinical syndrome, or d) decreased β-cell number and thus decreased potential cellular regeneration for tumor formation. Extensive literature review yielded 13 cases of metastatic insulinoma in patients with pre-existing diabetes in the past 50 years (**Supplementary Table 1**) (26–37). The majority had pre-existing T2DM (10/13) with mean age 59 years at diagnosis and equal sex distribution. The most common primary site was the pancreatic tail, and majority (7/8) pancreatic lesions were >4 cm in diameter, compared to mean lesion size of 3 cm in the largest published series of malignant insulinoma cases (8), potentially reflecting delayed diagnosis due to the initial requirement of excluding iatrogenic hypoglycemia. The most common metastatic sites were regional lymph nodes (8/13) and liver (11/13). Diazoxide (9/13) and somatostatin analogs (8/13) were the most common medical therapies utilized, whereas ^{177}Lu-DOTATATE has not been reported in a diabetic patient with metastatic insulinoma. Regarding outcomes, six patients died during follow-up, including two patients within 2 weeks from Diazoxide-related toxicity and otherwise due to treatment failure and progressive disease. Six patients had good outcome without recurrence of hypoglycemia; however, this observation is limited by short follow-up length of <1 year in the majority. Thus, our patient shares similarities with previously reported cases of metastatic insulinoma in pre-existing diabetes however is unique given his advanced age, use of ^{177}Lu-DOTATATE, and positive sustained outcome at extended 6-year follow-up.

Given that our patient's advanced age and extensive unresectable liver metastases, curative surgical resection was unsuitable. This led to the difficult medical management issue of recurrent hypoglycemia from metastatic insulinoma. Various review articles have summarized the available medical therapeutic options for managing recurrent insulinoma-induced hypoglycemia (2, 3, 9, 38). Briefly, these strategies are limited by lack of data particularly in patients with insulinoma, modest efficacy, and treatment-related intolerance and toxicity. Diazoxide is a nondiuretic benzothiazide analog, which opens the ATP-sensitive potassium channel on the pancreatic β-cell membrane, hence facilitating potassium cellular efflux and diminishing membrane depolarization and voltage-gated calcium-dependent exocytosis of insulin-containing vesicles (9). Diazoxide has approximately 50% efficacy in abating hypoglycemia in insulinoma but is not useful in controlling metastatic disease (hence not used in our patient) and is often limited by significant toxicity, e.g., fluid retention, renal/liver failure (3). Somatostatin analogs such as long-acting Octreotide and Lanreotide inhibit insulin release from pancreatic β-cells and have up to 50% efficacy in controlling hypoglycemia with modest tumor regression effect. Somatostatin analog use is often limited by gastrointestinal side effects and tachyphylaxis and in some cases worsens hypoglycemia due to concurrent inhibition of counter-regulatory glucagon release (9). Other less investigated but approved options in unresectable GEP NETs include chemotherapy (5-FU, Doxorubicin, Streptozotocin, Temozolomide, and Capecitabine), mTOR inhibitors such as Everolimus and multiple tyrosine kinase inhibitor Sunitinib, with data particularly scarce in metastatic insulinoma (9, 38).

We successfully trialed ^{177}Lu-DOTATATE in our patient, a form of peptide receptor radionuclide therapy (PRRT). Lutate (177-Lutetium-DOTA^0-Tyr^3-octreotate) is a radiolabeled somatostatin analog compound consisting of the somatostatin analog Tyr^3-octreotate, linked with a radioactive isotope, 177-Lutetium, with DOTA^0 acting as the linking agent. Intravenous infusion of ^{177}Lu-DOTATATE allows delivery of targeted cytotoxic ionizing radiation therapy specifically to neuroendocrine tumor cells, taking advantage of their somatostatin receptor (SST) overexpression (particularly SST2) and the low physiological SST expression in normal tissue. Compared to earlier radionuclides, 177-Lutetium emits diagnostic γ-radiation, allowing better dosimetry, emits therapeutic β-radiation that has a shorter tissue penetration range (2mm), limiting exposure to neighboring normal tissue, and has nine-fold higher affinity for SST2, and lower hematological/renal toxicity (10, 39).

The Rotterdam group in Netherlands has reported results of ^{177}Lu-DOTATATE in several patients with NETs in the past 20 years. Kwekkeboom et al. investigated 131 patients with metastatic GEP NETs (including two with insulinoma) with median follow-up 16 months (40). Results were favorable with 47% having an objective response, 35% stable disease, and 18% progressive disease, and ^{177}Lu-DOTATATE was considered safe with <2% experiencing serious hematological toxicity and two cases of serious liver/hepatic toxicity. Brabander et al. more recently assessed ^{177}Lu-DOTATATE efficacy in 443 patients with metastatic bronchial and GEP NETs (including 21 patients with functional panNETs) with 78-month median follow-up (41). Progression-free survival was 29 months, time to progression of 36 months, and overall survival of 63 months, with objective response (complete or partial) in 39% and stable disease reached in 43% of patients. Clinically significant hematological toxicity occurred in 10%, including acute leukemia (AL) in 0.7% and myelodysplastic syndrome (MDS) in 1.5% of patients with no treatment-related long-term renal or liver failure observed.

However, given the low prevalence of metastatic insulinoma, data exploring efficacy of ^{177}Lu-DOTATATE in these patients specifically are limited to small case series and single case reports. Our literature review revealed 33 published cases of metastatic insulinoma trialed on ^{177}Lu-DOTATATE therapy, with 32/33 having liver metastases (11, 42–49).

The largest such case series was conducted by Zandee et al. who investigated ^{177}Lu-DOTATATE safety and efficacy in 34 patients with metastatic functioning panNETs including 14 with insulinoma (11). Eight patients had pre-treatment with somatostatin analogs, five had surgery, and two had chemotherapy. Objective response, stable disease, and progressive disease occurred in 50%, 21.4%, and 28.6% of patients, respectively, with approximately 30 months mean progression-free survival and 67% experiencing reduction in hypoglycemia frequency.

In a cohort of 310 patients with GEP NETs managed with ^{177}Lu-DOTATATE between 2000 and 2006, Kwekkeboom et al. included five patients with metastatic insulinoma with partial response in three patients, stable disease in one patient, and progressive disease in the other (42).

Ong et al. described two men with inoperable metastatic insulinoma with severe hypoglycemia, who failed Diazoxide and somatostatin analog therapy (43). Both patients experienced control of hypoglycemia and reduction in size of liver metastases with ^{177}Lu-DOTATATE; however, one was co-treated with Everolimus and both with chemotherapy. One patient was hypoglycaemia-free at 10 months and the other had disease progression at 24 months.

Van Schaik et al. treated four patients with metastatic insulinoma and severe uncontrollable hypoglycemia failing conventional therapy including Octreotide (44). ^{177}Lu-DOTATATE achieved stable disease and euglycemia for mean 22 months (one patient still in remission at 20 months).

Magalhães et al. utilized ^{177}Lu-DOTATATE in four patients with unresectable metastatic insulinoma and refractory hypoglycemia all pre-treated with Diazoxide and Octreotide (45). Two patients had disease progression (mean 14 months) and mortality (mean 20 months), whereas two patients remained asymptomatic at mean follow-up 20 months.

Four single case reports have also outlined positive effects of ^{177}Lu-DOTATATE in metastatic insulinoma with refractory hypoglycemia, such as resolution of hypoglycemia and reduction in metastatic burden with all patients having ongoing disease control and radiological stability at mean follow-up interval of 15 months (46–49).

Hence, ^{177}Lu-DOTATATE is a potential effective and safe option in patients with unresectable metastatic insulinoma with perceived benefits including resolution of hypoglycemia and reduction in radiological metastatic burden. However, given likely positive publication bias and scarce data, further studies (ideally randomized and controlled) exploring^{177}Lu-DOTATATE efficacy in this subset of patients is certainly warranted.

Insulinoma, although a rare cause of hypoglycaemia in a patient with diabetes mellitus, is important not to miss and should especially be considered in patients on minimal glucose-lowering therapy or in whom hypoglycemia continues despite insulin cessation. Surgical management is the only cure and is preferred in suitable patients, whereas medical management in cases of unresectable insulinoma with recurrent hypoglycemia is extremely challenging due to poor prognosis and limitations of available treatment options such as diazoxide, somatostatin analogs, and chemotherapy. ^{177}Lu-DOTATATE, a form of PRRT, has an emerging evidence basis in patients with GEP NETs and shows promise as a potential effective and well-tolerated option in patients with recurrent hypoglycemia secondary to unresectable metastatic insulinoma; however, further studies are needed. Our patient had a successful and sustained response to ^{177}Lu-DOTATATE to the extent that he is now requiring exogenous insulin administration for his previously masked poor diabetic control. Despite not undergoing surgical management, ^{177}Lu-DOTATATE has provided him an exceptional outcome in terms of survival and quality of life considering his advanced age and extent of liver metastases.

PATIENT PERSPECTIVE

The patient declined to provide their perspective on the case report, however, provided signed written informed consent for this report to be published.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SK conceived the case report and drafted and critically reviewed the manuscript. MM drafted the manuscript. PR managed the patient and critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.906012/full#supplementary-material

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Reappraisal of a 2-cm Cutoff Size for the Management of Nonfunctional Pancreatic Neuroendocrine Tumors: A Population-Based Study

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Background: Expectant observation and aggressive surgery are both recommended for small nonfunctional pancreatic neuroendocrine tumors (NF-PanNETs). However, the optimal management of small NF-PanNETs remains disputable due to the heterogeneous clinical behavior.

Methods: Patients who were diagnosed with pancreatic neuroendocrine neoplasms (PanNENs) between 2000 and 2018 were identified from the surveillance, epidemiology, and end results (SEER) database and reviewed retrospectively. Tumor aggressiveness was defined as poor differentiation, lymph node involvement, liver involvement, and advanced stage. The best cutoff of tumor size associated with tumor aggressiveness was determined through the receiver operating characteristic (ROC) curve analysis. Univariate and multivariate analyses were used to identify prognostic factors in patients with tumors of ≤2 cm.

Results: A total of 5,172 patients with PanNENs were enrolled, including 1,760 (34.0%) tumors ≤2 cm and 3,412 (66.0%) tumors >2 cm. A 2.5-cm cutoff size was found to be associated with a satisfactory ability in predicting tumor aggressiveness. On multivariate analysis, age, gender, ethnicity, tumor grade, tumor number, and stage were independent prognostic factors for overall survival (OS) in patients with tumors less than or equal to 2 cm in size. A total of 1,621 patients were diagnosed with NF-PanNETs according to the WHO classification, of whom 1,350 underwent surgery, 271 performed active observation. The OS was significantly better in the surgery group compared to the observation group regardless of propensity score analysis. Additionally, a total of 407 patients were selected based on the multivariate Cox regression analysis, of whom 46 underwent observation, 361 underwent surgery, and the OS was comparable.

Conclusion: Expectant observation may be a reasonable alternative to aggressive surgical resection in highly selected small NF-PanNET patients. Also, the decision to observe versus surgery should not only be based on tumor size alone but also take into account other important clinicopathological factors.

Keywords: pancreatic neuroendocrine tumors, observation, surgery, tumor size, survival

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INTRODUCTION

Pancreatic neuroendocrine neoplasms (PanNENs) are among the heterogeneous group of neoplasms with the most rapidly increasing incidence recently (1, 2). This increase is largely attributed to the advances in diagnostic techniques, including computed tomography and endoscopy. Unlike pancreatic adenocarcinoma, the vast majority of PanNENs are considered clinically indolent diseases and associated with favorable prognoses (3, 4). Clinically, PanNENs are classified into functional and nonfunctional diseases. Different from functional PanNENs (F-PanNENs) that are combined with syndromes of hormone hypersecretion, nonfunctional PanNENs (NF-PanNENs) are not accompanied by clinically significant hormonal symptoms. With the wide use of cross-sectional imaging, a sizable fraction of patients are incidentally diagnosed with small, asymptomatic NF-PanNENs. To date, the natural history is, however, not well described. According to the WHO classification, PanNENs are classified into well-differentiated, low-to-intermediate-grade pancreatic neuroendocrine tumors (PanNETs) and poorly differentiated, high-grade pancreatic neuroendocrine carcinomas (PanNECs) (5, 6). The management of F-PanNETs has less controversies, while the treatment of NF-PanNETs, especially for tumors less than or equal to 2 cm in size, remains disputable (7–9). Incidentally diagnosed NF-PanNETs generally exhibit benign behaviors, making them suitable and feasible to undergo surveillance according to some guidelines. In addition, radical treatments such as pancreatectomy may carry a high risk of developing postoperative complications. However, there are limited data examining the safety of this conservative policy. Also, some studies found that NF-PanNETs are inclined to have lymph node involvement, which may compromise the survival results in patients who conduct a "wait-to-see" strategy (10, 11). In terms of the potential risks and uncertain benefits of the observation strategy, the recommendation for its use should be interpreted with caution.

Therefore, the purpose of the study was to identify the association between tumor size and aggressive behaviors in NF-PanNET patients as well as to compare the long-term survival outcomes between close observational monitoring and aggressive surgical resection among patients with NF-PanNETs ≤2 cm. In addition, we attempted to identify patients who were potential candidates for an observational treatment based on a large population database from the United States.

PATIENTS AND METHODS

In this retrospective study, patients diagnosed with PanNENs between 2000 and 2018 were identified from the surveillance, epidemiology, and end results (SEER) database. The evaluated variables included age at diagnosis, gender, year of diagnosis, ethnicity, marital status, tumor characteristics, functionality, treatment, and survival outcomes. The inclusion criteria for PanNENs based on the International Classification of Diseases for Oncology, third edition (ICD-O-3) were as follows: primary sites C25.0 to C25.9 with histological codes 8150, 8151, 8152, 8153, 8155, 8156, 8240, 8241, 8242, 8243, 8245, 8246, and 8249. Patients with unknown information on vital status and survival duration were excluded. The workflow of patient selection for this study is detailed in **Figure 1**. The long-term survival outcomes were compared between the observation and surgery cohorts by evaluating the overall survival (OS) and cancer-specific survival (CSS) before and after propensity score matching.

Statistical Analysis

All statistical analyses were performed using R software and SPSS with a two-sided significance level of 0.05. The categorical variables were presented as numbers (percentage) and were assessed between groups with the Chi-square (χ^2) test or Fisher's exact test, as appropriate. Continuous variables were described as mean ± standard deviation (SD) or median

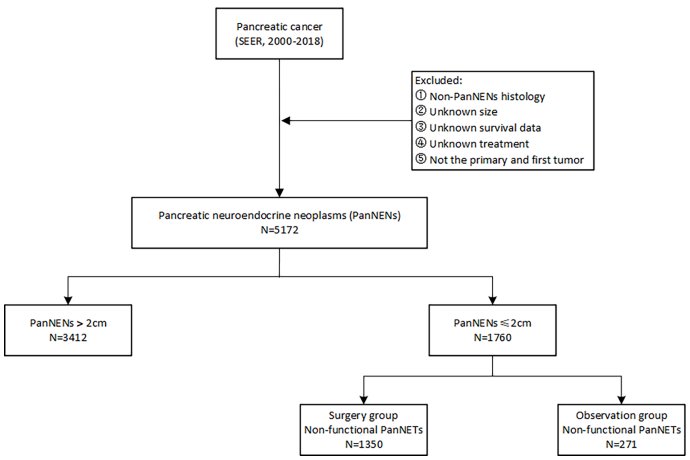

FIGURE 1 | The workflow of patient selection for this study.

(interquartile range) and were compared using the Student's *t*-test or the Mann–Whitney *U* test, as appropriate. Multivariate analysis examining who was more likely to perform surgery was conducted using predictive factors statistically significant to univariate analysis. Furthermore, Cox proportional hazard regression model was used to determine the prognostic variables in patients with tumors ≤2 cm. A propensity score matching (PSM) method using a logistic regression model was utilized to reduce the selection biases and balance confounding factors. In addition, an optimal cutoff value of tumor size for predicting tumor aggressiveness was defined as poor differentiation, lymph node involvement, liver involvement, and advanced stage using the receiver operating characteristics (ROC) method. Survival results were estimated with the Kaplan-Meier method and compared by the log-rank test between groups. In order to evaluate the efficacy of expectant observation in NF-PanNETs with less than or equal to 2 cm in size, OS and CSS rates were compared between observation and aggressive surgical resection cohorts before and after PSM.

RESULTS

Baseline Characteristics

Overall, a total of 5,172 patients with PanNENs between 2001 and 2018 were identified from the SEER database, including 1,760 (34.0%) tumors ≤2 cm and 3,412 (66.0%) tumors >2 cm. Among the cohort with PanNENs ≤2 cm, a vast majority of patients were white (76.1%), married (62.8%), younger than 65 years old (61.8%), well-differentiated (86.4%), and had the loco-regional disease at diagnosis (95.3%). Of note, about 82.7% of these neoplasms were surgically resected while only 0.7% received radiation and 2.1% received chemotherapy. As for patients with tumors larger than 2 cm, the baseline characteristics were significantly different from those with tumors ≤2 cm. Patients with PanNENs >2 cm presented with a more advanced tumor burden, including higher proportions of poor differentiation, lymph node involvement, liver involvement, and late tumor stage. Additionally, the rate of surgical treatment was significantly lower compared to that in patients with PanNENs ≤2 cm. The more detailed clinical characteristics are summarized in **Table 1**. Kaplan–Meier curves revealed that PanNENs >2 cm were associated with worse survival outcomes than PanNENs ≤2 cm (**Figure 2**).

Predictor of Aggressive Behavior: Role of Tumor Size

In order to determine the association between tumor size and aggressive behavior, the predictive ability of preoperative size in the subset of NF-PanNENs was evaluated using the ROC method. In our study, tumor aggressiveness was defined as poor tumor differentiation, lymph node involvement, liver involvement, and advanced tumor stage. In receiver operating characteristic curve analysis, tumor size resulted in an area under the curve (AUC) of 0.769 (95% CI, 0.755–0.782), showing a satisfactory ability to predict aggressiveness in patients with NF-PanNENs. The optimum tumor size cutoff value distinguishing tumor aggressiveness was 2.50 cm, resulting in 81.3% sensitivity and 63.3% specificity (**Figure 3**).

Characteristics of NF-PanNETs ≤2 cm Between Observation and Surgery Cohorts

Among the 5,172 patients with PanNENs enrolled in the SEER database, 1,621 patients were diagnosed with NF-PanNETs according to the WHO classification, of whom 1,350 underwent surgery and 271 performed a conservative treatment. Baseline demographics and clinicopathologic features were displayed in **Table 2**. As shown in the table, age at diagnosis, the rates of lymph node involvement and liver involvement, as well as tumor stage were significantly different between these two cohorts. In the surgery cohort, patients were more frequently presented with lymph node involvement and loco-regional disease.

Comparison of Survival Outcomes After Propensity Score Matching

After PSM, 259 patients were matched in each cohort, and the baseline characteristics were well-balanced (**Table 2**). With regard to survival results, the overall survival was significantly better in patients who underwent surgery regardless of the propensity score analysis. While the cancer-specific survival was comparable between these two groups after propensity score matching (**Figure 4**.)

Factors Associated With Patients With NF-PanNENs Who Underwent Surgery

On univariate logistic regression analysis, age at diagnosis, year of diagnosis, tumor size, functional status, lymph node status, liver involvement, and tumor stage were associated with patients who were more likely to receive surgical treatment. In multivariate analysis, diagnosis at early year, age (<65 years), size 1–2 cm, functional tumors, lymph node involvement, and liver involvement were significant predictors for patients treated with surgery (**Table 3**).

Analysis of Risk Factors for OS in Patients With PanNENs ≤2 cm

The estimated OS rates at 3, 5, and 10 years were 92.3%, 89.2%, and 75.6%, respectively, while the estimated CSS probabilities at 3, 5, and 10 years were 95.8%, 95.0%, and 89.6%, respectively. On multivariate analysis, age, gender, ethnicity, tumor grade, tumor number, and tumor stage were independent prognostic factors for OS in patients with small-sized PanNENs (**Table 4**).

Exploratory Analyses

In order to better define the appropriate indications for nonoperative management, we selected a cohort of patients based on the results of a multivariate survival analysis. Overall, a total of 407 patients were identified, of whom 46 underwent observation and 361 underwent aggressive surgery. In addition, the OS and CSS were comparable between these two groups (**Figure 5**).

TABLE 1 | Patient characteristics of PanNENs ≤2 cm versus >2 cm in the SEER database.

Variables	PanNENs (size ≤2 cm)	PanNENs (size >2 cm)	*p*-value
Total	1,760 (34.0%)	3,412 (66.0%)	
Age			0.004*
<65 years	1,087 (61.8%)	2,245 (65.8%)	
≥65 years	673 (38.2%)	1,167 (34.2%)	
Gender			<0.001*
Male	845 (48.0%)	1,934 (56.7%)	
Female	915 (52.0%)	1,478 (43.3%)	
Ethnicity			0.057
White	1,339 (76.1%)	2,667 (78.2%)	
Black	203 (11.5%)	397 (11.6%)	
Other	218 (12.4%)	348 (10.2%)	
Marital status			0.970
Married	1,105 (62.8%)	2,144 (62.8%)	
Other	655 (37.2%)	1,268 (37.2%)	
Tumor grade			<0.001*
Well differentiated	1,521 (86.4%)	2,054 (60.2%)	
Moderately differentiated	198 (11.3%)	892 (26.1%)	
Poorly differentiated	41 (2.3%)	466 (13.7%)	
Tumor number			0.021*
Single	1,572 (89.3%)	3,115 (91.3%)	
Multiple	188 (10.7%)	297 (8.7%)	
Tumor location			<0.001*
Head	433 (24.6%)	1,110 (32.5%)	
Body/tail	1,033 (58.7%)	1,677 (49.2%)	
Other	294 (16.7%)	625 (18.3%)	
Functional status			<0.001*
Functional	85 (4.8%)	345 (10.1%)	
Nonfunctional	1,675 (95.2%)	3,067 (89.9%)	
Lymph node involvement			<0.001*
Yes	148 (8.4%)	1,245 (36.5%)	
No	1,612 (91.6%)	2,167 (63.5%)	
Liver involvement			<0.001*
Yes	57 (3.2%)	735 (21.5%)	
No	1,676 (95.3%)	2,315 (67.9%)	
Unknown	27 (1.5%)	362 (10.6%)	
Tumor stage			<0.001*
Localized	1,481 (84.1%)	1,174 (34.4%)	
Regional	196 (11.2%)	1,082 (31.7%)	
Distant	83 (4.7%)	1,156 (33.9%)	
Surgery			<0.001*
Yes	1,456 (82.7%)	2,482 (72.7%)	
No	304 (17.3%)	930 (27.3%)	
Radiation			<0.001*
Yes	13 (0.7%)	171 (5.0%)	
No	1,747 (99.3%)	3,241 (95.0%)	
Chemotherapy			<0.001*
Yes	37 (2.1%)	699 (20.5%)	
No	1,723 (97.9%)	2,713 (79.5%)	
Primary endpoint: OS (months)			
Mean (95% CI)	168.3 (157.5–179.0)	124.0 (119.2–128.8)	<0.001[a,*]
Median (95% CI)	NE	129.0 (116.2–141.8)	<0.001[a,*]
Primary endpoint: CSS (months)			
Mean (95% CI)	188.4 (177.3–200.0)	141.3 (136.2–146.3)	<0.001[a,*]
Median (95% CI)	NE	176.0 (149.5–184.2)	<0.001[a,*]

*PanNENs, pancreatic neuroendocrine neoplasms; SEER, surveillance, epidemiology, and end results; OS, overall survival; CI, confidence interval. [a]Log-rank test. *Significance.*

DISCUSSION

Our study provides a comprehensive characterization of PanNENs and NF-PanNETs based on a large cohort of the population from the United States. Generally, the choice between observation and aggressive surgery should be on the basis of an accurate estimate of the malignant potential. However, our results indicate that using a 2-cm cutoff size alone to guide treatment decisions does not seem to be appropriate and safe. Surgery was found to be associated with survival advantages in patients with NF-PanNETs ≤2 cm compared to observation regardless of PSM analysis. Instead, patients with NF-PanNENs who were younger than 65 years old, of the female sex, white or other ethnicities rather than black, low-to-

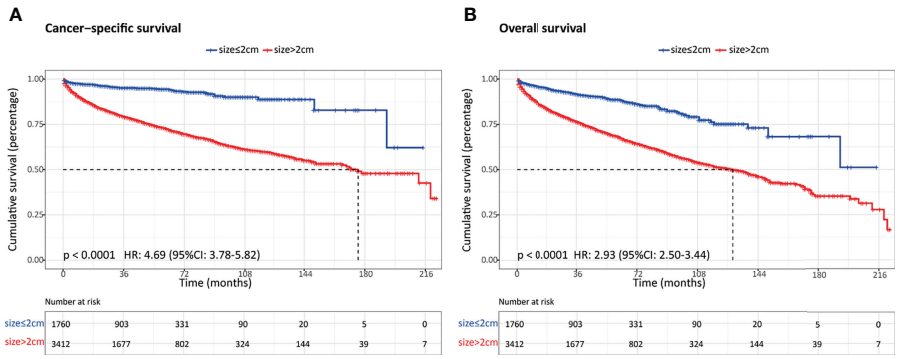

FIGURE 2 | Comparison of survival outcomes between patients with pancreatic neuroendocrine neoplasms (PanNENs) ≤2 cm and PanNENs >2 cm. **(A)** Cancer-specific survival. **(B)** Overall survival.

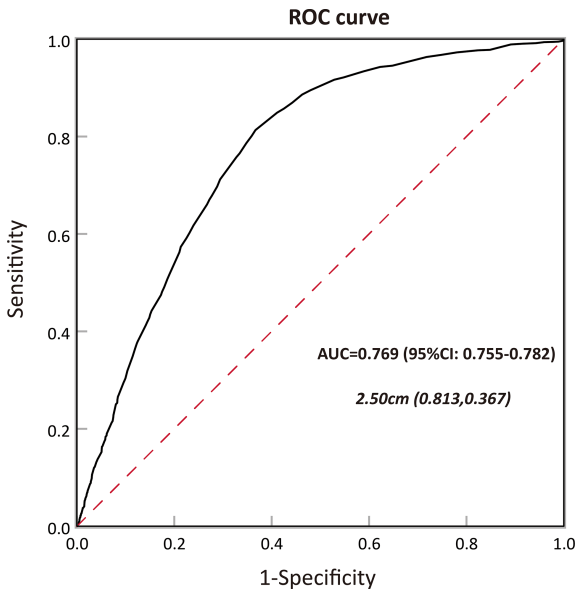

FIGURE 3 | Calculation of the cutoff value for tumor size in predicting tumor aggressiveness among patients with pancreatic neuroendocrine neoplasms (PanNENs) by receiver operating characteristic (ROC) analysis and the area under the curve (AUC).

intermediate grade, with single tumor, and loco-regional stage were the suitable candidates for active surveillance.

The optimal management of NF-PanNETs less than or equal to 2 cm in size represented an unsolved clinical challenge in recent years, especially with the steadily increasing incidence of these incidentally discovered tumors. Lacking adequately powered studies investigating their clinical features and identifying the prognostic factors, indications for expectant observation in treating small tumors remain ambiguous and inconsistent. A preoperative tumor size had been proposed to predict the malignant potential and help in clinical decision-making (12, 13). Some consensus recommendations suggest that observation can be considered an option due to the clinically indolent and benign course of tumors less than or equal to 2 cm. Both the European Neuroendocrine Tumor Society (ENETS) Consensus Guidelines and NCCN guidelines offer an active surveillance strategy for patients with low-grade tumors measuring less than 2 cm in size, with a comprehensive assessment of the individual patient characteristics (14, 15). Lee et al. retrospectively reviewed 133 incidentally detected, small NF-PanNETs patients (77 nonoperative, 56 operatives), and they found that nonoperative treatment may be advocated as these tumors commonly showed minimal or no growth during follow-up (16). In a matched case-control study, Ssdot et al. analyzed the natural history of small (<3 cm), asymptomatic PanNETs and evaluated the efficacy of surgical resection versus observation. Among the patients who were

TABLE 2 | Comparison of baseline characteristics before and after PSM in patients with NF-PanNETs ≤2 cm.

Variables	Before PSM			After PSM		
	Observation (*n* = 271)	Surgery (*n* = 1,350)	*p*-value	Observation (*n* = 259)	Surgery (*n* = 259)	*p*-value
Age			<0.001*			1.000
<65 years	128 (47.2%)	863 (63.9%)		122 (47.1%)	122 (47.1%)	
≥65 years	143 (52.8%)	487 (36.1%)		137 (52.9%)	137 (52.9%)	
Gender			0.081			0.429
Male	144 (53.1%)	639 (47.3%)		139 (53.7%)	130 (50.2%)	
Female	127 (46.9%)	711 (52.7%)		120 (46.3%)	129 (49.8%)	
Ethnicity			0.184			1.000
White	216 (79.7%)	1,012 (75.0%)		208 (80.3%)	208 (80.3%)	
Black	26 (9.6%)	158 (11.7%)		22 (8.5%)	22 (8.5%)	
Other	29 (10.7%)	180 (13.3%)		29 (11.2%)	29 (11.2%)	
Marital status			0.579			1.000
Married	167 (61.6%)	856 (63.4%)		160 (61.8%)	160 (61.8%)	
Other	104 (38.4%)	494 (36.6%)		99 (38.2%)	99 (38.2%)	
Tumor grade			0.863			0.537
Well differentiated	245 (90.4%)	1,225 (90.7%)		234 (90.3%)	238 (91.9%)	
Moderately differentiated	26 (9.6%)	125 (9.3%)		25 (9.7%)	21 (8.1%)	
Tumor number			0.736			0.588
Single	240 (88.6%)	1,205 (89.3%)		230 (88.8%)	226 (87.3%)	
Multiple	31 (11.4%)	145 (10.7%)		29 (11.2%)	33 (12.7%)	
Tumor location			0.071			1.000
Head	65 (24.0%)	320 (23.7%)		59 (22.8%)	59 (22.8%)	
Body/tail	150 (55.4%)	824 (61.0%)		148 (57.1%)	148 (57.1%)	
Other	56 (20.6%)	206 (15.3%)		52 (20.1%)	52 (20.1%)	
Lymph node involvement			0.002*			1.000
Yes	8 (3.0%)	109 (8.1%)		5 (1.9%)	5 (1.9%)	
No	263 (97.0%)	1,241 (91.9%)		254 (98.1%)	254 (98.1%)	
Liver involvement			<0.001*			1.000
Yes	19 (7.0%)	13 (1.0%)		9 (3.4%)	9 (3.4%)	
No	250 (92.3%)	1,325 (98.1%)		249 (96.1%)	249 (96.1%)	
Unknown	2 (0.7%)	12 (0.9%)		1 (0.4%)	1 (0.4%)	
Tumor stage			<0.001*			1.000
Localized	242 (89.3%)	1,165 (86.3%)		242 (93.4%)	242 (93.4%)	
Regional	8 (3.0%)	158 (11.7%)		7 (2.7%)	7 (2.7%)	
Distant	21 (7.7%)	48 (3.0%)		10 (3.9%)	10 (3.9%)	

*PSM, propensity score matching; NF-PanNETs, nonfunctional pancreatic neuroendocrine tumors. *Significance.*

initially observed, none of them developed distant metastases or died, with a median follow-up of 44 months (17). Similarly, Barenboim et al. identified 44 small asymptomatic NF-PanNETs treated with expectant observation between 2001 and 2018 and reported that no patients presented with regional or systemic disease progression or cancer-related death after a follow-up of 52.8 months. Considering the potential risks of preoperative morbidity and mortality, a strategy of conservative management seemed to be acceptable in selected patients (18). A systematic review including 5 retrospective literatures with 540 asymptomatic, small NF-PanNENs was conducted to evaluate the outcome between active surveillance with surgery. During the follow-up, the observation group did not occur disease-related deaths; therefore, they concluded that expectant management may be a reasonable alternative to aggressive surgery in highly selected patients (19). However, other studies have questioned the safety and feasibility of a conservative strategy and demonstrated that NF-PanNETs were associated with small but measurable malignant potential and aggressive surgical resection could provide long-term survival benefits (11). Gratian et al. found that 3 of 56 NF-PanNETs with tumors less than 2 cm developed metastatic disease and 2 of them died. In addition, tumor size was not related to distant metastasis or survival outcomes, which implied that it should not be used as an indication for treatment decisions (20). In a retrospective study including 3,243 cases with early-stage PanNETs ≤2 cm selected from the National Cancer Database, Chivukula et al. demonstrated a survival benefit of surgical resection for tumors 1 to 2 cm in size (21). Overall, the dilemma in managing patients with NF-PNETs ≤2 cm results from the benefits of surgery, which need to be weighed against the risks of possible disease progression, surgery-related morbidity, and comorbidities.

