The limited role of Chromogranin A as circulating marker for diagnosis and management of neuroendocrine neoplasms

Fame and flaws of Chromogranin A as a circulating marker for diagnosis and management of neuroendocrine neoplasms

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Abstract

Owing to the heterogeneity of neuroendocrine neoplasms (NENs), the availability of reliable circulating markers is critical for improving diagnostics, prognostic stratification, follow-up, and definition of treatment strategy. This review is focused on Chromogranin A (CgA), a hydrophilic glycoprotein present in large dense core vesicles of neuroendocrine cells. Despite being long identified as the most useful NEN-related circulating marker, clinical application of CgA is controversial as limited by several issues. CgA assays still lack of standardization, thus hampering not only clinical management but also the comparison between different assays. In the diagnostic setting, the role of circulating CgA is limited by a) the variety of oncological and non-oncological conditions affecting marker levels, which impairs specificity; b) the fact that a remarkable portion of NENs show normal CgA, which impairs sensitivity. The role of CgA as prognostic marker is still controversial, but some prospective data indicate prognostic value in advanced disease. By contrast, on the basis of prospective studies, the trend of circulating CgA does not represent a valid marker of morphological evolution and has therefore no utility for the follow-up phase. Although current knowledge about the role of the marker in the definition of treatment strategy has to be considered poor on the basis of few and mainly retrospective studies, there is some prospective evidence that a) baseline CgA is not useful for predicting the impact of a medical treatment on survival; b) CgA response is promising for the early prediction of medical treatments efficacy.

1. Introduction

Despite being rare diseases, neuroendocrine neoplasms (NENs) have shown a worldwide increase in the past several decades, with incidence rates rising from 1.52 to 7.41 cases per 100,000 from 1973 to 2012 (Leoncini et al. 2017). Therefore, physicians dealing with NENs urgently need better guidance as to clinical management, which is still empiric (Faggiano et al. 2012; Oberg 2012). Actually, the definition of NENs gathers an heterogeneous group of diseases, including malignancies from several anatomic areas, such as stomach, intestine, rectum, pancreas, lung, adrenals, and thyroid, and with variable evolution, from indolent to rapidly progressive (Baudin 2007; Yao et al. 2008a). The feature joining these tumours is that they arise from specialized cells spread throughout the body, belonging to the so-called diffuse neuroendocrine system, whom main ability is to produce, store, and release into the bloodstream bioactive molecules (Ferolla et al. 2008; Kaltsas et al. 2004; Langley 1994). This biological activity causes characteristic syndromes that represent the major factor affecting the clinical *scenario* of NENs, which are accordingly classified into functional and non-functional (Kulke et al. 2012). Indeed, the former are usually diagnosed at an earlier stage because of endocrine symptoms related to the hormonal production, whereas the non-functional ones remain silent for large part of their natural history and are frequently diagnosed when metastases have already occurred (Modlin et al. 2008). Owing to these observations, possible clinical application of tumour-related bioactive products, as detected in the serum or plasma, has represented the objective of a wide body of research. Particularly, researchers aimed to identify markers useful for: a) diagnosis anticipation and refining; b) prognostic stratification; c) disease evolution monitoring and response to treatment. Basing on the relationship with codified hormone-related syndromes, circulating markers of NEN are differentiated in common or broad *spectrum*, including chromogranin A (CgA), pancreatic polypeptide, and neuron-specific enolase, and specific or individual, including serotonin and its metabolite 5-

hydroxyindolylacetic acid, gastrin, glucagon, insulin, C-peptide, vasoactive intestinal peptide, somatostatin, histamine, calcitonin, parathyroid, somatotropic, adrenocorticotropic hormones, catecholamines and their metabolites, and neuropeptides (Ferolla et al. 2008). The present review is focused on CgA, a hydrophilic glycoprotein abundantly expressed in large dense core vesicles of neuroendocrine cells, whose main biological role is to regulate calcium-mediated exocytosis (Borges et al. 2010). Consistently with the definition of common marker, elevated levels of circulating CgA have been associated to almost all types of NEN, including those arising from the gastroenteropancreatic tract and the bronchopulmonary area, which represent the majority, but also pheocromocytomas/paragangliomas, medullary thyroid carcinoma, Merkel cell carcinoma of the skin, and (even if data are controversial) pituitary and parathyroid adenomas (Blind et al. 1992; Campana et al. 2007; Guignat et al. 2001; Kimura et al. 1997; Nobels et al. 1997; Sobol et al. 1986; Tomassetti et al. 2001b; Zatelli et al. 2007). Despite having a long recognized role for the histological definition of NEN (Solcia 2000), the actual use of CgA as circulating marker revealed to be far more tricky than expected (Modlin et al. 2014c). Indeed, clinical utility of this test is affected by a variety of issues, which will be strictly analyzed in our review.

2. CgA physiology: production and biological functions

CgA belongs to the granin family, which also includes chromogranin B (CgB), secretogranins II and III, and other proteins (7B2, NESP55, proSAAS, and VGF). All of them are involved in a series of biological pathways controlling protein (peptides, hormones, neurotransmitters, and growth factors) secretion upon secretagogue stimulation (Arvan et al. 1991). Besides being stored into secretory vesicles, the members of the granin family have many common properties, such as a similar acidic isoelectric point, the capacity to bind calcium ions, and the ability to form aggregates. Furthermore, their structure typically includes multiple dibasic cleavage sites, which allow the processing into smaller peptides, each displaying a differential function (Borges et al. 2010; Gerdes et al. 1988; Helle & Corti 2015; Mahata et al. 2010; O'Connor & Frigon 1984; Sanchez-Margalet et al. 2010). Human CgA is encoded by the *CHGA* gene, located on chromosome 14q32.12. This 12.192 base pairs long gene, encompassing 8 exons and 7 introns, is transcribed into a 2.041 base pairs long mRNA, which is in turn translated into a 439-amino-acid mature protein (Winkler & Fischer-Colbrie 1992) showing 10 dibasic sites for proteolytic cleavage (Konecki et al. 1987). CgA-derived peptides include vasostatins (VST I: hCgA1–76 and VST II: hCgA1–115) (Aardal et al. 1993), pancreastatin (PST: hCgA357–428) (Tatemoto et al. 1986), catestatin (CST: hCgA352–372) (Mahata et al. 1997), a 14 amino acid peptide with N-terminal tryptophan and C-terminal glutamic acid (hCgA324–337), and serpinin (bCgA 403–428) (Koshimizu et al. 2010). As shown in Figure 1, CgA is synthesized at the rough endoplasmic reticulum, where it is inserted via the N-terminal signal peptide, and then transported to the Golgi complex (Kuehn et al. 1998). CgA is then packaged together with other secretory proteins (i.e. hormones and peptides) into immature granules, where it may be cleaved into the various derived peptides by specific processing enzymes. Upon acidification, secretory granules mature, thus becoming ready for stimulation-induced release (Kim et al. 2006). CgA and the derived peptides display several biological functions. VST I (1-76) and VST II (1-115) have vasodilator and antimicrobial properties. VST I has also been

demonstrated to inhibit PTH secretion, promote cell adhesion, and inhibit VEGF-induced endothelial cell proliferation/migration (Belloni et al. 2007; Blois et al. 2006; Ferrero et al. 2004). Furthermore, it promotes calcium entry into neutrophils (Zhang et al. 2009), indicating an immune-endocrine crosstalk. PST (357-428) induces hyperglycemia by inhibiting glucose-stimulated insulin release from β-cells (Tatemoto et al. 1986) and glucose uptake in adipocytes and hepatocytes (Gonzalez-Yanes & Sanchez-Margalet 2000) and by stimulating glucagon secretion and glycogenolysis (Sanchez-Margalet et al. 1992a; Sanchez-Margalet et al. 1992b). In addition, it inhibits PTH release and stimulates histamine release. CST (352-372) is a potent endogenous antagonist of the nicotinic cholinergic receptor, being able to inhibit nicotine-induced catecholamine secretion (Mahata et al. 2004; Mahata et al. 1997). CST has also been demonstrated to inhibit lypolisis and fatty acid oxidation by regulating adrenergic and leptin signalling (Borges et al. 2013). Due to its capacity to stimulate histamine release, CST acts as potent vasodilator (Kruger et al. 2003). Furthermore, it was found to induce endothelial cell proliferation/migration and to reduce cardiac contractility. Intact CgA (1-439) controls dense core granule biogenesis as well as sorting and secretion of other proteins. Specifically, it prevents uncontrolled osmotic swelling of secretory vesicles, functioning as a matrix condenser for soluble intra-vesicular component (Borges et al. 2013). CgA has been demonstrated to participate to the regulation of cytosolic calcium stores, granule exocytosis in secretory cells (Yoo 2010; Yoo et al. 2010), and prohormone convertase activity. In addition, it is involved in blood pressure regulation through the stimulation of the sympathetic tone (Dimsdale et al. 1992; Takiyyuddin et al. 1991). Notably, CgA processing into CST induces opposite effects on blood pressure, since CST inhibits catecholamine secretion (as already discussed). This is a paradigm of the complexity of biological effects related to CgA, which depend on the balance between the intact and cleaved protein.

3. Methods for circulating CgA determination: issue of reproducibility

The assessment of circulating plasma or serum CgA levels can be performed by several commercially available kits, which differ in methodology but all rely on antibody-dependent assays such as enzyme-linked immunosorbent assay (ELISA), immunoradiometric assay (IRMA), radioimmunoassay (RIA) or immunofluorescent assay based on Time Resolved Amplified Cryptate Emission (TRACE). Recently, an alternative method has been described: Minamiki et al. were able to rapidly detect CgA with high sensitivity by means of an extended-gated organic field-effect transistor (OFET)-based immunosensor employing a non labeled monoclonal anti-CgA antibody (Minamiki et al. 2016). A study performed by measuring both plasma and serum CgA levels by a two-site chemiluminescence immunometric assay using a biotinylated monoclonal antibody displayed a strong positive linear correlation between the two measures ($r=0.9858$, $P<.0001$), suggesting that CgA levels could be reliably investigated in both sample types [Woltering EA, Hilton RS, Zolfoghary CM, et al. Validation of serum versus plasma measurements of chromogranin a levels in patients with carcinoid tumors: lack of correlation between absolute chromogranin a levels and symptom frequency. Pancreas 2006;33(3):250–4]. Similar results were reported by Glinicki et al. [Glinicki P, Jeske W, Kapuścińska R, Zgliczyński W. Comparison of chromogranin A (CgA) levels in serum and plasma (EDTA2K) and the respective reference ranges in healthy males. Endokrynol Pol. 2015;66(1):53-6. doi: 10.5603/EP.2015.0009] who found a good correlation between serum and plasma CgA ($r=0.8493$; $p<0.01$) by using the CIS-bio IRMA. However, plasma CgA levels were significantly higher as compared serum CgA level, probably due to matrix effect of each biological material. In addition, each assay is performed by using different antibodies, with varying sensitivity and specificity. As a consequence, different kits may lead to significantly different results, hampering the possibility to pool and/or compare data obtained by different research centers with different assays (Gut et al. 2016). Indeed, results from antibody-dependent assays are strikingly influenced by the employed antibodies. It has been reported that three different ELISA assays display different specificity for full-length CgA and its fragments, due to the use of the same capture antibody against CgA or VST I N-terminal regions coupled with

three different detection antibodies against epitopes located in the central region of CgA, or against the six C-terminal residues of full-length CgA, or the six C-terminal residues of VST I. Indeed, these assays could detect intact and processed CgA, only intact CgA, or only VST I, respectively (Helle & Corti 2015). Therefore, the detection of intact/cleaved CgA depends on the employed antibody. Sensitivity and specificity of available methods have been compared by a number of studies. Stridsberg et al. (Stridsberg et al. 2003) came to the conclusion that the best compromise between sensitivity and specificity is the use of RIA. On the other hand, a prospective multicenter study demonstrated that ELISA and IRMA methods display a good diagnostic performance, providing results that are comparable and showing a satisfactory correlation ($r= 0.843$, $p < 0.0001$) (Leon et al. 2005). However, these Authors also showed a 36% discordance rate between the two methods, confirming previous findings (Ferrari et al. 2004) and suggesting that they might provide partially different information. These results were further strengthened by a multi-center study comparing a two-step IRMA (IRMA; CGA-RIA CT, CIS-bio international-Shering, Gif-sur-Yvette, France) and an ELISA assay (DAKO Cytomation, Glostrup, Denmark). The employed IRMA assay is based on two monoclonal antibodies raised against CgA unprocessed central domain (CgA 145–245), thus detecting total human CgA. On the other hand, the employed ELISA assay measures more CgA fragments, since it is based on two polyclonal rabbit antibodies directed towards a 23 kDa C-terminal CgA fragment. The results of the two assays were found to correlate and a ROC analysis found a cutoff of 53 ng/ml for IRMA and 16 U/l for ELISA as discriminating between controls and patients with active gastroenteropancreatic NENs (sensitivity 71.3 and 84%; specificity 71 and 85%, respectively) (Zatelli et al. 2007).

