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**Molecular factors, diagnosis and management of gastrointestinal tract neuroendocrine tumors: An update**

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**Abstract**

The prevalence of gastrointestinal neuroendocrine tumors (GI-NETs) is increasing, and despite recent advances in their therapy, it remains inadequate in patients with advanced well-differentiated neuroendocrine tumors. These tumors present many challenges concerning the molecular basis and genomic profile, pathophysiology, clinicopathological features, histopathologic classification, diagnosis and treatment. There has been an ongoing debate on diagnostic criteria and clinical behavior, and various changes have been made over the last few years. <sup>6</sup>Neuroendocrine carcinoma of the gastrointestinal system (GI-NEC) is a rare but highly malignant neoplasm that is genetically distinct from gastrointestinal system NETs (GI-NETs). The diagnosis and management have changed over the past decade. Emerging novel biomarkers and metabolic players in cancer cells are useful and promising new diagnostic tools. Progress in PET-CT and scintigraphy with new radioactive agents (<sup>64</sup>Cu-DOTATATE or <sup>68</sup>Ga-DOTATATE) replacing enough octreoscan, has improved further the current diagnostic imaging. Promising results provide targeted therapies with biological agents, new drugs, chemotherapy and immunotherapy. However, the role of surgery is important, since it is the cornerstone of management. Simultaneous resection of small bowel NETs with synchronous liver metastases is a surgical challenge. Endoscopy offers novel options not only for diagnosis but also for interventional management. The

therapeutic option should be individualized based on current multidisciplinary information.

## **INTRODUCTION**

Gastrointestinal NETs are a genetically heterogeneous part of neoplasms originating from neuroendocrine cells distributed along the GI tract and exhibit different biological behaviors depending on location <sup>[1,2]</sup>. They are usually well-differentiated slow-growing lesions and may be functional or nonfunctional without hormone secretion and symptoms <sup>[3]</sup>. The vast majority of them are sporadic. However, at a rate less than 5%, they are found in genetic syndromes, mainly MEN 1 (multiple endocrine neoplasia), in the form of pancreatic islet cell neoplasms (gastrinoma, insulinoma, glucagonoma, in order) which carry a high rate of malignancy <sup>[4]</sup>.

There is a risk for the coexistence of GI-NETs with adenocarcinoma; also, it has been estimated that in patients with lung NETs, there was an increased risk for second primary malignancy compared to the general population <sup>[5]</sup>.

The neuroendocrine cells in general are located throughout the body, i.e., central nervous system, endocrine glands, pancreas, lungs, and gastrointestinal tract and secrete different hormones. These cells have a common embryological origin from the neural crest as well as common physiochemical properties such as amine precursor uptake and decarboxylation (APUD cells) producing amines. The amine precursors include 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine (DOPA). The neoplasms of the neuroendocrine cells or APUD cells were previously called APUDomas, but the term NETs now prevails <sup>[6]</sup>. They were divided into two categories, carcinoids in two-thirds and noncarcinoids in one-third, mainly in the pancreas, named PET (pancreatic endocrine tumors) of islet cell neoplasia. However, the traditional term carcinoids has been replaced widely by the recent term NETs <sup>[4]</sup>.

NETs were derived from the foregut (lungs-bronchi, thymus gland, esophagus, pancreas, stomach and duodenum), midgut (small intestine, vermiform appendix and right colon) and hindgut (distal third of transverse colon, descending colon, sigmoid

colon and rectum). The small intestine is the most frequently observed location, usually presenting in an advanced stage [4,7].

Their increasing incidence exhibits a fivefold increase within 30 years in the USA [3]; likewise, an increase has been reported in Japan. [8] The increased incidence may be partially attributed to a better understanding and recognition of the disease and to an improvement in diagnostic modalities [8]. This increase is in parallel with that of metabolic syndrome. It is not yet known whether the latter is involved in the etiology of GI-NETs or influences their consequences [1].

Mixed neuroendocrine neoplasms (MiNEN), neuroendocrine and nonneuroendocrine, are very rare, but with increasing incidence, heterogeneous lesions found in the gastrointestinal (GI) tract, which were previously known as mixed adenoneuroendocrine carcinomas, present a diagnostic and therapeutic challenge. Their histopathologic differentiation is mainly poor and less commonly well, which determines their biological behavior [9-12]. NEC (neuroendocrine carcinoma) with poor differentiation represents a more aggressive sub-group located mainly (over one-third) in the GI tract that requires careful assessment for surgical therapeutic excision [13].

The latest 2019 World Health Organization classification for gastroenteropancreatic neoplasms clarifies the issue histopathologically, taking into account the differentiation, mitotic rate and KI-67 proliferation index [14].

The diagnosis and management have changed over the past decade.

Novel diagnostic tests have contributed to the already promising advances [15].

Positron emission tomography-computerized tomography (PET-CT) combining functional metabolic and anatomical imaging as well as octreotide scintigraphy (Octreoscan), which is also called somatostatin receptor (SSTR) scintigraphy [16-18], are the preferred imaging diagnostic tools, although no modality alone offers the best results [19]. FDG-PET (fluorine-18-labeled deoxyglucose), gallium PET/CT, <sup>18</sup>F-FDOPA PET/CT (<sup>18</sup>F-fluorodihydroxyphenylalanine) and SPECT (single photon emission computerized tomography) scans are several new imaging tools. <sup>18</sup>F-FDOPA PET/CT is the most accurate novel tool for detecting metastasis from small bowel NETs [20].