Generally, tumor differentiation based on the WHO classification and the AJCC staging system were regarded as two main determinants in the selection of optimal management for patients with PanNETs. While both of these two elements were not easily obtained before surgery, the preoperatively available clinical variable, tumor size, was frequently used to predict the tumor metastatic progression and aid clinical decision-making. However, as NF-PanNETs are a heterogeneous group of entities that exhibit a broad spectrum of biological behavior, there is no clear cutoff for benign disease. The current study demonstrates that tumor size alone cannot differentiate whether patients with NF-PNETs less than 2 cm are the appropriate candidates for an expectant observation,

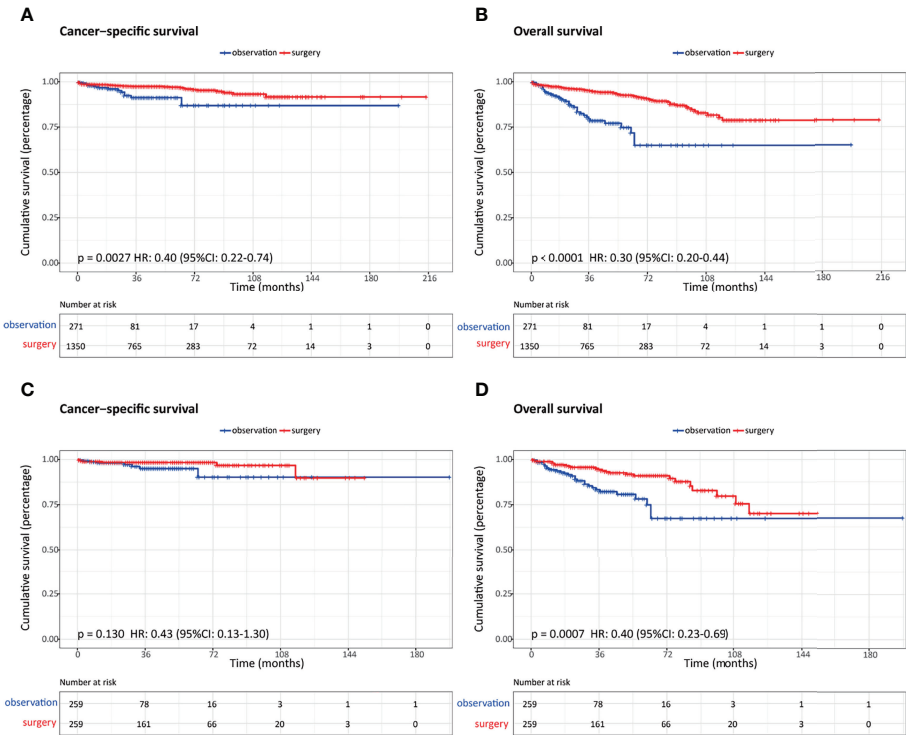

FIGURE 4 | Comparison of survival outcomes in patients with nonfunctional pancreatic neuroendocrine neoplasms (NF-PanNENs) ≤2 cm who underwent observation and surgery before and after propensity score matching (PSM). **(A)** Cancer-specific survival before PSM. **(B)** Overall survival before PSM. **(C)** Cancer-specific survival after PSM. **(D)** Overall survival after PSM.

TABLE 3 | Factors associated with patients with PanNENs ≤2 cm who underwent surgery in the SEER database.

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age				
<65 years	Ref		Ref	
≥65 years	0.50 (0.39, 0.65)	<0.001*	0.51 (0.39, 0.67)	<0.001*
Gender				
Male	Ref			
Female	1.25 (0.97, 1.61)	0.092		
Ethnicity				
White	Ref			
Black	1.34 (0.87, 2.06)	0.182		
Other	1.35 (0.89, 2.04)	0.160		
Year of diagnosis				
Per year	0.84 (0.80, 0.89)	<0.001*	0.79 (0.74, 0.84)	<0.001*
Marital status				
Married	Ref			
Other	0.89 (0.69, 1.16)	0.401		
Tumor grade				
Well differentiated	Ref			
Moderately differentiated	1.22 (0.79, 1.87)	0.368		
Poorly differentiated	0.52 (0.24, 1.14)	0.101		
Tumor size				
≤1 cm	Ref		Ref	
1–2 cm	1.64 (1.25, 2.14)	<0.001*	1.60 (1.20, 2.12)	0.001*

(Continued)

TABLE 3 | Continued

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Tumor number				
Single	Ref			
Multiple	0.93 (0.62, 1.39)	0.722		
Tumor location				
Head	Ref			
Body/tail	1.13 (0.83, 1.54)	0.441		
Other	0.72 (0.49, 1.05)	0.084		
Functional status				
Nonfunctional	Ref		Ref	
Functional	5.51 (1.73, 17.56)	0.004*	6.78 (2.01, 22.88)	0.002*
Lymph node involvement				
No	Ref		Ref	
Yes	2.68 (1.39, 5.17)	0.003*	4.43 (2.06, 9.52)	<0.001*
Liver involvement				
No	Ref		Ref	
Yes	0.16 (0.09, 0.31)	<0.001*	0.13 (0.08, 0.21)	<0.001*

*PanNENs, pancreatic neuroendocrine neoplasms; OR, odds ratio; CI, confidence interval; Ref, reference. *Significance.*

TABLE 4 | Factors associated with overall survival in patients with PanNENs ≤2 cm.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age				
<65 years	Ref		Ref	
≥65 years	3.50 (2.52, 4.84)	<0.001*	4.04 (2.86, 5.71)	<0.001*
Gender				
Male	Ref		Ref	
Female	0.69 (0.50, 0.94)	0.017*	0.65 (0.47, 0.90)	0.009*
Ethnicity				
White	Ref		Ref	
Black	1.68 (1.12, 2.53)	0.012*	2.26 (1.48, 3.44)	<0.001*
Other	0.64 (0.35, 1.16)	0.142	0.79 (0.43, 1.46)	0.459
Year of diagnosis				
Per year	0.95 (0.90, 1.01)	0.060		
Marital status				
Married	Ref			
Other	1.33 (0.98, 1.83)	0.072		
Tumor grade				
Well differentiated	Ref		Ref	
Moderately differentiated	1.43 (0.91, 2.25)	0.126	1.44 (0.91, 2.29)	0.124
Poorly differentiated	7.56 (4.58, 12.49)	<0.001*	6.10 (3.53, 10.53)	<0.001*
Tumor size				
≤1 cm	Ref			
1–2 cm	0.92 (0.65, 1.31)	0.652		
Tumor number				
Single	Ref		Ref	
Multiple	2.68 (1.91, 3.78)	<0.001*	2.33 (1.64, 3.31)	<0.001*
Tumor location				
Head	Ref			
Body/tail	1.00 (0.69, 1.46)	0.986		
Other	1.02 (0.63, 1.63)	0.952		
Functional status				
Nonfunctional	Ref			
Functional	1.25 (0.66, 2.38)	0.492		
Lymph node involvement				
No	Ref			
Yes	1.48 (0.94, 2.32)	0.093		

(Continued)

TABLE 4 | Continued

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Liver involvement				
No	Ref		Ref	
Yes	5.75 (3.47, 9.54)	<0.001*	1.03 (0.35, 3.01)	0.961
Tumor stage				
Localized	Ref		Ref	
Regional	1.30 (0.82, 2.06)	0.257	0.98 (0.60, 1.61)	0.933
Distant	5.61 (3.70, 8.52)	<0.001*	4.60 (1.73, 12.23)	0.002*

*PanNENs, pancreatic neuroendocrine neoplasms; HR, hazards ratio; CI, confidence interval; Ref, reference. *Significance.*

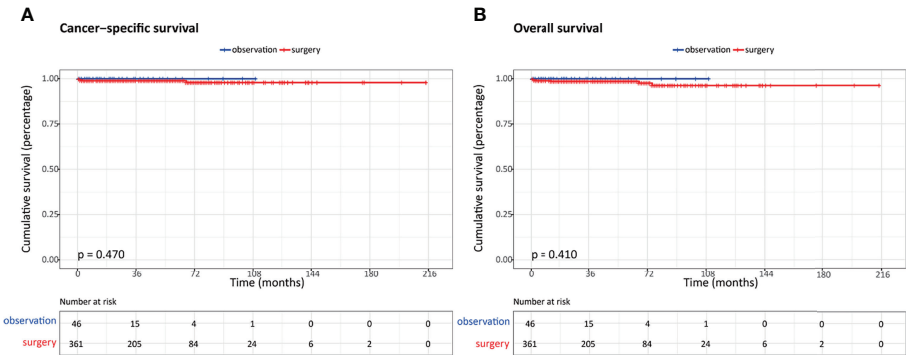

FIGURE 5 | Comparison of survival outcomes in patients with highly selected nonfunctional pancreatic neuroendocrine neoplasms (NF-PanNENs) ≤2 cm between observation and surgery cohorts after propensity score matching (PSM). **(A)** Cancer-specific survival. **(B)** Overall survival.

and other preoperatively available clinical features need to be taken into account as well, such as individual patient characteristics, comorbidities, and other risk factors for survival. In our study, a number of clinicopathological variables were identified to be associated with overall survival in NF-PanNENs ≤2 cm, including age, gender, ethnicity, tumor grade, tumor number, and tumor stage. Therefore, when considering the clinical factors that may inform the decision to perform observation or surgical resection, tumor size and these risk factors should act as a marker for the clinicians.

Our study had several limitations. The inherent biases with a retrospective design could not be completely eliminated even though we used propensity score matching. Secondly, the lack of important data in the SEER database may fail to incorporate some recognized prognostic parameters, such as the Ki-67 index and surgery-related complications. Last, the small size of patients after propensity score matching may therefore limit the generalization of the results.

In conclusion, expectant observation of small NF-PanNETs may be a reasonable alternative to aggressive surgical resection in highly selected patients. Also, the decision to observe versus surgery should not only be based on tumor size alone but also take into account other important clinicopathological factors. Further prospective multicentric studies and robust data are required to validate the benefit of this conservative policy.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. These data can be found here: https://seer.cancer.gov/.

AUTHOR CONTRIBUTIONS

GS contributed to the conception and designed the study. ZY drafted the manuscript. DZ conducted the statistical analysis. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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A patient with an ectopic sphenoid bone TSH secretory adenoma: Case report and review of the literature

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Ectopic thyroid-stimulating hormone (TSH)oma located outside the sella turcica is exceedingly rare and can be associated with significant diagnostic delay. The clinical presentation depends on the anatomical location and size of the ectopic tumor and the degree of thyrotoxicosis. A 71-year-old woman presented with goiter and thyrotoxicosis. Initial investigations revealed elevated free thyroxine (fT4) and tri-iodothyronine (fT3) with inappropriately high-normal TSH. Assay interference was unlikely, pituitary magnetic resonance imaging (MRI) scan was reported as "normal," and germline sequencing was negative for thyroid hormone receptor ß pathogenic variants. One year later, total thyroidectomy for enlarging symptomatic goiter and suspicious nodule revealed multifocal microscopic papillary thyroid carcinoma. Six years later, she presented to an ear, nose, and throat surgeon with nasal congestion, and a sphenoid bone mass was discovered on nasoendoscopy and imaging. Ectopic TSHoma was confirmed on surgical resection, and a review of the initial pituitary MRI scan revealed the mass which had initially been missed. This is the first reported case of an ectopic TSHoma located in the sphenoid bone. Ectopic TSHoma should be considered in patients with inappropriate TSH secretion when more common differentials are excluded including thyroid hormone resistance or pituitary TSHoma.

KEYWORDS

thyrotoxicosis, thyroid-stimulating hormone, TSHoma, ectopic TSHoma, thyrotropinoma, ectopic thyrotropin (TSH) secreting pituitary adenoma

Introduction

Pituitary adenomas originate from the adenohypophysis, and ectopic pituitary adenomas (EPAs) are extrasellar and are separate from the hypophysis and infundibulum (1, 2). EPA pathogenesis largely relates to the embryological development and migration of the adenohypophysis (1, 2). EPAs are rare with

approximately 180 cases described according to a recent extensive literature review (1), and clinical manifestations vary widely, depending on their secretory profile, size, and anatomical location. Symptoms often mimic those of the skull base or nasopharyngeal tumors and occur due to mass effect on adjacent structures, resulting in visual disturbance, facial paraesthesia, headache, and nasal congestion (2, 3). Specifically, nasopharyngeal and sphenoid sinus EPAs commonly present with epistaxis and nasal congestion, while sphenoid bone EPAs can also present with headache and cranial neuropathy (4). According to a review of published cases, most EPAs (85%) are hormonally active, of which ACTH (36%), prolactin (28%), and GH (22%) secretions are the most common (3, 4). TSH-secreting EPAs (ectopic TSHomas) are the rarest, and to our knowledge, only 13 such cases have been reported in the literature to date (5–17). TSHomas manifest as secondary hyperthyroidism, with elevated serum free thyroxine (fT4) and tri-iodothyronine (fT3) and non-suppressed serum TSH, and can present clinically with symptoms and signs of thyrotoxicosis and diffuse goiter which can often lead to misdiagnosis or delayed diagnosis. We present the first report of an ectopic TSHoma located within the sphenoid bone in a 71-year-old woman. A review of all reported cases of ectopic TSHoma provides key insights into the presentation, diagnosis, and treatment of this rare and often missed disorder.

Case description

A 71-year-old woman was referred to our endocrinology service in 2014 with a longstanding history of goiter for the past 10 years and recent intermittent dysphagia with solids without symptoms of thyrotoxicosis. She had a background of Hashimoto's thyroiditis, was born in Australia, and had no prior neck irradiation or family history of thyroid-related conditions. She had no history of atrial fibrillation or osteoporosis. Examination confirmed a large, palpable non-obstructive goiter. She was normocardic and in normal sinus rhythm.

Diagnosis, treatment, and outcomes

Thyroid function tests (TFTs) revealed elevated serum fT4 of 21.6 pmol/L (reference range: 9–19 pmol/L), high-normal fT3 of 5.8 pmol/L (reference range: 2.6–6.0 pmol/L), and inappropriately high-normal TSH of 3.79 mIU/L (reference range: 0.40–4.00 mIU/L). A review of previous biochemistry using different assays (including Roche and Abbott Architect) 6 years prior demonstrated a similar pattern of thyroid function derangement, and hence, assay interference was deemed unlikely. Other investigations in 2014 showed elevated anti-thyroid peroxidase antibodies of >1,000 IU/ml (reference range: 0–120 IU/ml) and elevated anti-thyroglobulin antibodies of 1,331 IU/ml (reference range: 0–80 IU/ml), consistent with Hashimoto's thyroiditis. Serum TSH α-subunit level was considered normal at 1.04 IU/L (postmenopausal reference range: 0–1.3 IU/L). The remainder of the pituitary panel was unremarkable: postmenopausal range follicle-stimulating hormone (FSH) of 61.3 IU/L (reference range: 25–130 IU/L) and luteinizing hormone (LH) of 24.7 IU/L (reference range: 5–62 IU/L), insulin-like growth factor 1 (IGF-1) of 11 nmol/L (reference range: 10–28 nmol/L), prolactin of 302 mIU/L (reference range: 85–500 mIU/L), cortisol of 353 nmol/L (reference range: 138–650 nmol/L), and adrenocorticotrophin hormone (ACTH) of 7.1 pmol/L (reference range: 0–12 pmol/L). Further investigations were undertaken to elucidate the underlying cause for inappropriate TSH secretion. No pathogenic variants were detected on germline DNA polymerase chain reaction (PCR) and Sanger sequencing of exons 7–10 and flanking intronic sequences of the thyroid hormone receptor ß (*THRß*) gene. Magnetic resonance imaging (MRI) of the pituitary suggested a normal pituitary gland and unperturbed infundibulum, optic chiasm, and cavernous sinuses.

One year later, she underwent total thyroidectomy for progressively enlarging symptomatic goiter and suspicious 2.2 cm right inferior thyroid nodule of Bethesda category III on fine needle aspiration biopsy. Histopathology revealed four foci of microscopic papillary thyroid cancer (micro-PTC) ranging between 0.5 and 3 mm in diameter with clear resection margins and no evidence of lymphovascular invasion or extrathyroidal extension. Post-thyroidectomy radioactive iodine ablation was not required. Thyroxine replacement was commenced but subsequent TFTs showed persistently elevated or high-normal serum TSH despite increasing doses of thyroxine to as much as 1,200 mcg/week (2.0 mcg/kg/day).

She then presented 6 years later with nasal congestion to an ear, nose, and throat surgeon who suspected a nasopharyngeal mass on nasoendoscopy. Computed tomography (CT) of the paranasal sinuses showed a 2.6-cm × 1.4-cm × 2.5-cm lytic lesion expanding the sphenoid bone and separate from the sella turcica. A subsequent MRI scan of the head demonstrated a 3.0-cm × 2.3-cm × 2.3-cm well-marginated T2-hyperintense and T1-hypointense lesion with heterogeneous contrast enhancement located within the sphenoid bone without extension into the sphenoid sinus or nasopharynx (Figure 1). Endonasal endoscopic resection and tumor histopathology confirmed an ectopic pituitary TSH-producing neuroendocrine tumor with invasion of the clivus and nasopharyngeal mucosa (Figure 2). Tumor cells had small amounts of lightly eosinophilic, granular cytoplasm and small–intermediate-sized round nuclei without any mitoses identified and with a Ki67

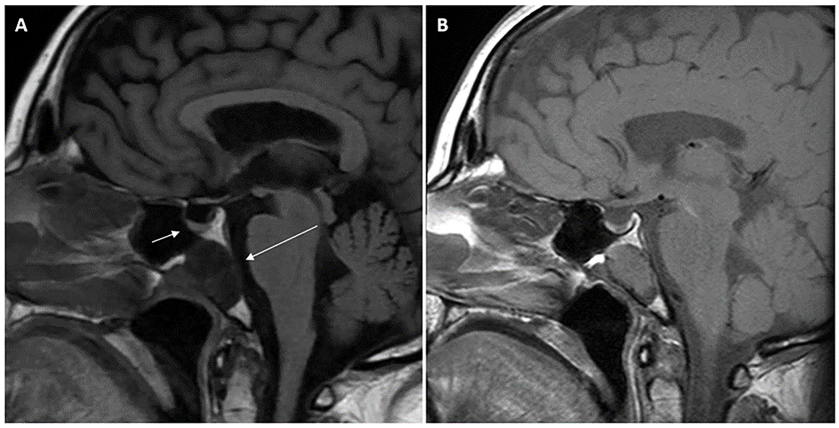

FIGURE 1
Magnetic resonance imaging (MRI) scans of the head demonstrating midline sphenoid bone mass. T1-weighted (FLAIR) sagittal view pituitary magnetic resonance imaging (MRI) scan performed in 2021 **(A)** demonstrating normal pituitary gland (short arrow) and adjacent midline T1-hypointense 3.0 cm × 2.3 cm × 2.3 cm mass (long arrow) located within the sphenoid bone extending to the clival, sphenoid sinus, and nasopharyngeal surfaces of the sphenoid bone with no extension into the sphenoid sinus or nasopharynx. Retrospective review of the T1-weighted sagittal view initial pituitary MRI scan performed in 2014 **(B)** demonstrated the same lesion within the sphenoid bone measuring 2.2 cm × 2.1 cm × 2.3 cm. Arrows are not included in **(B)** so the image is unperturbed and seen in the same way the radiologist viewed the scan.

index of 0.4%. Immunohistochemistry showed strong and diffuse staining for chromogranin A, synaptophysin, Pit1 transcription factor, TSH (Figure 2), and TSH α-subunit. Growth hormone and prolactin showed focal, weak staining, and transcription factors T-pit and SF1 were negative. Following tumor resection, her serum TSH decreased to 0.01 mIU/L. A retrospective review of her initial pituitary MRI scan images in 2014 revealed that a 2.2-cm × 2.1-cm × 2.3-cm mass within the sphenoid bone had initially been missed (Figure 1). At 5 months post-resection, thyroxine requirements were reduced to 800 mcg/week (1.3 mcg/kg/day) with normalized TFTs.

Timeline

February 2008	First biochemical evidence of TSH hypersecretion
March 2014	Endocrinology institution referral
October 2014	Initial pituitary MRI scan reported as "normal" Negative genetic testing for *THRβ* mutation
June 2015	Total thyroidectomy for obstructive goiter and suspicious thyroid nodule
August 2021	Ear, nose, and throat surgeon referral for investigation of nasal congestion Nasoendoscopy suggested nasopharyngeal mass
September 2021	MRI head scan identified sphenoid bone mass
October 2021	Resection of sphenoid bone mass and confirmation of the diagnosis of ectopic TSHoma

Discussion

The pathogenesis of EPAs relates to the embryological development of the hypophysis (13). Between the fourth and eighth week of gestation, Rathke's pouch develops from the ascending invagination of extracranial ectoderm from the primitive oral cavity, and it fuses with the descending neuroectoderm to form the adenohypophysis and neurohypophysis, respectively (3). During this process, embryological cells of the adenohypophysis migrate through the craniopharyngeal canal to the nasopharynx or sphenoid sinus before uniting with the neurohypophysis in the sella turcica of the sphenoid bone. Infrasellar EPAs can thus be a result of persisting embryological cells along the migration pathway, manifesting in the sphenoid sinus or nasopharynx (1, 2). Alternatively, suprasellar EPAs may be derived from cells of the supradiaphragmatic portion of the pars tuberalis in the suprasellar region (1, 9, 13). Another potential explanation is aberrant embryological cells migrating through the craniopharyngeal canal to both extracranial (infrasellar—nasopharynx, sphenoid sinus, clivus) and intracranial (supra- and parasellar) areas (2, 13). The most common location for EPAs is the suprasellar and sphenoid sinus followed by the nasopharynx.

A literature review yielded 14 published case reports of ectopic TSHoma including our report (Table 1), using PubMed/Medline search terms "ectopic" AND "TSHoma/thyrotropinoma," "ectopic" AND "TSH/thyrotropin" AND "pituitary," "ectopic" AND "TSH/thyrotropin" AND "adenoma," "ectopic" AND "TSH/thyrotropin" AND "pituitary" AND "adenoma." The first case was reported in 1996; however, 50% of the cases were published in the past 5 years

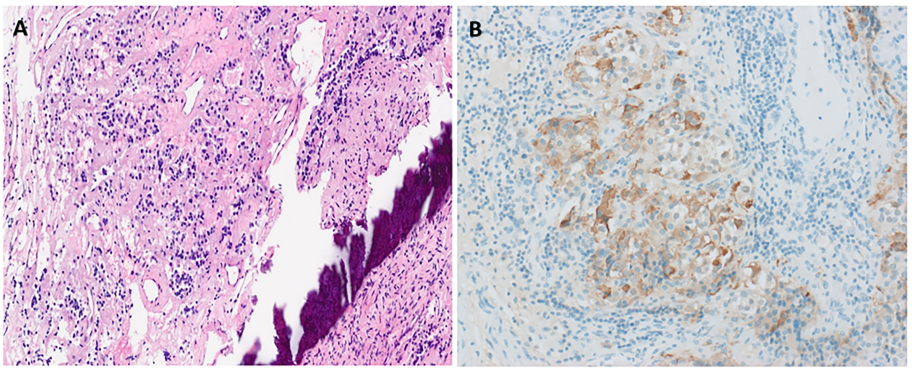

FIGURE 2
Histopathological confirmation of ectopic TSHoma. Histopathology (obtained from tumor resection) with hematoxylin and eosin staining **(A)** showing small irregularly shaped islands of cells separated by fibrovascular stroma beside the bone (dark purple streak). Cells have small amounts of lightly eosinophilic, granular cytoplasm and small–intermediate-sized round nuclei with no mitoses identified. Strong diffuse positive tumor cell staining on immunohistochemistry for TSH is also seen **(B)**.

suggesting greater recent awareness of ectopic TSHoma. The majority of the cases were women (9/14) and the median age at diagnosis was 48 years (range 10–78 years). Unlike other EPAs, the nasopharynx was the most common location (9/14), followed by the suprasellar (3/14), sphenoid sinus (1/14), and sphenoid bone (1/14) which may represent a unique nasopharyngeal predisposition of ectopic TSHoma. Tumor size was recorded in 10 cases, with a median maximal diameter of 2.0 cm (range 0.4–3.0 cm, one microadenoma). Symptomatic thyrotoxicosis (11/14) was the most common complaint followed by diffuse goiter (9/14) reflecting TSH hypersecretion. Mass effects differed based on tumor location with nasopharyngeal/sphenoid sinus/sphenoid bone lesions presenting with nasal congestion (6/11) and suprasellar lesions with visual impairment (2/3). Biochemically, the vast majority of patients had elevated fT4 and fT3, while 50% had elevated TSH (100% of the cases had non-suppressed TSH). Primary thyrotoxicosis/Graves' disease was the most common initial diagnosis (7/14) which led to inappropriate treatment with anti-thyroid drugs (8/14) and radioactive iodine ablation (2/14). Our patient is the first to undergo total thyroidectomy; however, this was performed for enlarging symptomatic goiter and suspicious nodule rather than inappropriate treatment of presumed Graves' disease. The median diagnostic delay from the initial presentation was 2.5 years (range <1 to 18 years). The single most important investigation which led to the correct diagnosis was an MRI brain scan (11/14) with the majority ordered as part of the investigation algorithm for inappropriate TSH secretion to exclude TSHoma. Three cases, including ours, required the identification of the mass by otolaryngological examination for nasal congestion to facilitate the correct diagnosis. The ectopic TSHoma was initially missed on an MRI brain scan in one case; however, this was rectified within <1 year. Our patient, however, experienced a 7-year diagnostic delay despite the midline sphenoid bone mass being visible in the same sagittal and coronal slice as the pituitary gland under investigation, suggesting a lack of awareness for ectopic TSHoma. The interval growth of the ectopic TSHoma may reflect natural history or could have been exacerbated by thyroidectomy and the resulting impaired feedback inhibition by the thyroid hormone. All patients underwent tumor resection with positive biochemical and structural outcomes at a median follow-up of 9 months (range 2 months to 7 years), likely reflecting the lack of concern for postoperative hypopituitarism allowing for more extensive complete surgical resection. Histopathological confirmation of ectopic TSH-secreting neuroendocrine tumor was achieved in all cases supported by features of the neuroendocrine tumor and positive tumor cell immunohistochemistry for TSH (14/14).

The differential diagnosis for inappropriate TSH secretion includes assay interference, thyroid hormone resistance, familial dysalbuminemic hyperthyroxinemia (FDH), and TSHoma (18). Laboratory assay interferences should first be excluded, such as the presence of heterophile antibodies, thyroid hormone antibodies, and high biotin levels (19). A useful approach to exclude assay interference is to repeat TFTs using a different assay in a different laboratory and, if other common differentials are excluded, to consider consultation with the biochemical department for exclusion of interfering antibodies. FDH occurs *via* autosomal dominant inheritance of pathogenic variants in the gene encoding albumin. The resulting mutant albumin has a higher binding affinity to T4 resulting in two- to three-fold elevated total T4 and mildly elevated total T3 concentrations. The mutant albumin also has greater binding to labeled T4 analogs, the assay tracer, and causes more antibodies to bind fT4 instead, giving abnormally high fT4 measurements (20). Thyroid hormone resistance is caused by autosomal dominant inheritance of mutations in the thyroid hormone receptor (THR) genes *THRα* and *THRß* which encode

TABLE 1 Summary of the 14 published cases to-date of ectopic TSHoma.

Author Year	Age Sex	Presentation	Initial investigations	Initial diagnosis	Initial treatment	Delay to diagnosis	LocationSize	Post-resection outcome and follow-up
Kumar et al. 2022	71 years Female	Intermittent dysphagia Diffuse goiter Re-presented with nasal congestion	Elevated fT4 High-normal fT3 High-normal TSH Normal-range TSH α-subunit Normal pituitary panel Negative *THRβ* mutation	Inappropriate TSH secretion	Total thyroidectomy for progressive goiter/ suspicious nodule	7 years	Sphenoid bone/ clivus 3.0 cm × 2.3 cm	TFTs normalized at 5 months
Li et al. 2021	10 years Female	Sweats, heat intolerance Diffuse goiter	Elevated fT4 Elevated fT3 Normal-range TSH Normal pituitary panel Elevated SHBG Positive somatostatin suppression test Negative *THRβ* mutation	Ectopic TSHoma	Surgical resection of the suprasellar mass	<1 year	Suprasellar 0.4 cm × 0.3 cm	Normalization of fT3, fT4, and TSH. Resolution of symptoms and no recurrence at 4 years
Ortiz et al. 2020	52 years Female	Weight loss, hyperdefecation Tachycardia, diffuse goiter	Elevated fT4 Elevated fT3 Elevated TSH Normal pituitary panel Elevated SHBG Positive somatostatin suppression test	Primary hypothyroidism	Thyroxine	2 years	Sphenoid sinus 2.4 cm × 2.4 cm	Initial recurrence requiring reoperation Subsequent normalization of TFTs and no structural recurrence at 2 years
Trummer et al. 2020	48 years Female	Palpitations, sweats	Elevated fT4 Elevated fT3 Elevated TSH Elevated alpha subunit/TSH ratio >1 Elevated SHBG Normal pituitary panel Positive TRH stimulation test	Inappropriate TSH secretion	Propylthiouracil Bisoprolol	1 year	Nasopharynx 2.0 cm × 1.8 cm	TFTs normalized No recurrence at 8 months
Kim et al. 2019	48 years Female	Palpitations, tremor, tachycardia Diffuse goiter	Elevated fT4 Elevated fT3 Normal-range TSH Elevated alpha subunit/TSH ratio >1 Diffusely increased uptake on thyroid uptake scan	Primary thyrotoxicosis	Methimazole Propylthiouracil Propranolol	4 years	Nasopharynx 1.1 cm × 0.8 cm	TFTs normalized No recurrence at 6 months

(Continued)

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TABLE 1 Continued

Author Year	Age Sex	Presentation	Initial investigations	Initial diagnosis	Initial treatment	Delay to diagnosis	LocationSize	Post-resection outcome and follow-up
Hanaoka et al. 2018	41 years Male	Visual impairment	NR	Craniopharyngioma	Surgical resection of the suprasellar mass	<1 year	Suprasellar NR	Normal TFTs and no structural recurrence at 7 years
Yang et al. 2017	27 years Female	Palpitations Diffuse goiter Nasal congestion	Elevated fT4 Elevated fT3 Elevated TSH Normal pituitary panel Negative T3 suppression test	Primary thyrotoxicosis	Methimazole Metoprolol Radioactive iodine ablation	10 years	Nasopharynx NR	TFTs normalized No recurrence at 3-years.
Wang et al. 2016	46 years Male	Palpitations, sweats, weight loss Visual impairment Diffuse goiter	Elevated fT4 Elevated fT3 Elevated TSH	Primary thyrotoxicosis	Propylthiouracil	1 year	Suprasellar 1.5 cm × 1.2 cm	TFTs normalized No structural recurrence at 6 months
Song et al. 2014	41 years Male	Palpitations, weight loss, atrial fibrillation Diffuse goiter	Elevated fT4 Elevated fT3 Elevated TSH Negative TRH stimulation test Positive somatostatin suppression test	Primary thyrotoxicosis	Propylthiouracil Propranolol	<1 year	Nasopharynx 1.9 cm × 1.7 cm	TFTs normalized No structural recurrence at 4 years
Nishiike et al. 2014	46 years Male	Sweats, palpitations Headaches Diffuse goiter	Elevated fT4 Elevated fT3 Normal-range TSH Negative TRH stimulation test Diffusely increased uptake on thyroid uptake scan	Inappropriate TSH secretion	Surgical resection of the nasopharyngeal mass	3 years	Nasopharynx 1.4 cm	TSH normalized No structural recurrence at 3 years
Tong et al. 2013	49 years Female	Nasal congestion Symptoms of thyrotoxicosis	Elevated fT4 Elevated fT3 Elevated TSH Normal pituitary panel Positive octreotide suppression test	Sinusitis Grave's disease	Methimazole	<1 year	Nasopharynx 2.0 cm × 2.0 cm	Recurrence-free at 3-month follow-up
Collie et al. 2005	50 years Female	Headaches, nasal congestion Non-suppressible TSH on thyroxine replacement	Non-suppressible TSH	Peripheral nerve sheath tumor	Surgical resection of the nasopharyngeal mass	NR	Nasopharynx 2.0 cm × 1.5 cm	TSH normalized No structural recurrence at 4 months
Pasquini et al. 2003	52 years Male	Sweats, palpitations, weight loss, atrial fibrillation Diffuse goiter Re-presented due to debilitating nasal congestion, rhinorrhea	Elevated fT4 Elevated fT3 Normal-range TSH Normal pituitary panel	Grave's disease	Methimazole	18 years	Nasopharynx NR	TFTs normalized initially Nasal congestion resolved Rising fT3 and fT4 at 10-month follow-up suspicious for incomplete resection

(Continued)

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TABLE 1 Continued

Author Year	Age Sex	Presentation	Initial investigations	Initial diagnosis	Initial treatment	Delay to diagnosis	LocationSize	Post-resection outcome and follow-up
			Diffusely increased uptake on thyroid uptake scan					
Cooper et al. 1996	74 years Female	Anxiety, tremors, weight loss Re-presented with nasal congestion	High-normal fT4 High-normal fT3 High-normal TSH Elevated alpha subunit/TSH ratio >1 Normal-range SHBG Positive TRH stimulation test	Grave's disease	Propylthiouracil Radioactive iodine ablation	9 years	Nasopharynx NR	TFTs normalized Nasal congestion resolved Recurrence-free at 2-month follow-up

fT4, free thyroxine; fT3, free tri-iodothyronine; TSH, thyroid-stimulating hormone; THR, thyroid hormone receptor; TRH, thyrotropin-releasing hormone; SHBG, sex hormone-binding globulin; NR, not recorded; TFT, thyroid function tests.

intranuclear T3 receptors, resulting in T3 receptor dysfunction. *THRß* mutation is by far the most prevalent subtype with biochemical findings of elevated fT4 and inappropriately normal or elevated TSH levels, biochemically indistinct from TSHoma (21). To help differentiate between the two conditions, family history should be sought and germline DNA sequencing for *THRß* mutations performed. Investigations that support the diagnosis of TSHoma over thyroid hormone resistance include elevated α-subunit/TSH ratio (>1.0) and serum sex hormone-binding globulin level (peripheral tissue marker of thyroid hormone action). Dynamic investigation results include the positive somatostatin suppression test (due to predominantly somatostatin receptor 2 expression), negative TRH stimulation test, and negative T3 suppression test (21, 22). It is unclear due to the rarity of cases whether the same conclusions can be drawn regarding ectopic TSHoma. However, we have observed certain clinical, biochemical, and radiological characteristics from the published case reports in comparison to pituitary TSHoma (Table 2).

