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NEUROENDOCRINE TUMORS: FROM ANATOMOPATHOLOGY TO CLINICAL PRESENTATION

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Abstract

Anatomopathological classification of Neuroendocrine tumors (NETs), today, covers a pivotal role in correctly identifying the disease and establish the right diagnostic and therapeutic approach it is needed in order to manage the patient. Depending on its grading and staging, NENs can have very different prognostic perspectives. Basing on WHO 2017 classification, in this paper will be explored their main characteristics, diving into main histotypes, dividing them into functional and non-functional tumors, keeping in mind their main locations: gastroenteropancreatic tract and lungs. Their typical clinical presentation and diagnostic strategies will be explained, mainly focusing on nuclear medicine and the importance of receptor overexpression (especially represented by somatostatin receptors, or SSTRs). This is the knowledge on which is based the diagnostic and therapeutic approach with peptide radiopharmaceuticals, especially 68Ga-DOTA-peptides (today, the gold standard in well-differentiated neuroendocrine neoplasms, only with the exception of insulinoma, that shows a low density of these molecules on its cellular surface).

Keywords: *Neuroendocrine tumors, carcinoid, microcytoma, small cell lung carcinoma, pheochromocytoma, neuroblastoma, medullary thyroid carcinoma, somatostatin receptors, peptides, octreotide, OctreoScan®*

Introduction

Along last decades, the effort to precisely classify NENs (Neuroendocrine Neoplasms) was very important, especially when WHO (World Health Organization) succeeded in organize these clinical entities in an adequate classification. (1)
NENs represent a vast and heterogenous group of neoplasms, mostly originating from neural crest. This kind of tumor has very unique peculiarities, such as the possibility to arise in every location into the organism, because of the widespread diffusion among all organs of APUD system cells, or in other words the originating cells of these neoplasms. (2)
Edition by edition, the classification became more and more precise and affordable, especially for physicians, because classifying in the right way the disease means also taking the right decisions in terms of diagnostic and therapeutic approach: these neoplasms can be insidious. Usually, they are well differentiated and low-graded, meaning that their growth rate is contained and tends to be slow, but sometimes they can also show an aggressive behavior and higher grading, implying a worse prognosis. (3) The correlation between the expression of certain membrane receptors, especially somatostatin receptors (SSTRs), and prognosis (expression of diagnostic and therapeutic approaches and possibilities) has been firmly defined. (4-8) In this context, a correct, precise and clinically useful anatomopathological classification starts to acquire a far more relevant importance.
In this paper, we are going to discuss about neuroendocrine neoplasms mainly from an anatomopathological perspective and the association to their specific clinical pictures, even when intercalated into a genetic/hereditary syndrome (such as MEN-1, VHL and Mahvash disease).

NETs' ANATOMOPATHOLOGICAL CLASSIFICATION AND TERMINOLOGY

The first edition of the classification of NENs was made by WHO in 2000 and 2004, focusing the attention on the gastrointestinal tract, that is the most frequent location of NENs (such that these neoplasms are also indicated as "GEP-NENs", or "Gastroenteropancreatic Neuroendocrine Neoplasms). (9) In this edition, there was the clear distinction between:

a. Well differentiated neoplasms (NET);
b. Poorly differentiated neoplasms (NEC).

Also, the distinction between "NET" (Neuroendocrine Tumor) as a well differentiated, localized clinical entity, in contrast to "NEC" (Neuroendocrine Carcinoma) as metastatic neoplasia clinical state.

Later, in 2010 a new WHO classification was published, enlightening several fundamental aspects of NENs. This time, WHO introduced also newer and more defined *grading* and *staging* tools. (10)

2017 WHO pancreatic neuroendocrine neoplasms classification, as the definition says, is particularly referred to PanNENs (Pancreatic Neuroendocrine Neoplasms), because in clinical practice this was the main location of well differentiated G3 NENs. The need of fine recognize and define them was very important to improve clinical management of these patients, so WHO intervened.
WHO defines morphologically well differentiated NENs with G3 range proliferation index as "*NET G3*", enlightening that they are *NETs* ("Tumors" stays for "morphologically well differentiated") and *G3* underlines their pronounced proliferation activity. Now the old "gray zone" of 2010 WHO classification (one of the most important limits of 2010 WHO classification) has finally an adequate recognition from an anatomopathological point of view, even if only ascribed to PanNENs (even if it is important to clarify that also the gastrointestinal tract could be the location of this specific type of neoplasia, although they are infrequent in comparison). (11)

Another new entry was the concept of "*MiNEN*" (Mixed Neuroendocrine Non-Neuroendocrine neoplasms), a peculiar form of neoplasms hosting both neuroendocrine and non-neuroendocrine cancer cells, showing also different grading and neoplasm types, meaning that they can include NET and NEC cells simultaneously, in combination of adenomatous or squamous tumor cells. (12)

NENs OVERVIEW: FROM EPIDEMIOLOGY TO CLINICAL PRESENTATION

Neuroendocrine cells have some common characteristics with neurons and endocrine cells, *in primis* regarding specific markers, very useful from an anatomopathological point of view: chromogranin (CgA) and synaptophysin are pivotal for histopathological exam and identifying the lesion. Anatomopathologists search for these markers in order to identify the cells, so they can make certainty diagnosis via the histopathological exam.
According Waldum et al., those cells are thought to originate from neural crest, but some studies suggest a more complex situation, finding different origins for several types of NE cells, such as an endodermic origin for C cells of thyroid. So, NE cells, probably, are not exclusively from the neural crest. (13)

Neuroendocrine neoplasms (NENs) are considered rare disease, because of low number of cases per year.
Gastrointestinal tract and pancreas are the most frequent locations of NENs ("GEP-NENs" or Gastroenteropancreatic Neuroendocrine Neoplasms), immediately followed by lungs, but there are also other less frequent organs where a NEN could origin, such as thyroid, prostate and genital tract, skin, adrenal glands.
Due to their epidemiologic frequency, P-NENs will be our most talked-about type of neuroendocrine neoplasia. But first, let's do some generic observation, that will turn out to be useful from a clinical and diagnostic point of view, trying to understand them deeply. (14)