Surgery is the cornerstone of management followed by interventional endoscopy, but new drugs, chemotherapy, immunotherapy and novel targeted therapy by biological agents provide promising results [21].

GI and pancreatic NETs are quite different in molecular pathology and treatment requiring distinct approach. In this extended narrative review focusing on GI NETs, we evaluate and summarize the modern data on pathogenesis and provide information on available treatments. Current information on contemporary aspects in genomic mutations, diagnosis, classification and therapeutic options will allow gastroenterologists to update their knowledge about gastrointestinal NETs.

### **Molecular factors – Genomic profile**

There is a rather familial predisposition for NET and second malignant development. Pathogenetic variants in the genes *ATM*, *RAD51C*, *MUTYH* and *BLM* have been found in a proportion of 9%-11% of NETs in the small intestine. The variants in these genes are associated with inherited susceptibility for malignancies. However, it is not yet known whether there is an association with NET development [22].

The cytokine *IL-17* (interleukin 17) gene that is expressed by the tumor only and the *TNFA* (tumor necrosis factor alpha) gene that is expressed by both the tumor and stroma promote the *pSTAT3* (phosphorylated signal transducer and activator of transcription 3) gene. A possible therapeutic option may be the targeting of these pathways [23].

Small intestine NET is the most common malignant neoplasia of the small bowel. Primary multifocal development arises from clonally independent cells [24].

A familial existence of small intestinal NETs has been reported. In a family with 16 affected individuals, a heterozygous deletion at 7q31.2 (cystic fibrosis locus) was found.

The deletion removed the TAP (topologically associating domain) border between *CFTR* (CF transmembrane conductance regulator) by inactivation and *WNT2* (Wnt family members 2) by activation. The predisposing genes were *IPMK* and *MUTYH*. This deletion is an incriminating event for small intestine NET and both small and large bowel adenocarcinoma [25].

Cancer cells develop mechanisms that increase nutrient uptake, since they require a vast amount of nutrients for their survival. Metabolic players could have a potential role in NETs. Specific mechanisms increase nutrient uptake of metabolic players *LAT-1* (amino acid transporter 1) and *GLUT-1* (glucose transporter 1) and perhaps may promote the proliferation and metastatic capacity of NETs. Immunohistochemical *GLUT-1* expression is elevated in GI NETs and is higher in grade 2 tumors than in grade 1 tumors. Additionally, there was a correlation between the *Ki-67 proliferation index* and *GLUT-1* expression. *LAT-1* expression is associated with increased GI NET malignancy and aggressiveness [26].

The correct identification of cell origin and an understanding of the mechanisms of tumorigenesis could open new horizons in prophylaxis and treatment. Currently, cancers are considered to originate from stem cells. Each cell division carries a slight risk of mutation; thus, any stimulation of proliferation has an increased risk of mutation. Some hormones (gastrin, estrogen, testosterone) and growth factors play a pivotal role in tumorigenesis by acting as signaling molecules. For example, increased gastrin levels may promote the growth of enterochromaffin-like (ECL) cells and induce a sequence of hyperplasia, dysplasia, type 4 gastric NETs, and possibly NECs or diffuse adenocarcinomas [27].

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Gene expression profiling can be used to classify small intestine NET subtypes and accurately predict metastasis. This might lead to individualized treatment.

Several transcribed genes are implicated in tumor development, metastasis and hormone secretion and can be useful in defining primary small intestine NETs and predicting metastasis occurrence. These genes include *MTA 1* (metastasis associated protein), *Ki-67 proliferation index*, *NAP1L1* (nucleosome assembly protein 1- like), *MAGE-D2* (melanoma antigen family D2), *FZD7* (frizzled homolog 7), *CgA* (chromogranin A), *survivin*, *NRP2* (neuropilin 2) and *Kiss 1* (Kiss 1 metastasis suppressor) [28].



Important genetic factors, such as the *RET* proto-oncogene and *tumor suppressor menin* and growth factors (VEGF, TGF- $\beta$ , IGF-1 and PDGF), are associated with cell differentiation regulation, tumor growth and hormone production [29].

GI-NECs are genetically different from GI-NETs. Alterations in the genes *TP53* and *RB1* are common in GI-NECs or in genes *CCNE1* (Cyclin E1) and *MYC* (MYC Proto-Oncogene, BHLH Transcription Factor). Alterations in the Notch gene family are characteristic of nonpancreatic NECs. The transcription factors, mainly the *SOX2* gene, are overexpressed in most GI-NECs [30].

*TP53*, *RB1*, <sup>1</sup> *KRAS* mutations have been detected in gastric and colorectal NECs, <sup>5</sup> although *BRAF* mutations have only been reported in colorectal NECs [31]. *BRAF* oncogene is located on chromosome 7q. *KRAS* or *BRAF* pathway-related signal transduction is very important in carcinogenesis. Activation of these oncogenes contributes to carcinogenesis. The most common mutation involves the *KRAS* oncogene on chromosome 12p, which exists in an inactive form. It activates the p21 protein, which causes cell transformation, proliferation, and invasion. Inactivation of tumor suppressor genes, such as the *TP53* (*tumor protein*) gene that promotes apoptosis (programmed cell death) and prevents cancer, leads to uncontrolled cell growth, proliferation and carcinogenesis. Additionally, the inactivation of maintenance genes, which regulate DNA damage repair, predisposes patients to cancer. The Notch signaling pathway maintains the required physiological balance among cell proliferation, differentiation and apoptosis. The mammalian target of rapamycin (mTOR) pathway is another cellular signaling pathway that is essential for physiological functions, such as cell growth and proliferation, but its hyperactivation may lead to carcinogenesis. Therefore, it is a target of novel therapeutic biological agents. [4,31-33].