Further studies compared different CgA assays. The results of the TRACE assay (KRYPTOR) have been compared with those of an ELISA kit (DAKO), which use is consolidated in clinical practice [Wolf M, Riedlinger I, Lehmann R, Häring HU, Schleicher E, Peter A. Comparison of the automated KRYPTOR chromogranin A assay with the DAKO ELISA. Clin Lab. 2014;60(12):2103-6]. The TRACE assay on the automated KRYPTOR immunoanalyzer (Thermo Fisher Scientific) is

a sandwich immunofluorescent assay, using two mouse monoclonal antibodies, based on a non-radioactive energy transfer between a donor (europium cryptate) and an acceptor (XL665). The study by Wolf et al. showed an excellent correlation between serum samples measured with the first generation KRYPTOR assay and those measured in plasma with the ELISA assay (r = 0.99). The workflow of the KRYPTOR assay was reported to be much faster than the ELISA assay, but the former method seems to be more sensitive to the storage temperature of the samples. In addition, the KRYPTOR assay issues different CgA levels depending on the sample origin (higher when starting from serum as compared to plasma), suggesting a possible interference by other analytes. The assay sensitivity has been explored by Popovici et al. [Popovici T, Moreira B, Schlageter MH, Bories PN. Automated two-site immunofluorescent assay for the measurement of serum chromogranin A. Clin Biochem. 2014 Jan;47(1-2):87-91.], who found that clinical sensitivity of KRYPTOR CgA for diagnosis of pheochromocytoma/paraganglioma and GEP NENs is 100 and 94%, respectively. In addition, the Authors found a good correlation between the KRYPTOR and RIA assay. A further study compared the results of the KRYPTOR assay with those of a solid-phase ELISA assay (CisBio) in serum samples [R.H.P. van der Knaap, D.J. Kwekkeboom, C.R.B. Ramakers, Y.B. de Rijke. Evaluation of a new immunoassay for chromogranin A measurement on the Kryptor system. Practical Laboratory Medicine, 1(2015): 5–11]. The Authors found that CgA levels measured with the KRYPTOR assay were significantly higher as compared to those measured by the ELISA CisBio assay, independently of gender, use of proton pump inhibitors, renal function, referral department and tumor location. Storage at low temperature (-20°C) seems to be crucial for the analyte recovery, also indicating a low stability of the first generation assay with time. The issue of the decay in CGA concentrations measured by the first generation KRYPTOR assay has been addressed by developing a second generation assay, which uses two monoclonal antibodies recognizing different CgA epitopes, with a reduced impact of protein folding on CgA measurement (Ferraro S, Borille S, Panteghini M. Reference intervals for the Kryptor second-generation Chromogranin A assay. Clin Chem Lab Med. 2016 Nov 1;54(11):e335-e337). In this

study, the Authors also investigated reference ranges of the most recent KRYPTOR assay, identifying an upper reference limit of 88 μg/L, similar to that found for the first-generation assay (94 μg/L) (Ferraro S, Mozzi R, Michelazzo C, Basco D, Panteghini M. Reference intervals for the new Brahms chromogranin A (CGA) assay. Biochim Clin 2013;37:S169). The utility of this improved assay has been also demonstrated in studies exploring the value of CgA as a predictive marker in tumors different from NENs [Niedworok C, Tschirdewahn S, Reis H, Lehmann N, Szücs M, Nyirády P, Romics I, Rübben H, Szarvas T. Serum Chromogranin A as a Complementary Marker for the Prediction of Prostate Cancer-Specific Survival. Pathol Oncol Res. 2017 Jul;23(3):643-650]. However, the application of this method in clinical practice is still limited.

The bottom line of all these studies is that different assays provide different information and therefore ideally the same assay should be used from diagnosis to follow-up in a single patient in order to obtain reliable and clinically useful results.

4. Circulating CgA in the diagnostic phase of NEN

The definition of CgA metrics in NEN diagnostics is not univocal, since it is hampered by the wide variability of analytical approaches applied by the various studies.

The most critical discrepancies were: a) the composition of the case-groups, with the strongest difference being represented by the fact that some studies included NEN from all sites (Bernini et al. 2001; Donica et al. 2010; Korse et al. 2012; Marotta et al. 2012; Molina et al. 2011; Tohmola et al. 2014) whereas some others tried to analyze more homogeneous set of patients, mainly selecting gastroenteropancreatic tumours (Belli et al. 2009; Modlin et al. 2013; Nehar et al. 2004; Peracchi et al. 2003; Tomassetti et al. 2001a; Zatelli et al. 2007) (Table 1.); b) the composition of the control-groups: although the majority of studies used healthy subjects as controls (Belli et al. 2009; Bernini et al. 2001; Campana et al. 2007; Donica et al. 2010; Korse et al. 2012; Molina et al. 2011; Nehar et al. 2004; Peracchi et al. 2003; Tomassetti et al. 2001b; Zatelli et al. 2007), which represents the best

approach to assess the diagnostic performance of a marker (Shapiro 1999), some researchers determined the metrics of circulating CgA by comparing NEN with non-NEN tumours (Nobels et al. 1998; Panzuto et al. 2004) or active versus disease-free NEN (Bajetta et al. 1999; Panzuto et al. 2004); c) the consideration of interfering factors: some authors tried to clean up the control-group from those conditions with known effect on CgA levels, thus obtaining a more pristine evaluation of marker specificity (Belli et al. 2009; Bernini et al. 2001; Campana et al. 2007; Molina et al. 2011; Nehar et al. 2004; Tomassetti et al. 2001b; Zatelli et al. 2007), whereas some others did not, thus providing data actually applicable into real-life practice (Donica et al. 2010; Korse et al. 2012; Marotta et al. 2012; Modlin et al. 2013; Peracchi et al. 2003; Tohmola et al. 2014) (Table 1.).

4.1 Specificity

A wide range of conditions, both benign and malignant (Table 2.), can induce NEN-unrelated CgA elevations, thus generating false-positive results (Ardill & O'Dorisio 2010). This strikingly hampers test specificity, which is considered as the major weakness of circulating CgA in the diagnostic setting of NEN (Kidd et al. 2016; Modlin et al. 2010a).

4.1.1 Non-oncological causes of CgA elevation

Real-life application of circulating CgA for NEN diagnostics is hampered by a variety of interfering non-oncological conditions, including benign diseases and iatrogenic causes, which are extremely common. These conditions strikingly affect test specificity and should mandatorily be considered by clinicians when interpreting CgA values.

Since secretion of CgA is ubiquitary (Lamberts et al. 2001), a variety of non-neoplastic processes inducing tissue damage and remodelling may produce elevations of the marker. These include a variety of gastrointestinal disorders, such as chronic atrophic gastritis (CAG) (Peracchi et al. 2005), Helicobacter pylori infection (Waldum et al. 1996), liver cirrhosis and chronic hepatitis (Spadaro et

al. 2005), pancreatitis (Malaguarnera et al. 2009), inflammatory bowel diseases (Sciola et al. 2009), and even irritable bowel syndrome (Sidhu et al. 2009). Among cardiovascular diseases, elevated CgA levels have been reported in hypertension, with higher levels being demonstrated in untreated patients (Takiyyuddin et al. 1995), chronic heart failure, where more accentuated elevations were detected in the fourth grade of the NHYA scale (Ceconi et al. 2002), and acute coronary syndromes, where higher concentrations predicted worsened outcome (Jansson et al. 2009). Other benign conditions in ducing an increase in CgA levels include some rheumatoid diseases such as giant cell arteritis, rheumatoid arthritis, and systemic lupus erythematosus (Di Comite et al. 2009a; Di Comite et al. 2009b) and pulmonary obstructive disease (Hoshino et al. 2008). Due to reduced clearance, elevation of circulating CgA also occurs in case of kidney and liver functional impairment (O'Connor et al. 1989). Particularly, the grade of renal dysfunction is directly related to CgA levels and may lead to concentrations as high as those detected in NEN patients (Hsiao et al. 1990). Ultimately, increased CgA levels have been also reported in endocrine disorders of non-neuroendocrine nature, such as hyperthyroidism, likely due to enhanced sympathetic activity which pairs to attenuation of the vagal tone (Al-Shoumer & Vasanthy 2009). The main iatrogenic cause of CgA elevation is use of proton pump inhibitors (PPIs) and other acid blocking drugs, which are largely administered by physicians (Fossmark et al. 2008). Indeed, inhibition of gastric acid production leads to compensative hypergastrinemia and G-cell hyperplasia, which in turn induce ECL-cell hyperplasia. Both G- and ECL- hyperplasia are responsible for CgA overproduction (Kuipers 2006). The role of the reported non-oncological conditions in affecting specificity of circulating CgA as diagnostic marker of NEN emerges when comparing Receiving-Operator Characteristics (ROC) analyses of studies trying to skim non-neoplastic controls for the presence of possible false-positives inductors with those not performing any selection (Table 1.). Indeed, authors applying the former approach reported remarkable specificity values, ranging from 95 to 100% (Belli et al. 2009; Bernini et al. 2001; Campana et al. 2007; Molina et al. 2011; Nehar et al. 2004; Tomassetti et al. 2001b), with the only exception of Zatelli et al. (Zatelli et al. 2007), who did

not rule out CAG. By contrast, specificity was less than 90% in the majority of studies where exclusion of interfering conditions was not performed (Donica et al. 2010; Marotta et al. 2012; Peracchi et al. 2003; Tohmola et al. 2014; Vezzosi et al. 2011). Furthermore, some authors specifically assessed the effect of benign conditions on test specificity by comparing the same cohort of NENs with separate groups of healthy subjects and patients carrying one or more interfering diseases (Table 1.). Campana et al. (Campana et al. 2007) selected a separate cohort of CAG patients reporting a remarkable drop, from 95.8 to 61.4%, of CgA specificity. More recently, Molina et al. (Molina et al. 2011) analyzed a separate group of patients with renal failure, gastric diseases, heart failure, liver cirrhosis, hypertension, and inflammatory bowel diseases, showing a dramatic reduction in test specificity, falling from 100% to less than 50%.

4.1.2 Oncological causes of CgA elevation

The actual impact of CgA elevation related to tumours other than NEN on test specificity is not univocal, due to the heterogeneity of available studies. However, the aim of future research should be to define performance of circulating CgA in differentiating NENs from those non-neuroendocrine malignancies posing an issue of differential diagnosis.

