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A Plasma Protein Biomarker Strategy for Detection of Small Intestinal Neuroendocrine Tumors

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Keywords
Neuroendocrine tumor · Biomarker · Diagnosis · Machine learning

Abstract
Background: Small intestinal neuroendocrine tumors (SI-NETs) are difficult to diagnose in the early stage of disease. Current blood biomarkers such as chromogranin A (CgA) and 5-hydroxyindolacetic acid have low sensitivity (SEN) and specificity (SPE). This is a first preplanned interim analysis (Nordic non-interventional, prospective, exploratory, EXPLAIN study [NCT02630654]). Its objective is to investigate if a plasma protein multi-biomarker strategy can improve diagnostic accuracy (ACC) in SI-NETs. ***Methods:*** At the time of diagnosis, before any disease-specific treatment was initiated, blood was collected from patients with advanced SI-NETs and 92 putative cancer-related plasma proteins from

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135 patients were analyzed and compared with the results of age- and sex-matched controls (n = 143), using multiplex proximity extension assay and machine learning techniques. ***Results:*** Using a random forest model including 12 top ranked plasma proteins in patients with SI-NETs, the multi-biomarker strategy showed SEN and SPE of 89 and 91%, respectively, with negative predictive value (NPV) and positive predictive value (PPV) of 90 and 91%, respectively, to identify patients with regional or metastatic disease with an area under the receiver operator characteristic curve (AUROC) of 99%. In 30 patients with normal CgA concentrations, the model provided a diagnostic SPE of 98%, SEN of 56%, and NPV 90%, PPV of 90%, and AUROC 97%, regardless of proton pump inhibitor intake. ***Conclusion:*** This interim analysis demonstrates that a multi-biomarker/machine learning strategy improves diagnostic ACC of patients with SI-NET at the time of diagnosis, especially in patients with normal CgA levels. The results indicate that this multi-biomarker strategy can be useful for early detection of SI-NETs at presentation and conceivably detect recurrence after radical primary resection.

Introduction

Small intestinal neuroendocrine tumors (SI-NETs) are rare neoplasms with a low but increasing incidence in the range from 0.5 to 1.5 per 100,000 inhabitants per year [1–5]. SI-NETs may be incidentally found during work up of other conditions, or patients may present with carcinoid syndrome (diarrhea and flushing) and with intermittent abdominal pain or acute bowel obstruction. Some of these symptoms are non-specific and often seen in irritable bowel syndrome. Therefore, correct SI-NET diagnosis is often delayed by several years [6]. At the time of diagnosis, many patients present with disseminated disease, making curative surgery impossible [7].

Circulating plasma chromogranin A (CgA), a glycoprotein released by neuroendocrine cells, and 5-hydroxyindolacetic acid (5-HIAA), a serotonin breakdown product that can be measured in blood and urine, have for decades been used as biomarkers in the diagnosis, treatment response, and follow-up of SI-NETs [8–11]. Neuron-specific enolase, another neuroendocrine related protein, has only shown low to moderate levels of sensitivity (SEN) and specificity (SPE) for GEP-NEN [12].

Most conclude that these biomarkers have relatively low SEN and SPE [13, 14]. Thus, there is a need for new biomarkers that would accurately detect SI-NETs at an early curable stage, both regarding primary diagnosis as well as for detection of recurrent and progressive disease [13, 15–17].

Newly discovered biomarkers such as miRNA have shown potential to improve the diagnostic SEN and SPE [18–20]. Several other novel biomarkers such as immune-molecules and circulating tumor cells are under investigation [20].

Previous studies in patients with NETs using RNA-based multi-biomarker approaches demonstrated improved tumor classification, metastatic profiling, as well as diagnostic accuracy (ACC) [21–23]. The proximity extension assay (PEA) technology has been used in several cancer studies to identify new biomarker candidates in patients with multiple myeloma [24], colorectal cancer [25], and ovarian cancer [26]. Edfeldt et al. [27] discovered new proteins that correlated with survival in patients with SI-NETs, using blood samples from 23 patients with SI-NETs that were analyzed with proximity ligation assay and PEA.

Machine learning is a scientific field with origins in computer science, artificial intelligence, and statistics. These techniques were introduced in cancer research >15 years ago [28]. The idea is to produce computer algorithms that learn how to correctly perform specific assignments in order to identify certain patterns present in a data set. Random forest (RF) is a well-established statistically supervised learning model and has been used to improve cancer diagnosis in previous studies [29, 30]. Adam et al. [31] demonstrated that multi-plasma protein analysis in combination with statistical machine learning techniques improved diagnostic ACC of prostate cancer by discriminating it from benign hyperplasia.