Loss in chromosomes was found in midgut carcinoids, such as in *18q22-qter* mainly and *11q-q23* in both primary tumors and metastases but also in *16q21-qter* only in metastases [32]. In ileac carcinoids, the loss of chromosome *18q* was reported, but gain of chromosomes *4q*, *5q*, *14q*, and *20q* was also found. Gain of chromosome *14q* was

considered to be a strong predictor for advanced disease and poor survival, and thus, its analysis was recommended in situ by fluorescence [33].

### **Diagnosis**

The diagnosis is based on clinical pictures, biochemical markers, endoscopy, and imaging and confirmed by histopathology [4,15,34,35].

### *Clinical presentation*

The clinical presentation is influenced by hormone secretion or not, therefore defining them as functional or nonfunctional.

Nonfunctional GI tract NETs are usually asymptomatic and revealed incidentally. They do not manifest specific symptoms initially but only display later symptoms that are related to tumor growth or metastases. Among the symptoms, the most common is obstruction and less often bleeding. The obstruction causes dysphagia in the esophagus, vomiting or early satiety in the stomach, incomplete or even complete intestinal obstruction in the small and large bowels with recurrent episodes of diffuse abdominal colicky pain and altered bowel habits (incomplete) or an acute episode of ileus (complete). The appendiceal location mainly on its tip is most commonly asymptomatic and is discovered incidentally by histopathology after appendectomy for acute appendicitis in up to 1% of removed specimens [4,36,37]. It is third in order after the rectum and small bowel and occurs mainly in young people, which has the most favorable prognosis among the NETs [35]. The location in the Vater's ampoule may cause episodes of acute cholangitis or acute pancreatitis. Metastases usually involving the liver are asymptomatic initially, but later they can present as malaise, nausea, anorexia, weight loss, anemia, jaundice or pain [4,15,35].

In the stomach, the most common type 1 (70% to 80%) occurs in cases of autoimmune atrophic gastritis with multiple small polypoid lesions (1-2 cm in diameter) having less aggressiveness and excellent prognosis. Type 2 (5% to 10%) with multiple small lesions (less than 1 cm in diameter) is associated with gastrinoma, often in the context of MEN 1 syndrome. Type 3 (10% to 15%) with solitary large lesions (more than 2 cm in diameter) by hepatic metastases (50% at the time of diagnosis) has the same prognosis as



adenocarcinoma. Type 4 enterochromaffin-like (ECL) cell NETs have similar characteristics to type 3. However, they are more aggressive and have a worse prognosis. These constitute either poorly differentiated NECs or variably MiNEN [35].

In small bowel locations, regardless of the primary lesion, solitary or multifocal lesions (50%) are usually under 1 cm in diameter, are grade G1 and G2 and grow slowly, with hepatic metastasis presence in approximately 80%-90% of cases at the time of diagnosis; likewise, lymph node metastasis and mesenteric fibrosis are present in approximately 50% of cases [35,38]. The expression of ESR1 (estrogen receptor 1) and AR mRNA (androgen receptor messenger RNA) was increased in primary NETs; likewise, mesenteric fibrosis was increased in men (71%), while mesenteric metastasis was increased with age in women above 70 years (72%). The combined increase in ER $\alpha$  (estrogen receptor alpha) and AR (androgen receptor) protein expression suggests the modulating effect of steroids [38].

Small and large bowel NETs or even stomach NETs may rarely manifest as acute intussusception [39-41].

In large bowel locations, the lesion is often large (more than 2 cm in diameter) with multiple metastases and has poor differentiation (grade 3) and aggressiveness. It manifests as adenocarcinoma, while carcinoid syndrome is extremely rare [35].

The location in the rectum may cause bleeding and tenesmus. Most lesions are small (less than 10 mm in diameter) and are discovered incidentally by colonoscopy between 50-60 years, and they can be managed by endoscopic resection without surgery [42,43].

Functional GI-NETs arise mainly from the most common location, i.e., the small intestine and can produce serotonin (5-hydroxytryptamine) or other vasoactive substances in various (small or large) amounts. The secreted amount beyond the hepatic capacity to metabolize serotonin causes episodes of carcinoid syndrome (flushing, bronchoconstriction, diarrhea, congestive heart failure, heartbeat feeling and peripheral edema). It usually occurs in extensive metastases and manifests itself in a paroxysmal occurrence that may be exacerbated by ingestion of food (chocolate, cheese) or alcohol (wine) [35].

### *Biomarkers*

Serum CgA (chromogranin alpha) has been the general biomarker for well-differentiated NETs for a long time. It is not associated with the tumor burden. Its diagnostic accuracy is now under debate. However, it is useful in follow-up. False-positive results occur in some circumstances, including drugs, inflammatory bowel disease, heart and renal failure, malignancy or **Novel biomarkers include circulating tumor cells, circulating tumor DNA, circulating microRNAs, and NETest. Liquid biopsy based on mRNA analysis in the serum is a useful novel biomarker. In particular, the NETest index is a new biomarker in peripheral blood that is based on the simultaneous assay of 51 neuroendocrine-specific marker genes by PCR (polymerase chain reaction). This test expresses the percentage of positivity of the genes involved. The results show a scale from 0 to 100% as an activity index. The cutoff point is 20%. An index between 20 and 40% indicates stable disease, while an index above 40% indicates progressive disease. Its diagnostic accuracy is high (99%) compared to that of CgA (21 to 36%). Additionally, it is very useful for follow-up after therapeutic excision or determining the response to drug therapy. It can reveal recurrence and may predict prognosis.** **A**  
**3** **new immunohistochemical marker is the transcription factor insulinoma-associated protein 1 (INSM1), which is more specific for the differentiation of NETs of the pancreas and rectum [4,35,44,45].**

