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NIKE: NEUROENDOCRINE TUMORS, INNOVATION IN KNOWLEDGE AND EDUCATION

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EDITORIAL
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Editorial—Special Issue: Foreword to the Special Issue on NIKE: Neuroendocrine Tumors, Innovation in Knowledge and Education

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Keywords: Neuroendocrine neoplasm (NEN), Neuroendocrine tumors (NET), endocrine syndromes, paraneoplastic syndromes, advances therapies, molecular targets, efficacy, toxicity

Editorial on the Research Topic

NIKE: Neuroendocrine Tumors, Innovation in Knowledge and Education

Neuroendocrine neoplasms (NENs) are heterogeneous tumors arising from the diffuse neuroendocrine system, showing a common phenotype but variable behavior and prognosis. They include the well-differentiated neuroendocrine tumors (NETs), which are classically slow-growing and potentially functioning and the poorly differentiated neuroendocrine carcinomas (NECs), which are highly proliferative and aggressive (1, 2). NENs represent a model for the multidisciplinary management of tumors because different specialists are required, both for diagnosis and therapy. Not only oncologists and surgeons but also gastroenterologists, radiologists, nuclear medicine physicians and endocrinologists are needed for patients presenting with a wide spectrum of symptoms, either related to hormonal hypersecretion or tumor growth (3, 4). On the other hand, pathologists are indispensable to achieve the diagnosis and define the histo-prognostic category (5). Despite recent advances in the comprehension of biology and clinical behavior of these tumors, as well as the development of new diagnostic tools and therapeutic agents, many unmet needs remain to be addressed (6–8). Furthermore, quality of life and management of treatment toxicities represent a challenge in these patients who are characterized by long survival even at a metastatic stage (9–12).

This special issue is a mirror of the activities of the NIKE project on NET innovation in knowledge and education. This project started in 2015 with the aim of identifying the different clinical and biological unmet needs of NENs. This NIKE issue is a collection of four case reports, four retrospective studies, one original study and one perspective which describe unusual NEN primary sites and paraneoplastic syndromes, adverse events to NEN therapy, new therapeutic targets and strategies, prognostic factors and finally the minimum and optional requirements for a pathology report of NENs.

Secretory activity is one of the main characteristics of NENs. Despite nonfunctioning tumors are more frequent, several NEN-related endocrine and paraneoplastic syndromes can occur and need to be known and managed. Giannetta et al. reported four NET patients presenting with paraneoplastic hypercalcemia, due to PTH-related peptide in three cases and 1-25-dihydroxy-vitamin-D hypersecretion in one case. Paraneoplastic hypercalcemia is a rare condition that can be difficult to manage in NET patients. Zhang et al. reported a case of ectopic Cushing's syndrome with unknown

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primary but presenting with lung lesions with a final diagnosis of nocardiosis. This is an opportunistic infection, usually related to immunosuppression, which represents a life-threatening condition of NET patients with ectopic Cushing's syndrome and could be a confounding factor in the diagnostic work-up of NET.

The spectrum of therapeutic approaches for NEN has been enlarged in the last years. In parallel, treatment-related toxicities and cumulative toxicities need to be managed to ensure a good quality of life in these patients. Gubbi et al. reported a case of primary hypothyroidism following the first cycle of peptide receptor radionuclide therapy (PRRT) with ^{177}Luthetium-DOTATATE for a metastatic paraganglioma. The intense thyroid uptake of ^{177}Luthetium suggests a direct effect of this radionuclide through somatostatin receptors expressed in thyrocytes, with consequent marked increases in anti-Tg and anti-TPO antibodies and rapid development of primary hypothyroidism. Chang et al. underlines that everolimus, a mTOR inhibitor indicated in progressive NETs, can be associated with fulminant hepatitis. A patient presenting with a metastatic NET of the pancreas died three months after starting everolimus 10 mg a day, because of fulminant hepatitis due to reactivation of a chronic HBV infection. A specific prophylaxis is suggested in HBV positive patients undergoing therapy with everolimus.

Despite NENs occurring mainly in gastroenteropancreatic and bronchial tracts, these tumors can show many other primary locations. Gallo et al. provide an accurate review of the main aspects of breast NENs, in order to better define an issue that has been subjected to subsequent changes in the classification criteria in the last years. Breast NENs are a rare subgroup of breast cancer but clear diagnostic criteria are still far from being established and the real size of this tumor could be underestimated.

Delving into the study of the mechanisms of NEN development, the fibroblast growth factor (FGF) pathway represents an intriguing oncogenic target. Vitale et al. analyzed its role in the development and progression of NENs, the occurrence of fibrotic complications and the onset of drug-resistance. Clinical trials with specific FGF-inhibitors are suggested to explore the role of the FGF pathway as molecular target for NEN therapy.

Di Molfetta et al. analyzed lights and shadows of immunotherapy in medullary thyroid cancer. This approach has been recently developed to induce an autoimmune response to the tumor and has found an excellent application in some cancer types like melanoma. In NEN, patient with merkelioma were found to be optimal candidates for immunotherapy, while less encouraging results have been observed in other NEN types. If available data on these new agents in medullary thyroid cancer are scarce at present, however some trials are now ongoing and a definitive conclusion on the role of immunotherapy in this setting will be addressed in the next years.

Lania et al. explore recent progress and future approaches of neoadjuvant therapy in NENs. This suggestive approach has been more and more relevant in many types of cancer, by using neoadjuvant chemo- or radiotherapy. In NEN there has been not a similar interest, likely for the peculiar biology and clinical course of these tumors which are, for the most part, slow growing, with low rates of proliferation and therefore less responsive to conventional anti-tumor therapies. A change in this view could be observed with PRRT, which is able to induce a significant rate of tumor shrinkage in NET expressing somatostatin receptors. Promising results are now available and other studies on this topic are ongoing.

An original study has been conducted by Barrea et al. on the role of metabolic syndrome and cardiometabolic indexes as prognostic factors in gastroenteropancreatic NETs. In the last years there is mounting evidence supporting the role of the metabolic syndrome in the pathogenesis of several tumors. In this study, the metabolic syndrome as well as the fatty liver index, a non-invasive tool for identifying individuals with non-alcoholic fatty liver disease, and the visceral adiposity index, a marker of adipose dysfunction, have been found to correlate with unfavorable clinicopathological characteristics.

Finally, Albertelli et al. provide a perspective article to analyze the main questions and relative answers, focusing on three main topics (i.e. morphology and classification, Ki67 and grading, immunohistochemistry), which were considered relevant for clinicians for understanding and correctly interpreting pathology reports on gastroenteropancreatic NENs. A minimum requirement in pathology report of NEN is also provided.

In summary, this special issue would be a support for endocrinologists, and for all other specialists involved in NEN management, by providing new insights in different fields of NEN, by suggesting new research lines and therapeutic targets and strategy, by reporting rare and unusual conditions related to NENs.

AUTHOR CONTRIBUTIONS

AF and AC both contributed to develop this article by resuming the results of all scientific manuscripts included in the Research Topic NIKE. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: Primary Hypothyroidism Associated With Lutetium 177-DOTATATE Therapy for Metastatic Paraganglioma

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Background: Lutetium 177 (^{177}Lu) - DOTATATE is a form of peptide receptor radionuclide therapy (PRRT) utilized in the treatment of neuroendocrine tumors. Data on ^{177}Lu-DOTATATE-induced thyroid dysfunction is limited.

Case Description: A 29-year-old male with *SDHB* positive metastatic paraganglioma enrolled under the ^{177}Lu-DOTATATE trial (NCT03206060) underwent thyroid function test (TFT) evaluation comprised of thyroid stimulating hormone (TSH) and free thyroxine (FT4) immunoassay measurements per protocol prior to ^{177}Lu-DOTATATE therapy. The TSH was suppressed [<0.01 μIU/ml (0.27–4.2 μIU/ml)], and FT4 was normal [1.3 ng/dl (0.9–1.7 ng/dl)]. The TSH receptor antibody and thyroid stimulating immunoglobulin index were undetectable [<1 IU/L (≤1.75 IU/L), and <1 (≤1.3) respectively], while the anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies were elevated [605 IU/ml (0.0–34.9 IU/ml), and 178 IU/ml (0.0-40.0 IU/ml) respectively]. Mass spectrometry on a stored (-80°C) plasma sample obtained one-month pre-PRRT revealed elevated total triiodothyronine (TT3) [235 ng/dl (65–193 ng/dl)] and FT4 [3.9 ng/dl (1.2–2.9 ng/dl)] levels. The patient was diagnosed with Hashimoto's thyrotoxicosis. However, the patient was asymptomatic. One month after the first dose of 200mCi ^{177}Lu-DOTATATE, the patient noted fatigue and a 2.6 Kg weight gain. The TSH (73.04 μIU/ml), anti-TPO antibodies (>1,000 IU/ml), and anti-Tg antibodies (668 IU/ml) had substantially increased, with reductions in FT4 (0.3 ng/dl) and TT3 [54 ng/dl (87–169 ng/dl)]. Diagnostic gallium 68 - DOTATATE positron emission tomography-computed tomography performed prior to ^{177}Lu-DOTATATE treatment revealed diffuse thyroid uptake. Post-therapy single-photon emission computed tomography also revealed diffuse uptake of ^{177}Lu-DOTATATE in the thyroid gland. Levothyroxine therapy was initiated, and the patient's symptoms resolved.

Summary: We report, for the first time, a patient with asymptomatic primary hyperthyroidism who rapidly developed symptomatic primary hypothyroidism 1 month after ^{177}Lu-DOTATATE therapy, accompanied by marked changes in TFTs and thyroid auto-antibody titers, with functional imaging evidence of diffuse uptake of ^{177}Lu-DOTATATE in the thyroid gland.

Conclusions: Thyroid dysfunction can be associated with PRRT. Thyroid uptake patterns on pre-treatment diagnostic somatostatin analog scans might predict individual susceptibility to PRRT-associated TFT disruption. Therefore, periodic evaluation of TFTs should be considered in patients receiving PRRT.

Keywords: DOTATATE, Lutathera, hypothyroidism, peptide receptor radionuclide therapy, paraganglioma

INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) is a form of targeted therapy that has demonstrated substantial efficacy in the treatment of neuroendocrine tumors (NETs) (1, 2). PRRT targets cells that possess high concentrations of somatostatin receptors (SSTRs), such as NETs, by utilizing a radiolabeled peptide (somatostatin) molecule (3). The somatostatin analog component of the molecule binds to the SSTRs and facilitates the delivery of the radionuclide directly to the tumor cells (2). Various analogs of PRRT possess theranostic properties, which is the ability to integrate diagnostic and therapeutic functions within the same pharmaceutical platform (4).

Lutetium 177 (^{177}Lu) - DOTA-DPhe1, Tyr3-octreotate (DOTATATE) (Lutathera®, Advanced Accelerator Applications, Saint-Genis-Pouilly, France) is a form of PRRT with the highest affinity to SSTR-2. It has been successfully utilized in treating gastroenteropancreatic NETs and was recently shown to markedly prolong progression-free survival among patients with advanced, well-differentiated midgut NETs in the NETTER-1 phase 3 trial (2). Additionally, this agent has demonstrated substantial safety and efficacy based on data from 1200 patients treated for gastroenteropancreatic and bronchial NETs (5). ^{177}Lu-DOTATATE has also been effectively utilized in the treatment of inoperable, metastatic pheochromocytomas and paragangliomas (PPGLs) (6, 7). ^{177}Lu radionuclide exerts its anti-tumor effects by emitting medium energy beta particles and has a maximal tissue penetration of 2 mm (8). ^{177}Lu-DOTATATE therapy is associated with several adverse effects, with nausea and vomiting being the most common adverse effects. Other adverse effects include fatigue/asthenia, abdominal pain, diarrhea, loss of appetite, musculoskeletal pain, headaches, flushing, dizziness, alopecia, cough, nephrotoxicity, hematotoxicity (leukopenia, thrombocytopenia, and lymphopenia), cardiotoxicity, hepatotoxicity, and acute hypertensive crisis (2, 7). ^{177}Lu-DOTATATE therapy has also been associated with the disruption of endocrine function, although these effects are extremely rare and is often transient (9). We report, for the first time, a patient with primary hyperthyroidism who rapidly progressed to primary hypothyroidism after the first dose of ^{177}Lu-DOTATATE therapy. A written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

CASE DESCRIPTION

A 29-year-old male with metastatic paraganglioma with succinate dehydrogenase subunit B (*SDHB*) germline pathogenic variant was enrolled in the ^{177}Lu-DOTATATE trial (ClinicalTrials.gov identifier: NCT03206060) for the treatment of inoperable, metastatic PPGL at our center. As a part of the protocol, the patient underwent baseline thyroid function test (TFT) comprised of thyroid stimulating hormone (TSH) and free thyroxine (FT4) evaluation on the day of the first cycle of therapy, just prior to ^{177}Lu-DOTATATE administration. The TSH level was suppressed [<0.01 μIU/ml (0.27–4.2 μIU/ml)], and the level was normal [1.3 ng/dl (0.9–1.7 ng/dl)] based on an immunoassay measurement. A repeat measurement of TSH and FT4 immunoassay revealed values of <0.01 μIU/ml and 1.2 ng/dl, respectively. TSH receptor antibody (TrAb) and thyroid stimulating immunoglobulin index were <1 IU/L (≤1.75 IU/L), and <1 (≤1.3), respectively. Anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies were 605 IU/ml (0.0–34.9 IU/ml), and 178 IU/ml (0.0-40.0 IU/ml), respectively. The patient complained of headaches, dizziness, and back pain, all of which were attributed to metastatic, biochemically active paragangliomas. The patient denied heat intolerance, weight loss, palpitations, sweating, diarrhea, changes in the appearance of his eyes, redness, eye pain or excessive tearing, neck pain, dysphagia, voice change, visual disturbances, skin changes, or diarrhea. Although multiple family members were affected with several non-thyroid malignancies, there was no family history of thyroid disorders or malignancies. The only form of radiation that the patient had received to the head and neck region was a computed tomography (CT) scan. His past medical history was relevant for SDHB-related metastatic paraganglioma (left glomus vagale, abdominal and hypopharyngeal lesions, and multiple bony metastases), Beckwith-Wiedemann syndrome (a genetic syndrome characterized by macrosomia and hemihypertrophy of the body, macroglossia, and increased risk for several embryonal tumors, and is usually caused due to cytogenetic abnormalities in chromosome 11p15) (10), and a chest neuroblastoma. The patient denied history of smoking, alcohol consumption, or drug use. Physical examination was unrevealing for lid lag, proptosis, conjunctival hyperemia or chemosis. The thyroid gland was of normal size without tenderness on

palpation, and there was no cervical lymphadenopathy. The skin examination and deep tendon reflexes were normal. The heart rate was normal (70 beats per minute) with regular rate and rhythm. There were no tremors observed either in the tongue or in the upper extremities. There was slight enlargement of left hand and foot from BW syndrome. The patient was initially diagnosed with subclinical hyperthyroidism and was monitored without therapy. After endocrine consultation was requested for the patient, it was noted that the total triiodothyronine (T3) values were not measured. Therefore, FT4 and total T3 were measured using mass spectrometry along with TSH using immunoassay on a plasma sample stored at −80°C that was obtained 1 month prior to ^{177}Lu-DOTATATE therapy. In this sample, the TSH was suppressed [<0.02 μIU/ml (0.27–4.2 μIU/ml)], FT4 was elevated [3.9 ng/dl (1.2–2.9 ng/dl)], along with an total T3 of 235 ng/dl (65–193 ng/dl), confirming a diagnosis of primary hyperthyroidism in retrospect, biochemically manifesting as thyrotoxic phase of Hashimoto's thyroiditis.

One month after the first cycle of 200mCi dose of ^{177}Lu-DOTATATE therapy, the patient developed new onset fatigue and experienced a weight gain of 2.6 kg. Physical examination revealed normal skin, heart rate, and deep tendon reflexes. The thyroid examination was unremarkable with no cervical lymphadenopathy. At this point, the TSH had substantially increased (73.04 μIU/ml), along with reduction in the levels of FT4 (0.3 ng/dl) on immunoassay, and reduction in total T3 levels [54 ng/dl (87–169 ng/dl)] on mass spectrometry. Anti-TPO antibodies were >1,000 IU/ml, and anti-Tg antibodies were 668 IU/ml (**Table 1**). Subsequently, weight-based levothyroxine therapy was initiated. On follow-up visits, the TFTs normalized and his symptoms improved. A diagnostic gallium 68 (^{68}Ga)-DOTATATE positron emission tomography-computed tomography (PET/CT) that was performed prior to the initiation of ^{177}Lu-DOTATATE therapy revealed diffuse increase in the uptake in the entire thyroid gland, with a maximum standardized uptake value (SUVmax) of 14.3 (**Figures 1A–D**). A post-treatment single-photon emission computed tomography (SPECT) scans revealed diffuse uptake of ^{177}Lu-DOTATATE in the thyroid gland (**Figures 1E, F**). Since then, the patient has continued his enrollment in the ^{177}Lu-DOTATATE trial and is on replacement levothyroxine therapy with serial monitoring of TFTs.

TABLE 1 | Thyroid function tests in the patient before and after ^{177}Lu-DOTATATE therapy.

Time course	TSH (0.27–4.2 μIU/ml)	Free T4 (0.9–1.7 ng/dl)	Total T3* (65–193 ng/dl)	Anti-TPO Ab (0.0–34.9 IU/ml)	Anti-Tg Ab (0.0-40.0 IU/ml)	TrAb (≤1.75 IU/L)	TSI index (≤1.3)
Before ^{177}Lu-DOTATATE therapy	<0.01 μIU/ml	1.3 ng/dl	235 ng/dl	605 IU/ml	178 IU/ml	<1 IU/L	<1
One month after the first dose of ^{177}Lu-DOTATATE therapy	73.04 μIU/ml	0.3 ng/dl	54 ng/dl**	>1,000 IU/ml	668 IU/ml	Not measured	Not measured

Total T3 measurements were performed using mass spectrometry. Free T4 and TSH values were measured using immunoassay.

***Total T3 at this time had a different reference range (87–169 ng/dl) due to a change in the mass spectrometry assays at our institution.*

TSH, Thyroid stimulating hormone; T4, Thyroxine; T3, Triiodothyronine; TPO, Thyroid peroxidase; Tg, Thyroglobulin; TrAb, TSH receptor antibody; TSI, Thyroid stimulating immunoglobulin.

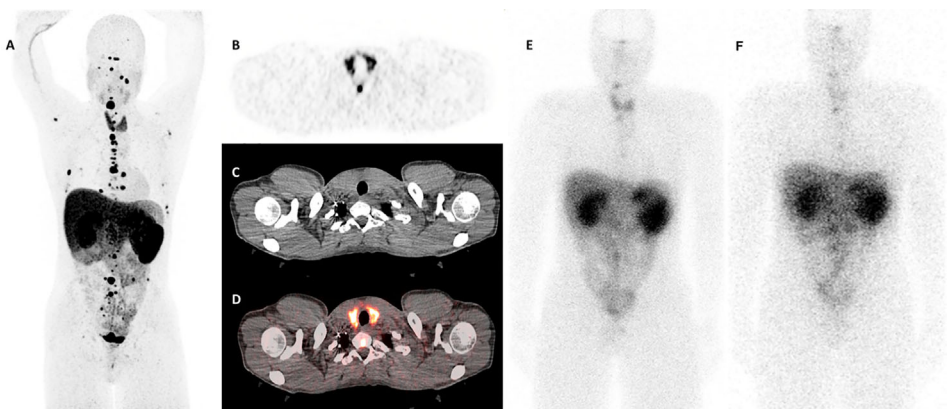

FIGURE 1 | Thyroid uptake patterns in the patient before and after ^{177}Lu-DOTATATE therapy. **(A)** A whole body (maximum intensity projection) view on the diagnostic ^{68}Ga-DOTATATE scan demonstrating multiple paragangliomas as well as a diffuse uptake in the thyroid gland with a maximum standardized uptake value (SUVmax) of 14.3. **(B–D)** Axial slices at the thyroid level: **(B)** Positron emission tomography (PET) scan only. **(C)** Computed tomography (CT) scan only. **(D)** Fused PET and CT images. Images **(B, D)** demonstrate diffuse uptake in the thyroid gland. **(E, F)** The 24-h post-treatment whole body single-photon emission computed tomography (SPECT) scans demonstrating diffuse uptake of ^{177}Lu-DOTATATE in the thyroid gland **(E)** After the first cycle of therapy, and **(F)** after the second cycle of therapy.

DISCUSSION

We report a patient with an initial diagnosis of asymptomatic primary hyperthyroidism who went on to develop overt, symptomatic primary hypothyroidism with marked changes in the TFTs and anti-thyroid antibody titers over a span of 1 month after the initiation of ^{177}Lu-DOTATATE therapy, along with the imaging evidence of increased thyroid uptake of ^{177}Lu-DOTATATE on SPECT imaging. Reports on sustained thyroid dysfunction following ^{177}Lu-DOTATATE therapy are exceedingly rare and, to the best of our knowledge, a switch from hyperthyroidism to hypothyroidism associated with ^{177}Lu-DOTATATE therapy, along with complementary functional imaging evidence has not been previously reported in the literature. However, it is not unlikely that the patient might have been on the natural course of Hashimoto's thyroiditis from thyrotoxic phase toward hypothyroid phase irrespective of ^{177}Lu-DOTATATE therapy, and the usual time course for such progression tends to be 1–24 months (11). However, the temporal association of marked changes in TFTs as well as a significant increase of anti-thyroid antibody titers associated with ^{177}Lu-DOTATATE therapy, along with evidence of increased ^{177}Lu-DOTATATE uptake on the SPECT imaging likely suggests a contribution of PRRT to thyroid disruption. Moreover, development of autoimmune thyroiditis has in fact been reported following ^{177}Lu-DOTATATE therapy (9). In a Dutch cohort treated with ^{177}Lu-DOTATATE for various forms of NETs, Teunissen et al. evaluated the pituitary-thyroid axis in 66 patients over a follow-up period of 12–24 months (9). The mean FT4 values changed from 1.38 ng/dl to 1.21 ng/dl over the treatment course, while there were no significant changes in TSH and total T3 levels. Two patients developed primary hypothyroidism: one patient developed anti-TPO antibody-positive hypothyroidism after the third cycle of treatment, while the other patient gradually developed hypothyroidism, needing hormone replacement 3.5 years after PRRT. However, patients with prior TFT abnormalities were excluded in this study.

The plausible mechanism for the rapid progression from hyperthyroid to hypothyroid phase in this patient could have been due to thyroid parenchymal destruction induced by ^{177}Lu-DOTATATE therapy followed by accelerated autoimmune destruction. Prior to the treatment with ^{177}Lu-DOTATATE, the patient had primary hyperthyroidism, and the presence of elevated anti-TPO and anti-Tg antibodies and an undetectable TrAb, suggested that the patient was likely in the thyrotoxicosis phase of Hashimoto's (autoimmune) thyroiditis. ^{177}Lu-DOTATATE therapy may have caused further damage to the thyroid follicles leading to increased exposure of thyroid parenchymal antigens (TPO, Tg), which in turn may have enhanced autoimmune-mediated destruction facilitating rapid progression toward overt hypothyroidism. This could explain the sudden increase in anti-TPO and anti-Tg antibodies, as well as TSH levels along with a concomitant reduction in FT4 and total T3 levels observed after ^{177}Lu-DOTATATE therapy. Another differential diagnosis to consider in this patient would be antibody-negative Graves' disease, which can be prevalent in 3%–5% of Graves' disease patients, especially among those with new diagnosis or those with milder forms of the disease (12).

The exact mechanism of ^{177}Lu-DOTATATE-associated thyroid disruption has not been elucidated. Cytotoxicity caused by the ^{177}Lu radionuclide is possible, as somatostatin analogs have been previously shown to localize in the thyroid gland (13). In a retrospective analysis on a cohort of 237 patients who underwent ^{68}Ga-DOTATATE imaging to localize unknown or metastatic NETs, 26 (11%) patients had an abnormal thyroid uptake, with 14 (54%) patients having focal uptake and 12 (46%) patients having diffuse thyroid uptake based on SUVmax measurements on the PET/CT (13). Among the patients with diffuse thyroid uptake, 42% had a history of hypothyroidism. Our patient was also found to have diffuse thyroid uptake on the diagnostic ^{68}Ga-DOTATATE obtained prior to ^{177}Lu-DOTATATE therapy, but he had hyperthyroidism at the time of diagnosis. ^{68}Ga-DOTATOC, a somatostatin analog that predominantly targets SSTR-2 and SSTR-5 has demonstrated increased uptake in five of eight cases of Hashimoto's thyroiditis (14). In the present case, although the thyroid uptake of ^{68}Ga-DOTATATE was less than paraganglioma uptake on the baseline DOTATATE PET scan (**Figure 1A**), this finding was reversed in the whole body scintigraphy performed at 24-h post ^{177}Lu-DOTATATE administration, with higher uptake in the thyroid gland compared to the paragangliomas (**Figures 1E, F**). This may point to a higher radiation dose to the thyroid than the baseline PET scan would suggest. This difference in uptake may be a reflection of slight changes in the biodistribution of the ^{68}Ga-chelated versus the ^{177}Lu-chelated DOTATATE agent, or due to differing radiopharmaceutical kinetics and organ/tumor washout times since the ^{68}Ga and ^{177}Lu images were acquired at different times post injection (1 and 24 h, respectively) (15). Furthermore, these findings could also be related to the differences in resolutions of PET imaging for ^{68}Ga-DOTATATE compared to planar imaging for ^{177}Lu-DOTATATE. These types of discrepancy between Ga-68 and Lu-177 DOTATATE have been previously reported (16).

Other somatostatin analogs such as indium 111 (^{111}In) pentetreotide are known to demonstrate physiological uptake into thyroid tissue (17). In addition, increased uptake of ^{111}In pentetreotide in a right lateral ectopic thyroid gland located at the carotid bifurcation has also been reported (18). This structure was surgically removed for the concerns of a paraganglioma, but final pathology revealed thyroid tissue with enlarged follicles, although evidence of any autoimmune destruction was not reported. These data may suggest that an increased uptake in the thyroid gland on a diagnostic somatostatin analog imaging study might predict potential thyroid disruption following ^{177}Lu-DOTATATE therapy or other forms of PRRT.

^{177}Lu-DOTATATE and other radionuclide somatostatin analogs demonstrate high affinity to SSTR-2 (19). SSTR-2 expression has been shown to be substantially prevalent in the thyroid, both in the normal tissue as well as in thyroid tumors (19). In a study by Druckenthaner et al., expression of *SSTR* mRNA was identified in 94% (16 out of 17) of the normal thyroid tissue samples, out of which 82% (14 out of 17) samples expressed *SSTR2* mRNA (19). Somatostatin analogs such as ^{68}Ga DOTATOC have also demonstrated uptake in normal thyroid glands (14). Therefore, it is likely that ^{177}Lu-DOTATATE may target those thyroid glands that harbor higher levels of SSTR-2.

Apart from pituitary-thyroid axis, endocrine disruption associated with ^{177}Lu-DOTATATE therapy has also been noted to affect pituitary-adrenal and pituitary-gonadal axes, albeit most of these effects are transient (9). These data may suggest that the endocrine system may be susceptible to PRRT-induced structural and functional disruption to a variable degree, and the risk of potential disruption might depend on the extent of SSTR-2 expression. In conclusion, ^{177}Lu-DOTATATE therapy can be associated with transient or permanent disruption of thyroid function. Identification of uptake patterns in the thyroid gland on pre-treatment diagnostic imaging studies (such as ^{68}Ga-DOTATATE) as well as presence of anti-TPO/anti-Tg antibodies may predict the susceptibility of a patient to PRRT-associated induction or exacerbation of thyroid dysfunction. In addition, patients with pre-existing autoimmune thyroid disease might be at a higher risk for accelerated destruction of the SSTR2-positive thyroid tissue following PRRT. Therefore, measurement of TFTs along with thyroid auto-antibody profile (anti-TPO, anti-Tg, and TrAb/TSI) should be considered prior to initiation of PRRT and periodically assessed throughout the course of therapy. Although not performed in our patient, thyroid ultrasonography along with color flow Doppler could serve as a useful tool to assess the physical characteristics as well as vascularity of the thyroid gland among patients with disrupted TFTs. Further studies with prospective data are needed to assess the spectrum, duration, and prognosis of PRRT-associated endocrine disruption, and to evaluate its association with pre-treatment imaging uptake patterns and with endocrine autoimmunity.

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 National Institutes of Health. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SG prepared the initial draft of the manuscript and revised the manuscript. MA-J, KP, and JK-G critically reviewed and revised the manuscript and provided input on the endocrine aspects of the manuscript. AJ, MK, JZ, BT, JC, EL, and FL critically reviewed and revised the manuscript and provided input on Lutathera therapy and radiological evaluation. JK-G and FL contributed equally toward the senior authorship of this manuscript. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Primary Neuroendocrine Neoplasms of the Breast: Still Open Issues

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Neuroendocrine breast tumors represent a rare subtype of breast cancer, accounting for less than 1% of all neuroendocrine neoplasms. Starting from their pathology definition, and going through their prevalence, prognosis and treatment, our knowledge is still really uncertain. In the present short review of the medical literature on this topic, we have evaluated in details their epidemiology, risk factors, pathogenesis, pathology, clinical presentation, radiographic aspects, prognosis, and therapy. We have thus been able to identify a number of open issues regarding primary neuroendocrine neoplasms of the breast that need to be clarified. Our ultimate aim was actually to try to understand whether neuroendocrine neoplasms of the breast can be considered a definite clinical entity and if neuroendocrine differentiation of breast tumors has a really clinical relevance.

Keywords: breast neuroendocrine neoplasms, breast carcinoma, review, neuroendocrine, breast cancer

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INTRODUCTION

Neuroendocrine neoplasms (NENs) are a group of heterogeneous tumors deriving from neuroendocrine cells. Neuroendocrine cells are scattered around the body. Therefore, NENs have been reported to arise in multiple sites, such as central nervous system, respiratory tract, larynx, gastrointestinal tract, thyroid, skin, breast, and urogenital system (1).

Primary neuroendocrine neoplasms of the breast (BNEN) are particularly rare, accounting for less than 1% of NENs. Furthermore, the definition of BNEN is still quite confused (2). In the 2003 World Health Organization (WHO) Pathology and Genetics of Tumours of the Breast and Female Genital Organs, BNEN were recognized as a distinct entity (3) requiring -as diagnostic criteria- the expression of NE markers (specifically chromogranin and synaptophysin) in more than 50% of cells (4). It was later revised and the term changed into carcinomas with NE features in the 2012 WHO Classification of Tumours of the Breast (5), with the 50% threshold for NE marker positivity considered arbitrary and therefore removed. In the more recent WHO classification published in 2019, BNEN can only be identified only when the proportion of neuroendocrine cells in samples is greater than 90% (6).

BNEN are overall heterogeneous in their definition, being characterized by a various grade of differentiation and histological overlap, and at present, they do not identify a definite clinical entity, and no specific prognosis or therapy have been recognized yet.

The aim of the present mini-review is to focus on light and shadow of our knowledge about BNEN, and to recognize the gaps to be filled in to better define this group of neoplasms as a precise clinical reality.

EPIDEMIOLOGY

Primary BNENs represent a rare and heterogeneous entity, whose real incidence is probably poorly understood because they are often under diagnosed (7–10). Its incidence among breast cancer has been reported to range from 0.1% to 5% (5, 9–12). After the WHO classification of breast tumors first recognizing BNEN as a separate unique entity in 2003, Gunhan-Bilgen et al. (2003) and Lopez et al. (2008) analyzed large series of breast cancers, including 1845 and 1368 cases respectively, and estimated that BNEN incidence varied between 0.3% and 0.5% of all breast cancers (11, 12). In 2012 the novel WHO Classification of Tumors of the Breast established that the diagnosis of a NE breast cancer can be made regardless of the percentage of tumor cells expressing NE markers. According to the 2012 WHO classification, primary BNEN would account for 2% to 5% of breast cancers (5, 13). However, Wang et al. analyzed the Surveillance, Epidemiology, and End Results (SEER) registries during 2003–2009 and reported 142 cases of primary BNEN, accounting for no more than 0.1% of total breast cancers, much less than the rate reported by the WHO (9). The relevant changes in the WHO classification criteria for BNEN over the years may explain the large differences in the incidence rate between one study and another. Consequently, further epidemiological studies should be performed by histologically revising tumor samples according to updated and uniform criteria.

Most patients with BNEN are postmenopausal women between their fifth and seventh decade of life (mostly aged >60 years), while the incidence in younger premenopausal women is still lower (5, 9, 14–17). Males are rarely affected by BNEN (9, 15–17). However, despite the overall low incidence, there are proportionally more males with BNEN than with other mammary cancers (9, 17). The distribution of ethnicity for BNEN seems to be similar to other mammary cancers (9).

The main risk factors for BNEN are currently believed to be the same as for non-neuroendocrine breast cancer, such as age and family history. Reproductive factors, as early menarche or late menopause, may also increase the risk of this disease, as well as significant exposure to estrogens, typical of patients taking oral contraceptives or undergoing hormone replacement therapy (HRT) (18). Nevertheless, the risk of breast cancer has been shown to significantly decrease after two years of discontinuation of HRT, as well as in women who stop taking oral contraceptives for more than 10 years (18–20). Evidence exists suggesting a link between high prolactin levels and risk of breast cancer development; however, it is unclear whether this is true for BNEN too. Prolactin has been shown to regulate breast stem cells and progenitor cells through its receptor (PRLR), acting as a pro-tumorigenic pathway (21). Moreover, PRL *via* PRLR is able to promote migration and invasion of breast cancer cells (22). Recently Zang et al. published two cases of BNEN associated with hyperprolactinemia, one patient suffering from mental disorder under antipsychotic drugs, and another one diagnosed with BNEN in late pregnancy, suggesting that hyperprolactinemia may represent a risk factor for the development of BNEN (23).

PATHOGENESIS

Current knowledge on natural history and pathogenic mechanisms of breast tumors with NE features is not clearly defined yet. There are different hypotheseson the pathogenesis of these neoplasms. The more controversial one suggests their origin from neoplastic transformation of native neuroendocrine cells constitutively present in the breast, despite little evidence of neuroendocrine cells detectable in benign breast tissue (24, 25). Conversely, according to another assumption, underlying the development of BNEN there would be an early splitting of the neoplastic stem cell differentiation into both neuroendocrine and epithelial lines (26). This assumption is consistent with the typical co-presence of neuroendocrine and exocrine cells in breast poorly differentiated neuroendocrine carcinomas (PD-NECs), and is supported by molecular studies which report a clonal correlation between neuroendocrine cells in PD-NEC and intraductal component of the tumor (25, 27). PD-NECs would therefore seem to originate from a neuroendocrine differentiation of breast cancer cells rather than from endocrine primitive cells.

CLINICAL ASPECTS

Considering the low frequency of BNEN, there is limited knowledge on their specific clinical presentation. There are neither specific clinical signs for this tumor nor differences in the clinical features of primary BNEN compared to other types of breast cancer. Published case series report a slow evolution of these tumors, having as the most frequent reason for consultation being an isolated breast nodule, which is sometimes associated with other local signs (painful axillary adenopathy, bloody nipple discharge, nipple retraction). Clinical presentation with symptoms due to metastatic diffusion (jaundice, hematuria, bone pain, respiratory symptoms, and neuralgia) has also been reported. Less frequently clinical presentations are isolated bloody nipple discharge, isolated skin retraction, anorexia, ulcerated breast masses, carcinomatous mastitis, and Paget like mass (28). In addition, a malignant lesion revealed by routine mammography, performed for family history of breast cancer, has been reported (17). Although extremely rare, a peculiarity of BNEN can be the occurrence of clinical manifestations from ectopic hormonal secretion. Sporadically, case reports of paraneoplastic syndrome with the ectopic production of adrenocorticotropic hormone (29), norepinephrine (30) and calcitonin (31) have been reported.

RADIOLOGY

As well as the clinical features, also the radiological characteristics are unspecific with radiological reports substantially similar to the other malignant breast lesions. Only single case reports (32–35) or small series (11, 17, 36) of BNENhave been reported with their imaging characteristics. Mammographic and ultrasonographic findings of reported BNEN are summarized in **Table 1**. The most common mammographic appearance is a hyperdense, irregularly shaped solitary mass. Margins are more commonly reported as indistinct, microlobulated, or spiculated. In most casescalcifications are absent. Ultrasound evaluation usually shows an irregular or microlobulated hypoechoic lesion, with homogeneous echo texture and no acoustic phenomena. However, posterior acoustic enhancement and, even more rarely, the phenomenon of acoustic shadowing have been reported.

PATHOLOGY

In the last WHO edition NENs of the breast are classified in neuroendocrine tumors (NETs) and neuroendocrine carcinomas (NECs) (6).

Mammary NETs grossly show clear or infiltrative border, and usually are larger than breast carcinoma of special (ST) or no special type (NST). Histologically, they are composed of large or small nests together with trabeculae, associated with numerous small fine vessels and a variably collagenized stroma, like well differentiated NETs of other organs. Those neoplasms with copious mucous production may also have tumor nests floating in large mucinous pools. Tumor cells range from oval to polygonal or plasmacytoid shape, or also spindled. However, they lack typical nuclear features of NETs, such as salt and pepper chromatin and even monotonous round or oval nuclei, more often displaying irregular appearance or the presence of nucleoli (37).