The aim of the first interim analysis from the EXPLAIN study was to investigate, in a real-life clinical setting, whether a plasma protein multi-biomarker strategy, measuring the relative concentrations of 92 putative cancer-related human proteins, in combination with machine learning, can provide new biomarkers to improve diagnostic ACC in SI-NETs.

Materials and Methods

This is an interim analysis from the Nordic EXPLAIN study (exploratory, non-interventional study for evaluating the diagnostic, prognostic, and response-predictive value of a multi-biomarker approach in metastatic GEP NETs [ClinicalTrials.gov: NCT02630654]).

The study was approved by the regional Ethics Committee in each participating country (Denmark, Finland, Norway, and Sweden), and the study complied with the Declaration of Helsinki and

Good Clinical Practice guidelines. All patients and the control group provided written informed consent after full explanation of the purpose and nature of all procedures used.

Patients

A total of 175 patients were initially recruited from 17 hospitals and screened to enable 150 patients with SI-NETs to enter the study. Thirty-five patients who were included had surgery prior to inclusion. Inclusion criteria were as follows: provision of written informed consent, suspected metastatic SI-NET (World Health Organization [WHO] grade 1 or 2, up to 10% Ki-67), and aged 18 years or older. SI-NET diagnosis was confirmed in all patients according to current clinical practice at each hospital. Exclusion criteria were as follows: NET not confirmed, previously treated for NET disease with anti-proliferative treatments, peptide receptor radionuclide therapy or other radiation therapy, other malignant diseases, chronic inflammatory diseases, and severe renal and/or liver disease. Patients who had surgery of the primary tumor but with residual metastatic disease could be included.

Clinical data were collected at each visit and as part of regular patient follow-up and entered into an electronic case report form (Viedoc; Pharma Consulting Group, Uppsala, Sweden). The use of proton pump inhibitors (PPIs) was registered. Patients using PPIs were not excluded, nor was the PPI treatment stopped before blood sample collection. Radiologic evaluation of metastatic disease at the different centers included contrast enhanced computed tomography, MRI, and somatostatin receptor positron emission tomography/computed tomography imaging.

A control group of 155 age- (±5 years) and sex-matched individuals was included for biomarker blood sampling. Exclusion criteria were as follows: malignant disease, chronic inflammatory disease, and renal or hepatic failure. The individuals were selected from the Karolinska University Hospital Clinical Pharmacology Trial Unit. Three control individuals were excluded due to the presence of chronic inflammatory disease.

Sample Collection and Analysis

A study blood sample (4 mL) was collected starting at the first visit and before any NET-specific treatment was initiated. Thereafter, blood samples were collected at visits that were part of the patients' normal follow-up. Samples were prepared by collecting blood in tubes containing ethylenediaminetetraacetic acid and placed on ice immediately after sampling. The samples were centrifuged at 2,500 g for 10 min at 4°C. After centrifugation, plasma was aspirated, aliquoted to new tubes (4 × 0.5 mL), and immediately hereafter stored at −80°C. Blood samples for the exploratory plasma protein biomarkers were transported on dry ice to SciLifeLab, Uppsala, Sweden, and analyzed using multiplex PEA by real-time PCR using the Fluidigm BioMark HD real-time PCR platform with the Olink Proseek Oncology II panel (Olink Proteomics, Uppsala, Sweden, http://www.olink.com/) as previously described [32, 33]. The multiplex PEA was developed from the proximity ligation assay technique by [32]. One pair of antibodies was used to target each specific protein. The antibodies were coupled to complementary oligonucleotides enabling DNA polymerase to amplify the double-stranded DNA, generating a PCR-reporter sequence by the proximity-dependent DNA polymerization event. The panel of proteins is selected by experts at Olink Proteomics after fulfilling several technical as well as biological criteria. The panel was selected on the basis that these proteins previously have been shown to be associated with mechanisms in neoplastic disease and classified according to UniProt, Human Protein Atlas, Gene Ontology, and DisGeNET [24, 25].

Table 1. Clinical characteristics for all patients with SI-NETs and for patients with SI-NETs and CgA ≤ ULN (≤5.3 nmol/L) at the time of diagnosis

