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The Diagnostic Value of Chromogranin A in Neuroendocrine Neoplasms is Potentiated by Clinical Factors and Inflammatory Markers

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Abstract: Objective: Neuroendocrine neoplasms (NENs) are a heterogeneous group of indolent tumors, with variable clinical behavior and steadily rising incidence. The aim of this study is to investigate the clinical and laboratory factors that contribute in predicting the aggressiveness and invasiveness of NENs. Special focus is given to clinical parameters that would enhance the diagnostic value of chromogranin A (CgA), via formalizing an integrated probability model, which would contribute to the timely and accurate identification of patients at high risk for metastatic disease at initial diagnosis. Designs and Methods: We identified a total of 93 patients with NENs, recruited at a specialized academic center in Athens, Greece. Anthropometric, clinical, laboratory, and pathological data were obtained from every patient before any therapeutic intervention. Results: Age over 50 years and male gender were accompanied by increased risk for metastases at the time of initial diagnosis. Additionally, when these parameters were combined with CgA levels, they were shown to enhance the predictive capacity of CgA. Different patient scenarios combining age, gender, and CgA levels are associated with different probabilities for metastatic disease, demonstrated schematically in a gradually escalating model, as age and CgA levels increase in both males and females. The lowest risk is observed in women aged <50 years old with CgA levels <200 ng/dl (6.5%), while the highest one is in males over 50 years old with CgA > 200 ng/dl (62.9%). Finally, it was shown that c-reactive protein (CRP) can predict disease extent at the time of diagnosis. Conclusions: CgA levels can not only be used as a direct predictor of tumor load in patients with NENs, but also, when interpolated with the effects of age and gender, cumulatively predict whether a NEN would be metastatic or not at the time of initial diagnosis, via a risk-escalating probability model.

Keywords: neuroendocrine neoplasms; chromogranin A; CRP; sex; age

1. Introduction

Neuroendocrine neoplasms (NENs) are a heterogeneous group of tumors arising from neuroendocrine cells in the endocrine and central nervous system, secreting various bioamines and peptides. They are rare tumors, responsible for 0.5% of all cancers [1], with a crude, age-adjusted incidence of approximately 6.98 cases/100,000 annually [2]. They are most commonly found in the

gastrointestinal system, but they can also originate in other areas, including the pancreas, lungs, ovaries, and the thyroid, pituitary, and adrenal glands [3].

Despite their identification over a century ago, the natural history of NENs remains inadequately understood. Their rarity, heterogeneity, and unique phenotypic, hormonally driven variation, expressed by nonspecific but debilitating symptoms, hamper efforts to totally understand their biological characteristics [2,4–6]. However, according to the largest and most recent population-based study of NENs conducted in the United States, the incidence and prevalence of NENs have continued to rise over time, rendering their closest investigation imperative [2].

There is a paucity of reliable clinical or biochemical parameters that can determine the clinical course of patients with NENs. Over the years, a variety of general and specific circulatory biomarkers has been developed for the diagnosis and follow-up of these patients [7]. Among them, chromogranin A (CgA), a hydrophilic glycoprotein abundantly expressed in large dense core vesicles of neuroendocrine cells, whose main biological role is to regulate calcium-mediated exocytosis, has been highlighted as the most useful NEN-related circulating marker [8]. CgA was shown to have a diagnostic, predictive, and prognostic role in this population of patients, due to its universal secretion by the majority of neuroendocrine cells that persist after malignant transformation [9,10].

However, in clinical practice, there are still limitations and flaws [8]. Specifically, CgA can be found elevated in various neoplastic, non-neoplastic, and iatrogenic situations, such as inflammatory bowel disease [11], chronic renal failure, and proton pump inhibitor (PPI) therapy, limiting its specificity as a NEN-related circulating biomarker. Furthermore, the majority of contemporary laboratory tests for CgA lack standardization, requiring special attention in the interpretation of results before making any therapeutic decision and warranting the conduction of further studies in order to enhance the clinical utility and prediction power of CgA [12]. Given all the above limitations, a blood-based multianalyte NEN-specific gene transcript analysis was recently developed, termed NETest. It represents a robust, reproducible PCR-based multianalyte test for the detection of NENs, encompassing the measurement of 51 neuroendocrine specific marker genes in peripheral blood. Its sensitivity and specificity are significantly higher compared to those of other monoanalytes, such as CgA. However, it is not yet widely available and needs further validation [13].