P-NENs origin from a neoplastic transformation of totipotent stem cells or differentiated mature endocrine cells within the exocrine component of the pancreas. They are not equal to each other. They can deeply differ from an anatomopathological point of view. Mainly, two pivotal characteristics are studied, especially to determine diagnosis and prognosis of patient:

1. grade of differentiation (G1-G3, defined by WHO classification, lastly updated in 2019 at the moment of the writing of this review)
2. hormone secretion (distinguishing functioning and non-functioning tumors)

Here we discuss more deeply these two aspects:

- The *grade of differentiation* can vary from primary lesion to other metastatic ones, implying significant consequences in clinical approach, because different lesions with different grading can express diverse molecular receptors, as well as a benign or malignant behavior. This can influence both diagnostic and therapeutic approach, because the lack of a certain receptor could make a radiopharmaceutical useless, due to the lack of its molecular target on the tumor cell surface, nullifying its purpose. As afterwards described, a different grading will guide the choice between a radiotracer rather than another one.

According to WHO classification, it is important distinguishing between *NET* [Neuroendocrine Tumors] for **G1-G2** neoplasms, and *NEC* [Neuroendocrine Carcinomas] for **G3** lesion.

- *Hormone secretion* allows the distinction in "functioning" (F-PNEN) and "non functioning" (NF-PNEN) neoplasms. It is typically associated to small NENs, in fact they show their presence as an endocrine clinical syndrome that alerts patients: in this way they can be diagnosed earlier than silent and slow-growing NENs, while big-sized NENs are epidemiologically far more frequent, but usually slow-growing and with an infiltrating attitude, being often lately diagnosed, when they already are in a metastatic condition. This type of neoplasia tends to debut with compression symptoms. To be precise, also NF-NENs can cause an increase of specific hormones in blood, but these situations are quite always subclinical, not enlightening any related symptom or sign.

Functioning NEN cells are able to synthetize and input hormones in the blood stream, such as insulin, gastrin, VIP, glucagon, somatostatin, ACTH, PP, serotonin (the latter mainly secreted by carcinoid NETs, causing so called "carcinoid syndrome"), bradykinin, histamine, causing clinical symptoms

and signs typically associated to an excess of their blood concentration (*i.e.* "hyper-secretive syndromes"). (15)

INSULINOMA

Speaking about NENs means debate of rare neoplasms, but among them insulinoma represents the most frequent functioning NEN (60% of cases). This neoplasia can secrete significant amount of insulin in the blood stream, causing a typical endocrine syndrome, *i.e.* hyperinsulinemic hypoglycemia: this condition displays with fasting hypoglycemia associated to neurological symptoms due to low glucose levels in blood (such as sleepiness, headache, sight troubles, confusion, behavior alterations, focal neurological symptoms, convulsions, coma), in addition to weight increase caused by the chronic excessive amount of insulin, that induces an intensified glucose uptake by insulin-depending cells (especially adipose cells). It is worth remembering that insulin causes also the cellular intake of potassium, exposing the patient to hypokalemya. This kind of clinical manifestation usually represents a typical way that brings to an early diagnosis, significantly reducing the odd of disease progression, ending with a better prognosis perspective.(16)

From a morphological point of view, insulinomas tend to be (90% of cases) a well differentiated, usually benign, well-delimited and solitary tumor, not exceeding 2 cm of diameter. When insulinomas arise in a syndromic contest, i.e. genetically determined disease as MEN-1 (Multiple Endocrine Neoplasia, also known as "*3P disease*", meaning "pancreas neuroendocrine tumors, pituitary gland adenomas, parathyroid hyperplasia"), they usually appear as multiple lesions, even if their behavior is typically benign. They can be located in any portion of pancreas, from head to tail. (17)

To diagnose an insulinoma imperatively needs a biopsy (making a histological exam possible, realizing a certainty diagnosis) and imaging. (18) In view of a surgical treatment (when feasible) or other therapeutic approaches, several techniques are employed to stage the patient: firstly, starting from the less invasive, CT or MRI are very useful techniques, that can localize 66% of lesions, with more difficult to visualize the smaller ones. (19) As a pre-surgical study, endoscopic ultrasonography (EUS) stands out in term of sensitivity (93%) and specificity (95%), but there is also the farther possibility of intraoperative ultrasonography, capable of visualize even EUS undetected lesions, since this technique would be performed without some interferences caused by the interposition of gases contained into the bowel between the organ examined and the ultrasonography probe (let's remind that air is one of the worst enemies in ultrasonography imaging, in terms of quality of returning signal, preventing the accurate visualization of retroperitoneal organs in abdominal US explorations, because of the interposition of bowel). (20-23)

From a medical nuclear imaging perspective, great progresses were made in last decades in NENs imaging techniques, including insulinomas. Nuclear medicine has experienced a number of unprecedented developments in recent years. (24-26) The modern era of radionuclide imaging and therapy is well into its seventh decade (27); nuclear medicine has experienced a great development within its practices and conventional nuclear medicine has benefited greatly from the application of hybrid imaging (28), achieving important results in many fields, such as brain (29,30), breast (31), prostate (32-35), liver (36) and many others. Regarding insulinoma, olecular biology studies have enlightened that this neoplasia, in contrast to other PNENs, does not express typical somatostatin receptors, preventing the use of SSA radiopharmaceuticals (i.e. ^{68}Ga-DOTA peptides PET/CT) in both diagnosis and therapy of these lesions. (37)