A variety of non-NEN malignancies are characterized by increased CgA levels (Glinicki & Jeske). The majority of them present histological pattern of neuroendocrine differentiation, including several digestive tumours, such as colorectal adenocarcinoma (Syversen et al. 1995), gastric and pancreatic cancer (Malaguarnera et al. 2009), and prostate adenocarcinoma (Angelsen et al. 1997). By contrast, there are some tumours showing CgA elevation where the presence of histological neuroendocrine differentiation has not been reported, such as primary hepatocellular cancer (Spadaro et al. 2005) and breast cancer (Giovanella et al. 2001). To date, the capability of circulating CgA in discriminating NEN from other malignancies has been evaluated by many studies, which reported controversial results characterized by wide variation of specificity values

(Marotta et al. 2012; Molina et al. 2011; Nehar et al. 2004; Nobels et al. 1998; Panzuto et al. 2004) (Table 3.). This was likely due to the heterogeneity of both NEN groups and neoplastic controls, with the latter including different tumour types. However, the mentioned papers do not provide a real picture of clinical practice, where what is actually required is to distinguish NENs from non-neuroendocrine malignancies posing to clinicians issues of differential diagnosis. This is particularly crucial for non-functional NENs, given the absence of the distinctive clinical and biochemical features related to hormone overproduction (Kulke et al. 2015). To date, poor data are available about this issue. Some authors focused on the possible role of circulating CgA in determining differential diagnosis between pancreatic NEN and the various pancreatic malignancies, such as ductal adenocarcinoma, cystic tumors, solid pseudopapillary tumors, acinar cell carcinoma, squamous cell carcinoma, lymphoma, and metastatic lesions (Mulkeen et al. 2006). This is a challenging clinical issue as, despite the high specificity demonstrated by CT and MRI (Ichikawa et al. 2000; Sundin et al. 2009), radiological phenotype of pancreatic NENs is variable, thus hampering instrumental diagnosis (Singhi et al. 2012). The retrospective analysis by Paik et al. (Paik et al. 2013) reported only 56% specificity of the marker in differentiating pancreatic NENs from other pancreatic masses, whereas the prospective study by Jun et al. (Jun et al. 2017), who specifically focused on patients suspected for a pancreatic NEN, showed a higher value, namely 77.8%, which even rose to 100% when selecting lesions larger than 4 cm. However, these data are not conclusive and the role of circulating CgA for pre-surgical diagnosis of pancreatic NENs needs to be addressed in other clinical series.

4.2 Sensitivity

Sensitivity of circulating CgA can be considered as acceptable for functional and advanced NENs and extremely poor for localized non-functional disease. Since diagnosis of the latter is the most challenging for clinicians, this strongly limits clinical utility of the test.

According to available studies (Table 1.), sensitivity of circulating CgA for the diagnosis of NENs vary from 60 to 100% (Oberg 2011). This represents a significant range of variation, which does not allow to define test sensitivity as acceptable or not. Actually, this is due to the fact that CgA levels are tightly related to disease-related features. Particularly, the main factors affecting the rate of abnormal CgA, which determines the sensitivity of the test, are tumour function and disease extent. Regarding the former, Janson et al. (Janson et al. 1997) firstly reported a remarkable rate of CgA elevation, namely 86.1%, in a large cohort of functional NENs from different sites. More recently, Nehar et al. (Nehar et al. 2004), focusing on a population of gastroenteropencreatic NENs, found CgA alterations in 70% of secreting tumours, whereas only 40% of the non-functional ones showed positivity for the CgA test. Regarding the role of disease extent, the Nehar study (Nehar et al. 2004) also found a dramatic difference in the rate of CgA elevation between metastatic and non-metastatic patients, namely 73 versus 26%. This dramatic impact on CgA sensitivity was further confirmed by Nikou et al. (Nikou et al. 2008), analyzing a cohort of non-functional pancreatic NEN, who found CgA alterations in the totality of patients with liver metastases, whereas the rate was much lower, 66.6%, in those subjects without liver involvement. According to these data, sensitivity of the CgA test can be considered as acceptable only for functional and advanced NENs. Recently, Jilesen et al. (Jilesen et al. 2014), analyzing a cohort of non-metastatic non-functional pancreatic NENs, found CgA elevation in only 27% of cases, further demonstrating the poor marker sensitivity in early non-functional disease. This strikingly limits clinical utility of circulating CgA as diagnosis of functional and metastatic NENs is mainly obtained by specific biomarkers and imaging modalities or biopsy, respectively (Jensen et al. 2012; Pavel et al. 2012), whereas a stronger support from biochemistry would be specifically required for localized non-secreting NENs, where diagnosis is more challenging.

4.3 Diagnostic role of circulating CgA in particular clinical settings

4.3.1 CAG and inflammatory bowel diseases

CAG and inflammatory bowel diseases, already defined as non-oncological causes of CgA elevation, represent for clinicians a diagnostic challenge as they also predispose to NEN development through stimulating proliferation of neuroendocrine cells (Ruszniewski et al. 2006; West et al. 2007). The possible role of circulating CgA in discriminating patients with CAG and inflammatory bowel diseases who develop gastric (type I) and intestinal NEN, respectively, has been tested by few studies, with not encouraging results. Peracchi et al. (Peracchi et al. 2005) found higher CgA levels in CAG-patients with gastric NEN, as compared with those without, but specificity of the test was extremely poor (23%), whereas Sciola et al. (Sciola et al. 2009) reported non-significant differences in CgA levels between inflammatory bowel diseases with and without concomitant intestinal NEN.

4.3.2 Multiple Endocrine Neoplasia (MEN)-1 related NENs

MEN-1 is an autosomal dominant hereditary syndrome predisposing to the development of a variety of NENs. The most common are pancreatic NENs, occurring in 40-70% of patients, whereas gastric, bronchial, and thymic tumours are less frequent (Thakker et al. 2012). Importantly, NENs represent the main cause of MEN-1 related death (Goudet et al. 2010). Therefore, a proper screening approach is required in order to detect NENs as early as possible, thus obtaining a reduction in MEN-1 related morbidity and mortality (Pieterman et al. 2009). To date, the standard of care suggest annual CgA determination, but this statement is defined as “low quality” (Thakker et al. 2012). Indeed, the actual value of circulating CgA as screening test for NEN diagnosis in patients with MEN-1 is still controversial as it has been assessed by very few studies. In 2003,

Peracchi et al. (Peracchi et al. 2003) found CgA alterations in the vast majority, 15 out of 16 cases, of MEN-1 patients affected with gastroenteropancreatic NENs, and this was consistent with a possible use of CgA as screening test. Nevertheless, 2 recent publications (de Laat et al. 2013; Qiu et al. 2016), analyzing larger cohorts of MEN-1 subjects and specifically focusing on the detection of pancreatic NENs, provided opposite results. Indeed, both research groups consistently demonstrated low diagnostic accuracy for CgA, also reporting poor sensitivity values, which make not feasible to use the marker as screening test.

5. Circulating CgA as prognostic marker in NEN

Despite having a demonstrated relationship with tumour load, the actual prognostic effect of circulating CgA is still controversial, due to the availability of a low number of prospective studies. However, available evidence is consistent with a significant prognostic impact of the marker in advanced disease.

Circulating CgA has long been used as an indirect survival predictor in clinical practice of NEN management. This was due to the well-demonstrated relationship with disease stage/extent, which represents the main predictor of clinical outcome (Ahmed et al. 2009). Indeed, the majority of authors demonstrated higher marker levels in patients with extensive metastases, as compared with those having localized disease or even limited hepatic involvement (Campana et al. 2007; Janson et al. 1997; Nehar et al. 2004; Nikou et al. 2008; Tomassetti et al. 2001b; Zatelli et al. 2007). Furthermore, Arnold et al. (Arnold et al. 2008) reported a direct correlation between CgA increase and the extent of liver involvement. Nevertheless, such correlation is not valid for all NEN types as CgA levels may be affected by many other clinico-pathological features. As an example, due to direct tumour secretion and gastrin-induced ECL-cell hyperplasia, non-metastatic gastrinomas show CgA levels as high as those reported in metastatic non-functional pancreatic NENs (Janson et al. 1997), therefore the association with disease load as well as the indirect prognostic significance are

lost in this case. Hence, the actual prognostic impact of circulating CgA can be assessed only analyzing the direct relationship with survival. To date, the vast majority of studies performing this kind of analysis are retrospective, thus providing low quality scientific evidence. However, all of them found that high serum CgA levels had a negative impact on survival (Arnold et al. 2008; Citterio et al. 2017; Ekeblad et al. 2008; Janson et al. 1997; Nanno et al. 2017; Nikou et al. 2008). Of note, studies focusing on advanced disease have to be considered the most important, since a proper prognostic stratification is mandatory in this setting. Arnold et al. (Arnold et al. 2008) showed that plasma CgA levels were related to survival time in a cohort of 344 patients with metastatic, well-differentiated NENs of gastroenteropancreatic origin. More recently, Citterio et al. (Citterio et al. 2017) evaluated a more homogeneous set of patients including 139 well-differentiated NENs with metastatic liver involvement. Authors identified basal CgA levels less than 200 ng/ml as a positive prognostic factor, and this result was confirmed after multivariate analysis. Actually, the prognostic significance of circulating CgA in advanced disease found some prospective confirmation when analyzing data from the interventional RADIANT-1, -2, and -3 studies. Considering the whole study populations and independently of the predictive role of response to the tested treatments, all these trials reported longer overall survival (OS) for patients with CgA levels less than 2 fold the upper normal limit (Pavel et al. 2017b; Yao et al. 2010; Yao et al. 2016; Yao et al. 2008b). Furthermore, a recent prospective assessment of prognostic factors of survival in NENs with metastatic liver involvement demonstrated normal values of circulating CgA as independent favourable prognosticator (Fairweather et al. 2017). To date, data about localized disease are extremely poor. Recently, Nanno et al. (Nanno et al. 2017) analyzed a cohort of resectable, well-differentiated pancreatic NENs finding that preoperative serum CgA levels were significantly higher in patients with post-operative recurrence, as compared to those without recurrence. However, these findings need further confirmation in independent series.

6. Circulating CgA in the follow-up phase of NEN

The follow-up phase of NENs essentially includes 2 clinical situations: a) patients being cured after surgery (R0 resection), where the objective is to identify relapses; b) subjects with more advanced tumour who do not or cannot achieve a disease-free status, where the objective is to monitor morphological evolution, in order to detect transition from stable to progressive disease (Modlin et al. 2010b). Since these patients can be subjected to a variety of treatments, which are usually administered sequentially or even simultaneously during the course of the disease, monitoring the tumour slope represents the mainstay for a proper clinical management. Here we report current evidence about the value of circulating CgA in each of the described settings.

6.1 Detection of tumour relapse after curative surgery

Evidence of the role of circulating CgA in this setting is still poor as based on few and controversial studies.

Initially, a retrospective study of 56 patients was consistent with a possible value of CgA as marker of disease recurrence in midgut NENs subjected to radical surgery. Authors showed that CgA rising above the normal range represented the first indicator of trecurrence, even anticipating 5-hydroxyindolylacetic acid increase and instrumental examinations (Welin et al. 2009). Therefore, a twice-a-year CgA determination together with transabdominal ultrasonography was proposed as a feasible follow-up scheme. In contrast to these findings, a recent study providing prospective evaluation of 15 R0-resected gastroenteropancreatic NENs reported no CgA elevation in the 2 subjects developing recurrence who had elevated pre-surgical levels (Modlin et al. 2016). Despite the low number of cases, this was consistent with a poor utility of the marker in the follow-up of NENs after curative surgery.

6.2 Assessment of morphological evolution in patients with non cured disease

On the basis of recently published prospective studies, the trend of circulating CgA over time cannot be considered as a valid marker of morphological evolution of NENs with persistent disease and its utility in the follow-up of these patients is therefore poor.

Initially, promising insights about the relationship between circulating CgA and morphological evolution of non cured NENs were provided by 2 retrospective studies (Bajetta et al. 1999; Nehar et al. 2004). Both research groups found high concordance between CgA changes and tumour slope, demonstrating that marker elevation higher than 25% was a highly sensitive predictor (83 and 89%, respectively) of tumour progression. In contrast to these findings, a more recent retrospective study by Walter et al. (Walter et al. 2012) found that marker changes were consistent with morphology in only 51% of cases and that a significant CgA elevation, defined as an at least 50% increase, was detectable in only 56% of patients with progressive disease. More definite evidence about this issue has emerged in last 2 years, with 2 prospective reports demonstrating the poor capability of CgA changes in reflecting morphological behavior of NENs. In 2015, Cwikla et al. (Cwikla et al. 2015) prospectively analyzed a cohort of 28 non cured gastroenteropancreatic NENs treated with somatostatin analogues (SSA). Considering an at least 25% increase as cut-off, authors found low concordance, namely 64%, between CgA modifications and tumour slope. Particularly, only 57% of progressing patients showed significant CgA increase. Similarly, a 2017 study by Pavel et al. (Pavel et al. 2017a) of 34 advanced gastroenteropancreatic NENs subjected to various treatments, which also considered 25% increase as cut-off, reported only 40% concordance between morphological behavior and CgA modifications.