Multiple data have supported the concept that inflammation is a critical component of tumor progression in different types of cancer [14,15]. Specifically, the tumor microenvironment, which is uniquely determined by inflammatory cells, catalytically modulates the neoplastic process, promoting proliferation, survival, and migration [16]. Thus, various inflammatory markers, including c-reactive protein (CRP), have been shown not only to be markedly elevated in malignancies, but also to share a prognostic importance [17–19]. Interestingly, recent findings have shown that the role of inflammation may be equally catalytic in NEN tumorigenesis, with several pro-inflammatory cytokines being implicated in the development of carcinoid tumors. Single-nucleotide polymorphisms (SNPs) in cytokine genes, which alter the regulation of their expression and function, have been detected in patients with NENs and have been associated with increased susceptibility to developing NENs. Furthermore, plasma levels of various cytokines have been evaluated in patients with NENs, with encouraging results regarding their clinical importance. For example, in a study by Berkovic et al, not only was interleukin-2 (IL-2) associated with increased susceptibility to NENs, but also, when serum levels were compared to established neuroendocrine markers such as CgA and 5-hydroxyindoleacetic acid (5-HIAA), IL-2 was more sensitive than 5-HIAA and CgA in NEN diagnostics [20]. In this context, pro-inflammatory cytokines may be linked with the basis of NEN etiology and, along with other biochemical markers, such as CgA, may be utilized as prognostic indexes in patients with NENs [21].

The purpose of this retrospective study is to evaluate the epidemiological, clinical, and pathological characteristics of patients with NENs, in order to predict the aggressiveness and invasiveness of NENs. Special focus is given to CgA, the clinical utility of which is cumulatively assessed with other parameters, such as age and sex, with the aim to propose an integrated probability model which would

contribute to the timely and accurate identification of patients at high risk for metastatic disease at initial diagnosis.

2. Materials and Methods

2.1. Subjects

Ninety-three (93) patients with NENs were recruited at the specialized, outpatient Medical Center of Neuroendocrine Tumors of the Endocrine Department of “Sotiria” University Hospital in Athens, Greece, during the period from September 2013 until the end of 2014. All patients were adults (>18 years), of Caucasian origin, and were newly or already diagnosed with NENs. In every visit, patients were requested to give a detailed medical history and undergo an extensive clinical evaluation. Overall, anthropometric, clinical, laboratory, imaging, and pathological data were obtained from every patient, including age, sex, personal history, primary tumor location, grading, staging, distant and locoregional lymph node metastases at diagnosis, hormonal activity, main symptoms, diagnostic modalities used, and therapeutic interventions made. The date of diagnosis was defined as the date of histological diagnosis of NENs.

The following primary NEN sites were described: stomach, small intestine (including duodenum), appendix, large intestine, rectum, pancreas, bronchus and lungs, other, and unknown. Rare NEN sites (e.g., skin, breast, head and neck, bladder, mediastinum) were included under the “other” category because a small number of cases were anticipated, while cases where the primary site was not determined were assigned to the “unknown” category. Small cell and large cell lung carcinoma, pheochromocytoma, paraganglioma, extra-adrenal paraganglioma, and medullary thyroid carcinoma were excluded because they represent biologically different diseases.

The grading of NENs was implemented based on the WHO 2010 criteria, while the staging was classified according to the American Joint Committee on Cancer and the International Union against Cancer (AJCC/UICC) 2009 criteria for well-differentiated tumors. Tumors with a Ki-67 index of $\leq 2\%$ were classified as G1, 3–20% as G2, and $>20\%$ as G3. Similarly, tumors with a mitotic rate of <2 per 10 high power fields (HPF) were classified as G1, 2–20/10 HPF as G2, and $>20/10$ HPF as G3. Gastroenteropancreatic NENs (GEP-NENs) were further classified as neuroendocrine tumor (NET) (G1 and G2) or neuroendocrine carcinoma (NEC) (G3), according to the 2010 WHO classification [22]. The results of immunohistochemical stain, lymphovascular invasion, perineural invasion, and lymph node metastasis were presented as either positive or negative.

