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Biomarkers in neuroendocrine tumors

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1. ABSTRACT

Here we review the role of clinical biomarkers (tissue and circulating markers) in the management of neuroendocrine tumors. These tumors may originate in different organs, from cells embriologically different but expressing common phenotypic characteristics, such as the immuno-reactivity for markers of neuroendocrine differentiation (defined as "pan-neuroendocrine"), the capacity to secrete specific or aspecific peptide and hormones, and the expression of some receptors, that are at the basis of the current diagnostic and therapeutic approach.

Table 1. NETs anatomo-pathological classification (WHO, 2000)

Well-differentiated endocrine carcinomas
Benign behavior
Uncertain behavior
Poorly differentiated endocrine carcinomas
Mixed exocrine-endocrine tumors

Table 2. Different categories of neuroendocrine markers tumors

Tissue markers
Secretion markers
Prognostic markers
Other markers

2. INTRODUCTION

Neuroendocrine tumors (NETs) constitute a heterogeneous and probably underestimated group of neoplasms accounting for at least 2% of neoplastic human proliferations. They are derived from diffuse neuroendocrine system (DNES), which is made up of peptide- and amine-producing cells with different hormonal profiles depending on their site of origin (1). The primitive tumor, not always identifiable, may originate from cells of the DNES in various organs (Figures 1, 2, 3). The most frequent sites of origin are the digestive and respiratory tracts. A typical feature of the DNES is their ability to secrete a wide spectrum of peptides. This provided the basis for the first hypothesis of the Amine Precursor Uptake Decarboxylation (APUD) system, postulated by Pearse in the mid-1960s (2). DNES cells have different embryological origins but share common secretory and/or neuroendocrine markers. In clinical practice, the diagnosis of NETs can be actually based on the detection of tissue and/or circulating neuroendocrine markers by using specific antibodies (3). These antibodies can be employed in radio-immunometric or immuno-enzymatic assays to detect circulating neuroendocrine markers, and in immuno-histochemical procedures to assess the neuroendocrine differentiation of a tissue. Their assessment may therefore play an important role in the diagnosis and management of NETs. In 2000 the World Health Organization (WHO) published a classification that takes into account morpho-histological, immuno-histochemical and clinico-pathological criteria (Table 1), making the role of neuroendocrine markers more relevant. The secretory pattern of NETs may differ according to the site of origin and grade of differentiation (4). However, most of the currently available circulating neuroendocrine markers are relatively aspecific. Furthermore, significant variations of circulating levels of these markers may not only result from active secretory cellular process but can also reflect cytolysis occurring during tumor growth or as a consequence of chemo, radio or radio-metabolic treatments. Therefore, the assessment of neuroendocrine markers may play a role in different steps of the NETs management: a) diagnosis, b) prognostic factors, c) therapeutic decision, d) evaluation of response-to-treatment, and d) follow-up. To simplify the role of neuroendocrine markers, they have been divided in: 1) Tissue markers 2) Secretion markers, 3) Prognostic markers, and 4) Other markers. The different spectrums of NETs are showed in the Table 2.

3. TISSUE MARKERS OF NEUROENDOCRINE DIFFERENTIATION.

3.1. Chromogranins (Cgs)

The chromogranin (Cg) family of glycoproteins, CgA, CgB and CgC, are stored, along with peptide hormones, in the granular vesicles of neuroendocrine and endocrine cells. They have a different molecular weight, ranging from 48 up to 115 kDa. Cgs constitute the main components of large secretory granules of neuroendocrine cells, where they are co-localized and secreted together with the other secretory peptides and hormones. There are other members of the Chromogranin family as secretogranin III (or 1B1075), secretogranin IV (or HISL-19), secretogranin V (or 7B2), and secretogranin VI (or NESP55) (5).

3.1.1. CgA

Human Chromogranin A (CgA), or parathyroid secretory protein 1 (gene name CHGA), is an acidic glycoprotein of 439 aminoacids and a molecular mass of 48 kDa, that is preceded by an 18-aminoacid signal peptide (6). Both C- and N-terminal regions of the molecule are greatly conserved, whereas the mid portion of the molecule shows considerable sequence variation between species. CgA is located in secretory vesicles of neurons and endocrine cells. Examples of cells producing chromogranin A are chromaffin cells of the adrenal medulla, enterochromaffin-like cells and beta cells of the pancreas. Chromogranin A is the precursor to several functional peptides including vasostatin, pancreastatin, catestatin and parastatin. These peptides negatively modulate the neuroendocrine function of the releasing cell (autocrine) or nearby cells (paracrine). Chromogranin A might promote the generation of secretory granules. CgA is the most used neuroendocrine differentiation marker for NETs in clinical practice. Its tissue expression can be revealed using specific antibodies or *in situ* hybridization techniques. Well-differentiated NETs are usually immunoreactive for CgA, while CgA immunoreactivity tends to decrease progressively until disappearance in less differentiated forms (Figure 1) (7).

3.1.2. CgB

Chromogranin B (CgB, secretogranin I) is a secretory granule matrix protein expressed in a wide variety of endocrine cells and neurons.

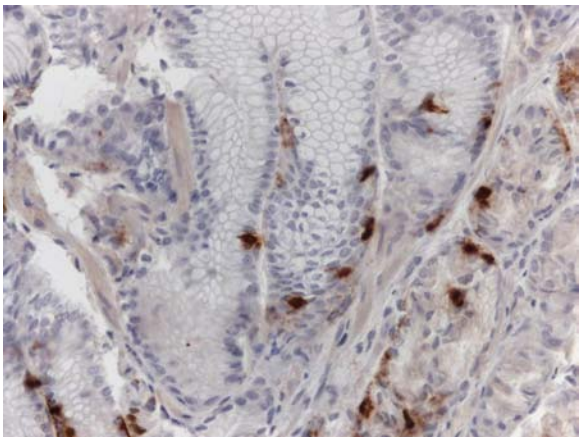

Figure 1. Normal distribution of neuroendocrine cells in the gastric mucosa. Immunostaining with polyclonal antibody to CgA. H/E counterstain. Original Magnification 40x.

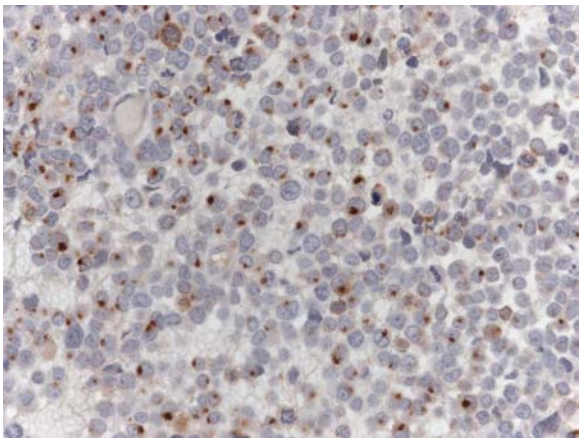

Figure 2. Poorly differentiated neuroendocrine carcinoma of the stomach. Dot-type immunostaining with polyclonal antibody to CgA. H/E counterstain. Original Magnification 40x.