7. Circulating CgA for the definition of treatment strategy

Currently, a variety of tools are available for NEN treatment. Besides surgery, radical or debulking, these include several medical therapies (SSA, interferon alfa, targeted agents such as tyrosine kinase and mTOR inhibitors, and chemotherapy), PRRT, and loco-regional treatments

(radiofrequency ablation and transarterial embolization) (Del Prete et al. 2014; Fiore et al. 2014; Frilling et al. 2012; Marotta et al. 2013; Modlin et al. 2010b; Oberg et al. 2010; Pavel et al. 2012; Ramundo et al. 2014). This poses 2 major challenges for clinicians, who are required a) to choose the best therapy for each specific patient; b) to test as early as possible effectiveness of the chosen approach in order to perform a prompt adjustment of treatment strategy. Here we analyze the current role of circulating CgA in this context.

7.1 Baseline CgA as predictive marker of treatment efficacy

The best available evidence, based on prospective placebo-controlled studies, shows no role of baseline CgA as predictive marker of response to treatment.

Actually, promising insights about this issue were initially provided from a dedicated analysis extrapolated from the phase II RADIANT-1 study of everolimus in advanced pancreatic NETs (Yao et al. 2011a). Indeed, authors demonstrated that CgA levels >2 fold the upper normal limit were associated to significant reduction in both PFS and OS. However, the absence of a placebo group strongly limited the validity of the reported relationship, which could be simply due to the prognostic effect of CgA rather than to an actual interaction with the treatment. This thesis was subsequently confirmed by RADIANT-2 and -3, which were randomized, placebo-controlled trials of everolimus in advanced NENs with carcinoid syndrome under SSA treatment and advanced pancreatic NENs, respectively (Pavel et al. 2011; Yao et al. 2011b). Indeed, recent *post-hoc* analyses of both studies specifically assessed whether baseline CgA levels were only prognostic or had actual capability of predicting treatment effect on OS (Pavel et al. 2017b; Yao et al. 2016; Yao et al. 2017). This was done by adjusting OS of the 2 study arms for pre-treatment CgA levels, which were imbalanced. Authors concluded that baseline CgA was not predictive of everolimus impact on outcome. Recently, the lack of predictive value of pre-treatment marker levels was also found for NENs subjected to PRRT, an established therapeutic modality mainly used for inoperable or

metastatic gastroenteropancreatic NENs (van der Zwan et al. 2015). Indeed, a recent prospective study by Bodei et al. (Bodei et al. 2016) showed no impact of elevated CgA (>600 ng/ml) on morphological response and PFS.

7.2 CgA response as predictive marker of treatment efficacy

Basing on subanalyses of some placebo-controlled studies, CgA response can be considered as a promising efficacy predictor of medical therapies. Regarding surgery and PRRT, available evidence shows no value of CgA changes as predictive marker of response, but the absence of a control group strongly limits the validity of these data.

The mentioned analysis from the RADIANT-1 reported that, among patients with elevated baseline levels, an early CgA response, defined as an at least 30% reduction of the marker at 4 weeks treatment, was predictive of morphological response, PFS, and OS (Yao et al. 2011a). This was consistent with the previous retrospective observation that an early CgA decrease was associated to improved RECIST response and clinical outcome in pancreatic NENs subjected to streptozocin-based chemotherapy (Kouvaraki et al. 2004). As previously discussed, these findings were intrinsically limited by the absence of a control group. However, the role of CgA reduction as predictive marker of response to medical therapies has found some confirmation through dedicated subanalyses of placebo-controlled trials. In 2011, a contribution to the European Society for Medical Oncology Congress, based on data from the RADIANT-2 trial, confirmed that early CgA responders had longer PFS, as compared with non-responders (Baudin et al. 2011). More recently, a subanalysis of the CLARINET study, a randomized phase III trial of lanreotide in advanced NENs, showed that a decrease in CgA was associated to reduced hazard of disease progression (Buil-Bruna et al. 2016). To date, no studies assessing predictive role of CgA modifications in NENs treated with surgery and PRRT include the presence of a control group of untreated patients. The mentioned Modlin study (Modlin et al. 2016), providing prospective evaluation of

gastroenteropancreatic NENs treated with surgery, found no significant post-surgical CgA changes between patients cured and non-cured. Similarly, the prospective Bodei study (Bodei et al. 2016) about PRRT found that the rate of CgA reduction was higher in non-responders than in responding cases (21 and 40%, respectively), thus demonstrating poor utility of CgA modifications in predicting treatment efficacy in these settings.

8. Conclusions

A wide body of research has been dedicated over the last 2 decades to define clinical application of circulating CgA in NENs. As all authors agree, the marker is intrinsically limited by the lack of assay standardization generating significant variations across different laboratories. This depends not only on the applied technique, but also on the employed antibody when using the same method (Modlin et al. 2010a), and hampers not only management of a single patient, but also the comparison between different studies, thus making hard to define the actual marker performance. Clinical value of CgA in the diagnostic setting is hampered by issues impairing both specificity and sensitivity. Regarding the former, the major problem is that several conditions other than NEN can affect CgA levels, therefore acting as confounding factors. These include some highly prevalent non oncological conditions, such as gastrointestinal and cardiovascular disorders or PPIs assumption, and a variety of non-NEN tumours. Among the latter, those with the highest impact in clinical practice are malignancies arising from anatomic areas where NENs occur more frequently, such as colorectal and pancreatic adenocarcinoma. Sensitivity of the test is intrinsically limited by the fact that a relevant portion of NENs, 30-50%, do not show elevated CgA levels (Lindholm & Oberg 2011). Due to the tight correlation of the marker with tumour function and disease extent, this issue mainly involves NENs with non-functional localized disease where CgA is normal in about 70% of cases (Jilesen et al. 2014). In this kind of patients, where the role of clinics and instrumental exams is limited and the need of an accurate biochemical marker is higher, diagnostic role of circulating

CgA is paradoxically marginal, due to the poor sensitivity. Despite these limitations, ENETS and other major societies dealing with NENs still recommend circulating CgA for the diagnostic definition (Caplin et al. 2015; Falconi et al. 2016; Jensen et al. 2012; Kloppel et al. 2009; Kulke et al. 2015; Kunz et al. 2013; Niederle et al. 2016; O'Toole et al. 2009; Oberg et al. 2012a; Oberg et al. 2012b; Ramage et al. 2012). Nevertheless, a recent Delphi consensus, focusing on the overall role of biomarkers in NEN, concluded that all monoanalyte tests, including CgA, do not meet the minimum required standard (defined as sensitivity and specificity higher than 80 and 90%, respectively) and no circulating biomarker can be considered, alone, as a supportable diagnostic tool (Oberg et al. 2015).

The role of CgA for NEN prognostic definition is still controversial as based on low quality evidence, mainly deriving from retrospective studies. However, a set of recently emerged prospective data focusing on NENs with advanced disease seem to outline significant prognostic value for baseline CgA levels.

Regarding the role of CgA for the definition of treatment strategy available evidence is overall poor as limited by the reduced number of dedicated studies and, also, by the retrospective nature of the majority of them. Despite needing further validation, there are some points that are gaining significant evidence: a) recent prospective studies (Cwikla et al. 2015; Pavel et al. 2017a) show that circulating CgA does not represent a valid marker of morphological evolution of disease and has therefore no utility for the follow-up phase; b) recent data from prospective placebo-controlled trials (Pavel et al. 2017b; Yao et al. 2016; Yao et al. 2017) show no CgA capability for predicting the actual impact of a medical treatment on survival; c) subanalyses of some prospective placebo-controlled trials (Baudin et al. 2011; Buil-Bruna et al. 2016) seem to outline a role for CgA response in the early prediction of medical treatments efficacy.

Independently of the real value in both the prognostic refining and treatment management, which needs to be further ascertained, it is important to remark that CgA, per definition, can be used as a

marker only in NENs showing abnormal serum levels. Since the portion of NENs with normal CgA is remarkable, as already discussed, this strongly limits the actual clinical application of the marker.

In conclusion, despite representing the best available monoanalyte marker related to NEN (Kulke et al. 2015; Modlin et al. 2010b), CgA carries the typical limitations of single-analyte measurements (Hood & Tian 2012), and is therefore unable to provide comprehensive evaluation of a heterogeneous entity such as NEN (Baudin 2007; Yao et al. 2008a). Hence, the new frontier seems to be represented by multianalytes approaches. Particularly, a blood-based algorithm including simultaneous determination of 51 NEN specific markers have been developed in recent years (Modlin et al. 2013), and all comparative studies were concordant in reporting significantly better metrics, as compared with CgA (Bodei et al. 2016; Modlin et al. 2014a; Modlin et al. 2014b; Modlin et al. 2016; Modlin et al. 2015; Pavel et al. 2017a).

Declaration of interest

Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure Legends

Figure 1: Physiology of production and secretion of CgA and related products.

References

Aardal S, Helle KB, Elsayed S, Reed RK & Serck-Hanssen G 1993 Vasostatins, comprising the N-terminal domain of chromogranin A, suppress tension in isolated human blood vessel segments. *J Neuroendocrinol* **5** 405-412.

Ahmed A, Turner G, King B, Jones L, Culliford D, McCance D, Ardill J, Johnston BT, Poston G, Rees M, et al. 2009 Midgut neuroendocrine tumours with liver metastases: results of the UKINETS study. *Endocr Relat Cancer* **16** 885-894.

Al-Shoumer KA & Vasanthy BA 2009 Serum chromogranin A concentration in hyperthyroidism before and after medical treatment. *J Clin Endocrinol Metab* **94** 2321-2324.

Angelsen A, Syversen U, Haugen OA, Stridsberg M, Mjolnerod OK & Waldum HL 1997 Neuroendocrine differentiation in carcinomas of the prostate: do neuroendocrine serum markers reflect immunohistochemical findings? *Prostate* **30** 1-6.

Ardill JE & O'Dorisio TM 2010 Circulating biomarkers in neuroendocrine tumors of the enteropancreatic tract: application to diagnosis, monitoring disease, and as prognostic indicators. *Endocrinol Metab Clin North Am* **39** 777-790.

Arnold R, Wilke A, Rinke A, Mayer C, Kann PH, Klose KJ, Scherag A, Hahmann M, Muller HH & Barth P 2008 Plasma chromogranin A as marker for survival in patients with metastatic endocrine gastroenteropancreatic tumors. *Clin Gastroenterol Hepatol* **6** 820-827.

Arvan P, Kuliawat R, Prabakaran D, Zavacki AM, Elahi D, Wang S & Pilkey D 1991 Protein discharge from immature secretory granules displays both regulated and constitutive characteristics. *J Biol Chem* **266** 14171-14174.

Bajetta E, Ferrari L, Martinetti A, Celio L, Procopio G, Artale S, Zilembo N, Di Bartolomeo M, Seregni E & Bombardieri E 1999 Chromogranin A, neuron specific enolase, carcinoembryonic antigen, and hydroxyindole acetic acid evaluation in patients with neuroendocrine tumors. *Cancer* **86** 858-865.

Baudin E 2007 Gastroenteropancreatic endocrine tumors: clinical characterization before therapy. *Nat Clin Pract Endocrinol Metab* **3** 228-239.

Baudin E, Wolin E, Castellano D, Kaltsas G, Panneerselvam A, Tsuchihashi Z, Saletan S, Yao JC & Gross D 2011 Correlation of PFS With Early Response of Chromogranin A and 5-hydroxyindoleacetic Acid Levels in Pts With Advanced

Neuroendocrine Tumours: Phase III RADIANT-2 Study Results. [abstract no. 6564]. *European Society for Medical*

Oncology, European Multidisciplinary Cancer Congress; Stockholm.

Belli SH, Oneto A, Aranda C, O'Connor JM, Domenichini E, Roca E, Mendez G, Bestani MC, Parma P, Giacomi N, et al. 2009 Chromogranin A as a biochemical marker for the management of neuroendocrine tumors: a multicenter study developed in Argentina. *Acta Gastroenterol Latinoam* **39** 184-189.

Belloni D, Scabini S, Foglieni C, Veschini L, Giazzon A, Colombo B, Fulgenzi A, Helle KB, Ferrero ME, Corti A, et al. 2007 The vasostatin-I fragment of chromogranin A inhibits VEGF-induced endothelial cell proliferation and migration. *FASEB J* **21** 3052-3062.