Plasma was obtained from whole blood draws before any therapeutic intervention. Chromogranin A, neuro-specific enolase (NSE), gastrin, CRP, and full blood count were recorded in every patient. In all subjects, use of PPIs was explicitly prohibited for at least two weeks before the plasma measurement. Chromogranin A and NSE measurements were obtained by radioimmunoassay and expressed in nanograms per milliliter (ng/mL). Chromogranin A normal levels were 19–98 ng/mL, whereas the normal cutoff level for NSE was <12.5 ng/mL. C-reactive protein measurements were performed via turbidimetric assay, with normal limits ranging below 5 mg/L.

The study was approved by the research ethics board of “Sotiria” University Hospital (Athens, Greece) (approval code: 21103/20.10.16) and the Scientific Committee of the Medical School of Athens (approval code: 8942/21-4-15). Signed consent was obtained from all subjects, after explanation of the nature and purpose of the study.

2.2. Statistical Analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 20 for Windows (SPSS, Inc., Chicago, IL, USA). Demographic characteristics of the study cohort are reported as means and standard deviations for continuous variables and as proportions and absolute counts for categorical variables. Normal distribution was tested by Shapiro–Wilks test. Comparison between quantitative data was performed using Student’s *t*-test, while comparison between categorical data was performed using the Chi-square test with Yates correction, Fisher’s exact

test, or McNemar's test as appropriate. Cut-off values of quantitative data were set based on the existing literature. Multiple logistic regression analysis was applied to investigate which laboratory data can predict the clinical behavior of NENs, and also to estimate the probability of metastases for different covariate configurations. Probability (*p*) values of <0.05 were considered statistically significant.

3. Results

We identified a total of 93 patients with NENs, 57 of which were female (61.3%) and 36 were male (38.7%). The mean age at the time of diagnosis was 57.57 ± 14.76 years old (range 26–82 years old). All patients were of Caucasian origin. Multiple endocrine neoplasia (MEN) was diagnosed in 1 patient (1.1%) (Table 1).

The majority of NENs were detected in the gastroenteropancreatic system. The most common primary site was stomach (38.7%, *n* = 36), followed by pancreas (15.1%, *n* = 14) and small intestine (10.8%, *n* = 10). In 12 patients (12.9%) the primary tumor remained unknown, despite extensive diagnostic workup. Primary tumor localization is more analytically described in Table 1.

Table 1. Anthropometric and tumor characteristics of the enrolled patients.

Anthropometric and Tumor Characteristics	Patients (N = 93)
Age (years), mean (SD)	57.57 (± 14.76)
Gender, <i>n</i> (%)	
Female, <i>n</i> (%)	57 (61.3%)
Male, <i>n</i> (%)	36 (38.7%)
Primary tumor localization, <i>n</i> (%)	
Appendix	4 (4.3%)
Colon	2 (2.2%)
Lung	1 (1.1%)
Pancreas	14 (15.1%)
Rectum	8 (8.6%)
Small intestine	10 (10.8%)
Stomach	36 (38.7%)
Unknown	12 (12.9%)
Other ^a	6 (6.5%)
MEN associated, <i>n</i> (%)	1 (1.1%)
Disease extent, <i>n</i> (%)	
Localized	62 (66.7%)
Regional	12 (12.9%)
Metastatic	19 (20.4%)
Presence of metastases, <i>n</i> (%)	
Non-Metastatic	74 (79.5%)
Metastatic	19 (20.4%)
Grading (WHO 2010), <i>n</i> (%)	
G1	68 (73.1%)
G2	22 (23.6%)
G3	3 (3.2%)

^a The "other" category includes rare neuroendocrine neoplasms (NENs), such as NENs of mediastinum, skin, bladder, and head and neck.

3.1. Disease Extent

Over half of the NENs (66.7%, $n = 62$) were localized at the time of diagnosis. Of the patients, 12.9% had regional disease, while 20.4% of the NENs were metastatic. Figure 1 demonstrates schematically the disease extent of NENs based on their site of origin. As shown, metastatic disease was mainly observed in NENs of unknown origin and pancreas (red color), while localized tumors (green color) mostly arose from stomach, colon, and the lungs.