Diagnosis requires immunohistochemistry (IHC) confirmation by tumor expression of neuroendocrine markers. The most widely used are chromogranin A and synaptophysin. Moreover, the insulinoma associated protein 1 (INSM1) has been shown to have a high sensitivity for NENs arising from different organs, and proposed as an additional valuable marker for BNEN (38, 39).

Breast NENs express hormone receptors (estrogen and progesterone receptors) more often than other breast carcinomas (40). On molecular subtyping, they usually belong to either luminal A or luminal B HER2 negative breast cancer (41–43). Breast NETs are graded in the same manner of breast invasive carcinoma of NST. Differential diagnosis can occur with other primitive mammary tumors that share expression of neuroendocrine markers: solid papillary tumor and mucinous carcinoma. Yet these tumors differ consistently from NETs on morphological grounds to be well separated (44).

Metastases to the breast from NENs of other organs can be distinguished through IHC: Cheratin 7, estrogen (ER) and progesterone receptors (PgR), GATA3 and Gross cystic disease fluid protein 15 (GCDFP15) are quite typical for breast NETs. Moreover, GCDFP15 often shows associated ductal carcinoma in situ, which lack in metastases (45).

Mammary NECs are of the small cell type and morphologically indistinguishable from NECs of other organs. Indeed they show small tumor cells densely packed with basophilic nuclei, scarce cytoplasm, and abundant necrosis. IHC will reveal at least focal positivity for one NE marker. They can express ER, PgR, GATA3, and GCDFP15 (46).

Despite NEC is a very rare neoplasm, its definition and features are clear enough and did never change in the last years. Conversely, NET definition has continuously changed in the last WHO classifications and some controversial issues on its nature still persist (47).

Current WHO classification considers breast NET as the extreme spectrum of mixed neuroendocrine non-neuroendocrine breast neoplasms (MiNeN), in which the non-neuroendocrine component is less than 10%. All mammary tumors in which the neuroendocrine component is between 10% and 90% are breast carcinomas of ST or NST and the neuroendocrine part is only worth mentioning.

PROGNOSIS

As far as the prognosis of BNEN is concerned, conflicting results have been reported, probably due to the low prevalence of this disease.

In a retrospective study, Lai et al. showed that in a sample of 224 patients with NENs, breast carcinomas expressing high levels of neuroendocrine markers and cytomorphologic features were characterized by a better prognosis. On the other side, a low level of expression of neuroendocrine markers was correlated with more aggressive clinical parameters, such as higher histological grade and pathological T and N stages. These results are suggestive of a better prognosis of invasive breast cancer with neuroendocrine features compared to their non-neuroendocrine counterparts (48). These data were confirmed in a prospective observational study performed on a cohort of 35 patients with BNEN reporting a lower recurrence rate in breast cancers with an expression of neuroendocrine markers >50% compared to those with focal expression (49). These findings provide a stratification based on neuroendocrine markers expression and may be helpful for detecting better treatment strategies, since all invasive breast carcinomas are currently treated according to non-endocrine tumor components guidelines. Furthermore, the study from Lai et al. provides an interesting food for thought, demonstrating a different behavior of CD56-only positive carcinomas compared to those expressing chromogranin and synaptophysin (48). In 2017, Rosen et al. demonstrated a longer overall survival (OS) and disease-free survival (DFS) in patients with BNEN treated with endocrine therapy/radiation therapy rather than chemotherapy, compared to those who did not receive treatment (45). Indeed, no real consensus has been reached on the prognosis for BNEN.

TABLE 1 | Mammographic (panel a) and ultrasonographic (panel b) findings in neuroendocrine neoplasms of the breast (BNEN).

Panel a

Reference	Patients series (nr.)	Location (nr.)	Parenchymal pattern (nr.)	Mass category (nr.)	Margin (nr.)	Shape (nr.)	Calcification (nr.)
Günhan-Bilgenl. et al. (11)	CS (5)	UOQ (4); UIQ (1)	fatty (1); heterogeneous density (4)	solitary (4); clustered several nodules (1)	indistinct (1); spiculated (2); microlobulated (1); lobulated (1)	irregular (1); round (4)	absent (5)
Fujimoto Y. et al. (33)	CR	nd	nd	nd	nd	nd	nd
Wu J. et al. (36)	CS (13)	UOQ (5); LIQ (1); UIQ (1); UQ (1); RETRO (2);	scattered fibro glandular density (7); Fatty (2); heterogeneous density (1)	solitary (5); focal asymmetry (12); clustered several nodules (1)	Indistinct (4); microlobulated (3)	irregular (4); round-ovoid (3)	a few, punctuate (1); solitary, punctuate (1); absent (7)
Chang E.D. et al. (32)	CR	UOQ, RETRO	high density	solitary	indistinct	irregular	absent
Yoon Y.S. et al. (34)	CR	LOQ	high density	solitary	Indistinct	round	absent
Hejjane L. et al. (35)	CR (2)	UQ (1); LQ (1); Right (2)	heterogeneous (2)	solitary (2)	irregular (2)	irregular (2)	absent (2)
Ozdirik B. et al. (17)	CS (5)	UOQ (3); LOQ(1); UQ (1) LQ (1)	high density (1)	solitary (1); clustered several nodules (1)	spiculated (1); indistinct (1)	irregular (1); round-ovoid (1)	absent (5)

Panel b

Reference	Patients series (nr.)	Margins (nr.)	Shape (nr.)	Echogenicity (nr.)	Echo texture (nr.)	Acoustic Phenomena (nr.)
Günhan-Bilgenl. et al. (11)	CS (5)	circumscribed (1); microlobulated (2); irregular (2)	irregular (2); round (3)	hypoechoic (5);	homogeneous (4); heterogeneous (1)	none (4); enhancement (1)
Fujimoto Y. et al. (33)	CR	indistinct	irregular	hypoechoic	homogeneous	enhancement
Wu J. et al. (36)	CS (13)	indistinct (4); circumscribed (3); microlobulated (1)	irregular (7); round-ovoid (2);	hypoechoic (9);	homogeneous (4); heterogeneous (5)	none (6); enhancement (2); shadowing (1)
Chang E.D. et al. (32)	CR	lobulated	irregular	hypoechoic	heterogeneous	enhancement
Yoon Y.S. et al. (34)	CR	irregular	round	hypoechoic	homogeneous	enhancement
Hejjane L. et al. (35)	CR (2)	irregular (1);	not reported	hypoechoic (2)	homogenous (2)	none (2)
Ozdirik B. et al. (17)	CS (5)	not reported	not reported	not reported	not reported	not reported

nr, number of cases; nd, not detected; CR, Case Report; CS, Case Series; RETRO, retro areola area; UOQ, upper outer quadrant; LIQ, lower inner quadrant; UIQ, upper inner quadrant; UQ, upper quadrant; LQ, lower quadrant; LOQ, lower outer quadrant.

THERAPY

There are no specific guidelines for the treatment of BNEN and the available data mainly descend from anecdotal and case reports. A standardized therapeutic scheme is extremely difficult to define since these are particularly rare and heterogeneous tumors. Currently most of the adopted strategies involve staging and treating BNEN similarly to conventional breast cancer (50), although it is recommended to consider their neuroendocrine origin, especially in the management of well-differentiated tumors (see **Table 2**).

The treatment of BNEN is primarily surgical, with partial or total mastectomy in relation to staging and tumor localization (51–54), possibly associated with axillary dissection and/or metastasectomy. Surgery isoften followed by adjuvant radiotherapy, especially for well and moderately differentiated BNEN (25).

Neoadjuvant or adjuvant chemotherapy is widely used in patients with high risk of recurrence or with metastatic or locally invasive disease (25). In clinical practice, well-differentiated BNEN and invasive breast carcinoma with neuroendocrine differentiation (IBC-NED) are usually treated with protocols commonly adopted for conventional breast cancer (eg, epirubicin and cyclophosphamide) and PD-NEC as for small cell lung cancer (eg, carboplatin and etoposide) (17, 52, 55–59, 61). Escape is frequent after a few months, but the neuroendocrine component can be controlled by anthracycline-based therapy (35).

TABLE 2 | Overview of therapeutic approaches for neuroendocrine neoplasms of the breast (BNEN).

Treatment		References
Surgery	Breast-conserving surgery/Mastectomy +/- Axillary lymph node dissection +/- Metastasectomy	(2, 51–54)
Radiotherapy	Adjuvant Radiotherapy	(25, 55)
Chemotherapy	Doxorubicin/Cyclophosphamide	(17, 34, 35)
	Carboplatin/Etoposide	(55–57)
	Cisplatin/Etoposide	(52)
	Fluorouracil/Epirubicin/Cyclophosphamide +/- Docetaxel	(58–60)
	Paclitaxel +/- Trastuzumab	(61, 62)
Endocrine therapy	Tamoxifen +/- Aromatase inhibitors	(12, 16, 35, 63)
	Somatostatin Analogues (SSAs)	(64)
	Peptide Receptor Radionuclide Therapy (PRRT)	(65)

However, a complete response, with a disease free period of thirty-six months after chemotherapy, was recently observed in one patient treated with six FEC 100 (5-fluorouracil, epirubicin, and cyclophosphamide) cycles followed by docetaxel (60). Another rare case of HER-2 positive NECB was treated with trastuzumab with a reported disease free period of 9 years (62).

Treatment with somatostatin analogues (SSAs) has not shown promising results as yet: this is probablyrelated to a non-negligible percentage of BNEN negative for somatostatin receptors (SSR), as well as to theconservative dosages mainly used and to the often advanced disease (64).

The use of peptide receptor radionuclide therapy (PRRT) is currently limited to the treatment of BNEN with somatostatin-receptor (SSR) positivity documented on imaging, after failure of conventional chemotherapy, or even as first-line treatment for advanced disease (65).

Anti-hormonal therapy (eg, aromatase-inhibitors), although not codified, has proven to be effective as adjuvant therapy in some cases of hormonal receptor positive BNEN (12, 35, 63).

Although other agents, such as mTOR inhibitors, are effective for the therapy of different types of NENs, and are also approved, in combination with aromatase-inhibitors, for the treatment of hormone receptor positive advanced breast cancer, currently the use of everolimus has only been hypothesized for BNEN.

Furthermore, Trevisi et al. have quite recently suggested the possibility of target therapy in BNEN, having been reported in a significant percentage of these tumors a mutation of PIK3CA, with alpelisib, as well as target therapy with sacituzumab govitecan targeting TROP-2 protein expressed in a small proportion of BNEN (66).

OPEN ISSUES AND CONCLUSIONS

Albeit BNEN were firstly described more than 40 years ago, and have been categorized more and more precisely thereafter, its rarity, together with still persisting diagnostic uncertainties, hampers drawing a precise clinical and prognostic picture. Furthermore, the lack of randomized controlled trials performed to compare different treatment strategies and their outcomes makes small case series the best available evidence on this issue, at present (67). Below, some of the main open issues relating to this entity are discussed.

Histogenesis—First of all, the same histogenesis of primary BNEN is still uncertain. Nowadays, according to the predominant assumption, they are thought to arise from metaplastic differentiation of a neoplastic epithelial progenitor cell during early carcinogenesis, rather than from a pre-existing neuroendocrine stem cell (53).

Standardization in neuroendocrine markers use—Neuroendocrine specific markers are not uniformly and/or routinely applied on every breast cancer sample examined. Only in the event that the pathologist suspects the presence of this type of tumor on histopathological analysis, the sample is subsequently evaluated for the expression of neuroendocrine markers.

Even if this was the case, the lack of consensus on the degree of neuroendocrine differentiation required for the diagnosis limited the uniformity of the diagnostic process, so far. Indeed, all BNEN are combined tumors composed of a heterogeneous mixture of exocrine and endocrine cells. Until 2003, a breast cancer could be classified as a primary neuroendocrine tumor when expressing chromogranin A, chromogranin B, or synaptophysin in more than 50% of the total cell population. Later, differently from the previous WHO classification, diagnosis could be done regardless of a cut-off value of tumor cells positively staining for neuroendocrine markers. Therefore, distinguishing a primary BNEN from a tumor with only partial neuroendocrine differentiation largely depends on the pathologist's judgement, lacking objective criteria to make diagnosis in case of uncertainties (43, 45, 68).

Diagnostic criteria—Consequently, the continuously evolving diagnostic criteria favored the lack of a uniform definition applied by different centers, affecting adequate comparisons and explaining the highly variable prevalence depicted by various case series (68).

Altogether, it is therefore very likely that these tumors are often under-diagnosed/under-reported, in routine pathological practice.

Prognostic and clinical implications—Moreover, the clinical and prognostic relevance of neuroendocrine differentiation of breast tumors is still questionable. Conflicting results have been reported on OS and DFS for BNEN vs other types of breast cancer. However, to the best of ourknowledge, none of the already published studies had analyzed primary BNEN based on their distinct histologic or molecular subtypes, rather considering these tumors as a single entity.

Actually, no therapeutic implications apply to the diagnosis of BNEN, and no standardized protocols for treatment of primary BNEN are available. Well-differentiated neuroendocrine breast tumors and invasive breast carcinomas with neuroendocrine differentiations are typically managed with cytotoxic chemotherapy after surgery, similar to more classical types of breast cancer, and poorly-differentiated neuroendocrine breast carcinomas with similar protocols as for small cell lung cancer, whereas hormonal therapy is used according on receptor status.

Relevance of somatostatin-receptor expression—Lastly, anecdotal experience concerning the relevance of SSR expression of BNEN for diagnostic (eg, Octreoscan and 68-Ga-DOTATATE PET) and therapeutic (PRRT) purposes represents another main criticism. Presently, PRRT has been recommended for SSR-positive tumors after failure of conventional therapy, but very few reports are available.

Because of all these issues, the discussion of each individual case in an interdisciplinary group of NEN experts is worthwhile in order to provide a tailored treatment for each individual patient (17).

In conclusion, it is likely that the cases diagnosed as primary BNEN are only minimally representative of their real prevalence. Only a systematic evaluation of neuroendocrine markers on all analyzed cases of breast cancer could give a reliable evaluation of the frequency of these tumors. Cancer registries centralizing uniform data collection, together with large multicentric studies, could sharpen our knowledge in the next future.

On the other hand, larger histologic and molecular-profiling studies of this rare but often under-reported cancer, with correlation with clinical data, would be warranted to shed light on BNEN. It is also possible that, at the end of these efforts, we will come to the conclusion that neuroendocrine features poorly add (if any) to the diagnosis and management of breast cancer, but the time has come to conclusively define the clinical relevance of neuroendocrine differentiation of breast tumors.

AUTHOR CONTRIBUTIONS

All authors have contributed equally to the conception and design of the review. MG, AF, and AC conceived the review. MG, SC, VV, NF, FC, EM, and RR reviewed published literature and drafted the article. AF and AC revised the manuscript critically. All authors contributed to the article and approved the submitted version.

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Cardio-Metabolic Indices and Metabolic Syndrome as Predictors of Clinical Severity of Gastroenteropancreatic Neuroendocrine Tumors

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Background: Obesity, mainly visceral obesity, and metabolic syndrome (MetS) are major risk factors for the development of type 2 diabetes, cardiovascular diseases, and cancer. Data analyzing the association of obesity and MetS with gastroenteropancreatic neuroendocrine neoplasms (GEP-NEN) are lacking. Fatty liver index (FLI) is a non-invasive tool for identifying individuals with non-alcoholic fatty liver disease (NAFLD). Visceral adiposity index (VAI) has been suggested as a gender-specific indicator of adipose dysfunction. Both indexes have been proposed as early predictors of MetS. This study aimed to investigate the association of FLI VAI as early predictors of MetS with gastroenteropancreatic neuroendocrine tumors (GEP-NETs).

Methods: A cross-sectional, case–control, observational study was carried out at the ENETS Centers of Excellence Multidisciplinary Group for Neuroendocrine Tumors, University "Federico II". VAI and FLI were calculated.

Results: We enrolled 109 patients with histologically confirmed G1/G2 GEP-NET (53 M; 57.06 ± 15.96 years), as well as 109 healthy subjects, age, sex- and body mass index-matched. Forty-four GEP-NET patients were G2, of which 21 were with progressive disease, and 27 patients had metastases. GEP-NET patients had a higher value of VAI ($p < 0.001$) and FLI ($p = 0.049$) and higher MetS presence ($p < 0.001$) compared with controls. VAI and FLI values and MetS presence were higher in G2 than in G1 patients ($p < 0.001$), in patients with progressive disease, and in metastatic *vs* non-metastatic patients ($p < 0.001$). In addition, higher values of VAI and FLI and higher MetS presence were

significantly correlated with the worst clinical severity of NENs. The cut-off values for the FLI and MetS to predict high grading of GEP-NETs and the presence of metastasis were also provided.

Conclusions: This is the first study investigating an association between VAI and FLI as early predictors of MetS and GEP-NET. Our findings report that the worsening of clinicopathological characteristics in GEP-NET is associated with higher presence of MetS, NAFLD, evaluated by FLI, and visceral adiposity dysfunction, evaluated by VAI. Addressing the clinical evaluation of MetS presence, NAFLD, and visceral adiposity dysfunction might be of crucial relevance to establish targeted preventive and treatment interventions of NEN-related metabolic comorbidities.

Keywords: gastroenteropancreatic neuroendocrine tumors, visceral adiposity index, fatty liver index, cardio-metabolic indices, metabolic syndrome

INTRODUCTION

Neuroendocrine neoplasms (NENs) represent a group of tumors characterized by a wide biological variability and clinical heterogeneity. NENs originate from the cells of the neuroendocrine system and they can arise in all tissues and organs; however, the gastroenteropancreatic (GEP) and respiratory tracts are the most frequently affected sites (1). Of all malignant cancers, NENs represent only 2%, although recent epidemiological data report a progressive increase in their incidence (2). When NENs are not associated with any endocrine syndrome, their diagnosis may be delayed for years by non-specificity of presenting, with the frequent progression to a metastatic stage prior to clinical diagnosis (3–5).

Very recently, the classification of the World Health Organization (WHO) recognized three forms of well-differentiated GEP-NENs, classified as G1, G2, and G3 neuroendocrine tumors (NETs), based on the proliferative activity expressed by the Ki67 index (6). GEP-NETs have a variable aggressiveness and are associated with a good to moderate survival, but poorly differentiated NENs, the so-called neuroendocrine carcinoma (NEC), are associated with a higher Ki67 index and a poorer prognosis (6).

The association of environmental factors, including obesity, mainly visceral obesity, metabolic syndrome (MetS), and non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of MetS (7), has been implicated as risk factors for different cancers (8, 9). However, the amount of evidence concerning their possible role as risk factors in NEN pathogenesis is still limited. There is an expanding interest towards the effect of diets on body composition, metabolic parameters, and oxidative status (10). Evidence suggests that there are multiple sources of oxidative stress in obesity, and it may have an influence on carcinogenesis (11). Polyphenols counteract oxidative stress, and the potential protective effect of substances as resveratrol has been investigated (12, 13). The link between obesity and cancer involves possible epigenetic modulators (14, 15), and it could impact on novel therapeutic approaches.

Growing results show the relationship between MetS or its components with several types of cancer development and cancer-related mortality (16, 17). It is suggested that the adipokines secreted from visceral adipocyte dysfunction, and the development of NAFLD play a key role in this association (18).

The gold standard for the diagnosis of NAFLD is the liver biopsy, even though non-invasive techniques, such as magnetic resonance imaging, computed tomography, and liver ultrasonography report adequate concordance with histological results (19). The fatty liver index (FLI), a simple algorithm based on parameters that are routine measurements in clinical practice, such as body mass index (BMI), waist circumference (WC), triglycerides (TGs) and glutamyltransferase (GGT), shows a high concordance with the liver imaging techniques and the histological criteria representing a useful tool to predict the presence of NAFLD (20). In addition, since most variables included in this algorithm are also traditional risk factors for cardiovascular diseases (CVD), FLI has also proved to be an early marker of CVD (21).

Visceral fat and liver inflammation are strictly associated in patients with NAFLD (22). Visceral Adiposity Index (VAI) is considered a marker of adipose tissue dysfunction based on BMI, WC in association with functional parameters such as TG and high-density lipoprotein cholesterol (HDL) (23–25). Similarly to FLI, VAI is associated with MetS (26) and several metabolic diseases, including type 2 diabetes mellitus (27). Of interest, both FLI and VAI have been used in several studies as predictors of incident cancer (28–31). In particular, in a very recent study, high FLI values have been reported to predict NAFLD and breast

Abbreviations: NEN, Neuroendocrine neoplasms; NET, Neuroendocrine tumors; GEP, gastro-entero-pancreatic; WHO, World Health Organization; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; FLI, fatty liver index; BMI, body mass index; WC, waist circumference; TGs, triglycerides; GGT, glutamyltransferase; CVDs, cardiovascular diseases; VAI, Visceral Adiposity Index; HDL, high-density lipoprotein cholesterol; SDs, standard deviations; NEC, neuroendocrine carcinoma; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; LDL, Low-Density Lipoprotein; MEN1, multiple endocrine neoplasia type 1; OR, Proportional Odds Ratio; IC, Interval Confidence; AIC, Akaike Information Criterion; ROC, Receiver operator characteristic; AUC, area under the curve.

cancer risk in postmenopausal women (29), while in a population-based longitudinal study VAI was reported as a predictor of incident colorectal cancer (32).

One of the relevant and as yet poorly investigated aspects of the pathogenesis of GEP-NEN is the possible involvement of metabolic dysfunctions, including NAFLD and MetS, particularly in GEP-NETs (G1 and G2), which have a natural history very different from NEC (33). Very recently, Santos AP et al. reported the highest presence of MetS and single risk factors, including WC, fasting plasma glucose, and fasting TG in 96 patients with GEP-NET compared with a control group cross-matched for age and gender (34). However, there is no evidence to date that has evaluated FLI and VAI in patients with NEN, either on their role as an early predictors of MetS.

Based on these premises, this case–control, cross-sectional study aims to investigate the alteration of cardio-metabolic indices, such as VAI and FLI, as early markers for the diagnosis of visceral adiposity dysfunction and NAFLD, respectively, in patients with GEP-NET. In addition, we investigated the possible association of VAI, FLI, and MetS on the clinical severity of NET. Finally, we provided specific cut-offs for cardiometabolic indices to predict grading, presence of metastases, and disease status.

MATERIALS AND METHODS

Design and Setting

This cross-sectional case–control observational study was carried out at the Department of Clinical Medicine and Surgery, Unit of Endocrinology, European Neuroendocrine Tumor Society (ENETS) Center of Excellence Multidisciplinary Group for Neuroendocrine Tumors, University "Federico II" of Naples. Both GEP-NET patients and controls were recruited from May 2017 to January 2020. The "Federico II" Medical School Ethical Committee has approved this cross-sectional case–control observational study (n. 201/17), which was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The purpose of the study was explained to all participants, and a written informed consent was obtained.

Population Study

The study has been conducted on 218 adult Caucasian subjects, in particular 109 GEP-NET patients and 109 healthy individuals as a control group enrolled among hospital volunteers and in the opera prevention project (35), and employees from the same geographical area.

The control group were matched by demographic and anthropometric characteristics, including sex, age, and BMI. In addition, none of the participants had a history of cancer, liver or renal failure, chronic inflammatory diseases, alcohol abuse, and none of them assumed medicaments. In addition, none of the individuals of the control group contemporarily participated in other clinical trials during the period of this study to avoid overlapping enrollment.

To improve the power and homogeneity of this study, only patients meeting the following criteria were included:

- Histological diagnosis of well-differentiated, low grade (G)1 and G2 GEP-NET, according to the classification of by the WHO (6);
- Patients with functioning GEP-NET: biochemically free of disease, without medical treatment, or after surgery performed more than 6 months before recruitment;
- Patients with non-functioning GEP-NET: at the moment of diagnosis treatment-naïve or after endoscopic surgery performed more than 6 months before the recruitment, or discontinuing Somatostatin Analogs (SSAs) for more than 6 months.

Instead were excluded GEP-NET patients meeting one or more of the following criteria:

- Well-differentiated/high grade G3 GEP-NET or poorly differentiated neuroendocrine carcinomas at histological diagnosis, according to WHO classification (6), since it has been reported that grade G3 GEP-NET patients were at risk of malnutrition (36);
- Diagnosis of Merkel cell carcinoma, pheochromocytoma/paraganglioma, medullary thyroid cancer, bronchial, or thymic NET;
- Ongoing medical treatment at the moment of the visit, including SSAs or targeted therapy, since it has been reported that these therapies could affect motor and absorptive functions, gastrointestinal secretory, or cause anorexia and hepatic toxicity (5);
- Individuals who underwent major surgery, since it could change the anatomy of the gastrointestinal tract;
- Patients with functioning GEP-NET who have not undergone gastrointestinal curative surgery for less than 6 months before recruitment and that were not pharmacologically treated at the moment of recruitment with drugs that affect the secretion of hormones (peptides and amines) which could cause dysfunction of the gastrointestinal tract, including altered motility, diarrhea, steatorrhea, and malabsorption (5);
- Based on a complete medical examination and laboratory investigation, the presence of clinical diseases that could influence metabolism, including liver or renal failure, acute or chronic inflammatory diseases, and history of other types of cancer;
- Abuse of alcohol intake defined by the DSM-V criteria (37).

Power Size Justification

The power of the sample was calculated by the difference of means ± standard deviation (SD) of the number of risk factors of MetS between GEP-NET and control group (2.06 ± 1.52 *vs* 0.97 ± 1.13; respectively). Considering that the number of cases required in GEP-NET and control group was 102, we have set at 109 the number of patients for GEP-NET and at 109 individuals for the control group.

The calculated power size was 95%, with a type I (alpha) error of 0.05 (95%), and a type II (beta) of 0.05. The calculations of

sample size and power were performed while using a sample size calculator Clinical Calc (38), as previously reported (39–42).

Physical Activity and Smoking Habits

Physical activity were evaluated by a standard questionnaire that expressed whether the participant habitually engaged at least 30 min/day of aerobic exercise (YES/NO), as already reported in several other previous studies (43–45). Similarly, through a standard questionnaire, individuals were considered as 'former smokers' when they stopped smoking at least one year before the interview, 'current smokers' when smoking at least one cigarette per day, and 'non-current smokers', as previously reported (46–48). Former and non-current smokers were considered as 'no-smokers' for the analyses.

Anthropometric Measurements

Anthropometric measurements were obtained with participants wearing light clothes and without shoes. Height and body weight were measured to the nearest 1 cm using a wall-mounted stadiometer and derived to the nearest 50 g using a calibrated balance beam scale, respectively (Seca 711; Seca, Hamburg, Germany). BMI was calculated by weight and height [weight (kg) divided by height squared (m^2), kg/m^2]. According to WHO's criteria, participants were classified by BMI as normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25.0–29.9 kg/m^2), grade I obesity (BMI 30.0–34.9 kg/m^2), grade II obesity (BMI 35.0–39.9 kg/m^2), grade III obesity (BMI ≥ 40.0 kg/m^2), as previously reported (49–52).

In line with the National Center for Health Statistics (NCHS), WC was measured to the closest 0.1 cm at the natural indentation or at a midway level between the lower edge of the rib cage and the iliac crest if no natural indentation was visible using a non-stretchable measuring tape (53).

Blood Pressure and Criteria to Define MetS

Systolic (SBP) and Diastolic (DBP) Blood Pressures were measured in all participants three times, and the mean of the second and third reading was recorded after the subject had been sitting for at least 10 min, with a random sphygmomanometer (Gelman Hawksley Ltd., Sussex, UK), as explained in other previous studies (54–56).

MetS was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III definition if three or more of the following five criteria are present: WC ≥102 cm (men) or 88 cm (women), blood pressure ≥130/85 mmHg, fasting TG level ≥150 mg/dl, fasting HDL cholesterol level ≤40 mg/dl (men) or ≤50 mg/dl (women), and fasting glucose ≥100 mg/dl (57).

Cardio-Metabolic Indices

VAI score has been calculated by the following sex-specific formula. Both triglycerides and HDL levels were expressed in mmol/L. Age-specific VAI cut-off values were used according to Amato MC et al. (23, 58).

Males: $VAI = [WC/39.68 + (1.88 * BMI)] * (TG/1.03) * (1.31/HDL)$

Females: $VAI = [WC/36.58 + (1.89*BMI)] * (TG/0.81) * (1.52/HDL)$

FLI was calculated with the formula: [FLI = eL/(1 + eL) × 100, L = 0.953 × loge TG + 0.139 BMI + 0.718 × logeγGT + 0.053 × WC-15.745]. FLI of 30 was considered as the cut-off value on the basis of Bedogni's criterion (59).

Assay Methods

After an overnight fast of at least 8 h, samples were collected in the morning between 8 and 10 a.m. and stored at −80°C until being processed. All biochemical analyses were performed with a Roche Modular Analytics System in the Central Biochemistry Laboratory of our Institution. Low-Density Lipoprotein (LDL) cholesterol and HDL cholesterol were determined by a direct method (homogeneous enzymatic assay for the direct quantitative determination of LDL and HDL cholesterol).

Clinicopathological Characteristics of the Tumor

In this study, we enrolled patients with G1–G2 GEP-NET, collecting data about primary tumor site, mitotic count, Ki67 index, tumor size, stage, genetic syndromes as multiple endocrine neoplasia type 1 (MEN1), presence of metastases or clinical functioning syndromes, comorbidities, and therapies for each patients.

Tumor size (mm) was calculated as the maximum diameter in the pathological specimen of the tumor or in the last imaging (computed tomography or magnetic resonance imaging) when surgery was not performed. In patients with multiple pancreatic lesions, as in MEN1, we considered the diameter of the biggest nodule. Only in a few cases (n = 3) the tumor size was not defined since the primary lesion was not found.

Tumor grade followed WHO 2010 classification, and tumor stage was defined according to the ENETS criteria, and patients were classified with localized disease (stages I–III) or advanced disease (presence of metastases, stage IV) (60). Immunohistochemistry for chromogranin A, synaptophysin, and Ki67 index was performed in all formalin-fixed paraffin-embedded tissue samples from biopsy or surgery of the primary tumor and/or metastases (61).

At the time of the evaluation, patients were classified as 'disease-free' when there was no biochemical or morphological evidence of disease, 'stable disease' or 'progressive disease' according to RECIST 1.1 criteria (62).

Statistical Analysis

The data distribution was evaluated by Kolmogorov–Smirnov test and the abnormal data (age, BMI, Ki67 index, SBP, DBP, fasting glucose, HDL-cholesterol, VAI, FLI, and MetS) were normalized by logarithm. The abnormal variables were logarithmically transformed and back-transformed for presentation in tables and figures.

The chi-square (χ^2) test was used to determine the significance of differences in the frequency distribution in gender, smoking, physical activity, difference in cardio-metabolic indices and MetS between patients and controls, difference in cardio-metabolic indices and MetS among G1 and

G2, NET patients free of the disease or with stable disease, and presence or absence of metastasis.

Student's paired *t*-test was used to analyze differences among age, anthropometric measurements, blood pressure, metabolic profile, cardio-metabolic indices, and MetS between GEP-NET patients and control group, for the difference among parameters included in this study with tumor grading (G1 *vs* G2), and presence/absence of metastasis, followed by Bonferroni *post hoc* analysis.

The differences among age, anthropometric measurements, blood pressure, metabolic profile, cardio-metabolic indices, and MetS with disease status (progressive disease, free disease, and stable disease) were analyzed by ANOVA test followed by the Bonferroni *post-hoc* test.

Proportional Odds Ratio (OR) models, *p*-value, 95% Interval Confidence (IC), and R^2 were performed to assess the association among quantitative variables (G1 *vs* G2 and presence/absence of metastasis). A multinomial logistic regression analysis, χ^2, *p*-value, and Akaike Information Criterion (AIC), and R^2 was performed to model the association among age, anthropometric measurements, blood pressure, metabolic profile, cardio-metabolic indices and MetS with the three groups of disease status (disease free, stable disease and progressive disease).

In addition, three multiple linear regression analysis models (stepwise method), expressed as R^2, beta (β), and *t*, with tumor grading, metastasis, and disease status as dependent variables were used to estimate the predictive value of VAI, FLI, and MetS.

Receiver operator characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity, criterion, standard error, and area under the curve (AUC), as well as cut-off values for MetS and FLI in detecting tumor grading (G2) and presence of metastasis in the GEP-NET patients. Variables with a variance inflation factor (VIF) >10 were excluded to avoid multicollinearity. Values ≤5% were considered statistically significant. Data were analyzed using the MedCalc® package (Version 12.3.0 1993- 2012, MedCalc Software bvba–MedCalc Software, Mariakerke, Belgium) and SPSS Software (PASW Version 21.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic, Clinical Characteristics, and Metabolic Parameters of GEP-NET Patients and Control Group

Demographic, clinical characteristics, and metabolic parameters of GEP-NET patients compared to controls were shown in **Table 1**. Of note, GEP-NET patients presented significant differences in comparison to the control group, in particular smoked less ($p < 0.001$), presented higher WC ($p = 0.004$) and SBP ($p = 0.007$), a worse metabolic profile, and had higher cardio-metabolic indices and MetS (**Table 1**). **Figure 1** shows the percentage differences in cardiometabolic indices, single risk factors of MetS, and presence of MetS in GEP-NET patients compared to controls. Considering age-and-gender specific cut-off points of VAI, most percentage of GEP-NET patients presented visceral adipose dysfunction ($p < 0.001$). Similarly, the percentage of presence of NAFLD in GEP-NET patients was higher than in the control group ($p = 0.009$). In addition, both single risk factors of MetS and the presence of MetS were more frequently diagnosed among GEP-NET patients than in controls (p=0.001), as reported in **Figure 1**.

TABLE 1 | Demographic, clinical characteristics, and metabolic parameters of GEP-NET patients compared to the control group.

Parameters	GEP-NET patients n. 109	Control Group n. 109	*p*-value
Demographic characteristics			
Gender (Females)	56 (51.4%)	56 (51.4%)	$\chi^2 = 0.018$, $p = 0.892$
Age (Years)	57.06 ± 15.96	56.16 ± 12.89	0.370
Clinical characteristics			
Smoking (Yes)	37 (33.9%)	67 (61.5%)	$\chi^2 = 15.46$, **$p < 0.001$**
Physical activity (Yes)	49 (45.0%)	54 (49.5%)	$\chi^2 = 0.29$, $p = 0.587$
Anthropometric measurements			
BMI (kg/m^2)	27.55 ± 5.33	28.15 ± 4.07	0.364
WC (cm)	93.87 ± 14.74	88.38 ± 10.93	**0.004**
Blood pressure			
SBP (mmHg)	125.18 ± 11.96	120.50 ± 12.41	**0.007**
DBP (mmHg)	76.74 ± 7.71	75.50 ± 7.93	0.209
Metabolic profile			
Fasting Glucose (mg/dl)	108.13 ± 15.49	92.35 ± 14.58	**<0.001**
Total cholesterol (mg/dl)	190.86 ± 41.78	158.97 ± 30.86	**<0.001**
HDL cholesterol (mg/dl)	46.75 ± 15.29	50.29 ± 8.05	**0.034**
LDL cholesterol (mg/dl)	118.70 ± 40.02	86.74 ± 31.22	**<0.001**
Triglycerides (mg/dl)	127.07 ± 51.55	109.70 ± 28.87	**0.003**
Cardio-Metabolic indices and MetS			
VAI	2.29 ± 1.57	1.53 ± 0.70	**<0.001**
FLI	52.15 ± 29.52	44.36 ± 23.32	**0.049**
MetS (number parameter)	2.06 ± 1.52	0.97 ± 1.13	**<0.001**

GEP-NET, Gastroenteropancreatic Neoplasm; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; MetS, Metabolic Syndrome. A p value in bold type denotes a significant difference (p < 0.05).