3.1.3. CgC

Chromogranin C (CgC) belongs to the family of neuroendocrine secretory proteins. In rodents the full length protein, secretogranin II, influences the packaging or sorting of peptide hormones and neuropeptides into secretory vesicles. The full length protein is cleaved to produce the active peptide secretoneurin, which has chemo toxic effects on specific cell types, and EM66, whose function is unknown. For clinical use, CgA is the most reliable markers of neuroendocrine differentiation. Both CgB and CgC are not frequently used in clinical practice.

3.2. Synaptophysin

Synaptophysin is an integral membrane glycoprotein (molecular weight 38 kDa) that occurs in pre-synaptic vesicles of neurons and small clear vesicles of normal and neoplastic neuroendocrine cells. It is expressed independently of the other neuroendocrine markers, notably secretory granule products. Other markers related to synaptophysin are SV2 and synaptobrevin.

3.3. Neural cell adhesion molecule (NCAM, CD56)

The neural cell adhesion molecule (NCAM, CD56) belongs to a group of cell-surface glycoprotein involved in direct cell–cell adhesion. It is expressed by neuronal and neuroendocrine cells (8). NCAM, however, is not only present in neuroendocrine cells but may also be expressed by non-endocrine normal tissues (renal tubules and thyroid follicle cells) and neoplastic tissues (non-small cell lung carcinomas and others).

3.4. Vesicular monoamine Transporter VMAT1 e VMAT2

Storage of monoamines in secretory granules of neurons or endocrine cells is critical for their regulated physiological secretion. Monoamine accumulation from the cytoplasm into storage organelles is mediated by vesicular monoamine transporters (WMATs) (9). Molecular cloning has identified two VMATs, which differ in terms of their functional properties and substrate specificity. In rat and human gastrointestinal tract, VMAT1 immunoreactive cells are rare in the stomach but abundant in the intestine. They were identified as enterochromaffin (EC) cells. In contrast, VMAT2 immunoreactive cells were exclusively located in the oxyntic mucosa of the stomach and proved to be enterchromaffin-like (ECL) cells (10). VMAT-2, and WMAT-1 are reliable markers for differentiation of gastric endocrine hyperplasia and neoplasia from ECL and EC cells, respectively. The significance of VMAT2 and VMAT1 as prognostic markers lies in the relatively poor prognosis for EComa compared to ECLoma, characterized by VMAT2-positivity. The absence of both VMAT2 and VMAT1 in neuroendocrine carcinoma may indicate poor prognosis (11).

3.5. Prohormone convertases (PC1, PC2)

Neuroendocrine tumors usually produce hormones in the form of prohormones, which must be processed into bioactive peptides by prohormone convertase (PC). PC2 and PC3 (also known as PC1) are members of a family of cellular endoproteolytic processing enzymes. Many regulatory peptides require the a-amidation of a carboxyl terminus for biologic activity or for binding to their receptors by peptidylglycine a-amidating mono-oxygenase (PGM) and peptidylamidoglycolate lyase, which are encoded in a single mRNA molecule. PC2, PC3, and PGM are also contained in the secretory granules of the neuroendocrine system and of the tumors derived from it. PC2 and PC3 have been reported to be useful markers for neuroendocrine cells (12).

3.6. Specific peptide hormone markers (gastrin, serotonin, somatostatin and others)

These can be exquisitely specific (provided that the tumor cell is producing the relevant peptide hormone in a form that reacts with the antibody), but are of little value as screen for the possibility of an endocrine cell tumor, unless the presence of a hormone hyper-secretion syndrome is known either clinically or bio-chemically in the patient in question. They also depend on the relevant peptide being

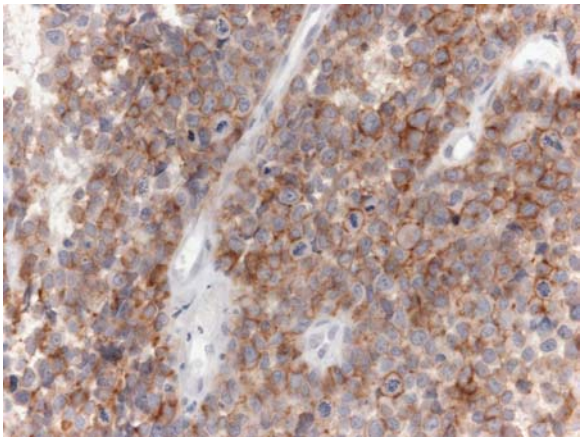

Figure 3. Poorly differentiated neuroendocrine carcinoma of the stomach. Cell membrane immunostaining with monoclonal antibody to CD56/123C3 (NCAM). H/E counterstain. Original Magnification 40x.

stored in the tumor cell, although this can be circumvented by the use of *in situ* hybridization for the specific mRNA.

Hormones that are highly specific for certain tumor localizations are luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin for pituitary adenomas, calcitonin for medullary thyroid carcinoma, parathyroid hormone for parathyroid adenoma, insulin and glucagon as well as pancreatic polypeptide for pancreatic tumors, serotonin and substance P for ileal and appendiceal neuroendocrine tumors, and vasoactive monoamine transporter-2 (VMAT2) for ECLomas.

3.7. Somatostatin receptors

Many NETs express somatostatin receptors. Five types of these receptors have been identified (e.g. SSTR1–5) (13). They can be demonstrated by autoradiography, by scintigraphic imaging (octreoscan) or by immunohistochemistry. The immunohistochemical expression of SSTR2, which shows a membranous pattern, correlates closely with the octreoscan signals (14). Serotonin-producing NETs and gastrinomas are more commonly positive for SSTR2/5 (up to 90%) than insulinomas (up to 60%).

3.8. Pancreatic polypeptide (PP)

PP is a polypeptide secreted by PP cells in the endocrine pancreas predominantly in the head of the pancreas. It consists of 36 amino acids and has a molecular weight of about 4200 Da. The function of PP cells is to self regulate the pancreas secretion activities (endocrine and exocrine), it also effects on hepatic glycogen levels and gastrointestinal secretions. Its secretion in humans is increased after a protein meal, fasting, exercise, and acute hypoglycaemia and is decreased by somatostatin and intravenous glucose. Increased PP cells are found in 20 to 67% of functioning and non-functioning tumors of the pancreas (15). There does not appear to be a relationship between the number of cells and their function because islet tumors containing subnormal, normal, or supernormal concentrations of PP compared with that in the normal pancreas may be associated with normal or high levels of circulating PP.