**5-HIAA (5-hydroxyindoleacetic acid) is the main metabolic product of serotonin. Its assessment in 24-hour urine determines serotonin levels in NETs originating from enterochromaffin cells mainly in the small bowel, secrete serotonin. It is associated with tumor burden. However, it may have false-positive results in some cases (foods, drugs or various diseases) [35].**

The serum NT-proBNP (N-terminal-proB-type natriuretic peptide) test, which analyzes the level of this hormone produced by the heart, is a useful biomarker in the case of carcinoid syndrome for the assessment of carcinoid heart disease [46].

Chemokines are a subgroup of cytokines that are secreted from epithelial and stromal cells and act as mediators of cellular functions, including signaling and migration.

Chemokine receptor 4 is expressed in GI-NETs. Its immunohistochemical expression is related to grade 3 and metastatic disease and represents a proper target therapy by antagonists of this receptor [47].

The new monoclonal antibody SP70 differentiates neoplasms of the gastric fundus, including NETs, from nonneoplastic lesions by immunostaining [48].

Ghrelin is secreted mainly by enteroendocrine cells in the oxyntic glands of the stomach. Serum levels are elevated in gastrointestinal tract malignancies and may be a prognostic factor or even a therapeutic target [49].

The very rare inflammatory myofibroblastic tumors are associated with malignancies such as malignant NETs of the small intestine with lymphatic and hepatic metastasis and a mesenteric location of the abovementioned tumor. In this case, TGF  $\beta$ 1 Levels are significantly increased, and it is a diagnostic marker, especially for follow-up [50].

Orthotopic liver transplantation for metastatic carcinoid tumors has a high recurrence rate with a poor prognosis. It is important to identify prognostic markers before performing this procedure. MIB-1 (Mindbomb homolog 1) is a recombinant monoclonal antibody that assesses Ki-67 cell proliferative activity by immunohistochemistry. It is a useful prognostic biomarker for such cases detecting tumors with more aggressive biological behavior, thus avoiding unnecessary transplantation [51].

#### *Endoscopy (diagnostic and interventional)*

Endoscopy with biopsy is the gold standard for NET diagnosis of the upper GI and colon, including the rectum. For the small intestine, video-capsule endoscopy and double-balloon endoscopy are other options when NETs cannot be detected by conventional imaging. Endoscopic ultrasound is another highly accurate diagnostic tool that, apart from size, echogenicity, and depth of invasion, can reveal lymph node involvement [17]. Additionally, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) or biopsy (EUS-FNB) is performed in submucosal lesions. The accuracy for lesions greater than 21 mm ranges between 49%-78% for the former and 76%-88% for the latter; endoscopic resection is suitable for localized NETs of the upper gastrointestinal tract and for other submucosal lesions of the GI tract less than 2 cm in

diameter [52,53]. The ESGE (European Society of Gastrointestinal Endoscopy) guidelines for subepithelial lesions, including NETs, recommend EUS as the best option to determine the features of the lesion; EUS-FNB or MIAB (mucosal incision-assisted biopsy) for tissue diagnosis for lesions equal to or greater than 2 cm in diameter; endoscopic resection for type 1 gastric neuroendocrine neoplasms if they are greater than 10 mm; and endoscopic resection for unknown histology gastric lesions less than 2 cm [54].

A case report of endoscopic submucosal dissection and en bloc removal for a small (< 5 mm), well-differentiated, G3, type 3 gastric NET with a Ki-67 index >80% has been reported [55].

Endoscopic resection has been recommended for duodenal NETs equal to or less than 10 mm. The outcomes of endoscopic submucosal dissection for duodenal NETs in 20 patients were assessed recently. They were grade 1, except for 3 cases of grade 2; were sized <15 mm, except for one >15 mm; and were located in the bulb except for one that was located in the 2<sup>nd</sup> part. En bloc removal was feasible in 90% of patients, but endoscopic complete resection was feasible in 95%. R0 resection was achieved in 75-90% of patients according to size, and R1 resection was achieved in 25%. The complication rate was 25% due mainly to perforation (4 cases) and bleeding (one case). No relapse was seen endoscopically after one year [56].

A recent meta-analysis and meta-regression including 1,360 rectal NETs compared ESD (endoscopic submucosal dissection) to EMR (endoscopic mucosal resection). Both were equivalent for lesions less than 1 cm. However, ESD was more effective (more complete resection and less microscopic margin invasion) than ESD for lesions equal to or less than 2 cm, despite its longer time and more potential bleeding [57].

Endoscopic submucosal dissection with myectomy (ESD-ME) for rectal NETs may overcome the risk of vertical margin invasion. Comparison of this new method with ESD alone has proven its safety and effectiveness for nonmetastatic lesions less than 16 mm [58].

In general, GI tract NETs less than 1 cm in diameter had a potential risk of lymphatic invasion exceeding 10 percent. In such a case, after endoscopic resection, a detailed follow-up and possible additional surgery are required [59].