In synthesis, one of the most important discoveries in NENs was the overexpression of somatostatin receptors (SSTR), classified in five different types (SSTR-1 to SSTR-5). This characteristic allowed to develop specific radiotracers (somatostatin analogues, like octreotide, labelled to radionuclides) that could have localized neoplastic cells expressing those specific receptors, because the latter can bind the radiopharmaceutical using the analogue ligand constituting the tracer itself. The main problem with insulinomas is their low expression of SSTR-2 (usually the mainly expressed somatostatin receptor in NENs), so a tool like OctreoScan® (a scintigraphy

techniques that uses octreotide, a somatostatin analogue with high affinity for SSTR-2) is quite useless. Surprisingly, more recent techniques as ^{68}Ga-DOTA-peptide PET/CT (used nowadays as gold standard diagnostic technique in well differentiated NENs) has demonstrated to be capable of identify lesions, both benign and malignant insulinomas, in 85% of patients. Other studies concluded that, because of this limitation, ^{18}F-FDG PET/CT would have been a better choice, since this technique showed satisfying results, instead. (38-40))
More and more studies are showing how more specific tracers, based on glucagon-like peptide-1 receptor (GLP-1), provide better imaging results in benign insulinomas, since they utilize a completely different molecular system to localize neoplastic cells expressing this particular receptor, even if SSTRs are low-expressed. More recent studies reported remarkable results in the employment of even other ligands combined to SPECT/CT, such as [Lys40(Ahx-DOTA-^{111}In)NH_2] exendin-4, [Lys40(Ahx-DTPA-^{111}In)NH_2] exendin-4, or [Lys40(Ahx-HYNIC-^{99m}Tc/EDDA)NH_2]exendin-4. (41)

The therapeutic approach of choice of NENs, when feasible and like majority of neoplasms disease, is the surgical approach, where performing a radical resection of the whole lesion would mean "recovery" from a clinical perspective. Although in patient with localized lesions it is a convenient approach, if at the presentation the insulinoma has metastasized or is defined as "unresectable" lesion, medical treatments are indicated as solution. (42)

GASTRINOMA

Gastrinoma is another functioning PNEN, the second one in frequency. Most frequently, it arises in pancreas (50-60% of cases) or in duodenum (40-50%). Typically, gastrinomas are sporadic, but they can associate to MEN-1 syndrome in up to 30% of the cases.
Since it is a functioning neoplasia, it is capable of hormone secretion: pro-gastrin and gastrin are intensively produced, causing a related endocrine syndrome, called *Zollinger-Ellison syndrome*: it is characterized by an abnormal secretion of gastric HCl, due to intense stimulation of parietal gastric cells in the stomach due to tumor-related hypergastrinemia, abdominal pain and diarrhea. The increase of gastric acidity means that the chyme, when reaches duodenum, is far more corrosive and irritant than expected, so it is prone to damage duodenal mucosa, causing peptic ulcers that are also treatment-resistant because of the persistent increased acidity condition (it is necessary to interrupt gastrin stimulation to get a consistent outcome). (43)

Primary lesions are usually malignant and node involvement is present in about 60-90% at diagnosis. In addition, they usually present liver metastasis at diagnosis, but the prognosis is variable, with a 5-year survival fluctuating between 62-75%.

Trying to diagnose gastrinomas basing on biochemical tests is very difficult, since the probability of other causes hiding behind a hypergastrinemia are several, like infections, PPI (Proton Pump Inhibitors) usage, renal failure or gastric obstruction; after all, provocative tests must be performed to confirm the results.
According to this, an imaging approach is mandatory, so if a suspected neoplastic lesion arises, it is possible to perform a biopsy and obtaining a sample, making certainty diagnosis possible with a histopathological exam.
First, to stage patients, diagnostic algorithms involve CT or MRI, Somatostatin analogue Receptor Scintigraphy (SRS) and EUS (endoscopic US): the goal is to find all (if multiples) neoplastic lesions and to evaluate the general extension of the neoplasia. (44)

Concerning therapeutic approach, surgery remains the best choice possible in terms of radicality. When feasible, in dependance of patient's staging, neoplasia enucleation is the best approach nowadays, associated to a complementary lymph node resection.
If surgery is not feasible, such as advanced/metastatic disease condition or highly aggressive malignant gastrinomas, a medical approach has to be adopted: it consists of PPI (that try to control symptoms related to gastrin hypersecretion) in addition to chemotherapy and/or other systemic strategies.

VIPOMA

Rare and usually solitary (70-80%) P-NET, VIPoma tends to locate into the pancreas (in 90% of cases), especially in its body or tail, but it is necessary to consider that majority of patients show also metastatic lesions at first diagnosis.

From a clinical point of view, this functional neoplasia causes Vasoactive Intestinal Peptide (VIP) increased secretion: this condition has been defined "WDHA syndrome" (the acronym is obtained from initials of following symptom terms), characterized by watery diarrhea (often resulting in hyperchloremic metabolic acidosis, because of the loss of bases with feces), hypokalemia and achlorhydria.
VIPoma has to be differentiated from other causes of chronic diarrhea (such as infective diseases or inflammatory bowel diseases), malabsorption conditions or laxative abuse. (45)

This neoplasia is usually easy diagnosed using CT or MRI, only in difficult cases or in localizing primary tumor SRS or DOTA-peptide PET/CT are used. (46)

The therapy is focused, firstly, on symptom control, recovering both the hydroelectrolytic and acid base disorders, with intravenous potassium and fluid administration; then, a surgical resection of tumor (if feasible) is very desirable, representing the treatment of choice; otherwise, chemotherapy or other systemic approaches are required if the disease is in an advanced/metastatic condition. (47)

GLUCAGONOMA

Usually located into pancreatic tail, this rare PNET varies in size, from 2 to 25 cm in diameter.

Glucagonoma, as the name suggests, is a functioning PNET causing glucagon hypersecretion, resulting in diabetes associated to symptoms involving cutaneous, gastrointestinal and nervous systems; in addition, patients present weight loss, anemia, stomatitis, thromboembolism. To summarize the most important clinical manifestations, it is useful to remember that glucagonoma causes the so-called "4D disease", i.e. Diabetes (but not ketoacidosis), Dermatitis (necrolytic migratory erythema), Depression, Deep vein thrombosis.

CT or MRI are the most used diagnostic techniques and SRS or DOTA-peptide PET/CT are relegated to functional evaluation in glucagonomas, especially because they often present in an advanced condition, usually showing metastasis located in the liver at presentation; so the involvement of more invasive instrument to find primary lesion, such as EUS, is quite rare. (48)

The therapy is based on Somatostatin Analogues (SSA, to control the hypersecretion of glucagon), surgical debulking, nutritional restoration and prophylaxis of embolism.