Bernini GP, Moretti A, Ferdeghini M, Ricci S, Letizia C, D'Erasmo E, Argenio GF & Salvetti A 2001 A new human chromogranin 'A' immunoradiometric assay for the diagnosis of neuroendocrine tumours. *Br J Cancer* **84** 636-642.

Blind E, Schmidt-Gayk H, Sinn HP, O'Connor DT & Raue F 1992 Chromogranin A as tumor marker in medullary thyroid carcinoma. *Thyroid* **2** 5-10.

Blois A, Srebro B, Mandala M, Corti A, Helle KB & Serck-Hanssen G 2006 The chromogranin A peptide vasostatin-I inhibits gap formation and signal transduction mediated by inflammatory agents in cultured bovine pulmonary and coronary arterial endothelial cells. *Regul Pept* **135** 78-84.

Bodei L, Kidd M, Modlin IM, Severi S, Drozdov I, Nicolini S, Kwekkeboom DJ, Krenning EP, Baum RP & Paganelli G 2016 Measurement of circulating transcripts and gene cluster analysis predicts and defines therapeutic efficacy of peptide receptor radionuclide therapy (PRRT) in neuroendocrine tumors. *Eur J Nucl Med Mol Imaging* **43** 839-851.

Borges R, Diaz-Vera J, Dominguez N, Arnau MR & Machado JD 2010 Chromogranins as regulators of exocytosis. *J Neurochem* **114** 335-343.

Borges R, Dominguez N, Smith CB, Bandyopadhyay GK, O'Connor DT, Mahata SK & Bartolomucci A 2013 Granins and catecholamines: functional interaction in chromaffin cells and adipose tissue. *Adv Pharmacol* **68** 93-113.

Buil-Bruna N, Dehez M, Manon A, Nguyen TX & Troconiz IF 2016 Establishing the Quantitative Relationship Between Lanreotide Autogel(R), Chromogranin A, and Progression-Free Survival in Patients with Nonfunctioning Gastroenteropancreatic Neuroendocrine Tumors. *AAPS J* **18** 703-712.

Campana D, Nori F, Piscitelli L, Morselli-Labate AM, Pezzilli R, Corinaldesi R & Tomassetti P 2007 Chromogranin A: is it a useful marker of neuroendocrine tumors? *J Clin Oncol* **25** 1967-1973.

Caplin ME, Baudin E, Ferolla P, Filosso P, Garcia-Yuste M, Lim E, Oberg K, Pelosi G, Perren A, Rossi RE, et al. 2015 Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. *Ann Oncol* **26** 1604-1620.

Ceconi C, Ferrari R, Bachetti T, Opasich C, Volterrani M, Colombo B, Parrinello G & Corti A 2002 Chromogranin A in heart failure; a novel neurohumoral factor and a predictor for mortality. *Eur Heart J* **23** 967-974.
Citterio D, Pusceddu S, Facciorusso A, Coppa J, Milione M, Buzzoni R, Bongini M, deBraud F & Mazzaferro V 2017 Primary tumour resection may improve survival in functional well-differentiated neuroendocrine tumours metastatic to the liver. *Eur J Surg Oncol* **43** 380-387.
Cwikla JB, Bodei L, Kolasinska-Cwikla A, Sankowski A, Modlin IM & Kidd M 2015 Circulating Transcript Analysis (NETest) in GEP-NETs Treated With Somatostatin Analogs Defines Therapy. *J Clin Endocrinol Metab* **100** E1437-1445.
de Laat JM, Pieterman CR, Weijmans M, Hermus AR, Dekkers OM, de Herder WW, van der Horst-Schrivers AN, Drent ML, Bisschop PH, Havekes B, et al. 2013 Low accuracy of tumor markers for diagnosing pancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1 patients. *J Clin Endocrinol Metab* **98** 4143-4151.
Del Prete M, Fiore F, Modica R, Marotta V, Marciello F, Ramundo V, Di Sarno A, Carratu A, di Roseto Cde L, Tafuto S, et al. 2014 Hepatic arterial embolization in patients with neuroendocrine tumors. *J Exp Clin Cancer Res* **33** 43.
Di Comite G, Previtali P, Rossi CM, Dell'Antonio G, Rovere-Querini P, Praderio L, Dagna L, Corti A, Doglioni C, Maseri A, et al. 2009a High blood levels of chromogranin A in giant cell arteritis identify patients refractory to corticosteroid treatment. *Ann Rheum Dis* **68** 293-295.
Di Comite G, Rossi CM, Marinosci A, Lolmede K, Baldissera E, Aiello P, Mueller RB, Herrmann M, Voll RE, Rovere-Querini P, et al. 2009b Circulating chromogranin A reveals extra-articular involvement in patients with rheumatoid arthritis and curbs TNF-alpha-elicited endothelial activation. *J Leukoc Biol* **85** 81-87.
Dimsdale JE, O'Connor DT, Ziegler M & Mills P 1992 Chromogranin A correlates with norepinephrine release rate. *Life Sci* **51** 519-525.
Donica H, Malecha-Jedraszek A, Stroslawska E, Burska A & Szubstarski F 2010 Significance of plasma chromogranin A determination in neuroendocrine tumour (NET) diagnosis. *Folia Histochem Cytobiol* **48** 603-610.
Ekeblad S, Skogseid B, Dunder K, Oberg K & Eriksson B 2008 Prognostic factors and survival in 324 patients with pancreatic endocrine tumor treated at a single institution. *Clin Cancer Res* **14** 7798-7803.
Faggiano A, Ferolla P, Grimaldi F, Campana D, Manzoni M, Davi MV, Bianchi A, Valcavi R, Papini E, Giuffrida D, et al. 2012 Natural history of gastro-entero-pancreatic and thoracic neuroendocrine tumors. Data from a large prospective and retrospective Italian epidemiological study: the NET management study. *J Endocrinol Invest* **35** 817-823.
Fairweather M, Swanson R, Wang J, Brais LK, Dutton T, Kulke MH & Clancy TE 2017 Management of Neuroendocrine Tumor Liver Metastases: Long-Term Outcomes and Prognostic Factors from a Large Prospective Database. *Ann Surg Oncol*.
Falconi M, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, Kos-Kudla B, Kwekkeboom D, Rindi G, Kloppel G, et al. 2016 ENETS Consensus Guidelines Update for the Management of Patients with Functional Pancreatic Neuroendocrine Tumors and Non-Functional Pancreatic Neuroendocrine Tumors. *Neuroendocrinology* **103** 153-171.
Ferolla P, Faggiano A, Mansueto G, Avenia N, Cantelmi MG, Giovenali P, Del Basso De Caro ML, Milone F, Scarpelli G, Masone S, et al. 2008 The biological characterization of neuroendocrine tumors: the role of neuroendocrine markers. *J Endocrinol Invest* **31** 277-286.
Ferrari L, Seregni E, Lucignani G, Bajetta E, Martinetti A, Aliberti G, Pallotti F, Procopio G, Della Torre S, Luksch R, et al. 2004 Accuracy and clinical correlates of two different methods for chromogranin A assay in neuroendocrine tumors. *Int J Biol Markers* **19** 295-304.
Ferrero E, Scabini S, Magni E, Foglieni C, Belloni D, Colombo B, Curnis F, Villa A, Ferrero ME & Corti A 2004 Chromogranin A protects vessels against tumor necrosis factor alpha-induced vascular leakage. *FASEB J* **18** 554-556.
Fiore F, Del Prete M, Franco R, Marotta V, Ramundo V, Marciello F, Di Sarno A, Carratu AC, de Luca di Roseto C, Colao A, et al. 2014 Transarterial embolization (TAE) is equally effective and slightly safer than

739 transarterial chemoembolization (TACE) to manage liver metastases in neuroendocrine tumors. *Endocrine*
740 **47** 177-182.
741 Fossmark R, Jianu CS, Martinsen TC, Qvigstad G, Syversen U & Waldum HL 2008 Serum gastrin and
742 chromogranin A levels in patients with fundic gland polyps caused by long-term proton-pump inhibition.
743 *Scand J Gastroenterol* **43** 20-24.
744 Frilling A, Akerstrom G, Falconi M, Pavel M, Ramos J, Kidd M & Modlin IM 2012 Neuroendocrine tumor
745 disease: an evolving landscape. *Endocr Relat Cancer* **19** R163-185.
746 Gerdes HH, Phillips E & Huttner WB 1988 The primary structure of rat secretogranin II deduced from a
747 cDNA sequence. *Nucleic Acids Res* **16** 11811.
748 Giovanella L, Marelli M, Ceriani L, Giardina G, Garancini S & Colombo L 2001 Evaluation of chromogranin A
749 expression in serum and tissues of breast cancer patients. *Int J Biol Markers* **16** 268-272.
750 Glinicki P & Jeske W Chromogranin A (CgA)--the influence of various factors in vivo and in vitro, and existing
751 disorders on it's concentration in blood. *Endokrynol Pol* **61** 384-387.
752 Gonzalez-Yanes C & Sanchez-Margalet V 2000 Pancreastatin modulates insulin signaling in rat adipocytes:
753 mechanisms of cross-talk. *Diabetes* **49** 1288-1294.
754 Goudet P, Murat A, Binquet C, Cardot-Bauters C, Costa A, Ruszniewski P, Niccoli P, Menegaux F, Chabrier G,
755 Borson-Chazot F, et al. 2010 Risk factors and causes of death in MEN1 disease. A GTE (Groupe d'Etude des
756 Tumeurs Endocrines) cohort study among 758 patients. *World J Surg* **34** 249-255.
757 Guignat L, Bidart JM, Nocera M, Comoy E, Schlumberger M & Baudin E 2001 Chromogranin A and the alpha-
758 subunit of glycoprotein hormones in medullary thyroid carcinoma and phaeochromocytoma. *Br J Cancer* **84**
759 808-812.
760 Gut P, Czarnywojtek A, Fischbach J, Baczyk M, Ziemnicka K, Wrotkowska E, Gryczynska M & Ruchala M 2016
761 Chromogranin A - unspecific neuroendocrine marker. Clinical utility and potential diagnostic pitfalls. *Arch*
762 *Med Sci* **12** 1-9.
763 Helle KB & Corti A 2015 Chromogranin A: a paradoxical player in angiogenesis and vascular biology. *Cell Mol*
764 *Life Sci* **72** 339-348.
765 Hood L & Tian Q 2012 Systems approaches to biology and disease enable translational systems medicine.
766 *Genomics Proteomics Bioinformatics* **10** 181-185.
767 Hoshino K, Suzuki J, Yamauchi K & Inoue H 2008 Psychological stress evaluation of patients with bronchial
768 asthma based on the chromogranin a level in saliva. *J Asthma* **45** 596-599.
769 Hsiao RJ, Mezger MS & O'Connor DT 1990 Chromogranin A in uremia: progressive retention of
770 immunoreactive fragments. *Kidney Int* **37** 955-964.
771 Ichikawa T, Peterson MS, Federle MP, Baron RL, Haradome H, Kawamori Y, Nawano S & Araki T 2000 Islet
772 cell tumor of the pancreas: biphasic CT versus MR imaging in tumor detection. *Radiology* **216** 163-171.
773 Janson ET, Holmberg L, Stridsberg M, Eriksson B, Theodorsson E, Wilander E & Oberg K 1997 Carcinoid
774 tumors: analysis of prognostic factors and survival in 301 patients from a referral center. *Ann Oncol* **8** 685-
775 690.
776 Jansson AM, Rosjo H, Omland T, Karlsson T, Hartford M, Flyvbjerg A & Caidahl K 2009 Prognostic value of
777 circulating chromogranin A levels in acute coronary syndromes. *Eur Heart J* **30** 25-32.
778 Jensen RT, Cadiot G, Brandi ML, de Herder WW, Kaltsas G, Komminoth P, Scoazec JY, Salazar R, Sauvanet A
779 & Kianmanesh R 2012 ENETS Consensus Guidelines for the management of patients with digestive
780 neuroendocrine neoplasms: functional pancreatic endocrine tumor syndromes. *Neuroendocrinology* **95** 98-
781 119.
782 Jilesen AP, Busch OR, van Gulik TM, Gouma DJ & Nieveen van Dijkum EJ 2014 Standard pre- and
783 postoperative determination of chromogranin a in resectable non-functioning pancreatic neuroendocrine
784 tumors--diagnostic accuracy: NF-pNET and low tumor burden. *Dig Surg* **31** 407-414.
785 Jun E, Kim SC, Song KB, Hwang DW, Lee JH, Shin SH, Hong SM, Park KM & Lee YJ 2017 Diagnostic value of
786 chromogranin A in pancreatic neuroendocrine tumors depends on tumor size: A prospective observational
787 study from a single institute. *Surgery*.
788 Kaltsas GA, Besser GM & Grossman AB 2004 The diagnosis and medical management of advanced
789 neuroendocrine tumors. *Endocr Rev* **25** 458-511.