3.9. CD57

CD57 (Leu-7) is a secretory granule associated marker with high specificity, but low sensitivity, in that their expression varies between different endocrine cell types and also depends on the presence of intracytoplasmic secretory granules, so that immunopositivity is often lost in poorly differentiated tumors (16).

3.10. CK19

The expression of cytokeratin 19, regarded as a marker of ductal epithelium, has been suggested as a marker of aggressiveness in NETs, which gives prognostic information independently from that obtained by WHO criteria (17).

3.11. Neuron Specific Enolase (NSE)

NSE, the gamma-gamma dimer of the glycolytic enzyme enolase, is the best-known cytosolic marker. Its reactivity is unrelated to the content of secretory granules in the cells. Enolases are homo- or heterodimers of the three subunits: alpha (46 kDa), beta (44 kDa), and gamma (46 kDa). The alpha-subunit is expressed in most tissues and the beta-subunit only in muscle. The gamma-subunit is expressed primarily in neurons, in normal and in neoplastic neuroendocrine cells. Co-expression of NSE and chromogranin A is common in neuroendocrine neoplasms. Gamma-gamma isozyme of enolase is found at elevated concentrations in small cell lung cancer and pediatric neuroblastoma. The immunohistochemically use of NSE is not recommended. The commercially available antibodies are of limited sensitivity because of unspecific staining of certain non-endocrine tissues, such as striated muscle. Moreover, it is well known that NSE has been recognized in some non-neuroendocrine tumor tissues, such as solid-pseudo papillary neoplasms of the pancreas and serous-cystic neoplasms of the pancreas. These disadvantages, therefore, suggest that NSE positivity should be used with caution as a neuroendocrine marker reaction in tumor diagnosis.

3.12. PGP 9.5

PGP 9.5 is another cytosolic marker that is expressed in neuroendocrine tumors but also in other tumors showing neural or melanocytic differentiation, and also in a wide range of other non-endocrine tumors. As NSE, PGP 9.5 is not recommended for immunohistochemical typing of NETs.

3.13. Neurofilaments

Neurofilaments are a type of intermediate filament that serves as major elements of the cytoskeleton supporting the axon cytoplasm They are the most abundant fibrillar components of the axon, being on average 3-10 times more frequent than axonal microtubules. Neurofilaments (10 nm) are built from three intertwined protofibrils which are themselves composed of two tetrameric protofilament complexs of monomeric proteins.

The neurofilament triplet proteins (68/70, 160, and 200 kDa) occur in both the central and peripheral nervous system and are usually neuron specific. The 68/70 kDa NF-L protein can self-assemble into a filamentous structure; however the 160 kDa NF-M and 200 kDa NF-H proteins require the presence of the 68/70 kDa NF-L protein to co-assemble. Neuromas, ganglioneuromas, gangliogliomas, ganglioneuroblastomas and neuroblastomas stain positively for neurofilaments. Although typically restricted to neurons, neurofilaments have been detected in paragangliomas and adrenal and extra-adrenal pheochromocytomas. Carcinoids, neuroendocrine carcinomas of the skin, and oat cell carcinomas of the lung also express neurofilaments. Neurofilament positivity in small cell carcinomas (neuroendocrine carcinomas) shows a characteristic paranuclear dotlike pattern.

3.14. Neuroendocrine secretory protein 55

This protein is a 241–amino acid polypeptide that belongs to the chromogranin family and is thus located within large dense core secretory granules (18). The value of neuroendocrine secretory protein 55 lies in its ability to stain pancreatic NETs (in addition to pheochromocytomas), whereas GI tract NETs are negative

3.15. Ghrelin

Ghrelin is a 28–amino acid peptide seen in oxyntic glands of the gastric mucosa. However, its expression has also been detected in neuroendocrine cells in the pancreas, pituitary, and heart. Ghrelin-producing NETs have been documented in the stomach and intestine

3.16. CDX2

The transcription factor CDX2, which belongs to the homeobox genes regulating the development of the epithelium of the small and large intestine, has proved to be a very reliable marker of all NETs arising in the midgut. In addition, some well-differentiated NETs from the lung, the pancreas and the rectum were also positive, while other NETs – in particular from the stomach, the thyroid and the paraganglia – were consistently negative (19, 20).

3.17. Thyroid transcription factor 1 (TTF-1)

TTF-1, a homeodomain containing transcription protein of the NKx2 gene family, is expressed in poorly differentiated neuroendocrine carcinomas of the lung (85%) and also in some pulmonary carcinoids (up to 60%), but usually not in well and poorly differentiated NETs of the gastrointestinal tract (21). However TTF-1 may be positive in extra pulmonary small cell carcinoma of different sites including also gastrointestinal tract (22). It is considered is a marker of pulmonary NETs aggressiveness (23).

3.18. Histidine decarboxylase

Histidine decarboxylase expression occurs in most neuroendocrine cells, but pancreatic NETs are most frequently positive for this marker (24).

3.19. Xenin

Xenin is a 25–amino acid peptide that appears to be specific to duodenal neuroendocrine cells. It has been shown that NETs from the duodenum, including non-functional, gastrin- and somatostatin-producing tumors, show xenin expression (25). In contrast, NETs from other sites in the GEP tract are negative. The utility of this marker may lie, therefore, in determining the site of origin in a NET that would otherwise be classified as “of unknown origin”.

3.20. Ki-67

The Ki-67 antigen is present in both proliferating normal and tumor cell populations, and thus, it has tremendous utility in the determination of a given cell population’s growth fraction, that is, the fraction of cells born into the proliferative category (26). The literature revealed a preponderance of studies in the last two decades supporting the validity of the Ki-67 proliferation index as a prognostic indicator for Gastroenteropancreatic-NETs (GEP-NETs). Between tested cut-off values of 2%, 5%, and 10%, non-functioning pancreatic endocrine tumors with a Ki-67 greater than 2% (27, 28), and greater than 5% were shown to have unfavorable prognosis in both univariate and multivariate analyses (29, 30). The Ki-67 has been endorsed by the WHO classification of NETs and, as such, for reproducibility and uniformity purposes, Ki-67 immunostaining should be routinely performed in the immunohistochemical work-up of NETs (31).

4. CIRCULATING MARKERS FOR NETS

NETs often produce and secrete a wide spectrum of hormones and pro-hormones. The secretory pattern depends on their embryological origin and stage of the disease. Correlation of serum markers with symptomatology and lesion location is important to facilitate accurate diagnosis. Markers common to many types of NETs include CgA, neuron-specific enolase, human chorionic gonadotrophin, alpha-fetoprotein, pancreatic polypeptide, calcitonin, urinary 5-hydroxyindoleacetic and 5-hydroxy-tryptamine serotinine / 5-hydroxy-tryptophane (Table 3). The measurement of neuroendocrine markers in the circulation of patients with NETs is of threefold importance. First, it establishes the diagnosis, secondly, it is useful in monitoring progression of disease and response to treatment and thirdly, it may assist as a prognostic indicator.