NON-FUNCTIONING P-NETs

NF-NENs are epidemiologically far more frequent, usually slow-growing and with an infiltrating attitude, being often lately diagnosed, when they already are in a metastatic condition. Due to their silent development, they can grow undisturbed, in fact when an early diagnosis occurs, often they appear as incidentalomas (meaning that the neoplastic lesion appears as an occasional finding, because the clinical investigation at which the patient underwent was performed for a completely different reason). In 90% of times, they act as malignant neoplasms at presentation, often with liver metastasis at first diagnosis.
They often present like adenocarcinomas, so a differential diagnosis is imperative, also because both prognosis and chemotherapeutic response of NENs tend to be better than the latter.

This type of neoplasia tends to debut with compression symptoms, due to its mass effect, but despite of this they anyway can present a sort of hormone secretion, the main difference with F-PNETs is that they do not tend to manifest a clinically detectable endocrine syndrome: usually this condition can be evaluated from a subclinical point of view, measuring these mild hormone increased levels only at a biochemical level, without any sort of endocrine-related symptom or sign.

Biopsy of both the primary lesion and other lesions (such as liver metastasis) is pivotal in diagnostic

procedure, because is the only way to have certain details about the nature of the lesion, since there are no specific hormone markers nor symptoms. A useful diagnostic support is represented by plasmatic dosage of specific NET marker, i.e. CgA, NSE (Neuron-Specific Enolase) and pancreastatin: if these markers are detectable at first diagnosis, they can be used as a follow-up method. After all, it is good practice dosing also pancreatic hormones, especially in incidentalomas, because (as mentioned before) also NF-PNETs can secrete hormones in blood stream, even if they are only subclinically evaluable, due to the lack of clear signs and symptoms referrable to an endocrine syndrome, so it could eventually be not evident but anyway present.
Imaging has in important role in staging the patient, using CT or MRI in first place, followed by function evaluation through SRS or DOTA-peptide PET/CT, finally also EUS is useful to guide biopsy of detected lesions, at least the primary one, allowing in-depth study of the neoplasia. (49)

GENETIC SYNDROMES RELATED TO PNETs

PNETs can arise as sporadic neoplasms or as a consequence of an underlying genetic syndrome, because hereditary genetical mutation, such as ones involving menin or VHL gene, are the substratum of these specific tumors. It is interesting noticing that, because of the strict monitoring to which undergo these patients, they are diagnosed usually benign tumors, with a better prognosis being equal in PNET histological type, maybe simply due to an earlier diagnosis and so an earlier treatment, with higher probability of success.

MEN-1

MEN-1 (Multiple Endocrine Neoplasia), also known as the "3P disease" (Pancreas neuroendocrine neoplasia, Pituitary gland adenoma, Parathyroid hyperplasia, summarizing the typical triad of this syndrome) is a hereditary disease, due to an autosomal mutation involving the gene of "menin" (the encoded protein), located on chromosome 11q13. Menin has a crucial pathogenic part, because it is a tumor suppressor protein with a role in regulating chromatin, it has surely a critic function in preventing tumorigenesis. After all, despite all extensive studies, his entire function is not completely clear.
MEN-1 has no correlation between genotype and phenotype, in fact it has been observed that different members of the same family, all affected by this disease, have shown different presentation.
It has been reported that small (or micro) PNETs are always described in affected patients with endocrine cell hyperplasia and dysplasia, larger PNETs are present in only half of the patients.
Studying in depth tissue micro sections, it was observed that the lack of one normal allele results in endocrine cell hyperplasia, instead the loss of the remaining normal allele ensues in micro PNETs and endocrine cell dysplasia; it was concluded that to develop gross PNETs, it is necessary an additional genetic mutation. (50)

MEN-1 diagnosis is suspected in a patient with at least one lesion that is part of the typical triad.
Known MEN-1 affected cases put the attention on the entire family, starting investigation also about other members: in fact, in a patient with a MEN-1 case in family, the arise of a typical neoplastic lesion marks him in turn as a mutation carrier; in contrast, when a mutation is known in a patient, he is considered MEN-1 affected even if he has no typical lesions.
As mentioned before, the majority of neoplasms in MEN-1 are benign, even if patient tend to present metastasis at the presentation and frequently the tumors have a considerable diameter (2-3 cm). The most frequent types are both functional (insulinomas and gastrinomas in first place) and non functional PNETs.
Once a diagnosis (it could be genetic or clinical) is defined, it is necessary for the patient to undergo lifelong biochemical and imaging surveillance, having the goal to early identify MEN-1 lesions, including gross PNETs. The best treatment of a diagnosed benign PNET has to be carefully evaluated for the patient, especially considering cost/effectiveness balance, because any surgical intervention could cause a variable grade of disfunction of both endocrine and exocrine pancreas. (51)

VHL

VHL (i.e. Von Hippel-Lindau) syndrome is an autosomal dominant hereditary disease caused by the mutation of the tumor suppressor VHL gene, located on chromosome 3p25-26.

This syndrome is characterized by the presence of multiple lesions, mainly meaning both malignant and benign neoplasms, involving primarily the CNS (Central Nervous System, most frequently affecting spinal cord, brainstem and cerebellum) and the eye (it can host several disorders, such as macular edema, glaucoma, sight loss and, more frequently, bilateral and multiple hemangioblastomas, potentially causing also retinal detach). Also, other visceral organs tend to host several types of lesions, especially cystic lesions are very common, often arising in pancreas and in kidneys, but there were described also carcinomas of kidney, pheochromocytoma, hemangioblastoma (in CNV and in the retina), in addition to PNETs. (47)

Focusing on PNETs, they usually present as solid pancreatic lesions, but they tend to be unusual in this syndrome context, because cystic pancreatic lesions appear to be far more frequent. When in a patient affected by VHL syndrome a PNET is diagnosed, usually it is:

- *Small*: gross PNETs arise in less than 20% of this kind of patients and they are typically non-functional;
- *Functioning*: even if they are not typically associated to hormone hypersecretion related syndrome like functional PNETs of non-VHL syndrome patients, sometimes these tumors can input into the blood stream some molecules anyway, such as hormones themselves and neuroendocrine markers. It is also possible, in rare cases, that an actual endocrine syndrome due to hormone hypersecretion could be described;
- *Benign*: in VHL syndrome, low growth rate and metastasis in less than 20% of cases are two pivotal factors that make the prognosis of these PNETs far better than patients with sporadic PNETs, especially because the latter are mainly non-functional PNETs, so they are silent from a clinical perspective till they have spread to the organism, resulting in metastasis that significantly worsen the future evolution of the disease. Another important factor that impacts prognosis is the stricter abdominal monitoring which these patients undergo, because of their known clinical condition, in contrast to patients with sporadic PNETs.