### *Imaging*

CT is the basic imaging tool commonly used for primary tumors and metastases in detecting lesions more than 0.5 cm [60]. It has difficulties and a limited role in imaging small lesions of the stomach, duodenum and small bowel. MRI is usually selected for preoperative staging and especially for evaluation of hepatic metastases (sensitivity 91%) [44,61] and their response to chemotherapy [62]. Diffusion weighted MRI and contrast enhanced MRI aid in the detection of small hepatic metastases. High resolution MDCT (multidetector computed tomography) is a new useful modality with a diagnostic accuracy of 90%. Nevertheless, it can localize the bowel NETs, guiding the surgery [63]. SPECT (photon emission computerized tomography) is another imaging option, but its radionuclides are inferior to those of PET [16]. As mentioned in Introduction section the novel diagnostic imaging includes PET-CT, FDG-PET, gallium PET-CT, FDOPA PET-CT (the most accurate) and SPECT [16-20]. Contrast enhanced ultrasound is used for follow-up.

Somatostatin receptor expression has been based since 1994 on the so-called octreoscan ( $^{111}\text{indium}$  pentetreotide scintigraphy), but in recent years, the novel functional imaging  $^{68}\text{Ga}$ -DOTATATE PET/CT ( $^{68}\text{Gallium}$ ) with its excellent detection rate (sensitivity 93–96% and specificity 85–100%) has been the method of choice for NETs and metastases. It uses the  $^{68}\text{Ga}$ -DOTA-peptides  $^{68}\text{Ga}$ -DOTATOC,  $^{68}\text{Ga}$ -DOTATATE, and  $^{68}\text{Ga}$ -DOTANOC and may possibly change our practice. The newer  $^{64}\text{Cu}$ -DOTATATE ( $^{64}\text{Curium}$ ) has higher diagnostic accuracy than  $^{68}\text{Ga}$ -DOTATATE [16,44,64,65].  $^{18}\text{F}$ -FDOPA PET/CT (6-[ $^{18}\text{F}$ ]-L-fluoro-L-3,4-dihydroxyphenylalanine) is valuable in the preoperative diagnosis of peritoneal carcinomatosis (possibility of 30%) due to GI-NETs mainly of the small intestine, thus determining the management strategy [20]. The new radiolabeled peptide  $^{99\text{mTc}}$ EDDA/HYNIC-TOC has a lower sensitivity (90.5%) than  $^{68}\text{Ga}$ -DOTA-peptides but greater specificity and diagnostic accuracy [17,66].



The role of nuclear medicine in diagnostic imaging is essential but has changed sufficiently in recent years. The somatostatin receptor scintigraphy by <sup>111</sup>indium pentetreotide (octreoscan) was the most used method for many years in clinical practice [67], but it is no longer the preferred imaging investigation method. However, despite important novel advances, its value may still be useful in some cases [18].

Apart from endoscopy, fluoroscopy is a valuable adjuvant option to assess the lumen of the upper GI tract, especially the duodenum, for NET existence or postoperative complications, obstructions and leakage [68].

The role of diagnostic endoscopic imaging has been mentioned above in detail.

#### *Histopathology staging*

The accurate histological assessment includes morphological evaluation, grading and immunohistochemistry results [34,69]. To establish the diagnosis of NETs, immunohistochemical staining for chromogranin A (CgA) and synaptophysin is necessary; <sup>3</sup> another promising new immunohistochemical neuroendocrine marker is the transcription factor insulinoma-associated protein 1 (INSM1) instead of synaptophysin [44].

The recent histopathology staging is based on the 2019 World Health Organization classification and grading that includes terminology, differentiation, grade, mitotic rate and KI-67 proliferation index, as shown in Table 1 [14].

Mixed neuroendocrine-nonneuroendocrine neoplasms (MiNENs) are rare aggressive neoplastic lesions in the gastrointestinal system with poor prognosis that usually consist of adenocarcinoma and neuroendocrine carcinoma. They are more frequently located in the colon, including the rectum. MiNENs of the ampulla are extremely rare. The main treatment is radical surgical resection followed by adjuvant chemotherapy [10]. Other locations include the esophagus and gastroesophageal junction, stomach, small intestine, appendix pancreas, liver, gallbladder and biliary tree. Currently, MiNENs also include neoplasms in the gastrointestinal system consisting of a nonneuroendocrine component and epithelial neoplasms other than adenocarcinoma. <sup>1</sup> There are 3 main subtypes, i.e., collision, combined and amphoteric lesions. They are

derived from a single precursor cell that has the capacity for dual differentiation after the initiation of carcinogenesis. Extensive surgery is not indicated in metastatic disease, especially in high-grade cases. Systemic chemotherapy is advocated in such cases [11].

For pathologic evaluation of small intestine NETs, the established guidelines include CgA and synaptophysin positive stain test as the minimum for making the diagnosis; tumor size more than 1 cm is classified as pT2; mesenteric tumor deposits equal or more than 2 cm are classified as pN2; and metastasis in 7 (from 12 previously) or more lymph nodes is classified as pN2. Findings of a recent study include only one positive test for CgA and synaptophysin [69].

#### *Prognostic factors*

For small intestine NETs, tumor size more than 1 cm predicts regional lymph node infiltration; presence of mesenteric tumor deposits predicts distant metastasis; and metastasis in 7 or more lymph nodes predicts disease progression [69].

Independent positive prognostic factors in cases of metastatic (stage IV) small intestine NETs include age less than 60 years, normal CRP levels, no diarrhea, metastatic liver involvement less than 50% and peptide radioreceptor treatment. Patients treated by both primary tumor resection and peptide radioreceptor treatment had the best survival benefit [45].