Previous cases of ectopic TSHomas have mostly been reported as a nasopharyngeal mass, and this is the first case where it was discovered in the sphenoid bone/clivus. Primary clival EPAs, as seen in our patient, are defined as EPAs being contained within the clivus and have been reported to be exceedingly rare (2). A clival tumor, however, has a broad differential diagnosis, including chordoma, chondrosarcoma, intraosseous meningioma, craniopharyngioma, lymphoma, myeloma, and solid organ metastases, with chordoma being the most prevalent (1). Clival EPAs have also been observed to be associated with malignant transformation, bone invasion, and a concurrent empty sella (1, 4), of which only bone invasion was observed in our patient. In addition, our patient also has a concurrent history of PTC, and this has only been described in another case report of ectopic TSHoma. Given the presence of goiter in other patients with ectopic TSHoma, the ectopic TSH is promoting the growth and proliferation of thyroid follicular cells, which may explain the association with PTC in our case and another case reported by Yang et al. (12). However, our patient's underlying Hashimoto's thyroiditis may also have been a contributing factor to PTC pathogenesis. Furthermore, incidental micro-PTC in thyroidectomy specimens is not uncommon, and the clinical relevance of this finding is unclear (23–25). To our knowledge, there is insufficient evidence at this stage to directly link ectopic TSHoma with PTC.

In summary, we present the first reported case of sphenoid bone ectopic TSHoma. Initial investigations revealed elevated fT3 and fT4, with an inappropriate high-normal TSH indicative of inappropriate TSH secretion. The initial diagnosis was not obtained in the setting of negative germline DNA testing for *TRHß* gene mutation, normal post-menopausal range TSH α-subunit level, and a pituitary MRI scan that was reported as "normal." The patient underwent total thyroidectomy 1 year later for progressive symptomatic goiter and suspicious nodule

TABLE 2 Clinical, biochemical, and radiological characteristics of pituitary and ectopic TSHoma.

	Pituitary TSHoma	Ectopic TSHoma
Clinical presentation	Hyperthyroidism, goiter Hypopituitarism	Hyperthyroidism, goiter ENT/visual symptoms
Location	Pituitary	Nasopharynx/suprasellar/sphenoid
Size	Macro > micro	Macro > micro
Thyroid function	High FT4, high FT3, normal/high TSH	High FT4, high FT3, normal/high TSH
Alpha subunit/TSH molar ratio	Increased/normal	Increased/normal
Sex hormone-binding globulin (SHBG)	Increased/normal	Increased/normal
Somatostatin suppression test	Positive	Positive
MRI pituitary	Pituitary adenoma	Normal pituitary
Surgical cure	<60% cure rate with macroadenoma	93% cure rate

TSH, thyroid-stimulating hormone; MRI, magnetic resonance imaging; fT4, free thyroxine; fT3, free tri-iodothyronine; ENT, ear nose & throat.

which revealed multifocal micro-PTC. Six years later, she presented with nasal congestion which directed the investigation to her nasopharynx, facilitating the discovery of a sphenoid bone mass. After surgical resection and histopathological tumor examination, the final correct diagnosis of ectopic TSHoma was obtained. Retrospective analysis of the initial pituitary MRI scan 7 years prior revealed that the sphenoid bone mass had been present since her initial presentation. Although ectopic TSHoma is exceedingly rare, we advocate that this diagnosis should be considered in patients with inappropriate TSH secretion and exclusion of other more common differential diagnoses such as assay interference, thyroid hormone resistance, and pituitary TSHoma, to minimize the diagnostic and therapeutic delay. Ectopic TSHoma may be clinically and biochemically indistinguishable from pituitary TSHoma, and one should have high clinical suspicion if no pituitary adenoma is identified on imaging and to look outside the sella turcica for an ectopic tumor in the nasopharynx, suprasellar, sphenoid sinus, and sphenoid bone.

Patient perspective

The patient declined to provide her perspective on the case report; however, she provided signed written informed consent for this report to be published.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SK conceptualized, drafted, and critically reviewed the manuscript. CP and HN assisted in drafting the manuscript. TD managed the patient and critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A real-life treatment cohort of pancreatic neuroendocrine tumors: High-grade increase in metastases confers poor survival

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Background: Tumor grade determined by the Ki67 index is the best prognostic factor for pancreatic neuroendocrine tumors (PanNETs). However, we often observe that the grade of metastases differs from that of their primary tumors. This study aimed to investigate the frequency of grade changes between primary tumors and metastases, explore its association with clinical characteristics, and correlate the findings with the prognosis.

Methods: Six hundred forty-eight patients with pancreatic neuroendocrine neoplasms treated at Fudan University Shanghai Cancer Center were screened for inclusion, and 103 patients with PanNETs who had paired primary tumors and metastases with an available Ki67 index were included. Re-evaluation of Ki67 was performed on 98 available samples from 69 patients.

Results: Fifty cases (48.5%) had a Ki67 index variation, and 18 cases (17.5%) displayed a grade increase. Metachronous metastases showed significantly higher Ki67 index variation than synchronous metastases (P=0.028). Kaplan–Meier analyses showed that high-grade metastases compared to low-grade primary tumors were significantly associated with decreased progression-free survival (PFS, P=0.012) and overall survival (OS, P=0.027). Multivariable Cox regression analyses demonstrated that a low-grade increase to high-grade was an unfavorable and independent prognostic factor for PFS and OS (P=0.010, and P=0.041, respectively).

Conclusions: A high-grade increase in metastases was an unfavorable predictor of PanNETs, which emphasized the importance of accurate

pathological grading and could provide a reference for clinical decision-making.

KEYWORDS

pancreatic neuroendocrine tumors, Ki67 index variation, grade increase, preoperative neoadjuvant treatment, prognosis

Introduction

Pancreatic neuroendocrine tumors (PanNETs) are a rare and heterogeneous group of tumors arising from the neuroendocrine system, and their prevalence has markedly increased over the last four decades (1–3). The clinical courses of PanNETs are highly variable, and their prognosis differs widely, ranging from indolent tumors with reasonable survival to distant metastases of an aggressive nature with a poor prognosis. Up to 80% of patients with PanNETs present with metastases at the time of diagnosis, mainly to the liver (4–6), whose median survival is only 23 months, compared to 124 months for localized disease (7). PanNETs with metastases require a multidisciplinary treatment approach, including surgery (8), somatostatin analogs (SSAs), targeted therapy, namely, everolimus and sunitinib (9, 10), chemotherapy (11), and/or peptide receptor radionuclide therapy (12). Surgery remains the only curative treatment for these patients, and patients can benefit from neoadjuvant treatment (NAT).

For better prognostic stratification, the European Neuroendocrine Tumor Society (ENETS) originally proposed a three-tiered grading system for gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) in 2006, and the World Health Organization (WHO) adopted the classification in 2010, which was mainly based on the Ki67 index and mitotic index (13–15). The WHO modified the classification in 2017 and divided pancreatic neuroendocrine neoplasms (PanNENs) into well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinoma (NEC). Given that the Ki67 index has been proven to be the most reliable and best prognostic factor of PanNENs (16), the 2017 WHO classification requires its use and strongly recommends careful evaluation of the Ki67 index. Accordingly, well-differentiated NETs are further divided into the following grades by the Ki67 index: G1: <3%; G2: 3–20%; G3: >20% (17, 18).

Grade currently plays a pivotal role in the clinical management of patients with NETs, especially PanNETs (19). However, concerns exist regarding Ki67 index variation and grade differences between the primary tumors and metastases (20–22). We also observed that the grade of metastases differed from that of their primary tumors in PanNET patients in clinical setting. Here, we set out to investigate the frequency of grade changes between primary tumors and metastases in PanNETs, to explore the association between clinical characteristics and grade changes and to determine whether grade changes correlate with patient outcomes. A better understanding of grade changes could add to our understanding of the heterogeneity between primary tumors and metastases in PanNETs and contribute to clinical decision-making.

Methods

Patient population

A total of 648 patients with pathology-confirmed PanNENs who were treated at Fudan University Shanghai Cancer Center (FUSCC) between January 1, 2006, and February 1, 2020, were screened for inclusion. Exclusion criteria for the study cohort were familial syndromes, no metastatic disease, and no pathology reports available or consultation with our institution. Then, 169 patients with metastatic PanNETs were included for further eligibility evaluation. Next, 103 patients who had paired primary tumors and metastases with an available Ki67 index were identified as the study cohort. To assess the selection bias due to the inclusion of patients who had paired primary tumors and metastases with an available Ki67 index, the remaining 66 patients who did not have paired tumors with an available Ki67 index were identified as the bias-control group. Additionally, to explore whether NETs evolved to NEC, 15 patients with metastatic PanNEC were served as additional data.

The study cohort consisted of 91 patients who underwent resection and 12 patients who underwent needle biopsy or laparotomy with biopsy. In the resection cohort, 74 and 17 patients had synchronous and metachronous metastases, respectively. The biopsy cohort consisted of 12 patients with synchronous metastases. Metastatic PanNETs patients with high-risk factors, including relatively large tumors, blood vessels and adjacent organ invasion were routinely received preoperative NAT. And surgical resection was considered after multidisciplinary discussion if the tumors stabilized or regressed. Pathology reports, including Ki67 index, size of the tumor, perineural, lymphatic, and microvascular invasion, and clinical data, including sex, age, operative procedures, treatment information, and follow-up information, were retrospectively reviewed from the medical record database.

Patients were observed at 3- to 6-month intervals following resection or biopsy and underwent physical examination, laboratory investigation and contrast-enhanced computed tomography or magnetic resonance imaging of the chest/abdomen/pelvis. PFS was defined as the time from the date of resection or biopsy to the date of progression. OS was defined as the time from the date of resection or biopsy to the date of either death (event) or the last follow-up (censored).

Tumors were restaged and regraded based on the 8th edition American Joint Committee on Cancer (AJCC 8th) tumor-node-metastasis (TNM) staging system and 2019 WHO classification. Metastatic diseases were divided into locoregional (nodal or mesenteric involvement) and distant metastases (liver, peritoneum or other sites). Liver metastases (LM) were classified into type I, II and III (23). Type I LM were confined to one liver lobe or two adjacent segments that could be removed by a standard anatomical resection. Type II LM primarily affected one lobe, with smaller satellites contralaterally, and could be managed surgically, including ablative approaches. Type III LM were diffuse, multifocal liver metastases that could not be treated surgically.

This retrospective study was approved by the institutional research ethics committee of FUSCC, and informed consent was obtained from the patients included in this study.

Immunohistochemistry and re-evaluation

Formalin-fixed, paraffin-embedded (FFPE) samples of 69 patients were available, including 63 primary tumors, 5 involved lymph nodes, and 30 LM. Slides of these samples were subjected to Ki67 immunohistochemical staining (MIB-1, Dako Corporation, CA, USA) using a two-step method as previously described (24–27). Detailed experimental procedures were described in the Supplementary Materials [available in a digital data repository (28)]. Additionally, TP53 mutation was associated with Ki67 index variation and might be a possible biologic mechanism for high-grade transition (22, 29, 30). Therefore, we selected FFPE samples of 27 patients for p53 immunohistochemical staining (ab1101, Abcam, Cambridge, UK). Abnormal p53 expression was defined as complete absence or more than 10% of tumor cells presented with moderate to strong nuclear positivity (30–32).

Statistical analyses

Statistical analyses were performed using SPSS (version 25.0, IBM, Armonk, NY, USA). Continuous variables were compared by nonparametric tests or Student's t-test. Categorical variables were compared by Pearson's chi-squared test or Fisher's test. The Kaplan–Meier method and log-rank test were used to estimate significant differences in survival. Cox regression analyses were performed to identify independent prognostic factors of PFS and OS. Variables significantly associated with PFS or OS in the univariable analysis were included in the multivariable analysis. Logistic regression models were used to control confounding variables. Two-sided *P values* of <0.05 were considered statistically significant. Figures were drawn with GraphPad Prism (version 8.0, San Diego, CA, USA, www.graphpad.com).

Results

Patient characteristics

A total of 103 patients who had paired primary tumors and metastases with an available Ki67 index were identified as the study cohort. These patients were divided into a resection cohort and a biopsy cohort (Figure 1). The clinicopathological characteristics and treatment information of the study cohort, resection cohort, and biopsy cohort were analyzed and are summarized in Table 1. In the study cohort, 4 patients had functional tumors, and their specific information is shown in Supplementary Table S1. The grade at first diagnosis was distributed as follows: G1 NET-10 (9.7%), G2 NET-76 (73.8%), and G3 NET-17 (16.5%). A total of 20 patients underwent pancreatoduodenectomy with or without LM resection, 66 patients underwent distal pancreatectomy with or without LM resection, 4 patients underwent total pancreatectomy with or without LM resection, and one patient underwent middle pancreatectomy with LM resection. Furthermore, 3 patients underwent laparotomy with biopsy, and 9 patients underwent needle biopsy. For sites of metastases, 92 patients (89.3%) had LM, 9 patients (8.7%) had nodal or mesenteric metastases, and 2 patients (1.9%) had peritoneum or other distant metastases. In addition, 86 patients (83.5%) were diagnosed with synchronous metastases, and 17 patients (16.5%) developed metachronous metastases.

Ki67 index variation and grade changes between primary tumors and metastases

In the study cohort, 50 cases (48.5%) showed variance in the Ki67 index between primary tumors and metastases, and the median variation in the Ki67 index in metastases compared to primary tumors was 0%, ranging from -14% to +29% (Table 2). The Ki67 variation led to grade changes between the primary tumors and metastases; 18 patients (17.5%) had a grade increase, and a grade decrease was observed in 6 patients (5.8%). Among those with grade increases, 7 patients (6.8%) changed from G1 to G2, 4 patients (3.9%) changed from G1 to G3, and 7 patients

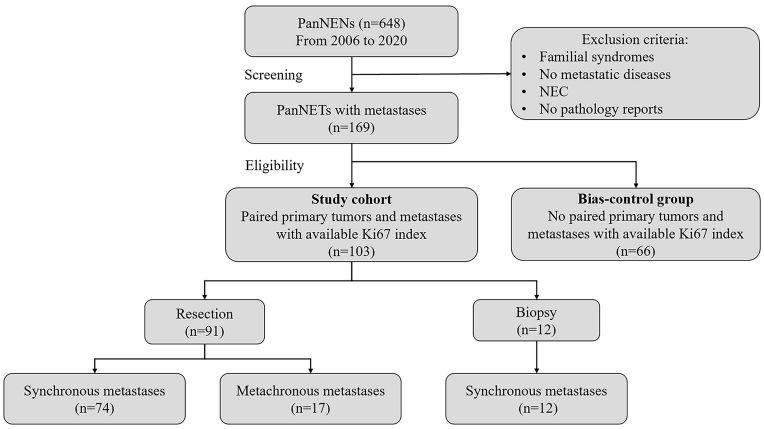

FIGURE 1
Flow diagram of the study cohort. Out of the 648 patients with PanNENs, 169 patients with metastatic PanNETs were included for further eligibility evaluation. Then 103 patients had paired primary tumors and metastases with an available Ki67 index were identified as the study cohort, and the remaining 66 patients were identified as the bias-control group. The study cohort consisted of 91 patients underwent resection and 12 patients experienced biopsy. In resection cohort, 74 and 17 patients had synchronous and metachronous metastases, respectively. And biopsy cohort consisted of 12 patients with synchronous metastases.

(6.8%) changed from G2 to G3 (Figures 2A–D). The characteristics of the eleven patients with G1 or G2 (G1/G2) increase to G3 are shown in Supplementary Table S2. In addition, grade changes between the primary tumors and metastases were found in 21 cases (23.1%) in the resection cohort and 3 cases (25.0%) in the biopsy cohort.

Association among clinical characteristics, Ki67 index variation, and grade changes

Metachronous metastases showed significantly higher Ki67 index variation than synchronous metastases (P=0.028, Figures 2Ea). Meanwhile, metachronous metastases had a higher proportion of G1/G2 increase to G3 than synchronous metastases, although the difference was not significant (P=0.060). Patients with AJCC 8th stage I/II showed a higher frequency of grade increase and G1/G2 increase to G3 than patients with AJCC 8th stage III/IV (P=0.033 and P=0.012, respectively, Figures 2Eb, c), which was consistent with the result that metachronous metastases showed higher Ki67 index variation. In the eleven patients with a G1/G2 increase to G3, all 4 patients with metachronous metastases and AJCC 8th stage II at first diagnosis showed a G2 increase to G3; among the 7 patients with synchronous metastases and AJCC 8th stage IV at first diagnosis, 4 patients had a G1 increase to G3, and the remaining 3 patients had a G2 increase to G3, indicating that patients with stage IV were more likely to have a more severe grade increase (Supplementary Table S2).

Ki67 index variation and grade changes in advanced patients who received NAT

Increasing evidence supports the application of NAT in advanced PanNETs (33, 34). Among the 68 advanced patients (AJCC 8th stage IV) who underwent surgical resection, 31 patients received NAT, and 37 cases did not receive NAT. The clinicopathological characteristics and treatment information of these patients are summarized in Supplementary Table S3. Of the 31 patients receiving NAT, 9 patients (29.0%) received SSAs with or without targeted therapy, 11 patients (35.5%) received capecitabine and temozolomide (CAPTEM) with or without targeted therapy, 7 patients (22.6%) received SSAs+CAPTEM with or without targeted therapy, a case (3.2%) received locoregional treatment, 2 patients (6.5%) received other chemotherapeutic regimens, and one patient (3.2%) received targeted therapy. All patients treated with NAT had LM, including 2 patients (6.5%) with type I LM, 2 patients (6.5%) with type II LM, and 27 patients (87.1%) with type III LM. Patients who received NAT presented a significantly higher proportion of type III LM than those who did not receive NAT (87.1% vs. 50.0%, P=0.009).

These patients achieved the effect of tumor stabilization or regression after receiving NAT and could be considered for surgery after multidisciplinary discussion. Among them, 8 patients underwent pancreaticoduodenal resection with LM, and 23 cases underwent distal pancreaticoduodenal resection with LM. Additionally, advanced patients who received NAT presented a significantly higher proportion of grade increase (25.8% vs. 5.4%, P=0.018) than those who did not receive NAT (Table S3).

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Table 1. Demographics and clinical characteristics of the study cohort, resection and biopsy cohort.

Characteristics	Study cohort (n=103)	Resection cohort (n=91)	Biopsy cohort(n=12)
	No. (%)	No. (%)	No. (%)
Gender			
Male	50 (48.5)	42 (46.2)	8 (66.7)
Female	53 (51.5)	49 (53.8)	4 (33.3)
Age, years, median	51	50.5	49.5
Tumor size, mm			
Mean (SD)	48.7 (3.0)	47.7 (3.0)	56.8 (3.4)
Median (range)	40.0 (8.0-160.0)	40.0 (8.0-160.0)	47.0 (20.0-120.0)
Location			
Head	28 (27.2)	22 (24.2)	6 (50.0)
Neck	6 (5.8)	5 (5.5)	1 (8.3)
Body	15 (14.6)	14 (15.4)	1 (8.3)
Tail	25 (24.3)	23 (25.3)	2 (16.7)
Body-Tail	29 (28.2)	27 (29.7)	2 (16.7)
Functional			
Yes	4 (3.9)	4 (4.4)	0 (0.0)
No	99 (96.1)	87 (95.6)	12 (100.0)
Lymph node positive	n=88	n=88	NA
Yes	48 (54.5)	48 (54.5)	
No	40 (45.5)	40 (45.5)	
Perineural invasion	n=84	n=84	NA
Yes	41 (48.8)	41 (48.8)	
No	43 (51.2)	43 (51.2)	
Microvascular invasion	n=84	n=84	NA
Yes	57 (67.9)	57 (67.9)	
No	27 (32.1)	27 (32.1)	
CgA[a]	n=102	n=90	n=12
Positive	97 (95.1)	86 (95.6)	11 (91.7)
Negative	5 (4.9)	4 (4.4)	1 (8.3)
Syn[a]	n=101	n=89	n=12
Positive	100 (99.0)	88 (98.9)	12 (100.0)
Negative	1 (1.0)	1 (1.1)	0 (0.0)
DAXX[a]	n=38	n=38	NA
Positive	34 (89.5)	34 (89.5)	
Negative	4 (10.5)	4 (10.5)	
ATRX[a]	n=36	n=36	NA
Positive	32 (88.9)	32 (88.9)	
Negative	4 (11.1)	4 (11.1)	
SSTR[a]	n=57	n=55	n=2
Positive	53 (93.0)	51 (92.7)	2 (100.0)
SSTR2	38 (66.7)	36 (65.4)	2 (100.0)
SSTR2+SSTR5	15 (26.3)	15 (27.3)	0 (0.0)
Negative	4 (7.0)	4 (7.3)	0 (0.0)
NSE classification	n=84	n=76	n=8
High	26 (31.0)	22 (28.9)	4 (50.0)
Low	58 (69.0)	54 (71.1)	4 (50.0)

(Continued)

Continued

Characteristics	Study cohort (n=103)	Resection cohort (n=91)	Biopsy cohort(n=12)
	No. (%)	No. (%)	No. (%)
PROGRP classification	n=46	n=43	n=3
High	8 (17.4)	7 (16.3)	1 (33.3)
Low	38 (82.6)	36 (83.7)	2 (66.7)
Metastases			
Site			
Liver	92 (89.3)	81 (89.0)	11 (91.7)
Nodal/mesenteric	9 (8.7)	9 (9.9)	0 (0.0)
Peritoneum/others	2 (1.9)	1 (1.1)	1 (8.3)
Type			
Synchronous	86 (83.5)	74 (81.3)	12 (100.0)
Metachronous	17 (16.5)	17 (18.7)	0 (0.0)
AJCC 8th TNM stage			
I	1 (1.0)	1 (1.1)	0 (0.0)
II	12 (11.7)	12 (13.2)	0 (0.0)
III	10 (9.7)	10 (11.0)	0 (0.0)
IV	80 (77.7)	68 (74.7)	12 (100.0)
WHO classification[b]			
G1	10 (9.7)	9 (9.9)	1 (8.3)
G2	76 (73.8)	68 (74.7)	8 (66.7)
G3	17 (16.5)	14 (15.4)	3 (25.0)
Operating methods			
Pancreatoduodenectomy	6 (5.8)	6 (6.6)	0 (0.0)
Distal Pancreatectomy	15 (14.6)	15 (16.5)	0 (0.0)
Total pancreatectomy	2 (1.9)	2 (2.2)	0 (0.0)
Total pancreatectomy with LM resection	2 (1.9)	2 (2.2)	0 (0.0)
Middle pancreatectomy with LM resection	1 (1.0)	1 (1.1)	0 (0.0)
Pancreatoduodenectomy with LM resection	14 (13.6)	14 (15.4)	0 (0.0)
Distal Pancreatectomy with LM resection	51 (49.5)	51 (56.0)	0 (0.0)
Laparotomy with biopsy.	3 (2.9)	0 (0.0)	3 (25.0)
Needle biopsy	9 (8.7)	0 (0.0)	9 (75.0)
Treatment			
Neoadjuvant treatment	n=33	n=33	NA
SSAs with or without targeted therapy	9 (27.3)	9 (27.3)	
CAPTEM with or without targeted therapy	11 (33.3)	11 (33.3)	
SSAs+CAPTEM with or without targeted therapy	7 (21.2)	7 (21.2)	
Locoregional treatment	1 (3.0)	1 (3.0)	
Other chemotherapy	3 (9.1)	3 (9.1)	
Targeted therapy	1 (2.0)	1 (3.0)	
Others	1 (3.0)	1 (2.0)	
Adjuvant treatment	n=78	n=78	NA
SSAs with or without targeted therapy	35 (44.9)	35 (44.9)	
CAPTEM with or without targeted therapy	6 (7.7)	6 (7.7)	
SSAs+CAPTEM with or without targeted therapy	6 (7.7)	6 (7.7)	
Locoregional treatment	12 (15.4)	12 (15.4)	
Other chemotherapy	4 (5.1)	4 (5.1)	
Targeted therapy	15 (19.2)	15 (19.2)	
Others	0 (0.0)	0 (0.0)	
Treatment for patients with biopsy	n=12	NA	n=12
SSAs with or without targeted therapy	4 (33.3)		4 (33.3)

(Continued)

Continued

Characteristics	Study cohort (n=103)	Resection cohort (n=91)	Biopsy cohort(n=12)
	No. (%)	No. (%)	No. (%)
CAPTEM with or without targeted therapy	2 (16.7)		2 (16.7)
SSAs+CAPTEM with or without targeted	3 (25.0)		3 (25.0)
therapy	1 (8.3)		1 (8.3)
Locoregional treatment	0 (0.0)		0 (0.0)
Other chemotherapy	2 (16.7)		2 (16.7)
Targeted therapy	0 (0.0)		0 (0.0)
Others			

CgA, chromogranin; Syn, synaptophysin; DAXX, death domain associated protein; ATRX, alpha-thalassemia/mental retardation X-linked; SSTR, somatostatin receptor; NSE, neuron specific enolase; PROGRP, progastrin releasing peptide; AJCC, American Joint Committee on Cancer; WHO, World Health Organization; [a]The expression in primary tumors; [b]Grade at first diagnosis; LM, liver metastases; SSAs, somatostatin analogs; CAPTEM, capecitabine and temozolomide; NA, not available.

Survival analyses

Next, we performed survival analyses to determine whether grade changes affect the patient prognosis. In the study cohort, the median follow-up time was 48.0 months [95% confidence interval (CI): 39.70-56.30], and the median PFS was 10.0 months (95% CI: 6.33-13.67). The Kaplan–Meier survival probability estimates of 1-year, 3-year, and 5-year progression-free survival were 41.7%, 18.4%, and 6.8%, respectively. The Kaplan–Meier survival probability estimates of 1-year, 3-year, and 5-year overall survival were 85.4%, 52.4%, and 28.2%, respectively (Figure S1). Of importance, G1/G2 increase to G3 was significantly associated with shorter PFS and OS than stable G1/G2 (P=0.012 and P=0.027, respectively, Figure 3). G2 increase to G3 was associated with shorter PFS and OS, although it was not statistically significant (P=0.115, and P=0.064, respectively, Supplementary Figures S2A, B). In addition, G2 decrease to G1 was associated with a longer OS, although the difference was not statistically significant (P=0.445, Supplementary Figure S2D).

Univariable analyses demonstrated that biopsy, NAT, and a low-grade increase to high-grade were associated with a shorter PFS (HR=3.443, 95% CI: 1.809–6.552, P=0.000; HR=2.186, 95% CI: 1.284-3.720, P=0.004, and HR=2.281, 95% CI: 1.162-4.479, P=0.017, respectively, Table 3). Biopsy and a low-grade increase to high-grade were associated with shorter OS (HR=6.862, 95% CI: 1.969-23.916, P=0.002; and HR=4.418, 95% CI: 1.051-18.578, P=0.043, respectively). Multivariable survival analyses indicated that NAT and a low-grade increase to high-grade were independent prognostic factors for PFS (HR=2.756, 95% CI: 1.474-5.153, P=0.001; and HR=2.695, 95% CI: 1.273-5.706, P=0.010, respectively). In addition, a low-grade increase to high-grade was an independent prognostic factor for OS (HR= 4.565, 95% CI: 1.063-19.612, P=0.041).

Association between p53 expression and Ki67 index variation

To further explore the association between high-grade transition and TP53 mutation, we performed p53 immunohistochemistry staining. Four out of 27 patients (14.8%) showed different p53 expression pattern between primary tumors and liver metastases, which was correlated

Table 2. Ki67 index variation and grade changes in metastases compared to primary tumors in PanNETs.