The surgical therapy is the best possible choice, guaranteeing a radical approach: it is necessary because of the high potential of malignant transformation of PNETs (even if they present as benign, they could always evolve in grading), so a pancreatic resection is performed; depending of the dimensions of the lesions, it can be chosen a more aggressive approach (total pancreas resection, if the lesions are >1 cm) or a more conservative one (partial pancreatic resection).

MAHVASH DISEASE

Mahvash disease a recently described (in 2008) autosomal recessive genetic syndrome, presenting a homozygous inactivating mutation hitting the glucagon receptor gene (GCGR).

The characteristics of this syndrome are:

- pancreatic neuroendocrine tumors (most frequently micro PNETs);
- very high glucagonemia;
- α pancreatic cells hyperplasia.

From a biochemical point of view, there is an increased plasmatic glucagon level in patient's blood, even if there are no clinical findings associated to a glucagonoma syndrome. It is interesting making a parallelism with the latter: in fact, while in typical glucagonoma-related clinical condition there is a primarily hyperproduction of glucagon made by the functioning tumor itself and this hormone interacts with normally expressed and working glucagon receptors, in Mahvash syndrome glucagon cannot properly interact with its receptor, because of a lower affinity of the receptor itself to its ligand and also a reduced production of cAMP (cyclic Adenosine MonoPhosphate), so there is a subsequent lower intracellular calcium release, compromising the intracellular molecular pathway of biosignaling. (47)

PNETs represent the main menace in this clinical condition, so after diagnosing the disease the patient undergoes to a long-term monitoring using diagnostic imaging, because new neuroendocrine neoplasms can arise even after previous surgical treatments aimed to perform resection on them.

OTHER NENs

Even if gastroenteropancreatic tract represent the most frequent localization of NETs, there are also other important histotypes in this tumor category: lung NETs surely represent the other most important localization, while other NETs are typical of the infant, such as neuroblastoma. Following, we are discussing more in-depth the aspects, both from an anatomopathological and clinical perspective, of these other tumor types.

LUNG NETs

Lung is the second most frequently localization of neoplasms among NETs themselves, representing about 30% of NET patients.
From an anatomopathological view, they are classified in benign and malignant variants. (52)

Benign phenotypes: they are called "**bronchial carcinoids**", originating from cells dispersed into the bronchial parietal structure. They are distinguished in typical (abbreviated in TC) and atypical carcinoids (AC). Bronchial carcinoids represent 1-5% of pulmonary neoplasms (20-40% of patients that are non-smokers). Clinically, signs and symptoms are related to intraluminal development and vasoactive amines production. Typical consequences of the first event are persistent cough, hemoptysis, bronchiectasis, emphysema, atelectasis, secondary infections due to luminal obstruction (facilitating germs proliferation, since there is no drainage of luminal secretions); classical carcinoid syndrome, instead, is characteristic of hormone secretion, causing intermittent diarrhea, face flushing and cyanosis. These types of neoplasms can be treated with surgery, achieving great results in terms of prognosis, especially because they rarely metastasize to other locations and, usually, they are non-functional tumors: typical carcinoids provide a 5-years and 10-years survival rate of 87%, while atypical carcinoids line up at, respectively, 56% and 35%.

Malignant phenotypes: these ones are divided in two main entities. The first one is **LCNEC** (Large Cell NeuroEndocrine Cancer), a subtype of the larger group of NSCLCs (Non-Small Cell Lung Cancer); secondarily, there is the lung **microcitoma** (also known as SCLC, i.e. Small Cell Lung Cancer). Given the higher incidence of the latter, it is necessary to spend a few more words about. Microcytoma is also known for its aggressivity, capable of silent and rapid spread, easily giving metastases (frequently micro-metastases, especially in the brain) that have a huge impact on the prognosis. If not treated, this type of neoplasia allows only few months of survival expectation.
From a clinical perspective, it arises with typical signs and symptoms related to pulmonary tumors: cough, weight loss, thoracic pain, dyspnea. In addition, especially given its neuroendocrine nature, it is frequently associated to paraneoplastic syndromes: it is about the tumor's capability of secreting hormones or hormone-like peptides, such as serotonin and bradykinin (these two hormones characterize the typical carcinoid syndrome), ADH (causing hyponatremia in the context of a syndrome of inappropriate secretion of ADH or SIADH), ACTH (determining Cushing syndrome), parathormone (implying hypercalcemia), calcitonin (causing the opposite condition in respect of the previous case, i.e. hypocalcemia), gonadotropins.
Furthermore, microcitoma has a very particular treatment response: when diagnosed, it is treated as a systemic disease *a priori*, that means it is always considered as a condition associated to distant metastases, implying the necessity of a chemotherapeutic approach (unique exception: the state of T1,2-N0,1-M0, where the current indications are represented by the combination of surgery and chemotherapy, in association with prophylactic cranial radiotherapy in case of signs of disease remission); successively to an eventual initial response, the tumor seams to involve, then it arises again in a chemotherapeutic-resistant form, making the previous oncologic treatment useless and needing for other chemotherapeutic strategies (beyond the first-line treatment). Overall, the prognosis of SCLC is very unsatisfactory, with a 5-

years survival rate of 31% in localized disease and less than 2% in metastatic conditions. (53)