Colon NET location in contrast with the rectum has a poor prognosis and is associated with poor survival. A recent large study from China including 1,196 patients determined 7 prognostic factors for worse outcome: age  $\geq 68$  years, female gender, tumor size  $\geq 3.5$  cm, grade  $> 2$ , without chemotherapy, N1 stage and M1 stage. The location in the vast majority (73.5%) concerned the right colon, and the median tumor size was 4.1 cm [70].

Extramural venous invasion is common in small intestine NETs and is an independent prognostic factor for distant hematogenous metastases, in mainly hepatic as well as colorectal carcinoma [71].

High lymphovascular invasion (24.8%), even in grade 1 cases (22.8%), was found in biopsy for resected GI-NETs. It is a prognostic factor of biological behavior for malignant tendency. Close follow-up is recommended in such cases [59].

The WHO classification has proven adequate in predicting the course of NETs, and the Ki-67 proliferation index is a better prognostic factor for survival than the degree of differentiation [72].

### **Management**

The standard management is surgical therapeutic resection of the primary tumor with wide lymph node clearance [73]. For small, well-differentiated lesions, therapeutic endoscopy has a key role, as mentioned above, that is based on the location [21,34]. For advanced disease, management includes surgical hepatic resection, techniques of local tumor destruction such as radiofrequency or microwave ablation, systemic chemotherapy, targeted therapy by biological agents, immunotherapy and drug therapy, mainly involving somatostatin analogs for symptom alleviation in inoperable lesions or suppressing tumor growth or recurrence after surgery [4,35].

<sup>1</sup> Endoscopic mucosal resection and endoscopic submucosal dissection are indicated for small ( $\leq 1$  cm) and superficial rectal NETs, provided that there is no involvement of muscularis propria or lymph nodes. For lesions above 2 cm in diameter, a low anterior resection or abdominal resection is recommended [4].

#### *Surgical excision*

The surgical excision depends on age, location, functional activity, size, grade, staging and sporadic or inherited origin. In general lesions  $>2$  cm require surgery [35].

The extremely rare esophageal NETs (1.3% of all GI-NETs) mainly in men (4/1) and in the lower third are metastatic in the majority at the time of diagnosis. Small lesions can be managed endoscopically, while large lesions ( $>2$  cm) require esophagectomy (Ivor Lewis or Mackeown) by wide lymphadenectomy [74].

For stomach location type 3 Lesions ( $>2$  cm) or type 2 (multiple small lesions) the indication is total or subtotal gastrectomy by wide lymphadenectomy. However, since

the majority of lesions are type 1, endoscopic excision is the method of first option, preferably by experienced gastroenterologists [21].

For duodenal location apart from bulb location, where endoscopic management is indicated, pancreatoduodenectomy or partial duodenectomy is often required [4,21].

For small intestinal location due to tumor local status a wide enterectomy by lymphadenectomy is indicated. The high rate (up to 50%) of central mesenteric involvement makes the surgical resection more complicated. A systematic review showed overall morbidity 13%, but severe 7% and 30-day mortality 2% [75]. Although, there has been a debate, for small intestine NETs the resection of the primary focus in case of inoperable distant metastases does not have any effect on survival and thus it is not indicated [76]. The opposite conclusion is withdrawn by a recent meta-analysis indicating survival benefit [7].

Colon location in the vast majority is managed as adenocarcinoma by colectomy mainly right or less common left and wide lymphadenectomy [35].

Rectum location in the most cases usually needs endoscopic management (ESD, EMR) as already mentioned [43]; apart from this, TEMS (Transanal Endoscopic Microsurgery) is another resection method. However for rectal lesions greater than 2 cm in size, grade 3, stage T3-T4 or by lymph node invasion low anterior resection or abdominoperineal resection may be necessary [21].

Treatment of appendiceal NETs depends on factors such as Ki-67 proliferation index, tumor size, location, mesoappendix invasion, and lymph node involvement. Ki-67 determines grading; it is in Grade 1  $\leq 2\%$ , Grade 2 between 3% to 20% and Grade 3  $>20\%$ . The vast majority of cases are Grade 1, with tumor size less than 1 cm in which a simple appendectomy is enough. Right hemicolectomy is mandatory for Grade 3 Lesions independently size, in lesions greater than 2 cm, location in basis and invasion of mesoappendix or lymph nodes. In intermediate cases of grey zone a meticulous investigation (imagine, biomarkers) is mandatory and the decision must be individualized [36]. However, given that in up to 18% of appendiceal NETs cases coexist with another neoplasm, colonoscopy is indicated in any case [4].

Nowadays, for liver metastasis hepatectomy is the undoubted management option offering chance of cure and long-term survival, alone or accompanied by adjuvant chemotherapy [77]. With the advances in energy based devices, it is feasible, safe and effective as one stage procedure; the simultaneous hepatectomy along with wide enterectomy for synchronous metastatic disease of small intestine NETs that is present on liver in half of patients, represents a recent achievement. Despite the high recurrence rate (81%), it will achieve prolonged survival, when metastases concern only the liver [78].

In case of functioning liver metastasis and no possibility for curative resection, cytoreduction or debulking surgery by removing the most tumor bulking (>90%) may alleviate the symptoms due to hormone hyper-secretion. It is recommended in combination with drug and chemotherapeutic agents [79]. Another option for extended bilobar metastases, but only functioning by uncontrolled symptoms or ineffective treatment, is the orthotopic liver transplantation. The 1-year, 3-year and 5-year actuarial survival rate after transplantation reaches 59%, 47% and 36%, respectively for carcinoids tumors; but it is worse and is not indicated for other NETs. Extended lymphadenectomy of the hepatoduodenal ligament during the total hepatectomy is necessary before the implant placement [80].