Ki67 index variation	Study cohort (n=103)	Resection cohort (n=91)	Biopsy cohort (n=12)
Patient number (%)	50 (48.5)	43 (47.3)	7 (58.3)
Delta Ki67 index, median (range)	0 (-14 to +29)	0 (-14 to +29)	0.5 (-5 to +27)
Tumor grade			
Stable, n (%)	79 (76.7)	70 (76.9)	9 (75.0)
Grade increase, n (%)	18 (17.5)	15 (16.5)	3 (25.0)
G1 increase to G2	7 (6.8)	6 (6.6)	1 (8.3)
G1 increase to G3	4 (3.9)	4 (4.4)	0 (0)
G2 increase to G3	7 (6.8)	5 (5.5)	2 (16.7)
Grade decrease, n (%)			
G2 decrease to G1	6 (5.8)	6 (6.6)	0 (0)

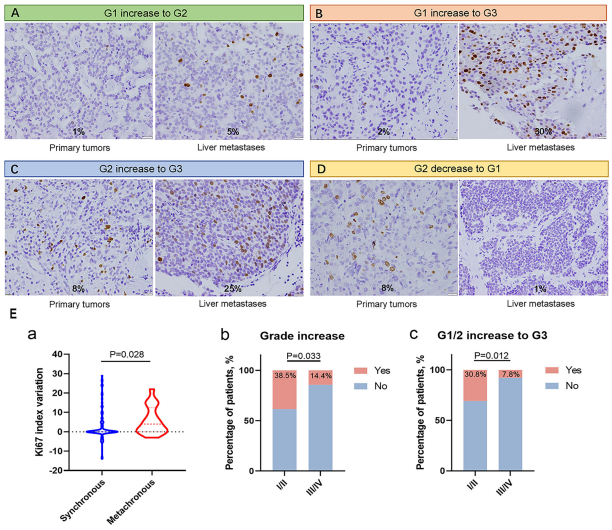

FIGURE 2
(A–D) Immunohistochemistry staining of Ki67 in available paired primary tumors and metastases, showing G1 increase to G2, G1 increase to G3, G2 increase to G3, and G2 decrease to G1, respectively. Magnification: 400×. **(E)** Association among clinicopathological characteristics, Ki67 index variation and grade changes. (a) Metachronous metastases showed higher Ki67 index variation than synchronous metastases. (b, c) Patients with AJCC 8th stage I/II showed higher frequency of grade increase, and G1/G2 increase to G3 than patients with AJCC 8th stage III/IV.

with higher Ki67 index variation (P=0.031, Supplementary Figure S5)

Discussion

In this retrospective study, 648 patients with PanNENs were screened, and 103 patients with advanced PanNETs who had paired primary tumors and metastases were identified as the study cohort. We described the Ki67 index variation and tumor grade changes in PanNETs, explored the association between the clinical characteristics and grade changes, and determined whether the grade changes predicted the clinical prognosis. The findings of this study could help improve our understanding of the heterogeneity of PanNETs and contribute to clinical decision-making.

In this study, 48.5% of cases had Ki67 index variation, 23.3% displayed grade changes, and 17.5% showed a grade increase. Of note, 10.7% of cases had high-grade metastases compared to low-grade primary tumors. For the associations among clinical characteristics, Ki67 index variation, and grade changes, metachronous metastases presented with higher Ki67 index variation than synchronous metastases. Patients with early-stage disease showed a higher frequency of grade increase and

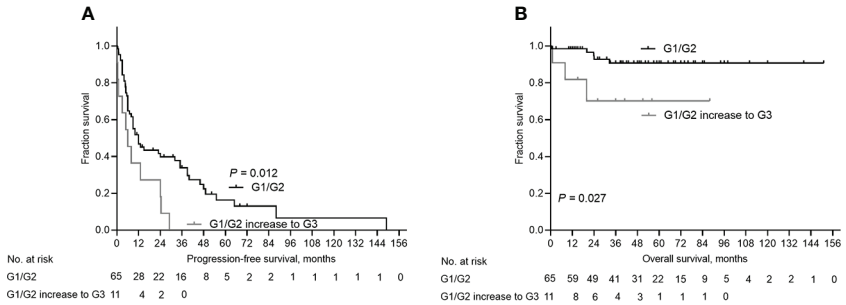

FIGURE 3
Kaplan-Meier curves depicting progression-free survival (PFS) and overall survival (OS) for patients G1/G2 increase to G3. **(A)** Patients with G1/G2 increase to G3 had decreased PFS than patients with stable G1/2 (P=0.012). **(B)** Patients with G1/G2 increase to G3 had decreased OS than patients with stable G1/2 (P=0.027).

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Table 3. Univariable and multivariable analysis of factors for progression-free survival and overall survival in the study cohort.

Factors	Progression-free Survival				Overall Survival			
	Univariable		Multivariable		Univariable		Multivariable	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender: male *vs* female	0.787 (0.505-1.227)	0.291			1.844 (0.563-6.040)	0.312		
Age: <51 *vs* ≥51 years	0.745 (0.480-1.156)	0.189			0.474 (0.146-1.543)	0.215		
Tumor size: <40 *vs* ≥ 40mm	1.135 (0.720-1.790)	0.584			0.785 (0.262-2.350)	0.665		
Tumor location: head *vs* neck, body and tail	1.582 (0.967-2.590)	0.068			1.058 (0.287-3.892)	0.933		
Functional: no *vs* yes	0.408 (0.126-1.321)	0.135			1.443 (0.185-11.283)	0.726		
Lymph node positive: no *vs* yes	1.128 (0.692-1.838)	0.628			1.892 (0.472-7.592)	0.368		
Perineural invasion: no *vs* yes	1.103 (0.668-1.823)	0.701			1.599 (0.376-6.798)	0.525		
Microvascular invasion: no *vs* yes	0.738 (0.431-1.263)	0.268			3.938 (0.483-32.072)	0.200		
CgA: negative *vs* positive	0.511 (0.206-1.271)	0.149			0.659 (0.084-5.158)	0.691		
Syn: negative *vs* positive	0.351 (0.048-2.575)	0.303			0.167 (0.021-1.320)	0.090		
DAXX: negative *vs* positive	1.411 (0.332-6.007)	0.641			23.321 (0-3.78E+10)	0.771		
ATRX: negative *vs* positive	2.470 (0.705-8.652)	0.157			31.539 (0-1.10E+7)	0.596		
SSTR: negative *vs* positive	0.512 (0.180-1.456)	0.210			22.359 (0-1.26E+10)	0.762		
Metastases site: liver *vs* nodal/mesenteric *vs* peritoneum/others	0.821 (0.479-1.409)	0.475			0.608 (0.096-3.864)	0.598		
Metastases type: synchronous *vs* metachronous	0.816 (0.462-1.443)	0.485			0.270 (0.034-2.125)	0.214		
Surgery: yes *vs* biopsy	3.443 (1.809-6.552)	**0.000**	NA	NA	6.862 (1.969-23.916)	**0.002**	4.254 (0.804-22.507)	0.088
Neoadjuvant treatment: no *vs* yes	2.186 (1.284-3.720)	**0.004**	2.756 (1.474-5.153)	**0.001**	0.743 (0.151-3.669)	0.716		
Grade changes: G1/G2 *vs* G1/G2 increase to G3	2.281 (1.162-4.479)	**0.017**	2.695 (1.273-5.706)	**0.010**	4.418 (1.051-18.578)	**0.043**	4.565 (1.063-19.612)	**0.041**

HR, hazard ratio; CI, confidence interval; CgA, chromogranin; Syn, synaptophysin; DAXX, death domain associated protein; ATRX, alpha-thalassemia/mental retardation X-linked; SSTR, somatostatin receptor; NSE, neuron specific enolase; PROGRP, progastrin releasing peptide; TNM, tumor–node–metastasis; NA, not available; bold values indicate statistical significance.

G1/G2 increase to G3 than patients with advanced-stage disease, indicating that early-stage tumors had more time to evolve, which was consistent with previous observations that NETs dedifferentiated over time. Additionally, G1 increase to G3 only presented in patients with stage IV and synchronous metastases, indicating that advanced tumors had a worse evolution than less advanced tumors. In the study cohort, all patients had only one primary tumor except one patient with two primary tumors, and the Ki67 values of the two primary tumors were both 5%. Therefore, the Ki67 index heterogeneity among primary tumors was not analyzed.

Previous studies reported that 35.3%-63% of metastatic GEP-NENs showed higher Ki-67 index at LM than primary site (35–37), and 7.5%-39% presented a grade increase (20, 21, 38–40). As for longitudinal increase, about 58.6%-65.1% of NEN patients showed an increase in Ki67 index, and nearly 28% showed upgrade when progression (41–44). Shi et al. reported that nearly two-thirds of small intestinal NETs had grade increase in LM (45). As for PanNENs, several studies described that 62.5%-63.6% patients with PanNETs had an increased grade when the disease evolved over time (22, 46). Alexandraki et al. analyzed 264 PanNENs and showed that 15 patients (5.7%) developed an increase in Ki67 during disease course (31). However, this study also included patients with multiple endocrine neoplasia type 1, and did not provide information about the 264-patient cohort. Given the strong heterogeneity of NENs, the results obtained in relatively homogeneous tumors would be more powerful. Therefore, this work aimed to explore the heterogeneity between primary tumors and metastases in a homogeneous and large-scale patient cohort of PanNETs, mainly to characterize the grade changes and correlate the findings with patient prognosis, also providing real-life data from the clinical treatment.

The "transformed" NETs that would evolve to either NET G3 or NEC has aroused interest. Several studies investigated this phenomenon (47–51) and proposed that the boundary between NETs and NEC was blurred. In this study, we observed that NET G1/G2 could evolve to NET G3, but did not find the transition from NETs to NEC. The concept that well-differentiated NETs would develop to poor-differentiated NEC probably needed further investigation.

Additionally, a suspicion of a grade increase in metastatic sites compared to primary tumors might constitute a negative prognostic factor (52). Therefore, we performed survival analyses and found that high-grade metastases compared to low-grade primary tumors were significantly associated with decreased PFS and OS. Multivariable survival analyses indicated that a low-grade increase to high-grade was an independent

prognostic factor for PFS and OS. Based on the above results, we concluded that PanNET patients with metastases, whether synchronous or metachronous, would have a grade increase in metastases compared to their primary tumors. Because of the longer clinical course of metachronous metastases, the probability of a grade increase was higher than among those with synchronous metastases. In addition, G1 increase to G3, a progression of two grades, but only in AJCC 8th stage IV tumors, suggesting that advanced tumors had a worse evolution than less advanced tumors.

Furthermore, Cox regression analysis showed that patients who received NAT had a shorter PFS, whereas there was no significant difference in OS. It should be noted that patients with high-risk factors, were routinely received preoperative NAT. Surgical resection was considered after multidisciplinary discussion if the tumors stabilized or regressed. It was obvious that patients with high-risk factors had a worse prognosis, but the results did not show any significant difference in OS compared to those without high-risk factors, indicating the potential effectiveness of preoperative NAT in patients with metastatic PanNETs.

Our results revealed the poor survival of patients with a high-grade increase in metastases, indicating the necessity of close follow-up and early intervention. Those patients with high-grade increase warrant different therapeutic strategies. In addition, this study also supported the use of multiple point puncture and accurate pathological grading for PanNET patients in metastatic lesions or when disease progression.

The possible underlying mechanism of Ki67 index heterogeneity and high-grade transition might due to the polyclonal tumor origin of NENs, with different mutational events, microenvironmental context, and/or epigenetic divergence between and within tumors (45). During the progression course, the selection of subclonal populations with a higher proliferative index (20), and de-reprograming would happen. In this study, higher Ki67 index variation was correlated with changes in p53 expression pattern, implying the TP53 mutation was probably involved in the high-grade progression. Additionally, treatment effects and therapy resistance might also contribute to the transition towards higher grade (44). The above hypotheses warrant further research.

Several limitations existed in the current study. First, all retrospective studies have inherent limitations. Second, the prognosis of most patients with PanNETs is good (53), but the median follow-up time of this study was relatively short. The follow-up data were available up until February 1, 2022, by which time only 13 cases (12.6%) had died. Those who had not died were considered right-censored, which might underestimate the overall survival time. Third, this study had selection bias of low to intermediate degrees due to the nonsignificant difference in survival between the study cohort and the bias control group. In addition, we did not explore the molecular mechanism of the clinical phenomenon that low-grade primary tumors would increase to high-grade metastases. To address this limitation, we are conducting another study to detect paired low-grade primary tumors and high-grade metastases using whole-exome sequencing to identify the mutant genes driving the grade increase of metastases and to investigate its molecular mechanism.

Overall, this study found that 17.5% of patients with metastatic PanNETs had a grade increase in metastases compared to their primary tumors. A high-grade increase in metastases was an unfavorable prognostic factor for PFS and OS, which could provide a useful reference for clinical decision-making.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by institutional research ethics committee of Fudan University Shanghai Cancer Center. The patients/participants provided their written informed consent to participate in this study.

Author contributions

W-HZ, JC, X-JY, X-WX and S-RJ conceived the original idea of the study. H-LG, W-SL, and YQ contributed to sample preparation and data collection. WH-Z, ZY, XL, and FW conducted all statistical analyses. WH-Z, Q-FZ, ZY, YZ, and X-MC contributed to the implementation of the research and interpretation of data. All authors discussed the results, helped prepare and shape the research and gave final approval of the manuscript to be published.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.941210/full#supplementary-material

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Targeting-intratumoral-lactic-acidosis transcatheter-arterial-chemoembolization for non-islet cell tumor hypoglycemia secondary to a liver metastatic solitary fibrous tumor: A case report and literature review

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Doege–Potter syndrome is a rare paraneoplastic syndrome characterized by non-islet cell tumor hypoglycemia secondary to a solitary fibrous tumor. Doege–Potter syndrome always presents with recurrent fasting hypoglycemia, which can occasionally be life-threatening. The best choice of treatment for Doege–Potter syndrome and solitary fibrous tumor is complete resection. However, when it is unfeasible, local-regional treatment can be used as a palliative therapy. Herein, we report a case of a 46-year-old man with Doege–Potter syndrome that occurred secondary to the liver and pancreatic metastatic solitary fibrous tumors. After he received six rounds of targeting-intratumoral-lactic-acidosis transcatheter-arterial-chemoembolization (TILA-TACE) treatment in our hospital, his hypoglycemia was clinically cured, and the liver metastatic tumor was well controlled. We suggest that

Abbreviations: WHO, World Health Organization; SFTs, solitary fibrous tumors; DPS, Doege–Potter syndrome; NTCTH, non-islet cell tumor hypoglycemia; TACE, transcatheter arterial chemoembolization; cTACE, conventional transcatheter arterial chemoembolization; TILA-TACE, targeting-intratumoral-lactic-acidosis transcatheter-arterial-chemoembolization; IGF, insulin-like growth factors; IGFBP, insulin-like growth factor binding protein; HPF, high-power field; MRI, magnetic resonance imaging; CT, computed tomography; HbA1c, hemoglobin A1c; AFP, alpha-fetoprotein; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen.

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TILA-TACE can be considered when curative resection is unfeasible for metastatic liver solitary fibrous tumors to help a patient obtain further surgery opportunities.

KEYWORDS

TILA-TACE, solitary fibrous tumor, Doege–Potter syndrome, non-islet cell tumor, hypoglycemia

Introduction

Solitary fibrous tumors (SFTs) are rare neoplasms that originate from mesenchymal spindle cells. They were first reported by Wagner in 1870, and their histopathological report was subsequently described by Klemperer and Rabin in 1931 (1). In 2013, based on the World Health Organization (WHO) classification of tumors of soft tissue and bone, SFTs were considered benign tumors with potential for malignant transformation (2). Most SFTs are inert, without local or distant recurrence. However, the 10-year disease-specific survival rate for pleural and extra-pleural SFT is about 73%–100%, and the 10-year recurrence rate is 10%–25% (3–8). Meningeal hemangiopericytoma in the central nervous system and SFT were classified as the same tumor by WHO. On the other hand, hemangiopericytoma/SFT is more aggressive; local recurrence is frequent and rapid, with the possibility of meningeal spread and early distant metastasis to the bone. According to the WHO classification, though most of SFTs are considered benign, some SFTs can be considered malignant with the following histopathologic characteristics: cytological atypia, hypercellularity, tumor necrosis, high mitotic rate (>4 per high-power field (HPF)), and/or infiltrative margins (9). At present, it is not recommended to use "benign" or "malignant" to evaluate the prognosis of patients; it is more applicable to apply the risk of recurrence/metastasis for SFTs. Patients with the following conditions are more likely to have tumor recurrence/metastasis: incomplete surgical resection, metastatic disease at the time of visiting, tumor greater than 10 cm, high mitosis rate (>4 mitotic images/10 HPF), and tumor necrosis (4, 10–14). Ki-67 greater than 5% is also considered a marker (15). The clinical manifestations of SFTs are usually nonspecific. Hypoglycemia occurs in approximately 5% of SFT cases, in a rare and challenging paraneoplastic syndrome known as the Doege–Potter syndrome (DPS), characterized as non-islet cell tumor hypoglycemia (NICTH) (16, 17). DPS usually manifests as a rare, refractory, and severe hypoglycemia.

For both SFTs and DPS, the best treatment is complete resection of the tumor. However, when curative resection is not feasible, short-term therapy, including continuous intravenous infusion of glucose and medical therapy, is beneficial. Other treatments include adjuvant chemotherapy and radiotherapy (18, 19). It is reported that pazopanib can effectively control the progression of the tumor and can be considered a first-line treatment for some advanced typical SFTs (20–23). Other drugs, like temozolomide and bevacizumab, could also play a role. However, more study is needed to verify the effects of these drugs (24). For primary and metastatic liver SFTs, local-regional treatments such as transcatheter arterial chemoembolization (TACE) have been reported (19, 25). Zhong et al. have recently reported a case of hepatic SFT in a patient who was treated with curative *ex situ* hepatectomy and liver autotransplantation (26). All of these approaches can play a part in treating hepatic SFTs.

Herein, we report the case of a patient with DPS caused by multiple metastatic SFTs in the liver and pancreas. Due to multiple liver metastases and serious hyperglycemia, he was not a suitable candidate for direct surgery. He had previously been treated with diet therapy, continuous infusion of glucose, low-dose glucocorticoid treatment, and sorafenib tosylate, as well as four cycles of conventional TACE (cTACE) in other hospitals, yet experienced little significant improvement. Fortunately, in our hospital, his hypoglycemia was clinically controlled after six cycles of targeting-intratumoral-lactic-acidosis (TILA)-TACE, and his metastatic liver tumors became almost necrotic after eight cycles of TILA-TACE treatment, which was first reported by our team (27). The patient then underwent a successful combined operation of the pancreatic body and tail resection, splenectomy, and left lateral lobe hepatectomy after his hypoglycemia was clinically cured and the further progression of SFTs was controlled.

In this case, our patient benefited from TILA-TACE rather than the original cTACE treatment. Hence, we suggest that for patients with primary and metastatic liver SFTs and who are not eligible for curative surgery, TILA-TACE may be a successful therapeutic alternative.

Case report

A 46-year-old Chinese man visited our hospital for recurrent paroxysmal unconsciousness occurring for 10 months (from January 2017). At first, his hypoglycemia usually occurred in the morning, and later it was irregular, accompanied by sweating, weakness, and occasional urinary incontinence, and these symptoms disappeared after eating. He had no history of

diabetes but had a history of drinking for 20 years and had been sober for 1 year before the onset of hypoglycemia. The results of laboratory test were as follows: serum blood glucose at 1.26 mmol/L (normal range: 3.89–6.11 mmol/L), serum insulin level at <3.48 pmol/L (normal range: 17.8–173.0 pmol/L), C-peptide at 0.03 nmol/L (normal range: 0.27–1.28 nmol/L), and insulin-like growth factors IGF-1 at < 25.0 ng/ml (normal range: 94.0–252 ng/ml). Glutamate acid decarboxylase antibodies, anti-insulin autoantibodies, and anti-islet cell antibodies were negative. Positron emission tomography showed scattered low-density lesions in the liver and an enlarged lymph node behind the pancreas with increased glucose uptake. Liver contrast-enhanced magnetic resonance imaging (MRI) was performed in May 2017 and revealed pancreatic and multiple liver masses, showing at least six large masses (diameter >5 cm) and multiple small metastases in the liver, the largest one of which was approximately 14 cm in diameter. All of the masses demonstrated a slightly high mixed signal on T2-weighted imaging and diffusion-weighted imaging and a slightly low mixed signal on T1-weighted imaging. In contrast, all these masses showed obvious enhancement. In addition, a mass was found located in the tail of the pancreas that measured 3.6 cm in diameter with short T1 and long T2 signals on MRI, and the mass showed evident enhancement after contrast. Neuroendocrine tumors were first considered (Figure 1). Later, liver mass biopsy and immunohistochemistry results showed positive staining with antibodies against STAT6 and CD34. Thus, the patient was diagnosed with SFT and DPS, and initially received four cycles of cTACE to reduce the side effects as direct surgery might be life-threatening. However, no improvement was observed, and the patient still had severe hypoglycemia. Previous medical history showed that he had resection of a right fossa pterygopalatine tumor in 2010, and reoperation in 2012 as the tumor recurred; a further pathology report showed a spindle cell tumor diagnosed as a hemangiopericytoma.

On physical examination, he was an overweight man with a body mass index of 29.01 kg/m^2. Facial changes included acrochordons and rhinophyma (Figure 2). Abdominal bulging was observed, and abdominal palpation revealed an enlarged liver. No other physical abnormalities were found.

Laboratory data showed fasting hypoglycemia, and the results were as follows: serum blood glucose at 1.25 mmol/L (normal range: 3.89–6.11 mmol/L), serum insulin level at <3.48 pmol/L (normal range: 17.8–173.0 pmol/L), C-peptide at 0.03 nmol/L (normal range: 0.27–1.28 nmol/L), IGF-2 at 1,964.33 ng/ml (normal range: 400–736 ng/ml), IGF-1 at <25.0 ng/ml (normal range: 94.0–252 ng/ml), pro-IGF-2 at 28.79 ng/ml, and IGF-2/IGF-1 >10. Tumor markers such as alpha-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9), and carcinoembryonic antigen (CEA) were negative. Routine biochemical indicators such as transaminase were normal in laboratory tests. Thyroid dysfunction, functional islet cell tumors, and adrenal dysfunction were excluded. Head MRI showed no obvious abnormalities except the VR space of the right cerebral peduncle and the lacunar foci in the right semioval area. Enhanced MRI of the nasopharyngeal area showed postoperative changes in the right pterygopalatine fossa tumor. There were multiple nodular abnormal signals in the right temporal fossa that may be a local recurrence. A contrast-enhanced computed tomography (CT) scan of the abdomen showed multiple nodules and masses of varying sizes in the liver, with unclear borders, which were enlarged compared to May 2017. The largest one was located in the eighth segment of the liver, with a diameter of 15.9 cm. The masses were unevenly enhanced in the arterial phase; in the portal phase and the delayed phase, the enhancement of the mass was weakened but

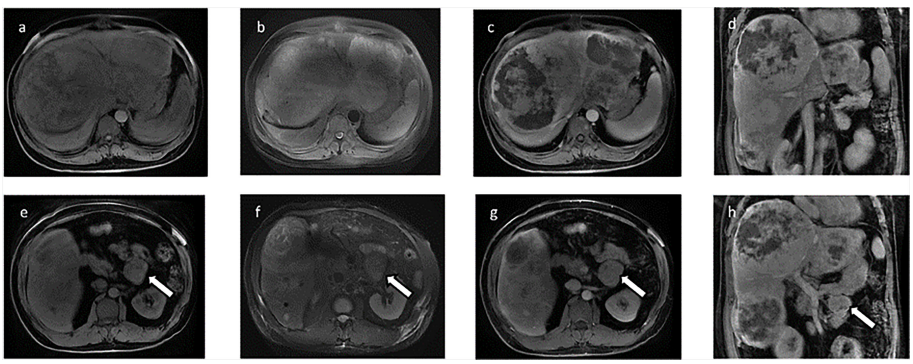


FIGURE 1
Contrast-enhanced magnetic resonance imaging (MRI) of the liver before TILA-TACE. Contrast-enhanced MRI of the liver showed at least six large masses in the liver, the largest one of which was approximately 14 cm in diameter. All of the masses demonstrated a slightly low mixed signal on T1WI **(A)** and a slightly high mixed signal on T2WI **(B)**; after contrast, all these masses were clearly enhanced **(C, D)**. In addition, a mass located in the tail of the pancreas that measured 3.6 cm in diameter was observed (arrow), with short T1 **(E)** and long T2 **(F)** signals on MRI, and the mass was evidently enhanced after contrast **(G, H)**.

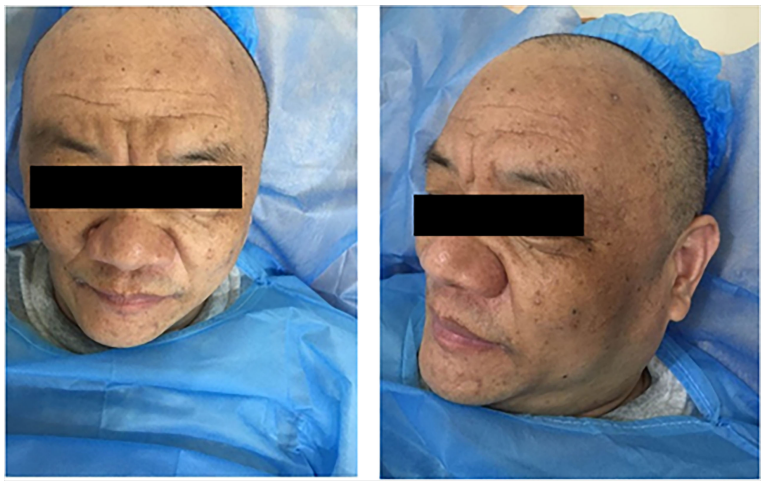


FIGURE 2
Facial changes of the patient, including acrochordons and rhinophyma.

still higher than the surrounding liver tissue. A mass in the posterior and lower parts of the pancreas was noted that was unevenly enhanced with a blurred border.

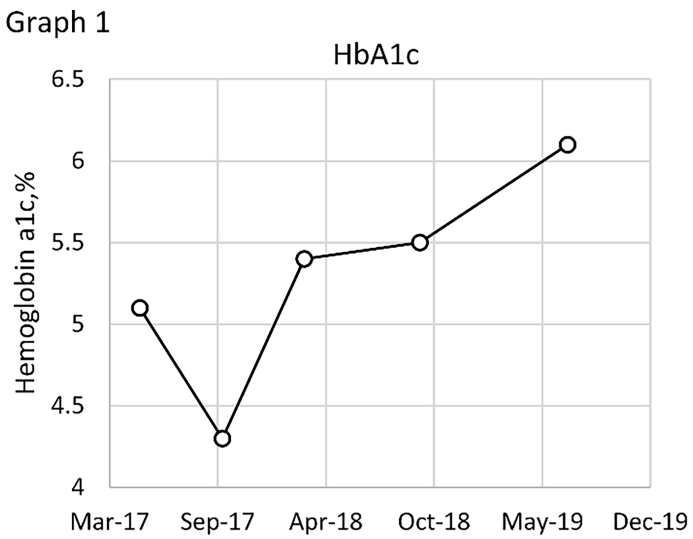
Based on the aforementioned evidence, the patient was diagnosed with metastatic liver and pancreatic SFT, along with the manifestation of DPS. We speculated that the right fossa pterygopalatine tumor was the primary SFT based on the WHO classification of hemangiopericytoma/SFT. Intravenous boluses of 10% dextrose were administered to correct his hypoglycemia, and his blood glucose was monitored through capillary tests and a blood glucose instantaneous sensor. There was a decrease in the frequency of the episodes of unconsciousness, but they occurred irregularly at least once a day.

The effects of diet and drugs were poor, cTACE did not work, and surgery was unavailable; thus, after a multidisciplinary discussion, he was treated with TILA-TACE therapy. Laboratory tests after the first TILA-TACE showed that big-IGF-2 and IGF-2 had obviously decreased, 18.05 and 922.33 ng/ml, respectively, and the serum blood glucose level had also significantly improved. However, hypoglycemia recurred after 3 days at a frequency and severity similar to those before TILA-TACE. One month later, after the second TILA-TACE cycle, the recurrence-free time had extended to 1 week. When the third TILA-TACE cycle was completed, the symptoms and the blood glucose level significantly improved, and no drug treatment was required. Hemoglobin A1c (HbA1c) during hospitalization before and after TILA-TACE treatment is shown in Graph 1. Insulin, C-peptide, IGF-1, HbA1c, and glucose levels during treatment are shown in Table 1. Contrast-enhanced MRI of the liver performed after the third cycle of TILA-TACE showed that almost all of the large liver lesions had complete necrosis (Figure 3). After six cycles of TILA-TACE, the patient's blood glucose was basically back to normal; no extra meals were required.

After the glucose level had stabilized, the patient underwent a combined operation of the pancreatic body and tail resection, splenectomy, and left lateral lobe hepatectomy after six cycles of TILA-TACE treatment. Due to multiple liver and pancreatic metastases, the surgeon did not suggest complete resection of the tumor. A pathological examination of the pancreas revealed a spindle cell tumor with an abundance of heterotypic cells. Microscopic examination with hematoxylin–eosin staining showed that the tumor had a high proliferation rate of four to five mitotic figures per 10 HPF, and the margin was negative. Immunohistochemistry findings revealed positive staining with antibodies against CD34, Bcl-2, STAT6, and CD31, and Ki-67 was 10% (Figure 4). After surgery, his blood glucose was basically back to normal (Table 1), and no extra meals were required. Unfortunately, we determined that the residual tumors had progressed, so two more cycles of TILA-TACE were performed after surgery.

Eight months after surgery, the patient was readmitted to our hospital because of instability while walking and left lower limb weakness. Contrast-enhanced MRI of the thoracic spine confirmed thoracic spine metastasis, and one metastatic nodule had projected into the spinal canal, compressing the spinal cord (Figure 5). A review of his previous imaging revealed that a metastatic thoracic spine tumor had been present in November 2017. After an uneventful thoracic spine tumor resection, he recovered well with no paraplegic symptoms. Histopathology also showed a spindle cell tumor (Figure 6).

Since 2017, the patient has received a total of eight cycles of TILA-TACE and has undergone a combined operation of the pancreatic body and tail resection, splenectomy, and left extrahepatic tumor resection and thoracic spine tumor



GRAPH 1
HbA1c during hospitalization, before and after TILA-TACE treatment.

resection in our hospital. He last visited our hospital for an evaluation in May 2020, and, according to his examination results, the disease was thought to be temporarily stable and his hypoglycemia clinically cured. There is no doubt that TILA-TACE is effective for metastatic liver SFTs and DPS. However, the subsequent treatment of the patient remains an open question because of multiple metastases. Due to economic and personal reasons, the patient did not want to receive further drug or surgical treatment for SFTs after March 2020. The patient died following tumor progression 7 months ago, before the submission of this paper. He had maintained stable blood glucose before his death, with no obvious hypoglycemia events occurring according to his family's description.

Discussion

DPS is mediated by several mechanisms. The first mechanism is the consumption of glucose by a large tumor (28). However, some studies have reported that hypoglycemia did not occur again when the tumor recurred and regrew to its former size, indicating that glucose consumption by the tumor may not be the main cause of hypoglycemia (28). Second,

TABLE 1 The patient's insulin, C-peptide, IGF-1, and hemoglobin A1c throughout the whole treatment.

Date	Glucose (mmol/L)	Insulin (pmol/L)	C-peptide (nmol/L)	IGF-1 (ng/ml)	Hemoglobin A1c (%)	
2017-05	1.26	<3.48	0.03	<25.0	5.1	
2017-10	1.25	<3.48	0.05	<25.0	4.3	After four times of cTACE
2017-11[a]	5.00	NA	NA	NA	NA	
2017-12[a]	4.80	NA	NA	NA	NA	
2018-02[a]	4.90	NA	NA	NA	NA	
2018-03	3.66	0.6	0.07	<25.0	5.4	1 week after the third round of TILA-TACE
2018-10	3.70	4.4	0.05	<25.0	5.5	2 months after the sixth round of TILA-TACE
2018-11	4.31	9.42	0.12	<25.1	NA	7 days after the surgery
2019-05[a]	6.81	NA	NA	NA	NA 6.1 (2019-07)	10 days after the last (eighth) round of TILA-TACE

[a]TILA-TACE operation time.