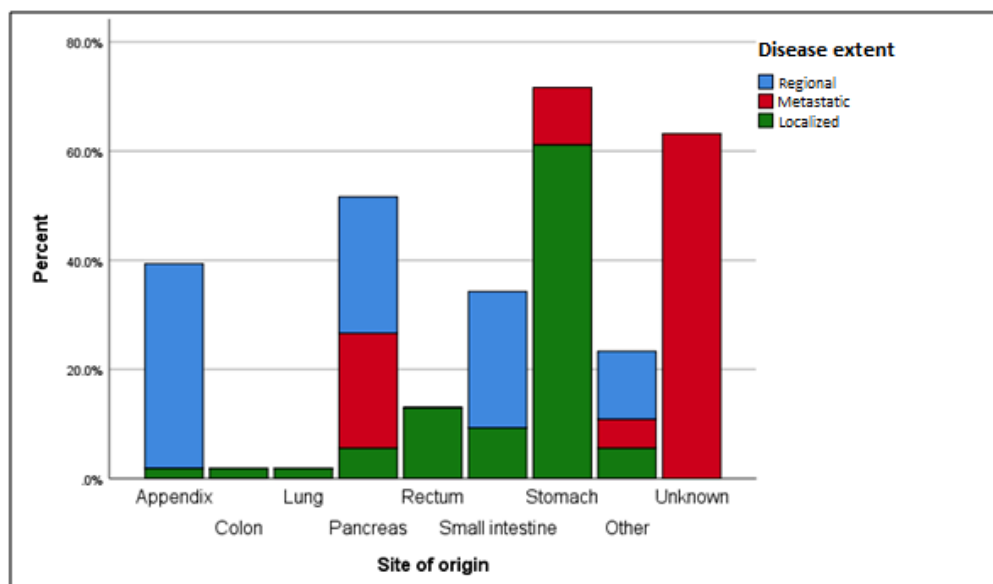


Figure 1. Disease extent of NENs based on their site of origin. Localized tumors are illustrated with green color, while regional and metastatic disease are illustrated with blue and red color, respectively. Metastatic disease was mainly observed in NENs of unknown origin and pancreas, while localized tumors mostly arose from stomach, colon, and the lungs.

3.2. Grading

Based on the Ki-67 index and/or mitoses number, the majority of ($n = 68$, 73.1%) NENs displayed a Ki-67 index of $\leq 2\%$, while G2–G3 classification with a high proliferation index was only observed in pancreatic, rectal, and rare NENs.

3.3. Gender and Age

Gender and age were assessed regarding their prediction power at the time of initial diagnosis. Multiple logistic regression analysis, after controlling for age and levels of CgA, showed that male patients with NENs displayed an elevated risk of having metastases at diagnosis (odds ratio—OR = 3.376, $p = 0.04$), compared to females. Similarly, patients over 50 years old were more likely to have developed regional NENs than localized ones (OR = 1.15, $p = 0.036$). The effects of both gender and age were independent of the effect of CgA. No correlation was found between these two variables and grading.

3.4. CgA and NSE

Chromogranin A and NSE were assessed as to whether these biochemical variables can share a predictive value in the course of NENs. Median values of CgA were compared between subgroups of patients based on disease extent and tumor grading using the Kruskal–Wallis test. It was found that median CgA levels differed significantly only between metastatic and non-metastatic NENs ($p < 0.01$) and not in grading subgroups ($p = 0.415$). Analogous findings were demonstrated in the subgroup of pancreatic NENs ($N = 14$), in which CgA levels were significantly associated with metastasis status.

Localized and regional cases of pancreatic NENs displayed statistically significant lower levels of CgA in comparison with metastatic cases.

Furthermore, in logistic regression analysis, controlling for age, gender, and grading, it was found that CgA predicts whether a NEN would be metastatic or not at the time of diagnosis. As CgA levels increase, it is more likely that we observe a metastatic NEN, with its relative risk ratio for every one-unit increase in CgA being 1.005 for having metastasis versus not. Likewise, when logistic regression analysis models were constructed to assess the effect of CgA in the grading of NEN, it was revealed that CgA cannot predict how aggressive its behavior can be (grading) after controlling for age, gender, and disease extent.

In an effort to further assess and maximize the clinical utility of the above-mentioned predicting effect of CgA in the disease extent of NENs, we used different cut-offs of CgA in order to determine the risk for having metastases, after adjusting for age and gender. As demonstrated in Figure 2, when CgA is >200 ng/dl, patients have almost 5 times higher risk for being metastatic at the time of diagnosis (OR = 4.82, $p = 0.008$, 95% CI: 1.5–7.46).