Kidd M, Bodei L & Modlin IM 2016 Chromogranin A: any relevance in neuroendocrine tumors? *Curr Opin Endocrinol Diabetes Obes* **23** 28-37.
Kim T, Gondre-Lewis MC, Arnaoutova I & Loh YP 2006 Dense-core secretory granule biogenesis. *Physiology (Bethesda)* **21** 124-133.
Kimura N, Miura W, Noshiro T, Mizunashi K, Hanew K, Shimizu K, Watanabe T, Shibukawa S, Sohn HE, Abe K, et al. 1997 Plasma chromogranin A in pheochromocytoma, primary hyperparathyroidism and pituitary adenoma in comparison with catecholamine, parathyroid hormone and pituitary hormones. *Endocr J* **44** 319-327.
Kloppel G, Couvelard A, Perren A, Komminoth P, McNicol AM, Nilsson O, Scarpa A, Scoazec JY, Wiedenmann B, Papotti M, et al. 2009 ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: towards a standardized approach to the diagnosis of gastroenteropancreatic neuroendocrine tumors and their prognostic stratification. *Neuroendocrinology* **90** 162-166.
Konecki DS, Benedum UM, Gerdes HH & Huttner WB 1987 The primary structure of human chromogranin A and pancreastatin. *J Biol Chem* **262** 17026-17030.
Korse CM, Taal BG, Vincent A, van Velthuysen ML, Baas P, Buning-Kager JC, Linders TC & Bonfrer JM 2012 Choice of tumour markers in patients with neuroendocrine tumours is dependent on the histological grade. A marker study of Chromogranin A, Neuron specific enolase, Progastrin-releasing peptide and cytokeratin fragments. *Eur J Cancer* **48** 662-671.
Koshimizu H, Kim T, Cawley NX & Loh YP 2010 Chromogranin A: a new proposal for trafficking, processing and induction of granule biogenesis. *Regul Pept* **160** 153-159.
Kouvaraki MA, Ajani JA, Hoff P, Wolff R, Evans DB, Lozano R & Yao JC 2004 Fluorouracil, doxorubicin, and streptozocin in the treatment of patients with locally advanced and metastatic pancreatic endocrine carcinomas. *J Clin Oncol* **22** 4762-4771.
Kruger PG, Mahata SK & Helle KB 2003 Catestatin (CgA344-364) stimulates rat mast cell release of histamine in a manner comparable to mastoparan and other cationic charged neuropeptides. *Regul Pept* **114** 29-35.
Kuehn MJ, Herrmann JM & Schekman R 1998 COPII-cargo interactions direct protein sorting into ER-derived transport vesicles. *Nature* **391** 187-190.
Kuipers EJ 2006 Proton pump inhibitors and gastric neoplasia. *Gut* **55** 1217-1221.
Kulke MH, Benson AB, 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, Choti MA, Clark OH, Doherty GM, Eason J, Emerson L, et al. 2012 Neuroendocrine tumors. *J Natl Compr Canc Netw* **10** 724-764.
Kulke MH, Shah MH, Benson AB, 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, Emerson L, Engstrom PF, Fanta P, Giordano T, et al. 2015 Neuroendocrine tumors, version 1.2015. *J Natl Compr Canc Netw* **13** 78-108.
Kunz PL, Reidy-Lagunes D, Anthony LB, Bertino EM, Brendtro K, Chan JA, Chen H, Jensen RT, Kim MK, Klimstra DS, et al. 2013 Consensus guidelines for the management and treatment of neuroendocrine tumors. *Pancreas* **42** 557-577.
Lamberts SW, Hofland LJ & Nobels FR 2001 Neuroendocrine tumor markers. *Front Neuroendocrinol* **22** 309-339.
Langley K 1994 The neuroendocrine concept today. *Ann N Y Acad Sci* **733** 1-17.
Leon A, Torta M, Dittadi R, degli Uberti E, Ambrosio MR, Delle Fave G, De Braud F, Tomassetti P, Gion M & Dogliotti L 2005 Comparison between two methods in the determination of circulating chromogranin A in neuroendocrine tumors (NETs): results of a prospective multicenter observational study. *Int J Biol Markers* **20** 156-168.
Leoncini E, Boffetta P, Shafir M, Aleksovska K, Boccia S & Rindi G 2017 Increased incidence trend of low-grade and high-grade neuroendocrine neoplasms. *Endocrine*.
Lindholm DP & Oberg K 2011 Biomarkers and molecular imaging in gastroenteropancreatic neuroendocrine tumors. *Horm Metab Res* **43** 832-837.
Mahata SK, Mahata M, Fung MM & O'Connor DT 2010 Catestatin: a multifunctional peptide from chromogranin A. *Regul Pept* **162** 33-43.
Mahata SK, Mahata M, Wen G, Wong WB, Mahapatra NR, Hamilton BA & O'Connor DT 2004 The catecholamine release-inhibitory "catestatin" fragment of chromogranin a: naturally occurring human

variants with different potencies for multiple chromaffin cell nicotinic cholinergic responses. *Mol Pharmacol* **66** 1180-1191.
Mahata SK, O'Connor DT, Mahata M, Yoo SH, Taupenot L, Wu H, Gill BM & Parmer RJ 1997 Novel autocrine feedback control of catecholamine release. A discrete chromogranin a fragment is a noncompetitive nicotinic cholinergic antagonist. *J Clin Invest* **100** 1623-1633.
Malaguarnera M, Cristaldi E, Cammalleri L, Colonna V, Lipari H, Capici A, Cavallaro A, Beretta M, Alessandria I, Luca S, et al. 2009 Elevated chromogranin A (CgA) serum levels in the patients with advanced pancreatic cancer. *Arch Gerontol Geriatr* **48** 213-217.
Marotta V, Franzese MD, Del Prete M, Chiofalo MG, Ramundo V, Esposito R, Marciello F, Pezzullo L, Carratu A, Vitale M, et al. 2013 Targeted therapy with kinase inhibitors in aggressive endocrine tumors. *Expert Opin Pharmacother* **14** 1187-1203.
Marotta V, Nuzzo V, Ferrara T, Zuccoli A, Masone M, Nocerino L, Del Prete M, Marciello F, Ramundo V, Lombardi G, et al. 2012 Limitations of Chromogranin A in clinical practice. *Biomarkers* **17** 186-191.
Minamiki T, Minami T, Sasaki Y, Wakida SI, Kurita R, Niwa O & Tokito S 2016 Label-Free Detection of Human Glycoprotein (CgA) Using an Extended-Gated Organic Transistor-Based Immunosensor. *Sensors (Basel)* **16**.
Modlin IM, Aslanian H, Bodei L, Drozdov I & Kidd M 2014a A PCR blood test outperforms chromogranin A in carcinoid detection and is unaffected by proton pump inhibitors. *Endocr Connect* **3** 215-223.
Modlin IM, Drozdov I, Alaimo D, Callahan S, Teixiera N, Bodei L & Kidd M 2014b A multianalyte PCR blood test outperforms single analyte ELISAs (chromogranin A, pancreastatin, neurokinin A) for neuroendocrine tumor detection. *Endocr Relat Cancer* **21** 615-628.
Modlin IM, Drozdov I & Kidd M 2013 The identification of gut neuroendocrine tumor disease by multiple synchronous transcript analysis in blood. *PLoS One* **8** e63364.
Modlin IM, Frilling A, Salem RR, Alaimo D, Drymousis P, Wasan HS, Callahan S, Faiz O, Weng L, Teixeira N, et al. 2016 Blood measurement of neuroendocrine gene transcripts defines the effectiveness of operative resection and ablation strategies. *Surgery* **159** 336-347.
Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsolakis AV & Kidd M 2010a Chromogranin A--biological function and clinical utility in neuro endocrine tumor disease. *Ann Surg Oncol* **17** 2427-2443.
Modlin IM, Kidd M, Bodei L, Drozdov I & Aslanian H 2015 The clinical utility of a novel blood-based multi-transcriptome assay for the diagnosis of neuroendocrine tumors of the gastrointestinal tract. *Am J Gastroenterol* **110** 1223-1232.
Modlin IM, Moss SF, Oberg K, Padbury R, Hicks RJ, Gustafsson BI, Wright NA & Kidd M 2010b Gastrointestinal neuroendocrine (carcinoid) tumours: current diagnosis and management. *Med J Aust* **193** 46-52.
Modlin IM, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, et al. 2008 Gastroenteropancreatic neuroendocrine tumours. *Lancet Oncol* **9** 61-72.
Modlin IM, Oberg K, Taylor A, Drozdov I, Bodei L & Kidd M 2014c Neuroendocrine tumor biomarkers: current status and perspectives. *Neuroendocrinology* **100** 265-277.
Molina R, Alvarez E, Aniel-Quiroga A, Borque M, Candas B, Leon A, Poyatos RM & Gelabert M 2011 Evaluation of chromogranin A determined by three different procedures in patients with benign diseases, neuroendocrine tumors and other malignancies. *Tumour Biol* **32** 13-22.
Mulkeen AL, Yoo PS & Cha C 2006 Less common neoplasms of the pancreas. *World J Gastroenterol* **12** 3180-3185.
Nanno Y, Toyama H, Matsumoto I, Otani K, Asari S, Goto T, Ajiki T, Zen Y, Fukumoto T & Ku Y 2017 Baseline plasma chromogranin A levels in patients with well-differentiated neuroendocrine tumors of the pancreas: A potential predictor of postoperative recurrence. *Pancreatology* **17** 291-294.
Nehar D, Lombard-Bohas C, Olivieri S, Claustrat B, Chayvialle JA, Penes MC, Sassolas G & Borson-Chazot F 2004 Interest of Chromogranin A for diagnosis and follow-up of endocrine tumours. *Clin Endocrinol (Oxf)* **60** 644-652.
Niederle B, Pape UF, Costa F, Gross D, Kelestimur F, Knigge U, Oberg K, Pavel M, Perren A, Toumpanakis C, et al. 2016 ENETS Consensus Guidelines Update for Neuroendocrine Neoplasms of the Jejunum and Ileum. *Neuroendocrinology* **103** 125-138.