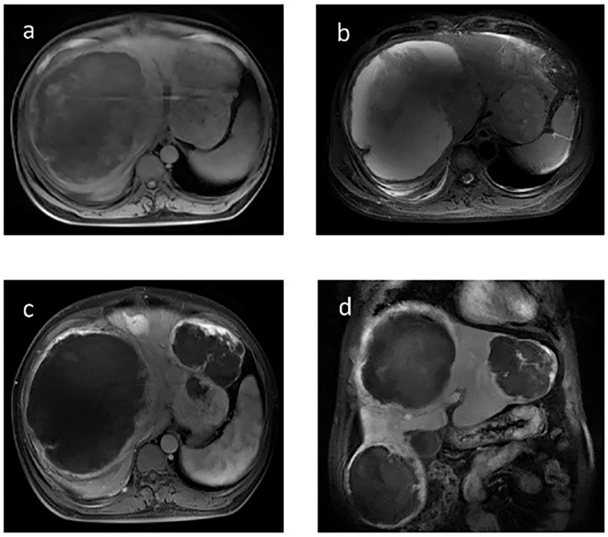


FIGURE 3
Contrast-enhanced MRI of the liver after the third TILA-TACE cycle showed that all of the masses demonstrated slightly low mixed signal on T1WI **(A)** and high mixed signal on T2WI **(B)**. After contrast, most of the masses were almost necrosed **(C, D)**.

abnormalities in the *EGR*-IGF system have been reported to lead to hypoglycemia (28). It is generally believed that the enhanced insulin-like effect is caused by oversecretion of big IGF-2 (29). Moreover, *IGF2* is an *EGR* target gene and is regulated by the chimeric transcription factor *NAB2-STAT6*, leading to abnormalities. NAB2-STAT6 gene fusion induced proliferation in cultured cells and activated the expression of EGR-responsive genes (30). The fusion of *NAB2* and *STAT6* produced the *NAB2-STAT6* chimeric transcription factor, which is located in the nucleus, where it is currently believed to drive tumorigenesis by constitutively activating *NAB2* target genes (30). The *NAB2-STAT6* fusion gene, as a unique molecular feature of SFT,

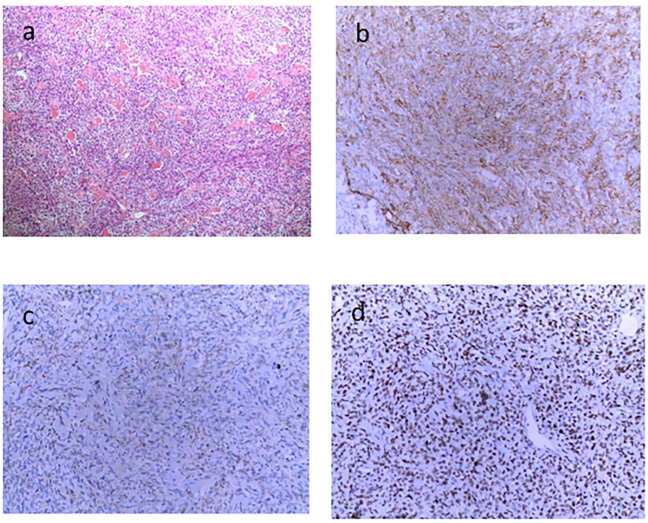


FIGURE 4
Microscopic examination of the pancreatic lesion (6.5 × 5 cm in size) demonstrated the presence of spindle-shaped cells, abundant tumor cells with atypia, approximately four to five mitotic figures/10 HPFs, and local infarction, in accordance with the World Health Organization grade III. **(A)** HE staining (×100). **(B)** CD34 immunohistochemical stain (×200). **(C)** BCL2 immunohistochemical stain (×200). **(D)** STAT-6 immunohistochemical stain (×200).

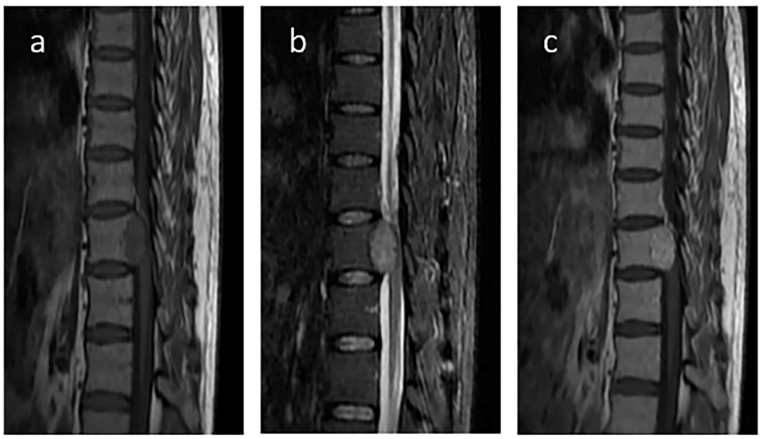


FIGURE 5
Contrast-enhanced magnetic resonance imaging (MRI) of the thoracic spine. Contrast-enhanced MRI of the thoracic spine confirmed thoracic spine metastasis and one metastatic nodule projected into the spinal canal and compressed the spinal cord. **(A)** T1WI; **(B)** T2WI; **(C)** T1WI postcontrast.

appears in up to 100% of cases and has not yet been detected in other tumors (31). RT-PCR detection can be used to identify the *NAB2-STAT6* fusion gene, but due to the diversity of fusion types, its sensitivity is much lower than that of STAT6. Therefore, STAT6 detection is more widely used in clinical settings (32–34).

The IGF system consists of two ligands, IGF-1 and IGF-2, as well as their two receptors. Normally, approximately 70%–80% of IGFs bind to insulin-like growth factor binding protein (IGFBP)-3 in serum, whereas residual IGFs bind to other IGFBPs, leaving less than 1% of IGFs free (28). This mechanism effectively protects IGFs from degradation and avoids hypoglycemia by limiting their binding to receptors (28, 35). Bertherat et al. studied the specific expression of the *IGF-2* gene in NICTH/DPS caused by pleural fibrosarcoma (36). They observed a loss of imprinting of parent alleles, which resulted in an excess expression of the *IGF-2* gene and an increase in incompletely processed IGF-2, termed big-IGF-2 or pro-IGF-2. Moreover, tumor cells did not seem to have enough enzymes to act on pro-IGF-2; thus, the excess pro-IGF-2 competes with IGF-1 and IGF-2 in binding to IGFBP to form a 40–50–kDa binary complex. In that case, the process results in an excess of free IGF-2 (37) and IGF-1 in the plasma (38, 39). The increased IGF-1 causes a negative feedback, leading to a decrease in the upstream GH secretion, followed by a decline in IGF-1 and IGFBP-3 levels. IGF-2, on the other hand, will not be reduced since its secretion is not regulated by feedback because it is automatic and paracrine (20). This leads to an increased ratio of serum IGF-2/IGF-1 and an inversely proportional relationship between IGFBP-3 and IGF-2. Meanwhile, the binary complex and free IGF-2 pass through capillary membranes relatively easily and bind to insulin receptors, subsequently causing

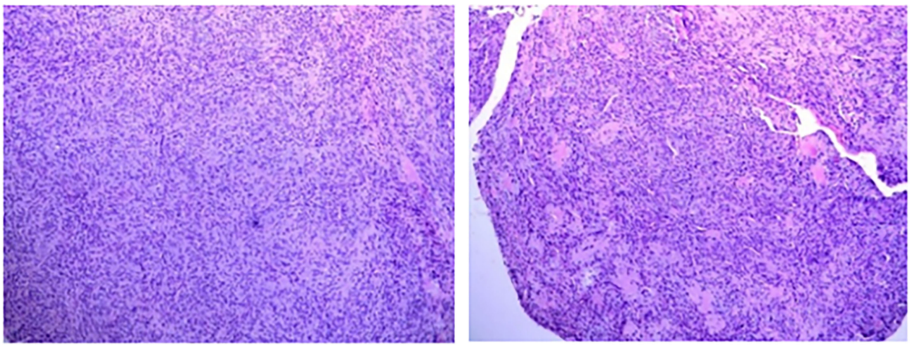


FIGURE 6
Microscopic examination of the thoracic spine metastatic tumor with HE staining (×100).

hypoglycemia (35). In DPS, as pro-IGF-2 tests have not been commercialized, the IGF-2:IGF-1 ratio is considered to be a surrogate marker for pro-IGF-2. DPS is diagnosed when the IGF-2:IGF-1 ratio is greater than 10 (40).

The best treatment for SFTs is complete resection of the tumor. Nevertheless, when curative resection is not available, metastasectomy, chemotherapy, and radiotherapy may be beneficial (18, 19). A retrospective work (n = 64) suggested that surgical resection of localized SFT can result in a 10-year overall survival (OS) of 58% when compared to that of metastatic SFT, which is 11% (21). Although there are various chemotherapies, no standard regimen has been recommended for metastatic SFTs. Several case reports and small sample studies have reported that pazopanib effectively controlled the progression of the tumor in metastatic SFTs, and the best median progression-free survival rate was 6.2 months (22). When it comes to metastatic liver SFTs, TACE may be a good alternative therapy. Velayati et al. summarized the safety and efficacy of the treatment of TACE (19). However, TACE may not always be effective. El-Khouli et al. reported the first use of cTACE: after three cycles of TACE treatments, no significant reduction in tumor size was observed (41). This is a condition similar to our patient. The reason for the low response of cTACE in metastatic liver SFTs needs further studies.

With regard to DPS, the therapies include the following: correct hypoglycemia immediately and treat the potential tumor or prevent recurrent episodes of hypoglycemia when the tumor cannot be controlled. There are several ways to correct hypoglycemia, but the best treatment is still surgery. Administrating quick-acting carbohydrates (such as glucose tablets, sugared fruit juices, or hard candy), intravenous glucose infusion, or injection of glucagon are good methods to correct hypoglycemia immediately (42). When the tumor secretes IGF, complete removal of the tumor can cure hypoglycemia (43). If surgery is not available, the methods mentioned above and glucocorticoid therapy can be considered alternatives. In spite of that, the efficacy is clear but not entirely curative. Several case reports have shown that TACE is a good choice for patients with liver SFT and DPS (25, 44). However, in these cases, there were no multiple metastases and some patients need multimodal treatment to control hypoglycemia after cTACE treatment (43). We speculated that cTACE treatment still has some limitations on both SFTs and DPS.

TILA-TACE is a powerful method to treat both SFTs and DPS. It can effectively induce tumor cell necrosis, which is more effective than that induced by cTACE. The mechanism of cTACE is by embolizing blood vessels and then creating a microenvironment with a low concentration of glucose, which affects energy metabolism and causes tumor cell death. However, in the condition of our patient, he had no improvement in blood glucose and tumor prognosis after four cycles of cTACE. The possible reasons were as follows: firstly, we know cTACE causes hypoxia and glucose deprivation, but hypoxia can simultaneously induce transcriptional activation of the *IGF-2* gene, leading to increased production of pro-IGF-2, which compromises the effect of tumor cell death (28); secondly, tumor cells can survive with the help of proton and lactate, which may lead to treatment failure (27). cTACE blocks tumor-feeding arteries; the amount of glucose in the embolized tumor is limited, and the low oxygen level speeds up glycolysis and glutaminolysis. As a result, lactate and proton accumulation create a chemical environment called lactic acidosis (high lactate concentration with acidic pH). When glucose is used up, lactate and protons accumulated together can rescue cancer cells from glucose deprivation-induced death. TILA-TACE, on the other hand, using bicarbonate to neutralize tumor bed, can convert intratumoral lactic acidosis to lactosis, which can effectively prevent tumor cells from using glucose and accelerate cell necrosis. This demonstrates a superior activity in the local control of large tumors (45). In our previous nonrandomized study, the 1-, 2-, and 3-year survival rates in patients with large hepatocarcinoma treated with cTACE were lower than those treated with TILA-TACE. The median survival of the former was 14 months, and the median survival time was 41 months for TILA-TACE ($p < 0.05$) (27). The hypothetical therapeutic mechanism of the novel treatment TILA-TACE is presented as a flowchart (Figure 7).

Considering the case of our patient, the primary lesion is likely to be intracranial SFT. The patient's overall survival was about 10 years, with about 40 months after the development of multiple metastatic SFTs. He had severe hypoglycemia caused by metastatic SFTs in the liver and pancreas. Laboratory examinations revealed that fasting hypoglycemia, GH, and IGF-1 levels were lower than the measurable values, along with a low fasting C-peptide level. Additionally, levels of IGF-2, pro-IGF-2, and the molar ratio of IGF-2 to IGF-1 were increased. After cTACE treatment, the symptoms and laboratory tests showed no improvement, and abdominal CT even indicated an enlarged liver tumor. However, after TILA-TACE treatment, most of the metastatic liver SFTs had completely undergone necrosis and the serum levels of pro-IGF-2 and IGF-2 had decreased. Thereafter, his hypoglycemia was significantly improved, and, after tumor partial resection, his blood glucose had improved to normal levels. No other extra meals were required. Overall, TILA-TACE played a vital role in the treatment of our patient: it cured hypoglycemia and provided our patient with surgical opportunities for SFT metastases.

Therefore, it is reasonable to believe that the main cause of DPS in our patient was the excess of IGF-2 and pro-IGF-2. TILA-TACE is not only an effective measure for the treatment of metastatic SFTs but is also applicable to patients with DPS. TALI-TACE can effectively reduce tumor size, control tumor progression, provide patients with surgery opportunities and prolong survival in metastatic SFTs. Meanwhile, based on the good effect of TALI-TACE on the rapidly growing giant liver

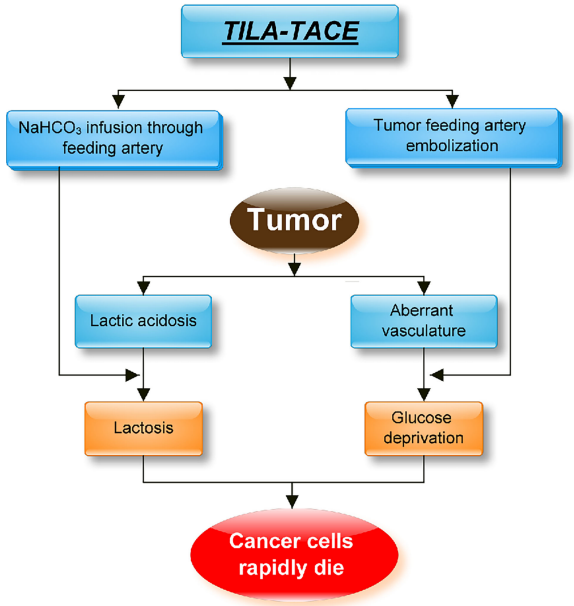


FIGURE 7
The hypothetical approach of TILA-TACE to treating large liver metastatic solitary fibrous tumors by targeting intratumoral lactic acidosis. cTACE embolizes tumor-feeding arteries that block glucose supply but also create a hypoxia condition and trap lactic acidosis. Intratumoral lactic acidosis rescues cancer cells from glucose deprivation. Hypoxia-enhanced angiogenesis could also significantly contribute to tumor survival. On the contrary, TILA-TACE is designed for neutralizing lactic acidosis by bicarbonate, which rapidly kills cancer cells before revascularization and thus significantly improves the therapeutic efficiency.

tumor, we also speculate that TALI-TACE is suitable for patients with primary SFTs in the liver. Overall, the effect of intratumoral lactic acidosis on tumor cells in a combination of hypoxia-enhanced revascularization significantly contributes to the cTACE therapeutic bottleneck (46). Whereas, destroying intratumoral lactic acidosis by TILA-TACE will be a potential protocol for hypervascular primary tumor in the liver and neuroendocrine tumor liver metastasis. Further prospective clinical trials are needed to verify the effectiveness of TILA-TACE.

Conclusion

In summary, DPS is a rare paraneoplastic syndrome associated with SFTs, characterized by NICTH. We report the first case of metastatic SFTs in the liver, pancreas, and thoracic spine with DPS, which was successfully treated with TILA-TACE. This led to a clinical cure of DPS in a 3-year follow-up and subsequently earned the opportunity for tumor resection. Therefore, we suggest that TILA-TACE be recommended not only for patients with SFTs and DPS but also for patients with hypervascular primary or metastatic liver tumors when curative surgery is not applicable.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine and was performed in accordance with the Declaration of Helsinki of 2013 for Human Research. Written informed consent for publication of the clinical details and images was obtained from the patient.

Author contributions

XS and MC conceptualized the study. KJ analyzed and interpreted all data and wrote the manuscript. SZ and LL collected and assembled data and wrote the manuscript. JW and YW analyzed the data. LL and WC interpreted study data and revised the manuscript. WG revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case Report: Composite pheochromocytoma with ganglioneuroma component: A report of three cases

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Composite pheochromocytoma (CP) is a very rare tumor originating from neural crest cells, predominantly composed of pheochromocytoma (PCC), a chromaffin cell tumor arising in adrenal medulla, and ganglioneuroma, a tumor derived from autonomic ganglion cells of the nervous system. Moreover, CP may be present in the hereditary syndromes of which pheochromocytoma is part. Literature offers scarce data on this subject, and particularly about its biological behavior, clinical evolution, and molecular profile. We report the phenotype and outcome of three cases of CP (PCC and ganglioneuroma components), followed up at the Endocrine Service of the Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, UFRJ, Rio de Janeiro, Brazil. Two nonsyndromic patients (cases 1 and 2) were negative to germline mutations in genes *VHL*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127*, and *MAX*, while the third case (case 3) had clinical diagnosis of neurofibromatosis syndrome. Cases 1, 2, and 3 were diagnosed at 29, 39, and 47 years old, respectively, and were followed up for 3, 17, and 9 years without no CP recurrence. All cases had apparent symptoms of catecholaminergic excess

secreted by PCC. Ganglioneuroma, the neurogenic component present in all three cases, had a percentage representation ranging from 5% to 15%. Tumors were unilateral and large, measuring 7.0 cm × 6.0 cm × 6.0 cm, 6.0 cm × 4.0 cm × 3.2 cm, and 7.5 cm × 6.0 cm × 4.5 cm, respectively. All cases underwent adrenalectomy with no recurrence, metastasis, or development of contralateral tumor during follow-up. Genetic testing has been scarcely offered to CP cases. However, a similar frequency of genetic background is found when compared with classic PCC, mainly by the overrepresentation of NF1 cases in the CP subset. By literature review, we identified a notorious increase in cases reported with CP in the last decade, especially in the last 3 years, indicating a recent improvement in the diagnosis of this rare disorder in clinical practice.

KEYWORDS

pheocromocytoma, ganglioneuroma, composite pheocromocytoma, molecular study, pathology, paraganglioma

Introduction

Composite pheochromocytoma (CP) is a very rare clinical condition with almost a hundred cases reported in the medical literature and constitutes less than 3% of all adrenal gland neoplasms and sympathoadrenal pheochromocytomas. CP is characterized by the coexistence of pheochromocytoma (PCC) or paraganglioma (PGL) with other neurogenic tumors, such as ganglioneuroma, ganglioneuroblastoma, neuroblastoma, schwannomas, malign peripheral nerve sheath tumors, such as sustentaculoma, rhabdomyosarcoma, and neuroendocrine carcinoma. Ganglioneuroma is the most common tumor among neurogenic tumors in the context of CP, accounting for 75% of the cases reported (1–8).

Of note, most patients with CP present clinical manifestations of catecholamine excess (three-quarters), with no distinctive clinical or radiological feature from usual PCC or PGL. Thus, the diagnosis of CP is based only on pathological findings (1, 9).

Of value, the diagnosis of CP should be warranted if at least 5% of one of the endocrine or neural components is documented, as it is not uncommon for some mature ganglion cells or sparsely dispersed Schwannian-like stroma to occur in typical PCC or PGL (8). The prompt and accurate diagnosis of neural and endocrine components may be important, mainly if one of them is associated with undifferentiated or poorly differentiated tumors compromising the prognosis of these patients as, for example, presence of neuroendocrine carcinoma or spindle cell sarcoma (8, 10).

Pheochromocytoma may be considered the neoplasia of higher genetic variability known as more than 35% of the patients harbor a germline mutation found in one of at least 16 possible genes, and it is amplified if somatic mutations were considered (11, 12). In contrast, only a few studies have supported genetic testing to CP and, thus, the molecular profile of these cases is not fully known, except by an apparent overrepresentation of neurofibromatosis (NF1) (4, 7).

In the present study, we report the phenotypic features and outcome of three cases documented with CP by coexistence of PCC and ganglioneuroma. Furthermore, investigation of germline allelic variants, directed by phenotype, was applied to two of them with sporadic and nonsyndromic presentation. In addition, an extensive review of the current literature of previously reported cases, an apparently peculiar genetic profile, and suspected underdiagnosis of this rare condition are presented and discussed.

Materials and methods

Clinicopathologic information

After giving informed consent, as established in the approval by the Ethics Committee of our Institution, three consecutive patients with CP, diagnosed between 2004 and 2018, were enrolled in this study. Patient outcomes on follow-up and clinicopathologic information, including serum catecholamine levels and disease associations, were obtained from the medical record.

Pathology and immunohistochemistry

The surgical specimens obtained from the adrenalectomy of the three enrolled patients were fixed in formalin and embedded

in paraffin (FFPE), and sections were stained with hematoxylin–eosin (HE). Tissue samples representative of the different macroscopic aspects identified in the large adrenal tumors of cases 1 and 3 were collected and included. In turn, the surgical specimen from case 2 was entirely included.

The pheochromocytoma of the adrenal gland scaled score (PASS score) was established for all the cases. Representative tissue blocks were selected for each case. Immunohistochemical (IHC) analysis was done with the following antibodies: chromogranin A (CgA), clone 5H7, obtained from Leica Biosystems (dilution: 1:1,000); neurofilament (NF), clone 2F11, from Dako Cytomation (1:3,000); S100, from Dako Cytomation (1:5,000); and Ki-67, clone SP6, from Spring Bioscience (1:300). Five areas of greater Ki-67 expression intensity (hot spots) were selected and counted to define label index.

Genetic analyses

DNA samples from all patients were obtained from peripheral blood using the modified Miller method at the Carlos Chagas Filho Biophysics Institute (UFRJ). Subsequently, the material was prepared at the Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP) for sequencing of the following genes: *VHL*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127*, and *MAX*. The polymerase chain reaction (PCR) was used to amplify the exons and exon–intron boundaries of the mentioned genes. The PCR reactions were carried out using genomic DNA (100–500 ng), 200 µM of each deoxynucleotide (dNTP), 10–30 pmol of each primer, 2.5 U of Taq DNA polymerase enzyme, and 5 µl of the buffer supplied by the manufacturer (Amersham Pharmacia, Uppsala, Sweden) and H_20 MiliQ to be completed for a final volume of 25 µl.

The amplification protocol consisted of denaturation at 94°C for 5 min, followed by 35 to 40 cycles (with hybridization temperature according to oligonucleotides) of 94°C for 40 s, annealing (55°C–60°C) for 40 s and 72°C for 50 s, followed by a final extension cycle of 72°C for 7 min. The amplified fragments were subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide (0.5 µg/ml) (Invitrogen™ Life Technologies, Carlsbad, CA, USA). PCR products were purified using the QIAquick PCR purification Kit (Qiagen Str. 1, 40724 Hilden, Germany) according to the manufacturer's recommendations. Sequencing reactions were performed with the ABI Prism BigDye Terminator Kit (Life Technologies, Carlsbad) and the fragments were run in an ABI prism 3,100 and 3,500 xL Genetic Analyser (Life Technologies, Carlsbad).

In addition to the coding region of *SDHB*, we have also used a primer pair (forward: AGCGCCAATTGTGGAAATAG; reverse: GCCTGAGGCAGATAGTAGGG) for specifically detecting the previously described *SDHB* 15678bp deletion (c.1-10413_73-3866del; g.17043962_17059585del) that removes the promoter region and exon 1 of this gene (4).

Case reports

Case 1

A 29-year-old woman presented with abdominal pain, asthenia, and diarrhea. She had no signs of arterial hypertension, despite extensive investigation. Blood tests revealed increased levels of free plasmatic epinephrine and norepinephrine (Table 1). Abdominal computed tomography showed an expansive and well-delimited lesion with heterogeneous enhancement by means of contrast, located in the topography of the right adrenal gland, measuring 7.0 cm × 7.4 cm × 6.6 cm, contiguous to the right hepatic lobe and the superior renal pole, without plans to cleave with the inferior vena cava. Morphology and dimensions of the left adrenal gland were normal (Table 1). With preoperative alpha-adrenergic blocker, patient was submitted to open right adrenalectomy without postoperative complications. A complete remission of

TABLE 1 Genetic, radiological, hormonal, and clinical profiles and outcomes of three women with composite PCC.

Cases/ sex	Age at diagnosis/ current age (years)	Follow-up (years)[a]/ recurrence	Clinical features	Hormonal hypersecretion	Adrenalectomy (L, left; R, right)	Radiologic features (L, left; R, right)	Molecular analysis[b]
1/F	29/31	3/No	Abdominal pain; asthenia; diarrhea; no hypertension	Free plasmatic epinephrine and norepinephrine	Open (R)	R, 7.0 × 7.4 × 6.6 cm L, normal	Negative
2/F	39/56	17/No	Hypertension, sweating, weight loss	Urinary epinephrine and norepinephrine	Laparoscopic (L)	L, 3.9 cm R, normal	Negative
3/F	47/55	8/No	Hypertension; tachycardia; sweating; low left back pain; type 2 diabetes; NF1	NA	Laparoscopic (L)	L, 5.6 × 5.2 × 6.0 cm R, normal	–[c]

PCC, pheochromocytoma; F, female; NF1, neurofibromatosis type 1; NA, not available. [a]No evidence of new PCC or even paraganglioma or any tumor during follow-up. [b]The following genes were studied in cases 1 and 2: VHL, SDHB, SDHC, SDHD, SDHAF2, TMEM127, and MAX. [c]Genetic analysis was not performed as the patient had an irrefutable clinical diagnosis of NF1.

symptoms and normal hormonal measurements were noticed after surgery, with no recurrence during 3 years of follow-up.

Case 2

A 39-year-old woman presented with severe paroxysmal systemic arterial hypertension, sweating, and progressive weight loss. Hormonal evaluation revealed high values of urinary epinephrine and norepinephrine (Table 1). Magnetic resonance image showed a lesion of 3.9 cm in the left adrenal gland with normal morphology and dimensions of the right adrenal (Table 1). She also received alpha-adrenergic blockade before laparoscopic adrenalectomy without any perioperative relevant intercurrences. A complete remission of symptoms was noticed after adrenalectomy, with no recurrence after 17 years of follow-up.

Case 3

A 47-year-old woman, with type 2 diabetes mellitus, systemic arterial hypertension, and a previous clinical diagnosis of neurofibromatosis type 1 (NF1), presented with persistent left low back pain, tachycardia, and sweating (Table 1). Computed tomography showed a nodular formation, with soft tissue density, heterogeneous, with intervening hypodense areas and tenuous parietal calcifications, with early heterogeneous contrast enhancement, measuring 5.6 cm × 5.2 cm × 6.0 cm, located in the left adrenal, suggesting pheochromocytoma. The morphology of the contralateral adrenal was normal (Table 1). A baseline hormonal profile was not performed, and the patient was referred to our service only after laparoscopic left adrenalectomy. Also, there was no previous clinical history of use of preoperative medication preparation (alpha blockade) or of early peri- and postoperative complications. Also, there was no recurrence of CP during 9 years of follow-up (Table 1).

Pathological features

A histopathological study of the three cases revealed a composite PCC whose neurogenic component was a ganglioneuroma in all of them (Figure 1). The percentage representation of ganglioneuroma component in the anatomic specimen was 15%, 10%, and 5% in cases 1, 2, and 3, respectively. This neurogenic component was basically present in the middle of cystic and hemorrhagic areas of the tumor in cases 1 and 3 or like a peripheral ridge in the tumor of case 2 (Figure 1). Cases 1 and 2 had Ki-67 of <3% and case 3 had >3%. PASS score was 7, 3, and 11 in cases 1, 2, and 3 (Table 2), respectively.

In all cases, by IHC, chromogranin A (CgA) highlighted the cytoplasm of PCC cells and aggregated or diffuse neuronal cells showed cytoplasmatic positivity of neurofilament (NF) (Figure 2). In turn, IHC to S100 protein had strong positivity in sustentacular cells of PCC as in Schwann cells from ganglioneuroma. Thus, the pathological diagnosis of CP in all three cases was defined by a combination of microscopic findings from sections stained by HE and IHC features.

Case 1

The right adrenal tumoral specimen measured 7.0 cm × 7.4 cm × 6.6 cm, weighed 120 g, and had a thick capsule and a smooth brown surface. Neoplasia was composed by large cells with focal nuclear pleomorphism and nucleoli with vesicular chromatin, <3 mitosis/10 high power fields (HPF), without atypical mitosis or necrosis or angioinvasion or extra capsular extension.

Case 2

The tumor size was 6.0 cm × 4.0 cm × 3.2 cm, weighed 50 g, and had fibroelastic consistence and a brownish color. Microscopy revealed a lobular arrangement neoplasia with pleomorphic cells of poorly delimited granular cytoplasm, clear nuclei, and predominantly eosinophilic nucleoli.

Case 3

The tumoral mass measured 7.5 cm × 6.0 cm × 4.5 cm, weighed 90 g, with a well-defined lobular arrangement composed of cells with mild or moderate atypia and a rare anaplasia focus, with angioinvasion focus and extracapsular extension area to adjacent adipose tissue.

Genetic analysis

Genetic analysis was negative for germline pathogenic variants in all genes studied (*VHL*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127*, and *MAX*) of the two nonsyndromic cases (cases 1 and 2) (Table 1). Benign/likely benign variants were identified in *TMEN127* gene of these cases: case 1, silent variant c.621G>A p.(Ala207Ala; dbSNP:rs3852673); and case 2, missense variant c.268G>A p.(Val90Met; dbSNP: rs121908823). Case 3 was exempted from genetic analysis due to the irrefutable clinical diagnosis of NF1 syndrome.

Discussion

In the present study, we reported the phenotype and outcome of three cases diagnosed with composite pheochromocytoma (CP)

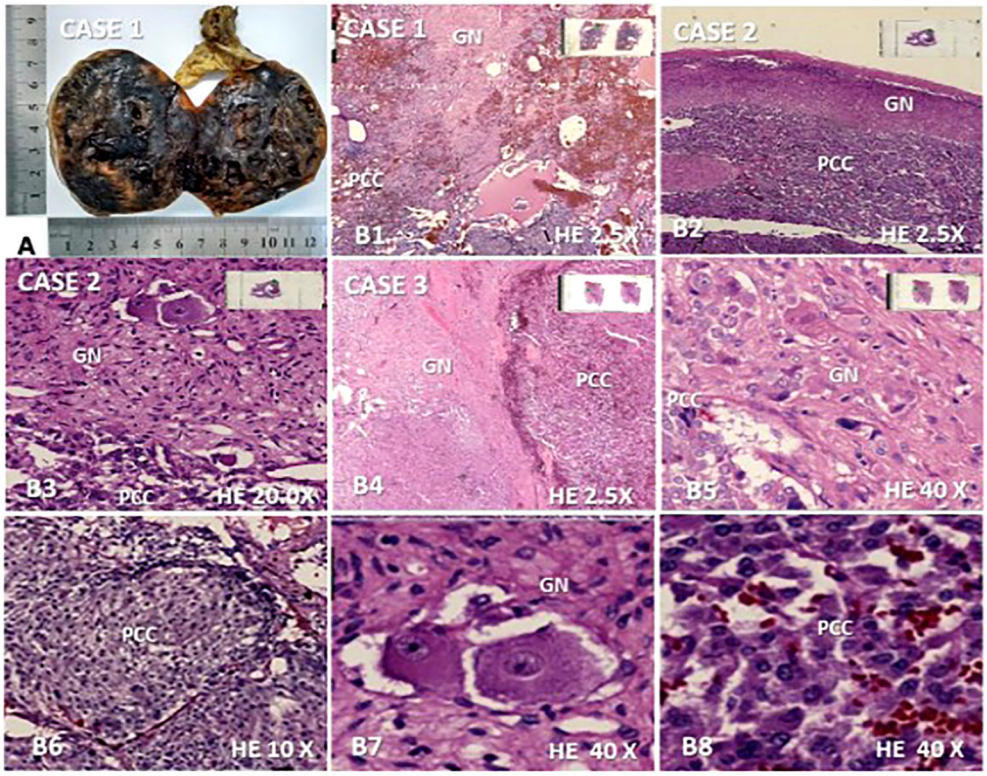

FIGURE 1
Macroscopic and microscopic features found in the three composite pheochromocytoma (CP) cases with ganglioneuroma as a neurogenic component. **(A)** Opened surgical specimen of case 1 measuring 7.0 ×6,0 × 6.0 cm; **(B1–B8)**, microscopy (hematoxylin-eosin) revealing the presence of both components of CP in cases 1, 2 and 3: **(B1)**. ganglioneuroma (GN) interspersed between pheochromocytoma (PCC) and cystic/hemorrhagic areas (2.5x); **(B2)**. ganglioneuroma presenting as a peripheral ridge adjacent to the chromatin tumor in case 2 (2.5x); **(B3)**. GN dispersed in most part of the figure with clear margin of frontier with PC component located in inferior region (20x); **(B4, B5)**. well-delimited neurogenic component of the CP of the case 3 is shown on the left side **(B4)** (2.5x) and in most part of the figure **(B5)** (40x) while PCC is seen, on the right side **(B4)** (2.5) and in the left inferior region **(B5)** (40x); **(B6)**. A nodular area of PC is represented (10x); **(B7)**. Ganglion cells dispersed in Schwan stroma; **(B8)**. Chromaffin cells network of PCC.

between 2004 and 2018. All of them had an association of pheochromocytoma (PCC) and ganglioneuroma.