More interestingly, in order to reproduce the cumulative effect of age, gender, and levels of CgA in predicting whether a NEN would be metastatic or not at the time of first diagnosis, an integrated probability model analysis was conducted, as depicted in Figure 3. More analytically, different patient scenarios combining age, gender, and CgA levels are associated with different probabilities for metastatic disease, demonstrated schematically in a gradually escalating model, as age and CgA levels increase in both males and females. The lowest risk is observed in women aged <50 years old with CgA levels of <200 ng/dl (6.5%), while the highest one is in males over 50 years old with CgA > 200 ng/dl (62.9%).

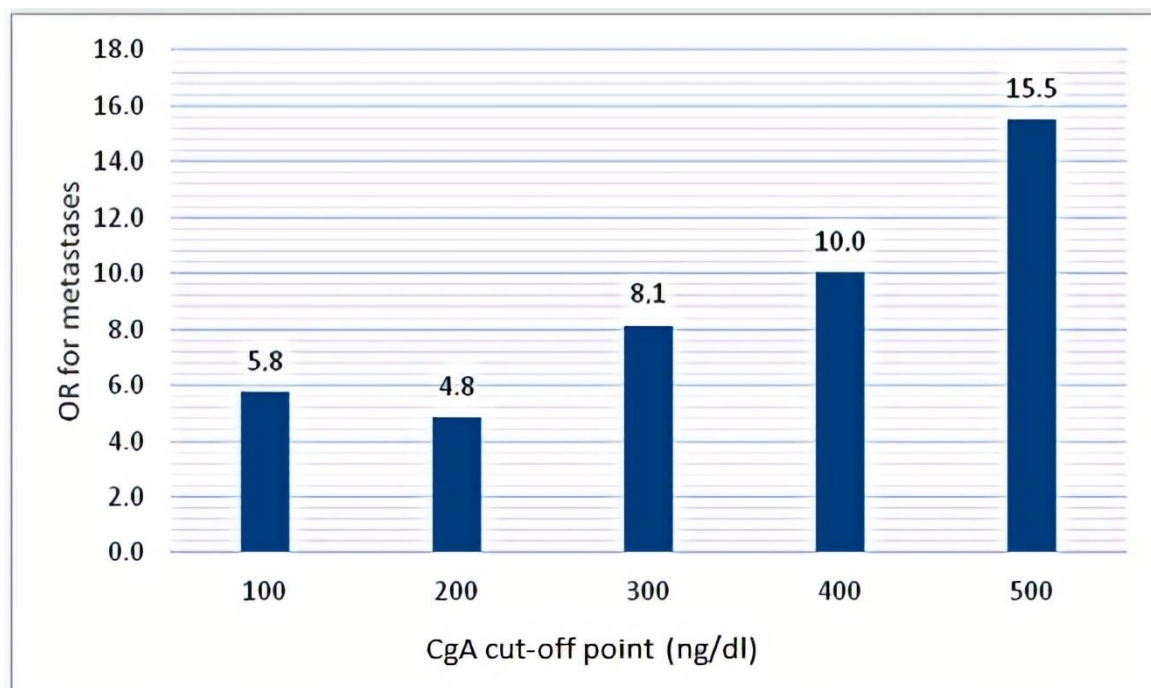


Figure 2. Using multiple logistic regression models, odds ratio (OR) for metastatic disease was calculated for different chromogranin A (CgA) (ng/dl) cut-off points, after adjusting for gender and age.