892 Nikou GC, Marinou K, Thomakos P, Papageorgiou D, Sanzanidis V, Nikolaou P, Kosmidis C, Moulakakis A &
893 Mallas E 2008 Chromogranin a levels in diagnosis, treatment and follow-up of 42 patients with non-
894 functioning pancreatic endocrine tumours. *Pancreatology* **8** 510-519.
895 Nobels FR, Kwekkeboom DJ, Bouillon R & Lamberts SW 1998 Chromogranin A: its clinical value as marker of
896 neuroendocrine tumours. *Eur J Clin Invest* **28** 431-440.
897 Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, De Herder WW, Krenning EP,
898 Bouillon R & Lamberts SW 1997 Chromogranin A as serum marker for neuroendocrine neoplasia:
899 comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. *J Clin Endocrinol*
900 *Metab* **82** 2622-2628.
901 O'Connor DT & Frigon RP 1984 Chromogranin A, the major catecholamine storage vesicle soluble protein.
902 Multiple size forms, subcellular storage, and regional distribution in chromaffin and nervous tissue
903 elucidated by radioimmunoassay. *J Biol Chem* **259** 3237-3247.
904 O'Connor DT, Pandlan MR, Carlton E, Cervenka JH & Hslao RJ 1989 Rapid radioimmunoassay of circulating
905 chromogranin A: in vitro stability, exploration of the neuroendocrine character of neoplasia, and
906 assessment of the effects of organ failure. *Clin Chem* **35** 1631-1637.
907 O'Toole D, Grossman A, Gross D, Delle Fave G, Barkmanova J, O'Connor J, Pape UF & Plockinger U 2009
908 ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: biochemical markers.
909 *Neuroendocrinology* **90** 194-202.
910 Oberg K 2011 Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours. *Endocr Relat*
911 *Cancer* **18 Suppl 1** S17-25.
912 Oberg K 2012 Neuroendocrine tumors of the digestive tract: impact of new classifications and new agents
913 on therapeutic approaches. *Curr Opin Oncol* **24** 433-440.
914 Oberg K, Akerstrom G, Rindi G & Jelic S 2010 Neuroendocrine gastroenteropancreatic tumours: ESMO
915 Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* **21 Suppl 5** v223-227.
916 Oberg K, Hellman P, Ferolla P & Papotti M 2012a Neuroendocrine bronchial and thymic tumors: ESMO
917 Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* **23 Suppl 7** vii120-123.
918 Oberg K, Knigge U, Kwekkeboom D & Perren A 2012b Neuroendocrine gastro-entero-pancreatic tumors:
919 ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* **23 Suppl 7** vii124-130.
920 Oberg K, Modlin IM, De Herder W, Pavel M, Klimstra D, Frilling A, Metz DC, Heaney A, Kwekkeboom D,
921 Strosberg J, et al. 2015 Consensus on biomarkers for neuroendocrine tumour disease. *Lancet Oncol* **16**
922 e435-446.
923 Paik WH, Ryu JK, Song BJ, Kim J, Park JK, Kim YT & Yoon YB 2013 Clinical usefulness of plasma chromogranin
924 a in pancreatic neuroendocrine neoplasm. *J Korean Med Sci* **28** 750-754.
925 Panzuto F, Severi C, Cannizzaro R, Falconi M, Angeletti S, Pasquali A, Corleto VD, Annibale B, Buonadonna A,
926 Pederzoli P, et al. 2004 Utility of combined use of plasma levels of chromogranin A and pancreatic
927 polypeptide in the diagnosis of gastrointestinal and pancreatic endocrine tumors. *J Endocrinol Invest* **27** 6-
928 11.
929 Pavel M, Baudin E, Couvelard A, Krenning E, Oberg K, Steinmuller T, Anlauf M, Wiedenmann B & Salazar R
930 2012 ENETS Consensus Guidelines for the management of patients with liver and other distant metastases
931 from neuroendocrine neoplasms of foregut, midgut, hindgut, and unknown primary. *Neuroendocrinology*
932 **95** 157-176.
933 Pavel M, Jann H, Prasad V, Drozdov I, Modlin IM & Kidd M 2017a NET Blood Transcript Analysis Defines the
934 Crossing of the Clinical Rubicon: When Stable Disease Becomes Progressive. *Neuroendocrinology* **104** 170-
935 182.
936 Pavel ME, Baudin E, Oberg KE, Hainsworth JD, Voi M, Rouyrre N, Peeters M, Gross DJ & Yao JC 2017b
937 Efficacy of everolimus plus octreotide LAR in patients with advanced neuroendocrine tumor and carcinoid
938 syndrome: final overall survival from the randomized, placebo-controlled phase 3 RADIANT-2 study. *Ann*
939 *Oncol*.
940 Pavel ME, Hainsworth JD, Baudin E, Peeters M, Horsch D, Winkler RE, Klimovsky J, Lebwohl D, Jehl V, Wolin
941 EM, et al. 2011 Everolimus plus octreotide long-acting repeatable for the treatment of advanced
942 neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-
943 controlled, phase 3 study. *Lancet* **378** 2005-2012.

Peracchi M, Conte D, Gebbia C, Penati C, Pizzinelli S, Arosio M, Corbetta S & Spada A 2003 Plasma chromogranin A in patients with sporadic gastro-entero-pancreatic neuroendocrine tumors or multiple endocrine neoplasia type 1. *Eur J Endocrinol* **148** 39-43.
Peracchi M, Gebbia C, Basilisco G, Quatrini M, Tarantino C, Vescarelli C, Massironi S & Conte D 2005 Plasma chromogranin A in patients with autoimmune chronic atrophic gastritis, enterochromaffin-like cell lesions and gastric carcinoids. *Eur J Endocrinol* **152** 443-448.
Pieterman CR, Schreinemakers JM, Koppeschaar HP, Vriens MR, Rinkes IH, Zonnenberg BA, van der Luijt RB & Valk GD 2009 Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome. *Clin Endocrinol (Oxf)* **70** 575-581.
Qiu W, Christakis I, Silva A, Bassett RL, Jr., Cao L, Meng QH, Gardner Grubbs E, Zhao H, Yao JC, Lee JE, et al. 2016 Utility of chromogranin A, pancreatic polypeptide, glucagon and gastrin in the diagnosis and follow-up of pancreatic neuroendocrine tumours in multiple endocrine neoplasia type 1 patients. *Clin Endocrinol (Oxf)* **85** 400-407.
Ramage JK, Ahmed A, Ardill J, Bax N, Breen DJ, Caplin ME, Corrie P, Davar J, Davies AH, Lewington V, et al. 2012 Guidelines for the management of gastroenteropancreatic neuroendocrine (including carcinoid) tumours (NETs). *Gut* **61** 6-32.
Ramundo V, Del Prete M, Marotta V, Marciello F, Camera L, Napolitano V, De Luca L, Circelli L, Colantuoni V, Di Sarno A, et al. 2014 Impact of long-acting octreotide in patients with early-stage MEN1-related duodeno-pancreatic neuroendocrine tumours. *Clin Endocrinol (Oxf)* **80** 850-855.
Ruszniewski P, Delle Fave G, Cadiot G, Komminoth P, Chung D, Kos-Kudla B, Kianmanesh R, Hochhauser D, Arnold R, Ahlman H, et al. 2006 Well-differentiated gastric tumors/carcinomas. *Neuroendocrinology* **84** 158-164.
Sanchez-Margalet V, Calvo JR & Goberna R 1992a Glucogenolytic and hyperglycemic effect of 33-49 C-terminal fragment of pancreastatin in the rat in vivo. *Horm Metab Res* **24** 455-457.
Sanchez-Margalet V, Calvo JR, Lucas M & Goberna R 1992b Pancreastatin and its 33-49 C-terminal fragment inhibit glucagon-stimulated insulin in vivo. *Gen Pharmacol* **23** 637-638.
Sanchez-Margalet V, Gonzalez-Yanes C, Najib S & Santos-Alvarez J 2010 Metabolic effects and mechanism of action of the chromogranin A-derived peptide pancreastatin. *Regul Pept* **161** 8-14.
Sciola V, Massironi S, Conte D, Caprioli F, Ferrero S, Ciafardini C, Peracchi M, Bardella MT & Piodi L 2009 Plasma chromogranin a in patients with inflammatory bowel disease. *Inflamm Bowel Dis* **15** 867-871.
Shapiro DE 1999 The interpretation of diagnostic tests. *Stat Methods Med Res* **8** 113-134.
Sidhu R, McAlindon ME, Leeds JS, Skilling J & Sanders DS 2009 The role of serum chromogranin A in diarrhoea predominant irritable bowel syndrome. *J Gastrointestin Liver Dis* **18** 23-26.
Singhi AD, Chu LC, Tatsas AD, Shi C, Ellison TA, Fishman EK, Kawamoto S, Schulick RD, Wolfgang CL, Hruban RH, et al. 2012 Cystic pancreatic neuroendocrine tumors: a clinicopathologic study. *Am J Surg Pathol* **36** 1666-1673.
Sobol RE, O'Connor DT, Addison J, Suchocki K, Royston I & Deftos LJ 1986 Elevated serum chromogranin A concentrations in small-cell lung carcinoma. *Ann Intern Med* **105** 698-700.
Solcia E, kloppel , G., Sobin, LH et al. 2000 Histological Typing of Endocrine Tumours (WHO. World Health Organization. International Histological Classification of Tumours)
Spadaro A, Ajello A, Morace C, Zirilli A, D'Arrigo G, Luigiano C, Martino F, Bene A, Migliorato D, Turiano S, et al. 2005 Serum chromogranin-A in hepatocellular carcinoma: diagnostic utility and limits. *World J Gastroenterol* **11** 1987-1990.
Stridsberg M, Eriksson B, Oberg K & Janson ET 2003 A comparison between three commercial kits for chromogranin A measurements. *J Endocrinol* **177** 337-341.
Sundin A, Vullierme MP, Kaltsas G & Plockinger U 2009 ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: radiological examinations. *Neuroendocrinology* **90** 167-183.
Syversen U, Halvorsen T, Marvik R & Waldum HL 1995 Neuroendocrine differentiation in colorectal carcinomas. *Eur J Gastroenterol Hepatol* **7** 667-674.
Takiyyuddin MA, Baron AD, Cervenka JH, Barbosa JA, Neumann HP, Parmer RJ, Sullivan PA & O'Connor DT 1991 Suppression of chromogranin-A release from neuroendocrine sources in man: pharmacological studies. *J Clin Endocrinol Metab* **72** 616-622.

Takiyyuddin MA, Parmer RJ, Kailasam MT, Cervenka JH, Kennedy B, Ziegler MG, Lin MC, Li J, Grim CE, Wright FA, et al. 1995 Chromogranin A in human hypertension. Influence of heredity. *Hypertension* **26** 213-220.
Tatemoto K, Efendic S, Mutt V, Makk G, Feistner GJ & Barchas JD 1986 Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* **324** 476-478.
Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, Melmed S, Sakurai A, Tonelli F & Brandi ML 2012 Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab* **97** 2990-3011.
Tohmola N, Itkonen O, Sane T, Markkanen H, Joenvaara S, Renkonen R & Hamalainen E 2014 Analytical and preanalytical validation of a new mass spectrometric serum 5-hydroxyindoleacetic acid assay as neuroendocrine tumor marker. *Clin Chim Acta* **428** 38-43.
Tomassetti P, Migliori M, Lalli S, Campana D, Tomassetti V & Corinaldesi R 2001a Epidemiology, clinical features and diagnosis of gastroenteropancreatic endocrine tumours. *Ann Oncol* **12 Suppl 2** S95-99.
Tomassetti P, Migliori M, Simoni P, Casadei R, De Iasio R, Corinaldesi R & Gullo L 2001b Diagnostic value of plasma chromogranin A in neuroendocrine tumours. *Eur J Gastroenterol Hepatol* **13** 55-58.
van der Zwan WA, Bodei L, Mueller-Brand J, de Herder WW, Kvols LK & Kwekkeboom DJ 2015 GEPNETs update: Radionuclide therapy in neuroendocrine tumors. *Eur J Endocrinol* **172** R1-8.
Vezzosi D, Walter T, Laplanche A, Raoul JL, Dromain C, Ruszniewski P, d'Herbomez M, Guigay J, Mitry E, Cadiot G, et al. 2011 Chromogranin A measurement in metastatic well-differentiated gastroenteropancreatic neuroendocrine carcinoma: screening for false positives and a prospective follow-up study. *Int J Biol Markers* **26** 94-101.
Waldum HL, Arnestad JS, Brenna E, Eide I, Syversen U & Sandvik AK 1996 Marked increase in gastric acid secretory capacity after omeprazole treatment. *Gut* **39** 649-653.
Walter T, Chardon L, Chopin-laly X, Raverot V, Caffin AG, Chayvialle JA, Scoazec JY & Lombard-Bohas C 2012 Is the combination of chromogranin A and pancreatic polypeptide serum determinations of interest in the diagnosis and follow-up of gastro-entero-pancreatic neuroendocrine tumours? *Eur J Cancer* **48** 1766-1773.
Welin S, Stridsberg M, Cunningham J, Granberg D, Skogseid B, Oberg K, Eriksson B & Janson ET 2009 Elevated plasma chromogranin A is the first indication of recurrence in radically operated midgut carcinoid tumors. *Neuroendocrinology* **89** 302-307.
West NE, Wise PE, Herline AJ, Muldoon RL, Chopp WV & Schwartz DA 2007 Carcinoid tumors are 15 times more common in patients with Crohn's disease. *Inflamm Bowel Dis* **13** 1129-1134.
Winkler H & Fischer-Colbrie R 1992 The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* **49** 497-528.
Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, et al. 2008a One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* **26** 3063-3072.
Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruszniewski P, Hoosen S, St Peter J, Haas T, Lebwohl D, et al. 2010 Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. *J Clin Oncol* **28** 69-76.
Yao JC, Pavel M, Lombard-Bohas C, Van Cutsem E, Voi M, Brandt U, He W, Chen D, Capdevila J, de Vries EG, et al. 2016 Everolimus for the Treatment of Advanced Pancreatic Neuroendocrine Tumors: Overall Survival and Circulating Biomarkers From the Randomized, Phase III RADIANT-3 Study. *J Clin Oncol*.
Yao JC, Pavel M, Phan AT, Kulke MH, Hoosen S, St Peter J, Cherfi A & Oberg KE 2011a Chromogranin A and neuron-specific enolase as prognostic markers in patients with advanced pNET treated with everolimus. *J Clin Endocrinol Metab* **96** 3741-3749.
Yao JC, Phan AT, Chang DZ, Wolff RA, Hess K, Gupta S, Jacobs C, Mares JE, Landgraf AN, Rashid A, et al. 2008b Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. *J Clin Oncol* **26** 4311-4318.
Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, et al. 2011b Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med* **364** 514-523.
Yao JC, Voi M, Lincy J & Pavel M 2017 Reply to V. Amoroso et al. *J Clin Oncol* **35** 1488-1489.