Our cases represent the most frequently reported phenotype of CP, with ganglioneuroma being the main neurogenic component in 65%–71% of the cases and PCC being the dominant endocrine component (4, 7) in over 70% of the cases reported (6).

While PCCs/PGs are tumors that originate from the chromaffin cells, ganglioneuroma represents the mature spectrum of tumors from autonomic ganglion cells or their

TABLE 2 Pathological features of three cases with composite PCC.

Cases	Age at the adrenalectomy	Pathological features of composite PCC[a, b]			
		Weight (g) [(size (cm)]	PASS	Ki-67	Neurogenic component (%)
1	29	120 7.0 × 6.0 × 6.0	7/20	<3%	Ganglioneuroma (15%)[c]
2	39	50 6.0 × 4.0 × 3.2	3/20	<3%	Ganglioneuroma (10%)[d]
3	47	90 7.5 × 6.0 × 4.5	11/20	>3%	Ganglioneuroma (5%)[c]

PCC, pheochromocytoma. [a]All cases had positive immunohistochemistry (IHC) to chromogranin (CgA) at the cytoplasm of PCC cells and to neurofilament (NF) in the cytoplasm of neuronal cells (aggregated or diffuse presentation). [b]IHC of S100 protein was positive in sustentacular cells of PCC as well as in Schwann cells from ganglioneuroma. [c]It was present in the middle of cystic and hemorrhagic areas of the tumor. [d]It was present as a peripheral ridge in the tumor of case 2.

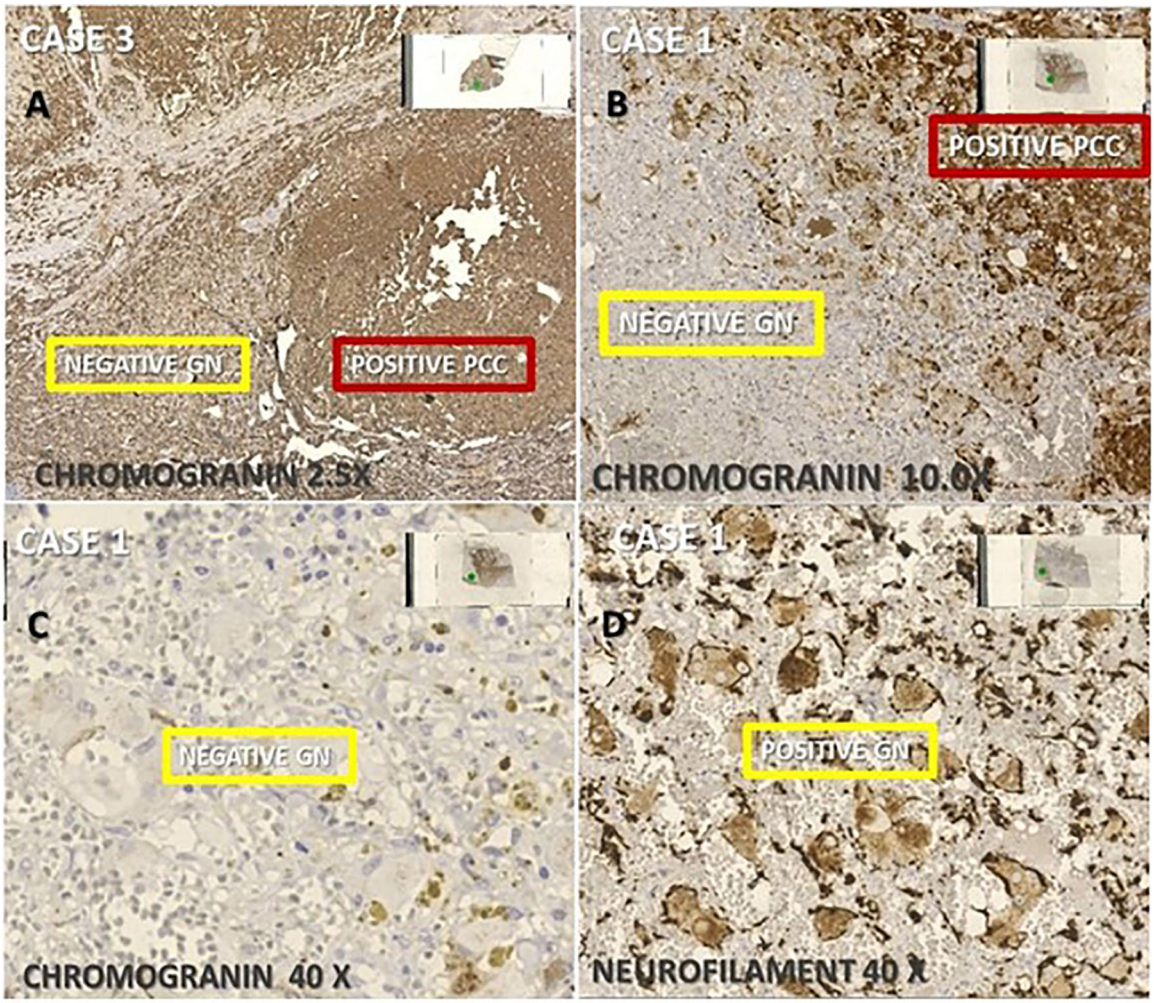

FIGURE 2
Immunohistochemistry (IHQ) of the three composite pheochromocytoma (CP) cases associated with ganglioneuroma, **(A)** ×2.5 and **(B)** ×10. IHQ was positive for chromogranin in pheochromocytoma (PCC) and negative ganglioneuroma (GN); **(C)** negative expression of chromogranin in GN; and **(D)** positive IHQ for neurofilament in GN.

precursors. Embryologically, both chromaffin and ganglion cells are derived from neural crest cells. Any disturbance in the migration or development of the neural crest may result in the development of composite tumors (2, 4). The malignant potential of the PCC component is extremely rare in the composite tumors associated with ganglioneuroma, occurring in only 3% of the cases (4). In fact, these tumors usually have a benign behavior with no recurrence or metastatic disease, as seen in our cases (6). In turn, most cases with malign disease are represented by neurogenic tumors arising of immature ganglion cells as ganglioneuroblastoma, neuroblastoma, malignant peripheral nerve sheath tumor, or neuroendocrine carcinoma (2, 4, 6).

Clinical presentation depends on whether the tumor is functional or nonfunctional, although the majority of cases present as functional at diagnosis (4, 13). Paroxysmal or sustained arterial hypertension appears at diagnosis with a frequency varying between 48% and 72%, and headaches, tachycardia, and sweating are present in 50% of CP cases (2, 13). In our report, two out of three patients presented arterial hypertension. However, a recent review documented arterial hypertension in one-quarter of CP cases (18/74) (7). Thus, one of our cases had PCC discovered incidentally, as seen in the case reported by Rao et al., which also had CP with elements of both PCC and ganglioneuroma (2).

The age at the time of diagnosis of our three cases was 29, 39, and 47 years old, respectively, which is consistent with the literature, where the majority of cases are diagnosed between the ages of 40 and 60 (4, 6, 7). The youngest and oldest cases diagnosed with CP were 4 and 86 years old, respectively (7). The

frequency of CP was mildly higher in women (57% vs. 43%) in the 90 CP cases reported (7). Our presented three cases were all women.

CPs are mostly unilateral, as seen in our patients who presented unilateral lesions measuring between 6.0 and 7.5 cm in greatest diameter. In fact, bilateral PCC occurred in only 5% of the 56 cases with CP. These three bilateral PCCs all had genetic syndrome, with two having NF1 and the third having MEN2A (7). Our NF1 case did not develop contralateral PCC during 8 years of follow-up.

Exceptionally, PGL may be the tumor arising from chromaffin cells in CP as recently documented in a case with *MAX* germline mutation and retroperitoneal PGL diagnosed at 20 years old followed by unilateral multifocal PCC at 28 years old (14). PGL in CP was reported in less than 20 cases so far (15).

Immunohistochemically, the components of CP resemble their counterparts in normal tissue or in pure tumors of the same type. Therefore, staining patterns help to identify neuroblastomata's foci and to distinguish immature neurons from neoplastic chromaffin cells of the similar size. Schwann cells and sustentacular cells stain for S100 protein, whereas axon-like processes stain for neurofilament proteins (NFPs). PCC is composed of polygonal to spindled cells arranged in an alveolar, trabecular, or solid pattern, often with an atypical Zellballen appearance (16). Also, PCC cells contain many secretory granules and stain strongly for chromogranin A and synaptophysin, whereas neurons contain relatively sparse granules and show weak or focal staining, often in a linear or punctuate pattern corresponding to cell processes (17). Immunohistochemical markers play a central role in PCC routine diagnosis. They are essential in the definition of the cell proliferation with the Ki-67 label index, when the differential diagnosis between PCC and adrenocortical tumors is an issue, and in detecting *SDHB* mutations.

CP is a very rare condition, with less than 100 cases reported so far (1, 7). Since 2000, we diagnosed 20 patients with PCC and three of them were CP, which is not a negligible percentage. Interestingly, reviewing the literature, a remarkable increase in the diagnosis of CP has been reported in the last decade, mainly in the last 3 years, suggesting that the diagnosis of the disorder may have been neglected. Thus, in a long period of 70 years—between 1940 and 2010—45 CP patients have been reported so far (4, 7). In the subsequent 10 years, the number of cases reported with CP doubled (96). Of value, in a short span of 24 months—between 2020 and 2022—44 new cases were recognized, indicating that CP has been better diagnosed more recently (Figure 3; Supplementary Table S1).

It is possible that the underdiagnosis suspected of this disorder may have been associated with the absence of microscopic representation of the neurogenic component during macroscopic selection of large tumors, lack of embedding of the entire adrenal gland in paraffin or few cuts for microscopic representation of CP, increasing the risk of absence of microscopic representation of cases with a low percentage of the neurogenic component. One of our cases had only 5% of this neurogenic component, reinforcing the potential risk of underdiagnosing CP.

Also, it is relevant to note that CP have been described in the literature with a heterogeneous aspect of cystic or hemorrhagic areas and that the secondary neurogenic component is usually scant and sparse. Thus, we would like to draw attention to the importance of a careful assessment of the macroscopic aspect of tumors and the representation of areas with different aspects to ensure a proper diagnosis of the tumor. This is especially important in those cases of CP associated with neoplasms of greater biological aggressiveness such as neuroblastoma, neuroendocrine carcinoma, and malignant nerve sheath tumor (6, 7, 18). Overall, in an extensive review of the literature,

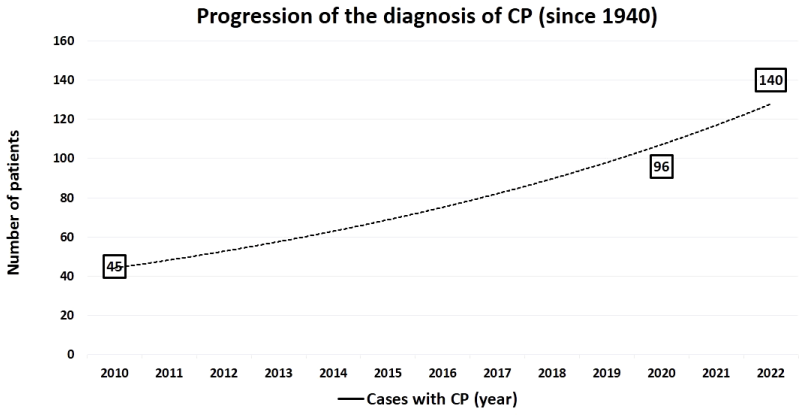

FIGURE 3
Increasing diagnosis of composite pheochromocytoma (CP) during last eight decades (1940-2022), including the three casesof the present study. CP, composite pheochromocytoma.

Dhanasekar et al. noticed that most patients with CP had ganglioneuroma (61,6%), followed by ganglioneuroblastoma (15.1%), neuroblastoma (10.11%), schwannoma (1.1%), and others (7.7%) (7).

Despite the identification of multiple genetic causes of PCC and PGL in the last decade, around two-thirds of these tumors remain without molecular diagnosis, suggesting that other susceptibility genes could be implicated (7, 19, 20). Using a wide-genomic approach, PCC/PGL was clustered into two major groups depending on their global transcription profiles. Cluster 1 includes *VHL*, *SDHx*, *FH*-mutated tumors, and a part of the sporadic PCC/PGL. Cluster 1 was composed of tumors without clear mutations or sporadic tumors; they showed signatures of pseudohypoxia, angiogenesis, and decreased oxidoreductase response. This profile links these tumors with hypoxia-inducible factor (HIF) role, which is supported by the overexpression of HIF1α and HIF2α found in this cluster (14, 19–21). A second group (cluster 2) of PCC/PGL is related to mutations in *RET*, *NF1*, *KIF1β*, and *TMEM127* genes and to undefined tumors enriched for kinase receptor signaling pathways, translation initiation, protein synthesis, and genes involved in neural/neuroendocrine identity (20, 21).

It is recognized that CP can be associated with genetic disorders such as NF1, von Hippel–Lindau disease, and multiple endocrine neoplasia (16, 17, 21–25). In a recent systematic review, genetic syndromes were associated with CP in 28% of the patients (26/94), being: NF1 (19%), MEN2A, (4%), Von Hippel–Lindau (VHL) (2%), and watery-diarrhea hypokalemia–achlorhydria (WHDA) syndrome (2%). This prevalence was similar to that described in PCC alone (7). However, the prevalence of NF1 seems to be overrepresented in CP when compared with NF1 in large series of PCC/PGL (19% vs. 3%) (7, 25). One of our cases had NF1, reinforcing that CP may be more prevalent in this genetic syndrome.

Thus, we recommend extra attention during macroscopic analysis by the pathologist to provide microscopic representation of heterogeneous regions of the surgical specimen in PCC or PGL and, when it is possible, the full inclusion of the tumor in paraffin, especially in NF1 cases. It is possible that with this strategy, more cases with CP will be diagnosed. With this caution, immature forms of neurogenic component could eventually be revealed or excluded. This rationale is reinforced by extremely variable percentual representation of both components of CP ranging from 10% to 80% (26), suggesting that the diagnosis of some cases of CP are missed.

Reviewing the literature, only a few studies have offered genetic testing to cases with CP. (Supplementary Table S2). We supported genetic investigation of the germline for the main PCC-related genes. The genetic analysis of NF1 in case 3 was not performed as this patient had an irrefutable clinical diagnosis of this inherited disorder. We are not able to identify any pathogenic variants in *VHL*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127*, or *MAX* genes in our two nonsyndromic CP cases. The *RET* analysis was waived in these two cases based on phenotypic features of them. In fact, MEN2A or MEN2B phenotype was absent in these cases. Also, PCC is rarely the first clinical manifestation of MEN2 and, when this occurs, is most frequently associated with *RET* mutations in the codon 634, whose patients invariably have medullary thyroid carcinoma at the ages at which they were diagnosed with PCC. Furthermore, during outcome, MTC was not diagnosed, and familial history remained negative for this syndrome. Finally, the probability to presence of a *RET* mutation in a large series of PCC is 5%. Considering the phenotype and outcome of our two nonsyndromic cases, the probability of *RET* mutation would be extremely low and, if it was present, they would be defined as anecdotical MEN2 cases (25, 27, 28).

One of the limitations of our study is that germline mutations in other genes more rarely associated with PCC/PGL could be the underlying genetic cause of our cases. Thus, the *SDHA* gene, representing 1% of all PCC cases and others very rarely associated with PCC, could not be excluded (25). As of now, only one CP case has been investigated by a genetic panel based on next-generation sequencing (NGS), and it resulted in the description of the first CP case with germline MAX mutation (14).

Of value, somatic mutation analysis was not a focus of the present paper. From our knowledge, only two CP cases had tumors studied by an extensive NGS panel (26). Thus, genes causing somatic mutations, such as *ATRX* and others, were not investigated in our cases. Of value, Chen et al. (2021) revealed that protein expression of *ATRX* and *SDHB* was normal in 18 CP cases (26). Importantly, these authors discovered that 20.0% (3/15) of the tumors studied by CP (PCC and ganglioneuroma) had *BRAF* mutations (K601E and K601N) and 46.7% (7/15) had *HRAS* mutations (Q61R, Q61L, G13R) based on genetic findings from the two cases studied by the NGS panel. These mutation frequencies were both significantly higher than those reported in ordinary PCC/PGL, suggesting that the underlying molecular mechanism of CP/PG are different from those reported in PCC/PGL alone (26). Thus, further studies focusing on the genetic causes of CP should be warranted based on the apparently higher frequency of NF1, differential expression of *ATRX*, and higher frequencies of *BRAF* and *HRAS* somatic mutation.

Very recently, exome and transcriptome analyses were conducted on a composite tumor of a 5-year old boy having as components PCC and neuroblastoma. Interestingly, most mutations (80%) were shared by all samples in both components, indicating that NB and PCC evolved from the same clone. Also, the presence of mutation and focal amplification of the *FGFR1* oncogene in both components suggests that this gene may be a primary driver of this tumor (29).

Prognostic factors of PCC include Ki-67 label index, histological pattern, cellularity, coagulative necrosis, vascular/capsular invasion, and type of catecholamine production. Ki-67 index greater than 3% indicates malignant behavior (1). Only the case presenting a phenotype compatible with NF1 presented a Ki-67 higher than 3%. However, during a long follow-up time, the patient remained asymptomatic with no evidence of recurrent PCC. Our patients with PCC and ganglioneuroma without immature components presented a good prognosis during the outcome, as previously reported (4). The presence of distant metastasis is the only criterion for malignancy in CP, which is usually derived from immature component (2, 3, 23). Considering that recurrence may occur in a few cases, long-term follow-up is required in CP (3).

Little is known about the biological potential, evolution, and genetic molecular profile of CP, although they may be associated with reported genetic syndromes, similarly to classic PCC. Indolent behavior has been described, as seen in the follow-up of our patients so far. However, there have been descriptions of metastasis associated with CP with ganglioneuroblastoma (16, 22, 23). Hence, the importance of a perfect pathological diagnosis of this association should be provided.

CP is mainly represented by the association of PCC and ganglioneuroma. Overall, patients have a favorable clinical course. However, there are cases with a malignant neurogenic component. Considering the percentage variability in the presence of both components (5%–80%), careful anatomopathologic examination is essential to avoid underdiagnosis of this rare condition. Notably, by reviewing the literature, there has been an important increase in the diagnosis of CP in the last decade, especially in the last 3 years, indicating a recent improvement in the diagnosis of this disorder in clinical practice. The prevalence of germline mutation in CP seems to be similar to that of PCC. However, genetic testing has been scarcely documented in these cases. Despite this, the genetic background of CP seems to be different from that described in regular PCC by the overrepresentation of the NF1 syndrome. More extensive genetic analysis of CP, including investigation of the germline and somatic tissues, is warranted to improve knowledge of this genetic background.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving human participants were reviewed and approved by Comite de Etica e Pesquisa da Faculdade de Medicina e do Hospital Universitario Clementino Fraga Filho - UFRJ. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PA and MC: main authors, both contributed equally to this manuscript. APV: pathologist responsible for the study of tumors, JN and JW: data collection and figure creation; SHCN surgeon e patient`s doctor, EN, FC, RF, DC: reviewers; VL: sample collection and DNA extraction; DL: reviewer and contributor for the presentation of the manuscript format; PS and JL responsible for the molecular study; AHV: coordinator, patient's doctor and responsible for submitting the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.903085/full#supplementary-material

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Risk factors, survival analysis, and nomograms for distant metastasis in patients with primary pulmonary large cell neuroendocrine carcinoma: A population-based study

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Introduction: Pulmonary large cell neuroendocrine carcinoma (LCNEC) is a rapidly progressive and easily metastatic high-grade lung cancer, with a poor prognosis when distant metastasis (DM) occurs. The aim of our study was to explore risk factors associated with DM in LCNEC patients and to perform survival analysis and to develop a novel nomogram-based predictive model for screening risk populations in clinical practice.

Methods: The study cohort was derived from the Surveillance, Epidemiology, and End Results database, from which we selected patients with LCNEC between 2004 to 2015 and formed a diagnostic cohort (n = 959) and a prognostic cohort (n = 272). The risk and prognostic factors of DM were screened by univariate and multivariate analyses using logistic and Cox regressions, respectively. Then, we established diagnostic and prognostic nomograms using the data in the training group and validated the accuracy of the nomograms in the validation group. The diagnostic nomogram was evaluated using receiver operating characteristic curves, decision curve analysis curves, and the GiViTI calibration belt. The prognostic nomogram was evaluated using receiver operating characteristic curves, the concordance index, the calibration curve, and decision curve analysis curves. In addition, high- and low-risk groups were classified according to the prognostic monogram formula, and Kaplan–Meier survival analysis was performed.

Results: In the diagnostic cohort, LCNEC close to bronchus, with higher tumor size, and with higher N stage indicated higher likelihood of DM. In the prognostic cohort (patients with LCNEC and DM), men with higher N stage, no surgery, and no chemotherapy had poorer overall survival. Patients in the high-risk group had significantly lower median overall survival than the low-risk group.

Conclusion: Two novel established nomograms performed well in predicting DM in patients with LCNEC and in evaluating their prognosis. These nomograms could be used in clinical practice for screening of risk populations and treatment planning.

KEYWORDS

Large cell neuroendocrine carcinoma (LCNEC), the Surveillance, Epidemiology, and End Results (SEER) database, nomogram, distant metastasis (DM), predictive model

Introduction

In 1991, the first report of pulmonary large cell neuro endocrine carcinoma (LCNEC) occurred. (1) In 2015, LCNEC was removed from the pathological classification of the large cell carcinomas and placed under pulmonary neuroendocrine neoplasms (NENs), which was revised by the World Health Organization and carried over to the latest 2021 edition. (2, 3) LCNEC is an uncommon pathologic type that accounts for 3% of all lung malignancies. (4) Recent reports indicated that its incidence increased year by year, from 0.01/100,000 people in 1990 to 1.8/100,000 people in 2010, with its annual mortality doubling between 2004 and 2015. The survival time and rates for patients with stage I–III LCNEC were close to those of patients with non-small cell lung cancer (NSCLC), whereas those of patients with stage IV were more like those of patients with small cell lung cancer (SCLC). (4, 5) In NENs, LCNEC is similar to SCLC and is a high-grade, rapidly progressing, easily metastatic malignancy. (6) The incidence of brain metastases in patients with LCNEC was significantly higher than in patients with SCLC or NSCLC. (4, 7) Sex, age, primary tumor site, TNM stage, surgery status, and chemotherapy have been shown to be independent risk factors for the prognosis of LCNEC in previous studies. (8–12) However, there is still controversy around the clinical management and treatment of LCNEC, such as using radiotherapy, and there are no standardized treatment approaches, especially for patients with LCNEC and distant metastasis (DM). As a result, a novel clinical predictive model is needed to assess the risk variables for incidence and prognosis of LCNEC with DM so that early intervention may be provided to this high-risk population.

Nomograms have been widely utilized in the prognostic analysis of cancer because of its capacity to graphically and intuitively show risk factors related to prognosis. (13, 14) Moreover, it has been used to assess metastasis in patients with osteosarcoma, with good results. (15) Therefore, we used the Surveillance, Epidemiology, and End Results (SEER) database to access public data and to evaluate the risk variables related to DM in patients with *de novo* (primary) LCNEC and to conduct further prognostic analysis. We present here two nomograms that can be used.

Materials and methods

Study population

We obtained data from SEER*Stat software v8.3.9.2, released on 20 August 2021. (16) Data were extracted from the sub-database "Incidence–SEER Research Plus Data, 18 Registries, Nov 2020 Sub (2000–2018)". Due to the limitations of the SEER database, the years of diagnosis were limited to 2004–2015 to ensure consistency in TNM staging. Then, according to the International Classification of Diseases for Oncology, Third Edition (ICD-O-3)/World Health Organization 2008, "Lung and Bronchus" was selected as the primary tumor site. Based on histologic type (ICD-O-3: 8013/3, 3762) patients with "LCNEC" were selected, and their original data were downloaded. Further data filtering was then performed in R software (version 4.1.2). (17) The exclusion criteria included (1): multiple primary tumors (2), age < 18 years, and (3) pathology grade I or II (low-grade), as the LCNEC is a high-grade neuroendocrine carcinoma. In addition, relevant clinicopathological characteristic, including age, sex, race, primary tumor site, laterality, pathology grade, TNM stage, and tumor size were required to be available. We enrolled 959 patients in the diagnostic cohort (Supplementary Figure 1A) and further excluded (1): patients with no DM and (2) survival time < 1 month, and (3) patients for whom data on surgery, chemotherapy, or radiotherapy status were not included. Then, 272 patients were enrolled in the prognostic cohort (Supplementary Figure 1B).

The study population was randomly split into training (70%) and validation groups (30%) in the diagnostic cohort, with a 7:3 ratio. The training and validation groups of the prognostic cohort were derived from the diagnostic cohort without regrouping. In each cohort, to investigate the factors associated with the incidence and the prognosis of DM in LCNEC, we

created two nomogram models and performed a survival analysis. Both models were constructed using the training groups and validated using the validation groups.

Variables collected

As part of the diagnostic cohort, the following variables were assessed: sex, age, race, laterality, T stage, N stage, primary site, pathological grade, and tumor size. In the prognostic cohort, variables included those in the diagnostic cohort as well as surgery, chemotherapy, and radiotherapy status. Further subgroup analysis was performed in the diagnostic and prognostic cohorts to prepare for nomogram establishment. Meanwhile, survival analysis was conducted in the prognostic cohort, with overall survival (OS) as the primary endpoint; OS was defined as the period from the initial diagnosis of LCNEC and death of any cause.

Statistical analysis

The study cohort was randomly grouped to form a training and a validation group. All variables were reclassified as categorical variables, and the clinicopathological characteristics of LCNEC patients were compared using the Chi-squared test in the training and validation groups, some using Fisher's exact test.

In the diagnostic cohort, we utilized logistic regression analysis to analyze risk variables of DM in patients with LCNEC patients. First, the univariate analysis was performed, with a two-sided $P < 0.05$ regarded as statistically significant, to identify risk factors. Then, significant variables were incorporated into the multivariable risk model, and odds ratios (ORs) and 95% confidence intervals (CIs) were computed. The independent risk factors selected by the model were then incorporated into a nomogram for visualization and clinical predictive analysis. Finally, we compared the novel nomogram with each individual risk variable using the receiver operating characteristic (ROC) curves of the training and validation groups and computed the area under the curve (AUC) to assess the validity of the novel nomogram. Decision curve analysis (DCA) and GiViTI calibration belt were used to assess the reliability of the nomogram.

In the prognostic cohort, the risk variables for OS in patients with LCNEC with DM were assessed using Cox proportional hazards regression analysis. The variables with statistical significance (2-sided $P < 0.05$) from the univariate analysis were applied to the multivariable analysis to screen individual risk factors related to prognosis. Hazard ratios (HRs) and 95% CIs were also calculated at the same time. According to the results of the univariate and multivariable analyses, a prognostic nomogram was established. The validity of the nomogram was assessed using the concordance index (C-index), as well as time-dependent ROC curves at 1, 2, and 3 years, based on the nomogram and individual prognostic risk factors. The reliability of the nomogram was evaluated using DCA curves and the calibration curves at 1, 2, and 3 years. All validations were carried out in the training group and the validation group. In addition, the nomogram algorithm was used to determine the individual risk score of risk variables. Based on the median risk score, the prognostic cohort was separated into high- and low-risk groups to prepare for the survival analysis. The Kaplan–Meier method was applied to assess the OS of the two risk groups, and the log-rank test was used to obtain P-values in the training and validation groups.

R software and associated packages, including "table1", "regplot", "pROC", "ROCR", "givitiR", "rms", "ggDCA", "survival", "survminer", and "survivalROC" were used for the aforementioned statistical analyses.

Results

Baseline characteristics of the diagnostic and prognostic cohorts

Our study included two major study cohorts, the diagnostic cohort of patients with LCNEC and the prognostic cohort of patients with LCNEC with DM. Baseline characteristics are presented in Tables 1 and 2. Among the 959 patients with LCNEC patients, patients were most commonly elderly and male. The most common primary tumor site was in the lung, and the most common tumor size was ≤3 cm. Among the tumor stages, T2 and N0 were the most common, and 308 patients had DM (M1). In the diagnostic cohort (Table 1), all patients with LCNEC were randomized into a training and a validation group. The Chi-squared test (some using Fisher's exact test) revealed no significant differences in any of the covariates between the two groups, indicating that the grouping was completely random. The training and validation groups had mean ages of 64.93 years (range, 18–92 years; interquartile range, 58–72 years) and 65.62 years (range, 45–90 years; interquartile range, 59–73 years), respectively. In the prognostic cohort (Table 2), the grouping was entirely consistent with the diagnostic cohort. There were 272 patients with LCNEC with DM, most of them were elderly men as before. The most common tumor stages were T4 and N2. The primary tumor site was still common in the lung. For treatment, most patients received chemotherapy and radiotherapy, but few underwent surgery.

TABLE 1 Baseline characteristics of pulmonary large cell neuroendocrine carcinoma (LCNEC) patients (in the diagnostic cohort).