RGS (Radioguided Surgery) based on preoperative radionuclides (<sup>111</sup>indium-111 pentetreotide, gallium-68 DOTA-peptides or technetium-99m EDDA/HYNIC-peptides) may improve the intraoperative reveal of GI tract NETs location [81].

#### *Drug therapy- Immunotherapy*

Somatostatin analogs such as octreotide or lanreotide have been used after surgery to suppress tumor growth or recurrence and in inoperable cases for symptom palliation; also, they are included in the first-line therapy of NETs not amenable to curative surgery [17,35,82,83].

Telotristat is an inhibitor of tryptophan hydroxylases (TPH 1 and TPH2) that limits serotonin biosynthesis and relieves manifestations of carcinoid syndrome. Telotristat together with a long-acting somatostatin analog is currently recommended for



uncontrolled carcinoid syndrome diarrhea by the USA NCCN (National Comprehensive Cancer Network) [84].

The NCCN, the North American Neuroendocrine Tumor Society and the Society of Nuclear Medicine and Molecular Imaging guidelines recommend somatostatin analogs as first-line treatment for positive somatostatin receptor grades 1 and 2 gastroenteropancreatic NETs [82].

In such patients with progressive advanced disease, for first-line treatment apart from somatostatin analogs, the therapeutic choices include  $^{177}\text{Lu}$ -DOTA-TATE, everolimus, chemotherapy or palliative radiotherapy for painful bone metastases [82].

$^{177}\text{Lu}$ -DOTA-TATE is a PRRT (radiolabeled peptide receptor radionuclide therapy) approved by the USA FDA (Food and Drug Administration) in January 2018 and used for NETs with positive somatostatin receptors [82]. PRRT has superiority over somatostatin analogs (SSAs) and is the first choice of treatment for patients with advanced well-differentiated NETs (effectiveness 96%). PRRT is followed by SSA plus bevacizumab (effectiveness 86%) and SSA plus IFN- $\alpha$  (interferon alpha) (effectiveness 78%); all three have similar serious side effects [85].

There have been five somatostatin receptor (SSTR) subtypes, i.e., SSTR1, 2A and 2B, 3, 4, and 5. Somatostatin receptor antagonist therapy targets these subtypes by exerting antineoplastic activity. Peptide receptor radionuclide therapy (PRRT) uses radiolabeled SSA radionuclides ( $^{90}\text{Y}$  or  $^{177}\text{Lu}$ ), which are firmly connected with a transport vehicle that binds directly to tumor cells, similar to a Trojan horse. It may be the most important anti-SSTR2 (most frequently expressed in lung and gastroenteropancreatic NETs) treatment for NETs in recent years [86].

The vast majority of target treatments include the above-mentioned agents against somatostatin receptors and also against paramycin pathways (mTOR) such as everolimus [87]. New drugs (ganitumab or cixutumumab) that increase the efficacy of everolimus have been reported [86].

After failure of standard treatment, targeted therapy by sintilimab, a monoclonal antibody against programmed cell death protein 1 (PD-1), has been assessed with

**promising results.** The response rate was 20.8% for GI tract NETs and 27.8% for NECs [88].

Target therapy by EZH2<sup>8</sup> (enhancer of zeste homolog 2), which is a histone methyltransferase catalyzing trimethylation of histone H3 Lysine 27 (H3K27me3), represents a novel promising biological agent with an important role in small bowel NETs [89].

Surufatinib, previously known as sulfatinib, is a novel, oral, tyrosine kinase inhibitor that inhibits receptors of VEGF (Vascular Endothelial Growth Factor), FGF (Fibroblast Growth Factor) and CSF (Colony Stimulating Factor 1) and exhibits antineoplastic activity, even in advanced progressive well-differentiated NETs [90].

Sunitinib and everolimus have been used as target therapies for NETs among other uses since they inhibit tumor growth, vasculature and spread capacity [46,91]. Sunitinib is a tyrosine kinase inhibitor approved initially as a target therapy for renal cell cancer and GISTs (gastrointestinal stromal tumors) resistant to imatinib treatment. Everolimus is an inhibitor of mTOR (mammalian target of rapamycin) that was approved as an immunosuppressor, to avoid rejection after transplantation and as a target therapy in renal cell carcinoma and other neoplasms (91).

The combination of the biological agents bevacizumab, an anti-VEGF monoclonal antibody, with atezolizumab, an anti-PD-L1 monoclonal antibody (against the protein programmed cell death-ligand 1), in patients with advanced NETs showed promising results that were compatible with other therapies [92].

Novel monoclonal antibodies as immunotherapy alone or in combination have been investigated for NETs with limited effectiveness and limited data. Several trials are still ongoing. They include pembrolizumab, spartalizumab, toripalimab, avelumab, regorafenib, lenvatinib, tislelizumab and others [93].

Systemic chemotherapy is recommended in cases that worsen despite first-line treatment with somatostatin analogs, or involve increasing tumor burden or rapid growth. Chemotherapy has limited efficacy in extrapancreatic NETs, including GI tract NETs, and thus, it is not indicated routinely. It includes choices such

as streptozotocin with 5-fluorouracil or its precursor form capecitabine, temozolomide, which tends to replace streptozotocin, doxorubicin, interferon- $\alpha$ , platinum based agents (oxaliplatin with better safety or cisplatin), dacarbazine and irinotecan. In addition to hepatic artery embolization (spongostan, gel-foam) or preferably chemoembolization by doxorubicin, cisplatin is helpful in hepatic metastases. Chemoembolization in combination with systemic chemotherapy is related to better outcomes [94,95].