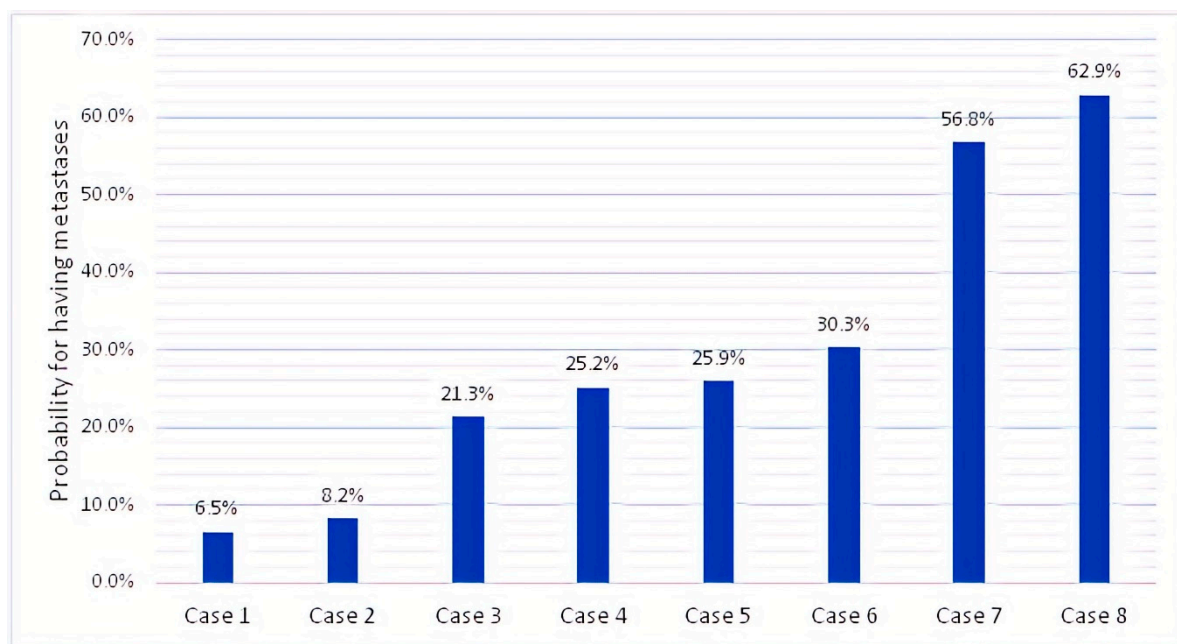


Figure 3. Probability of metastases for eight different models: Case 1 is a female less than 50 years old with CgA < 200 ng/dl, Case 2 is a female above 50 years old with CgA < 200 ng/dl, Case 3 is a male less than 50 years old with CgA < 200 ng/dl, Case 4 is a female less than 50 years old with CgA > 200 ng/dl, Case 5 is a male above 50 years old with CgA < 200 ng/dl, Case 6 is a female above 50 years old with CgA > 200 ng/dl, Case 7 is a male less than 50 years old with CgA > 200 ng/dl, Case 8 is a male above 50 years old with CgA > 200 ng/dl. The lowest risk is observed in women aged <50 years old with CgA levels of <200 ng/dl (6.5%), while the highest one is in males over 50 years old with CgA > 200 ng/dl (62.9%).

In contrast to CgA, when median values of NSE were compared between subgroups of patients based on disease extent and tumor grading using the Kruskal–Wallis test, we found that median NSE levels were comparable to those of all subgroups in both disease extent and grading classification ($p = 0.355$ and $p = 0.287$, respectively). NSE was able to predict neither disease extent nor grading at the time of first diagnosis.

Finally, focusing on the subgroup of gastric NENs, the majority of gastric NENs were multiple, localized, well-differentiated, displaying a Ki-67 < 2%, and mainly detected in the body of the stomach (66.6%), followed by fundus and pyloric antrum. Median gastrin levels were elevated (median gastrin = 238 pg/mL), but they were not found to be positively correlated with either CgA or NSE levels.

3.5. CRP and Other Inflammatory Markers

The prognostic utility of markers of systemic inflammatory response, including CRP and white blood count (WBC), was evaluated. C-reactive protein was found to be positively correlated with CgA ($p = 0.291$, $p = 0.045$). Furthermore, CRP was shown to have a significant effect on disease extent. Specifically, as CRP increases, it is much more likely that we will observe a metastatic NEN versus a non-metastatic one, with this effect being independent of CgA. The relative risk ratio for every unit increase of CRP is 1.493 for a patient being metastatic at the time of diagnosis (OR = 1.493, $p = 0.008$. 95% CI: 1.1–2.05). Analogously to CgA, CRP was not able to predict the grading of NEN.

After analyzing WBC as well as other parameters, such as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelets-to-lymphocyte ratio (PLR), and mean platelet volume (MPV), that have been studied in the literature, we were not able to detect any predictive effect either in disease extent or in grading (Table 2).

Table 2. The prognostic utility of markers of systemic inflammatory response in predicting the disease extent of NENs, as expressed in relative risk ratio in a multivariate analysis, after controlling for age, gender, and CgA.