4.1. Chromogranin A (CgA)

CgA is physiologically released by exocytosis and may be detected in the blood. In particular, when a tumor develops in an endocrine tissue, it becomes the main source of circulating CgA (32). High CgA levels have been demonstrated in the serum or plasma of patients with different types of endocrine tumors such as pheochromocytoma, medullary thyroid carcinoma, and enterochromaffin and pancreatic islet cell tumors (33-35). The physiological functions of CgA are gradually elucidated, although many questions still remain (36). In particular, circulating CgA levels have been claimed to be useful markers for NETs, with a high specificity and a sensitivity ranging from 27% to 81% (37, 38) and it is commonly agreed that CgA is the most useful general circulating marker for NETs (37). Levels of circulating CgA are increased in 60-80% of gastroenteropancreatic

Table 3. Main general markers of secretion of NETs

	Sensitivity	Specificity
CgA	60-90% (except MTC:<50%)	68-100%
NSE	38-70% (except PD NETs:>70%)	30-85%
Alpha-HCG	25-40%	about 25%
AFP	N.R.	Less than 20%
PP	30% (GI NETs), 70% (pancreatic NETs)	67%
CT	9-28% (except MTC: about100%)	about 100%
5-HIAA	5-50% (except ileal NETs: 65-85%)	about 100%

MTC: medullary thyroid carcinoma; PD: poorly differentiated; GI: gastrointestinal; N.R.: not reported.

(GEP) NETs, particularly in NETs of foregut origin, in which it shows a higher sensitivity (with the exception of insulin-secreting pancreatic NETs, more associated with CgB hyper secretion) while in NETs of midgut origin its sensitivity is similar to 5-HIAA. Moreover, its role in rectal NETs still needs better defining (39). The CgA levels have an inverse correlation with the grade of differentiation of NETs and may be increased in epithelial tumors with a neuroendocrine phenotype (prostate, breast, thymus, uterus and colon rectum) (40). Some authors reported that the highest levels are recorded in subjects with metastatic neuroendocrine tumors, with extreme elevations up to 1000 times the upper limit of normal in cases of metastatic carcinoid tumor (41, 42). In contrast, serum concentrations of CgA are rarely elevated in subjects with small neuroendocrine tumors, such as insulinomas, paragangliomas and pituitary adenomas (41-43). To date the relationship between CgA levels, tumor mass, tumor progression and response to therapy is still a matter debate (39, 44, 45). CgA circulating levels seem poorly correlated with tumor mass during therapy with somatostatin analogues. Increased levels of CgA also occur in non-NETs pathological conditions, such as chronic atrophic gastritis, kidney and hepatic failure, inflammatory chronic diseases and arterial hypertension (46). Moreover patients treated with long-term histamine type 2 receptor antagonists or proton pump inhibitors have raised circulating CgA (46). Because of all these, gastric hypochlorhydric and achlorhydric states are common; care must be taken in the interpretation of CgA results. Circulating CgA concentrations of more than twice the reference range are usually indicative of NETs, whereas concentrations lower than this, are inconclusive. CgA is significantly suppressed by somatostatin analogue treatment in the majority of NETs patient and this suppression appears poorly correlated with tumor mass during this kind of therapy. This suppression may bring concentrations of CgA previously elevated to within the reference range. Concerning methodology, the results obtained using radioimmunoassay and enzyme-linked immunosorbent assay methods do not seem completely comparable, suggesting the opportunity to compare results only when obtained with the same method.

4.2. Neuron-specific enolase (NSE)

NSE is the neuron-specific isomer of the glycolytic enzyme 2-phospho-D-glycerate hydrolyase or enolase with a 90 kDa weight. This isomer is present in neurons and neuroendocrine cells and can therefore serve as a biochemical marker for tumors derived from these cells. Serum NSE levels are frequently elevated in patients with several NETs. Like CgA, NSE as a general neuroendocrine marker cannot differentiate between different subtypes of NETs. Elevated NSE levels were exclusively associated with poor tumor differentiation (47). NSE has a high sensitivity and specificity in small cell lung poorly differentiated neuroendocrine carcinomas (48). Elevated NSE circulating levels has been correlated with dismal prognosis, in these patients (40). The role of circulating NSE can be used to evaluate the responsiveness to therapy and in the follow-up to detect disease relapse early on. In all the other kinds of NETs, NSE has lower sensitivity and sensibility than CgA. During the antiblastic treatment, the circulating NSE levels can be increased as a consequence of tumor cell lysis.

4.3. Human chorionic gonadotrophin (HCG)

HCG is a glycoprotein hormone synthesized during pregnancy by the trophoblastic cells of the placenta. It consists of a alpha and beta-subunit. The beta-subunit is specific for HCG, whereas the alpha-subunit is also common to the other hormones of the glycoprotein family: luteinizing hormone (LH), follicole stimulating hormone (FSH), and thyroid stimulating hormone (TSH). Although only the intact hormone is biologically active, the uncombined subunits are also released in the circulation. HCG only fulfils a physiological role during pregnancy, where it is responsible for the preservation of the function of the corpus luteum. It is a well-established marker tumoral trophoblastic, or germ-cell tissue in hydatiform mole, choriocarcinoma or testicular cancer. Ectopic secretion of alpha-subunits, not of intact HCG, is frequently encountered in NETs. The serum levels of HCG alpha-subunits are generally much lower than in trophoblastic tumors. Prevalence reported in the literature varies considerably for similar NETs, probably because of differences in patients' selection and analytical methods. The clinical usefulness of alpha-subunits as a marker for NETs is limited, however, because most subjects with elevated levels also have elevated CgA concentrations (47). The beta-subunit of HCG is positive in about 30% of patients with foregut NETs while it does not seem to have any utility in midgut and hindgut NETs (40).

4.4. Alpha-Fetoprotein (AFP)

Alpha-Fetoprotein is a fetal serum glycoprotein encoded by a gene located on chromosome 4. Its molecular weight is calculated to be 70 kDa, with 4% carbohydrate. Changes in AFP sugar chains have been noted, suggesting micro heterogeneity of AFP. Elevated serum concentrations of AFP during fetal life are observed. AFP has been localized in hepatocytes, yolk sac and mucous membrane gastrointestinal cells. In the fetus, AFP is a transport protein that peaks, in the 16th week of pregnancy. In, healthy subjects, serum AFP levels show high levels in newborns and then decline gradually, reaching levels of less than 10 ng/mL in 300 days. Raised serum levels of AFP are observed during pregnancy, and in patients with mucoviscidosis, acute hepatitis (30-50%), chronic hepatitis (15-50%), cirrhosis (11-47%) and other malignancies (gastrointestinal, pancreatic, biliary, non-seminomatos germ-cell testicular, and germ-cell ovarian). Moreover Shat T *et al* observed that AFP is elevated only in a minority

NETs patient, and also he demonstrated the ability of AFP to highlight a group of NETs patients with aggressive, high-grade tumors and poor prognosis (47).