Neuroendocrine carcinomas exhibit very aggressive behavior with a median overall survival of up to 12 months. Combination chemotherapy, as in non-small cell lung carcinoma of cisplatin (CDDP) with etoposide (VP-16), has been the classical approach [95].

A recent study from the California Cancer Registry including 154 patients with GI-NETs and liver metastases showed that liver-directed therapy is associated with improved overall and disease-specific survival compared to systemic therapy alone [96].

### **Prognosis**

Appendiceal NETs have the most favorable prognosis [97]. The prognosis of stomach NETs for type 1 is excellent with the 5-year survival rate being greater than 95%, while that for type 2 is good and for type 3 is poor, being equal to or less than 50%. Nonfunctioning duodenal NETs generally have a better prognosis than adenocarcinoma, while high-grade lesions have a worse prognosis. Rectal NETs have the best prognosis among GI-NETs because they are small and without invasion in the majority of cases. In T1, the 5-year survival is 98%-100% for localized lesions, 54%-74% for regional metastases and 15%-37% for distant metastases [21].

Large bowel NETs, in contrast with those of the rectum, have a much worse prognosis and one of the worst survival outcomes among the other GI tract NETs due to their greater size, T stage, grade and lymphatic invasion [70].

Small bowel NETs, as slow-growing lesions, have a rather favorable prognosis in general, but uncommonly, there are some cases with unfavorable prognoses. Overall survival is more favorable than in other cancers. It was estimated that the 5-year

survival rate was 67% and the 10-year survival rate was 37%. A meta-regression analysis found that younger age, mainly, or perhaps primary tumor resection is related to better prognosis [98]. The presence of preoperative symptoms determines the prognosis. Those induced by hormone secretion predict advanced disease and poor prognosis [99,100].

It is debatable whether resection of the primary tumor in metastatic small bowel NETs is valuable for improving prognosis. A recent study showed that it does not affect overall survival and thus is not recommended as a standard in such cases [74]. However, a recent meta-analysis supports the opposite, i.e., Noncurative primary site excision offers increased survival (5-year rate of 74%, 10-year rate of 44%) [79].

A NET nomogram (Modlin Score) can be used for prognosis and consists of 15 variables: age, sex, ethnicity, symptoms, tumor size, invasion, metastasis, histology, Ki-67 index, urinary 5-HIAA, serum HgA, liver function tests, carcinoid heart disease, surgical management or long-acting somatostatin analog therapy [101]. Malnutrition and neoplastic cachexia influence the outcome of cancers affecting survival; they are also associated with the nutritional status of gastrointestinal NETs. Consequently, nutritional assessment or even more precisely ghrelin molecular expression is necessary in such cases to improve the prognosis [102].

NECs (neuroendocrine carcinomas) in the rectum are very uncommon and involve aggressive neoplasms with a dismal prognosis. Treatment options include surgery, chemotherapy and radiotherapy. A large retrospective study including 805 cases confirmed the poor outcome even after radical excision. This indicates cautious and circumspect selection of patients by poor differentiation and high-grade rectal NETs. Systemic chemotherapy may improve the outcome [103].

The biological behavior of MiNENs (mixed neuroendocrine non-neuroendocrine neoplasms) is similar to that of pure NECs, both characterized by poor prognosis [104].

The role of artificial intelligence and machine learning and a deep understanding of the diagnosis and management of NENs of the gastrointestinal system have been recently

evaluated. A standard of practice has not yet been established; however, it may serve as a useful adjunct in current practices [105].

## **CONCLUSION**

The gastrointestinal tract and pancreas are the most common locations of neuroendocrine tumors (NETs). The assessment of NETs includes determining the site, grade, stage and secretion capacity. New imaging techniques, histopathology assessment and classification have been valuable in making the diagnosis and planning therapeutic management. Liquid biopsy and the NETest gene index is a useful novel biomarker in peripheral blood. The somatostatin receptor scintigraphy is no longer the preferred imaging investigation.  $^{68}\text{Ga}$ -DOTATATE PET/CT or the newest  $^{64}\text{Cu}$ -DOTATATE PET/CT are the preferred imaging. Surgical or endoscopic resection is usually indicated for localized lesions depending on the tumor size (surgery is necessary for tumors 2 cm or more in size). Hepatectomy or even liver transplantation has well-defined indications as well as debulking surgery in secreting NETs. Long-acting release somatostatin analogs is the first-line treatment for inoperable advanced disease, followed in unresponsive cases by chemotherapy or targeted immunotherapy by novel biological agents. Somatostatin receptor antagonist therapy exerts antineoplastic activity. Radiolabeled peptide receptor radionuclide therapy and somatostatin analogs plus bevacizumab or IFN- $\alpha$  are novel therapeutic options. Telotristat treatment is indicated for persistent diarrhea in carcinoid syndrome. The therapeutic option should be individualized based on current multidisciplinary information. The gastroenterologists play an important role in diagnosis and management. Future assessments should be focused on targeted biological agents of the implicated molecular factors, new effective chemotherapy drugs, gene therapy and the use of artificial intelligence in the diagnosis and management of these diseases to open new horizons for therapeutic strategies.



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