Inflammatory Marker	Mean \pm SD	Relative Risk Ratio (RR)	p-Value
C-reactive protein (CRP) (mg/L)	6.95 \pm 7.57	1.493	0.008
White blood count (WBC)	9743.24 \pm 7172.8	1.00	0.546
Neutrophil-to-lymphocyte ratio (NLR)	2.67 \pm 1.92	2.222	0.279
Platelet-to-lymphocyte ratio (PLR)	133.39 \pm 113.5	1.020	0.336
Lymphocyte-to-monocyte ratio (LMR)	5.66 \pm 4.81	0.501	0.382
Mean platelet volume (MPV)	10.52 \pm 10.6	7.1	0.506

4. Discussion

The main findings of this study are focused on the predictive effect of CgA, especially when combined with other parameters, such as age and sex. More specifically, it is highlighted that the prognostic capacity of CgA in patients with NENs can be significantly strengthened when it is combined with age and sex, contributing ultimately to a potentially time-saving, individualized, and cost-effective diagnosis and therapeutic approach.

Both gender and age were shown to share predictive value at the time of the initial diagnosis, as male gender and age over 50 years are associated with an increased risk for having metastases (OR = 3.376, p = 0.04 for gender and OR = 1.15, p = 0.036 for age). In the literature, a meta-analysis conducted by Jensen et al. showed that advanced age at the time of diagnosis and male gender indicated a poor prognosis [23]. Likewise, in a study of GEP-NENs, male gender and increased age at the time of diagnosis were associated with poorer five-year survival rates [24]. Although these parameters may represent poor prognosis in the majority of cancer entities [25], in the present original research, it is highlighted that both gender and age not only are emerging as important risk factors for advanced disease extent, but also, when combined with CgA, can enhance its predictive effect in the detection of patients with NENs at increased risk for advanced disease (Figure 3). All the above findings constitute clinically valuable information that can potentially determine our therapeutic interventions when we first evaluate patients with NENs.

Chromogranin A is a hydrophilic glycoprotein commonly involved in a series of biological pathways controlling protein (peptides, hormones, neurotransmitters, and growth factors) secretion upon secretagogue stimulation [26]. In the unique cohort of NENs, CgA has been established as a universal laboratory marker, as its elevated levels have been associated with almost all types of NENs. However, as a consequence of the ubiquitous co-secretion of CgA with other regulatory peptides, there are multiple causes of CgA elevation that are unrelated to NENs, limiting its sensitivity and specificity in the diagnostic setting [8]. Therefore, integrating the prognostic effects of other parameters such as age and sex with CgA could probably contribute to surpassing these limitations and enhancing the isolated diagnostic and prognostic value of CgA.

Circulating CgA has long been used as an indirect survival predictor in clinical practice of NEN management. In fact, there is a plethora of studies in the literature demonstrating that higher levels of CgA are associated with advanced disease and enhanced tumor load [27–30]. Concerning our analysis, it was shown that median CgA levels differed significantly between metastatic and non-metastatic NENs (p < 0.01); simultaneously, in logistic regression analysis, after controlling for age, gender, and grading, CgA was able to predict whether a NEN would be metastatic or not at diagnosis. In fact, as CgA levels increase, there is a concomitant increase in the relative risk ratio for having metastasis versus not. Focusing on the subgroup of pancreatic NENs, CgA was also found to be significantly elevated in the metastatic cases, whereas patients with localized or regional disease displayed either normal or mildly elevated levels. This finding is in concordance with existing literature, according to

which plasma CgA levels are associated with tumor size, metastasis status, and tumor stage in patients with pancreatic NENs and, therefore, should be used cautiously in the diagnosis and evaluation of treatment response of early stage pancreatic NENs [12].

Furthermore, in the present study, it was highlighted that when CgA is >200 ng/dL, patients have almost 5 times higher risk of being metastatic at the time of diagnosis (OR = 4.82, $p = 0.008$, 95% CI: 1.5–7.46). In a study by Citterio D et al., a similar cut-off of CgA was used in a more homogeneous set of patients of 139 well-differentiated NENs with metastatic liver involvement, in which basal CgA of ≥ 200 ng/mL was found to be an independent negative prognostic factor for survival [31]. Analogous findings were provided by the prospective RADIANT-1, -2, and -3 trials, in which patients with circulating levels of CgA higher than twice the upper normal limit demonstrated shorter overall survival [32,33].