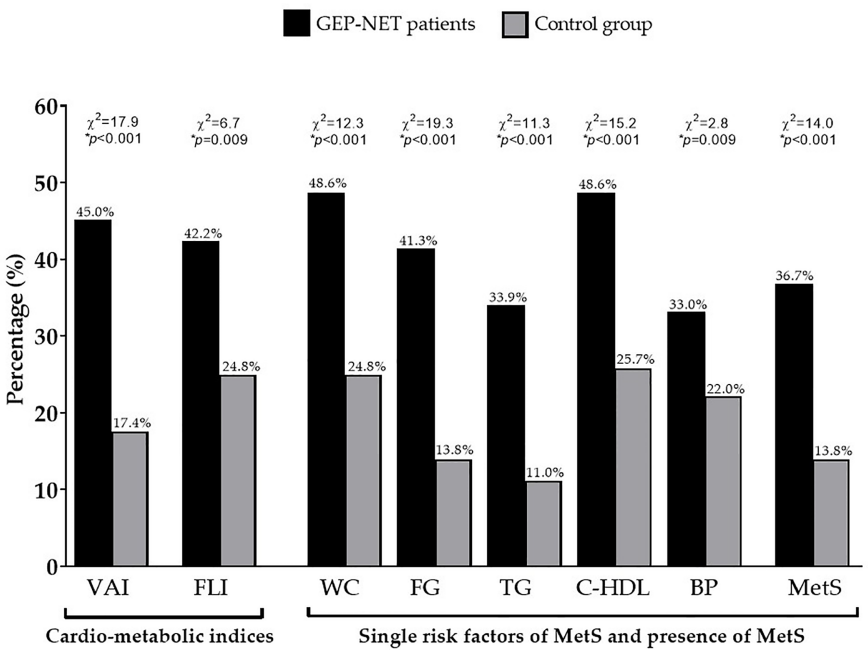

FIGURE 1 | Percentage differences in cardiometabolic indices, single risk factors of MetS, and presence of MetS in GEP-NET patients compared to controls. Most percentage of GEP-NET patients presented visceral adipose dysfunction ($p < 0.001$). Similarly, the percentage of presence of NAFLD in GEP-NET patients was higher than in the control group ($p = 0.009$). In addition, both single risk factors of MetS and the presence of MetS ($p < 0.001$) were more frequently diagnosed among GEP-NET patients than in controls. GEP-NET, Gastroenteropancreatic Neoplasm; VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; WC, Waist Circumference; FC, Fasting glucose; TG, Triglycerides; C-HDL, Cholesterol-High Density Lipoprotein; BP, Blood Pressure; MetS, Metabolic Syndrome.

Tumor Characteristics of GEP-NET Patients

A total of 109 patients (F:M = 56:53) affected by GEP-NET were included in the study. The mean size of the tumor was 24.58 ± 22.71 mm. Primary NETs were located in the pancreas (n = 54, 49.5%), stomach (n = 17, 15.6%), intestine (n = 30, 27.6%), and in few cases the primary site was unknown (n = 8, 7.3%). The majority of patients had non-functioning GEP-NET (n = 97, 89.0%). Twenty-two NET patients (20.2%) had a MEN1 syndrome. All GEP-NET patients were classified according to the pathological parameters with the mitotic rate and Ki67 index, as well differentiated tumor G1 (n = 65, 59.6%) or G2 (n = 44, 40.4%); the mean of Ki67 index was 3.88 ± 4.08%. At diagnosis, 27 patients (24.8%) had metastases (stage IV), the majority of them in the liver. At the time when the patients were enrolled in the clinical study, most of them (n. 51, 46.8%) had stable disease, 37 patients (33.9%) were disease free, and the remaining 21 patients (19.3%) had progressive disease, according to the RECIST1.1 criteria.

Cardio-Metabolic Indices and MetS in GEP-NET Patients According to Tumor Grading, Presence of Metastasis, and Disease Status

Differences in demographic, anthropometric measurements, blood pressure, metabolic parameters, and cardio-metabolic indices and MetS in the GEP-NET patients grouped by grading G1/G2 were summarized in **Table 2**.

Interestingly, GEP-NET G2 patients in comparison to patients with localized GEP-NET G1, had significant higher WC ($p = 0.003$), SBP and DBP ($p < 0.001$ and $p = 0.006$, respectively), and the worst metabolic parameters, except HDL cholesterol. Of interest, GEP-NET G2 patients showed the highest value of cardio-metabolic indices and MetS (number parameter) (**Table 2**). Similarly, **Figure 2** reported the difference of VAI, FLI, and MetS according to specific cut-off points. As observed, GEP-NET G2 patients presented the highest percentage of cardio-metabolic indices, single risk factors of MetS, and presence of MetS ($p < 0.001$) compared to GEP-NET G1 patients.

Similar data were observed also when these parameters were grouped by disease status (**Table 3**). A significant worse metabolic profile, cardio-metabolic indices, and MetS were shown in GEP-NET patients with progressive disease, in comparison to patients who were free of disease or with stable disease (**Table 3**).

Even when we considered the difference of VAI, FLI, and MetS according to specific cut-off points, GEP-NET patients with progressive disease had the highest percentage of cardio-metabolic indices, single risk factors of MetS and presence of MetS ($p = 0.014$) compared to GEP-NET patients free of the disease or with stable disease, **Figure 3**.

TABLE 2 | Differences in demographic, anthropometric measurements, blood pressure, metabolic parameters, and cardio-metabolic indices and MetS in the GEP-NET patients according to tumor grading.

Parameters	G1 n. 65	G2 n.44	*p*-value
Age (years)	55.32 ± 17.26	59.64 ± 13.61	0.149
Anthropometric measurements			
BMI (kg/m^2)	27.23 ± 5.64	28.02 ± 4.88	0.439
WC (cm)	90.58 ± 15.15	98.73 ± 12.77	**0.003**
Blood pressure			
SBP (mmHg)	121.85 ± 10.52	130.11 ± 12.37	**<0.001**
DBP (mmHg)	75.08 ± 7.47	79.20 ± 7.47	**0.006**
Metabolic profile			
Fasting Glucose (mg/dl)	102.71 ± 14.22	116.14 ± 13.84	**<0.001**
Total cholesterol (mg/dl)	178.52 ± 32.34	209.09 ± 47.51	**<0.001**
HDL cholesterol (mg/dl)	48.97 ± 13.21	43.48 ± 17.59	0.066
LDL cholesterol (mg/dl)	105.97 ± 29.27	137.49 ± 46.28	**<0.001**
Triglycerides (mg/dl)	117.91 ± 43.98	140.61 ± 59.01	**0.023**
Cardio-Metabolic indices and MetS			
VAI	1.89 ± 1.05	2.88 ± 1.99	**0.001**
FLI	42.93 ± 28.15	65.77 ± 26.27	**<0.001**
MetS (number parameter)	1.42 ± 1.12	3.00 ± 1.56	**<0.001**

GEP-NET, Gastroenteropancreatic Neoplasm; G, grading; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; MetS, Metabolic Syndrome. A p value in bold type denotes a significant difference (p < 0.05).

Differences in demographic, anthropometric measurements, blood pressure, metabolic parameters, and cardio-metabolic indices and MetS in the GEP-NET patients according to the presence/absence of metastasis were summarized in **Table 4**. The worse WC ($p = 0.003$), blood pressure, metabolic profile, cardio-metabolic indices, and MetS were presented in the presence of metastasis, and the latter GEP-NET patients also presented the highest percentage of cardio-metabolic indices, single risk factors of MetS, and presence of MetS ($p = 0.035$), **Figure 4**.

Correlation Between Tumor Aggressiveness and Metabolic Profile, Cardio-Metabolic Indices, and MetS in GEP-NET Patients

To assess the correlation of grading and metastasis, a bivariate proportional OR model with demographic, anthropometric measurements, blood pressure, metabolic profile, cardio-metabolic indices, and MetS was performed (**Table 5**). A part age, BMI, and HDL cholesterol for grading all other parameters were significantly associated with the highest grading G2 and with the presence of metastasis; **Table 5**.

A multinomial logistic regression model to assess the association between patients with progressive disease and demographics, anthropometric measurements, blood pressure, metabolic profile, cardio-metabolic indices, and MetS, was performed (**Table 6**). Progressive disease was associated with higher values of age ($p = 0.012$), WC ($p = 0.005$), blood pressure ($p = 0.007$ and $p = 0.004$ for SBP and DBP, respectively), fasting glucose ($p = 0.015$), triglycerides ($p = 0.029$), VAI ($p = 0.001$), FLI ($p = 0.009$), MetS ($p < 0.001$), and lower HDL cholesterol ($p = 0.001$); **Table 6**.

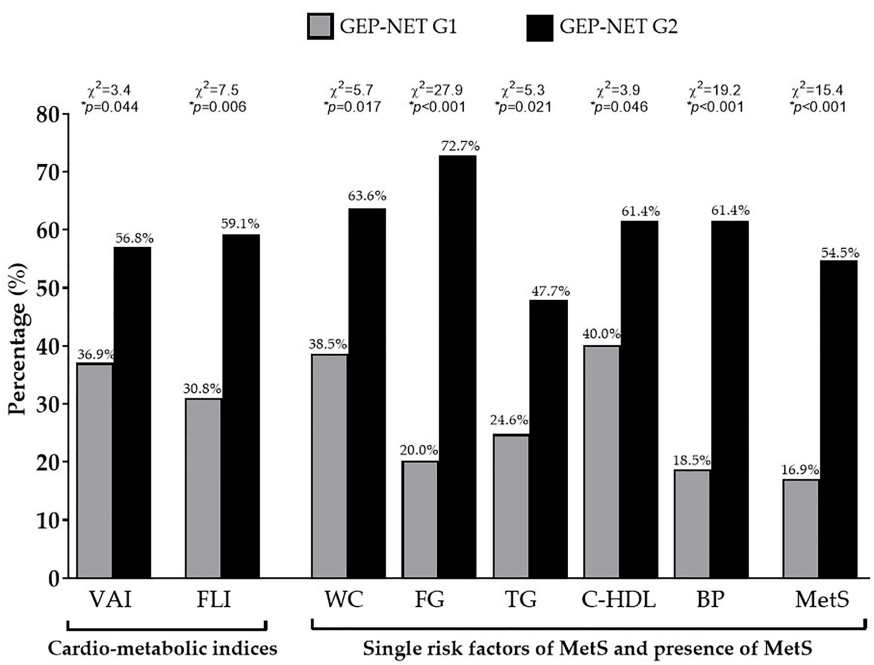

FIGURE 2 | Difference of VAI, FLI, and MetS according to the grading. GEP-NET G2 patients presented the highest percentage of cardio-metabolic indices ($p = 0.044$ and $p = 0.006$ for VAI and FLI, respectively), single risk factors of MetS, and presence of MetS ($p < 0.001$) compared to GEP-NET G1 patients. GEP-NET, Gastroenteropancreatic Neoplasm; G, Grading, VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; WC, Waist Circumference; FC, Fasting glucose; TG, Triglycerides; C-HDL, Cholesterol-High Density Lipoprotein; BP, Blood Pressure; MetS, Metabolic Syndrome.

TABLE 3 | Differences in demographic, anthropometric measurements, blood pressure, metabolic parameters, and cardio-metabolic indices and MetS in the GEP-NET patients according to disease status.

Parameters	Progressive Disease n. 21 (19.3%)	Free Disease n. 37 (33.9%)	Stable Disease n. 51 (46.8%)	*p*-value
Age (years)	56.95 ± 13.51	55.05 ± 17.37	58.57 ± 15.95	0.598
Anthropometric measurements				
BMI (kg/m^2)	29.07 ± 4.81	27.26 ± 4.83	27.13 ± 5.85	0.353
WC (cm)	98.64 ± 13.75	92.53 ± 15.62	92.88 ± 14.35	0.256
Blood pressure				
SBP (mmHg)	127.62 ± 12.61	122.70 ± 12.22	125.98 ± 11.40	0.263
DBP (mmHg)	78.33 ± 8.99	75.41 ± 7.85	77.06 ± 7.01	0.354
Metabolic profile				
Fasting Glucose (mg/dl)	119.81 ± 17.42	104.65 ± 12.93	105.84 ± 14.31	**<0.001**
Total cholesterol (mg/dl)	215.05 ± 42.99	193.86 ± 41.00	178.73 ± 37.66	**0.003**
HDL cholesterol (mg/dl)	41.81 ± 16.35	42.27 ± 13.46	52.04 ± 14.64	**0.004**
LDL cholesterol (mg/dl)	142.70 ± 44.65	124.32 ± 37.98	104.74 ± 34.06	**<0.001**
Triglycerides (mg/dl)	152.71 ± 62.74	136.38 ± 43.69	109.76 ± 46.32	**0.002**
Cardio-Metabolic indices and MetS				
VAI	3.12 ± 2.02	2.69 ± 1.67	1.66 ± 0.93	**<0.001**
FLI	69.24 ± 31.58	51.97 ± 28.13	45.24 ± 27.22	**0.006**
MetS (number parameter)	3.19 ± 1.78	2.11 ± 1.34	1.55 ± 1.27	**<0.001**

GEP-NET, Gastroenteropancreatic Neoplasm; G, grading; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; MetS, Metabolic Syndrome. A p value in bold type denotes a significant difference (p < 0.05).

To compare the relative predictive power of the cardio-metabolic indices and MetS, three multiple linear regression analysis models with oncological parameters (tumor grading, metastasis, and disease status) were performed and reported in **Table 7**. Model 1 compared the relative predictive power of grading G1/G2 on cardio-metabolic indices and MetS. In this model MetS entered at the first step ($p < 0.001$), followed by FLI ($p < 0.001$); VAI was excluded. Model 2 compared the relative predictive power of metastasis on cardio-metabolic indices and MetS. In this model, MetS entered at the first step ($p < 0.001$),

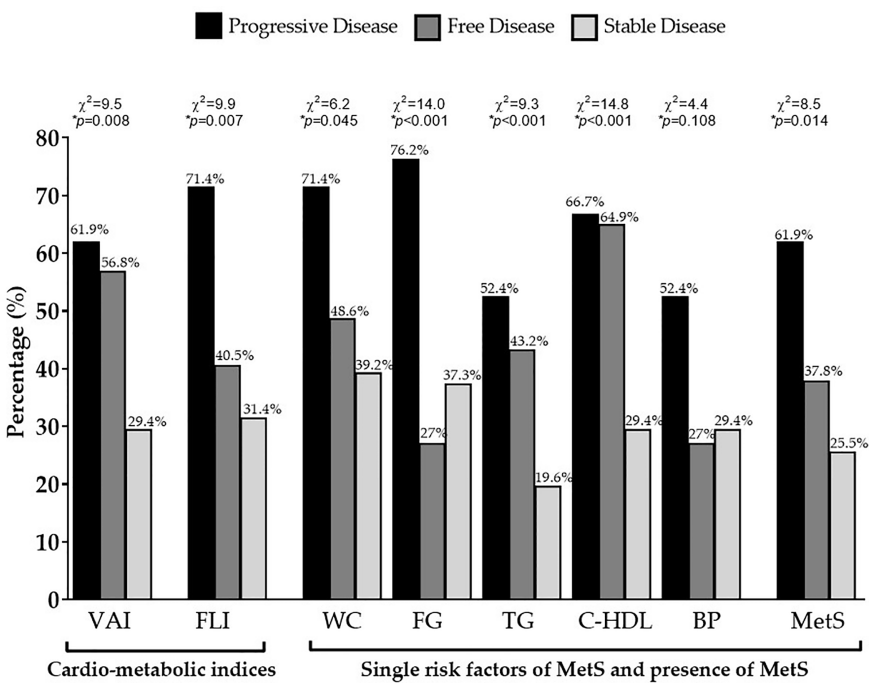

FIGURE 3 | Difference of VAI, FLI, and MetS according to status of disease. GEP-NET patients with progressive disease had the highest percentage of cardio-metabolic indices ($p = 0.008$ and $p = 0.007$ for VAI and FLI, respectively), single risk factors of MetS, and presence of MetS (p=0.014) compared to GEP-NET patients free of disease or with stable disease. VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; WC, Waist Circumference; FC, Fasting glucose; TG, Triglycerides; C-HDL, Cholesterol-High Density Lipoprotein; BP, Blood Pressure; MetS, Metabolic Syndrome.

TABLE 4 | Differences in demographic, anthropometric measurements, blood pressure, metabolic parameters, and cardio-metabolic indices and MetS in the GEP-NET patients according to the presence/absence of metastasis.

Parameters	Absence of Metastasis n. 82	Presence of Metastasis n. 27	*p*-value
Age (years)	56.48 ± 16.86	58.85 ± 12.94	0.448
Anthropometric measurements			
BMI (kg/m^2)	27.17 ± 5.44	28.70 ± 4.93	0.182
WC (cm)	92.16 ± 14.63	99.08 ± 14.05	**0.033**
Blood pressure			
SBP (mmHg)	123.59 ± 11.66	130.00 ± 11.76	**0.018**
DBP (mmHg)	75.73 ± 6.94	79.81 ± 9.14	**0.016**
Metabolic profile			
Fasting Glucose (mg/dl)	105.43 ± 15.12	116.29 ± 13.87	**0.001**
Total cholesterol (mg/dl)	184.64 ± 36.84	209.74 ± 50.29	**0.006**
HDL cholesterol (mg/dl)	48.59 ± 14.73	41.14 ± 15.88	**0.027**
LDL cholesterol (mg/dl)	112.09 ± 34.20	138.75 ± 49.53	**0.002**
Triglycerides (mg/dl)	119.78 ± 46.84	149.22 ± 59.39	**0.009**
Cardio-Metabolic indices and MetS			
VAI	2.00 ± 1.20	3.14 ± 2.17	**0.001**
FLI	46.59 ± 28.42	69.04 ± 26.62	**<0.001**
MetS (number parameter)	1.74 ± 1.35	3.00 ± 1.64	**<0.001**

GEP-NET, Gastroenteropancreatic Neoplasm; G, grading; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; MetS, Metabolic Syndrome. A p value in bold type denotes a significant difference (p < 0.05).

followed by FLI ($p < 0.001$); VAI was excluded. In model 3, the disease status was better predicted by VAI ($p = 0.014$); MetS and FLI were excluded (**Table 7**).

Four ROC analyses were performed to determine the cut-off values of the MetS and FLI predictive of high grading (G2) and presence of metastasis, respectively. A MetS> 2 ($p < 0.001$, sensitivity 65.9%, specificity 83.1%, AUC 0.78, standard error 0.046; **Figure 5A**) and a FLI >64.8 ($p < 0.001$, sensitivity 59.1%, specificity 76.9%, AUC 0.72, standard error 0.050; **Figure 5B**), could serve as thresholds for significant increased risk of G2 tumor. A MetS >1 ($p < 0.001$, sensitivity 81.5%, specificity 52.4%, AUC 0.72, standard error 0.059; **Figure 5C**) and a FLI >61.2 ($p = 0.001$, sensitivity 74.1%, specificity 70.3%, AUC 0.72, standard error 0.058; **Figure 5D**) could serve as a threshold for significantly increased risk of presence of metastasis.

DISCUSSION

In this cross-sectional, case–control, observational study, we evaluated the associations of VAI and FLI, as cardiometabolic indices, and MetS with tumor clinicopathological characteristics in a selected group of GEP-NET patients. The main result of the study is the positive association between the cardiometabolic indices and MetS with the clinicopathological characteristics of NET, independently of age and BMI. In addition, we have

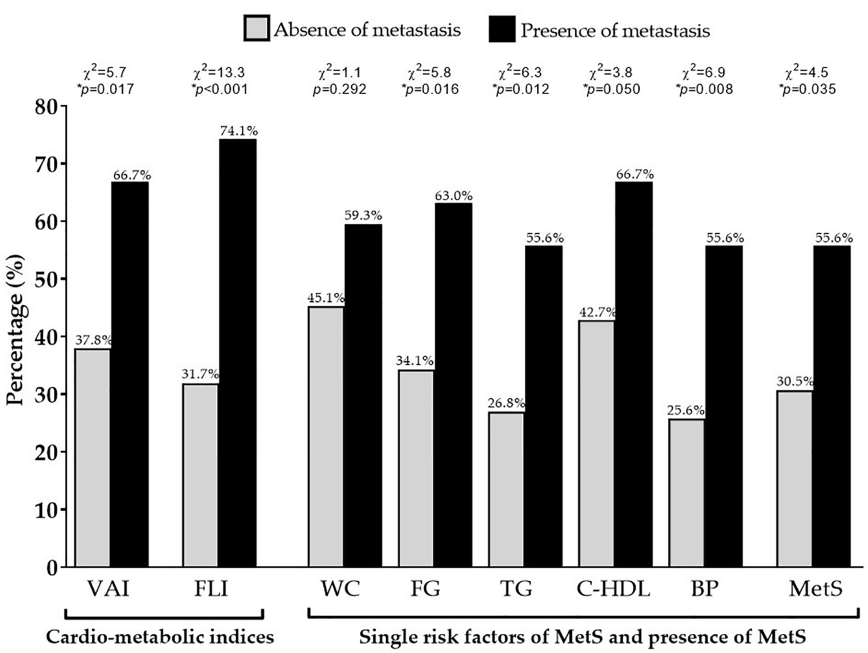

FIGURE 4 | Difference of VAI, FLI, and MetS according to metastasis. GEP-NET patients with the presence of metastasis had the highest percentage of cardio-metabolic indices ($p = 0.017$ and $p < 0.001$ for VAI and FLI, respectively), single risk factors of MetS (except for the WC, $p = 0.292$), and presence of MetS ($p = 0.035$) compared to GEP-NET patients with the absence of metastasis. VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; WC, Waist Circumference; FC, Fasting glucose; TG, Triglycerides; C-HDL, Cholesterol-High Density Lipoprotein; BP, Blood Pressure; MetS, Metabolic Syndrome.

TABLE 5 | Bivariate proportional odds ratio model performed to assess the association of tumor aggressiveness with demographic, anthropometric measurements, blood pressure, metabolic profile, cardio-metabolic indices, and MetS.

Parameters	Grading G2				Metastasis (presence)			
	OR	*p*-value	95% CI	R^2	OR	*p*-value	95% CI	R^2
Age (years)	1.02	0.168	0.993–1.04	0.02	1.01	0.501	0.98–1.04	0.01
Anthropometric measurements								
BMI (kg/m^2)	1.03	0.250	0.957–1.11	0.01	1.05	0.206	0.97–1.14	0.02
WC (cm)	1.04	**0.006**	1.01–1.07	0.07	1.03	**0.038**	1.00–1.07	0.04
Blood pressure								
SBP (mmHg)	1.08	**0.001**	1.03–1.11	0.12	1.05	**0.018**	1.00–1.09	0.05
DBP (mmHg)	1.07	**0.007**	1.02–1.14	0.07	1.08	**0.019**	1.01–1.14	0.05
Metabolic profile								
Fasting Glucose (mg/dl)	1.07	**<0.001**	1.04–1.11	0.19	1.05	**0.003**	1.02–1.08	0.09
Total cholesterol (mg/dl)	1.02	**<0.001**	1.01–1.03	0.13	1.02	**0.008**	1.00–1.03	0.07
HDL cholesterol (mg/dl)	0.98	0.070	0.95–1.00	0.03	0.96	**0.032**	0.93–0.99	0.05
LDL cholesterol (mg/dl)	1.02	**<0.001**	1.01–1.03	0.15	1.02	**0.004**	1.00–1.03	0.08
Triglycerides (mg/dl)	1.00	**0.026**	1.00–1.02	0.05	1.01	**0.012**	1.00–1.02	0.06
Cardio-Metabolic indices and MetS								
VAI	1.56	**0.003**	1.17–2.09	0.10	1.56	**0.003**	1.16–2.09	0.09
FLI	1.03	**<0.001**	1.01–1.05	0.14	1.03	**0.001**	1.01–1.05	1.11
MetS (number parameter)	2.30	**<0.001**	1.63–3.23	0.25	1.77	**<0.001**	1.29–2.43	0.12

BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; MetS, Metabolic Syndrome. A p value in bold type denotes a significant difference (p < 0.05).

TABLE 6 | Multinomial logistic regression model to assess the association between disease status with age, anthropometric measurements, blood pressure, metabolic profile, cardio-metabolic indices, and MetS.

Parameters	Progressive disease			
	χ^2	*p* value	AIC	R^2
Age (years)	134.68	**0.012**	178.05	0.709
Anthropometric measurements				
BMI (kg/m^2)	239.50	0.094	239.49	0.889
WC (cm)	189.59	**0.005**	212.11	0.824
Blood pressure				
SBD (mmHg)	38.85	**0.007**	79.07	0.300
DBD (mmHg)	32.18	**0.004**	65.46	0.256
Metabolic profile				
Fasting Glucose (mg/dl)	132.94	**0.015**	176.48	0.705
Total cholesterol (mg/dl)	183.17	0.172	208.65	0.814
HDL cholesterol (mg/dl)	138.19	**0.001**	176.07	0.719
LDL cholesterol (mg/dl)	222.86	0.109	231.18	0.871
Triglycerides (mg/dl)	208.99	**0.029**	224.25	0.853
Cardio-Metabolic indices and MetS				
VAI	122.86	**0.001**	131.18	0.771
FLI	122.81	**0.009**	129.18	0.766
MetS (number parameter)	51.39	**<0.001**	80.10	0.376

BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; MetS, Metabolic Syndrome. A p value in bold type denotes a significant difference (p < 0.05).

TABLE 7 | Multiple regression analysis models (stepwise method) with tumor aggressiveness and cardiometabolic indices and MetS.

Parameters	Multiple Regression analysis			
Model 1—Tumor Grading-	R^2	*β*	*t*	*p* value
MetS	0.257	0.514	6.19	**<0.001**
FLI	0.138	0.381	4.27	**<0.001**
	Variable excluded: VAI			
Model 2—Metastasis-	R^2	*β*	*t*	*p* value
MetS	0.120	0.358	3.97	**<0.001**
FLI	0.100	0.330	3.61	**<0.001**
	Variable excluded: VAI			
Model 3—Disease Status-	R^2	*β*	*t*	*p* value
VAI	0.031	0.336	2.49	**0.014**
	Variable excluded: MetS and FLI			

VAI, Visceral Adiposity Index; FLI, Fatty Liver Index; MetS, Metabolic Syndrome. A p value in bold type denotes a significant difference (p < 0.05).

provided the cut-off values for the FLI and MetS to predict high grading of GEP-NET and the presence of metastasis.

Given the rarity and heterogeneity of GEP-NET, clinical trials designed to investigate the role of metabolic risk factors for these tumors are lacking. To the best of our knowledge, to date, this is the first study reporting differences in cardiometabolic indices in a selected group of GEP-NET patients compared to healthy controls matched for age, gender, and BMI.

The current prevalence of GEP-NET is 6.4 cases/100,000 inhabitants, with an increased incidence over the last four decades (2, 63). This increase was initially attributed to the improvement of diagnostic skills with the widespread use of advanced imaging techniques. However, the role of metabolic mechanisms underlying the etiology of GEP-NET has not yet been investigated before. Still, the potential contributions of different environmental factors, including metabolic dysfunctions, were mostly neglected as most evidence focused primarily on the genetics or molecular pathways of NET (64–66). Epidemiological data suggest that beyond the genetic influences, also environmental factors are involved in the increased incidence in GEP-NET (67). Indeed, only few retrospective evidence has addressed the potential association between MetS and GEP-NET (65, 68, 69), and these few studies were predominantly limited to pancreatic neuroendocrine tumors only (70, 71). In a recent case–control study, however, single risk factors of MetS, including visceral

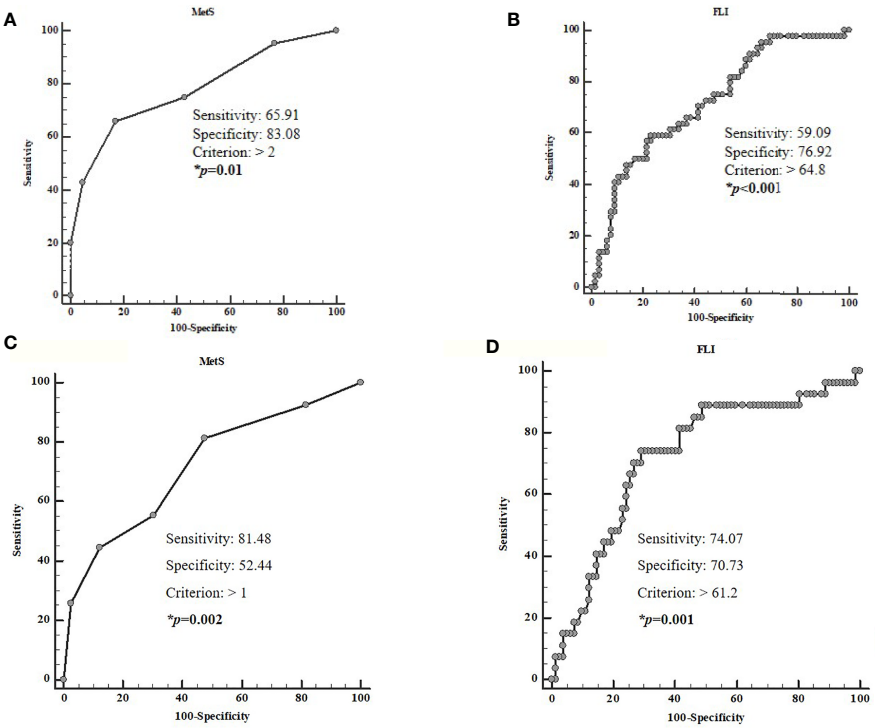

FIGURE 5 | ROC analysis to determine the cut-off values of the MetS and FLI predictive of high grading **(A, B)** and the presence of metastasis **(C, D)**. A MetS >2 ($p < 0.001$, sensitivity 65.9%, specificity 83.1%, AUC 0.78, standard error 0.046; **A**) and a FLI > 64.8 ($p < 0.001$, sensitivity 59.1%, specificity 76.9%, AUC 0.72, standard error 0.050; **B**) could serve as thresholds for significant increased risk of G2 tumor. A MetS > 1 ($p < 0.001$, sensitivity 81.5%, specificity 52.4%, AUC 0.72, standard error 0.059; **C**) and a FLI> 61.2 ($p = 0.001$, sensitivity 74.1%, specificity 70.3%, AUC 0.72, standard error 0.058; **D**), could serve as a threshold for significantly increased risk of presence of metastasis. MetS, Metabolic Syndrome; FLI, Fatty Liver Index.

adiposity, high triglyceride levels, or hyperglycemia, were more present in GEP-NET patients compared to the control group (34).

NAFLD and MetS are well-established risk factors for different tumors; nevertheless, if these metabolic conditions are also risk factors for GEP-NET or if these conditions are able to negatively influence the clinicopathological characteristics of NET and consequently, disease behavior is yet to be fully established.

To the best of our knowledge, this is the first observation of GEP-NET patients with the highest values of VAI and FLI, and the presence of MetS are more likely to have higher-grade tumors or present advanced-stage disease at diagnosis with metastasis. In addition, VAI, FLI, and MetS were significantly associated with the three clinicopathological characteristics of GEP-NET included in this study.

These findings suggest that accurate metabolic profiling should be an integral part of the clinical evaluation of patients with GEP-NET and support a role for adiposity dysfunction and NAFLD, evaluated by VAI and FLI, respectively, and the presence of MetS as relevant risk determinants in GEP-NET patients. Similar associations were also shown for other types of tumors, such as esophageal cancer (72), colon and rectal cancer (73), thyroid cancer (74, 75), and prostate cancer (76).

However, this study has some limitations and some strengths that must be considered. Among the limitations, the cross-sectional nature of this study did not allow identification of any causal association between cardio-metabolic indices or MetS and GEP-NET characteristics and to clearly determine their prognostic value to predict GEP-NET clinical severity. Furthermore, the suggested cut-off value of FLI and MetS to identifying tumor aggressiveness should be viewed with caution until data in larger populations become available to perform an appropriate cross-validation. Moreover, we recognize how the liver biopsy is the gold-standard technique for identifying NAFLD. Hepatic biopsy is an invasive procedure burdened with rare but potentially life-threatening complications. However, FLI, although it is a surrogate marker of NAFLD, has largely proved to represent an easy and reliable screening tool to identify NAFLD (77, 78). The lack of a liver biopsy may prompt us to further investigate the association between cardiometabolic indices and MetS in GEP-NET patients.

However, the strengths of this study are several. First, the sample size was sufficiently large. In fact, we have calculated the sample size using 95% power, and the number of participants required was 102 (51 cases and 51 controls), while we used 218 (109 GEP-NET patients and 109 controls) individuals *i.e.* more than double those required. Second, the homogeneity of our sample population further strengthens the power of the study. In fact, in order to improve the power of this study, we increased the

homogeneity of the cohort of NET patients by including only patients who were biochemically free of disease for more than 6 months without medical treatment, or treatment-naïve patients with non-functioning GEP-NET. In addition, all GEP-NET patients had a diagnosis of well-differentiated G1/G2 and were matched for age, sex, and BMI with a well-characterized control group.

CONCLUSIONS

In conclusion, our findings report that the worsening of clinicopathological characteristics in GEP-NET is associated with visceral adiposity dysfunction, evaluated by VAI, NAFLD, evaluated by FLI, and the presence of MetS. These novel results, although requiring confirmation in larger scale clinical trials, help to fulfil an unmet clinical need and provide a breakthrough toward understanding the putative mechanisms leading to GEP-NET progression and increased prevalence. Finally, to address the clinical evaluation of cardiometabolic indices in GEP-NET patients might be of crucial relevance to establish targeted preventive and treatment interventions of NET-related metabolic comorbidities.

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 "Federico II" Medical School Ethical Committee has approved this cross-sectional case–control observational study (n. 201/17), which was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, LB, SS, and GM. Data curation, GM, GP, RMo, and RMi. Formal analysis, LB, GM, and SS. Funding acquisition, LB, AF, AC, and GM. Investigation, LB, GM, BA, RMo, RMi, and GP. Methodology, LB and GM. Project administration, LB and GM. Resources, LB. Software, LB. Supervision, LB, GM, AC, SS. Validation, AF, AC, SS, RMo, RMi, and LB. Visualization, LB. Writing—original draft, LB and SS. Writing—review & editing, LB, GM, AC, and SS. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Cushing's Syndrome With Nocardiosis: A Case Report and a Systematic Review of the Literature

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Objective: To analyze and summarize the clinical characteristics, treatments, and prognosis of Cushing's syndrome (CS) with nocardiosis.

Methods: A patient in our hospital and additional 17 patients of CS with nocardiosis in the English literature were included in this study. Clinical characteristics, laboratory data, imaging studies, treatments, and prognosis were evaluated.

Results: A 41-year-old man with CS was diagnosed and treated in our hospital. He had co-infections of nocardiosis and aspergillosis. Together with 17 patients of CS with nocardiosis in the English literature, 2 patients (11.1%) were diagnosed as Cushing's disease (CD) while 16 (88.9%) were diagnosed or suspected as ectopic ACTH syndrome (EAS). The average 24hrUFC was 7,587.1 ± 2,772.0 μg/d. The average serum total cortisol and ACTH (8 AM) was 80.2 ± 18.7 μg/dl and 441.8 ± 131.8 pg/ml, respectively. The most common pulmonary radiologic findings in CT scan were cavitary lesions (10/18) and nodules (8/18). Co-infections were found in 33.3% (6/18) patients. The CS patients with co-infections had higher levels of ACTH (671.5 ± 398.2 *vs* 245.5 ± 217.1 pg/ml, $P = 0.047$), and 38.9% (7/18) patients survived through the antibiotic therapy and the treatment of CS. Patients with lower level of ACTH (survival *vs* mortality: 213.1 ± 159.0 *vs* 554.7 ± 401.0 pg/ml, P = 0.04), no co-infection, underwent CS surgery, and received antibiotic therapy for more than 6 months, had more possibilities to survive.

Conclusions: Nocardia infection should be cautioned when a patient of CS presented with abnormal chest radiographs. The mortality risk factors for CS with nocardiosis are high level of ACTH and co-infections. We should endeavor to make early etiological diagnosis, apply long-term sensitive antibiotics and aggressive treatments of CS.

Keywords: Cushing's syndrome, nocardiosis, infection, ectopic ACTH syndrome, Cushing's disease

INTRODUCTION

Endogenous Cushing's syndrome (CS) is characterized by excessive elevation of glucocorticoid concentrations produced by adrenal cortex. It is generally divided into adrenocorticotropic hormone (ACTH)-dependent and ACTH-independent CS. The most common cause of CS is corticotropin-secreting pituitary adenoma that leads to Cushing's disease (CD). The ectopic ACTH syndrome (EAS) accounts for 10 to 20% of ACTH-dependent CS (1).

Nocardiosis most frequently presents with pulmonary disease, followed by disseminated disease, extra-pulmonary disease [such as in the central nervous system and primary skin and soft tissue disease (2)]. Nocardiosis is regarded as an opportunistic infection, with the majority of infections occurring in immunocompromised patients, including those with long-term corticosteroid exposure, malignancy, human immunodeficiency virus (HIV) infection, and history of transplantation (3–7), associated with high mortality of 34.5–40% (3, 4). Xu L. et al. (8) reviewed 12 patients of nocardiosis in EAS patients. However, reports of nocardiosis in patients with other forms of CS were not included.

In this study, we presented a patient of nocardiosis with suspected EAS in our hospital and analyzed 17 patients of nocardiosis in CS reported in the literature to summarize the clinical characteristics, treatments, and prognosis of CS with nocardiosis.

METHODS

Medical Information of This Case

We collected the clinical characteristics, laboratory data, imagings, and microbiology results of a patient of CS with nocardiosis in Peking Union Medical College Hospital (PUMCH). This study was approved by the Ethics Committee of PUMCH.

Literature Review

A systematic literature review was conducted through searched PubMed, Web of Science, and Embase, finding all relevant and available articles published in English. MeSH terms included "Cushing's syndrome," "Nocardia Infections," or "nocardiosis." Original research, case reports, case series, or review articles published until October, 2020 with detail medical history and laboratory data were included. Studies which analyzed cases of exogenous CS were excluded.