4.5. Pancreatic polypeptide (PP)

PP is produced by normal pancreatic islets, but its levels are increased in approximately 80% of patients with pancreatic tumors and in about half of patients with carcinoid tumors. For neuroendocrine tumor patients with strongly elevated PP levels, the term PPoma has been introduced (5, 48, 49). Because of the significant response to food, it is essential that PP be measured only after an overnight fast (a minimum of 8 h). Circulating PP rises with increasing age and is higher in males than in females (50). Before methods for the measurement of CgA were available, PP used as a general marker for neuroendocrine tumors, although it is poorly specific. PP measurement is now useful in the diagnosis and monitoring of endocrine tumors where no other general specific marker is raised and in PPomas. Moreover PP is a rather unspecific marker which may be elevated in patients with diarrhea of different etiology and also in patients with diabetes mellitus.

4.6. Calcitonin (CT)

CT is measured by radioimmunoassay or by MIA. It is stable in blood and a fasting specimen is not required. Calcitonin is raised in medullary thyroid cancer (MTC) where concentrations may be more than a thousand-fold the reference range. In monitoring the progression of disease, attention should be paid to the method used for assay. Twenty five percent of MTC cases are familial where there is germ-line mutation on chromosome 10 (51). For this reason family members were screened for MTC and a pentagastrin stimulation test may be used to stimulate the secretion of Calcitonin in such subjects. MTC tumors frequently arise as a part of the multiple endocrine neoplasia type 2 (MEN2)-syndrome. Calcitonin may also be arisen in some pancreatic endocrine tumors, particularly those that are multi-hormone producing and in some non-endocrine cancers (52).

4.7. Urinary 5-hydroxyindoleacetic acid

Midgut carcinoid tumors are characterized by the production of serotonin. Serotonin, however, has proved difficult to measure reliably in the circulation. Traditionally the excretory products of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxy-triptamine have been measured in a 24-h urine collection from patients suspected for carcinoid. The 5-HIAA increases in typical carcinoid syndrome and therefore represents the crucial marker of ileal NETs associated with carcinoid syndrome Agranovich *et al*, found that patients with gastrointestinal carcinoids and an elevated 5-HIAA level had shorter survival than those with normal levels (53). Janson *et al* observed a significantly reduced survival among patients with carcinoid tumors who had an urinary 5-HIAA concentration above 300 micromol/day (54). In agreement, a study of patients with midgut carcinoid syndrome demonstrated that urinary 5-HIAA levels above 500 micromol/day were associated with a shorter survival (55). The degree of elevation of urinary 5-HIAA is also correlated with the severity of carcinoids symptoms, with the highest levels being observed in patients with carcinoid heart failure (55). The sensitivity in ileal NETs varies between 65% and 85%, depending on the series, while its specificity can reach 100%, when interfering drugs or foods are avoided in the 3 days preceding the urine collection. Patients must exclude items that are rich in serotonin from their diet before and during the urine collection. Banana, avocado, aubergine, pineapple, plums, walnut, paracetamol, fluorouracil, methysergide, naproxen and caffeine may cause false positive results. Levodopa, aspirin, adenocorticotrophic hormone (ACTH), methyldopa and phemothiazines may give a false negative result (5). Three-quarters of patients with midgut carcinoid excrete urinary 5HIAA as do approximately one-third of patients with foregut carcinoid. Patients with hindgut carcinoid do not excrete these products. The gold-standard method is high precision liquid chromatography, even if colorimetric or fluorimetric methods have been used in the past and are still used in non-referral laboratories as a first screening. Recently, an assay for the evaluation of plasmatic 5-HIAA, comparable with urinary methods, has been described, but has the advantage of being more acceptable and convenient for the patient (56, 57).

4.8. 5-hydroxy-tryptamine serotonin (5-HT) / 5-hydroxy-tryptophane (5-HTP)

NETs originating from foregut are rarely associated with 5-HT hyper-secretion, while secretion of 5-HT precursors such as 5-HTP, histamine or peptidic hormones may be more common. The atypical carcinoid syndrome can be considered the clinical counterpart of these secretions and may represent a life-threatening condition (40). It accounts for about 5% of these cases of carcinoid syndromes, and it is very often recognized with some delay, due to the variability and aspecificity of the clinical presentations. Some therapeutic procedures (chemo-embolization, laser treatment and others) may precipitate an atypical syndrome crisis and therefore its suspect and treatment is essential before invasive procedures. NETs of midgut often secret high levels of 5-HT and can be associated with secretion of many other vasoactive substances, such as tachynin A, prostaglandins, a kallikrein. These markers play a crucial role in the pathogenesis of flushing (usually less responsive to somatostatin analogues therapy). NETs of hindgut secrete 5-HT more rarely but can secrete many other GEP peptides and hormones (58).

4.9. Other circulating markers

Other markers play an interesting role in the secretory activity of NETs. To date it is possible to evaluate the circulating levels of the following hormones: vasoactive intestinal peptide (VIP), used in the diagnosis of VIPoma; gastrin, used in the diagnosis of gastrinoma; glucagon used in the diagnosis of glucagonoma and usually its concentration may be 10-100 times the reference range; insulin and pro-insulin are used in the diagnosis of insulinoma; somatostatin is raised in the circulation of patients with duodenal endocrine tumors (20%) (58) or hindgut carcinoid (30-35%) (59). Where elevations are two to four times the reference range. Somatostatin is grossly elevated in patients with somatostatinoma. This is a rare tumor most commonly originating in the pancreas and

frequently presents at a late stage with widespread metastases, due to the mild symptoms associated with this tumor; and ghrelin, a novel gastrointestinal hormone involved in several metabolic functions. Several studies have demonstrated elevated ghrelin levels in patients with NETs. In some patients with NETs strongly elevated levels of ghrelin have been found which prompted a new name "ghrelinomas" for these tumors, but the clinical picture of ghrelinoma is as yet unclear (60, 61).

5. PROGNOSIS ASSESSMENT

Apart from Ki-67 and CK19, a number of other prognostic markers have been evaluated by immunohistochemistry in NETs, with variable results. However, accuracy in the prognosis assessment may also derive from the application of the new proposed TNM classification system, specifically aimed to improve the histological classification of NETs and to define exactly and comparably the tumor-specific variables that are important for the prognosis and development of treatment plans (62). Moreover, DNA microarray technology emerged as a tool to better understand gene expression patterns that underlie tumor development. Considering neuroendocrine tumors, novel genes may be expressed in a highly differential manner, may have biological relevance in tumor-genesis and may be proved to be important also for tumor progression. A planned second-step protein analysis may, then, allow correlation with genes of potential interest in both a non-targeted and targeted manner.