	Training (N=671)	Validation (N=288)	Overall (N=959)	χ2	P
Sex				2.523	0.112
Female	321 (47.8%)	121 (42.0%)	442 (46.1%)		
Male	350 (52.2%)	167 (58.0%)	517 (53.9%)		
Age, years				0.498	0.919
≥18 and <60	194 (28.9%)	83 (28.8%)	277 (28.9%)		
≥60 and <70	247 (36.8%)	101 (35.1%)	348 (36.3%)		
≥70 and <80	181 (27.0%)	80 (27.8%)	261 (27.2%)		
≥80	49 (7.3%)	24 (8.3%)	73 (7.6%)		
Race				3.523	0.172
Black	85 (12.7%)	26 (9.0%)	111 (11.6%)		
Other	26 (3.9%)	8 (2.8%)	34 (3.5%)		
White	560 (83.5%)	254 (88.2%)	814 (84.9%)		
Laterality					0.374
Left	300 (44.7%)	126 (43.8%)	426 (44.4%)		
Right	371 (55.3%)	161 (55.9%)	532 (55.5%)		
Bilateral	0 (0%)	1 (0.3%)	1 (0.1%)		
Primary site					0.770
Lung	640 (95.4%)	272 (94.4%)	912 (95.1%)		
Bronchus	23 (3.4%)	12 (4.2%)	35 (3.6%)		
Overlapping lesion of lung	8 (1.2%)	4 (1.4%)	12 (1.3%)		
Grade				0.677	0.411
III	506 (75.4%)	225 (78.1%)	731 (76.2%)		
IV	165 (24.6%)	63 (21.9%)	228 (23.8%)		
T				1.041	0.791
T1	195 (29.1%)	80 (27.8%)	275 (28.7%)		
T2	275 (41.0%)	114 (39.6%)	389 (40.6%)		
T3	41 (6.1%)	22 (7.6%)	63 (6.6%)		
T4	160 (23.8%)	72 (25.0%)	232 (24.2%)		
N				0.996	0.802
N0	347 (51.7%)	158 (54.9%)	505 (52.7%)		
N1	87 (13.0%)	36 (12.5%)	123 (12.8%)		
N2	184 (27.4%)	71 (24.7%)	255 (26.6%)		
N3	53 (7.9%)	23 (8.0%)	76 (7.9%)		
M				1.114	0.291
M0	448 (66.8%)	203 (70.5%)	651 (67.9%)		
M1	223 (33.2%)	85 (29.5%)	308 (32.1%)		
Tumor size, cm				1.190	0.755
≤3	285 (42.5%)	122 (42.4%)	407 (42.4%)		
>3 and ≤5	176 (26.2%)	84 (29.2%)	260 (27.1%)		
>5 and ≤7	107 (15.9%)	41 (14.2%)	148 (15.4%)		
>7	103 (15.4%)	41 (14.2%)	144 (15.0%)		

Diagnostic predictive model of DM in patients with LCNEC

In the diagnostic cohort, the results of logistic regression analysis are shown in Table 3. First, a univariate analysis found five variables that may be associated with DM in LCNEC, including tumor size, primary tumor site, T stage, N stage, and sex. However, we excluded the T stage as it could be contradictory to clinical practice. These variables were then further incorporated into a multivariable analysis, which ultimately revealed three independent risk factors associated with DM, namely, a primary site of LCNEC closer to bronchus, and patients were more likely to have DM with larger tumor size and higher N stage. Additionally, to access

TABLE 2 Baseline characteristics of pulmonary large cell neuroendocrine carcinoma (LCNEC) patients with distant metastasis (in the prognostic cohort).

	Training (N=196)	Validation (N=76)	Overall (N=272)	χ2	P
Sex				2.008	0.157
Female	90 (45.9%)	27 (35.5%)	117 (43.0%)		
Male	106 (54.1%)	49 (64.5%)	155 (57.0%)		
Age, years				2.116	0.549
≥18 and <60	60 (30.6%)	22 (28.9%)	82 (30.1%)		
≥60 and <70	72 (36.7%)	30 (39.5%)	102 (37.5%)		
≥70 and <80	47 (24.0%)	21 (27.6%)	68 (25.0%)		
≥80	17 (8.7%)	3 (3.9%)	20 (7.4%)		
Race					0.584
Black	25 (12.8%)	12 (15.8%)	37 (13.6%)		
Other	7 (3.6%)	1 (1.3%)	8 (2.9%)		
White	164 (83.7%)	63 (82.9%)	227 (83.5%)		
Laterality					0.043
Left	79 (40.3%)	39 (51.3%)	118 (43.4%)		
Right	117 (59.7%)	36 (47.4%)	153 (56.3%)		
Bilateral	0 (0%)	1 (1.3%)	1 (0.4%)		
Primary site					0.804
Lung	175 (89.3%)	67 (88.2%)	242 (89.0%)		
Bronchus	18 (9.2%)	7 (9.2%)	25 (9.2%)		
Overlapping lesion of lung	3 (1.5%)	2 (2.6%)	5 (1.8%)		
Grade				0.194	0.660
III	148 (75.5%)	60 (78.9%)	208 (76.5%)		
IV	48 (24.5%)	16 (21.1%)	64 (23.5%)		
T					0.728
T1	25 (12.8%)	10 (13.2%)	35 (12.9%)		
T2	83 (42.3%)	27 (35.5%)	110 (40.4%)		
T3	9 (4.6%)	3 (3.9%)	12 (4.4%)		
T4	79 (40.3%)	36 (47.4%)	115 (42.3%)		
N				0.537	0.911
N0	53 (27.0%)	23 (30.3%)	76 (27.9%)		
N1	25 (12.8%)	10 (13.2%)	35 (12.9%)		
N2	84 (42.9%)	29 (38.2%)	113 (41.5%)		
N3	34 (17.3%)	14 (18.4%)	48 (17.6%)		
Tumor size, cm				0.236	0.972
≤3	51 (26.0%)	18 (23.7%)	69 (25.4%)		
>3 and ≤5	55 (28.1%)	23 (30.3%)	78 (28.7%)		
>5 and ≤7	40 (20.4%)	15 (19.7%)	55 (20.2%)		
>7	50 (25.5%)	20 (26.3%)	70 (25.7%)		
Surgery				0.036	0.849
No	164 (83.7%)	65 (85.5%)	229 (84.2%)		
Yes	32 (16.3%)	11 (14.5%)	43 (15.8%)		
Chemotherapy				0.091	0.763
No	59 (30.1%)	25 (32.9%)	84 (30.9%)		
Yes	137 (69.9%)	51 (67.1%)	188 (69.1%)		
Radiotherapy				0.030	0.863
No	84 (42.9%)	31 (40.8%)	115 (42.3%)		
Yes	112 (57.1%)	45 (59.2%)	157 (57.7%)		

TABLE 3 Analyses of distant metastasis in LCNEC patients using univariate and multivariate logistic regression.

	Univariate analysis			Multivariate analysis		
	OR	95%CI	P	OR	95%CI	P
Sex						
Female	Reference			Reference		
Male	1.336	1.062-1.683	0.038	1.102	0.850 -1.430	0.537
Age, years						
≥18 and <60	Reference					
≥60 and <70	0.897	0.677-1.189	0.526			
≥70 and <80	0.817	0.602-1.107	0.274			
≥80	0.938	0.588-1.477	0.820			
Race						
Black	Reference					
Other	0.740	0.359-1.465	0.478			
White	0.824	0.584-1.172	0.359			
Laterality						
Left	Reference					
Right	1.071	0.851-1.348	0.624			
Bilateral	NA	NA	0.967			
Primary site						
Lung	Reference			Reference		
Bronchus	7.777	4.100 -15.948	<0.001	3.192	1.582 -6.914	0.009
Overlapping lesion of lung	1.646	0.597 -4.312	0.398	0.925	0.303 -2.714	0.906
Grade						
III	Reference					
IV	0.918	0.699-1.199	0.600			
T						
T1	Reference					
T2	2.653	1.910-3.729	<0.001			
T3	1.679	0.930-2.938	0.137			
T4	7.893	5.561-11.345	<0.001			
N						
N0	Reference			Reference		
N1	2.436	1.677-3.519	<0.001	2.206	1.497 -3.231	<0.001
N2	4.759	3.590-6.332	<0.001	3.864	2.882 -5.194	<0.001
N3	12.760	8.102-20.575	<0.001	8.754	5.452 -14.350	<0.001
Tumor size, cm						
≤3	Reference			Reference		
>3 and ≤5	2.118	1.569-2.864	<0.001	1.759	1.274-2.429	0.004
>5 and ≤7	3.265	2.317-4.607	<0.001	2.313	1.592-3.358	<0.001
>7	5.510	3.908-7.811	<0.001	3.588	2.456-5.258	<0.001

NA, Not applicable.

the risk of DM, three independent risk variables were combined into a novel diagnostic predictive model, and a nomogram was generated in the training group (Figure 1A). Then, the ROC curves were drawn, with AUCs of 0.761 and 0.773 for the training and validation groups, respectively (Figures 1B, E). DCA both in the training and validation groups (Figures 1C, F) demonstrated the reliability of the nomogram. Moreover, we plotted the GiViTI calibration belts, which showed that the 95% CI did not cross the diagonal bisector at 45 degrees, and the P-values for the training and validation groups were 0.101 and 0.065, respectively (Figures 1D, G), indicating that the nomogram was reliable for predicting DM. (18) Meanwhile, for each individual risk factor, ROC curves were created, and the diagnostic nomogram outperformed any single factor in the training and validation groups (Figures 2A, B).

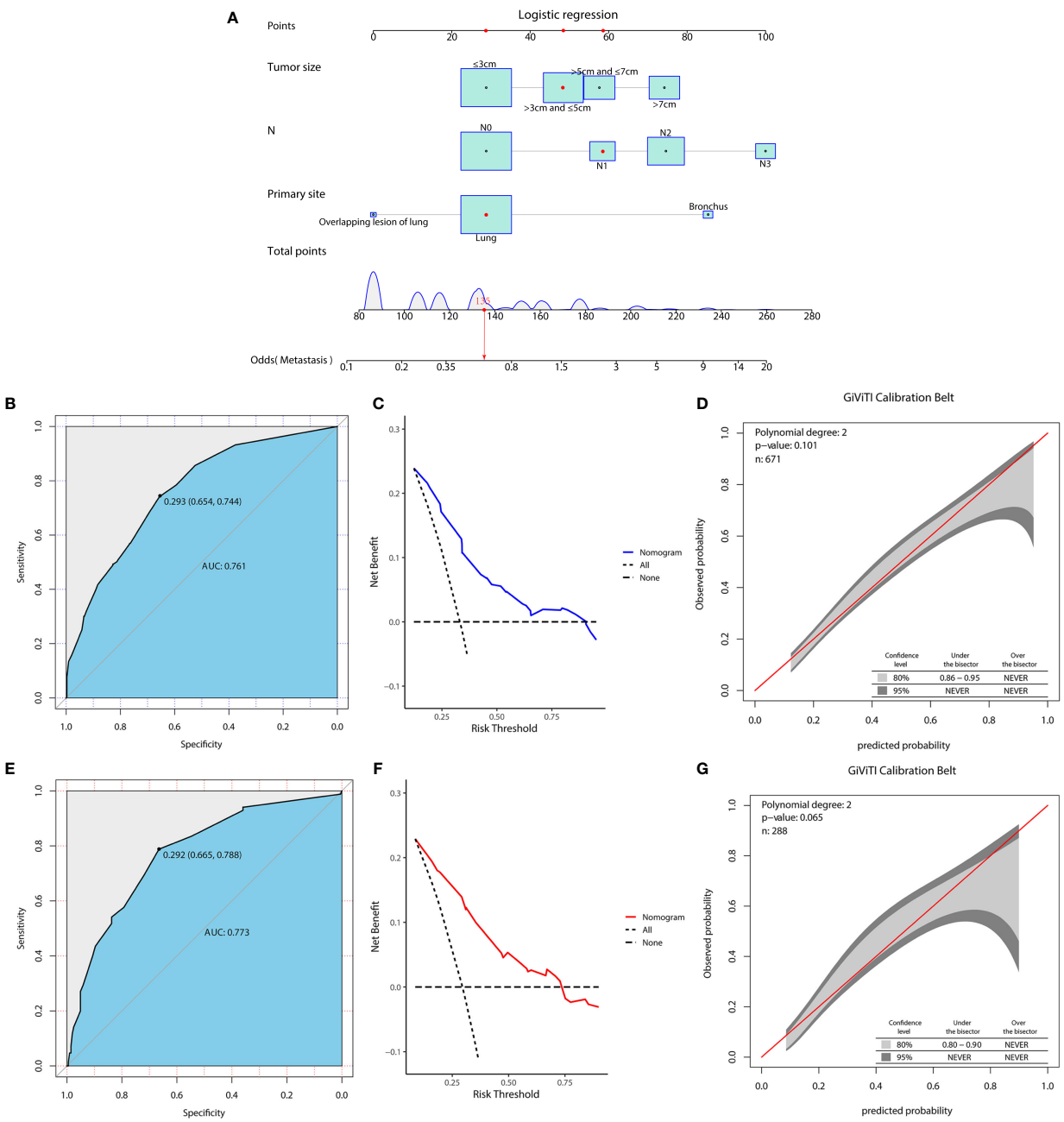

FIGURE 1
A diagnostic nomogram was developed for predicting the risk of distant metastasis in patients with LCNEC **(A)**. The receiver operating characteristic (ROC) curve **(B)**, decision curve analysis (DCA) curve **(C)**, and the GiViTI calibration belt **(D)** of the training group, and the ROC curve **(E)**, DCA curve **(F)**, and the GiViTI calibration belt **(G)** of the validation group were used to evaluate the validity and reliability of the nomogram.

Prognostic predictive model of patients with LCNEC with DM

In the prognostic cohort, univariate and multivariable Cox proportional hazards regression analyses were performed to search for factors linked with OS in patients with LCNEC with DM (Table 4). Four variables were selected, including sex, N stage, surgery, and chemotherapy status. Specifically, male sex, no surgery, no chemotherapy, and a higher N stage were independent risk factors, highly associated with worse OS. Then, in the training group, we created a prognostic nomogram based on these four risk variables (Figure 3) and validated it in the validation group. First, in patients with LCNEC with DM, the nomogram could be utilized to predict

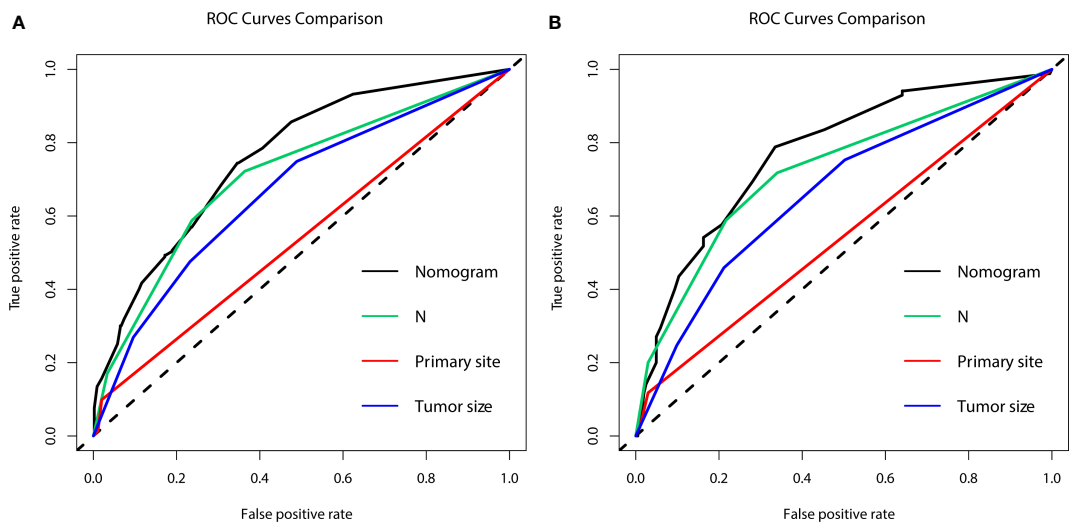

FIGURE 2
The area under the receiver operating characteristic curves (AUCs) were compared for the diagnostic nomogram in the training group **(A)** and validation group **(B)** with all independent variables, including N stage, primary site, and tumor size.

OS at 1, 2, and 3 years. In the training and validation groups, the appropriate DCA (Figures 4A–C, 5A–C) and calibration curves (Figures 4D–F, 5D–F) are shown. These results suggested the prognostic nomogram was a feasible predictive model. Second, the time-dependent ROC curves at 1, 2, and 3 years of the nomogram proved the model performed well in prognostic analysis, with respective AUCs of 0.809, 0.876, and 0.926 in the training group (Figure 6A) and 0.748, 0.790, and 0.840 in the validation group (Figure 6B). Together with a concordance index of 0.723, on the one hand, these results confirmed the validity of the prognostic nomogram, and, on the other hand, the nomogram seemed to be better at predicting long-term survival. Additionally, the ROC curves of the prognostic nomogram were compared to those of all individual risk variables, and it was shown that the prognostic nomogram outperformed any single factor at 1, 2, and 3 years in the training (Figures 7A–C) and validation groups (Figures 7D–F).

Outcomes of survival analysis

According to the prognostic nomogram, we then utilized the Kaplan–Meier method to evaluate the OS of both the high- and low-risk groups. Median survival time in the high- group and low-risk groups was 4 and 11 months, respectively, in the training group (Figure 8A), and 4 months and 10 months, respectively, in the validation group (Figure 8B). Compared to the low-risk group, the high-risk group had significantly lower OS (training group, $p<0.0001$; validation group, $p=0.00057$).

Discussion

Pulmonary LCNEC shows a high prevalence of lymph node metastases (60-80%) and DM (40%) at the time of diagnosis, with a median survival time for individuals with pulmonary LCNEC who develop DM of about five months. (19, 20) Therefore, we must take effective measures to diagnose DM of LCNEC as early as possible to provide appropriate treatment time. In the present study, to screen for high-risk groups, we developed two nomograms for the diagnostic and prognosis analysis of patients with LCNEC with DM and categorized them according to the risk score produced by the model. First, the larger the primary tumor and the closer the tumor to bronchus, the more likely it was to metastasize. Second, the prognosis of patients with LCNEC who had DM was improved by surgery and chemotherapy, but it was worse in male patients than in female patients. Third, regional lymph node metastasis was a significant risk factor affecting the prognosis of patients with LCNEC, which was related to the occurrence of DM and the prognosis of patients with LCNEC patients with DM.

Diagnostic cohort

Recently, studies focusing on clinical characteristics and prognosis of LCNEC have been published. Lowczak et al. showed that LCNEC, as with SCLC, was frequently associated with male sex, heavy smoking, and advanced age (median age of 65 years). (21) Cao et al. indicated that, although fewer older

TABLE 4 Analyses of overall survival in LCNEC patients with distant metastasis using univariate and multivariate Cox regression.

	Univariate analysis			Multivariate analysis		
	HR	95%CI	P	HR	95%CI	P
Sex						
Female	Reference			Reference		
Male	1.427	1.158-1.757	0.005	1.468	1.179-1.828	0.004
Age, years						
≥18 and <60	Reference			Reference		
≥60 and <70	1.277	0.994-1.640	0.109	1.134	0.876-1.467	0.423
≥70 and <80	1.407	1.067-1.856	0.042	1.346	1.014-1.788	0.085
≥80	2.272	1.488-3.469	0.001	1.451	0.934-2.254	0.165
Race						
Black	Reference					
Other	0.936	0.491-1.785	0.866			
White	1.172	0.869-1.581	0.384			
Laterality						
Left	Reference					
Right	1.278	1.039-1.572	0.051			
Bilateral	NA	NA	0.205			
Primary site						
Lung	Reference			Reference		
Bronchus	1.546	1.090-2.194	0.041	1.550	1.066-2.256	0.054
Overlapping lesion of lung	1.808	0.855-3.823	0.193	2.379	1.113-5.082	0.060
Grade						
III	Reference					
IV	1.084	0.854-1.376	0.579			
T						
T1	Reference					
T2	1.129	0.813-1.569	0.542			
T3	1.066	0.600-1.893	0.855			
T4	1.855	1.332-2.583	0.002			
N						
N0	Reference			Reference		
N1	1.085	0.769-1.531	0.696	1.181	0.827-1.688	0.442
N2	1.591	1.237-2.045	0.002	1.479	1.138-1.921	0.014
N3	1.857	1.351-2.552	0.001	1.730	1.231-2.432	0.008
Tumor size, cm						
≤3	Reference					
>3 and ≤5	1.272	0.964-1.679	0.154			
>5 and ≤7	1.268	0.938-1.713	0.195			
>7	1.687	1.265-2.251	0.003			
Surgery						
No	Reference			Reference		
Yes	0.424	0.318-0.566	<0.001	0.320	0.229-0.445	<0.001
Chemotherapy						
No	Reference			Reference		
Yes	0.499	0.400-0.621	<0.001	0.292	0.226-0.378	<0.001
Radiotherapy						
No	Reference					
Yes	1.016	0.828-1.247	0.899			

NA, Not applicable.

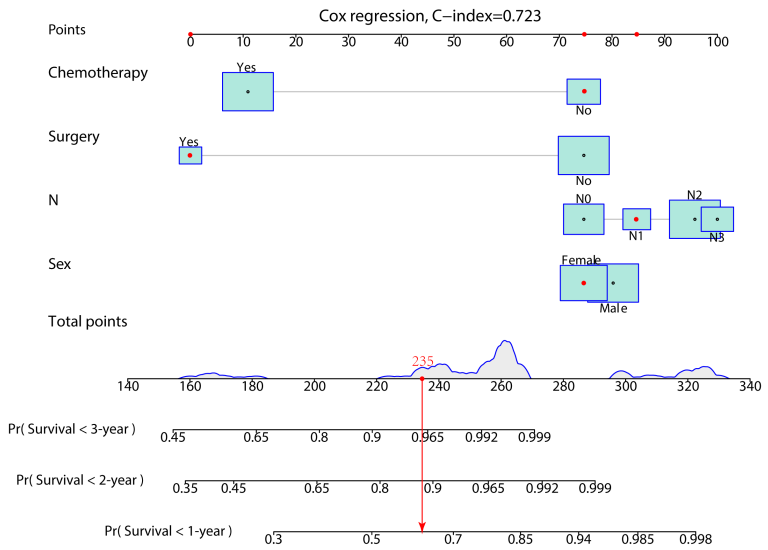

FIGURE 3
A prognostic nomogram was developed for predicting the 1-, 2-, and 3-year OS of patients with LCNEC with distant metastasis.

individuals with pulmonary LCNEC underwent surgery, chemotherapy, or radiation therapy, aggressive and effective treatment could increase survival time dramatically. (22) This corresponds with our study, which showed patients with LCNEC were more commonly older men. However, because the SEER database lacks smoking information, we did not evaluate the relationship between smoking and DM. Interestingly, in the present study, although in the prognostic cohort, the majority of LCNEC patients with DM are still elders (≥60 years old, nearly 70%), age was not a risk factor for DM; this requires further

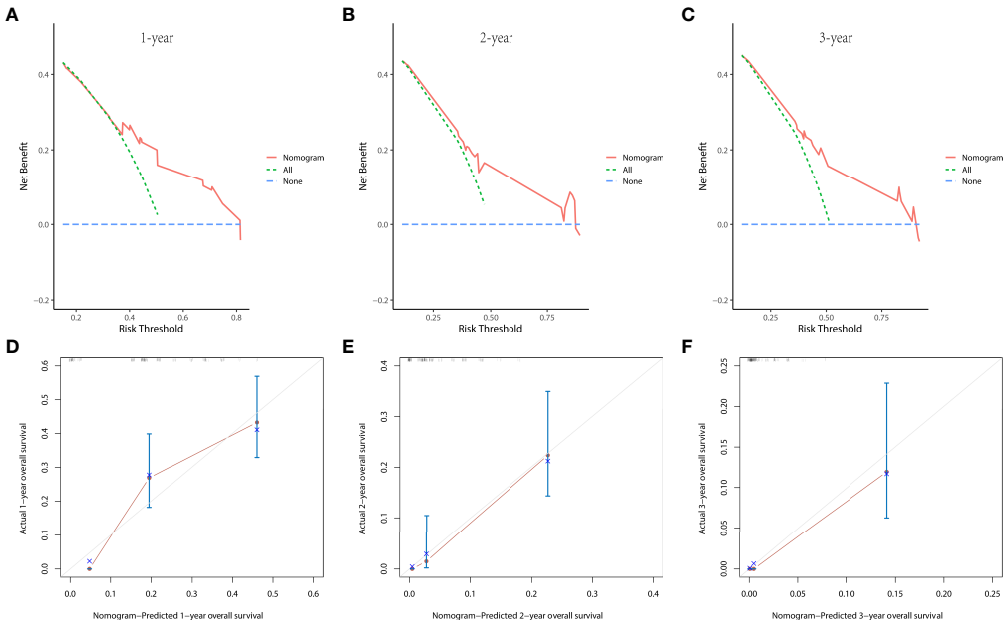

FIGURE 4
The decision curve analysis (DCA) curves at 1 **(A)**, 2 **(B)**, and 3 years **(C)** and the calibration curves at 1 **(D)**, 2 **(E)**, and 3 years **(F)** in the training group were used to evaluate the reliability of the prognostic nomogram.

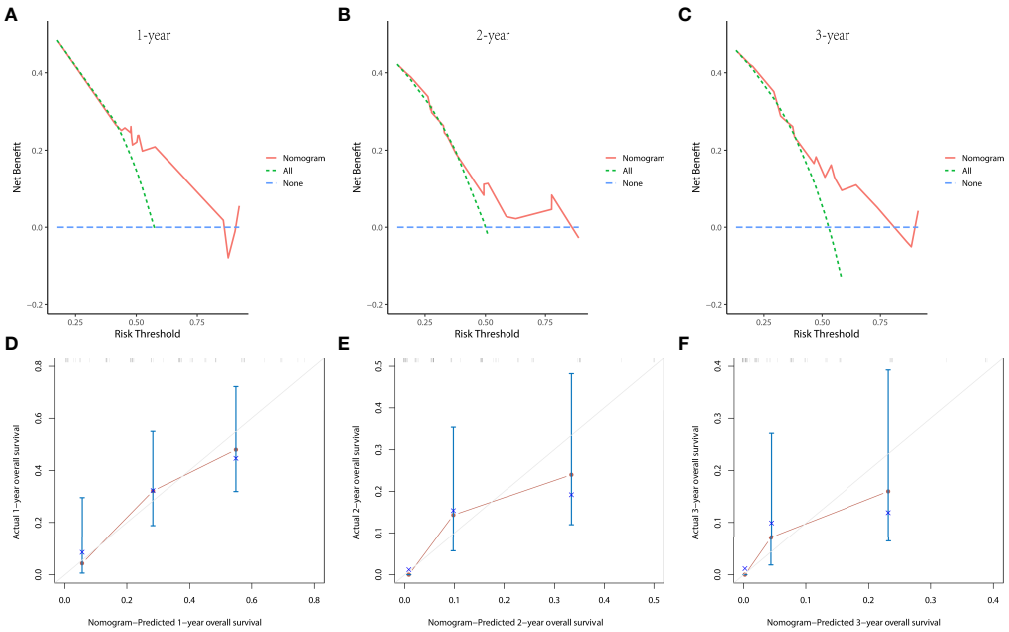

FIGURE 5
The decision curve analysis (DCA) curves at 1 **(A)**, 2 **(B)**, and 3 years **(C)** and the calibration curves at 1 **(D)**, 2 **(E)**, and 3 years **(F)** in the validation group were used to evaluate the reliability of the prognostic nomogram.

study to validate. In addition, the results in the T3 staging seem to be contrary to clinical practice. On the one hand, since the TNM staging in this study was the 6th edition staging, this may be due to deficiencies in the staging itself; an updated staging system may solve this problem, suggesting that it may be better to evaluate whether a patient with LCNEC is susceptible to DM based on tumor size rather than T staging. Additionally, since our study focused on patients with LCNEC with DM, the patients with an advanced tumor stage may have been more similar to SCLC in terms of features and prognosis. However, recent studies have shown that not all LCNEC harbors the neuroendocrine profile of SCLC, implying that some LCNECs have features of NSCLC, especially molecular features. (23) Rekhtman et al. found commonly genomic alterations in

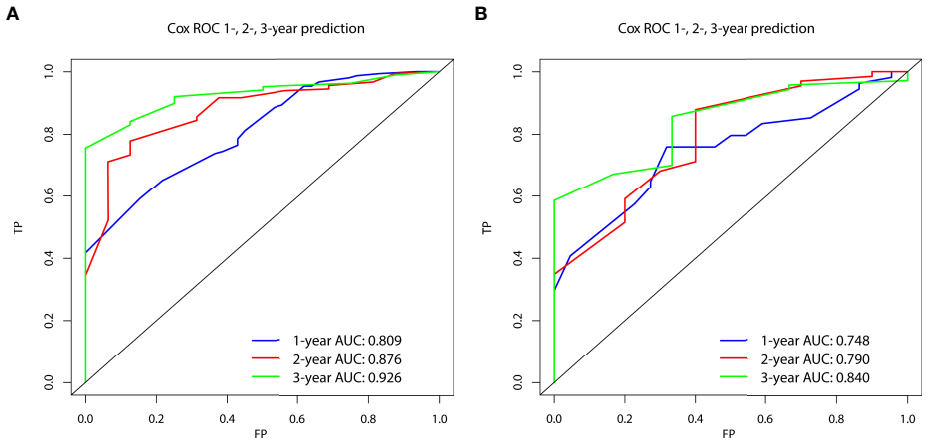

FIGURE 6
The time-dependent receiver operating characteristic (ROC) curves at 1, 2, and 3 years in the training group **(A)** and in the validation group **(B)** were used to evaluate the validity of the prognostic nomogram.

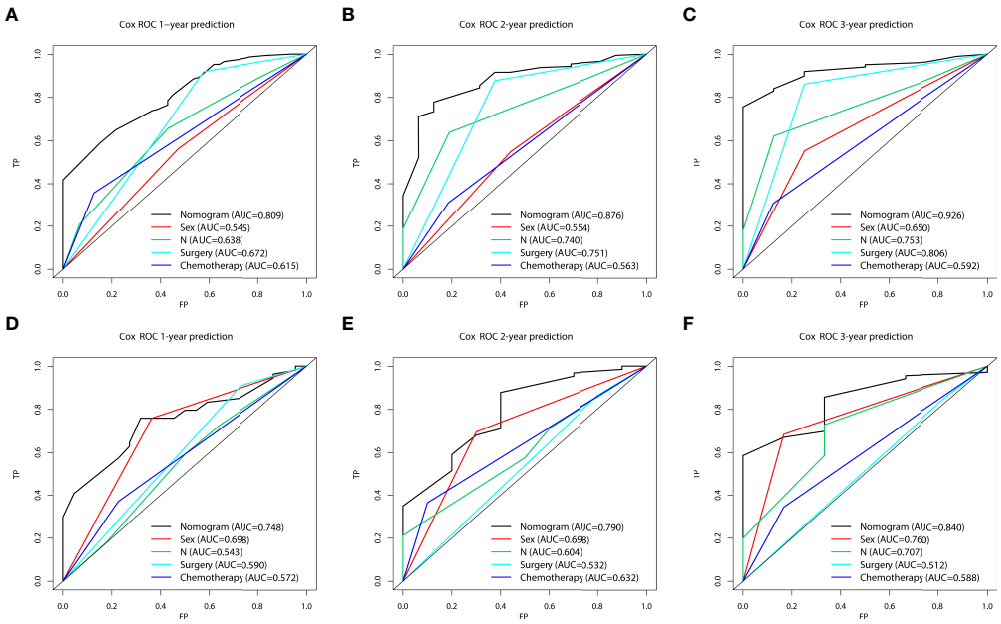

FIGURE 7
The area under the receiver operating characteristic curves (AUCs) were compared for the prognostic nomogram in the training and validation groups with all independent variables, including Sex, N stage, Surgery, and Chemotherapy at 1 **(A, D)**, 2 **(B, E)**, and 3 years **(C, F)**.

LCNEC including the genes for p53 (TP53; 78%), retinoblastoma (RB1; 38%), serine/threonine kinase 11 (STK11; 33%), kelch-like ECH associated protein 1 (Keap1; 31%) and the Kirsten rat sarcoma viral oncogene (KRAS) (22%). In addition, NSCLC-like LCNEC exhibited more frequent mutations in NOTCH family genes (28%), which may be key regulators of neuroendocrine differentiation. (24) Accordingly, LCNEC could be divided into two major subsets, one with SCLC-like mutations, including the biallelic inactivation of tumor protein RB1 and TP53, and the other with NSCLC-like mutations, including biallelic inactivation of KEAP1/STK11. (25) A refined classification of LCNEC will influence diagnosis, prognosis, and treatment decisions, (26) and may be useful in assessing the presence of DM.