PHEOCHROMOCYTOMA / PARAGANGLIOMA

Pheochromocytoma is a rare neoplasia of adrenal medulla, originating from chromaffin cells, that are able to produce catecholamines (noradrenaline and adrenaline), making this organ the major source of these hormones in the entire body. Pheochromocytomas can be divided into intra-adrenal and extra-adrenal, depending on their primary location. When chromaffin cells undergo to a neoplastic transformation and tumor arises, they maintain the capability of catecholamines secretion (important note: extra-adrenal tumors are able to secrete only noradrenaline, while intra-adrenal ones can input into blood stream both adrenaline and noradrenaline), causing a typical endocrine syndrome dominated by arterial hypertension, characteristic of this tumor's clinical presentation.
There is also another type of cells, really similar to chromaffin cells, that belong to the so-called paragangliar system: the latter is constituted by cellular groups and nodules strictly connected to sympathetic and parasympathetic paraganglia, including the adrenal medulla itself. These structures can also host neuroendocrine tumors, named **paragangliomas**, that can be located from the base of the skull to urinary bladder, presenting a functional or non-functional behavior (depending on their hormone secretion activity, obviously consisting in catecholamines). The prototype of (parasympathetic) paraganglioma is the tumor of carotid glomus, but it can also arise in thoracic, abdominal and pelvic (sympathetic) paraganglia. (54)

Pheochromocytoma has a prevalence of about 0.5% in hypertensive patients and typical symptoms, reaching about 4-6.5% of adrenal incidentalomas (i.e. incidental lesions found in the adrenal medulla during an imaging investigation performed for a completely different reason).
Usually, these tumors are able to secrete catecholamines and are diagnosed as lesion with a diameter larger than 2 cm, occupying only one of the two adrenal glands. On the other hand, 10-20% of cases are, malignant, multiple or located outside the adrenal medulla.

There are also some hereditary syndromes involving pheochromocytoma, such as MEN-2A (Multiple Endocrine Neoplasia type 2A or Sipple syndrome, with the association of medullary carcinoma of thyroid, pheochromocytoma and parathyroid hyperplasia), MEN-2B (also known as MEN 3, characterized by medullary carcinoma of thyroid, pheochromocytoma, mucosal ganglioneuromas and marfanoid habitus), VHL syndrome and neurofibromatosis type 1 (von Recklinghausen syndrome).

Focusing on the clinical presentation, pheochromocytomas and functional paragangliomas cause an endocrine syndrome related to excessive plasmatic levels of catecholamines, while non-functional paragangliomas tend to stay clinically silent for a longer period of time.
A catecholamine-secreting tumor is suspectable when the patient presents arterial hypertension (the most frequent sign), hyperidrosis, increased general metabolic activity, hyperglycemia, headache. Crises are typical of this condition, arising as sudden attacks of headache, sweating and palpitations, in addition to paroxysmal arterial hypertension: these phenomena are usually due to trigger factors, like the compression of the tumor, physical activity, psychological stress *et similia*.
Arterial hypertension (or HBP, i.e. High Blood Pressure) tends to be nearly always treatment-resistant and with severe blood pressure values. Complications HBP-related can occur, such as cerebral hemorrhage, ventricular hypertrophy or hypertensive retinopathy; in addition, other condition can arise, as cardiac arrhythmias, cardiac angina or infarction (due to increased oxygen consumption caused by the increased contractile activity of the heart), stypsis, dyspnea, hyperthermia, paresthesia, mydriasis. (55)

From a diagnostic point of view, the first step is represented by some biochemical measurements, evaluating catecholamines and their metabolites (metanephrines) concentration: as a side note, free plasmatic metanephrines levels have shown a better sensibility than catecholamines and vanillylmandelic acid.

Other pharmacological exams usually performed are the suppression test with clonidine (that is able to decrease catecholamines' level in essential arterial hypertension, while it has no effect in hyper-catecholaminemia associated to pheochromocytoma) and a provocation test with glucagon, the most dangerous one.
Levels of chromogranin A (CgA) are also elevated in about 80% of pheochromocytomas, because of their neuroendocrine nature; other markers are also evaluable, such as synaptophysin and NSE (Neuron Specific Enolase).
Imaging in this pheochromocytoma is absolutely needed: CT and MRI represent first level techniques, showing a great sensibility of 100%, but it is important to note that CT decreases its accuracy in patients with MEN-2A/2B, recurrent, metastatic or extra-adrenal tumors; additionally, specificity of CT and MRI is not as good, presenting an approximate value of 70%.
Nuclear imaging is pivotal in depicting lesions (useful in pheochromocytomas and paragangliomas), both intra- and extra-adrenal ones, mainly using ^{131}I and ^{123}I-MIBG in combination with scintigraphy or SPECT (^{123}I-MIBG SPECT should be the procedure of choice, thanks to its higher sensibility). Other radiopharmaceuticals are studied in combination with PET, with the purpose of further improving the results: radiotracers like ^{11}C-HED, ^{18}F-DOPA and ^{18}F-FDA are providing better and better outcomes, according to studies, especially when associated to hybrid machineries (i.e. PET/CT). (56)

NEUROBLASTOMA

Neuroblastoma belongs to a specific neoplasms' category, called "neuroblastic tumors", originating from primordial cells coming from neural crest and localized into adrenal medulla (40% of cases) or in sympathetic ganglia, in other words it is a neoplastic transformation of sympathetic nervous cells precursors, not sparing the brain itself.
Among extracranial neoplasms, it is the most frequent malignant solid tumor in childhood, with 18 months as mean age which the tumor is diagnosed at; additionally, 40% of these cases are identified in children. In the majority of patients, neuroblastoma is a sporadic neoplasm, but in1-2% of them it is part of a hereditary condition (ALK gene mutation represents the most important predisposing factor) involving both adrenal glands or arising in multiple locations in the context of sympathetic nervous system.