Recently, for instance, the full malignant phenotype of pancreatic NETs has been proved to have four most highly up-regulated genes (among 112): FEV (member of the ETS family of oncogenic transcription factors), ADCY2 (adenylate cyclase 2) NR4A2 (nuclear receptor subfamily 4, group A, member 2), GADD45b (growth arrest and DNA-damage-inducible, beta) (63).
In sum, by improving the molecular classification of NETs, it is possible to enhance the ability to predict tumor behavior, provide important new insights into the molecular biology and tumor pathogenesis, and design the next generation of targeted therapies

6. CONCLUSIONS AND PERSPECTIVES

NETs are uncommon although various group of tumors. The correct correlation between tissue and circulating markers play a crucial role in the diagnosis and in the appropriate therapeutic approach. Moreover, some of these markers (tissue and circulating) play also a prognostic role. New molecular classification may provide further delineation of prognostic and therapeutic profiles. We believe that, considering the complexity and rarity of these tumors, it is necessary the development of centers of excellence to coordinate their studies and researches.

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8. REFERENCES

1. Ronald A DeLellis. The neuroendocrine system and its tumors: an overview. *Am J Clin Pathol* 115, S5 (2001)

2. Antony G Pearse. The cytochemistry and ultrastructure of polypeptide-hormone producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. *J Histochem Cytochem* 17, 303-313 (1969)

3. Carlo Capella, Philip U Heitz, Heinz Höfler, Enrico Solcia, Gunter Klöppel. Revised classification of neuroendocrine tumors of the lung, pancreas and gut. *Virchows Arch* 425, 547-560 (1995)

4. Eric Baudin, Jean-Michel Bidart, Philippe Rougier, Vladimir Lazar, Pierre Ruffié, Jacques Ropers, Michel Ducreux, Frédéric Troalen, Jean-Christophe Sabourin, Etienne Comoy, Philippe Lasser, Thierry DeBaere, Martin Schlumberger. screening for multiple endocrine neoplasia type 1 and hormonal production in apparently sporadic neuroendocrine tumors. *J Clin Endocrinol Metab* 84, 69-75 (1999)

5. Wouter W de Herder. Biochemistry of neuroendocrine tumors. *Best Pract Res Clin Endocrinol Metab* 21, 33–41 (2007)

6. Daniel T O'Connor, Ronald P Frigon. Chromogranin A, the major catecholamine storage vesicle solution protein. Multiple size forms, subcellular storage and regional distribution in chromaffin and nervous tissue elucidated by radioimmunoassay. *J Biol Chem* 259, 3237-3247 (1984)

7. Barry S Wilson, Ricardo V Lloyd. Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. *Am J Pathol* 115, 458-468 (1984).

8. Ricardo V Lloyd. Practical markers used in the diagnosis of neuroendocrine tumors. *Endocrine Pathology* 14, 293–301 (2003)

9. Shimon Schuldiner, Anat Shirvan, Michal Linial. Vesicular neurotransmitter transporters: from bacteria to humans. *Physiol Rev* 75, 369-392 (1995)

10. Jeffrey D Erickson, Martin K-H Schafer, Tom I Bonner, Lee E Eiden, Eberhard Weihe. Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proc Natl Acad Sci* 93, 5166-5171 (1996)

11. Rolf Eisselea, Martin Anlaufb, Martin K.-H. Schäferb, Lee E. Eidenc, Rudolf Arnolda, Eberhard Weiheb. Expression of vesicular monoamine transporters in endocrine hyperplasia and endocrine tumors of the oxyntic stomach. *Digestion* 60, 428-439 (1999)

12. Lucio Scopsi, Maria Gullo, Franco Rilke, Sean Martin, David F Steiner. Proprotein convertases (PC1/PC3 and PC2) in normal and neoplastic human tissues: their use as markers of neuroendocrine differentiation. *J Clin Endocrinol Metab* 80, 294-301 (1995)

13. Marco Volante, Francesca Bozzalla-Cassione, Mauro Papotti. Somatostatin receptors and their interest in diagnostic pathology. *Endocrine Pathology* 15, 275-292 (2004)

14. Hasan Kulaksiz, Rolf Eissele, Dirk Rössler, Stefan Schulz, Volker Höllt, Yalcin Cetin, Rudolf Arnold. Identification of somatostatin receptor subtypes 1, 2A, 3 and 5 in neuroendocrine tumors with subtype specific antibodies. *Gut* 50, 52-60 (2002)

15. Thomas O'Dorisio, Aaron Vinik. Pancreatic polypeptide and mixed peptide-producing tumors of the gastrointestinal tract. In Contemporary Issues in Gastroenterology. Eds: Cohen S, Soloway RD. Churchill-Livingstone, NY (1985)

16. Gareth T Williams. Endocrine tumors of the gastrointestinal tract-selected topics. *Histopathology* 50, 30-41 (2007)

17. Anja M Schmitt, Martin Anlauf, Valentin Rousson, Sonja Schmid, Andreas Kofler, Florian Riniker, Juliane Bauersfeld, Andre Barghorn, Nicole M Probst-Hensch, Holger Moch, Philipp U Heitz, Guenter Kloeppel, Paul Komminoth, Aurel Perren. WHO 2004 criteria and CK19 are reliable prognostic markers in pancreatic endocrine tumors. *Am J Surg Pathol* 31, 1677-1682 (2007)

18. Amitabh Srivastava, Osvaldo Padilla, Reiner Fischer-Colbrie, Arthur S Tischler, Yogeshwar Dayal. Neuroendocrine secretory protein-55 (NESP-55) expression discriminates pancreatic endocrine tumors and pheochromocytomas from gastrointestinal and pulmonary carcinoids. *Am J Surg Pathol* 28, 1371-1378 (2004)

19. Lori A Erickson, Bettina Papouchado, Haitham Dimashkieh, Shiwu Zhang, Naotoshi Nakamura, Ricardo V Lloyd. Cdx2 as a marker for neuroendocrine tumors of unknown primary sites. *Endocrine Pathology* 15, 247-252 (2004)

20. Stefano La Rosa, Elena Rigoli, Silvia Uccella, Anna Maria Chiaravalli, Carlo Capella. CDX2 as a marker of intestinal EC-cells and related well-differentiated endocrine tumors. *Virchows Archiv* 445, 248-254 (2004)

21. Yun-Cai Cai, Barbara Banner, Jonathan Glickman, Robert D Odze. Cytokeratin 7 and 20 and thyroid transcription factor 1 can help distinguish pulmonary from gastrointestinal carcinoid and pancreatic endocrine tumors. *Human Pathology* 32, 1087-1093 (2001)