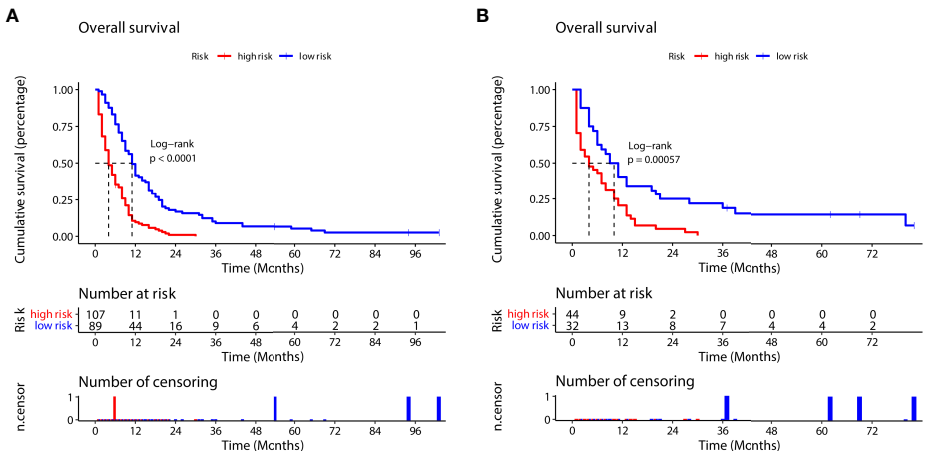

FIGURE 8
Survival outcomes in the training group **(A)** and validation group **(B)** for the high-risk and low-risk groups (according to the prognostic nomogram formula).

Prognostic cohort

In our study, sex was not a factor associated with the development of DM in patients with LCNEC, but was a factor affecting the prognosis of those with DM. The prognosis of male patients was worse than that of women. Recent studies found that lifestyle, tobacco use, secondhand smoke exposure, several occupational exposures, treatment type received, duration of anticancer treatment after diagnosis, endogenous circulating levels of sex hormones, and expression and mutation rates of several related genes (including EGFR, KRAS, and P53) had differences between men and women, and that sex differences have important implications for lung cancer development, prognosis, and treatment preferences. (27, 28) In addition, one immunohistochemistry marker, the Ki-67 proliferation index (PI), may have an effect on the prognosis of LCNEC, and recent studies have shown that Ki-67 PI≥55% was strongly associated with poor survival. (29, 30) Hermans et al. showed that patients with stage IV LCNEC with a solitary brain metastasis and N0/N1 disease more commonly had a Ki67 PI ≤ 40%, and these patients had better prognosis than those with Ki67 PI>40%. (31) However, Walts et al. suggested that a blanket use of 20%, 40%, or any other Ki-67 cut-off to diagnose LCNEC or analyze prognosis was inaccurate. (32) Unfortunately, the lack of Ki-67 data in the SEER database prevented further exploration in the present study, and it is hoped that large multicenter studies will be available to assess this.

Regarding treatment modalities, although previous studies have explored the treatment of LCNEC, the results were limited, contradictory, and rare for patients with LCNEC with DM. In our analyses, to investigate the positive effects of surgery and chemotherapy on patients with LCNEC with DM, we used multivariate Cox regression analysis and survival analysis, but, due to limited information in the SEER database, we were unable to conduct further analysis. The main findings of previous studies are as follows. First, primary surgical treatment significantly improved survival in patients with LCNEC patients, even in those with stage IV. (10) However, LCNEC had a high postoperative recurrence rate, with more than half relapsing within one year, although the R0 resection margin and N0 status (no lymph node metastasis) improved the time to recurrence. (33) As a result, even for LCNEC patients with an earlier stage, surgery alone was insufficient. (34) Second, chemotherapy alone could be more beneficial than other treatments, even for patients in stage IV. (8) The best treatment approaches are still being explored. Fisch et al. suggested that aggressive systemic therapy for metastatic LCNEC, including platinum doublets and immunotherapy, could improve OS. (35) Genomic, such as cell free DNA analysis and next-generation sequencing, subtyping was helpful for therapeutic decision-making and prognostication of patients with LCNEC. (36, 37) However, Hadoux et al. found that, in patients with LCNEC receiving platinum–etoposide chemotherapy, retinoblastoma protein (Rb) status had no influence on prognosis. (38) Therefore, the relationship between gene expression and treatment regimens requires further study. Third, there is still controversy about radiotherapy. On the one hand, radiotherapy could prolong the survival of patients with LCNEC, including those in stage IV, especially those who have received chemotherapy or have not undergone surgery. (39) However, radiotherapy may shorten the survival time of individuals undergoing surgery. (40–42) On the other hand, it is interesting to note that the metastatic pattern of LCNEC is similar to NSCLC, but the prognosis is similar to that of SCLC. (43)The brain was the most common metastatic site, so prophylactic cranial irradiation is an effective treatment and might be improve survival time. (44) In patients with LCNEC with brain metastases, stereotactic radiosurgery is superior to whole brain radiation treatment. (45) Moreover, Girelli et al. reported that patients with LCNEC with lymph node metastasis had a poor prognosis, and more active multidisciplinary approaches were needed. (46) Overall, surgery combined with chemotherapy may be an appropriate treatment for LCNEC with DM, especially in patients with regional lymph node metastasis.

Advantages and shortcomings

Previous studies on patients with LCNEC with DM were limited, and most of them were single-center studies with a lack of validation. The advantages of the present study are that the data came from the SEER database, the sample size was large, and the follow-up period was long. We created an entirely new nomogram for visualization, to predict independent risk factors for the occurrence and prognosis of DM in individuals with LCNEC, that could be used for screening high-risk patients and guiding personalized treatment in clinical practice.

Nevertheless, there are some shortcomings in the present study. First, the number of patients with LCNEC with DM was only 272, and as this was a retrospective study, this may have led to potential bias. Second, although our nomograms have been internally validated in both the training and validation groups, more data is needed to determine the wider applicability of the external validation model. Third, there is a lack of key information in the SEER database that may be relevant to survival, for example, smoking history, performance status, tumor biomarkers status, genetic testing results, specific treatment modality; these data can help further refine our model. In particular, the recent increase in use of immunotherapy and targeted therapy in lung cancer may offer new hope for patients with LCNEC with DM. Kim et al. showed that the PD-1/PD-L1 pathway was found to be activated in the LCNEC microenvironment and associated with a high mutation burden. (47) Vrontis et al. suggested that treatment and

management of patients with advanced LCNEC could be achieved with SCLC approaches, such as platinum–etoposide–atezolizumab chemotherapy, which can improve prognosis. (48) Additional prospective randomized controlled studies are needed.

Conclusions

In the present cohort study, individual risk variables and prognostic factors for DM in patients with LCNEC were identified using two regression analysis approaches and related variables were applied to establish a new predictive model and perform further survival analysis. Meanwhile, two novel nomograms were developed, including a diagnostic nomogram and a prognostic nomogram, and these could be reliable tools for clinical screening of risk populations and for optimizing treatment.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: Surveillance, Epidemiology, and End Results (SEER) Program. SEER*Stat Software version 8.3.9.2. https://seer.cancer.gov/seerstat/download. Accessed 12 Mar 2022.

Ethics statement

This study was exempt from the approval processes of the Institutional Review Boards because the SEER database patient information is de-identified. Therefore, a patient consent form was not applicable.

Author contributions

All authors contributed to the study conception and design. Data collection and analysis were performed by ZS. The first draft of the manuscript was written by ZS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.973091/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Flow diagram for the diagnostic cohort **(A)** and the prognostic cohort **(B)**.

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Case report: Two sisters with a germline *CHEK2* variant and distinct endocrine neoplasias

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Genetic testing has become the standard of care for many disease states. As a result, physicians treating patients who have tumors often rely on germline genetic testing results for making clinical decisions. Cases of two sisters carrying a germline *CHEK2* variant are highlighted whereby possible other genetic drivers were discovered on tumor analysis. *CHEK2* (also referred to as CHK2) loss of function has been firmly associated with breast cancer development. In this case report, two siblings with a germline *CHEK2* mutation also had distinct endocrine tumors. Pituitary adenoma and pancreatic neuroendocrine tumor (PNET) was found in the first sibling and pheochromocytoma (PCC) discovered in the second sibling. Although pituitary adenomas, PNETs, and PCC have been associated with *NF1* gene mutations, the second sister with a PCC did have proven germline *CHEK2* with a pathogenic somatic *NF1* mutation. We highlight the clinical point that unless the tumor is sequenced, the real driver mutation that is causing the patient's tumor may remain unknown.

KEYWORDS

CHEK2, PNET, PCC, NF1, germline, somatic, tumor

Introduction

With advances in genetics, more patients are being referred to genetic counseling to help identify pathogenic mutations they may be harboring that could affect their own management and identify at-risk family members. However, even when a germline mutation is discovered, it may not always be the driver mutation causing the patient's tumors. In this case series, we describe two sisters with a germline *CHEK2* mutation in addition to endocrine tumors that may have driver somatic mutations that could give rise to endocrine neoplasms. *CHEK*2 encodes for a serine threonine kinase involved in the response to DNA damage. Loss of CHEK2 function has been documented in breast cancer (1, 2) colon cancer, renal cell carcinoma (RCC), prostate cancer, ovarian cancer, and some cases of Li-Fraumeni syndrome (3, 4), a familial syndrome more commonly associated with the tumor suppressor gene *p53*. Patients with Li-Fraumeni syndrome are at risk for breast cancer, soft tissue sarcomas, and adrenal cortical carcinoma (ACC). When CHEK2 is activated, it inhibits CDC25C phosphatase, thereby stabilizing the p53 tumor suppressor protein and causing cell cycle arrest in G1 (5). Recently, *CHEK2* mutations were described in the context of endocrine cancer: one patient with a germline *CHEK2* mutation with ACC was reported (6), and a pathogenic variant of the gene was documented in a patient with multiple endocrine gland tumors (7). Moreover, associations between *CHEK2* and pancreatic neuroendocrine tumors (PNETs) have been detected from whole genome sequencing (8). Here we describe a patient with a history of colon polyps, pituitary adenoma, PNET, and a *CHEK2* c.1100delC germline mutation (9, 10). The index patient's sister, who had a history of pheochromocytoma (PCC) was also found to carry the same germline mutation in *CHEK2* and a known pathogenic *NF1* somatic mutation (p.Lys1444Glu) (11–13).

PCCs arise from the chromaffin cells of the adrenal medulla and have high heritability with a large roster of germline or somatic mutations. PCCs associated with germline gene mutations are currently categorized into three main clusters: pseudohypoxia (Cluster I), kinase signaling (Cluster II), and the third cluster characterized by expression of the *CSDE1* and *UBTF-MAML3* genes associated with an active Wnt signaling pathway (Cluster III) (14). Germline mutations in the neurofibromatosis gene (*NF1*) belong to Cluster II and account for approximately 3% of all PCC cases (15, 16), while the frequency of somatic mutations in PCC is estimated to be 20-30%; both germline and somatic *NF1* mutations have been amply described in PCC (17–19).

Objective

Here we report that both sisters have a germline *CHEK2* mutation, and in at least one of the tumors, a somatic mutation that may be the driver for their endocrine neoplasias was identified in the *NF1* gene. The connection between *NF1* and *CHEK2* mutations in PCC remains to be explored. Although clinical relevance of *CHEK2* mutations in these cases cannot be confirmed, we hypothesize that it may play a role in both cases. This report illustrates how sequencing the actual tumor can elicit the driver mutation that germline testing alone would have been unable to identify.

Description of cases and diagnostic assessment

Case 1

58-year old woman with a history of type 2 diabetes, polyneuropathy, colon polyps, hypertension, and obesity was found to have hypercortisolism. Hypercortisolism was suspected when she continued to have rapid weight gain despite following a strict diet and regular exercise. A 1mg dexamethasone suppression test showed an 8AM cortisol of 28 µg/dL (nl<1.8 µg/dL). Her 24 hr urine free cortisol was 89.8 µg/24 hrs (nl<50 µg/24 hrs). The ACTH value was unsuppressed at 69 pg/mL. Pituitary MRI showed a 4mm pituitary adenoma. Inferior petrosal sinus sampling localized the source of ACTH to the pituitary. Patient underwent transphenoidal resection of the pituitary tumor and the histomorphology was consistent with a pituitary adenoma (Figure 1A), and immunohistochemical stains confirmed ACTH reactivity. Due to back pain, she underwent a CT scan, which showed a cystic pancreatic mass along the pancreatic body. Fine needle aspiration of this mass was consistent with a PNET. This mass is currently being monitored. Due to patient's history of pituitary adenoma and PNET, she underwent genetic testing, which revealed *CHEK2* c.1100delC, and no other susceptibility mutation was identified. Figure 2 depicts her family pedigree, which shows both the patient and her younger sister have the *CHEK2* mutation. We obtained DNA from archival sections of the pituitary adenoma and confirmed the presence of the *CHEK2* mutation, but no evidence of loss of heterozygosity. However, the presence of normal stromal cells may have confounded this analysis.

Case 2

Initially at the age of 39-years old, Case 1's younger sister was evaluated for nephrolithiasis. At that time, a CT scan detected an incidental 4cm left adrenal mass, which was biochemically confirmed to be a PCC (20) with elevated plasma metanephrines 0.86 nmol/L (<0.50) and normetanephrines 3.09 nmol/L (<0.90). The 24 hr urine metanephrine were 973 mg/24 hrs (<400), normetanephrines

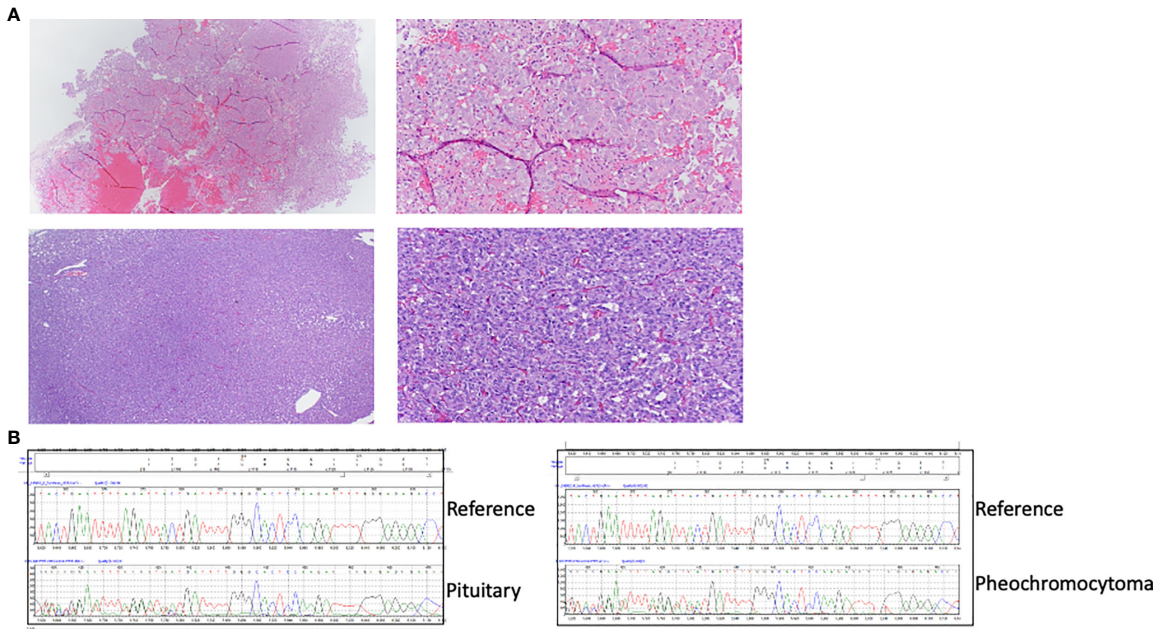

FIGURE 1
(A) HE images of the proband's pituitary adenoma (top) and her sister's pheochromocytoma (bottom). Images on the left were taken at lower (40X) magnification and those on the right at higher (100X) magnification. **(B)** *CHEK2* sequence traces from DNA obtained from the proband's pituitary adenoma (top) and the sister's pheochromocytoma(bottom), obtained from formalin-fixed paraffin embedded (FFPE) tissue, showing the presence of a frameshift variant (c.1000delC) and consistent with retention of both alleles or the presence of nontumoral admixed cells in the DNA. Reference wild-type sequence is shown for comparison.

1120 mg/24 hrs (<900), dopamine 200 mg/24 hrs (65–400), norepinephrine 37 mg/24 hrs (15–80), epinephrine 12 mg/24 hrs (0–20). The patient did not have hypercalcemia and had a normal PTH level, without any evidence of other tumors (20). She underwent a left adrenalectomy, and the pathology was consistent with a PCC (Figure 1B). The patient was tested for mutations in PCC-associated genes ((21): *RET*, *TMEM127*, *MAX*, *VHL*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *NF1*, and no

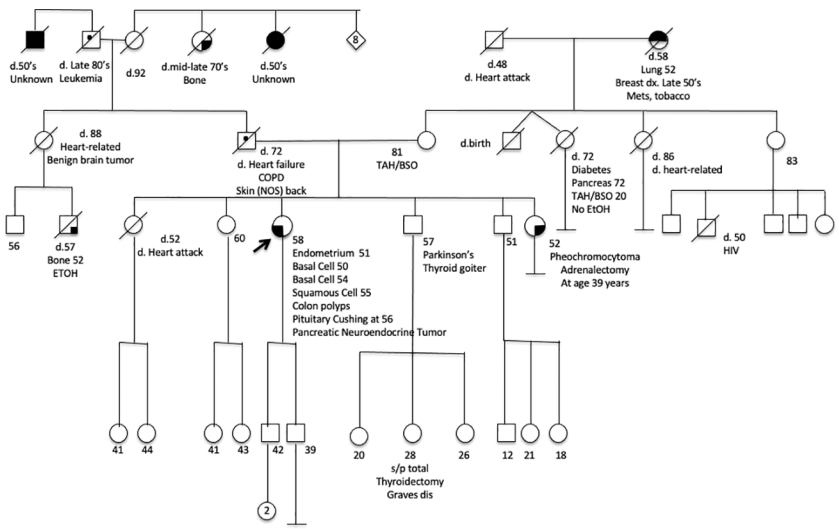

FIGURE 2
A pedigree chart of the affected family.

documented variants were identified. The presence of the *CHEK2* c.1100delC mutation in DNA derived from her PCC and archival PCC tumor tissue was subsequently determined. In addition, using the hPheo1 cell line developed from the patient's PCC (20), the *CHEK2* locus was analyzed for the presence of c.1100delC using an allele-specific PCR assay using primers Chk2ex10f (5'-TTAATTTAAGCAAAATTAAATGTC), Chk2ex10r (5-GGCATGGTGGTGTGCATC), and Chk2delC (5'-TGGAGTGCCCAAAATCATA). PCR products were separated in 2–3% agarose gels, cloned, and sequencing was used to confirm this mutation (Figure 3A). No evidence of loss of heterozygosity was observed. DNA isolated from both the patient's PCC tumor and hPheo1 cell line derived from it was further analyzed by targeted next generation sequencing and Sanger sequenced and determined to harbor a *KIF1B* variant T827I, considered by PolyPhen-2 to be likely benign. Importantly, this analysis uncovered a pathogenic mutation in the *NF1* gene, NM_001042492.3(NF1): c.4330A>G (p.Lys1444Glu) (Figure 3B), associated with predominance of the variant allele (MAF > 70%), and suggestive of loss of heterozygosity of the wild-type allele. These findings support the notion that this *NF1* somatic variant is the driver event underlying this tumor. The patient has not had recurrence fourteen years after adrenalectomy.

Discussion

In this report, we describe two sisters sharing a *CHEK2* c.1100delC mutation and presenting with endocrine tumors in a

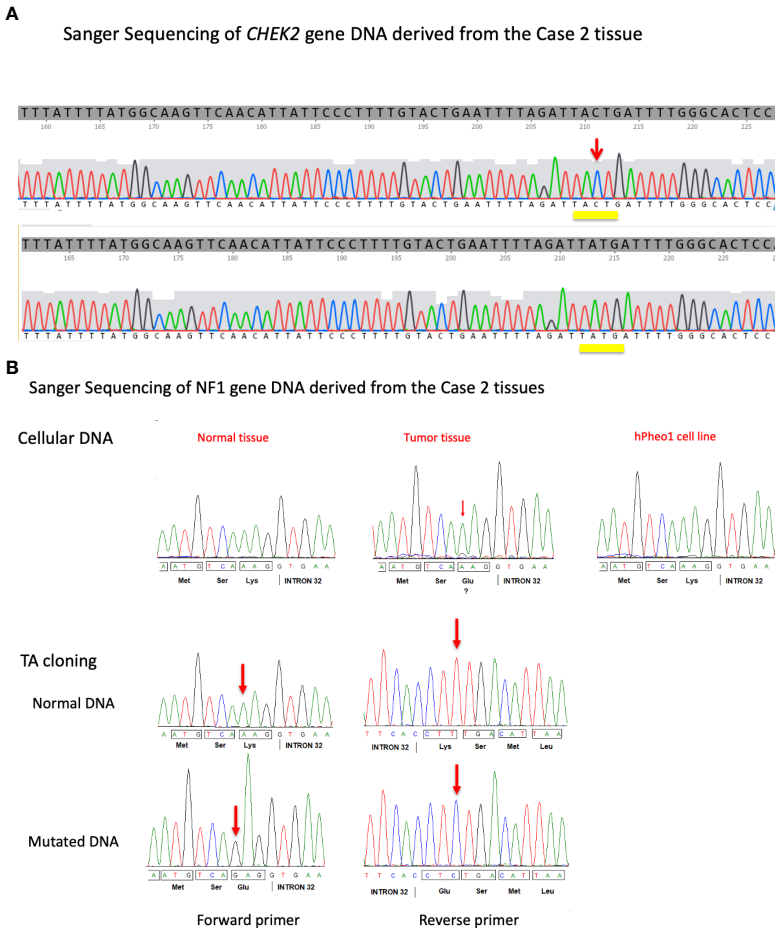

FIGURE 3
(A) DNA sequence traces obtained from the individual clones carrying PCR fragments derived from the hPheo1 cell line derived from the proband's sister's pheochromocytoma tumor. Isolates carrying either a wild-type version (*top*) or the mutant version c.1100delC (*bottom*) of the *CHEK2* gene were obtained. The position of the 1100C nucleotide is indicated by a red arrow. **(B)** Formalin-fixed paraffin-embedded tissue DNA from different slides was amplified for *NF1* exon 32 and sequenced along with hPheo1 DNA (*top*). Additionally, PCR products were TA cloned and isolates sequenced (*bottom*). The position of the mutation is indicated (red arrow).

pattern not typical of classic multiple endocrine neoplasia. Although it was well known that *CHEK2* mutations are associated with breast and colon cancers, within the last few years, it has been recognized to also be associated with PNETs (8). The first sister does have a PNET. Of note, none of the sisters have a history of primary hyperparathyroidism. In this report, Case 1 had Cushing disease and is being followed for her non-functional PNET. The discovery of her *CHEK2* mutation and the absence of other pathogenic mutations in other susceptibility genes raised the question of whether this variant could be related to this patient's syndromic phenotype. Pathological studies in Case 1 were not able to show loss of heterozygosity (LOH) in the pituitary tumor sample, as the tumor size was very small and normal stromal cells may have confounded the interpretation of the results (Supplementary Figure1).

The commonality between the pathophysiology of *CHEK2*, and other mutations such as *PALB2* –also associated with PNETs– is that they have an association with DNA repair (Figure 4). With DNA damage, CHEK2 is activated, affecting BRCA1/PALB2/RAD51 complex needed for DNA repair (23). Interestingly, Case 1's younger sister was found to have a PCC and testing of germline from this patient demonstrated the *CHEK2* c.1100delC mutation, but no mutation in PCC susceptibility genes, such as *RET*, *TMEM127*, *MAX*, *VHL*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *NF1*. Case 2 did not have any other endocrine tumors such as hyperparathyroidism or medullary thyroid cancer consistent with MEN2 syndrome, or other PCC-related syndromes. As the patient's tumor produced both metanephrines and normetanephrines, it was predicted that her tumor belongs to the Cluster II classification in PCC genes associated with kinase signaling including *RET, NF1, and TMEM127*, as opposed to Cluster I PCC tumors that are associated with pseudohypoxia signaling pathway (21). Indeed, subsequent evaluation of DNA from the patient's PCC tumor and hPheo1 cell line detected the pathogenic mutation in the *NF1* gene, NM_001042492.3 (NF1): c.4330A>G (p.Lys1444Glu) (Figure 3B). A second variant identified in primary PCC and hPheo1 was *KIF1B* variation T827I (c.2480C > T, rs121908162), which is predicted to be benign by PolyPhen-2 (24). Other mutation, previously reported for hPheo1 line, in addition to *KIF1B*, also includes NRAS Q61K (24) which was not detected in the patient's tumor DNA, and may represent an adaptation of the cell line in culture (data not shown).

Recently, Dietlein et al. developed an algorithm to identify previously unsuspected cancer driver genes based on the presence of an excess of mutations in unusual nucleotide contexts (25). In their approach, they evaluated whole exome sequencing data from over 11000 tumors of distinct tissue types and identified *CHEK2* as a candidate driver gene in PCCs (25). LOH studies on Case 2's PCC sample were inconclusive due to the possible presence of normal admixed cells. Therefore, it was difficult to demonstrate that Case 2's PCC was indeed due to a *CHEK2* driver mutation. The somatic pathogenic *NF1* mutation in Case 2 tumor was associated with a classic second-hit (loss of the wild-type allele), similar to other conventional somatic loss of function *NF1* mutations. Somatic analysis of the pituitary and PNET, which might offer potential insights on somatic events that may have occurred in these tumors, was not feasible. Interestingly, a case in the literature with a different germline *CHEK2* p.R180C with

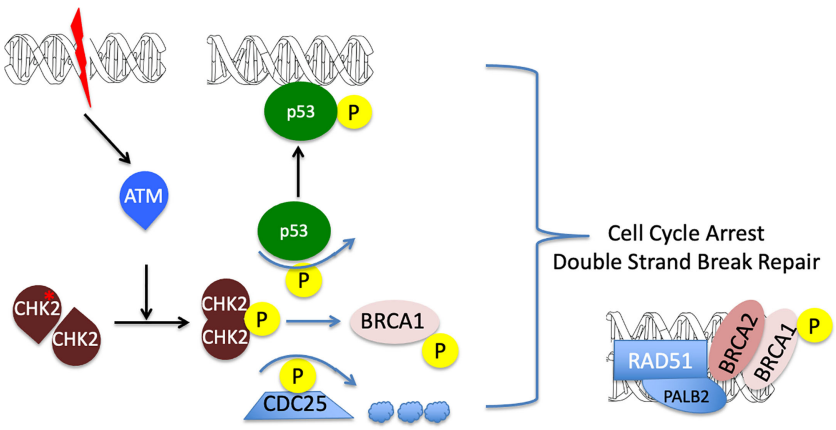

FIGURE 4
A diagram illustrating CHEK2 function in cell cycle arrest and DNA repair. Double strand break in the DNA leads to ATM activation and subsequent CHEK2 phosphorylation and dimerization. Activated CHECK2 in turn mobilizes p53 and BRCA1 and promotes degradation of the CDC25 phosphatase. The resulting formation of active p53 and RAD51/PALB2/BRCA1/2 DNA complexes, together with a down-regulation of CDC25, leads to the initiation of double strand break repair and cell cycle arrest. Decrease in CHEK2 kinase activity results in the loss of cell cycle control and promotes genomic instability (reviewed in (22)).

NF1 mutation has been described. However no endocrine tumors were reported (26).

Future studies with other patients and families will be instructive to assess whether *CHEK2* variants may have any impact as a potential modifier in endocrine neoplasias. Clinically, if a patient has a tumor such as seen in both cases, and genetic testing reveals a germline mutation, the clinician must be aware that the patient's tumor will need to be sequenced to identify the driver mutation causing neoplastic formation.

Take-away lessons

In this report, two siblings with a germline *CHEK2* mutation and atypical endocrine tumor associations are described. One sister has a pituitary tumor causing Cushing Disease as well as a PNET. The other sister was found to have a PCC carrying a pathogenic somatic *NF1* mutation. Although all three endocrine tumors (pituitary adenoma, PNET, PCC) can be associated with *NF1*, it was difficult to demonstrate this for the pituitary adenoma and PNET. *CHEK2* is known to be associated with PNET. Whether *CHEK2* mutation could be a contributing factor in a new syndrome of pituitary, PNET, and PCC tumors remains to be determined. Although the clinical relevance of *CHEK2* mutations in these cases cannot be confirmed, we hypothesize that it may play a role in both cases. These two cases underscore the need for resected tumor to be further analyzed, to give a complete picture of whether the germline mutation is solely responsible for tumor formation or there is an additional somatic mutation that serves as a driver. This information can be very important clinically: if the driver mutation is consistent with aggressive disease that would affect the patient's surveillance and the physician's ability for early detection of a recurrence with metastatic potential. Solely relying on germline mutation may affect clinical decision-making.

Patient perspective

Sister 1

"Now that I am aware of the *CHEK2* variant, I am more diligent with all of my healthcare. I make the recommended cancer screenings a priority. Additionally, since having a pituitary tumor removed, my overall health has significantly improved. I walk four miles a day and have lost 50 pounds. I was able to return to work in the Fall of 2019 and have made great strides in recovering my mental health, affected by Cushings. I have a pancreatic neuroendocrine tumor that is being followed. I have annual CT/MRIs and follow up appointments with the pancreatic oncologist. I believe my experiences with critical and rare health situations has helped me to be more aware of my specific issues and my overall health."

Sister 2

"Due to genetic testing I recently found out that I have the *CHEK2* gene. I'm so grateful to have this information because it will most likely save my life. I am now scheduled for several check-ups that I would not have scheduled prior to my genetic testing. My pheochromocytoma was found by accident 14 years ago. Had we known then what we know now my life could be quite different. Genetic testing is a great tool in the business of saving lives."

Data availability statement

The variant information has been deposited into ClinVar (www.ncbi.nlm.nih.gov/clinvar) under the accession number SCV002571738.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

RV, YD, KH, JB, SM, AA, ST, MT, OG, PS, PD, HG contributed to the clinical and scientific experiments of the manuscript. RV, YD, KH, JB, SM, AA, ST, MT, OG, PS, PD, HG reviewed the manuscript. HG, ST, OG, PS, PD wrote the initial draft of the manuscript. HG and PD conceptualized the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

HG has received royalties from the University of Texas Southwestern Medical Center.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1024108/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Sanger sequencing traces on *CHEK2* of the proband's pituitary adenoma, obtained from formalin-fixed paraffin embedded (FFPE) tissue, showing the presence of a frameshift variant (c.1000delC, arrow). Representation of the normal, wild-type (WT) allele and the variant allele are shown, indicating an almost 50-50 distribution, consistent with retention of both alleles or the presence of nontumoral admixed cells in the DNA.

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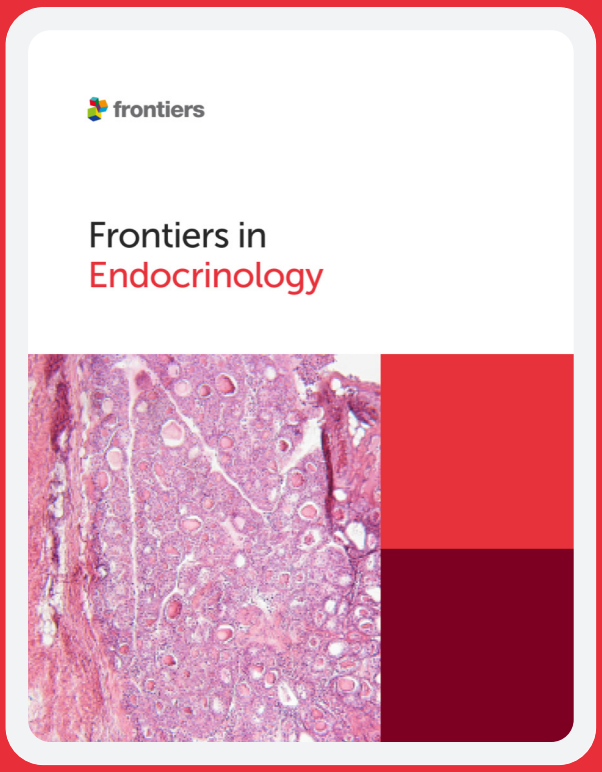

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