From a clinical perspective, the presentation deeply varies depending on the age. In patients with less than 2 years, the tumor appears as a huge abdominal mass in association to fever and weight loss (so, it goes in differential diagnosis with Wilms' tumor). In older children the neoplasm can also stay unnoticed, reaching medical attention only when other findings arise, such as bone pain, respiratory and/or gastrointestinal symptoms. The tumor tends has a strong attitude to metastasize through blood and lymph, in particular to bones (both cortical bone and bone marrow), liver, lymph nodes and lungs.
The patient can show proptosis and periorbital ecchymosis (due to the frequent involvement of periorbital region), or bladder and intestinal disfunction caused by the involvement of nerve bundles (usually due to the presence of a paraspinal neuroblastoma). (57)
Another peculiar characteristic of neuroblastomas is the production of catecholamines, responsible of an endocrine syndrome frequently associated to arterial hypertension, but it is far milder in terms of seriousness and frequency in comparison with pheochromocytoma. Catecholamines are a pivotal diagnostic instrument: the measurement of plasmatic catecholamines and their metabolites concentrations (vanillylmandelic acid and homovanillic acid) represent an important confirming test of the supposed diagnosis. The use of radiolabeled MIBG is also an established procedure in diagnostic path, characterized by an accuracy of 90% in depicting primary, residual and metastatic lesions; today, MIBG imaging is the technique of reference in neuroblastoma, utilized in disease staging and restaging, search of postsurgical residual tumors, treatment response monitoring and even early diagnosis in recurrent neoplasms during follow-up. Finally, MIBG diagnostics also preludes to ^{131}I-MIBG (i.e. radiometabolic therapy). (58)

Prognosis is poor (40% of survival rate 5 years after diagnosis), despite therapeutic improvements.

Although, the younger the patient, the better tends to be the prognosis: older patients have a poorer prognosis in comparison to children affected by a neuroblastoma of same seriousness.

MEDULLARY THYROID CARCINOMA

Thyroid is an endocrine gland characterized by a follicular histologic structure, mainly constituted by colloid surrounded by follicular (thyrocytes, producing thyroid hormones T_3 and T_4) and parafollicular cells (or C cells, secreting calcitonin), which derive from neural crest. The latter are the cells which MTC (Medullary Thyroid Carcinoma) originates from, meaning that this tumor is able to secrete calcitonin, CEA (CarcinoEmbryonic Antigen), VIP (VasoIntestinal Peptide) and SS (SomatoStatin).
This neoplasm represents 3-12% of oncologic diseases in thyroid. MTC can be a sporadic (75-80%) or hereditary tumor (20-25%), often presenting an aggressive and invasive behavior, frequently associated with metastases.
MTC is sporadic in 70% of cases, whereas remnant patients can have or not a familiar history of MEN association: in the first case it is about MEN 2A or 2B, but there is also a clinical entity called FMTC (i.e. Familiar Medullary Thyroid Carcinoma) which is not associated to a multiple neuroendocrine neoplasia.
RET oncogene mutation covers a key role in tumorigenesis, both the sporadic and the familiar ones. MEN 2A/2B patients that show a MTC tend to be younger, while sporadic and familiar MTCs are usually older (40-60 years old). (58)

The clinical picture of a sporadic MTC is different from a familiar MTC or a MEN-associated one.
Sporadic MTCs present a cervical mass sometimes associated to dysphonia or dysphagia, due to the invasive behavior of the neoplasms. The first symptom is related to the recurrent nerve's infiltration, also known as inferior laryngeal nerve (a branch of vagus nerve), that provides motor innervation to the entire laryngeal intrinsic musculature, except for cricothyroid muscle; in this way the phonation process is damaged, because of vocal chords' paralysis: depending on the infiltration of one or both nerves, the paralysis itself can be partial (involving only one nerve and one chord) or complete (when the tumor infiltrates both nerves and vocal chords). Dysphagia, instead, is related to the infiltration of the esophagus. Sometimes, the first clinical manifestation of the neoplasm is a paraneoplastic syndrome, associated to the secretion of VIP- or ACTH-like peptides (potentially causing diarrhea or Cushing's syndrome, respectively). Regarding hypocalcemia, it is not a relevant sign even if calcitonin levels are significantly increased because of its hypersecretion (related to the tumor itself). (59)
In patient with a FMTC, symptoms and signs are usually related to the thyroid itself (localized symptoms) and they can display other endocrine tumors.
Regarding patients affected by MENs, there is the frequent association of multiple endocrine tumors in addition to MTC; in MEN 2B, medullary thyroid carcinoma is the most aggressive and invasive in comparison of other ones arising in other clinical contexts (such as MEN 2A, FMTC or sporadic MTC). Patients with mutated RET oncogene are suggested to undergo to a prophylactic thyroidectomy, in which context are usually found micro-MTC (<1 cm) or simple parafollicular cells hyperplasia, even if their clinical picture is absolutely asymptomatic: the reason of this strategy lies in the certainty of future development of MTC. (60)

Plasmatic calcitonin levels represent a pivotal instrument of diagnosis in MTC (in addition, it is implied also in follow-up of the patient, in this way every abnormal increase of this hormone represents an alert for the physician). Another important clinical and prognostic marker is CEA, especially from a surgical point of view, because it indicates the tumor mass entity, otherwise it is quite useful in diagnostics of tumors non-secreting calcitonin.
Nuclear medicine represents a fundamental instrument in following-up the patient after the surgical intervention, while in preoperative phase it is not much useful.
In this context, MIBG scintigraphy is a useful procedure able to depict adrenal medulla hyperplasia or eventual pheochromocytomas, in MEN-2 patients (in addition to a MTC), in this way ^{131}I-MIBG radiometabolic therapy can be considered.
To define a patient surgically cured, it is needed the evidence of normal calcitonin and CEA levels. (61)

OVERVIEW OF NUCLEAR MEDICINE APPLIED TO NENs: FROM CURRENT TO FUTURE DIAGNOSTIC TECHNIQUES AND THERAPEUTIC APPROACH

NENs have represented and represent still today a diagnostic and therapeutic challenge. Intensive and numerous efforts have been done in order to significantly improve the quality of detection and clinical management of these neoplasms.