22. Anna M O'Kane, Mark E O'Donnell, Rajeev Shah, Declan P Carey, Jack Lee. Small cell carcinoma of the appendix. *World J Surg Oncol* 6, 1-4 (2008)

23. Anjali Saqi, Diane Alexis, Fabrizio Remotti, Govind Bhagat. Usefulness of CDX2 and TTF-1 in differentiating gastrointestinal from pulmonary carcinoids. *Am J Clin Pathol* 123, 394-404 (2005)

24. Akihide Tanimoto, Yasumasa Matsuki, Tatsuo Tomita, Takakazu Sasaguri, Shohei Shimajiri, Yasuyuki Sasaguri. Histidine decarboxylase expression in pancreatic endocrine cells and related tumors. *Pathol Int* 54, 408-412 (2004)

25. Gerhard E. Feurle, Martin Anlauf, Gerd Hamscher, Rudolf Arnold, Günter Klöppel, Eberhard Weihe. Xenin-immunoreactive cells and extractable xenin in neuroendocrine tumors of the duodenal origin. *Gastroenterology* 123, 1616-1626 (2002)

26. Malcolm R Alison. Assessing cellular proliferation: what's worth measuring? *Hum Exp Toxicol* 14, 935-944 (1995)

27. Stefano La Rosa, Fausto Sessa, Filippo Bosi, Andrea Fabbri, Carlo Capella. Prognostic factors in nonfunctioning pancreatic endocrine tumors. *Lab Invest* 72, 54A (1995)

28. Gildas Rigaud, Edoardo Missiaglia, Patrick S. Moore, Giuseppe Zamboni, Massimo Falconi, Giorgio Talamini, Anna Pesci, Antonella Baron, Daniele Lissandrini, Guido Rindi, Piergiovanni Grigolato, Paolo Pederzoli, Aldo Scarpa. High resolution allelotype of nonfunctional pancreatic endocrine tumors: identification of two molecular subgroups with clinical implications. *Cancer Res* 61, 285-292 (2001)

29. Giuliano Pelosi, Giuseppe Zamboni, Claudio Doglioni, Stefania Rodella, Enrica Bresaola, Calogero Iacono, Giovanni Serio, Antonio Iannucci, Aldo Scarpa. Immunodetection of proliferating cell nuclear antigen assesses the growth fraction and predict malignancy in endocrine tumors of the pancreas. *Am J Surg Pathol* 16, 1215-1225 (1992)

30. Ayumu Goto, Tomoya Niki, Yuichi Terado, Junko Fukushima, Masashi Fukayama. Prevalence of CD99 protein expression in pancreatic endocrine tumors (PETs). *Histopathology* 45, 384-392 (2004)

31. Mina Jamali, Runjan Chetty. Predicting prognosis in gastroentero-pancreatic neuroendocrine tumors: an overview and the value of Ki-67 immunostaining. *Endocr Pathol* 19, 282-288 (2008)

32. Daniel T O'Connor, Leonard J Deftos. Secretion of chromogranin A by peptide-producing endocrine neoplasms. *N Engl J Med* 314, 1145-1151 (1986)

33. Leonard J Deftos. Chromogranin A: Its role in endocrine function and as an endocrine and neuroendocrine tumor marker. *Endocr Rev* 12, 181-187 (1991)

34. Gregor Schürmann, Ulrich Raeth, Bertram Wiedenmann, Heinrich Buhr, Christian Herfarth. Serum Chromogranin A in the diagnosis and follow-up of

neuroendocrine tumors of the gastroenteropancreatic tract. *World J Surg* 16, 697-701 (1992)

35. Laurence Guignat, Jean Michel Bidart, Maryalice Nocera, Emmanuel Comoy, Martin Schlumberger, Elisabeth Baudin. Chromogranin A and the alpha-subunit of glycoprotein hormones in medullary thyroid carcinoma and pheochromocytoma. *Br J Cancer* 84, 808-812 (2001)

36. Anna L Iacangelo, Lee E Eiden. Chromogranin A: a current status as a precursor for bioactive peptides and a granulogenic/sorting factor in the regulated secretory pathway. *Regul Pept* 49, 497-528 (1995)

37. Paola Tomassetti, Marina Migliori, Patrizia Simoni, Riccardo Casadei, Rossella De Iasio, Roberto Corinaldesi, Lucio Gullo. Diagnostic value of plasma chromogranin A in neuroendocrine tumors. *Eur J Gastroenterol Hepatol* 13, 55-58 (2001)

38. Kjell Oberg, Eva T Janson, Barbro Eriksson. Tumor markers in neuroendocrine tumors. *Ital J Gastroenterol Hepatol* 31, S160-S162 (1999)

39. Eric Baudin, Jean Michel Bidart, Anne Bachelot, Michel Ducreux, Daniel Elias, Pierre Ruffié, Martin Schlumberger. Impact of chromogranin A measurement in the work-up of neuroendocrine tumors. *Ann Oncol* 12, s79-82 (2001)

40. Piero Ferolla, Giuliano Faggiano, Gelsomina Mansueto, Nicola Avenia, Maria G Cantelmi, Paolo Giovenali, Maria Laura Del Basso De Caro, Francesco Milone, Giorgio Scarpelli, Stefania Masone, Fausto Santeusanio, Gabriela Lombardi, Gloria Angeletti, Annamaria Colao. The biological characterization of neuroendocrine tumors: The role of neuroendocrine markers. *J Endocrinol Invest* 31, 277-286 (2008)

41. Daniel T O'Connor, Leonard J Deftos. Secretion of chromogranin A by peptide-producing endocrine neoplasms. *N Engl J Med* 314, 1145-1151 (1986)

42. Frank R. E. Nobels, Dik J. Kwekkeboom, Willy Coopmans, Christiaan H. H. Schoenmakers, Jan Lindemans, Wouter W. De Herder, Eric P. Krenning, Roger Bouillon, Steven W. J. Lamberts. Chromogranin A as serum for neuroendocrine neoplasia: comparison with neuron-specific enolase and the α-subunit of glycoprotein hormones. *J Clin Endocrinol Metab* 82, 2622-2628 (1997)

43. Frank R. E. Nobels, Dik J. Kwekkeboom, Willy Coopmans, Ronnie Hoekstra, Roger Bouillon, Steven W. J. Lamberts. A comparison between the diagnostic value of the measurement of gonadotropins α-subunit and chromogranin A and their response to TRH in clinically non-functioning, alpha-subunit secreting and gonatroph pituitary adenomas. *J Clin Endocrinol Metab* 183, 200-213 (1993)

44. Eva T Janson, Lars Holmberg, Mats Stridsberg, Barbro Eriksson, Elvar Theodorsson, Erik Wilander, Kjell Oberg. Carcinoid tumors: analysis of prognostic factors and survival in 301 patients from a referral center. *Ann Oncol* 8, 685-690 (1997)