Statistical Analysis

Data management and analysis were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Data were presented as proportions for categorical variables and mean SD or median (interquartile range) for continuous variables. Significant differences between groups for continuous variables were tested using a *t*-test or the nonparametric Mann–Whitney *U* test, as appropriate. χ^2 tests were used for comparisons of categorical data.

RESULTS

Case Presentation

A 41-year-old man developed fatigue for 2 months with progressive polydipsia and polyuria. A month ago, he went to the local hospital and had the examination revealed that his blood pressure was 150/100 mmHg and his fasting blood-glucose was 17 mmol/L. He was given insulin treatment afterwards. However, he could not uphold the regular treatment and his blood glucose could not be well controlled. He manifested fever of 38.4°C and cough with yellow phlegm following catching a cold 6 days ago. The patient gradually presented with mental disorders of mania and aggressive behavior for 4 days. He was urgently referred to our hospital. On admission, the man appeared weak and confused, thinning of the skin with pigmentation, bruising and edema of face and both lower extremities but no red-purple striae. He presented with mild moon face, no obvious buffalo hump. In laboratory examinations, serum glucose was 24.8 mmol/L, sodium 164 mmol/L, potassium 2.6 mmol/L, albumin 21g/L, creatine 102 μmol/L. Arterial blood gas analysis demonstrated of metabolic alkalosis. The X-ray of chest showed patch shadows of left middle lobe and right upper lobe. Insulin therapy, potassium supplements (oral potassium chloride, 9.0 g/d), spironolactone 60 mg/d, intravenous fluids, and empirical antibiotics moxifloxacin were applied. The patient's consciousness, serum glucose, and sodium returned to normal 2 days later. He got a normal body temperature and less cough with phlegm 5 days later. On the 6^{th} day, chest CT showed patchy infiltration and small nodules of bilateral lung lobes (**Figures 1A, B**).

Examinations for CS were performed a week after his admission when the patient's condition was improved. His serum ACTH was 171 pg/ml (normal range <46 pg/ml), 24-h urinary free cortisol (24hrUFC) was 3,522 μg (normal range 12.3–103.5 μg/24 h), repeated 24hrUFC was 2746 μg. The baseline serum cortisol was 51.21 μg/dl (normal range 4.0–22.3 μg/dl), after overnight 1 mg dexamethasone suppression test (DST) and high-dose DST were 58.76 and 62.74 μg/dl respectively. CS was diagnosed according to the clinical practice guideline of the diagnosis of CS (9). MRI scanning of pituitary gland showed no abnormal signal. In consideration of diabetes and hypertension in young age, mild physical appearance of CS, repeated high levels of 24hrUFC, and no suppression in 1 mg DST, the diagnosis of CS was established. Furthermore, because of rapid onset and severe conditions of the patient with the extreme cortisol excess, markedly elevated ACTH level, no suppression in high-dose overnight DST, no space-occupying lesion in pituitary gland, ectopic ACTH syndrome (EAS) was suspected. But CT scanning of chest and abdomen and ^{99m}Tc-octreotide scintigraphy gave no clue for the ectopic location of ACTH-secreting tumor.

Chest CT on the 15^{th} day demonstrated multiple enlarged nodules, partial cavitary lesions (**Figures 1D, E**). Lung cancer was suspected but no tumor cell was founded in lung tissues from biopsy. GM test was positive. Modified acid-fast stains of sputum and lung tissues from percutaneous lung needle biopsy showed filamentous branching organisms. Sputum culture after 72 h

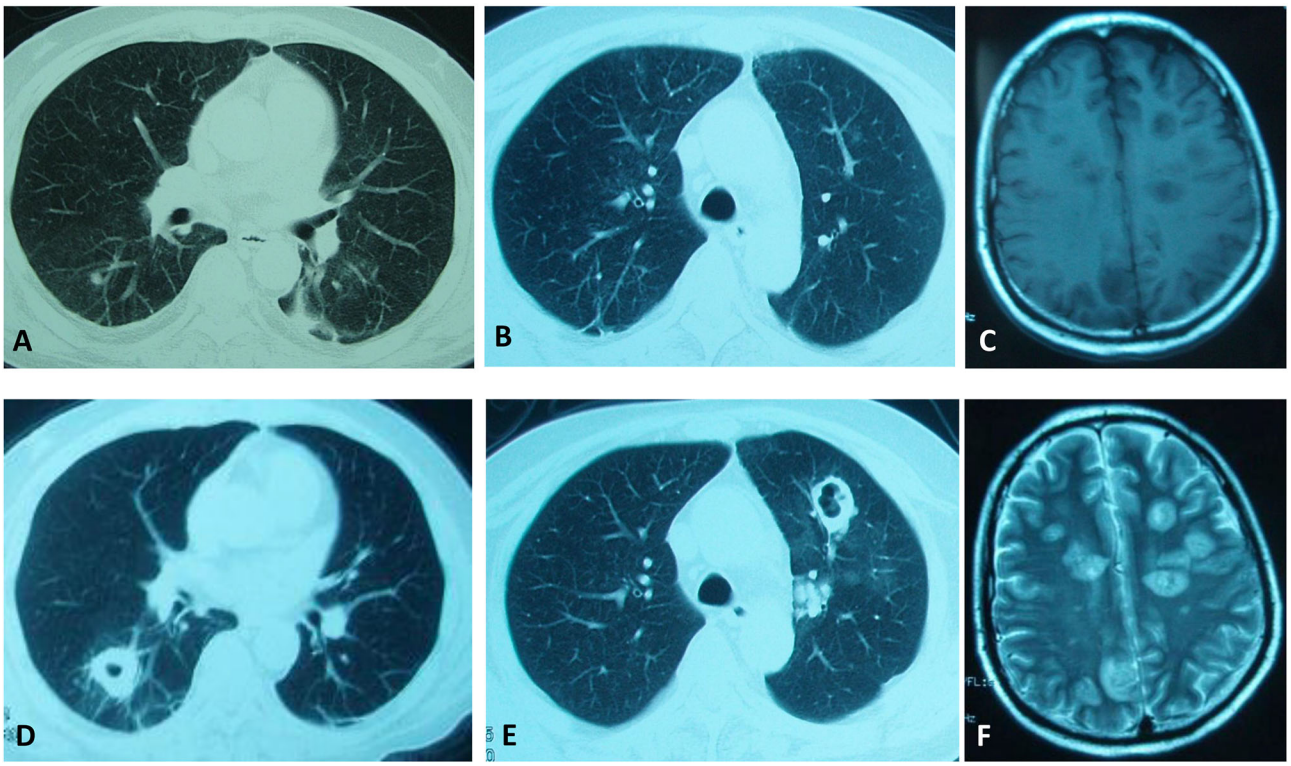

FIGURE 1 | Clinical images of our patient. CT scanning of the chest showing continuous development to a large cavitary mass in both lung lobes. **(A, B)** were taken on the 6th day. **(D, E)** were taken on the 15th day. **(C, F)** MRI scanning showing multiple long T1/T2 signal lesions in the brain on the 24th day.

grew *Aspergillus fumigates* and *Nocardia cyriacigeorgica*. Lung tissue culture after 48 h grew *Nocardia cyriacigeorgica*. Although trimethoprim-sulfamethoxazole (TMP-SMZ), ceftriaxone combined amphotericin B were used, the patient's situation deteriorated with head MRI scanning displaying multiple long T1/T2 signal lesions suggesting multiple brain abscesses on the 24th day (**Figures 1C, F**). We suspected the patient had the brain Nocardia or Aspergillus infections. Unfortunately, he refused further medication and died after auto discharge.

Literature Review

Seventeen CS with nocardiosis patients from 15 published reports (8, 10–23) were reviewed. Together with our case, 18 CS patients (11 male, 7 female) were identified. Patients were HIV negative and had no history of organ transplantation, no use of immunosuppression therapy. The clinical characteristics were summarized in **Table 1**. The average age of patients was 41.9 ± 5.0 years. Eight patients (44.4%) had hypertension and 14 patients (77.8%) had diabetes mellitus. The causes of CS were all ACTH-dependent. Two patients (11.1%) were diagnosed as CD while nine patients (50.0%) diagnosed as EAS and seven patients (38.9%) with suspected as EAS of unknown origin including our patient. The ectopic ATCH originated from bronchial carcinoid (3/18), pancreatic neuroendocrine tumor (2/18), small cell lung carcinoma (1/18), paraganglioma (1/18), neuroblastoma (1/18), and small cell carcinoma in rib (1/18). The average 24hrUFC was 7,587.1 ± 2,772.0 μg/d. The average serum total cortisol and ACTH (8 AM) was 80.2 ± 18.7 μg/dl and 441.8 ± 131.8 pg/ml, respectively. Serum cortisol levels of 11 EAS and 2 CD were above 43.1 μg/dl. The 24hrUFC levels of 11 EAS were above 2,000 μg/d. Totally, serum cortisol or 24hrUFC levels were above these levels in 83.3% (15/18) patients.

The pulmonary nocardiosis related symptoms varied including fever, cough, expectoration, dyspnea, chest pain, and hemoptysis. Seven patients were confused and six patients progressed to respiratory failure that required intubation and mechanical ventilation. Three patients had only chest imaging changes with no fever or any pulmonary symptoms. The pulmonary radiologic findings included cavitary lesions (10/18), nodules (8/18), infiltration (3/18), consolidation (2/18), and pleural effusion (2/18) (**Table 1**). Multiple pulmonary radiologic findings manifested in one patient. The diagnosis of nocardiosis was established by modified acid-fast and/or methenamine silver stain and culture from sputum, bronchoalveolar lavage fluid (BALF), or biopsy tissues of lung, skin, and brain lesions. Pulmonary nocardiosis was diagnosed in all patients. Other infection sites of Nocardia were brain (5/18), skin (2/18), blood (1/18), and paravertebral site (1/18). Co-infections were found in 33.3% (6/18) patients. Co-infected microorganisms included *Pneumocystis jirovechi* (3/18), *Aspergillus* (3/18), *Escherichia coli* (2/18), *Clostridium difficile* (1/18), *Enterococcus* (1/18), *Pseudomonas* (1/18), and *Staphylococcus aureus* (1/18) (**Table 1**). Diagnosis time of Nocardia was variant.

TABLE 1 | Clinical characteristics, diagnosis, treatments, and outcomes of 18 patients of Cushing's syndrome with nocardiosis.

Authors	Country	Age (year)	Gender	HTN	DM	24hr UFC (μg)	ACTH (pg/mL)	Serum total cortisol (μg/dl)	Cause of Cushing's syndrome	Infection sites	Chest imaging	Co-infection	Antibiotics	Treatment of Cushing's syndrome	Outcome
Petersen DP, 1981 (10)	U.S.	72	F	No	No	NA	elevated	elevated	EAS origin from pulmonary carcinoid tumor	lung and skin	nodules	none	TMP-SMZ	Mitotane	mortality
Natale RB, 1981 (11)	U.S.	24	M	No	Yes	1,1820	902	110	EAS origin from bronchial carcinoid	lung	infiltrated and cavitary lesion	*Pneumocystis carinii*	TMP-SMZ	metapyrone and bilateral adrenalectomy	mortality
Higgins TL, 1982 (12)	U.S.	47	M	No	Yes	882	1,128	44	EAS origin from pancreatic neuroendocrine tumor	lung	nodules	*E. coli* and *Pseudomonas*	intravenous sulfadiazine and oral cycloserine	metyrapone, aminoglutethimide,5-fluorouracil, streptozocin, and Cytoxan	mortality
Findlay JC, 1992 (13)	U.S.	71	F	Yes	Yes	NA	NA	47.8	Cushing's disease	lung and brain	cavitary lesion	none	sulfadiazine	aminoglutethimide and metyrapone	mortality
Boscaro M, 1994 (14)	Italy	27	M	No	No	980	48.5	27	occult EAS	lung, brain, and abdomen	infiltration	none	TMP-SMZ	metyrapone + aminoglutethimide followed by bilateral adrenalectomy	survival
Huang TP, 1994 (15)	China	25	M	No	Yes	8,454	725	62	EAS origin from rib small cell carcinoma	lung	nodules and cavitary lesion	none	NA	ketoconazole	mortality
Beinart GA, 2003 (16)	U.S.	68	M	Yes	Yes	4,322	519	82	EAS origin from metastatic small cell lung carcinoma	lung	consolidation and cavitary lesion	*Aspergillus*, *Clostridium difficile* colitis, enterococcal bacteremia	TMP-SMZ	carboplatin, etoposide, ketoconazole	mortality
Chrysanthidis T, 2010 (17)	Greece	52	F	No	Yes	>1812	79	20.3	occult EAS	lung, brain, and skin	infiltration	none	meropenem, gentamicin, and minocycline	ketoconazole	mortality
Sutton BJ, 2011 (18)	U.S.	42	F	No	No	NA	152	NA	EAS origin from pulmonary carcinoid tumor	lung	nodules	none	TMP-SMZ	RFA of the carcinoid tumor	survival
Chowdry RP, 2012 (19)	U.S.	48	F	No	Yes	16,340	296	106.2	EAS origin from pancreatic neuroendocrine cancer	lung and blood	nodules and pleural effusion	*E. coli* and *Pneumocystis jirovechi*	TMP-SMZ	ketoconazole	mortality
Momah N, 2012 (20)	U.S.	42	M	Yes	Yes	21,469	1,013	130	occult EAS	lung and brain	cavitary lesion	methicillin-sensitive *Staphylococcus aureus*, Pneumocystosis and brain aspergillosis	TMP-SMZ	ketoconazole, octreotide, and radical thymectomy and mediastinectomy	mortality
Rizwan A, 2014 (21)	Pakistan	53	M	Yes	Yes	2,000	68.5	20	occult EAS	lung	cavitary lesion	none	TMP-SMZ	bilateral adrenalectomy	survival
Rizwan A, 2014 (21)	Pakistan	54	M	Yes	Yes	27,216	159	134	occult EAS with multiple metastasis	lung	cavitary lesion	none	TMP-SMZ	none	mortality

(Continued)

TABLE 1 | Continued

Authors	Country	Age (year)	Gender	HTN	DM	24hr UFC (μg)	ACTH (pg/mL)	Serum total cortisol (μg/dl)	Cause of Cushing's syndrome	Infection sites	Chest imaging	Co-infection	Antibiotics	Treatment of Cushing's syndrome	Outcome
Rizwan A, 2014 (21)	Pakistan	38	M	Yes	Yes	9,088	255	192	occult EAS	lung	consolidation and pleural effusion	none	TMP-SMZ	ketoconazole	survival
Xu L, 2016 (8)	China	35	M	Yes	Yes	3,118.08	372	>50	EAS origin from mediastinal paraganglioma	lung	nodules and cavitary lesion	none	TMP-SMZ	resection of the mediastinal tumor	survival
Kobayashi K, 2018 (22)	Japan	52	F	No	No	NA	469	59.6	EAS origin from olfactory neuroblastoma	lung	nodules	none	TMP-SMZ	metyrapone and mitotane	survival
Mylonas CC, 2019 (23)	Greece	40	F	Yes	Yes	NA	126.9	61.5	Cushing's disease	lung	nodules and cavitary lesion	none	TMP-SMZ	transsphenoidal pituitary surgery	survival
Our case, 2020	China	41	M	No	Yes	3,522	171	51.2	occult EAS	lung and brain	cavitary lesion	*Aspergillus*	TMP-SMZ, ceftriaxone and amphotericin B	none	mortality

The shortest time for identification of Nocardia was 3 days after symptoms onset. In some patients, the Nocardia identification time lasted for several weeks, even after the patients' death.

Fourteen patients were treated with TMP-SMZ for nocardiosis. Due to the resistance of TMP-SMZ, one patient was treated with meropenem and gentamicin. The duration of antibiotic therapy lasted from 3 days to 1 year. The treatment of CS included surgery and medical therapy. Transsphenoidal pituitary surgery was performed in one patient, resection or radiofrequency ablation (RFA) of EAS tumor in three patients, bilateral adrenalectomy in three patients. Drugs that reduced cortisol levels, including ketoconazole, metapyrone, mitotane, and cytotoxic drugs, were used in 12 patients. In terms of prognosis, 11/18 (61.1%) patients died. Eight patients died of infections and three patients died of progression of malignancy. The average ACTH, cortisol, and 24hr UFC level of mortality and survival were 554.7 ± 401.0 and 213.1 ± 159.0 pg/ml ($P = 0.04$), 78.8 ± 39.5 and 72.0 ± 69.6 μg/dl, 9,369.3 ± 9,560.8 μg, and 3,796.5 ± 3,634.1 μg, respectively. The patients that had co-infections had higher ACTH level (671.5 ± 398.2 *vs* 245.5 ± 217.1 pg/ml, $P = 0.047$). Patients with lower level of ACTH, no co-infection, underwent CS surgery and received antibiotic therapy for more than 6 months had more possibilities to survive (**Table 2**).

DISCUSSION

Although nocardiosis in EAS patients has been reported, our review presented 18 nocardiosis with CS patients (16 EAS and 2 CD) and emphasized the possibility of Nocardia infection in other forms of CS. In addition, according to the clinical characteristics, treatments, and prognosis of these 18 patients,

TABLE 2 | Comparison of clinical characteristics between distinct outcomes of patients of Cushing's syndrome with nocardiosis.

		Survival	Mortality	P value
n		7	11	
Age (year)		41.0 ± 9.2	49.5 ± 16.5	0.24
Gender	Female	3	4	0.78
	Male	4	7	
24hrUFC (μg)		3,796.5 ± 3,634.1 (n = 4)	9,369.3 ± 9,560.8 (n = 6)	0.31
F (μg/dl)		72.0 ± 69.6 (n = 5)	78.8 ± 39.5 (n = 10)	0.81
ACTH (pg/ml)		213.1 ± 159.0 (n = 7)	554.7 ± 401.0 (n = 9)	0.04
Cause of Cushing's syndrome	CD	1	1	1.00
	EAS	6	10	
DM	Yes	4	10	0.25
	No	3	1	
Extrapulmonary nocardiosis	Yes	1	6	0.09
	No	6	5	
Co-infections	Yes	0	6	0.02
	No	7	5	
Surgery of CS	Yes	5	2	0.02
	No	2	9	
Treatment duration of antibiotics	≥6 months	6	2	0.002
	<6 months	0	8	

we put forward the risk factors for mortality in CS patients with nocardiosis.

Opportunistic infections in endogenous CS were predominantly observed in patients with severe cortisol excess (24). Previous reports (16, 25) had shown that high levels of endogenous glucocorticoids above the cut-off levels of serum cortisol, 43.1 μg/dl and 24hrUFC, 2,000 μg/d, were reliable indicators for severe infections in EAS patients. Fifteen of 18 (83.3%) patients including 13 EAS and 2 CD patients in our series were detected of high levels of serum cortisol or 24hrUFC exceeded these cut-off values. In addition, our review showed that CS patients with higher level of ACTH had more risks for co-infections and mortality. It was suggested that we should give more concern to avoiding infections in CS patients with extremely high ACTH concentrations. We did not find the difference in serum cortisol or 24hrUFC between patients of survival and mortality maybe because of the relatively small sample size. Hypercortisolism impaired cellular and humoral immunity. CS patients show significant lymphopenia, especially the reduction in the CD4+ subset, the reduction in the CD4/CD8 ratio are predictors for opportunistic infections (26). However, there was no record of lymphocytes subsets analysis in our review.

Pulmonary infection was the most common manifestation in nocardiosis. The clinical characteristics and symptoms of pulmonary nocardiosis were non-specific. Some patients had no pulmonary symptoms while some patients experienced respiratory failure rapidly. The radiologic findings were variable. Nodules, masses, cavitations, infiltration, consolidation, and pleural effusion could be radiographic presentations of pulmonary nocardiosis. Xu L. et al. (8) proposed cavity lesions, consolidation/infiltration, and nodule/mass were the major findings for EAS patients. The most common findings were cavitary lesion and nodules in our review. It was noted that these radiologic findings could also be the presentation of fungal, mycobacterial infections, and malignancies including both primary and metastatic lung cancers. Lung nodules were suspected to be tumors of EAS in five patients (27.7%) (19, 21–23) in our series including our patient. Biopsies of suspicious lung nodules were performed. Histological and cytologic examination of the biopsy showed no evidence of malignancy but inflammation. Nocardia infection was confirmed by the biopsy. Therefore, rapid changes of chest imagings indicated an infective etiology rather than malignancy and Nocardia infection should be carefully cautioned (19).

Aggressive diagnostic approaches were warranted in individuals suspected of infections. Broncho-alveolar lavage (BAL), brushing by bronchoscopy, or percutaneous lung fine-needle aspiration from the cavitated nodule might be the drawing location for cytology examinations and culture to establish the diagnosis of pulmonary nocardiosis. We should pay adequate attention in order to make early etiological diagnosis.

In our CS review, 11 of 18 patients (61.1%) died. The mortality rate was similar with that reported in EAS patients of 66.7% (8). It is seemed that the mortality rate of nocardiosis in CS patients is higher than that in other immunocompromised patients of 34.5–40% (3, 4). Mortality appeared to be correlated with multiple sites of infections and was reported as high as 100% in patients with disseminated diseases (4, 27). We did not find the extrapulmonary nocardiosis had impacts on mortality. The reason might be the small sample of our case series. Co-infections with other microorganisms have been found to attribute to mortality in nocardiosis (28). Our patients and the other two patients (16, 20) with nocardia and aspergillus co-infections had bad outcomes. Moreover, 33.3% (6/18) patients had co-infections in our series. All of them died afterwards. CS patients with marked high levels of ACTH are prone to have co-infections. Clinicians need to be mindful of opportunistic co-infections in patients with CS.

Reducing the cortisol level was essential for the treatment of CS with nocardiosis (29, 30). Resection of primary tumor that induced over-secretion of ACTH was an efficient and rapid strategy. However, EAS can be a diagnostic challenge with the hormonal source difficult to find. Seven patients (38.9%) had occult EAS in our series. ^{68}Ga-conjugated somatostatin receptor targeting peptide positron emission tomography (^{68}Ga-SSTR-PET/CT) contributes to localization of primary tumor of EAS (31). If the primary tumor couldn't be found, bilateral adrenalectomy might be of value (32). Anticortisolic drugs also provided decrease of hypercorticism (33).Patients who underwent CS surgery had better prognosis than those treated by medicines only. Moreover, it was worth mentioning that patients with nocardiosis generally needed 6 to 12 months of antibiotic therapy, depending on their immunological status and the organs infected (34). The survived patients received antibiotic drugs for more than 6 months in our review.

There are some limitations in this study. Firstly, EAS was suspected without definite localization of primary tumor produced excess hormone in our patient. Secondly, this is a retrospective study. In addition, the sample size is relatively small as Nocardia infection in CS is incredibly rare reported. Future research is required to improve the prognosis of CS with nocardiosis.

In conclusion, Nocardia infection should be cautioned when a patient with CS presents abnormal chest radiographs. The mortality risk factors of CS with nocardiosis are high level of ACTH and co-infections. We should endeavor to make early etiological diagnosis. Long-term application of sensitive antibiotics and aggressive treatments of CS are beneficial for prognosis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Peking Union Medical College 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

DZ and YJ designed the study, and DZ and HF participated in data collection. DZ performed the systematic review and drafted the manuscript. YJ edited and reviewed the manuscript. LL, ZL, WX, and XX partially conceived the research idea. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Everolimus Related Fulminant Hepatitis in Pancreatic Neuroendocrine Tumor With Liver Metastases: A Case Report and Literature Review

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Background: Everolimus, an immunosuppressant, is approved for the treatment of advanced renal cell carcinoma, metastatic hormone receptor-positive breast cancer, and pancreatic neuroendocrine tumors (P-NETs) but has been reported to be related to hepatitis B reactivation. Here, we present the first case of fatal fulminant hepatitis B reactivation in a man with P-NET accompanied by multiple liver metastases who received everolimus and octreotide long-acting repeatable (LAR).

Case Presentation: A 45-year-old male had a history of chronic hepatitis B infection. He was found to have a complicated liver cyst incidentally, and then he underwent biopsy, which disclosed a grade 2 neuroendocrine tumor (NET). Subsequent MRI of the abdomen and PET revealed a solid mass at the pancreatic tail with numerous liver tumors favoring metastases and peripancreatic lymph node metastases. Transarterial chemoembolization (TACE) of the right lobe of the liver was performed, and he started to take 5 mg everolimus twice a day and 20 mg octreotide LAR every month 8 days after the 1st TACE. No hepatitis B virus (HBV) prophylaxis treatment was administered. He then underwent laparoscopic distal pancreatectomy and splenectomy three and half months after the initial treatment of everolimus. He continued everolimus 5 mg twice a day and octreotide 20 mg every month after the operation. Three months later, hepatic failure occurred due to acute hepatitis B flare-up–related fulminant hepatic failure since other possible causes of hepatic failure were excluded. Five days after hepatic failure presented, hepatic failure was apparent, and pulseless ventricular tachycardia occurred. The patient expired after failed resuscitation.

Conclusion: A literature review of everolimus-related hepatitis B reactivation was conducted. In P-NET patients with chronic hepatitis B who will undergo everolimus treatment, HBV prophylaxis should be considered since fatal hepatitis B reactivation might occur under rare conditions.

Keywords: acute hepatitis B flare-up, everolimus, fulminant hepatitis B, neuroendocrine tumor (NE tumor), pancreatic neuroendocrine tumor (pNET)

BACKGROUND

Everolimus, at type of mammalian target of rapamycin (mTOR) inhibitor, is approved for the treatment of advanced renal cell carcinoma, metastatic hormone receptor-positive breast cancer, and pancreatic neuroendocrine tumors (P-NETs). However, as an immunosuppressant, everolimus has been reported to be related to hepatitis B reactivation. Previous publications have proposed fatal hepatitis B reactivation in patients receiving everolimus for metastatic breast cancer and advanced renal cell carcinoma. Here, we present the first case of fatal fulminant hepatitis B reactivation in a man with P-NET accompanied by multiple liver metastases who received everolimus and octreotide long-acting repeatable (LAR).

CASE REPORT

A 45-year-old male had a history of chronic hepatitis B infection and hypertension and a mild increase in glucose levels. He had been regularly followed up in a gastrointestinal outpatient clinic for chronic hepatitis B infection. Half a year before admission, a complicated liver cyst was incidentally found on liver sonography in another hospital, where computed tomography (CT)-guided biopsy of the complicated liver cyst was performed. The pathology report revealed grade 2 neuroendocrine tumors (NETs) with a mitotic figure of 7 in 10 high-power fields. Immunohistochemical staining confirmed the diagnosis of NETs with positive chromogranin A, synaptophysin, and CD 56 staining. The lesion was negative for CK-7 and hepatocellular carcinoma markers, including Hepar-1, arginase-1, and glypican-3. Metastatic NET was considered. During this period, there was no abdominal pain, flushing, or diarrhea but mild cold sweating.

He was then hospitalized in our hospital. The hemogram and biochemistry investigations were normal, including serum carbohydrate antigen 19-9 (CA 19-9) levels (<0.6 IU/L, normal: <37 IU/L), carcinoembryonic antigen (CEA) levels (1.36 ng/mL, normal: <5 ng/mL), and alpha-fetoprotein levels (3.8 ng/mL, normal: <9 ng/mL). Serum chromogranin A levels were elevated (119.80 ng/mL, normal: <101.9 ng/mL), but adrenal function (cortisol 12.87 μg/dL, normal: 7-9AM 4.2-22.4 μg/dL, 3-5PM 3.1-16.7 μg/dL; adrenocorticotropic hormone (ACTH) 35.10 pg/mL, normal: 7.2-63.3 pg/mL), gastrin levels (44.2 pg/mL, normal: 28-185 pg/mL), and C-peptide levels (4.5 ng/mL, 1.1-4.4 ng/mL) were all within the normal range, as were urinary vanillylmandelic acid (VMA) (8.1 mg/day, normal: 1.9-9.8 mg/day) and 5-hydroxyindoleacetic acid (5-HIAA) (4.8 mg/day, normal: 2-6 mg/day) levels. Hepatitis B surface antigen (HBsAg) (7069.00, nonreactive: <0.9, equivocal: 0.9-10, reactive: >10), anti-HBs antibody (528.50 IU/L, nonreactive: <10), and anti-HBc antibody (0.005, non-reactive: >1.0) were all reactive but nonreactive to anti-hepatitis C virus (HCV) antibody. Subsequent abdominal CT showed a pancreatic body cystic tumor approximately 1.5 cm in size (**Figure 1A**) and a suspected pancreatic tail tumor associated with liver tumors in the right and left lobes (**Figure 1B**). Magnetic resonance imaging (MRI) of the abdomen revealed a solid mass at the pancreatic tail with numerous liver tumors favoring metastases. The pancreatic body tumor was a cyst (**Figure 2A**). Positron emission tomography (FDG-PET) with MRI also showed peripancreatic lymph node metastases (**Figure 2B**). Transarterial chemoembolization (TACE) of the right lobe of the liver was performed during this admission. He was discharged 3 days after TACE.

He started to take 5 mg everolimus twice a day and 20 mg octreotide LAR every 28 days, starting from 8 days after the 1st TACE. No hepatitis B virus (HBV) prophylaxis treatment was administered. After he took everolimus for three months and octreotide 3 times, and subsequent abdominal CT showed stationary pancreatic NETs but decreased liver metastasis

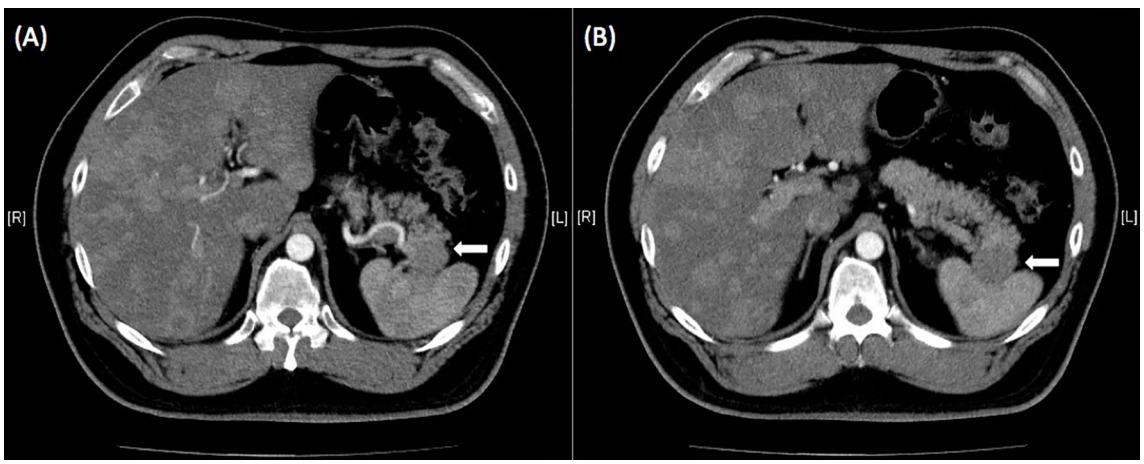

FIGURE 1 | **(A)** Abdominal computed tomography showed a pancreatic body tumor approximately 1.5 cm in size associated with liver tumors in the right and left lobes. Adrenal glands were normal without enlargement. **(B)** A suspected tumor at the pancreatic tail was disclosed on enhanced abdominal computed tomography (white arrow).

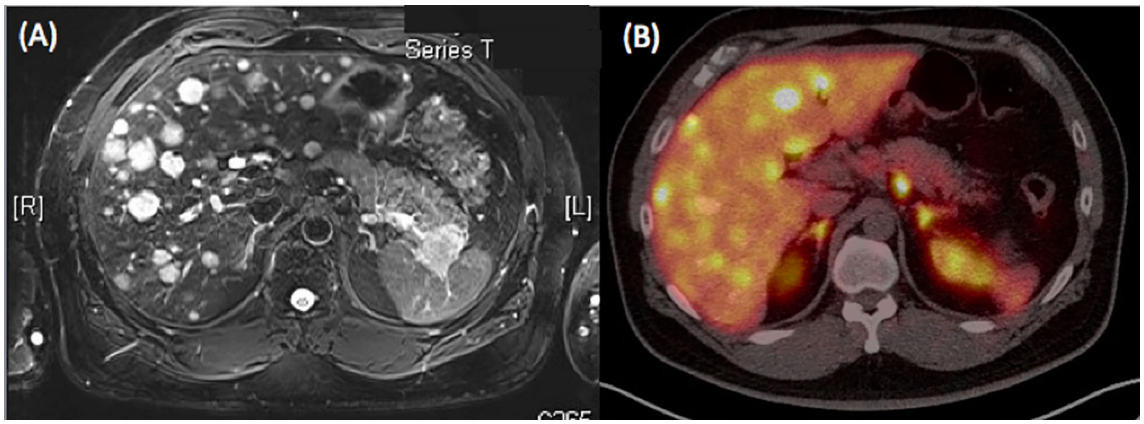

FIGURE 2 | **(A)** MRI of the abdomen revealed a solid mass at the pancreatic tail with numerous liver tumors favoring metastases. The pancreatic body tumor was a cyst. **(B)** PET with MRI also showed peripancreatic lymph node metastases.

nodule numbers, suggesting partial response to concurrent everolimus (**Figures 3A, B**). Therefore, he underwent laparoscopic distal pancreatectomy and splenectomy three and half months after the initial treatment of everolimus. The pathology report revealed grade 2 NETs of the pancreas with direct invasion of the spleen. Immunohistochemical analysis revealed that tumor cells were positive for CD56, chromogranin A, synaptophysin and beta-catenin on the membrane and negative for insulin. Ki-67 index was 15% (**Figures 4A, B**). The postoperative course was uneventful, and he was discharged 7 days after the surgery.

He continued everolimus 5 mg twice a day and octreotide 20 mg every 28 days after the operation. No HBV prophylaxis treatment was administered as before. Two and half months after the surgery, he underwent abdominal CT for follow-up, which disclosed no evidence of local recurrence at the pancreas and regression of the liver tumor where previous TACE was performed; the other liver metastases appeared as stable disease.

Approximately 2 weeks after the last abdominal CT, he had general weakness with an icteric look and then coma. He visited another hospital abroad, and was initially hospitalized there; hepatic failure progressed there. Four days later, he was transferred back to our hospital and admitted to the intensive care unit (ICU). The hemogram and biochemistry study showed coagulopathy but no thrombocytopenia (international normalized ratio (INR) 2.9, platelet count 22,6000/μL), jaundice (total bilirubin 11.1 mg/dL, direct bilirubin 6.9 mg/dL), abnormal liver function test (aspartate aminotransferase (AST) 824 U/L, alanine aminotransferase (ALT) 636 U/L), azotemia (blood urea nitrogen (BUN) 21.2 mg/dL, creatinine 5.87 mg/dL) or elevation in ammonia level (408 μg/dL). The HBV DNA level was 1.832509 million cps/ml, and the anti-hepatitis B e antibody was reactive, so entecavir was prescribed. Anti-hepatitis C antibody, CMV-IgM, HIV antigen, EB-VCA IgM, and RPR were all nonreactive. Acute hepatitis B flare-up–related fulminant hepatic failure was diagnosed since other possible causes of hepatic failure were

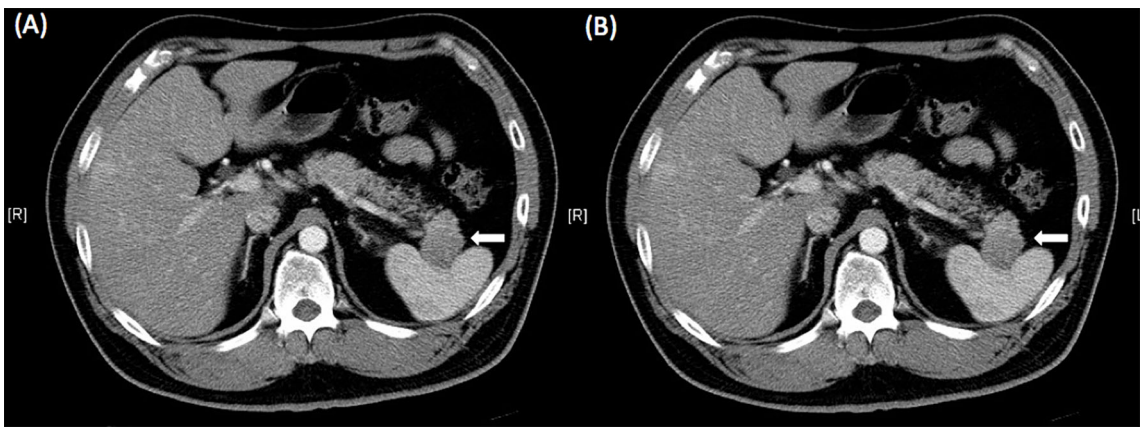

FIGURE 3 | Abdominal computed tomography after 3 months of treatment with everolimus showed stationary pancreatic neuroendocrine tumors (**A**, white arrow) but decreasing liver metastasis nodule numbers **(B)**, suggesting a partial response to everolimus.