Apart from setting a cut-off level for CgA crucial for prognosis, we also took into account the effects of age and gender in order to reproduce a cumulative effect in predicting whether a NEN would be metastatic or not at the time of initial diagnosis. Figure 3 schematically depicts how the risk of metastasis escalates as age and CgA levels increase in both males and females. To our knowledge, this is the first time that specific cumulative relative risk ratios for metastasis of NENs, based on age, gender, and CgA, have been proposed in literature. Further, well-designed, prospective studies are necessary to establish these risk ratios, as they constitute information with major clinical value for clinicians managing patients with NENs.

On the other hand, in our study cohort, no predicting effect was documented between CgA and grading. This is in agreement with a larger and more homogenous study of 1159 patients with pancreatic NENs, in which CgA levels did not correlate with histological differentiation, mitotic rate, or pathologic disease stage [34]. Chromogranin A is more frequently elevated in well-differentiated tumors, as compared with poorly differentiated ones, probably due to the reduced functional integrity of the neuroendocrine secretory system in poorly differentiated tumors [26].

Likewise, in our cohort, NSE was able to predict neither disease extent nor grading at the time of first diagnosis. This might be attributed to the small number of poorly differentiated tumors, with high Ki-67 levels, included in the cohort, as NSE is particularly elevated in patients with poorly differentiated NENs [35,36].

Moreover, we additionally proceeded to evaluate whether inflammation can be a significant factor in the pathophysiology of NENs. Indeed, inflammatory biomarkers could probably help clinicians assess prognosis in patients with NENs, since testing for these markers is cheap, sensitive, and easily done via a routine blood test. Thus, in the present study, CRP levels were measured before any therapeutic intervention and were found to be positively correlated with CgA. Furthermore, it was highlighted not only that CRP had a significant predicting effect on disease extent (the relative risk ratio for every unit increase of CRP is 1.493 for a patient being metastatic at the time of diagnosis), but also that this effect is independent of CgA. There are studies in the literature addressing this area in NENs, as well. Specifically, Wiese D et al., in a retrospective analysis of 149 patients with pancreatic NENs, showed that CRP is an independent prognostic factor for survival in these patients and suggested that pretreatment CRP measurements should be considered for incorporation into prospective studies of outcome in patients with pancreatic NENs and clinical trials of systemic therapies for these tumors [37]. More recently, it was shown that CRP can be a poor prognostic marker of survival in the whole group of gastroenteropancreatic NENs, with each increase of 1 mg/L in the CRP value being accompanied by an increase of the risk of death by 1.5% [24]. However, to our knowledge, the present study is the first time that the independent effect of CRP on tumor burden has been documented in a cohort of NENs. Overall, given the small sample size of our cohort and the limited data in the literature, the clinical utilization of CRP as a prognostic biomarker in assessing the natural course of NENs should be exercised with caution until more validated data and reproducible cut-off levels are established.

Finally, peripheral inflammatory hematologic parameters of WBC, such as NLR, LMR, PLR and MPV, were not found to be correlated with disease extent and grading in our cohort. These results were

in discordance with two studies of patients with pancreatic NENs showing that these inflammatory markers are adversely associated with overall survival [38,39]. This could probably be attributed to the small sample size in the study by Gaitanidis A et al. or to the fact that only patients with advanced disease state were enrolled in the study by Qi Q et al. Indeed, in a recent meta-analysis, it was highlighted that NLR could be utilized as an adverse prognosis factor only in pancreatic NENS, as the majority of included cases were patients with pancreatic NENs, while the prognostic value of other hematologic parameters deserves further investigation [40].

The findings of this study must be viewed in light of the following limitations. The primary limitation is the small sample size of these rare tumors, which should be considered in the context of this specific geographic and ethnic background. Another limitation is the lack of clinical outcomes, such as survival or disease progression rates.

In conclusion, to summarize the main findings of this study, circulating CgA levels not only can be used as a direct predictor of tumor load in patients with NENs, but also, when interpolated with the effects of age and gender, can cumulatively predict whether a NEN would be metastatic or not at the time of initial diagnosis, via a risk-escalating probability model. Simultaneously, CRP, one of the most widely used inflammatory markers in routine clinical practice, can predict disease extent at the time of diagnosis and be useful both in treatment planning and monitoring during follow-up of all NENs. Further studies are necessary to verify whether these findings can be used as a clinical tool in defining therapeutic management and evaluating survival rates.

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