Today, the combination of a combined involvement of both traditional radiology (i.e. CT and MRI) and nuclear imaging (SRS, SPECT and PET) provides great outcomes in comparison to the past, especially because the evolution of techniques culminates, nowadays, in hybrid procedures, such as PET/CT, PET/MRI and SPECT/CT.

The idea on which nuclear medicine and, in particular, hybrid techniques base on is simple and effective at the same time: combining data coming from these machineries, it is possible to extrapolate a huge amount of information, from morphostructural to functional/biological ones. The key role, in this case, is covered by a type of drugs very familiar to nuclear physicians: radiopharmaceuticals.

In this field, great progresses have been made: it all started with octreotide (the forefather of somatostatin analogues, or SSAs) labelled to ^{111}In through a bifunctional chelator (DTPA), realizing ^{111}In-DTPA-octreotide (also known as ^{111}In-penteteotride) or OctreoScan®, of which functional mechanism was totally based on the capability of binding SSTRs (somatostatin receptors). (62)

These receptors are overexpressed on the neoplastic cells' surface, representing an ideal target of radiopharmaceuticals: it is about a membrane receptor (more precisely, they are GPCRs, or G-Protein Related Receptors) that can bind somatostatin (or an analogues molecule, like somatostatin analogues [SSAs] or somatostatin antagonists [SS-ANTs]) to form a ligand-receptor complex and then it is internalized into the cell via an endocytosis process. The interaction between the drug and the receptor is the pivotal phenomenon that allows not only the localization of the neoplastic cells (due to the physical connection established by the ligand-receptor complex, allowing the anchorage of radiopharmaceutical, so it can emit radiations from a stable and precise point in the organism), but also the phenomenon of internalization, better explained later, when we will briefly talk about PRRT.

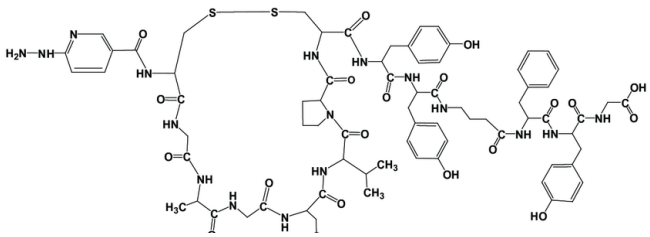
SSTRs have been deeply studied, allowing researchers to cloning 5 different subtypes (named SSTR-1 to SSTR-5) and they show some biological differences, especially in terms of internalization of the ligand-receptor complex; on the other hand, not all SSAs or SS-ANTs have the same affinity for each subtype, setting the complexity level of their approach even higher. The role of SSTRs became more and more determining in the efficacy and accuracy of imaging techniques. (623

Since the synthesis of octreotide, several progresses occurred, conducting us to ^{68}Ga-DOTA-peptides PET/CT, considered today the gold standard in well-differentiated NENs' diagnostics. Nowadays, DOTA-peptides can easily be defined the most used somatostatin analogue type in NENs' nuclear medicine investigations. Many studies have been led to enlighten the strengths and the drawbacks of these ligands, allowing to delineate a precise pharmacodynamic and pharmacokinetic profile of each one of them. (64,65)

Basing on the same biological principles of nuclear imaging, radiopharmaceuticals are today employed also in the therapeutic approach of neuroendocrine tumors: PRRT (Peptide Receptor Radionuclide Therapy) is a therapeutic strategy that aims to bring radionuclides into a neoplastic cell, promoting its cytoplasmatic accumulation, allowing the collection of certain amounts of α or β^- radiations that are able to kill the cell from inside, deeply and irreversibly damaging DNA and other vital structures, inducing its death. (66) In this case the conception of "radiopharmaceutical internalization" is pivotal; regarding this topic, it is important to stress the conception that not all receptors perform equally in terms of internalization and the same happens for SSAs, therefore studies are today focused on comparisons between different radiopharmaceuticals and their receptor interactions. (67) It is important to notice that preliminary nuclear diagnostics using somatostatin-based radiopharmaceuticals is absolutely mandatory before starting PRRT, because the functional

imaging allows the demonstration of SSTRs' expression on neoplastic cells. (68)

As in every medical and, generally speaking, scientific branch, progress is incessant, so it is in the research of further improvements in NENs' imaging. One of the new radiotracers that are gaining more and more interest, there is ^{99m}Tc-EDDA/HYNIC-TOC SPECT/CT (also known as ^{99m}Tc-Tektrotyd ®). (52)



HYNIC-peptide chemical structure

Firstly, ^{99m}Tc is the most utilized radionuclide in oncologic nuclear imaging, in general. The reason lies in its cost-effective production (as it is a generator's product) and its physical characteristic (a half-life of 6 hours while it is able to emit γ-rays, the radiation that is detected by an appointed machinery, i.e. gamma camera), in addition to its ease of labelling to other molecules using a bifunctional chelator, such as HYNIC (6-hydrazinonicotinamide).
In synthesis, a radiopharmaceutical, as previously described, is constituted by a radionuclide (^{99m}Tc), a bifunctional chelator (HYNIC) and a binding molecule (TOC, a somatostatin agonist derived from octreotide, which has the highest affinity to SSTR-5 among all of them) that has the ability to interact with a given receptor (in this case somatostatin receptors, precisely). To improve compound's stability, a co-ligand was added: firstly, it was tricine [or N-(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine], but results were unsatisfactory, so, later, it was substituted by EDDA (Ethylenediamine-N,N'-Diacetic Acid), providing a more stable radiotracer and with less isomers.
Gaining more and more consensus, ^{99m}Tc-EDDA/HYNIC-TOC rapidly became the most used radiopharmaceutical in SPECT/CT applied to NETs, at least in Europe, providing far better results in diagnostics than ^{111}In-DTPA-octreotide (OctreoScan®) in this field. (69)

The path of progress is never ending and further new radiopharmaceuticals and techniques are in development, with the main purpose of improving accuracy and sensitivity, otherwise to make PRRT even more efficient, because only in this way it will be possible to drastically change NET's clinical progress, allowing a better perspective for the patient, even in an advanced condition of disease. (70)

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