45. Dan Granberg, Mats Stridsberg, Rein Seensalu, Barbro Eriksson, Gudmar Lundqvist, Kjell Öberg and Britt Skogseid. Plasma chromogranin A in patients with multiple endocrine neoplasia type 1 *J Clin Endocrinol Metab* 84, 2712-2717 (1999)

46. Joy ES Ardill. Circulating markers for neuroendocrine tumors of the gastroenteropancreatic tract. *Ann Clin Biochem* 45, 539-559 (2008)

47. Tapan Shah, Rajaventhan Srirajaskanthan, Meetu Bhogal, Christos Toubanakis, Timothy Meyer, Amanda Noonan, Caroline Witney-Smith, Tarek Amin, Pamela Bhogal, Nat Sivathasan, Brian Warner, Daniel Hochhauser, Martyn E Caplin. Alpha-Fetoprotein and human chorionic gonadotrophin-b as prognostic markers in neuroendorine tumor patients. *Br J Canc* 99, 72-77 (2008)

48. Barbro Eriksson, Kjell Oberg, Mats Stridsberg, Tumor markers in neuroendocrine tumors. *Digestion* 62, 33-38 (2000)

49. Francesco Panzuto, Carola Severi, Renato Cannizzaro, Massimo Falconi, Silvia Angeletti, Alessandra Pasquali, Vito D Corleto, Bruno Annibale, Angela Buonadonna, Paolo Pederzoli, Gianfranco Delle Fave. Utility of combined use of plasma levels of chromogranin A and pancreatic polypeptide in the diagnosis of gastrointestinal and pancreatic endocrine tumors. *Journal of Endocrinol Invest* 27, 6-11 (2004)

50. Marion M O'Hare, John G Daly, Kyle D Buchanan. Radioimmunoassay for pancreatic polypeptide, and its age-related changes concentration. *Clin Chem* 29, 1923-1927 (1983)

51. Elizabeth A Fialkowski, Jeffrey F. Moley. Current approaches in medullary thyroid carcinoma, sporadic and familial. *J Surg Oncol* 2006, 9:737-47.

52. Hitoshi Ito, Jun Hata, Hiroshi Yokozaki, Eiichi Tahara. Calcitonin in human gastric mucosa and carcinoma. *J Cancer Res Clin Oncol* 112, 50-56 (1986)

53. Alexander L. Agranovich, George H. Anderson, Mohamed Manji, Brian D. Acker, Walter C. Macdonald, William J. Threlfall, Carcinoid tumor of the gastrointestinal tract: Prognostic facto and disease out come. *J Surg Oncol* 47, 45-52 (1991)

54. Eva T Janson, Lars Holmberg, Mats Stridsberg, Barbro Eriksson, Elvar Theodorsson, Eric Wilander, Kiell Oberg Carcinoid tumors: analysis of prognostic factors and survival in 301 patients from a referral center. *Ann Oncol* 8, 685-690 (1997)

55. Gunnel Westberg, Bo Wängberg, Hakan Ahlman, Claes-Hakan Bergh, Marie Beckman-Suurküla, Kenneth

Caidahl. Prediction of prognosis by echocardiography in patients with midgut carcinoid syndrome. *Br J Surg* 88, 865-872 (2001)

56. Tim J Degg, Keith R Allen, Julian H Barth. Measurement of plasma 5 hydroxyindoleacetic acid in carcinoid disease as an alternative to 24 h urine collection? *Ann Clin Biochem* 37, 724-726 (2000)

57. Joy ES Ardill, Bo Erikkson. The importance of the measurement of circulating markers in patients with neuroendocrine tumors of the pancreas and gut. *Endocr Relat Canc* 10, 459-462 (2003)

58. Irvin M Modlin, Mark Kidd, Igor Latich, Michelle N Zikusoka, Michael D Shapiro. Current status of gastrointestinal carcinoids. *Gastroenterology* 128, 1717-1751 (2005)

59. Barbro Eriksson, Kjell Oberg. Peptide hormones as tumor markers in neuroendocrine gastrointestinal tumors. *Acta Oncol* 30, 477-483 (1991)

60. Sabrina Corbetta, Maddalena Peracchi, Valentina Cappiello, Andrea Lania, Eleonora Lauri, Luca Vago, Paolo Beck-Peccoz, Anna Spada. Circulating ghrelin levels in patients with pancreatic and gastrointestinal neuroendocrine tumors: identification of one pancreatic ghrelinoma. *J Clin Endocrinol Metab* 88, 3117-3120 (2003)

61. Apostolos V Tsolakis, Guida M Portela-Gomes, Mats Stridsberg, Lars Grimelius, Anders Sundin, Barbro K Eriksson, Kjell E Öberg, Eva T Janson. Malignant gastric ghrelinoma with hyperghrelinemia. *J Clin Endocrinol Metab* 89, 3739-3744 (2004)

62 Ulrich-Frank Pape, Henning Jann, Jacqueline Müller-Nordhorn, Angelina Bockelbrink, Uta Berndt, Stefan N Willich, Martin Koch, Christoph Röcken, Guido Rindi, Bertram Wiedenmann Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors. *Cancer* 113, 256-265 (2008)

63. Eva-Maria Duerr, Yusuke Mizukami, Aylwin Ng, Ramnik J Xavier, Hirotoshi Kikuchi, Vikram Deshpande, Andrew L Warshaw, Jonathan Glickman, Matthew H Kulke, Daniel C Chung. Defining molecular classifications and targets in gastroenteropancreatic neuroendocrine tumors through DNA microarray analysis *Endocrine-Related Cancer* 15, 243-256, (2008)

Abbreviations NETs: Neuroendocrine tumors; DNEs: diffuse neuroendocrine system ; APUD : Amine Precursor Uptake Decarboxylation; WHO: World Health Organization; CGs: Chromogranins; NCAM, CD56: Neural cell adhesion molecule; WMATs : Vesicular monoamine transporters; EC: Enterochromaffin; ECL: Enterchromaffin-like cells; PCs: Prohormone convertase; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; NSE: Neuron-specific enolase; TTF-1: Thyroid transcription factor 1; GEP: Gastroenteropancreatic; NSE: Neuron-specific enolase; HCG : Human chorionic gonadotrophin; AFP: Alpha-fetoprotein; PP: Pancreatic polypeptide; CT: Calcitonin; 5-HIAA: Urinary 5-hydroxyindoleacetic acid; 5-HT: 5-hydroxy-tryptamine serotinine; 5-HTTP: 5-hydroxy-tryptophane; GEP: Gastroenteropancreatic; LH: Luteinizing hormone; FSH: Follicole stimulating hormone; TSH: Thyroid stimulating hormone; MTC: Medullary thyroid cancer; MEN2: Multiple endocrine neoplasia type 2

Key Words Neuroendocrine tumors, Clinical Biomarkers, Cancer, Diagnosis, Review

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