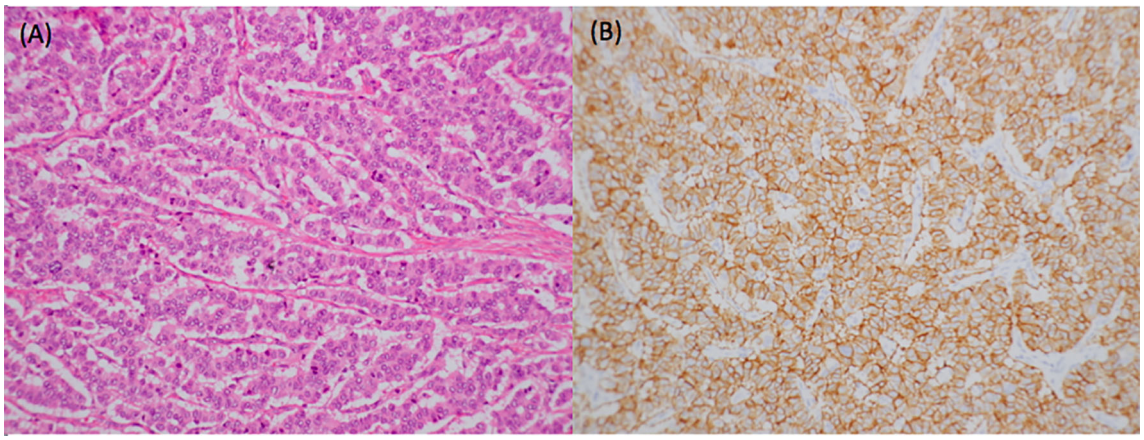

FIGURE 4 | Pathology from distal pancreatectomy showed a neuroendocrine tumor with numerous mitotic figures **(A)**. Immunohistochemical analysis revealed that tumor cells were all positive for CD56, chromogranin A, synaptophysin, and beta-catenin on the membrane but negative for insulin. Ki-67 index was 15%. Therefore, it was a grade 2 neuroendocrine tumor **(B)**.

excluded. However, general tonic clonic seizures occurred in the ICU, but brain CT showed no obvious lesions. Five days after hepatic failure was apparent, pulseless ventricular tachycardia occurred. The patient expired after failed resuscitation.

DISCUSSION

Everolimus is approved as a single agent for the treatment of advanced renal cell carcinoma and P-NETs and as combination treatment with exemestane for the treatment of hormone receptor-positive breast cancer (1–3). Pavel et al. published the randomized, double-blind, placebo-controlled, phase 3 RADIANT-2 study in which median progression-free survival (PFS) improved for 5.1 months with everolimus plus octreotide LAR compared with placebo plus octreotide LAR in patients with low- or intermediate-grade advanced NETs and a history of carcinoid symptoms (4). Despite no significant improvement in PFS, the latest result of the final overall survival from the RADIANT-2 study was positive with a hazard ratio of 1.08 (5), and everolimus plus octreotide LAR was still considered an effective approach for these patients. This finding was supported by our patient having stable disease of the original P-NET tumor but significant improvement in liver metastases, in which the tumor burden seemed decreased under the control of everolimus plus LAR. However, acute flare-up of hepatitis B leading to fulminant hepatic failure occurred when everolimus plus LAR was used for 5.5 months. One out of 204 patients in the RADIANT-2 trial developed fatal HBV reactivation (4). A similar event has been reported in a patient with metastatic breast cancer who received treatment with everolimus plus exemestane for a 15-day period (6). Everolimus-related acute hepatitis reactivation has also been reported in renal cell carcinoma, in which one event occurred after a 3-month period of everolimus use and another after a 5-month period (7, 8). The latter patient died of fulminant hepatitis. **Table 1**

TABLE 1 | Case reports about everolimus related HBV reactivation.

Year	Disease	Medicine used	Duration of treatment till HBV reactivation	Treatment of HBV reactivation	Outcome
2013 Sezgin et al. (7)	RCC with lung and axillary metastases	Everolimus 10mg daily then tapering to 5mg daily	5 months	Tenofovir	Resolved
2013, Shinta et al. (8)	RCC with lung metastases	Everolimus	5 months	Entecavir and steroid pulse therapy (methylprednisolone, 1000 mg/day for 3 days with gradual tapering)	Died
2013, Eleonora et al. (6)	Breast cancer with lung, bone, pancreas, intramuscular metastases	Everolimus 10mg daily+ exemestane 25mg daily	24 days	Tenofovir	Died
2016, Olivier et al. (3)	Breast cancer with bone metastases	Everolimus 10mg daily+ exemestane 25mg daily	3 months	Entecavir	Resolved
2021 Chang et al.	P-NET accompanied by multiple liver metastases	Everolimus 5mg twice a day + long acting octreotide 20mg every month	5.5 months	Entecavir	Died

RCC, Renal cell carcinoma.

summarizes the case reports on everolimus-related HBV reactivation. According to the literature review, this rare but fatal event seems to occur from 0.5 to 6 months after the start of use of everolimus. In breast cancer, fatal events seem to occur earlier (0.5 months and 3 months), and in renal cell carcinoma, they seem to occur later (both 5 months). The fatal event in our case tended to occur later (5.5 months), which is similar to that in renal cell carcinoma. However, since the number of case reports is still limited, the risk factors and the duration of HBV reactivation after everolimus use still need further investigation.

Based on recent estimates, approximately 350 million people worldwide suffer from chronic hepatitis B infection (9). HBV reactivation is defined as a sudden and rapid increase in the HBV DNA level by at least 100-fold in patients with previously detectable HBV DNA or the reappearance of HBV DNA viremia in individuals who did not have viremia before the initiation of immunosuppressive or biological modifier therapy or cancer chemotherapy. Five stages have been proposed regarding HBV reactivation related to immunosuppressive or biological modifier therapy or cancer chemotherapy (9). HBV reactivation should particularly be paid attention to when people are exposed to cancer chemotherapy, immunosuppressive therapy, or biologic therapies for the management of malignancies or benign conditions, such as rheumatologic conditions, inflammatory bowel disease, dermatologic conditions, or solid-organ or bone marrow transplantation (9, 10). **Table 2** summarizes cytotoxic and immunosuppressive agents that have been reported to be related to HBV reactivation.

Everolimus is an mTOR inhibitor, and other similar medicines include rapamycin. The immunosuppressive properties of everolimus may predispose patients to opportunistic infections and/or the reactivation of previous infections. As expected, infective pneumonia and other bacterial and invasive fungal infections have been reported in patients treated with everolimus, as well as the reactivation of viral infections (7, 12), including hepatitis E virus (13–16). Another mTOR inhibitor, sirolimus, has also been reported to be associated with the reactivation of hepatitis B with octreiotide previously (17). The possible mechanism was that octreotide has been proposed to significantly reduced hepatic blood flow that decreased the liver metabolic activity in patients with hepatitis B surface antigen positive cirrhotic patients, which might be relevant to the reactivation of hepatitis B (18). In addition, somatostatin has been be hypothesized to be related to autocrine and paracrine regulatory role, and *via* neuro-endocrine modulation of the immune response, it might represent a direct regulatory relation between the nervous and immune system (19). Consequently, as an analogue of somatostatin, octreotide might play similar role. In contrast, several mechanisms have been proposed regarding the relationship between HBV inactivation, instead of activation, from mTOR inhibitors (20–22). Consequently, the mechanism by which fulminant hepatitis B originates from everolimus remains unclear.

Due to the increased risk of the reactivation of hepatitis B in patients who will receive immunosuppressive or cytotoxic therapies, many institutes have suggested screening before treatment is initiated, with at least HBsAg, anti-HBc, and anti-HBs (9, 10, 23–28); all those who are negative for HBsAg, anti-HBc, and anti-HBS should be vaccinated against HBV. Although no guidelines are available concerning the feasibility of antiviral prophylaxis combined with everolimus in treating P-NET or breast cancer, lamivudine, entecavir or tenofovir were suggested for anti-HBV prophylaxis in advanced renal cell carcinoma patients receiving everolimus (29). The duration of prophylaxis remains inconclusive. However, according to the European Association for the Study of the Liver (EASL) 2017 clinical practice guidelines, HBV prophylaxis should continue for at least 12 months and 18 months for rituximab-based regimens after the cessation of immunosuppressive treatment and discontinued only if the underlying disease is under remission. Close follow-up is also suggested, including liver function tests and HBV DNA during prophylaxis lasting for at least 12 months after antiviral agent withdrawal, since HBV reactivation might develop after antiviral agent discontinuation (26).

In conclusion, in P-NET patients who will receive everolimus plus octreotide LAR, HBV reactivation might occur, though the incidence is low. The duration of everolimus use for HBV reactivation is still inconclusive. The mechanism between everolimus and hepatitis B reactivation remains unclear. The protocol for HBV prophylaxis in everolimus is not well established. However, since fatal reactivation events have been reported in advanced renal cell carcinoma and metastatic breast cancer, clinicians should consider routine HBV screening and antiviral prophylaxis before everolimus therapy is initiated for P-NET patients receiving everolimus plus octreotide LAR.

TABLE 2 | Cytotoxic or immunosuppressive agents associated with HBV reactivation (6, 11).

Alkylating agents	Cyclophosphamide, Chlorambucil, Cisplatin, Temozolomide, Procarbazine
Alkaloids	Vincristine, Vinblastine
Antimetabolites	Cytarabine, Fluorouracil, Gemcitabine, Mercaptopurine, Methotrexate, Thioguanine
Monoclonal antibodies	Rituximab (anti-CD20) Alemtuzumab (anti-CD52) Mogamulizumab (anti CC-chemokine receptor 4) Anti-TNF-alpha (infliximab, adalimumab, golimumab, and certolizumab)
Other cytotoxic agents	Docetaxel, Etoposide, Fludarabine, Mitomycin, Bleomycin
Tyrosine kinase inhibitor (TKI)	Imatinib, Nilotinib, Dasatinib, Erlotinib, Ibrutinib
Other	Interferon

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 patient's family for the publication of this case report and any accompanying images.

AUTHOR CONTRIBUTIONS

C-YT, K-HL and S-YW: discussion and review about the organization of this article, and also deal with the pathology and image of this article. J-TH and T-SY: assist with the revised of article. All authors contributed to the article and approved the submitted version.

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frontiers in Endocrinology

MINI REVIEW
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Immune Checkpoint Inhibitors: New Weapons Against Medullary Thyroid Cancer?

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Medullary thyroid carcinoma is a rare neuroendocrine neoplasm that originates from thyroid C cells. Surgery, with complete resection of the tumor, is the only curative approach. However, in most cases, the tumor recurs at locoregional or metastatic level. In this setting, the management remains challenging. In recent years, the immune checkpoint inhibitors have provided promise for changing the cancer treatment paradigm through the application of new approaches that enhance the body's natural antitumor defenses. The aim of this review is to summarize and discuss available data on efficacy and safety of the Food and Drug Administration-approved immune checkpoint inhibitors in patients with medullary thyroid carcinoma. After an extensive search, we found 7 useful data sources (one single-case report, one short article with very preliminary data, five ongoing registered clinical trials). Despite the lack of published evidence regarding the use of immune check point inhibitors, it must be considered that all the ongoing registered clinical trials saw first light in the last three years, thus indicating a growing interest of researchers in this field. Results coming from these trials, and hopefully, in the next future, from additional trials, will help to clarify whether this class of drugs may represent a new weapon in favor of patients with medullary thyroid carcinoma.

Keywords: medullary thyroid carcinoma, immune checkpoint inhibitors, avelumab, durvalumab, ipilimumab, nivolumab, pembrolizumab

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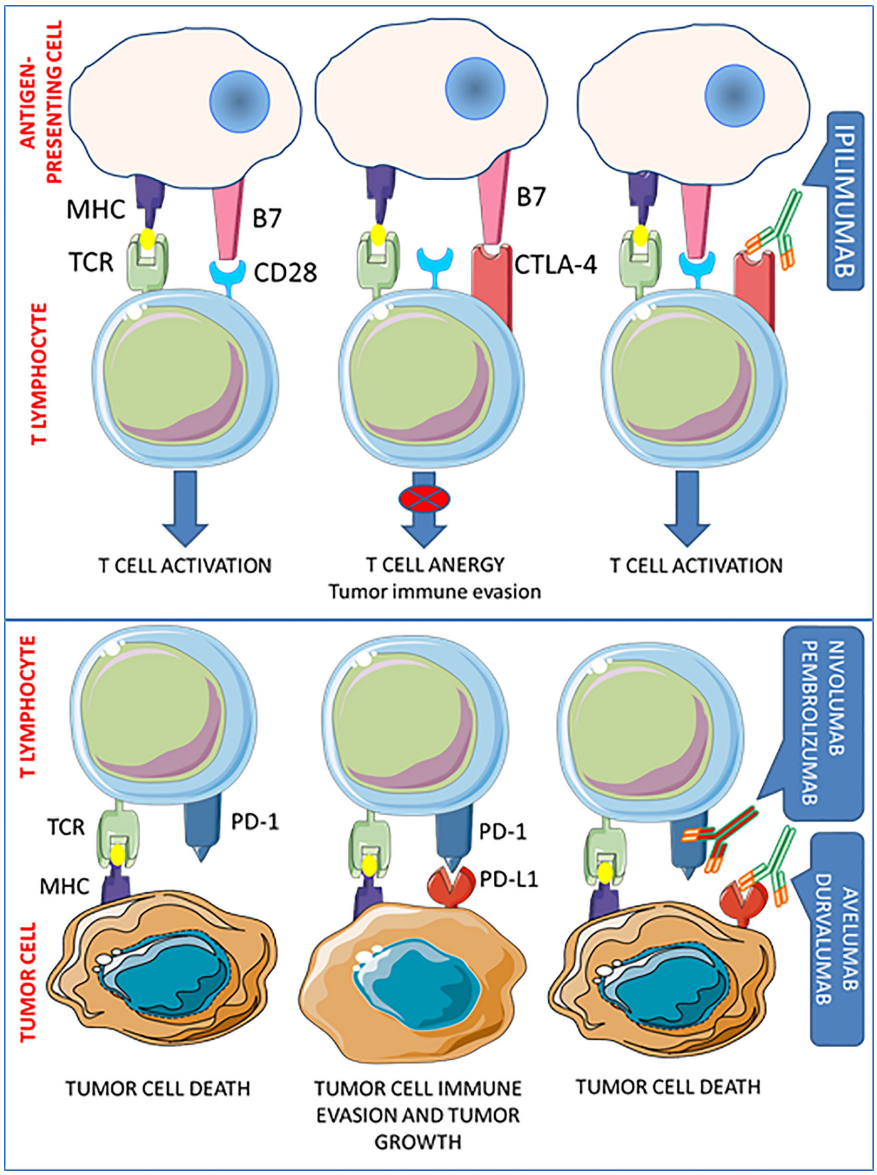
Abbreviations: AEs, adverse events; APCs, antigen-presenting cells; ATC, anaplastic thyroid cancer; DTC, differentiated thyroid carcinoma; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DoR, duration of response; FDA, Food and Drug Administration; ICIs, immune checkpoints inhibitors; KO, knock-out; MTC, medullary thyroid carcinoma; NEN, neuroendocrine neoplasm; ORR, overall response rate; OS, overall survival; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand-1; PD-L2, programmed death-ligand-2; PFS, progression-free survival; RCTs, registered clinical trials; RET, rearranged during transfection; TME, tumor microenvironment; TCR, T cell receptor; TKIs, tyrosine kinase inhibitors; Tregs, regulatory T cells.



GRAPHICAL ABSTRACT |

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine neoplasm (NEN) that originates from thyroid C cells.

Surgery, with complete resection of the tumor, is the only curative approach (1). However, in many patients, the tumor displays an aggressive behavior, resulting in persistence or locoregional and distant disease recurrence. In this setting, the management remains challenging (1, 2).

Tyrosine kinase inhibitors (TKIs) vandetanib and cabozantinib have shown to improve progression-free survival (PFS), and are currently available as approved agents for the treatment of progressive MTC. However, both drugs may cause grade III or IV adverse events (AEs), classified according to the Common Terminology Criteria for Adverse Events of the National Cancer Institute (3, 4).

In 2020, the new generation TKIs selpercatinib and praseltinib gained the Food and Drug Administration (FDA) approval in patients with advanced/metastatic rearranged during transfection (RET) gene-mutant MTC who require systemic therapy, therefore widening the spectrum of available therapies. However, also for these drugs severe AEs have been reported (5, 6).

Therapeutic options also include radionuclide therapy, such as peptide receptor radionuclide therapy (i.e. lutetium-177 and yttrium-90 labeled somatostatin analogs) (7), and iodine-131-metaiodobenzylguanidine (8). However, radionuclide therapy is not approved for MTC treatment.

In recent years, immunotherapy has provided promise for changing the cancer treatment paradigm through the application of new approaches that enhance the body's natural antitumor defenses.

One of the main mechanisms by which tumors escape host immune surveillance is the so-called cancer immunoediting. Acting on immune checkpoints, tumor cells promote the development of an immunosuppressive environment, to prevent the activation of T cell cytotoxicity. Thus the interfering with immune checkpoint signaling, to restore T cell functioning, is nowadays considered one of the most effective novel antitumor treatment goals (9). To date, antibodies targeted against the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (i.e. ipilimumab), the programmed cell death protein-1 (PD-1) (i.e. cemiplimab, nivolumab, and pembrolizumab), and the programmed death-ligand-1 (PD-L1) (i.e. atezolizumab, avelumab, and durvalumab), have been approved by the FDA for human use with the aim to re-activate patient antitumor immunity (**Figure 1**). These drugs, referred as immune checkpoints inhibitors (ICIs), demonstrated significant clinical effectiveness in the treatment of advanced solid tumors and a favorable safety profile, so that entered in the standard clinical practice for several malignancies (10) (**Table 1**).

The inhibitory co-receptor CTLA-4 is constitutively expressed by immunosuppressive regulatory T cells (Tregs), but it can be induced in T cells when activated by antigen-presenting cells (APC). In resting T cells, CTLA-4 is intracellularly localized in endosomes, but, upon T cell receptor (TCR) and CD28 costimulatory signaling activation, CTLA-4 translocates to the cell membrane (11). When exposed on cell surface, CTLA-4 prevents CD28 binding to B7.1 and B7.2 on APCs, thus

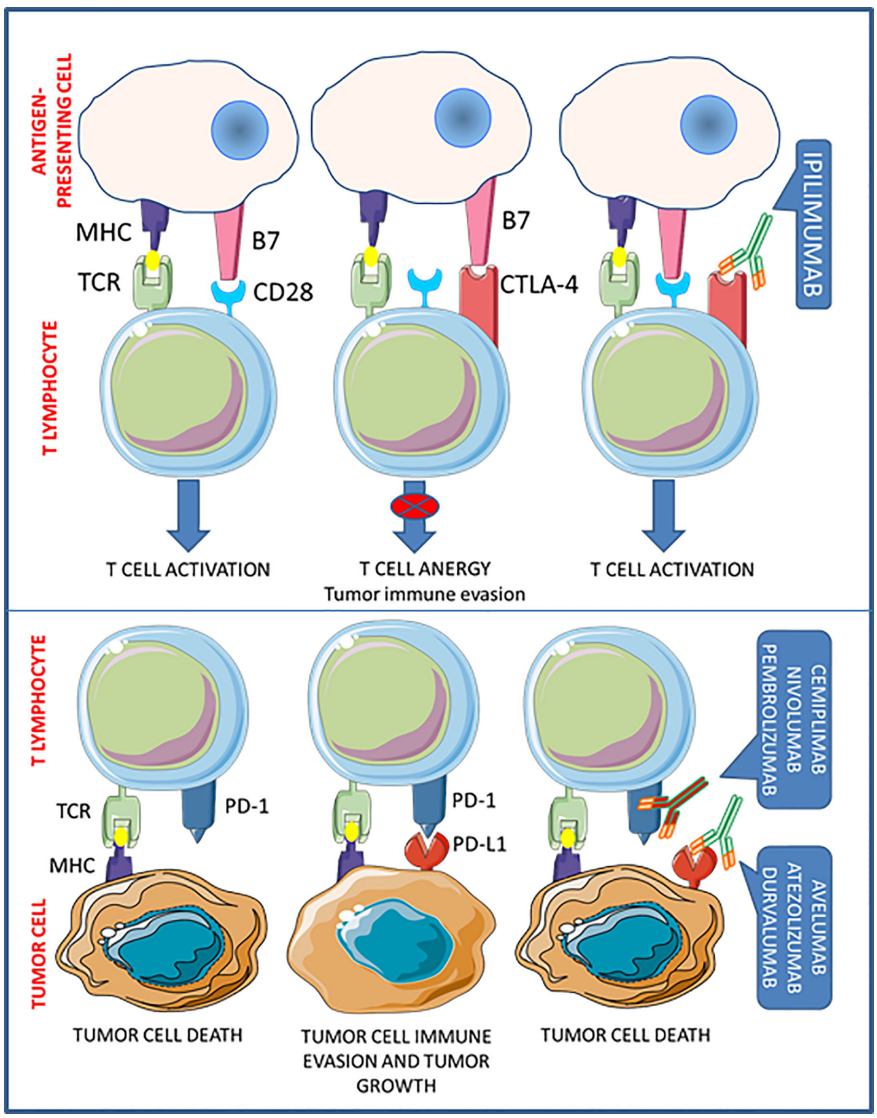


FIGURE 1 | Mechanisms of action for FDA-approved immune checkpoint inhibitors.

TABLE 1 | FDA-approved immune checkpoint inhibitors.

Drug	Brand Name	U.S. Approval	Molecular target	Antibody Description	Indications	Most common adverse reactions
Atezolizumab	Tecentriq®	October 2016	PD-L1	Humanized monoclonal antibody (IgG1-kappa)	Urothelial carcinoma, NSCLC, triple-negative breast cancer	Fatigue, nausea, constipation, cough, dyspnea, and decreased appetite
Avelumab	Bavencio®	March 2017	PD-L1	Fully human monoclonal antibody (IgG1-lambda)	MCC, urothelial carcinoma, RCC	Fatigue, musculoskeletal pain, diarrhea, nausea, infusion-related reaction, rash, decreased appetite, peripheral edema, and urinary tract infection
Cemiplimab	Libtayo®	September 2018	PD-1	Fully human monoclonal antibody (IgG4-kappa)	cSCC	Fatigue, rash and diarrhea
Durvalumab	Imfinzi®	May 2015	PD-L1	Fully human monoclonal antibody (IgG1-kappa)	Urothelial carcinoma, NSCLC	Fatigue, musculoskeletal pain, constipation, decreased appetite, nausea, peripheral edema, urinary tract infection, cough, pneumonitis/radiation pneumonitis, upper respiratory tract infections, dyspnea, rash and alopecia
Ipilimumab	Yervoy®	March 2011	CTLA-4	Fully human monoclonal antibody (IgG1-kappa)	Melanoma, RCC	Fatigue, diarrhea, pruritus, rash, and colitis. Additional AR at high doses include nausea, vomiting, headache, weight loss, pyrexia, decreased appetite, and insomnia
Nivolumab	Opdivo®	December 2014	PD-1	Fully human monoclonal antibody (IgG4-kappa)	Melanoma, NSCLC, SCLC, RCC, cHL, HNSCC, urothelial carcinoma, MSI-H or dMMR colorectal cancer, HCC	Fatigue, rash, musculoskeletal pain, pruritus, diarrhea, nausea, asthenia, cough, dyspnea, constipation, decreased appetite, back pain, arthralgia, upper respiratory tract infection, pyrexia, headache, abdominal pain, and vomiting
Pembrolizumab	Keytruda®	September 2014	PD-1	Humanized monoclonal antibody (IgG4-kappa)	Melanoma, NSCLC, HNSCC, cHL, PMBCL, urothelial carcinoma, MSI-H cancer, gastric cancer, cervical cancer, HCC, MCC	Fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, constipation, pain, and abdominal pain

cHL, classical Hodgkin lymphoma; cSCC, cutaneous squamous cell carcinoma; dMRR, deficient mismatch repair; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; MCC, Merkel cell carcinoma; MSI-H, high level microsatellite instability; NSCLC, non-small-cell lung carcinoma; PMBCL, primary mediastinal B-cell lymphoma; RCC, renal cell carcinoma. Data source, FDA prescribing information.

precluding activation and proliferation of T cells (12). In fact, CTLA-4 inhibition causes a major immunostimulation, as experimentally shown in CTLA-4 knock-out (KO) mice, which die after few months due to uncontrolled lymphoproliferative disorders (13) and, in a clinical setting, by the reactivation of T cell-mediated tumor rejection.

Binding of PD-1 to its ligands, PD-L1 and programmed death-ligand-2 (PD-L2), also prevents T cell activation. PD-L1 is an inducible protein expressed in innate and adaptive immune cells, mesenchymal cells, and cancer cells (14), while PD-L2 is mainly expressed by APCs. PD-1 binding to PD-L1 significantly prevents immune responses directed against cancer cells, thereby altering T cell cytokine release, inhibiting TCR signaling, and abridging T cells/APCs interactions (15). The relevant role of this system in controlling T cell activity was demonstrated in PD-1-KO mice, which develop a spontaneous lupus-like disease caused by unrestrained autoreactive T cells (16). On the other hand, the inhibition of PD-1/PD-L1 binding in cancer can promote T cell activation and proliferation, ultimately leading to cytotoxicity in tumors.

MTC is reported to exhibit low PD-L1 expression in both tumor cells and tumor-infiltrating immune cells (17–19) and no microsatellite instability, irrespective of the presence/absence of either desmoplasia, lymph node metastases and/or RET mutation (18, 20).

However, PD-L1 positivity is associated with aggressive clinicopathological features (e.g., larger tumor size, lymph node or distant metastasis and higher TNM stage) (18, 19) and accounted as a predictor of structural recurrence and biochemical recurrence/persistent disease (19), and CTLA-4 expression is also correlated with advanced staging and structural recurrence-free survival (21), thus suggesting a possible prognostic role in the management of MTC (22).

AIM

The aim of this review is to summarize and discuss available data on efficacy and safety of FDA-approved ICIs in patients with MTC.

MATERIALS AND METHODS

Published Articles

We performed a literature search in the international online databases (PubMed, Web of Science, Scopus, and Embase) using the following terms: "immune checkpoint inhibitors", CTLA-4, PD-L1, PD-1, atezolizumab, avelumab, cemiplimab, durvalumab, ipilimumab, nivolumab, pembrolizumab, "medullary thyroid cancer", "medullary thyroid carcinoma", "thyroid cancer", "multiple endocrine neoplasia type 2".

The search was last updated February 14, 2021.

Registered Clinical Trials

By using the same keywords adopted for reviewing published articles, we conducted an in-depth search in the ClinicalTrials.gov registry, European Clinical Trials Database, and China Clinical Trials Register.

The search was last updated February 14, 2021.

RESULTS

Published Articles

The initial literature search revealed a total of 108 published articles, two of which were pertinent to the study objectives.

Del Rivero et al. have recently reported the case of a 61-year-old male with recurrent MTC (23) showing sharp decline in serum calcitonin level while on avelumab. The patient had been successfully treated with off-label sunitinib for 5 years, but he was forced to withdraw the drug due to relevant side effects. He was then enrolled on a clinical trial with a yeast-based, CEA-targeted, therapeutic cancer vaccine (GI-6207) (24), and his calcitonin doubling time improved in 3 months. He then chose to have elective surgery to remove a neck lymph node and, per protocol, the vaccine was discontinued. Three months after surgery, his calcitonin level was still rising and he was enrolled on a phase I, open-label, multiple-ascending dose trial of avelumab (Avelumab in Metastatic or Locally Advanced Solid Tumors [JAVELIN Solid Tumor], NCT01772004). Thereafter, his calcitonin level decreased > 40% on 5 consecutive evaluations, and response assessment by RECIST v1.1 criteria (25) reported stable disease. However, an immune-related AE (i.e., asymptomatic grade 3 rise in lipase) ultimately led to mandatory treatment discontinuation. A subsequent analysis of a patient's lymph node (resected post-vaccination) revealed that the tumor was PD-L1 positive.

Very preliminary results of a phase II trial evaluating nivolumab plus ipilimumab in patients with aggressive thyroid cancer (NCT03246958) are also available (26). Indeed, 7 patients with progressive MTC and prior TKI failure were included in an exploratory cohort of the study and assessed for radiographic response based on RECIST v1.1 criteria. Lack of partial response is reported for all the 7 patients, without giving further detail. Also, no safety information is provided for MTC as a single cohort (please see the *Registered Clinical Trials* section for more comprehensive description of the trial design).

Registered Clinical Trials (RCTs)

We found 37 registered clinical trials (RCTs), five of which fully matched the aim of this review (**Table 2**).

NCT03753919 (A Phase II Study of Durvalumab (MEDI4736) Plus Tremelimumab for the Treatment of Patients With Progressive, Refractory Advanced Thyroid Carcinoma - The DUTHY Trial) is a prospective, multi-center, open label, stratified, exploratory phase II study whose aim is to evaluate the following outcomes in patients affected by advanced thyroid cancer (estimated enrollment: 46 patients). Primary outcomes are PFS rate at 6 months and overall survival (OS) rate at 6 months; secondary outcomes comprise overall response rate (ORR), duration of response (DoR), median PFS, incidence of treatment-emergent AEs, median OS, and response status after start of study treatment. According to the primary histotype, patients are divided in three cohorts: i) advanced, radioiodine-refractory differentiated thyroid carcinoma (DTC), including papillary, follicular, Hürtle cell and poorly-differentiated thyroid carcinoma (Cohort 1); ii) advanced MTC (Cohort 2); iii) anaplastic thyroid cancer (ATC) (Cohort 3). Each cohort is planned to receive durvalumab plus tremelimumab (anti-CTLA-4 antibody, not yet approved by FDA) every 4 weeks up to 4 cycles followed by durvalumab alone every 4 weeks until progression, unacceptable toxicity or withdrawal. The study started in April 2019, with the estimated study completion date being July 2021. The present study status is "Recruiting".

NCT03246958 (A Phase 2 Study of Nivolumab Plus Ipilimumab in RAI Refractory, Aggressive Thyroid Cancer With Exploratory Cohorts in Medullary and Anaplastic Thyroid Cancer) is a phase II clinical trial evaluating nivolumab in combination with ipilimumab, as a possible treatment for thyroid cancer, focusing on effectiveness (estimated enrollment: 53 patients). The primary endpoint is radiographic response rate as determined by RECIST v1.1 (i.e. partial response plus complete response), whereas secondary outcomes are PFS, OS, and tolerability at two years. This trial is designed to recruit patients with metastatic, progressive, RAI refractory DTC with exploratory cohorts in ATC (7 patients), and incurable, progressive MTC with prior TKI failure (10 patients). Participants aged ≥18 years are divided in two experimental arms: the first arm will be administered nivolumab alone for two weeks followed by nivolumab plus ipilimumab, whereas the second arm ipilimumab alone for two weeks followed by nivolumab/ipilimumab combination therapy. The study started in September 2017. The estimated study completion date is set for March 2025. The present study status is classified as "Active, not recruiting". As above reported, very preliminary results of this trial have been recently published recently (26).

NCT04514484 (Pilot Trial of Nivolumab Plus Cabozantinib for Advanced Solid Tumors in Patients With HIV Infection) is a phase I trial that aims at defining in HIV-positive patients with advanced/metastatic solid cancer (estimated enrollment: 18 patients) the incidence of dose limiting toxicities during cycle 1 of therapy with cabozantinib and nivolumab (primary outcome). Secondary outcomes include the assessment of immune status (CD4 and CD8 cell counts) at each time point from baseline, HIV viral loads, changes in serum markers of immune activation, in immune checkpoint markers, in angiogenesis markers, and in infiltrating immune cell markers. According to the protocol, patients ≥18 years old receive cabozantinib on days 1-28 and nivolumab on day 1. Cycles repeat every 28 days for up to 1 year or 1 year after a partial response is achieved, or 6 months after a complete response is achieved in the absence of disease progression or unacceptable toxicity. The study started in November 2020. The estimated study completion date is November 2025. The present study status is "recruiting".

TABLE 2 | Registered clinical trials evaluating FDA-approved immune checkpoint inhibitors in medullary thyroid carcinoma.

ClinicalTrials.gov Identifier	Molecule	Trial name	Study phase	Medical condition under investigation	Assigned intervention	Primary outcome(s)	Estimated enrollment, n	Estimated study completion date	Trial status
NCT03753919	Durvalumab	A Phase II Study of Durvalumab (MEDI4736) Plus Tremelimumab for the Treatment of Patients With Progressive, Refractory Advanced Thyroid Carcinoma - The DUTHY Trial	Phase II	Metastatic thyroid cancer, including differentiated thyroid carcinoma, medullary thyroid carcinoma, and anaplastic thyroid cancer	Durvalumab 1500 mg plus tremelimumab 75 mg every 4 weeks up to 4 cycles followed by durvalumab 1500 mg every 4 weeks until disease progression, unacceptable toxicity or patients' decision. Cohort 2 is composed by patients affected by advanced medullary thyroid carcinoma	Progression-free survival rate at 6 months [time frame: 6 months] according to RECIST 1.1 criteria Overall survival rate at 6 months [time frame: 6 months]	46	July 2021	Recruiting
NCT03246958	Nivolumab +ipilimumab	A Phase 2 Study of Nivolumab Plus Ipilimumab in RAI Refractory, Aggressive Thyroid Cancer With Exploratory Cohorts in Medullary and Anaplastic Thyroid Cancer	Phase II	Thyroid cancer (radioactive iodine-refractory, aggressive thyroid cancer with exploratory cohorts in medullary thyroid carcinoma and anaplastic thyroid cancer)	Arm I: ipilimumab will be administered *via* IV infusion, starting two weeks after nivolumab alone. Arm II: nivolumab will be administered *via* IV infusion, starting two weeks after ipilimumab alone	Radiographic response rate [time frame: 2 years], as determined by RECIST v1.1 (partial response+complete response)	53	March 2025	Active, not recruiting
NCT04514484	Nivolumab	Pilot Trial of Nivolumab Plus Cabozantinib for Advanced Solid Tumors in Patients With HIV Infection	Phase I	17 listed advanced, refractory, or metastatic solid tumors, including medullary thyroid carcinoma	Patients receive cabozantinib on days 1-28 and nivolumab on day 1. Cycles repeat every 28 days for up to 1 year or 1 year after a partial response is achieved, or 6 months after a complete response is achieved in the absence of disease progression or unacceptable toxicity	Incidence of dose limiting toxicities [time frame: 28 days], defined during cycle 1 of therapy	18	November 2025	Recruiting
NCT03072160	Pembrolizumab	Phase II Trial of Pembrolizumab in Recurrent or Metastatic Medullary Thyroid Cancer	Phase II	Medullary thyroid carcinoma	Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks for two years	Determine whether a PD-1 inhibitor will permit a decline in calcitonin levels or response on imaging [time frame: one year]	17	November 2019	Completed (Results submitted)
NCT03012620	Pembrolizumab	Secured Access to Pembrolizumab for Patients With Selected Rare Cancer Types	Phase II	Sarcoma, ovarian neoplasm, central nervous system neoplasm, thyroid neoplasm (including medullary thyroid carcinoma), neuroendocrine carcinoma, germ cell and embryonal neoplasms, NK/T-cell lymphoma	Pembrolizumab 200 mg on day 1 of every 21 day cycle	Objective response rate [time frame: measured at the first scheduled disease assessment following study treatment initiation (day 84 ± 7 days)] according to RECIST v1.1	350	December 2023	Recruiting

NCT03072160 (Phase II Trial of Pembrolizumab in Recurrent or Metastatic Medullary Thyroid Cancer) is a phase II, open label, single center clinical trial aimed to determine, in patients having or not having undergone previous vaccine therapy (estimated enrollment: 15 patients in each cohort), whether a PD-1 inhibitor may allow for a decline in calcitonin levels or radiographic response (primary outcome); secondary outcomes include impact of previous therapeutic cancer vaccine on response rates, evaluation of immune responses in each cohort, changes in CEA and calcitonin kinetics, PFS, OS and safety. All patients will receive pembrolizumab 200 mg every 3 weeks. The study started in June 2017 and was completed in November 2019, and indeed the present study status is "completed". On 11 February 2021, very preliminary results appeared in the Study Results section of the ClinicalTrials.gov registry. Thirteen patients were enrolled in the cancer vaccine arm (2/13 patients completed the trial), and 4 patients were enrolled in the control arm (none completed the trial). Disease progression was observed in 1/13 patients of the first arm, and in 1/4 patients of the second arm.

NCT03012620 (Secured Access to Pembrolizumab for Patients With Selected Rare Cancer Types) is a phase II, 2, non-randomized, open-label, multicenter study which aims to investigate the efficacy and safety of pembrolizumab in 7 different cohorts of patients with unresectable/locally advanced/metastatic rare cancers for which no other treatment options are available (estimated enrollment: 350 patients). Primary outcome is ORR, whereas secondary outcomes comprise PFS, OS, DoR, time to response, frequency and severity of AEs, and ORR/PFS/OS in subgroups of subjects with high versus low expression of PD-L1, CD4, FOX3 and other immune markers. According to the protocol, cohort 4 features rare thyroid cancer patients of ≥18 years, including MTC; these patients, same as for all other cohorts, are planned to receive pembrolizumab 200 mg on day 1 of every 21-day cycle. The study started in July 2017 and its estimated completion date is December 2023. The present study status is "recruiting".