1047 Yoo SH 2010 Secretory granules in inositol 1,4,5-trisphosphate-dependent Ca2+ signaling in the cytoplasm
1048 of neuroendocrine cells. *FASEB J* **24** 653-664.
1049 Yoo SH, Huh YH & Hur YS 2010 Inositol 1,4,5-trisphosphate receptor in chromaffin secretory granules and
1050 its relation to chromogranins. *Cell Mol Neurobiol* **30** 1155-1161.
1051 Zatelli MC, Torta M, Leon A, Ambrosio MR, Gion M, Tomassetti P, De Braud F, Delle Fave G, Dogliotti L &
1052 degli Uberti EC 2007 Chromogranin A as a marker of neuroendocrine neoplasia: an Italian Multicenter
1053 Study. *Endocr Relat Cancer* **14** 473-482.
1054 Zhang D, Shooshtarizadeh P, Laventie BJ, Colin DA, Chich JF, Vidic J, de Barry J, Chasserot-Golaz S, Delalande
1055 F, Van Dorsselaer A, et al. 2009 Two chromogranin a-derived peptides induce calcium entry in human
1056 neutrophils by calmodulin-regulated calcium independent phospholipase A2. *PLoS One* **4** e4501.
1057
1058

1059 Figure 1

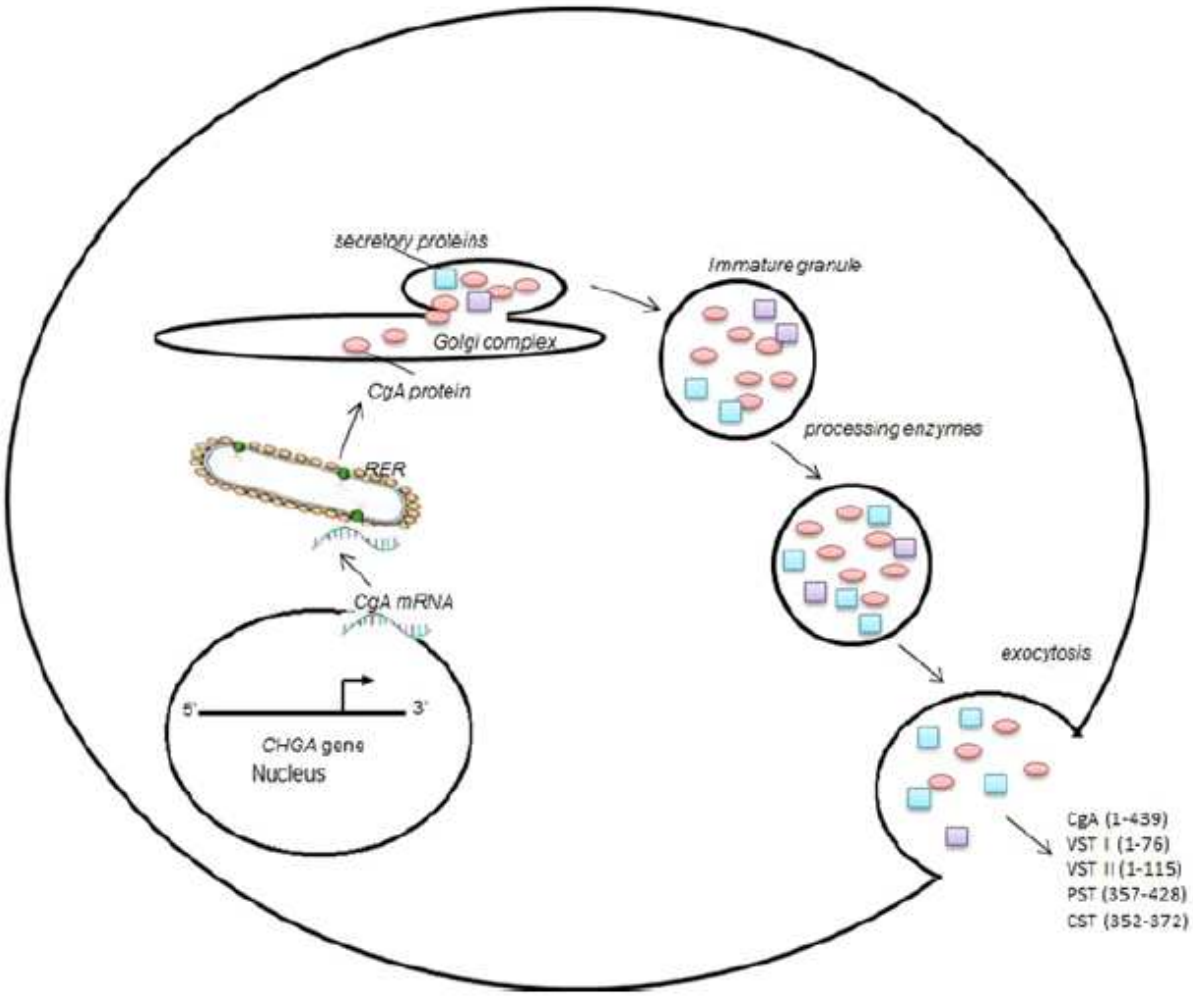

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Table 1 Patients features, methodological approach and results from studies assessing circulating CgA as a diagnostic marker of NEN.

Study (year)	NEN cohort			Comparison group	Interfering diseases	PPIs assumption	Assay	Cut off values	Sensitivity (%)	Specificity (%)
	N	Primary location	Stage IV (N)							
Tomassetti *et al.* (2001*a*,*b*)	80	GEP	NA	28 HS	Excluded	Not considered as exclusion criterium	ELISA	17 U/L	56	100
Bernini *et al.* (2001)	48	All sites	NA	130 HS 48 HP	Excluded –	Not considered as exclusion criterium	RIA	100 ng/mL	75	100 97.9
Peracchi *et al.* (2003)	61	GEP	28	50 HS	Not considered as exclusion criteria	Not considered as exclusion criterium	ELISA	20 U/L	92	83
Nehar *et al.* (2004)	124	GEP	97	50 HS	Excluded	Not considered as exclusion criterium	IRMA	100 µg/L	66	100
Zatelli *et al.* (2007)	81	GEP	NA	129 HS	Excluded (with the exception of CAG)	Excluded	IRMA ELISA	53 ng/mL 16 U/L	71.3 71	84 85
Campana *et al.* (2007)	238	GEP, lung	95	48 HS 42 CAG	Excluded –	Excluded –	ELISA	18 U/L 53 U/L	85.3 66.5	95.8 71.4
Belli *et al.* (2009)	119	GEP	84	39 HS	Excluded	Excluded	RIA	2.8 nmol/L	92.3	100
Donica *et al.* (2010)	41	All sites	30	15 HS	Not considered as exclusion criteria	Not considered as exclusion criterium	ELISA	18 U/L	71	87
Molina *et al.* (2011)	66	All sites	45	52 HS 98 IDs	Excluded –	Not considered as exclusion criterium –	IRMA ELISA RIA IRMA ELISA RIA	90 ng/mL* 60 ng/mL* 6 nmol/L* 90 ng/mL* 60 ng/mL* 6 nmol/L*	80.3 83.3 65.2 80.3 83.3 65.2	100 100 100 35.7 46.9 31.6
Vezzosi *et al.* (2011)	184	GEP	44	No comparison group	Not considered as exclusion criteria	Not considered as exclusion criterium	IRMA	98.1 ng/mL	71	Not available
Marotta *et al.* (2012)	42	All sites	8	100 HS (benign nodular goiter)	Not considered as exclusion criteria	Not considered as exclusion criterium	IRMA	Not identified	Not defined (failed ROC analysis)	Not defined (failed ROC analysis)
Korse *et al.* (2012)	573**	All sites	388	282 HS	Not considered as exclusion criteria	Not considered as exclusion criterium	RIA	87 µg/L	69	95
Modlin *et al.* (2013)	81	GEP	NA	94 HS	Not considered as exclusion criteria	Not considered as exclusion criterium	ELISA	19 U/L	32	99
Tohmola *et al.* (2014)	41	All sites	NA	26 HS	Not considered as exclusion criteria	Not considered as exclusion criterium	RIA	6 nmol/L	51	86

CAG, chronic atrophic gastritis; GEP, gastroenteropancreatic; HP, hypertension; HS, healthy subjects; IDs, interfering diseases; N, number; NEN, neuroendocrine neoplasm; PPIs, proton-pump inhibitors; ROC, receiving-operator characteristics.
*Cut-off values extrapolated from HS to obtain 100% specificity; **the cohort included a majority (293 cases) of grade 3 patients.

Table 2 Conditions affecting CgA-circulating levels.

Non-oncological		Oncological
Benign diseases	Iatrogenic causes	
Gastrointestinal: chronic atrophic gastritis, Helicobacter pylori infection, liver cirrosi, chronic hepatitis, pancreatitis, inflammatory bowel diseases, irritable bowel	Proton pump inhibitors	Colorectal carcinoma
Cardiovascular: hypertension, heart failure, acute coronary syndromes	Histamine 2 receptor antagonists	Gastric carcinoma
Renal and hepatic dysfunctions	Serotonin reuptake inhibitors	Pancreatic carcinoma
Others: giant cell arteritis, rheumatoid arthritis, systemic lupus erythematosus, pulmonary obstructive disease, hyperthyroidism		Prostate carcinoma
		Breast carcinoma
		Hepatocellular carcinoma
		Ovarian carcinoma

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Table 3 Composition of the study groups and results from studies assessing circulating CgA as a diagnostic marker between NEN and non-NEN tumors.

Study (year)	**NEN group**	**Non-NEN group**	**Sensitivity** (%)	**Specificity** (%)
Nobels *et al.* (1997)	211 from all sites	180: breast carcinoma, non-small cell lung cancer, pancreatic carcinoma, adenocarcinoma of unknown origin, non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, meningioma, and astrocytoma	53	93
Panzuto *et al.* (2004)	68 GEP	24: gastric, colorectal, and pancreatic carcinoma	84	63
Nehar *et al.* (2004)	124 GEP	77: thyroid carcinoma, non-endocrine pancreatic tumors, others unspecified	62.9	97.4
Molina *et al.* (2011)	66 from all sites	94: non-small cell lung cancer, colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, prostatic carcinoma, hepatocellular carcinoma, ovarian carcinoma, breast carcinoma endometrial carcinoma, astrocytoma, melanomas, sarcoma, and bladder carcinoma	83.3	41.5
Marotta *et al.* (2012)	42 from all sites	120: prostate carcinoma, colorectal carcinoma, lung cancer (unspecified histology), hepatocellular carcinoma, gastric carcinoma, papillary thyroid carcinoma	Not defined (failed ROC analysis)	Not defined (failed ROC analysis)

1064 GEP, gastroenteropancreatic; NEN, neuroendocrine neoplasm; ROC, receiving-operator characteristics.