DISCUSSION

Our review shows, despite very limited published evidence, an increasing attention to the possibility of treating MTC with ICIs, and indeed we found 5 ongoing RCTs with FDA-approved drugs that collectively involve nearly 500 patients with solid tumors, including MTC.

As an additional sign of interest, two trials investigating camrelizumab, a novel PD-1 inhibitor recently approved in China for the treatment of relapsed/refractory classical Hodgkin lymphoma (27, 28), are also intended to recruit patients with MTC, i.e. the NCT04612894 (The Efficacy and Safety of Anti-PD-1 Antibody Camrelizumab Combined With Apatinib for Neoadjuvant Therapy in Locally Advanced Thyroid Cancer: a Phase II Study), and NCT04521348 (A Phase II Study to Explore the Safety and Efficacy of Multiple Target Kinase Inhibitor (mTKI) Combined With Anti-Programmed Death-1(PD-1) Antibody in the Treatment of Advanced Thyroid Cancer) trials.

Notably, PD-1 and CTLA-4 have non-redundant immunosuppressive effects, paving the way for the development of clinical protocols with antibodies targeting the two pathways (29). Combination therapy with anti-PD-1 and anti-CTLA-4 drugs (durvalumab plus tremelimumab, NCT03753919 trial; nivolumab plus ipilimumab, NCT03246958 trial) is giving rise to great expectations in MTC. Overall, there is reliable evidence supporting a greater efficacy of the combined PD-1/CTLA-4 blockade over the two monotherapies in reversing tumor immune inhibition (30–32).

A number of different new scenarios could be opened by combinations or sequential schemes with other anti-tumor treatment modalities.

Systemic chemotherapy has been proposed to exert synergistic effects when combined with PD-1/PD-L1 blocking drugs in non-small-cell lung carcinoma (33). Indeed, chemotherapeutic agents may affect antitumor immunity both indirectly stimulating the immune system through immunogenic death of tumor cells, and directly regulating immune cell subsets, thereby reducing immunosuppression in the tumor microenvironment (TME) (34).

Second- or third-line treatment with ICIs has become increasingly common for patients with advanced disease who have already received other types of anticancer therapies (35).

It has been hypothesized that previous administration of cancer vaccines can drive immune cells to the TME and upregulate PD-L1 expression in the tumor cells due to cytokine release in the TME, thus giving a chance for anti-PD-L1/PD-1 drugs in patients who may not have otherwise benefited from such immunotherapies (36, 37). Interestingly, in the above-mentioned case report by Del Rivero et al. (23), the 61-year-old male showing >40% decrease in calcitonin level while on avelumab had previously undergone a 3-month trial with the GI-6207 cancer vaccine. Although a subsequent analysis of a patient's lymph node (resected post-vaccination) revealed that the tumor was PD-L1 positive, no information about PD-L1 status before vaccination is available. As a further complication in this case's assessment, the patient had been previously treated with the TKI sunitinib, which is acknowledged to deplete Tregs, and may have affected PD-L1 status as well (38).

The therapeutic potential of FDA-approved atezolizumab, avelumab, ipilimumab and pembrolizumab has also been investigated in NENs other than MTC (39, 40), thereby confirming a strong interest for ICI therapy in this subset of tumors.

CONCLUSION

Despite the lack of evidence regarding the use of ICIs in MTC, it should be considered that all the aforementioned RCTs saw first light in the last three years, thus indicating a growing interest of researchers in this field. Results coming from these trials, and hopefully from additional ones in the next future, will help clarify whether these drugs may represent a new weapon in favor of patients with MTC, and determine their position in the treatment algorithm.

AUTHOR CONTRIBUTIONS

SDM, AD, GF, TFl, and TFe were responsible for the design, the methodology, the draft preparation, the reviewing and editing. AC and AF were responsible for the supervision. All authors contributed to the article and approved the submitted version.

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Role of FGF System in Neuroendocrine Neoplasms: Potential Therapeutic Applications

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Neuroendocrine neoplasms (NENs) are a heterogeneous group of tumors originating from neuroendocrine cells dispersed in different organs. Receptor tyrosine kinases are a subclass of tyrosine kinases with a relevant role in several cellular processes including proliferation, differentiation, motility and metabolism. Dysregulation of these receptors is involved in neoplastic development and progression for several tumors, including NENs. In this review, we provide an overview concerning the role of the fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR) system in the development and progression of NENs, the occurrence of fibrotic complications and the onset of drug-resistance. Although no specific FGFR kinase inhibitors have been evaluated in NENs, several clinical trials on multitarget tyrosine kinase inhibitors, acting also on FGF system, showed promising anti-tumor activity with an acceptable and manageable safety profile in patients with advanced NENs. Future studies will need to confirm these issues, particularly with the development of new tyrosine kinase inhibitors highly selective for FGFR.

Keywords: neuroendocrine neoplasms, FGFR (fibroblast growth factor receptor), FGF (fibroblast growth factor), VEGF - vascular endothelial growth factor, VEGFR - vascular endothelial growth factor receptor

INTRODUCTION

Neuroendocrine neoplasms (NENs) are a heterogeneous group of tumors originating from neuroendocrine cells dispersed in different organs (1–5).

Receptor tyrosine kinases are a subclass of tyrosine kinases with a relevant role in several cellular processes including proliferation, differentiation, motility and metabolism. Dysregulation of these receptors plays a relevant role in neoplastic development and progression for several tumors, including NENs (6, 7).

In this review, we provide an overview concerning the role of the fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR) system in NENs.

FGF SYSTEM IN HEALTH AND CANCER

FGFs and related receptors are members of a large family with a wide range of effects. This system is involved in organogenesis (during development), homeostasis and repair of adult tissues. Moreover, FGF family promotes angiogenesis, growth, differentiation and migration of cells mainly through the activation of RAS-MAPK, PI3K-AKT and PLCγ pathways, with a relevant role in the development and progression of several tumors (8). These effects are mediated by the interaction of FGFs with four tyrosine kinase receptors: FGFR1, FGFR2, FGFR3 and FGFR4, which are composed by an extracellular domain, a transmembrane domain and an intracellular domain. The binding of ligands induces conformational changes that lead to a dimerization of these receptors. This event activates the intracellular tyrosine kinase domain, which in turn triggers the signalling cascade (8). FGFs, based on their biochemical functions, sequence similarity and evolutionary relationships, are classified into different subfamilies: FGF1, FGF4, FGF7, FGF8, FGF9, FGF15/19 and FGF11.

FGF1 and FGF2 are members of the FGF1 subfamily. FGF1 is the only FGF that can activate all FGFRs splice variants. It is involved in cell cycle regulation, cell differentiation, survival and apoptosis. FGF1 plays a central role in neuroprotection and axon regeneration and appears to improve functional recovery after spinal cord injury (9). FGF2 has known angiogenic properties (10, 11). The FGF4 subfamily (FGF4, 5,6) can activate FGFR1-3 (IIIc) and FGFR4. These molecules are fundamental in embryonic development and muscle regeneration (8, 9). FGF7 subfamily (FGF3, 7, 10, 22) preferentially activates FGFR2(IIIb), although FGF3 and FGF10 can also interact with FGFR1(IIIb). FGF3 is involved in the neural development, while FGF7 is required for lung, kidney and neuronal synapses development. The development of epithelial components, such as limb and lungs, and mammary gland requires epithelial-mesenchymal interactions granted by FGF10. Finally, FGF22 regulates the circuit remodeling in the injured spinal cord (12–15). FGF8 subfamily members (FGF8, 17 and 18) activate FGFR4 and FGFR1-3(IIIc). They are involved in the skeletal and brain development and in odontogenesis (8, 14, 16–18). The FGF9 subfamily (FGF9, 16, 20) interacts with FGFR1-3(IIIc), FGFR3 (IIIb) and FGFR4. These proteins are involved in a proper heart, kidney and skeletal development (8, 14, 19, 20). The FGF15/19 subfamily comprises FGF15/19, 21 and 23. FGF15/19 bind FGFR1-3(IIIc) and FGFR4. FGF21 can activate FGFR1(IIIc) and 3(IIIc), as well as FGF23, which can also interact with FGFR4. This subfamily acts as hormones and regulates hepatocyte and adipocyte metabolism (8, 14). FGF11 subfamily members (FGF11, 12, 13, 14) are known as intracellular FGFs. These peptides are not secreted and interact with the cytosolic carboxy terminal tail of voltage gated sodium channels. They cover an important role in the development of the nervous system (8, 21).

A deregulation of the FGF/FGFR system can be involved in cancer development and progression through modulation of cell proliferation, migration and angiogenesis (22).

Besides its role in physiological angiogenesis, FGF2 is implied in tumor-induced angiogenesis and metastatic process and appears to direct tumor-associated macrophages toward a pro-tumorigenic state (23–25). FGF4 promotes cancer cell proliferation, invasion and migration by causing a switch of the receptor FGFR2-IIIb, a splice variant expressed in epithelial cells, into FGFR2-IIIc, expressed in mesenchymal cells and able to induce epithelial-mesenchymal transition (26). FGF5 can promote osteosarcoma proliferation by activating the MAPK signaling pathway (27) and the FGF5/FGFR1 axis contributes to melanoma progression (28). FGF6 can stimulate proliferation of prostate cancer cells through the activation of FGFR4 (29). Among the FGF7 subfamily, FGF3 and FGF7 have been reported to be highly expressed in breast cancer (30, 31) and gastric adenocarcinoma (32), respectively. In addition, the FGF10/FGFR-IIb signaling appears to have a role in breast and pancreatic tumors (15, 33). Although the mechanism is unclear, Jarosz et al. observed a potential role of FGF22 in skin tumorigenesis (34). In a recent study, FGF22 and its receptor FGFR-IIb appear to be associated with the development of lung adenocarcinoma through the MAPK and Rap I signaling pathways (35). A deregulation of FGF18, caused by an altered expression of its negative regulator miR-590-5p, is able to stimulate proliferation and epithelial-mesenchymal transition, with enhanced invasion abilities, in gastric cancer cells (36). In HER^+ breast cancer cell lines, overexpression of FGF18 stimulates the expression of genes involved in migration and cancer metastasis through Akt/GSK3β pathway (37). By the interaction with FGFR2 and FGFR3 and the activation of the ERK/Akt pathway, FGF18 is able to induce proliferation and invasion in endometrial carcinoma (38).

The FGF/FGFR pathway has also a key role in the onset of drug-resistance (39). FGF/FGFR pathway is the first compensatory mechanism in tumors resistant to drugs targeting the vascular endothelial growth factor (VEGF) system (40–42). Indeed, VEGF-dependent vessels are suppressed during prolonged anti-VEGF therapy, while the expression of FGF2 is increased, leading to a novel angiogenesis dependent on FGF2 signaling pathway. This condition drives the tumor toward drug-resistance (42). Boichuk et al. (43) showed that FGF signaling is activated in gastrointestinal stromal tumors after the acquisition of imatinib resistance. Interestingly, the use of a potent FGF inhibitor markedly reduced cell growth in resistant cells compared to imatinib-sensitive cells. This effect increased when the two molecules were combined in resistant cells, showing also that the FGF-inhibitor can restore sensitivity to imatinib.

FGF SYSTEM IN NEUROENDOCRINE NEOPLASMS

The role of the FGF/FGFR system has been analyzed also in NENs and several lines of evidence support its function in the modulation of tumor fibrosis, proliferation, angiogenesis and drug resistance, through a dynamic cross talk between NEN cells, fibroblasts, endothelial cells and inflammatory cells (44).

Bordi et al. identified FGF2 by immunohistochemistry in endocrine cells of the gastric oxyntic mucosa and mRNA of FGF2 in enterochromaffin-like carcinoid tumors (45). Immunohistochemical studies demonstrated the staining for FGF-2 in 100% of NEN cells from the midgut and the pancreas, while FGF2 receptors were observed only in the stromal component (46). La Rosa et al. found cytoplasmic immunoreactivity for FGF1 in 26 (43%) out of 60 GEP-NENs and FGFR1-4 were found in 68-88% of tumors with tumor microenvironment components also expressing FGFRs (47). The authors observed also that normal endocrine cells of the gut rarely expressed FGFRs thus hypothesizing that in normal mucosa the FGF/FGFR system has not an autocrine role on modulating endocrine cells functions. Therefore, *de novo* expression of FGFRs by NEN cells may play a role in the autocrine/paracrine signaling responsible of tumorigenesis, stromal fibrosis and tumor-induced angiogenesis.

NEN are often characterized by the development of fibrosis, local or distant. The best-known fibrotic complications are carcinoid heart disease, which develops in about 20% of patients with carcinoid syndrome (48), and mesenteric fibrosis, which affects up to 40-50% of small bowel NENs (49, 50). Less known complications are represented by retroperitoneal fibrosis (50), scleroderma (51), infiltration of the pleura (52) or alveoli (53) and fibrosis of the bladder (54). Although the pathogenesis of fibrotic complications is unclear, serotonin, with a relevant mitogenic power on fibroblasts, mesangial cells, smooth muscle cells, endothelial cells and NEN cells, may have a role in these events (55). The FGF system appears to be also implicated in the mechanism of gastrointestinal NEN fibrosis (56). In fact, Bordi (45) showed that among the 10 patients suffering from type 3 gastric NEN and with positive immunohistochemistry for FGF2, some had diffuse stromal fibrosis. Another study (57), which analyzed a pool of 41 gastrointestinal NENs, showed a positive correlation between FGF1 and the amount of fibrous stroma in tumors. The FGF is responsible of cell proliferation and stroma formation and its action is potentiated by serotonin (58). Moreover, FGF may activate also the expression of the connective tissue growth factor genes that regulate myofibroblast differentiation, collagen synthesis and fibroblast proliferation (59).

The mRNA expression of FGF receptor was found more frequently in functioning NENs (including gastrinomas and insulinomas) than in functionally inactive NENs (53.6% vs. 22.2%) (60). Although this difference was not statistically significant (p=0.10), speculating on the association between FGFR expression and hormone production may be not totally irrational, but further evidence is required to corroborate these findings.

The FGFR4-G388R single-nucleotide polymorphism was investigated in 71 patients with pancreatic NEN (61). The authors observed that FGFR4-R388 allele was independently associated with liver metastases. To further analyze the impact of the FGFR4 SNP, the same authors transfected BON1 cells with either FGFR4-G388 or FGFR4-R388 and injected them in SCID mice. They found that xenografts expressing FGFR4-R388 displayed a more aggressive biological behavior and were resistant to everolimus treatment. This latter aspect was investigated also among 17 patients previously treated with everolimus in a clinical trial. Patients harboring FGFR4-R388 allele achieved a worse tumor response (9% vs. 25%) and a reduced median PFS (4.8 vs. 16.6 months) and OS (9.3vs 40 months) compared to patients homozygous for FGFR4-G388. Although decreased drug response was related to persistently high mTOR and STAT3 phosphorylation despite of everolimus treatment, these data were not confirmed by Cros et al., who reported no modification of the mTOR pathway in patients with pancreatic or ileal NENs harboring FGFR4-R388 allele (62). This apparent inconsistency corroborates the need for further studies validating the identification of molecular parameters useful to predict drug efficacy and resistance (63).

The FGF/FGFR system collaborates with the VEGF signaling pathway in the initiation and maintenance of tumor angiogenesis. These mechanisms have been demonstrated in allograft transplantation experiments and in mouse model of pancreatic NEN (the Rip1Tag2 transgenic mice), where interfering with the FGF function by a soluble form of the FGFR2 IIIb significantly inhibited tumor-induced angiogenesis and tumor growth (64). The FGF system acts as a second proangiogenic circuit, indeed VEGF is the main regulator of angiogenesis but, as reported by Casanovas et al., experiments in the Rip1Tag2 model of pancreatic islet carcinoma documented that initial inhibition of the angiogenesis achieved by VEGF signaling blockade was restored by the upregulation of the FGF system (65). Therefore, blocking both VEGF and FGF signaling pathways may reveal synergic antiangiogenic effects and inhibit tumor progression secondary to compensatory feedback loops driving tumor revascularization. For instance, Allen et al. investigated the effect of brivanib, a selective inhibitor targeting both VEGF and FGF receptors, in a mouse model of pancreatic NEN. Brivanib was effective not only as first-line therapy, but also as second-line treatment after failure of two agents inhibiting VEGF receptors (DC101 and sorafenib) (66).

FGFRs AS THERAPEUTIC TARGET IN NENs

In the last few years, the therapeutic approach for NENs has changed following the approval of several innovative targeted treatments such as tyrosine kinase inhibitors (TKIs). Although no specific FGFR kinase inhibitors have been evaluated in NENs, several clinical trials on multitarget TKIs, acting also on FGF, are ongoing and few published studies have demonstrated their efficacy in NENs (44). The interest in FGF pathway inhibitors relies also in the possibility to overcome resistance to VEGF inhibition that may arise after long term use of these drugs or could be intrinsic in tumor expressing FGF2 (67–69). The results of clinical trials in NENs evaluating multitarget TKI, acting also on FGF, are described below (**Table 1**).

Surufatinib is a potent TKI targeting VEGF receptors (VEGFR) 1, 2, and 3, FGFR1, and CSF-1R. In preliminary phase I and Ib/II studies surufatinib showed encouraging anti-tumor activity in advanced NENs (81, 82).

TABLE 1 | Clinical trials evaluating the effects of multitarget tyrosine kinase inhibitors, acting also on FGFR, in patients with NENs.

Ref	Therapy and dose	Molecular target	Study design (Trial name)	Tumors	Number of patients (placebo)	Median follow- up (placebo)	Primary outcome	Results	Main AE (%)
(70)	Surufatinib 300 mg/day	VEGFR 1,2,3 FGFR1 CSF-1R	Randomised, double-blind, placebo-controlled, phase 3 (SANET-EP)	Advanced extrapancreatic NETs (G1-G2)	129 (69)	13.8 months (16.6 months)	PFS	Median PFS: 9.2 months (surufatinib) vs. 3.8 months (placebo)	Hypertension (36%); proteinuria (19%)
(71)	Surufatinib 300 mg/day	VEGFR 1,2,3 FGFR1 CSF-1R	Randomised, double-blind, placebo-controlled, phase 3 (SANET-P)	Advanced pancreatic NETs (G1-G2)	113 (59)	19.3 months (11.1 months)	PFS	Median PFS: 10.9 months (surufatinib) vs. 3.7 months (placebo)	Hypertension (38%); proteinuria (10%); hypertriglyceridemia (7%)
(72)	Surufatinib 300 mg/day	VEGFR 1,2,3 FGFR1 CSF-1R	Dose escalation/expansion study	Heavily pre-treated progressive NETs	32	19 weeks	ORR	9.4%	Hypertension, fatigue, diarrhea
(73)	Surufatinib 300 mg/day	VEGFR 1,2,3 FGFR1 CSF-1R	Phase 2, open label, two stage design study	Advanced MTC	27	–	ORR	22.2%	hypertension (20.3%), proteinuria (11.9%), hypertriglyceridemia (5.1%)*
(74)	Lenvatinib 24 mg/day	VEGFR 1-3 FGFR1-4	Prospective multicohort phase 2 (TALENT)	Advanced pancreatic and gastrointestinal NETs (G1-G2)	111	19 months	ORR	42.3% pancreatic 16.3% gastrointestinal	Hypertension (22%); fatigue (11%); diarrhea (11%)
(75)	Lenvatinib 24 mg/day	VEGFR 1-3 FGFR1-4	Phase 2, multicenter, open-label, single-arm clinical trial	Unresectable or metastatic progressive MTC	59	–	ORR	36% (all PR)	Diarrhea (14%); hypertension (7%); decreased appetite (7%)
(76)	Lenvatinib 24 mg/day	VEGFR 1-3 FGFR1-4	Nonrandomized, open-label, multicenter, phase 2 study	Progressive MTC	9	9.6 months	Safety	100% of patients ≥1 AE; 1.7% of patients AE leading to discontinuation	Decreased appetite (100%); hypertension (89%); palmar-plantar erythrodysesthesia (89%)
(77)	Lenvatinib 24 mg/day	VEGFR 1-3 FGFR1-4	Prospective, post-marketing observational study	UnresectableMTC	28	12 months	Safety	100% pts ≥1 AE	Hypertension; proteinuria; palmar-plantar erythrodysesthesia
(78)	Nintedanib	VEGFR 1,2,3 FGFR2	Multicenter phase 2 study	Advanced progressing carcinoid on stable dose SSA for ≥3 months	30	16 weeks	PFS	PFS at 16 weeks 86.7% in 26 pts	Diarrhea (18%); increase in GGT (18%); lymphopenia (18%)
(79)	Anlotinib 12 mg/day	VEGFR 2-3 FGFR1-4	Single-arm phase 2 study	Advanced or metastatic MTC	58	9.8 months	PFS	PFS at 48 weeks 84.5%	Hand-foot syndrome (79.3%); hypertriglyceridemia (46.5%); elevated cholesterol levels (43.1%)
(80)	Anlotinib 12 mg/day	VEGFR 2-3 FGFR1-4	Multicenter, randomized, double-blind, placebo-controlled phase IIB trial (ALTER01031)	Advanced or metastatic MTC	62 (29)	–	PFS	Median PFS: 20.67 months (anlotinib) vs 11.07 months (placebo)	Hand-foot syndrome; hypertension; hypertriglyceridemia

AE, adverse events; FGFR, fibroblast growth factor receptor; MTC, medullary thyroid carcinoma; NET, neuroendocrine tumor; ORR, overall response rate; PFS, progression free survival; pts, patients; SSA, somatostatin analogs; VEGFR, Vascular Endothelial Growth Factor Receptor.

**data reported for the overall population (differentiated thyroid cancer and MTC).*

Two randomized phase III placebo controlled trials evaluated safety and efficacy of surufatinib in patients with well differentiated NENs of extra-pancreatic (SANET-ep) and pancreatic (SANET-p) origin (70, 71).

In SANET-ep study (70) 198 patients were randomly assigned to surufatinib 300 mg/day (n=129) or placebo (n=69). Median progression-free survival (PFS) was 9.2 months in the surufatinib group versus 3.8 months in the placebo group. The overall response rate (ORR) was 10% in the surufatinib group versus zero in the placebo group. The most common treatment-related adverse events (AE) of grade ≥ 3 were hypertension (36% surufatinib vs 13% placebo) and proteinuria (19% vs. 0%). In SANET-p study (71) 113 patients were randomly assigned to surufatinib (300 mg/day) and 59 to placebo. The median PFS was 10.9 months for surufatinib versus 3.7 months for placebo; ORR was 19% in the surufatinib group and 2% in the placebo group. The most common AE of grade ≥ 3 were hypertension (38% surufatinib *vs.* 7% placebo), proteinuria (10% *vs.* 2%) and hypertriglyceridemia (7% *vs.* none).

Another study evaluated the effect of surufatinib dose escalation/expansion in 32 patients with heavily pre-treated progressive NENs, 16 patients with pancreatic NENs and 16 with extra-pancreatic NENs. Nineteen patients remained on active treatment (13 extra-pancreatic and 6 pancreatic), 9 patients discontinued due to disease progression, 2 withdrew consent and 2 discontinued due to AE. An ORR of 9.4% was observed (72).

An open label phase II study evaluated efficacy and tolerability of surufatinib (300 mg/day) in 27 patients with progressive medullary thyroid cancer (MTC). Objective response was observed in 22.2% of patients with MTC, and the majority (88.9%) achieved disease control. The therapy was well tolerated (73).

Therefore, surufatinib demonstrated promising anti-tumor activity with an acceptable and manageable safety profile in advanced NENs.

Lenvatinib is a potent VEGFR1-3 and FGFR1-4 inhibitor. The TALENT trial, a prospective phase II study, evaluated efficacy, safety and tolerability of lenvatinib (24 mg once daily) in G1/G2 advanced pancreatic (n=55) and gastrointestinal (n=56) NENs resistant to previous targeted agents. The ORR was 29% (42.3% for pancreatic NENs and 16.3% for gastrointestinal NENs). PFS and overall survival (OS) for pancreatic NENs were 15.5 months and 29.2 months, while for gastrointestinal NENs were 15.4 months and not reached, respectively. The most frequent grade 3/4 AE were hypertension (22%), fatigue (11%) and diarrhea (11%) (74). Thus, lenvatinib showed a promising PFS and OS in a pretreated population.

A phase II, multicenter, open-label, single-arm clinical trial evaluated efficacy and tolerability of lenvatinib (24-mg daily, 28-day cycles) in 59 patients with MTC. ORR was 36%, all PR. Disease control rate (DCR) was 80%, 44% had SD. Median time to response was 3.5 months. Median PFS was 9.0 months. Grade 3/4 AE included diarrhea (14%), hypertension (7%), decreased appetite (7%), fatigue, dysphagia and increased alanine aminotransferase levels (5% each) (75).

Another phase II study evaluated lenvatinib treatment in 9 patients with MTC. The most frequently reported AE were decreased appetite (100%), hypertension (89%), palmar-plantar erythrodysesthesia (89%), diarrhea (89%), fatigue (78%) and proteinuria (67%). Median PFS was 9.2 months. Median OS was 12.1 months. ORR was 22% and DCR was 100% (76).

Recently, a prospective, post-marketing observational study evaluated, in daily clinical practice, the safety and effectiveness of lenvatinib in 28 patients with MTC. Hypertension, proteinuria and palmar-plantar erythrodysesthesia syndrome were the most frequently reported AE. The 12-months OS rate was 83%. ORR was 45% (77).

Nintedanib is a dual inhibitor of VEGFR1, -2, and -3 as well as FGFR2 and showed both antiangiogenic and antitumor activity in the RIP1-Tag2 transgenic mouse model of tumorigenesis for pancreatic NEN (44). A multicenter phase II study evaluated efficacy, safety and tolerability of nintedanib in 30 patients with unresectable/metastatic carcinoids on stable dose of SSA for ≥3 months. PFS at 16 weeks was 86.7% in 26 patients. PR was observed in 4%, SD in 83%, disease progression in 8% of patients. Quality of life was maintained or improved in at least 50% of subjects. The most common grade 3 AE were hypertension and decreased appetite (78).

A prospective randomized double-blind phase II study evaluated the efficacy and tolerability of nintedanib in progressing MTC after prior TKI treatment. The study was stopped due to slow accrual with 32/67 patients enrolled, without reaching the targeted statistical power. The most common AE were diarrhea (18%), nausea (9%), GGT increase (18%) and lymphopenia (18%) (83).

Anlotinib is a novel TKI targeting VEGFR2-3 and FGFR1-4 with high affinity. Anlotinib has previously shown promising antitumor activity on MTC in preclinical models and a phase I study (84). A phase II clinical trial showed a relevant antitumor activity of anlotinib (12 mg once daily, two weeks on/one week off) in 58 patients with advanced MTC. PFS rates at 24, 36, and 48 weeks were 92.2%, 87.8% and 84.5%, respectively. Significant decreases in serum calcitonin (≥50%) occurred in 57.5% of patients. The most common AE included hand-foot syndrome (79.3%), hypertriglyceridemia (46.5%), hypercholesterolemia (43.1%), fatigue (41.4%), proteinuria (39.7%), hypertension (39.7%), sore throat (37.9%), diarrhea (34.5%) and anorexia (34.5%) (79).

These data have been confirmed in a phase IIb study (ALTER01031), enrolling a larger cohort of patients (80). Ninety-one patients with advanced MTC were randomized: 62 to anlotinib arm and 29 to placebo arm (12 mg/die from day 1 to 14 of a 21-day cycle). Median PFS was 20.7 months in anlotinib arm vs. 11.1 months in placebo arm. The most common AE after anlotinib arm were hand-foot syndrome, hypertension, hypertriglyceridemia and diarrhea (80).

Several clinical trials on the use of multi target TKI, with an action also on FGFR, in patients with NENs are currently ongoing. **Table 2** reports the main characteristics of trials registered on clinicaltrials.gov.

TABLE 2 | Ongoing clinical trials evaluating the effects of multitarget tyrosine kinase inhibitors, acting also on FGFR, in patients with NENs.

Identifier	Therapy	Molecular target	Study design	Tumors	Estimated sample size	Primary outcome	Start date	Estimated Completion Date
NCT02399215	Nindetanib	FGFR VEGFR PDGFR	Multicenter open label phase II study	Well or moderately differentiated (G1, G2) NEN not pancreatic	30	PFS	May 2015	October 2020
NCT04207463	Anlotinib + AK105 *(anti PD1)*	FGFR VEGFR PDGFR c-kit	Multicenter multi-cohort open label phase II study	G1 or G2 GEP NET (cohort 5)	150 (all cohorts)	ORR	June 2020	December 2020
NCT02259725	Regorafenib	FGFR VEGFR1-3 TIE2 KIT RET RAF-1 BRAF BRAFV600E PDGFR	Multicenter multi-cohort open-label phase II study	Carcinoid (cohort A) or pancreatic islet cell tumors (cohort B)	48	PFS	August 2016	August 2021
NCT03950609	Lenvatinib + Everolimus *(mTOR inhibitor)*	FGFR1-4 VEGFR1-3	Single center open-label phase II study	Unresectable well differentiated carcinoid tumors	32	ORR	July 2019	May 2021
NCT03475953	Regorafenib + Avelumab *(anti PD-L1)*	FGFR VEGFR1-3 TIE2 KIT RET RAF-1 BRAF BRAFV600E PDGFR	Multicenter, open label phase I/II study	G2 or G3 GEP NEN (cohort G)	362	ORR (Phase 2)	May 2018	November 2020
NCT02657551	Regorafenib	FGFR VEGFR1-3 TIE2 KIT RET RAF-1 BRAF BRAFV600E PDGFR	Open-label phase II study	Metastatic medullary thyroid cancer	33	PFS	January 2016	October 2022
NCT03008369	Lenvatinib	FGFR1-4VEGFR1-3	Open-label phase II study	Metastatic PPGLs	25	TRR	May 2017	December 2020

FGFR, fibroblast growth factor receptor; GEP, gastro-entero-pancreatic; NA, not available; NEN, neuroendocrine neoplasm; NET, neuroendocrine tumor; ORR, objective response rate; PD1, Programmed cell death protein 1; PDGFR, Platelet-derived growth factor receptor; PD-L1, Programmed cell death ligand 1; PFS, progression free survival; PPGL, Pheochromocytoma and Paraganglioma; pts, patients; TRR, tumor response rate (complete response and partial response); VEGFR, Vascular Endothelial Growth Factor Receptor.

CONCLUSIONS AND FUTURE PERSPECTIVES

In the last years there is mounting evidence supporting the role of FGF/FGFR system in the development and progression of NENs and probably in the occurrence of fibrotic complications (mesenteric and/or retroperitoneal fibrosis). In addition, the FGF/FGFR pathway could also have a key role in the onset of drug-resistance. Indeed, FGF/FGFR pathway is a main compensatory mechanism in anti-VEGF-therapy-resistant tumors.

Currently no specific FGFR kinase inhibitors have been evaluated in patients affected by advanced NENs. Although recent clinical trials have reported a significant antitumor activity and manageable safety profile of several multitarget TKIs, which are able to block many molecular pathways including FGFR, it is not possible to isolate the efficacy of FGFR inhibition alone. Future studies should better confirm these issues and clarify the role of FGF/FGFR pathway in promoting drug-resistance in NENs. The development of new TKIs, highly selective for FGFR and with less toxicity, may open an innovative therapeutic strategy to be integrated into a personalized approach for this heterogeneous class of tumors. In addition, recent preclinical studies showed a potent inhibition in tumor growth both in hepatocellular carcinoma (85) and in ovarian cancer (86), through the simultaneous blockade of mTOR and FGFR pathways. Considering the pivotal role of deregulated mTOR signaling activation in the proliferation of NENs, particularly in pancreatic tumors, combining mTOR inhibitors and TKIs targeting FGFRs could represent a future therapeutic approach in NENs.

AUTHOR CONTRIBUTIONS

GV, AlC, PM, RM, GP, and DS conceptualized and wrote the manuscript. AF and AnC contributed to draft the manuscript. All authors contributed to the article and approved the submitted version.

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Pathology Reporting in Neuroendocrine Neoplasms of the Digestive System: Everything You Always Wanted to Know but Were Too Afraid to Ask

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During the 5th NIKE (Neuroendocrine tumors Innovation in Knowledge and Education) meeting, held in Naples, Italy, in May 2019, discussions centered on the understanding of pathology reports of gastroenetropancreactic neuroendocrine neoplasms. In particular, the main problem concerned the difficulty that clinicians experience in extrapolating relevant information from neuroendocrine tumor pathology reports. During the meeting, participants were asked to identify and rate issues which they have encountered, for which the input of an expert pathologist would have been appreciated. This article is a collection of the most rated questions and relative answers, focusing on three main topics: 1) morphology and classification; 2) Ki67 and grading; 3) immunohistochemistry. Patient management should be based on multidisciplinary decisions, taking into account clinical and pathology-related features with clear comprehension between all health care professionals. Indeed, pathologists require clinical details and laboratory findings when relevant, while clinicians require concise and standardized reports. In keeping with this last statement, the minimum requirements in pathology datasets are provided in this paper and should be a baseline for all neuroendocrine tumor professionals.

Keywords: neuroendocrine neoplasms (NENs), neuroendocrine classification, immunohistochemistry, pathology, morphology, grade, Ki67

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INTRODUCTION

During the 5th NIKE (Neuroendocrine tumors Innovation in Knowledge and Education) meeting, held in Naples, Italy, in May 2019, discussions centered on the understanding of pathology reports in gastroenetropancreactic (GEP) neuroendocrine neoplasms (NENs). In particular, the main problem concerned the difficulty clinicians (be they experts or not) have, in extrapolating relevant information from neuroendocrine tumor pathology reports. As the famous publication entitled "Clinicians are from Mars and pathologists are from Venus" (1), perfectly summed up, this is not a new issue. During the meeting, participants were asked to identify issues which they have encountered, for which the input of an expert pathologist would have been appreciated. This article is a collection of the most rated questions, focusing on three main topics: 1) morphology and classification; 2) Ki67 and grading; 3) immunohistochemistry.

METHODS

A series of questions on various aspects of pathology were proposed to a panel of 36 experts in the field of GEP-NENs (including endocrinologists, pathologists, oncologists, gastroenterologists, surgeons, radiologists and laboratory clinicians; see acknowledgment section). All questions are summarized in **Table 1S**, along with the rate of votes obtained during the poll (participants could select a total of 8 questions), while the top scored questions are answered below.

MORPHOLOGY AND CLASSIFICATION: QUESTIONS AND ANSWERS

Question 1: What Classification System for Neuroendocrine Neoplasms of the Digestive System Should I Be Expecting in a Pathology Report and Does the Category NET G3 Really Exist?

Classification systems for NENs have varied over time, each one emphasizing different aspects including function, morphology, site, size and extension of primary tumor, presence of metastases.

With the 2010 GEP NEN WHO classification (2), a morphology and proliferation-based classification system was introduced. It focused on the morphologic distinction between well differentiated (WD neuroendocrine tumors - NET) and poorly differentiated (PD neuroendocrine carcinomas - NEC) neoplasms, as already suggested in the WHO 2000 classification (3). WD-NETs are composed of uniform neoplastic cells, with organoid, trabecular or ribbon-like architecture, round/oval nuclei with "salt and pepper" chromatin and with low nuclear-cytoplasmic ratio. They present secretory granules responsible for intense and diffuse staining for general neuroendocrine markers (synaptophysin and chromogranin) (**Figures 1A–D**). Nucleoli are inconspicuous and little or no atypia is seen. Mitoses are rare/uncommon and necrosis is also generally absent. PD-NECs, are either of large cell or small cell type (or mixed), with pleomorphic and atypical nuclei, solid growth pattern and abundant non-ischemic necrosis, arranged to form either "map-like" or "spotty" necrosis. Mitoses are plentiful and often atypical (4) and proliferation index is extremely high (**Figures 1E–H**).

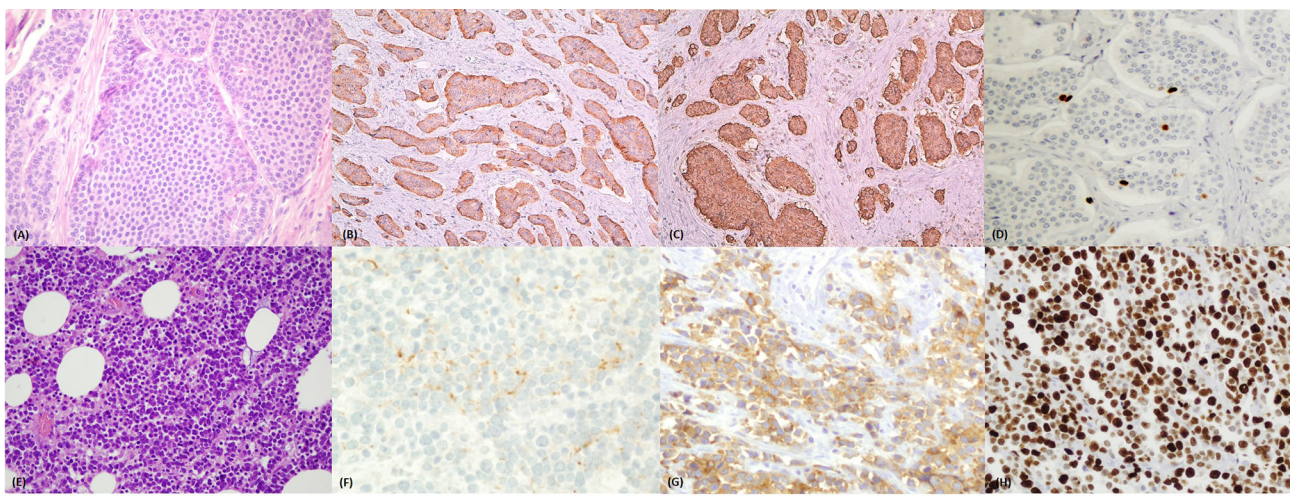

FIGURE 1 | **(A–D)** Well differentiated neuroendocrine tumor of the ileum. **(A)** Haematoxylin and eosin stained section (magnification 40x) of a well differentiated ileal neuroendocrine tumor with organoid insular architecture and monomorphic cells with ample eosinophilic cytoplasm and uniform nuclei. **(B)** Chromogranin A positivity and **(C)** synaptophysin positivity by immunohistochemistry. **(D)** Ki67 immunostaining showing rare positive nuclei (stained brown) with <3% proliferation ratio – grade 1. **(E–H)** Poorly differentiated neuroendocrine carcinoma of the colon. **(E)** Haematoxylin and eosin stained section (magnification 40x) of a poorly differentiated neuroendocrine carcinoma showing solid structure and small/moderate atypical cells with scanty cytoplasm and hyperchromatic nuclei. **(F)** Focal dot like positivity for Chromogranin A but diffuse, cytoplasmic expression of synaptophysin **(G)**. **(H)** Ki67 immunostaining showing diffusely positive nuclei (stained brown) with 90% proliferation ratio – grade 3.

The second aspect of the 2010 WHO classification, which has now become paramount for patient management, is grade, based on mitotic index and/or Ki67 index (see question 3). Initially, categories comprised G1 and G2 WD-NETs and G3 PD-NECs. Some G3 neoplasms were, however, found to be morphologically well differentiated (perhaps with focal areas of greater atypia) but with proliferation indexes greater than 20% [around 45% (5), usually no higher than 50-60%] (6, 7). Subsequent studies have shown that WD G3 neoplasms are a separate category showing better survival compared to PD G3 carcinomas (but worse compared to G2 NET) (8), somatostatin receptor positivity (9), Gallium-PET positivity (with associated possible FGD-PET positivity) and, at the molecular level, mutation profiles similar to WD G1/G2 tumors (10). The landmark study by Sorbye et al (11) reported differences in response to chemotherapy between G3 NENs with Ki67 < 55% compared to > 55%; this study unfortunately failed to review the morphology of the accrued cases.

A revised common classification system of GEP-NENs was therefore proposed for pancreatic NENs in 2017 (12) and extended to all digestive NENs in 2019 (13).

With regards to stage, the 2017 edition of the UICC/AJCC staging manual has specified site specific TNM systems for well differentiated GEP NETs including gastric, duodenal/ampullary, jejunal/ileal, appendiceal, colonic/rectal and pancreatic NETs. The use of this updated system should be standard in all pathology reports.

Question 2: How Should I Interpret a Pathology Report Showing a Digestive System Mixed Neuroendocrine Non-Neuroendocrine Carcinoma?

Mixed neuroendocrine/non-neuroendocrine neoplasms have been described in all organs of the digestive system, with highest frequency in the colon and a diagnostic requirement is that both components be at least 30% of the lesion (though this cut off is arbitrary and not evidence-based). The WHO 2010 classification recommended the term mixed adeno-neuroendocrine carcinoma (MANEC) (2) for such tumors however, this term, does not adequately cover the heterogeneity of possible combinations of neuroendocrine (WD or PD) and non-neuroendocrine (adenocarcinoma, squamous cell carcinoma or adenoma for example) phenotypes.

For this reason, the 2017-2019 WHO classifications changed the term to mixed neuroendocrine and non-neuroendocrine neoplasms (MiNEN). These neoplasms can be stratified into different prognostic categories according to the grade of malignancy of each component: low-grade MiNENs (adenoma and a WD-NET, called MANETs (14); high-grade MiNENs, (PD-NEC with adenocarcinoma, called MANEC or squamous carcinoma in the esophagus or anal canal); intermediate grade neoplasms, composed of adenocarcinoma and NET (15, 16).

In general, the most aggressive cell population drives clinical behavior and this should be considered for therapeutic strategy (17). Recent studies on digestive system MiNENs have shown that prognosis is driven mostly by the NEC component when present, and often, it is this component which metastasizes (18). The Ki67 proliferative index of the neuroendocrine component appears to be the key prognostic factor with differences in survival if Ki67 is above 55% (17). Similarly, Ki67 of 55% seems to be important in composite lung large cell neuroendocrine carcinomas also (19).

With regards to origin, a few studies have demonstrated that both the high-grade NEC component and the non-neuroendocrine component probably derive from the same precursors as they show similar mutation profiles (20).

GRADING AND KI67 EVALUATION: QUESTIONS AND ANSWERS

Question 3: Why Is Grade so Important in NENs and How Reliable Is Ki67 Evaluation on Cytological or Small Tissue Samples?

Grade represents a major prognostic factor (21, 22) and is evaluated on the basis of mitotic index and proliferative index (Ki67 immunostaining) evaluated on sections of tumor. Ki67 is a nuclear protein expressed in the active phases of the cell cycle (G1/S1/G2/M phases) and its function is as a biological surfactant to disperse mitotic chromosomes (23). Discordance between grade assessed by mitotic counting or by Ki67 index is often seen (about 30% of cases), and grade is usually higher when assessed by Ki67 (24, 25). In WHO 2017-2019 (12, 13), grade cut offs have been slightly modified between G1 and G2 so that no grey zone (between 2 and 3%) exists; the distinction between G1 and G2 tumors is now <3% Ki67 index and <2 mitosis/10 high power fields (HPF).

The suggested number of cells in hot spots of expression which should be counted has changed over the years, from 2000 cells in the WHO 2010 to 400-500 cells in the WHO 2017-2019. Furthermore, methods of evaluation of Ki67 have come under scrutiny in recent years as not all methods are equally reliable (26). 'Eye-ball' estimation has proved to be unreliable while optimal methods include automated counting by image analyser, manual eye-counting and manual count of camera-captured image. The accuracy and reproducibility of these methods vary in different studies (27, 28).

Besides technical aspects, other possible limitations of Ki67 index assessment derive from the small quantity of tissue available, such as small biopsies (29, 30) and, even more so, in case of cytologic samples. Several studies have focused on the comparison of grading evaluation using endoscopic ultrasound-guided fine needle aspiration and surgical pathology in pancreatic NEN, identifying the correct identification of grade G2 NET as the principal limitation of cytology with both over and undegrading of lesions (31–34). Overall, agreement between cytology and definitive histologic examination was extremely variable in all studies ranging from as low as 34% (31) to close to 100% (35). While it is true that cytology may not be able to accurately predict Ki67 proliferation index in the intermediate range (distinction between G1 from G2 WD-NETs), it is reliable

in identifying very proliferative tumors (36) and clinicians should be aware of this.

Question 4: Is There Intra/Intertumoral Heterogeneity in Grade and Can Grade Change Between Sites and Over Time?

With regards to intratumoral heterogeneity of grade in NENs, this can be seen (up to 77% of patients in a study in small bowel NENs (37)) and may be related to multifocality and size, when primary tumor > 1 cm, making the staining of Ki67 sufficient only in the largest lesion (38).

When considering change in grade, this has been shown to occur between the primary and metastatic sites and between synchronous/metachronous metastases (39). The first published study on this topic identified 49 patients with metastatic GEP-NEN, showing a discrepancy in grade between sites in 39% of cases, especially in distant compared to locoregional metastases (39). Further studies have demonstrated an overall discordance rate between primary and metastatic tumour of between 1/3-1/2, both with regards to increase (including from G1 to G3) and decrease in grade from primary to metastatic sites (40). Importantly, increased grade in metastatic sites is associated with lower progression free survival and overall survival (41–43).

In conclusion, it is very important for the clinician to be aware of the possibility of change of grade between sites and over time and it may become useful to re-evaluate grade on a new biopsy.

IMMUNOHISTOCHEMISTRY: QUESTIONS AND ANSWERS

Question 5: Is it Necessary to Evaluate SSRs on Neoplastic Cells by Immunohistochemistry?

Five somatostatin receptor (SSTR) subtypes have been identified; moreover, two forms of the SSTR2, A and B, are transcripted by alternative splicing, with SSTR2A being the most highly expressed (44). SSTR2 and SSTR5 are the most expressed subtypes and their expression on the membrane of neoplastic cells is the rationale for the use of somatostatin analogues (SSA) and peptide receptor radionuclide therapy in WD-NENs (45, 46). In most cases, functional imaging with 68-Ga-DOTATOC/DOTANOC/DOTATATE PET CT permits the *in vivo* evaluation of receptor expression (47); as an alternative, the presence of SSTR can be demonstrated by immunohistochemistry. SSTR2A monoclonal antibody has shown high sensitivity/specificity and can be used in formalin-fixed and paraffin-embedded tissues. To standardize the interpretation of immunostaining, Volante et al. proposed a score considering the subcellular pattern and the extension of positive neoplastic cell population (48) with demonstrated high interlaboratory and interobserver SSTR2A immunostaining agreement (49).

Clinicians should be aware of the availability of SSTR2 receptor evaluation in those patients who have not undergone pre-operatory nuclear imaging when, for example, the diagnosis of NEN is made after surgery as recommended by ENETS (36).

SSTR2A expression has been shown to be higher in low-grade NENs and decreased in high grade lesions, both in digestive (50) and in lung (51) neoplasms. Studies have proposed a correlation between the downregulation of SSTR2 expression and NEN growth and progression (52) as well as differences in expression in metastatic sites compared to primary (53). SSTR2A expression may also be correlated with prognosis [WD-NETs with high expression of SSTR2 are associated with longer overall survival (54–56)].

While several studies have evaluated the expression of all SSTR subtypes in NEN (57, 58), this profiling is not part of the routine immunohistochemical evaluation. Notwithstanding this, two aspects seem very promising for future applications: the expression of SSTR5, for predicting the additional value of new SSA pasireotide (59) and the identification of the truncated variant of SSTR5 which seems associated with worse prognosis and low response to SSA (60).

Question 6: How Sensitive/Specific Are Site of Origin Markers (TTF1, CDX2, PAX8, ISL1, PDX1)?

A frequent clinical setting (between 9-19% of NENs) is a patient with multiple liver metastases which show WD-NET and for which the clinician requires, not only a diagnosis of histotype and grade, but an indication of origin as well (61).

Determining the origin of the tumor by histologic features alone is often impossible. The typical neuroendocrine markers used in clinical practice, chromogranin and synaptophysin, do not indicate a specific primary, therefore, further immunohistochemical testing may be required to help pathologists identify primary site. Only in WD-NETs are transcription factors useful and these may be differentially expressed in the bowel (CDX2), lung (TTF1) or pancreas (PAX8, ISL1, PDX1). PD-NECs do not express transcription factors with reliability and these should not be used to identify origin (e.g. TTF1 is often expressed in PD-NECs of any site, including the digestive system).

CDX2 is a nuclear homeobox transcription factor responsible for development of all (neuroendocrine and non-neuroendocrine) intestinal epithelial cells. High prevalence of CDX2 expression was found in ileal (86%) and colonic (75%) NETs while no expression was found in NETs of gastric origin, lung, skin, ovary and thymus (62). CDX2 expression has however been reported in a low percentage of pancreatic NETs (pNETs) (15-26%) (62, 63). Worthy of note is that CDX2 has been shown to be expressed in up to 98% of appendiceal and rectal NETs which originate from enterochromaffin cells (serotonin producing) but not from L-cell NETs (which may be found at both sites) (64).

TTF1 is a transcriptional factor expressed in tissues from the thyroid and lung. Immunohistochemical TTF1 staining is commonly used to identify NET of pulmonary origin as it is highly specific (100%) for pulmonary NET with a lower sensibility, ranging from 35% to 53% (62, 63).

OPT – orthopedia homeobox (65) is an extremely useful lung NET marker which is positive in 80% of bronchopulmonary carcinoids and shows much higher sensitivity (80.2% sensitivity and 99.4% specificity) compared to TTF1.

Paired-box genes (PAX) encode a family of nine transcription factors (PAX1-9) important for embryogenesis and organogenesis. PAX8 was found to be expressed in 56-74% of pNET (66, 67), However, specificity is hindered by PAX expression in NETs from the duodenum (75%), stomach (10%) (67, 68).

ISL1 is a transcription factor expressed in pancreatic islet cells and has been shown to be expressed in primary GEP-NETs and, less so, in pulmonary NENs: 59-90% pancreatic, 89% duodenum, 0-16% lung, 0-16% ileum, 0% gastric (69–71). Overall, ISL1 should not be considered entirely specific for pNETs, (overall sensitivity - 69-90% and specificity - 78-88%) considering that sensitivity ranges fall to 67-76% in metastatic pNETs (while specificity increases to 89-98%).

Finally, the sensibility and sensitivity of Pancreatic and Duodenal Homeobox 1 (PDX1) and its role in characterization of NETs is discordant. While some studies found a relatively high specificity and sensibility of PDX1 for pNET (72% expression in primary pNET and 100% in metastatic pNET, with a specificity of 92% and 75% respectively) (72), others demonstrated staining of PDX1 in the rectum, stomach, duodenum, appendix (and rarely in the lung and small bowel) and low percentages of expression in pNET (30%) (62, 73).

An important issue with pancreatic markers is that appendiceal/rectal L-cell tumors often express pancreatic markers such as ISL1, PDX1 and PAX8, as shown above. To overcome this potential pitfall, recent studies have shown that special AT-rich sequence binding protein-2 (SATB2), a transcription factor binding protein, may be used as a specific marker for appendiceal/rectal NETs (it is not expressed in pancreatic/duodenal NETs) (74). Lastly, to distinguish rectal and appendiceal L-cell NETs, positivity for prostatic acid phosphatase confirms rectal origin.

Question 7: How Should These Markers Be Used (Immunopanels to Identify Sites of Origin)?

Various immunopanels have been proposed in the literature to identify site of origin, based on differential use of transcription factors and hormone/amine products (61, 69, 73, 75, 76). An immunohistochemical panel demonstrating TTF1 positivity, negativity for CDX2, ISL1 and PDX1 supports a diagnosis of pulmonary NEN. In this setting calcitonin and CEA expression study can help pathologist to distinguish medullary thyroid carcinoma and pulmonary NEN (61, 69). Conversely, an immunohistochemical panel showing strong and diffuse positive staining for CDX2 and negativity for TTF1, ISL1 and PDX1 favors a midgut origin (usually ileal or appendiceal) (61, 69, 73). An immunohistochemical panel demonstrating TTF1 negativity, negative or weak staining for CDX2, ISL1 and PDX1 positivity suggests a NEN originating from the pancreas or duodenum (61, 72) (the distinction between a pancreatic versus a duodenal NEN is challenging). An immunohistochemical panel demonstrating TTF1 and PDX1 negativity, negative or weak staining for CDX2 and ISL1 positivity suggests a L-cell NEN (61, 70, 72). Despite the use of multiple markers primary tumor detection often remains challenging and requires clinical and radiologic information to reach the final diagnosis.

Question 8: Are There Other Immunohistochemical Prognostic Markers for NEN Apart From Ki67?

New prognostic immunomarkers, have been recently proposed in NEN. Most of these markers have been principally investigated in pNET and their role in NENs of different sites still remains to be established.

Cytokeratin-19 (CK19) has been shown to be a prognostic factor for NEN even though its prognostic role seems to vary depending on the subtype of pNET. Indeed, CK19 has been identified as a prognostic factor in pNET, excluding insulinomas, with evidence of correlation between CK19 expression and a more aggressive phenotype (77). CK19 has been shown to be an independent prognostic factor (78) with a 5-year survival of all CK19 negative cases of 100%, with a drop to 47% in CK19 positive neoplasms as confirmed by a recent meta-analysis (79).

Insulinoma associated protein 1 (INSM1), a nuclear transcription factor, is a sensitive and well-validated marker for neuroendocrine differentiation (80). Preliminary studies suggest the potential utility

TABLE 1 | Minimum and optional requirements for a pathology report of gastroenteropancreatic neuroendocrine neoplasm [adapted from Volante et al. (87)].

Minimum Requirements	WHO used (2017-2019) for pathology report Differentiation and WHO tumor type (NET, NEC, MINEN), if NEC large or small cell, if MINEN, histotype of NE and non-NE components Tumor Grade (<3% for G1, 3-20% G2, > 20% G3) for NET Ki-67 index as precise value (%) Size and location Depth of invasion Lympho-vascular invasion (present/absent) Perineural invasion (present/absent) Lymph node status (number evaluated nodes, number of positive nodes) R status and description of margins Immunohistochemical markers used for identification of primary, in case of biopsy Immunohistochemical markers performed and relative results pTNM stage (AJCC/WHO/UICC)
Optional Requirements	Ki-67% on different site (primary and metastases) Mitotic index as value (x2 mm^2) If positive lymph node, description of presence/absence of extra nodal extension Hormone positivity on immunohistochemistry Somatostatin receptor immunohistochemistry (not for routine patology report)

NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; MINEN, mixed neuroendocrine-non-neuroendocrine neoplasms; AJCC, American Joint Commission on Cancer; WHO, World Health Organization; UICC, Union for International Cancer Control.

of INSM1 as a prognostic factor, as INSM1 expression seems to correlate with more malignant behavior and with greater propensity of metastasis in gastrointestinal NENs (81).

c-KIT, a tyrosine kinase receptor of the platelet derived growth factor subfamily, was found to be a negative independent prognostic marker in pNET with adverse prognosis in c-KIT positive NENs (82).

The prognostic role of DAXX/ATRX expression is more controversial. Some studies have shown loss of expression of DAXX/ATRX to be associated with more aggressive behavior and shorter disease-free survival (83, 84). In contrast, other observations appear to show an improved overall survival in tumors showing loss of DAXX/ATRX (85, 86).

CONCLUSIONS

In conclusion, patient management should be based on multidisciplinary decisions based on precise and specific comprehension of information and communication. Clinicians require an understanding of classification systems (which change over time) and the importance of novel markers which may aid in diagnosis and prognosis as well as concise and standardized pathology reports. In keeping with this last statement, an example of the minimum requirements in pathology datasets is shown in **Table 1** and should be a baseline for all neuroendocrine tumor professionals (87).

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.

AUTHOR CONTRIBUTIONS

MA and FG conceived the study, wrote and finalized the manuscript. GP, FL, and CR contributed to the collection of information and references, writing of the manuscript and approval of the final manuscript. AF and AC supervised and 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.2021.680305/full#supplementary-material

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CASE REPORT
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Case Report: Unmasking Hypercalcemia in Patients With Neuroendocrine Neoplasms. Experience From Six Italian Referral Centers

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Background: Hypercalcemia is a common paraneoplastic syndrome which can occur in up to 10% of patients with advanced neoplasms. Paraneoplastic parathyroid hormone-related protein (PTHrP) represents the most frequent cause of this syndrome. In neuroendocrine neoplasms (NENs) paraneoplastic hypercalcemia is rare.

Case Series: The present series includes all patients with NENs and paraneoplastic hypercalcemia from four Italian centres: **(I)** A 40-year-old man was hospitalized for repeated episodes of falls, hyposthenia and drowsiness. Severe hypercalcemia was found. Metastatic pancreatic G2 NEN and PTHrP-related hypercalcemia were diagnosed. The patient started therapy with somatostatin analogs (SSA) and Denosumab. After disease progression peptide receptor radionuclide therapy (PRRT) was started with an objective response associated with PTHrP reduction and normocalcemia. **(II)** A 45-year-old man was referred for pancreatic G2 NEN. SSA and subsequently everolimus were administered for metastases occurrence. Hypercalcemia occurred and PRRT and Denosumab were started for disease progression with the onset of bone metastases. Despite disease stability after four cycles of PRRT the patient's performance status worsened until death. **(III)** A 49-year-old woman was hospitalized for psychic slowdown, confusional state, sensory dullness. A severe hypercalcemia, associated with a pancreatic G1 NEN was diagnosed and treated with haemodialysis, bisphosphonates injections and continuous infusion of calcitonin. 1,25-dihydroxyvitamin D was high, PTHrP was undetectable. After surgery serum calcium levels and 1,25-dihydroxyvitamin D were normalized. **(IV)** A 69-year-old man was hospitalized after the onset of shortness of breath and dyspnea, asthenia and weight loss. Computed Tomography (CT) and ^{68}Ga DOTATOC Positron Emission Tomography (PET)-CT

revealed a left pulmonary nodule. Hypercalcemia and markedly elevated PTHrP levels were detected. The histological examination revealed an atypical carcinoid. After surgery, calcium levels were normalized, PTHrP was significantly reduced with an improvement of general conditions.

Conclusion: In our series, paraneoplastic PTHrP-related hypercalcemia occurred in pancreatic NEN and in one bronchial carcinoid representing the third case in the literature. Our case associated with 1,25-dihydroxyvitamin D secretion represents the fourth case in the literature. PTHrP secretion should be considered in NENs' patients with hypercalcemia. Acute treatment should be focused on lowering calcium levels, and long-term control can be achieved by tumor cytoreduction inhibiting PTHrP release.

Keywords: paraneoplastic hypercalcemia, parathyroid hormone-related protein, pancreatic NEN, bronchial carcinoid, 1,25-dihydroxyvitamin D

INTRODUCTION

Hypercalcemia of malignancy is a severe clinical condition which can occur in 20–30% of patients with advanced neoplasms (1, 2). The prognosis of these patients is poor; indeed 50% patients die within a month and 75% within 3 months (3). It is caused by bone osteolysis due to metastases (20% of cases), paraneoplastic secretion of parathyroid hormone-related protein (PTHrP) (80%), configuring a humoral hypercalcaemia of malignancy (HHM), and rarely by ectopic parathyroid hormone (PTH) (<1%) or 1,25-dihydroxyvitamin D secretion (<1%) (1). In the presence of hypercalcemia, where bone metastases are absent, an endocrine cause should be suspected (4). On the whole, endocrine paraneoplastic hypercalcemia can occur in up to 10% of neoplastic patients (5).

Pathogenic mechanisms of endocrine paraneoplastic hypercalcemia are related to bone resorption, renal and intestinal calcium reabsorption. PTHrP and PTH stimulate bone resorption *via* receptor activator of nuclear factor-B (RANK)/RANK ligand (RANKL) system activation (4). Besides bone resorption, PTH and PTHrP also stimulate renal reabsorption of calcium (6). Moreover, PTH, but not PTHrP, increases intestinal reabsorption of calcium *via* induction of 1,25-dihydroxyvitamin D synthesis (6). Calcitriol-mediated hypercalcemia results from increased intestinal reabsorption of calcium and increased bone resorption (7).

HHM is diagnosed in presence of elevated PTHrP, suppressed PTH, low phosphorus and low-normal 1,25-dihydroxyvitamin D levels (7). Ectopic PTH production is characterized by high PTH levels, low phosphorus, and high 1,25-dihydroxyvitamin D levels (7). Hypercalcemia due to calcitriol secretion is diagnosed in the presence of high levels of 1,25-dihydroxyvitamin D, associated with low PTH levels, and high phosphorus levels (7).

Neuroendocrine neoplasms (NENs) are a heterogeneous group of relatively rare malignancies deriving from the neuroendocrine system (8). NENs are capable to secrete peptide hormones and amines which can provoke specific clinical syndromes (8). Besides bone metastases, primary hyperparathyroidism, which is part of multiple endocrine neoplasia type 1 (MEN1) and type 2 (MEN2) syndrome, can be a cause of hypercalcemia in patients with NENs, in detail pancreatic, duodenal, gastric, pulmonary, and thymic NENs (9) or medullary thyroid carcinoma (10). Thus, primary hyperparathyroidism should be considered in differential diagnosis. Indeed, in NENs paraneoplastic hypercalcemia is rare (11), it is mostly associated with gastro-entero-pancreatic (GEP) NENs, specifically pancreatic NENs (pNEN) (12), and mainly related to PTHrp secretion (11). We present a series including all patients with NENs and paraneoplastic hypercalcemia from four different NEN Italian centers in the last 15 years. Clinical features of the four cases are summarized in **Figure 1**, laboratory data and symptoms are reported in **Table 1**.

CASE 1

On July 2019 a 40-year-old man with personal history of brain arteriovenous malformations (AVM) and thyroidectomy in 2000 for papillary thyroid carcinoma with post-surgical permanent hypoparathyroidism, was hospitalized after repeated episodes of falls due to marked hyposthenia and drowsiness. Additionally, he reported a feeling of early satiety that has arisen two months before, he also reported weight loss in the last three months (from 110 kg to the current 87 kg). Brain computed tomography (CT) and magnetic resonance (MR) imaging were negative. Blood test showed hypercalcemia (15.5 mg/dl, range 8.4–10), elevation of cholestasis markers gamma-glutamyltransferase (874 UI/l, range 8–61), and alkaline phosphatase (526 UI/l, range 40–129). The oral treatment with calcium carbonate and

Abbreviations: PTHrP, parathyroid hormone-related protein; HHM, hypercalcaemia of malignancy; PTH, parathyroid hormone; RANK, receptor activator of nuclear factor-B; RANKL, RANK ligand; NENs, neuroendocrine neoplasms; MEN1, multiple endocrine neoplasia type 1; MEN2, multiple endocrine neoplasia type 2; GEP, gastro-entero-pancreatic; pNENs, pancreatic NENs; AVM, arteriovenous malformations; CT, Computed Tomograph; MR, magnetic resonance; PET, Positron Emission Tomography; PRRT, peptide receptor radionuclide therapy; NSE, neuron specific enolase; NET, neuroendocrine tumor; pNET, pancreatic NETs; SSAs, somatostatin analogs.

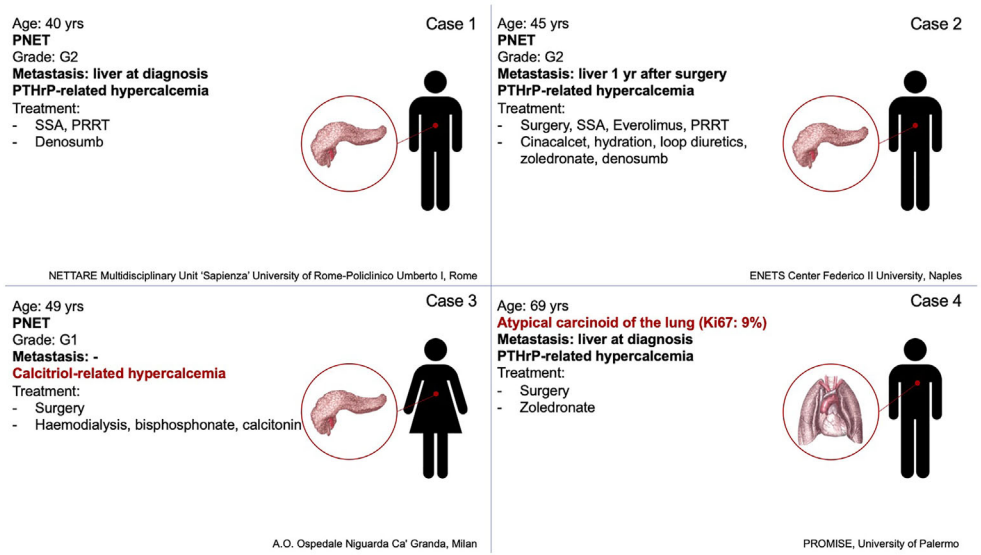

FIGURE 1 | Summary of tumor's characteristics, hypercalcemia's origin, and lines of therapy of the four cases.

TABLE 1 | Summary of laboratory data and clinical presentation of the four cases at paraneoplastic hypercalcemia diagnosis.

Case	Laboratory data							Symptoms
	Calcium	*PTHrp*	*PTH*	*25-OH Vitamin D*	*1,25-dihydroxyvitamin D*	*Calcitonin*	*Chromogranin A*	
1	15.5 mg/dl (8.4–10)	451 ng/l (<20)	1.2 pg/ml (15–65)	16 ng/ml (>20)	NA	958 pg/ml (<10)	470 ng/ml (<100)	Hyposthenia and drowsiness
2	11.8 mg/dl (8.4–10.2)	NA	13.2 pg/ml (10–79)	30 ng/ml (>20)	NA	NA	307 ng/ml (<110)	Abdominal pain
3	21 mg/dl (8.4–10)	NA	12 pg/ml (10–90)	8 ng/ml (>20)	85 pg/ml (16–55)	3,079 pg/ml (01.1–15)	222 UI/l (<20)	Psychic slowdown, confusional state, sensory dullness
4	14.4 mg/dl (8.4–10)	109 ng/ml (<20)	4.7 pg/ml (15–65)	NA	NA	NA	184.9 ng/ml (<100)	Shortness of breath and dyspnea, asthenia and weight loss

calcium citrate plus calcitriol was withdrawn without a decrease in calcium levels.

During the diagnostic work-up an ultrasound of the upper abdomen was performed and showed numerous hyperechoic solid formations of likely metastatic significance in the liver. Subsequently, total body CT confirmed the presence of multiple liver metastases, affecting 50% of the left hepatic lobe and 30% of the right lobe, and found a voluminous lesion with regular margins, sized 95 × 85 × 75 mm, in the pancreatic tail. Moreover, more metastatic implants, with a maximum size of 24 × 20 mm, were observed in the left subdiaphragmatic area. The patient was then subjected to liver biopsy, the histological examination revealed a liver localization of well differentiated NEN, Ki67 index 5%. Immunohistochemistry was positive for CK 8/18, CD56, synaptophysin, and weakly positive for CDX2 and chromogranin A. Among circulating neuroendocrine markers, calcitonin (958 pg/ml, normal values <10) and chromogranin A (470 ng/ml, normal values <100) levels were found elevated. The patient underwent ^{68}Ga DOTATOC Positron Emission Tomography (PET)-CT which showed uptake of the radiotracer in the pancreatic lesion, and in hepatic and nodal metastases. Subsequently, he started therapy with Lanreotide Autogel 120 mg every 28 days. Given the hypercalcemia, possibly of paraneoplastic origin, Denosumab 120 mg every 28 days was started. To investigate hypercalcemia origin, PTHrP was dosed and was found markedly elevated (451 ng/l, normal values <20). Three months later, CT imaging showed hepatic disease progression. Thereby, peptide receptor radionuclide therapy (PRRT) with ^{177}Lu-LUTATHERA 7400 MBq was prescribed. The patient performed four cycles of therapy from November 2019 to June 2020. The treatment was well tolerated (except for mild leukopenia), and clinical (improved general conditions) and biochemical (stabilization of calcium levels) responses were good. After the first cycle of treatment, PTHrP declined from 451 to 150 ng/l (see **Figure 2**). Moreover, an objective tumor response was observed at CT evaluation after three cycles of treatment: hepatic lesions were reduced at VIII segment (44 × 36 *vs* 53 × 48 mm), IVa segment (25 × 23 *vs* 30 × 27 mm), and VI segment (26 × 19 *vs* 32 × 26 mm) (see **Figure 2**).

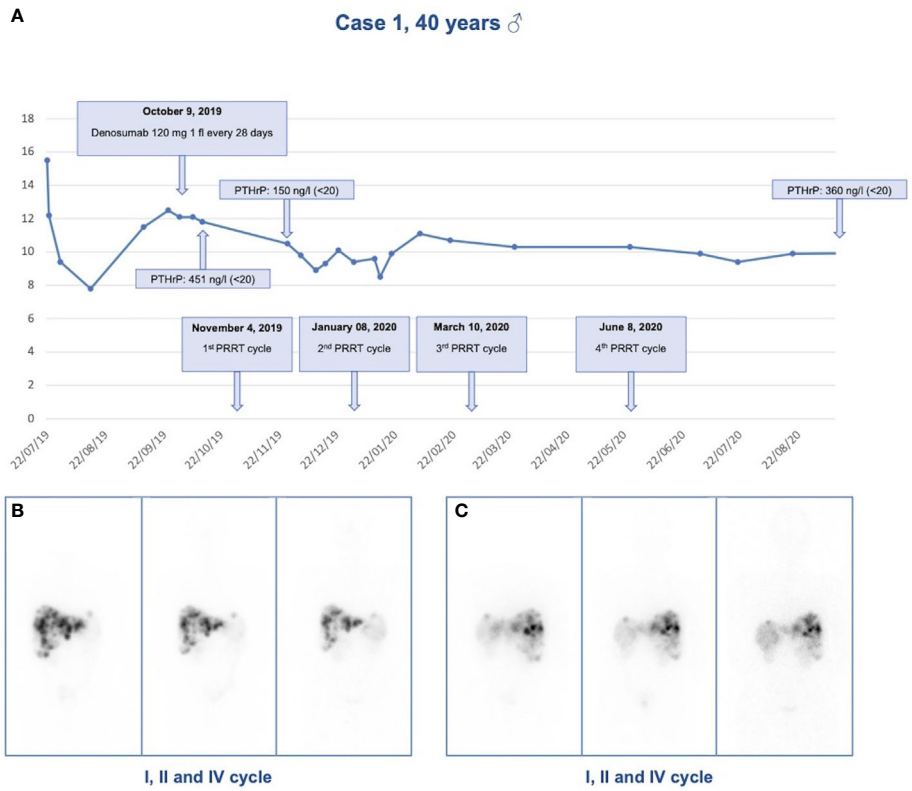

FIGURE 2 | Response to treatment in Case 1. **(A)** Calcium levels in relationship to denosumab administration and PRRT cycles in case 1; **(B)** Anterior view of ^{177}Lu-LUTATHERA scintigraphy after I, II, and IV cycles of PRRT; **(C)** Posterior view of ^{177}Lu-LUTATHERA scintigraphy after I, II, and IV cycles of PRRT.

CASE 2

A 45-year-old male with history of multinodular goiter and hypertension underwent distal pancreatectomy and splenectomy in 2006 due to the detection of a caudal pancreatic mass 4.5 cm in diameter. Histological examination revealed a G2 pancreatic neuroendocrine tumor (NET) without lymph node metastases, Ki67 was 5% according to the WHO 2010 criteria. Immunohistochemistry showed positivity for synaptophysin, chromogranin, neuron specific enolase (NSE), pan-cytokeratin, and calcitonin. One year later, therapy with somatostatin analogs (SSAs) was started due to onset of multiple liver metastases, and in 2010 Everolimus was introduced due to hepatic progression. Mild serum calcium elevation appeared in 2014, with normal renal function, PTH, and 25-hydroxyvitamin D levels. Neck ultrasound, performed for follow up of a multinodular goiter, revealed a hypoechoic lesion resembling a parathyroid adenoma. Serum calcium levels progressively increased (11.8 mg/dl normal range 8.4–10.2 mg/dl), thus Cinacalcet was introduced at the dosage of 30 mg/daily and increased up to 60 mg/daily. However, it was withdrawn because of its inefficacy. No evidence of disease progression was recorded, but clinical conditions rapidly deteriorated, and hypercalcemia was treated with intravenous saline hydration and loop diuretics to obtain rapid restoration. Subsequently, intravenous bisphosphonate Zoledronate was introduced, 4 mg monthly for 12 months (from October 2018 to October 2019) with normalization of calcium levels. PTH levels remained within normal reference range, while neck ultrasound did not confirm the suspected parathyroid lesion. Clinical and biochemical results strongly support the hypothesis of a hypercalcemia ascribable to a secretion of PTHrP, since all other causes of hypercalcemia were excluded, but unfortunately the assay for PTHrP was not available. In 2018 the dose of Everolimus was lowered from 10 to 5 mg/daily due to hematologic side effects and the total body CT scan showed progressive liver disease, and centimetric bilateral hip and vertebral sclerotic bone metastases, with positive ^{68}Ga DOTATOC PET-CT. The slight bone metastatic involvement without osteolysis could not explain recurring hypercalcemia. Everolimus and Zoledronate were suspended, and the patient began PRRT and Denosumab 120 mg monthly. Disease stability was obtained, but unfortunately osteonecrosis of the jaw occurred and Denosumab was suspended after only two administrations. Calcium levels increased again at 12.1 mg/dl, and intravenous saline hydration and loop diuretics were administered. Prednisone 20 mg daily was needed to normalize calcium levels. Despite disease stability after four cycles of PRRT the patient's performance status worsened and the patient deceased in 2019.

CASE 3

In July 2007 a 49-year-old woman with a huge pNEN associated with severe hypercalcemia was referred to Niguarda Hospital (Milan, Italy). The medical history of the woman was silent until 4 months earlier, since she had 20 kg weight loss, asthenia, and hyperglycemia. One month before she arrived at our medical facility the patient went to the emergency room of another hospital because of the onset of neurologic symptoms (psychic slowdown, confusional state, sensory dullness). Emergency room blood exams showed severe hypercalcemia (21 mg/dl). The patient was hospitalized in the nephrology unit where she underwent hemodialysis (single treatment), bisphosphonates injections, and continuous infusion of calcitonin to control hypercalcemia. Neoplastic mass embolization through spirals positioning in the splenic artery was performed. Calcium levels normalized in a few days and at the same time a gradual improvement of neurologic symptoms was observed until a complete recovery. Imaging studies showed a 12 cm mass of pancreatic body and tail. A needle biopsy of the mass was diagnostic for a well differentiated NEN. ^{111}In-pentetreotide scan was performed and showed tracer uptake in the abdominal lesion. Patient was discharged and referred to our hospital for further investigations and surgery. When the patient arrived at our facility her calcium levels were between 8.5 and 8.7 mg/dl (normal). Diabetes and anemia requiring blood transfusions were observed. Hormonal examinations showed low-normal PTH levels (12 pg/ml, normal values 10–90), high levels of calcitonin (3,079 pg/ml, normal values 0.10–15.00) and chromogranin A (222 UI/l, normal values <20). Vitamin D metabolites were determined showing low levels of 25-hydroxyvitamin D (8 ng/ml, normal values >20) and high levels of 1,25-dihydroxyvitamin D (85 pg/ml, normal values 16.0–55.0). An abdomen MR confirmed an expansive 12 cm lesion of the body and tail of the pancreas, highly vascularized, with a central necrotic area, with apparent infiltration of the fundus of the stomach and the splenic ileum, and showed thrombus of the splenic vein jutting out within the portal mesenteric confluence. The patient underwent distal pancreatectomy, splenectomy, resection of the gastric fundus, and removal of the neoplastic thrombus. Pathological examination of the pancreatic mass was diagnostic for a well differentiated G1 NET, angioinvasive, massively infiltrating the gastric wall. Immunohistochemical staining for chromogranin A, synaptophysin and somatostatin was observed; MIB1 was <1%. Twenty-four hours after surgery reduction of serum calcium levels was observed, which in the following days dropped to 5.4 mg/dl despite calcium (both intravenous and oral administration) and calcitriol supplementation, post-surgical ionized serum calcium was 1.08 mmol/l (1.18–1.29). A week after surgery, improvement until normalization of serum calcium was gradually observed and calcium and calcitriol supplementations were reduced. The postoperative course was also characterized by pleural effusion treated with drainage placement and antibiotic therapy. Soon after surgery, glycemia, calcitonin, and chromogranin A normalized, whereas PTH levels increased to 180 pg/ml (normal values 10–90), to then return into the normal limits in the following months. Thirteen years after surgery the patient was in good general condition, calcium and PTH levels were in normal range, and there was no evidence of disease recurrence.

CASE 4

In August 2016 a 69-year-old man with personal history of dilatative cardiomyopathy due to ischemic heart disease was hospitalized after the onset of shortness of breath and dyspnea, asthenia, and weight loss (from 98 to 75 kg). Chest radiograph showed a pulmonary nodule of about 40 mm. Total body CT confirmed a pulmonary nodule of 32 × 43 mm located at the apical segment of the lower left lobe, and partly leaning and compressing some bronchial branches, with a small calcification. In addition, a solid liver lesion of 10 mm was detected. Blood tests showed hypercalcemia (14.4 mg/dl, range 8.4–10) and low PTH (4.7 pg/ml, range 15–65). A ^{68}Ga DOTATOC PET-CT was performed showing an uptake of the radiotracer in the left pulmonary lesion. A lung biopsy showed a histological report of a poorly differentiated neuroendocrine carcinoma, Ki67 index 10%. Immunohistochemistry was positive for chromogranin A, CK7, and weakly and focal positive for TTF1. Circulating neuroendocrine markers showed high NSE (107 ng/ml, range 1–16) and chromogranin A (184.9 ng/ml, normal values <100). Liver biopsy showed a hemangioma. Therapy with Zoledronate 4 mg intravenously every 28 days was immediately started, given the hypercalcemia, possibly of paraneoplastic origin. To investigate hypercalcemia origin, PTH-rP was measured and found to be markedly elevated (109 ng/ml, normal values <20). After stabilization of hypercalcemia, a lower left lung lobectomy was performed in line with guideline (13).

The histological examination was well differentiated NET positive for chromogranin A and synaptophysin, of the lower lateral lobe, mitosis >2HPF, Ki67 9%. After surgical lung lobectomy, calcium levels were normalized, PTH-rP was significantly reduced from 109 to 5 ng/ml and an improvement of the general conditions was achieved.

DISCUSSION

This case series includes all patients with NET and paraneoplastic hypercalcemia from six Italian centers. 847 patients (517 GEP-NETs and 119 pulmonary NETs) were evaluated. In line with other series (11), there were four cases of paraneoplastic hypercalcemia (0.5%), respectively in three pancreatic NETs (pNET) G2 (n. of cases: two) or G1 (n. of cases: one), and in one lung NET (atypical carcinoid). The rarity of the association of paraneoplastic hypercalcemia in NETs of the respiratory tract represents the first peculiarity of this series. In the two G2 pNETs and in the lung carcinoid, hypercalcemia was likely associated with high PTHrP; in the G1 pNET hypercalcemia was associated with elevated 1,25-dihydroxyvitamin D, a rare cause of paraneoplastic hypercalcemia in NENs. In the cases of the atypical carcinoid and the G1 pNET, where PTHrP and 1,25-dihydroxyvitamin D caused respectively the hypercalcemia, a

normalization of the calcium levels was achieved after surgery. In the two cases of G2 pNET with probable PTHrP-related hypercalemia we observed: in the first case, the normalization of calcium levels after therapy with Denosumab and PRRT; in the second case, patient died due to worsening of his performance status despite the different lines of treatment used to achieve disease stability (SSAs, Everolimus, PRRT) and to control hypercalcemia (hydration, loop diuretics, Zoledronate, corticosteroids, Denosumab). In all cases, the antitumor treatment for NEN associated with the specific treatment for paraneoplastic hypercalcemia led to serum calcium normalization. In the past decades, paraneoplastic PTHrP ectopic secretion was associated with poor prognosis (3) and reduced overall survival (12). The consequent hypercalcemia needs to be controlled. Supportive treatment approach includes the standard management for the correction of hypercalcemia: intravenous isotonic saline, bisphosphonates, and Denosumab (4). SSAs may help to improve symptom control slowing the tumor growth, but it's not sufficient to control hypercalcemia, above all in patients with tumor progression, as in our cases (12, 14). The most successful treatment options for PTHrP-producing NETs were SSAs and PRRT with ^{177}Lu-DOTATATE, as previously described (12). Interestingly, calcitonin levels were high in two cases of metastatic pNEN. In a recent literature review on calcitonin-producing pNENs no case of concomitant paraneoplastic hypercalcemia was reported (15). The real prevalence of calcitonin production by pNENs could be underestimated by the lack of specific symptoms (16). However, recent evidence shows that it is not an exceptional event and seems to not identify a separate clinical entity (17).

NENs with paraneoplastic hypercalcemia are poorly described in the literature. Nevertheless, they represent a condition deserving an early differential diagnosis from hypercalcemia due to bone metastases and a specific therapeutic framework (1). Observations coming from this series were: (i) the paraneoplastic hypercalcemia syndrome occurred mainly in P-NENs, according to the literature (12); (ii) one of the four cases reported represents, to the best of our knowledge, the third case in the literature of PTHrP secretion from a bronchial carcinoid (18, 19); (iii) while in one it was due to secretion of 1,25-dihydroxyvitamin D, which represents, to the best of our knowledge, the fourth case in literature of calcitriol-related paraneoplastic hypercalcemia in NENs (20–22).

NEN-associated hypercalcemia occurs rarely and generally in patients with advanced metastatic cancer and with a poor prognosis. Given that the principal mechanisms of hypercalcemia in cancer patients are related to the secretion of PTHrP by tumor cells and very rarely to the secretion of calcitriol, it is mandatory to identify promptly the hypercalcemia-related symptoms and the underling paraneoplastic secretion. The clinical features of hypercalcemia include nausea, vomiting, lethargy, renal failure, and coma. The severity of symptoms depends not only on the degree of hypercalcemia (calcium levels >14 mg/dl are considered severe), but also on the rapidity of onset. The laboratory assessments for the diagnosis of hypercalcemia include serum levels of calcium and ionized calcium, evaluation of PTH, PTHrP, and 1,25-dihydroxyvitamin D. In case of a paraneoplastic hypercalcemia, laboratory findings include elevated calcium levels, low-to-normal PTH levels, and often high PTHrP levels. Ionized calcium levels should be dosed or calculated as following: corrected calcium (mg/dl) = measured calcium (mg/dl) + [0.8 × (4.0 − albumin (mg/dl)]. The optimal approach to control paraneoplastic hypercalcemia is the treatment of the underlying tumor. To control hypercalcemia, it is important to discontinue medications that contribute to it (e.g., calcium supplements, vitamin D, thiazide diuretics, calcium-containing antacids, and lithium). The first-line approach to persistent hypercalcemia is fluid repletion with normal saline; loop diuretics may be added after adequate volume restoration. Intravenous bisphosphonates, inhibiting osteoclast bone resorption, are also used (1).

CONCLUSION

In conclusion, this is the first Italian series of patients with paraneoplastic hypercalcemia from GEP and respiratory tract NETs. PTHrP secretion should be considered in patients with NETs and hypercalcemia associated with low PTH levels. 1,25-dihydroxyvitamin D should always be evaluated to exclude a paraneoplastic secretion. Management of malignant hypercalcemia secondary to PTHrP-secreting NETs is challenging. Acute management should be focused on lowering calcium levels, and long-term control can only be achieved by tumor cytoreduction and inhibition of PTHrP release. Optimal therapy depends on the extent of metastatic disease and tumor grade. Cytoreduction of metastasis should be accomplished when possible. SSAs, systemic antineoplastic therapy can all be helpful, and PRRT using radiolabeled SSAs seems to be more effective, given the extensive impact it can achieve on neoplastic tissue. It is of notice that the aggressive nature of some tumors with Ki67 >5% and high PTHrP levels may suggest a worse prognosis, indicating the need for an early diagnosis.

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

EG, AC, and AF conceived and designed the case series. FS, RM, EMG, and VG shared the four cases, collected the data, and

wrote the cases. EG, FS, RM, EMG, VG, AR, and IZ co-wrote the MS. AC and AF contributed to the revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Neoadjuvant Therapy for Neuroendocrine Neoplasms: Recent Progresses and Future Approaches

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Neuroendocrine neoplasms (NENs) are a heterogeneous group of tumors, their treatment being challenging and requiring a multidisciplinary approach. Though the only curative treatment is surgery, up to 50% of patients are diagnosed with metastatic disease. In the last years, neoadjuvant chemo(radio)therapy has become part of the standard of care in the treatment of different cancer types. However, evidence of its efficacy and safety in NEN patients has not yet been confirmed in the literature. The aim of the present review is to perform an extensive review of the scientific evidence for neoadjuvant therapy in patients with gastroenteropancreatic and thoracic NENs.

Keywords: neuroendocrine tumors, somatostatin (analogs and derivatives), peptide receptor radionuclide therapy, everolimus, capecitabine, temozolomide, chemotherapy

INTRODUCTION

Although neuroendocrine neoplasms (NENs) are considered rare malignancies, their incidence has rapidly increased in the last decades. Since several patients are diagnosed with metastatic disease, curative surgery is usually not an option (1), palliative surgical intervention possibly being effective in controlling clinical symptoms and improving patient's quality of life (2–4). Neoadjuvant therapy, with the aim of reducing tumor size and disease burden, can potentially change the clinical scenario making it suitable for curative surgery as already demonstrated in other cancer types (5–8). While it is conceivable that neoadjuvant chemo-and radiotherapy might be effective in NENs (9), reliable evidence is still lacking in this field and study results are difficult to compare due the heterogeneity of both neoadjuvant therapies used, and series studied. Moreover, although the available therapeutic options (i.e., somatostatin analogs, everolimus, chemotherapy, sunitinib, and peptide receptor radionuclide therapy PRRT) are currently not included in any therapeutic algorithm with specific neoadjuvant purpose, most of them have been used with this intent, even successfully (9). Neoadjuvant chemotherapy, radiotherapy, and PRRT have been shown to provide variable results in terms of tumor down-sizing (10). The aim of the present minireview is to perform an

extensive review of the scientific evidence for neoadjuvant therapy in patients with pancreatic, gastrointestinal, and thoracic NENs.

PANCREATIC NEUROENDOCRINE NEOPLASMS

Pancreatic neuroendocrine neoplasms (pNENs) account for 1 to 2% of all pancreatic tumors and most of them are sporadic and nonfunctioning. Their incidence has been increasing and survival rates, although improved, remain poor compared with other primary sites, with an overall survival of 3.6 years (1). Often pNENs present with advanced disease at diagnosis and the treatment of metastatic unresectable pNENs remains debated and the role of neoadjuvant therapies is still uncertain. However, among gastrointestinal NENs, data on the possible role of neoadjuvant therapy are mainly related to pNENs even if the results seem to be somewhat contradictory and difficult to interpret due to the extreme heterogeneity of the data and the incompleteness of the information provided (**Table 1**). In a large series of high-grade gastrointestinal neuroendocrine carcinomas, where pancreas was the most common primary site (361 patients), neoadjuvant or adjuvant therapy resulted in better overall survival (OS) in patients with early-stage disease compared with those treated with resection alone. Details of neoadjuvant therapies were not available, but single and multi-agent chemotherapy besides radiotherapy were included. The positive effect of neoadjuvant or adjuvant therapy on OS seems to suggest the importance of these treatments to lower the incidence of both micrometastases and possibly to enhance tumor resection thus lowering the risk of local and systemic recurrences (26).

Patients who received neoadjuvant or adjuvant therapy had better over-all survival, suggesting that high incidence of micrometasta-sis contributes to the poor surgical outcomes. In

TABLE 1 | Neoadjuvant therapies in gastroenteropancreatic NENs.

Year	Article type	Neoadjuvant treatment	Outcome	Reference
Pancreas				
2019	research article	no details available	no changes in OS	(11)
2008	research article	PRRT	Partial response and R0 resection	(12)
2009	case report	PRRT	Partial response and R0 resection	(13)
2010	case report	PRRT	Partial response and R0 resection	(14)
2011	research article	PRRT	Stabilization or partial response	(15)
2012	case report	PRRT	Partial response and R0 resection	(16)
2015	research article	PRRT	Better PFS and lower morbidity	(17)
2015	research article	PRRT	Improved PFS	(17)
2017	research article	PRRT	Improved PFS	(18)
2018	case report	PRRT	Partial response and R0 resection	(19)
2015	research article	chemo	Improved survival	(20)
2016	research article	chemo	Improved OS	(21)
2017	research article	chemo	no changes in tumor size	(22)
2018	research article	chemo	Improved OS and RFS	(23)
2011	case report	chemo, radio	R0 resection	(24)
2017	research article	chemo, radio	Stabilization or partial response and R0 resection	(25)
2018	research article	chemo, radio	Low recurrence risk and improved OS	(26)
2012	research article	chemo, radio	No effects	(27)
2012	research article	PRRT, chemo	Radiological response	(28)
2020	research article	PRRT, chemo	Stabilization or partial response	(29)
2020	research article	PRRT, chemo	Improved PFS and OS	(30)
Esophagous				
2018	case report	chemo	complete response, complete resection	(31)
1989	case report/review	chemo	almost complete response, complete resection	(32)
1995	case report	chemo	almost complete response, complete resection	(33)
1999	case report	chemo	almost complete response, complete resection	(34)
2002	case report	chemo	partial response, reduced tumor burden	(35)
2003	case report	chemo	partial response, reduced tumor burden	(3)
Rectum				
2017	research article	chemo, radio	almost complete response, complete resection	(36)
2018	case report	chemo	reduction of primary lesion/grading	(4)
Miscellanea				
2009	research article (midgut tumor)	PRRT	partial response, partial resection	(15)
2012	research article (midgut tumor)	PRRT	partial response, complete resection	(37)
2012	research article (midgut tumor)	SSA-PRRT	partial response, partial resection	(38)
2012	research article (duodenal tumor)	PRRT	partial response	(29)
2015	case report (small bowel tumor)	PRRT	no response	(39)

Chemo, chemotherapy; Radio, radiotherapy; PRRT, peptide receptor radionuclide therapy; SSA, somatostatin analogs; OS, overall survival; R0, microscopically margin-negative resection; PFS, progression-free survival; RFS, relapse free survival.

addition, the relatively high proportion of margin-positive resection raises the question of whether there is a role of downstaging with neoadjuvant therapy, aimed at enhancing resection and lowering risk of systemic recurrence.

Xie et al. recently described the largest group of pNENs in whom neoadjuvant therapies were used. The study included 4,892 patients who underwent curative-intent surgical resection. Authors showed that neoadjuvant therapy was mainly prescribed in patients <65 years, with grade 3 pNENs localized in the head of the pancreas and associated to the presence of metastasis. In this setting, Authors did not find any significant improvement in OS even in patients with grade 1 and 2 pNENs thus suggesting that neoadjuvant therapy should be used with caution given the lack of conclusive data (11). It is worth noting that, the main limitation of the Study was the lack of any detail regarding the type of neoadjuvant therapies used.

PRRT with radiolabeled somatostatin analogues, over the years, has evolved as an important therapeutic option for the treatment of inoperable or metastasized, well/moderately differentiated, NETs, particularly of the GEP (**Table 1**) (9). Conversely, neoadjuvant PRRT based on either 177Lu-octreotate or 90Y-DOTATATE have been shown to provide variable results as tumor downsizing possibly due to the heterogeneous inclusion criteria, the variable length of follow-up and the different response criteria used (10). Kwekkeboom et al. described a series of 310 patients with pNENs treated with 177Lu-octreotate PRRT. A partial response was observed in four of them, all undergoing a subsequent R0 resection (12). Similar results were confirmed by other case reports and small series (13, 14, 16, 19). Van Vliet et al. described the results of 177Lu-octreotate as neoadjuvant therapy in 29 patients with borderline, unresectable or oligometastatic nonfunctioning pNENs (14). After PRRT, successful surgery was performed in nine patients and all resection specimens showed fibrosis/sclerosis or necrosis thus confirming the effects of 177Lu-octreotate on tumor tissues. As a result, the median PFS was 69 months for patients with successful surgery and 49 months for the other patients (14). On the same line, other case reports and case series confirmed a beneficial effect of neoadjuvant PRRT with 90Y-DOTATATE on tumor and/or metastases downsizing in patients with pNENs, thus leading to a successful surgical intervention (15, 17). Recently, Partelli et al. reported a series of 23 resectable or potentially resectable G1-G2 pNENs who underwent neoadjuvant PRRT (177Lu-octreotate in three and 90Y-DOTATATE in 20 patients) compared to 23 patients who underwent upfront surgical operation. PRRT did result in a reduction of primary tumor size and a reduction in the number of positive lymph nodes compared with controls. Interestingly, though both the rates of disease-specific survival and median PFS from time of diagnosis were similar between groups, in the subgroup of patients who underwent an R0 resection, a trend toward a prolonged PFS was observed in the PRRT group (18).

The administration of chemotherapy prior to surgical resection is increasingly used for patients with adenocarcinomas of the pancreas with the aim to improve surgical results. Similarly, different regimens of chemotherapies have been described as neoadjuvant treatment in pNENs, all these studies being biased by their retrospective design and by the heterogeneity of chemotherapy regimens used (**Table 1**). Dumont et al. evaluated the effect of different neoadjuvant chemotherapy regimens (i.e., 5-fluorouracil, streptozotocin, doxorubicin, cisplatin, etoposide, and oxaliplatine) on segmental portal hypertension (SHP), the feasibility of surgery and the prognostic influence of a complete surgery in 42 patients with G1/G2 locally advanced pNETs associated to SHP. A complete resection was achieved in 13 out of 28 cases underwent surgery and a not statistically significant trend towards improved 5-year survival was observed in patients with R0 resections compared to R1/R2 resections and no resection at all (20). A retrospective analysis of 59 patients with a histologic diagnosis of pancreatic neuroendocrine carcinomas (pNECs) described five patients who underwent neoadjuvant treatment with etoposide and cisplatin before surgery. Four of them had a curative resection and one patient with stage IV disease remained with residual small liver metastases (21). A further retrospective observational study analyzed the efficacy of neoadjuvant 5-fluorouracil (5-FU), doxorubicin, and streptozocin (FAS) chemotherapy in 29 patients with non-metastatic locally advanced well-differentiated pNENs. In this series, neoadjuvant FAS did not induce a clinically significant change in the size of the primary tumor in up to 90% of treated patients thus suggesting that localized disease does not benefit from this preoperative treatment in terms of tumor downstaging (22). Preoperative FAS treatment has been further evaluated in a retrospective series of 27 patients with pancreatic neuroendocrine liver metastases (NELM) who underwent liver resection. Despite being associated with higher rates of synchronous disease, lymph node metastases, and larger tumor size, patients who underwent preoperative FAS had similar overall survival OS and RFS as patients who did not. Similarly, in patients who presented with synchronous liver metastases the median OS and RFS were significantly greater among patients who received preoperative FAS. Authors concluded that preoperative FAS could be considered for patients with advanced synchronous pancreatic NELM (23).

Few data are available on the effects of association of chemotherapy and radiotherapy as neoadjuvant therapy in pNENs, all these data being mainly obtained from case reports (**Table 1**). A poorly differentiated pNEC metastatic to the breast and lung was successfully managed with neoadjuvant chemotherapy (5-FU treatment followed by carboplatin and etoposide) and radiotherapy, followed by radical surgical resection (24). Among 33 patients with pNENs undergoing surgical resection with curative intent, 16 underwent surgery alone, while 17 underwent adjuvant or neoadjuvant external beam radiotherapy in addition to surgery. Fluoropyrimidine-based chemotherapy was delivered concurrently in 14 patients receiving radiotherapy. Local control in patients receiving combined modality therapy was like those who had surgery alone. Although the Authors conclude that the role of neoadjuvant radiotherapy remains unclear, it has been hypothesized that patients who underwent radiotherapy had more aggressive or extensive disease than the surgery alone

group thus explaining the lack of any significant effect of combined neoadjuvant treatments (27).

Capecitabine combined with temozolomide (CAPTEM) has been frequently used in the treatment of pNENs. In particular, Strosberg et al. demonstrated that CAPTEM regimen was extremely effective for treatment of metastatic pNENs, resulting in an objective response rate of 70% and median PFS of 18 months (40). These data have been strengthened by a recent metanalysis confirming that capecitabine combined with temozolomide is effective for treating patients with advanced NENs, disease control rate being 72.8% (41). Neoadjuvant CAPTEM regimen with or without radiation has been successfully applied in six pNENs with borderline resectable disease. All patients had radiological evidence of tumor regression after neoadjuvant treatment (two partial responses and four stabilization) and all of them could undergo successful resection of the primary tumor with negative margins in 4/6 patients (25). The efficacy of CAPTEM regimen in the neoadjuvant setting was further confirmed by Ostwal et al. who studied 30 patients with locally advanced pNENs or pancreatic neuroendocrine hepatic metastases receiving neoadjuvant CAPTEM. Partial response was observed in 13 of them, while a stable disease was found in 16 patients thus suggesting that neoadjuvant CAPTEM might improve the radicality of the surgical procedure (29).

The association of PRRT and chemotherapy has also been used as neoadjuvant therapy in pNENs, taking advantage from the radiosensitising effects of 5-FU. In this respect, the combination of PRRT with 177Lu-octreotate and 5FU chemotherapy was found to be effective in five nonfunctioning pancreatic and one duodenum NEN with inoperable disease, resulting in radiological response in all pNENs. Only one patient underwent surgery successfully after treatment and remained 12 months postoperatively alive and free of disease (28). Finally, combined PRRT and chemotherapy sandwiching two cycles of CAPTEM between two cycles of PRRT, has been proposed in neoadjuvant setting. This regimen resulted in favorable response rates with effective control of symptoms and longer PFS and OS in NEN patients with aggressive, both FDG- and SSTR-avid, metastatic progressive disease (30).

GASTROINTESTINAL NEUROENDOCRINE NEOPLASMS

As for pNENs, neoadjuvant treatments have been proposed for other gastrointestinal NENS, data being mainly based on few case reports (**Table 1**). Neoadjuvant chemotherapy can be effective in patients with esophageal neuroendocrine carcinoma (ENEC), which are rare but aggressive neoplasms. In 2018, Yamamoto et al. reported the case of a patient with an ENEC who received neoadjuvant chemotherapy using etoposide and cisplatin. One course of chemotherapy led to tumor downstaging at endoscopy and to the absence of FDG accumulation at PET-CT examination. Seven weeks after chemotherapy, a thoracoscopic esophagectomy was performed and the histopathological examination of the resected specimen revealed no residual cancer cells, demonstrating a complete response with neoadjuvant treatment (31). Other few cases of ENECs treated with neoadjuvant chemotherapy have been reported (32–35). In three patients, cisplatin or combination chemotherapy caused an almost complete regression of the neoplasm with evidence of only microscopic foci of tumor in the resected esophageal specimen (32–34), while in other three cases treated with carboplatin/etoposide or combination chemotherapy a significant reduction in tumor burden was observed (35).

Neoadjuvant chemotherapy or chemoradiotherapy has been anecdotally reported to be effective also in rectal NENs (**Table 1**). In a study reporting on the management of patients with high grade rectum or anal canal neuroendocrine carcinomas, two cases were treated with preoperative pelvic chemoradiation. One of them received radiotherapy followed by consolidative cisplatin/5-FU, low anterior resection, and postoperative cisplatin/etoposide, while the second patient received induction oxaliplatin/irinotecan, followed by radiotherapy, trans anal excision, and additional oxaliplatin/irinotecan. Both patients had only microscopic foci of residual carcinoma at surgery (36). In another case report, a 50-year-old woman diagnosed with a liver mass and a G3 rectal NEN was treated with two cycles of neoadjuvant chemotherapy with etoposide and nedaplatin, this treatment being effective in rectal tumor but not liver metastasis shrinkage. Subsequently, the patient was switched to irinotecan plus nedaplatin, associated to octreotide LAR 30 mg/month because of neuroendocrine symptoms and MRI abdomen scan showed no significant changes in lesions size. Therefore, surgery was suggested and histopathological examination showed that the tumor downgraded from G3 to G2 thus suggesting that neoadjuvant chemotherapy may be effective in reducing primary lesion size and possibly grading, offering favourable conditions for less demolitive and more effective surgery.

The possible role of neoadjuvant PRRT and PRRT + chemotherapy combination was evaluated in small series of advanced gastrointestinal NENs (**Table 1**). Sowa-Staszczak et al. reported on neoadjuvant 90YDOTA-TATE treatment of five patients with foregut tumors, including three with pancreatic, and one with midgut NEN. According to RECIST criteria, disease stabilization was observed in four and partial responses in two patients, one with pancreatic and the second with the midgut NEN. In this latter case, tumor size decreased from 11 to 7.2 cm one month after PRRT. Five months later, a further reduction in tumor size was observed, enabling qualification for a laparotomy, which was performed 11 months after PRRT. However, only partial removal of the tumor was possible due to infiltration of the large vessels (15). In another study on 89 patients with disseminated and inoperable gastrointestinal NENs, Authors described one patient with a midgut tumor who was successfully treated with PRRT in a neoadjuvant setting, thus enabling an effective surgical intervention (37). The case of a 43-year-old man complaining of abdominal pain, vomiting, weight loss and flushes, who underwent CT examination that revealed upper and middle abdomen tumor was reported by Sowa-Staszczak et al. (38). Histopathological examination of tumor specimen obtained during exploratory laparotomy showed a well-differentiated NET according to the 2000 WHO classification. The patient received five cycles of chemotherapy (streptozocin and 5-FU) without any

response and then he underwent PRRT with 90Y-DOTA-TATE. The subsequent CT scan revealed a reduction in tumor size and the patient was therefore candidate to a second laparotomy for a partial excision of the tumor. Then he was treated with long-acting SSA and two additional courses of 90Y-DOTA-TATE that induced a further reduction of tumor size, potentially enabling a further laparotomy for curative surgery (38). Barber et al. reported their experience with PRRT as neoadjuvant treatment in five patients with NENs, one of them being diagnosed with a locoregional recurrence of a duodenal tumor. The patient was treated with one cycle of 177 Lu-DOTATATE, with a partial scintigraphy and biochemical response (28). On the other hand, Frilling et al. reported the case of a patient with a small-bowel well differentiated NEN metastasised to the root of the mesentery, who underwent four cycles of neoadjuvant PRRT with 177Lu-DOTATATE. A following 68Ga-DOTATATE PET/CT demonstrated high tracer uptake in the mesenteric and aortocaval tumor foci with significantly higher SUV than pre-treatment imaging with no change in size of either the mesenteric or the aortocaval lesions. The patient then underwent a modified liver free multivisceral transplantation (39).

Overall, the few reported experiences would suggest that neoadjuvant chemotreatment can be a successful management strategy in esophageal NEN, while too little data are available about chemo/radio-treatment of other non-pancreatic gastrointestinal NENs in a neoadjuvant setting. However, PRRT seems represent an option in selected cases in this context.

THORACIC NEUROENDOCRINE NEOPLASMS

Lung neuroendocrine neoplasms represent approximately 20–30% of all NENs. Based on clinical, histological and molecular data, lung NENs are classified in two main categories well differentiated neuroendocrine tumors (carcinoids) and poorly differentiated neuroendocrine carcinomas (NECs). Furthermore, lung carcinoids (LC) are classified in typical (low grade) and atypical carcinoids (intermediate grade) and lung NECs in large-cells and small-cells carcinomas (LCNEC and SCLC, respectively). Only low-quality evidence guides the therapeutic management of LC, and everolimus is the only approved drugs. However currently used systemic therapeutic options include somatostatin analogues, alkylating- and oxaliplatin-based chemotherapies and PRRT. Few data are available on the efficacy of neoadjuvant treatment in thoracic NENs and all these data come from small series and case reports (**Table 2**).

Srirajaskanthan el al. reported two patients with lung NENs that received a preoperative chemotherapy with 5-FU, cisplatin and streptozotocin, that induced a good response with consequently a curative resection, both patients being disease free at 36 months after surgery (42). A multicentric study by Daddi et al. reported six of 247 patients with atypical carcinoids treated with neoadjuvant chemotherapy as an initial diagnosis of SCLC was performed on fine needle aspiration biopsy. Though no data on the results of neoadjuvant treatment were clearly shown, Authors found an association between adjuvant and neoadjuvant treatments and a worse prognosis. These data do not support the efficacy of neoadjuvant therapies in terms of complete regression of the metastatic disease. However, these treatments might be effective in alleviating clinical signs and symptoms (43). The same Authors reported five patients with poorly differentiated NECs who underwent to induction therapy and surgery without disease recurrence at 5 years, but no information was available on the chemotherapy regimens used (44).

More data are available on the role of neoadjuvant therapies in LCNEC. In the multicenter retrospective study by Veronesi et al., 15% of 144 patients who underwent surgical resection for LCNEC, received neoadjuvant chemotherapy (i.e., platin/etoposide, gemcitabine, vinorelbine and taxol). In this study no association was found between neoadjuvant chemotherapy and survival except for stage I patients in whom induction or post-operative chemotherapy tended to be associated to a longer OS (OS rate at 3 years 100% vs 58%) (45). Sarkaria et al. retrospectively analyzed 100 patients with LCNEC operated at Memorial Sloan-Kettering Cancer Center. Twenty-four patients received neo-adjuvant platinum-based chemotherapy and 68% showed a partial response and 31% were characterized by a stable disease. The correlation analysis did not show any association between OS and neoadjuvant or adjuvant chemotherapy. The authors also

TABLE 2 | Neoadjuvant therapies in lung NENs.

Year	Article type	Lung NENs	Neoadjuvant treatment	Outcome	Reference
2009	case report	undefined	chemo	complete response and resection	(42)
2014	research article	atypical carcinoids	chemo	no response, worse prognosis	(43)
2004	research article	NEC	chemo	complete resection	(44)
2006	research article	LCNEC	chemo	longer OS (stage I)	(45)
2011	research article	LCNEC	chemo	longer OS (stage IB-IIA)	(46)
2010	research article	LCNEC	chemo	higher 5-year survival rate	(47)
2019	research article	LCNEC, SCLC	chemo	higher 5-year survival rate	(48)
2018	case report	LCNEC	chemo	downsizing of the tumor, complete resection	(49)
2019	case report	LCNEC	chemo	downsizing of the tumor, complete resection	(50)
2010	research article	LCNEC	chemo	worse 5-year OS	(51)
2015	research article	Thymus NENs	chemo or radio	no effects	(52)
2008	case report	Thymus NENs	chemo	downsizing of the tumor, complete resection	(53)

NEC, neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell neuroendocrine carcinoma; Chemo, chemotherapy; radio, radiotherapy; OS, overall survival.

performed a subgroup analysis in patients with completely resected advanced stage (IB–IIIA) disease and, in these patients, neoadjuvant and adjuvant chemotherapy resulted in an improved OS (2 vs 7.4 years) and 5 years OS rate (37% vs 51%) (46). Saji et al. retrospectively confirmed a positive effect of perioperative chemotherapy on survival in 45 patients with LCNEC. In this study, seven patients received a neoadjuvant chemotherapy (cisplatin and paclitaxel in four and cisplatin/topotecan in three, respectively) thus leading to a statistically significant higher 5-year survival rate (87.5% vs 58%) (47). Similar results were obtained by Ogawa et al. retrospectively evaluating a series LCNEC and SCLC who underwent complete resection. Seventy patients (31 with LCNEC and 32 with SCLC) received perioperative platinum-based chemotherapy and a significant improvement of the 5-year OS rates was observed (74.5% vs. 34.7%). Multivariate analysis revealed that perioperative chemotherapy, sublobar resection, and lymph node metastasis were independently associated with survival (48). The efficacy of perioperative chemotherapy in patients with LCNEC has been further confirmed by some case reports (49, 50). In particular, Mauclet et al. reported a case of a 41-years old women with a large LCNEC with mediastinal involvement. After an ineffective first line chemotherapy with cisplatin etoposide, patient underwent to palliative radiotherapy and second line therapy with Nivolumab that led to a downsizing of the tumor. Patient underwent surgery with the complete removal of the tumor and histology showed an absence of viable tumor cells, while necrosis and fibrosis were observed (50). Finally, the retrospective analysis of 63 patients with LCNEC showed that neoadjuvant platin-etoposide based chemotherapy was associated with a trend towards a worse 5-year OS rate despite a partial response in 12 cases was observed. Authors suggested that the negative association between neoadjuvant chemotherapy and survival could be due to the fact that only patients with stage III tumors received induction chemotherapy (51).

NENs of the thymus are very rare tumors, accounting for 0.4% of all carcinoid tumors. Based on WHO 2015, also thymic NENs are classified in two main histopathological and clinical categories: well differentiated tumors, typical and atypical carcinoids and poorly differentiated tumors, small cell and large cell carcinoma. These tumors could be associated to ectopic hormonal secretion, in particular adrenocorticotropic hormone secretion or to multiple endocrine neoplasia type 1. The prognosis of patients with thymic NENs is poor because of the high incidence of local recurrence and distant metastasis and 5-year OS vary from 30–70%. As for other lung NENs, few data are available on neoadjuvat therapy in thymic NENs (**Table 2**). Few data are available on neoadjuvant therapy in thymic NENs. Filosso et al. reported 25 patients with primary thymic NENs treated by induction therapy (19 by chemotherapy and six by radiotherapy), these treatments having no impact on survival (52). Dham et al. described a clinical case of a 40-year-old man with unresectable typical carcinoid of the thymus. Three weeks of treatment with sunitinib 50 mg per day for 4 weeks with 2 weeks off and octreotide LAR 30 mg very 4 weeks, induced a tumor shrinkage that led to curative surgery of the mediastinal mass and no evidence of disease recurrence was evident 12 months after surgery (53).

CONCLUSIONS

Data on neoadjuvant treatment of NEN patients are scanty, mainly based on inhomogeneous and often incomparable retrospective studies of limited numbers of patients and prospective studies are necessary to clarify the role of neoadjuvant therapy in this clinical setting. Available literature on pancreatic NEN patients suggests PRRT to be variably successful as a neoadjuvant approach, as well as chemotherapy to be more promising in patients with advanced synchronous pancreatic NELM, while—in the same setting—combined chemo/radio/PRRT-therapies would not be supported by sufficient evidence. Neoadjuvant PRRT, chemo or chemoradio-therapies have been anectodotally reported to be effective in non-pancreatic gastrointestinal NENs. Among thoracic NENs, neoadjuvant chemotherapy has been inconsistently reported to be beneficial in LCNC patients, also in relationship to disease stage, while little evidence would suggest neoadjuvant treatment to be negligible in thymic tumors. Therefore, it is advisable to use a neoadjuvant approach with caution, as the effects on quality of life and long-term results in terms of prolonged survival remain yet to be confirmed and the choice of this therapeutic approach should be discussed for each single patient in a multidisciplinary setting.

AUTHOR CONTRIBUTIONS

AL conceived and authored the final draft of the manuscript. FF, MR, and RM provided further content, added key references, and authored sections of the manuscript. AF and AC revised the manuscript. All authors contributed to the article and approved the submitted version.

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