	All patients with SI-NETs (n = 135)	Patients with SI-NETs and CgA ≤ ULN (≤5.3 nmol/L) (n = 30)
Primary tumor location, *n* (%)		
Small intestine	122 (90.4)	27 (90.0)
Unknown	13 (9.6)	3 (10.0)
Regional lymph nodes, *n* (%)		
Nx	10 (7.4)	0 (0)
N0	5 (3.7)	7 (23.3)
N1	120 (88.9)	23 (76.7)
Distant metastases, *n* (%)		
Mx	13 (9.6)	1 (3.3)
M0	31 (23.0)	20 (66.6)
M1	91 (67.4)	9 (30.0)
NET grade, *n* (%)		
Missing	5 (3.7)	3 (10.0)
G1	61 (45.2)	17 (56.7)
G2	69 (51.1)	10 (33.3)
No. of metastases		
Mean (SD)	9.4 (8.6)	4.2 (4.2)
Median (range)	7 (1–36)	2 (1–13)
Ki-67%		
Mean (SD)	4.8 (4.1)[a]	1.8 (2.7)[c]
Median (range)	4 (1–17)[a]	1.5 (0.3–10)[c]
Plasma CgA, nmol/L		
Mean (SD)	46.8 (85.7)	3.7 (1.1)
Median (range)	48 (2–620)	3.6 (2.0–5.3)
Urine/serum 5-HIAA (%ULN)		
Median (range)	168 (16–5,953)[b]	73 (16–148)[d]

CgA, chromogranin A; ULN, upper limit of normal; SI-NET, small intestinal neuroendocrine tumor. [a] n = 126. [b] n = 83. [c] n = 26. [d] n = 21.

Chromogranin A was analyzed centrally at Akademiska Laboratoriet, Uppsala, Sweden, with the NEOLISA™ Chromogranin A assay (Euro Diagnostica). Urine/serum 5-HIAA was analyzed at each individual hospital using methods according to clinical routine and presented as percent of upper limit of normal (%ULN). Serum 5-HIAA was measured at 3 clinics in Finland, morning urine 5-HIAA was used in 1 clinic in Norway, and 13 clinics used 24-h urine samples. A total of 135 patients with SI-NETs and 143 age- and sex-matched controls with complete biomarker information were retained for the data analysis.

Statistical Analysis

Levels of biomarkers were compared between patients with SI-NETs and the control group. Folded F test was used to test for evidence of equal variance between the 2 populations. If the results

Table 2. Performance metrics generated with random forest for model 1 and model 2

	Model 1				Model 2	
	including all 92 biomarkers and CgA		including 12 top biomarkers		including all 92 biomarkers	
	mean (SD), %	min–max, %	mean (SD), %	min–max, %	mean (SD), %	min–max, %
PPV	90 (4.4)	85–96	91 (6.6)	82–100	90 (13.7)	75–100
NPV	88 (7.1)	83–100	90 (5.9)	86–100	90 (8.6)	79–100
SEN	87 (8.1)	81–100	89 (6.4)	85–100	56 (32.2)	20–100
SPE	91 (4.7)	86–97	91 (6.3)	83–100	98 (2.2)	96–100
ACC	89 (3.6)	84–93	90 (4.9)	84–96	89 (8.1)	78–100
AUC	99 (0.5)	98–100	99 (0.4)	99–100	97 (3.0)	90–100
MR	11 (3.6)	7–16	10 (4.9)	4–16	11 (8.1)	0–22

CgA, chromogranin A; PPV, positive predictive value; NPV, negative predictive value; SEN, sensitivity; SPE, specificity; ACC, accuracy; AUC, area under the curve; MR, misclassification rate; model 1, all patients with SI-NETs (n = 135); model 2, patients with SI-NETs, and CgA ≤ ULN (≤5.3 nmol/L) (n = 30).

of folded F indicated the presence of unequal variance, the Welch-Satterthwaite t test was applied. Otherwise, the equal variance t test was used.

RF, a commonly used machine learning algorithm to classify data, was the approach retained for the current study. Preliminary machine learning analysis has shown that RF yielded better ACC to classify between the control group and patients with SI-NETs than support vector machine [34].

Two classification analyses were performed. First, model 1 included all the selected participants, that is, 135 patients with SI-NETs and 143 the control group, the 92 plasma protein biomarkers, and CgA. CgA values were log-transformed to match the already log-transformed biomarkers. The second RF analysis (model 2) included the control group (n = 143) and patients with SI-NETs with CgA values ≤ULN (n = 30). CgA-log was excluded from the analysis since the marker has normal values for the 2 groups of patients.

To validate the RF algorithm, a 5-fold cross-validation was performed. Thus, the complete data set was partitioned into equally (or near equally) sized folds or segments. This gave an approximate split between training and validation sets of 80 and 20%, respectively. The 5-fold cross-validation strategy was chosen to avoid having small groups of patients in the validation sets. This cross-validation strategy was applied to all the models. Software JMP® Pro 14.1.0 and the random forest boosted tree method were used to perform the classification analysis. The classifier constructs a predictive model by adding a sequence of decision trees where each of the trees is fit on the residuals of the previous tree (JMP® 14 Predictive and Specialized Modeling 2018, SAS Institute Inc., Cary, NC, USA). A maximum of 50 layers and 3 splits per tree were allowed. Performance of the different models was evaluated by comparing the following metrics: negative predictive value (NPV), positive predictive value (PPV), SEN, SPE, and ACC. These metrics were calculated from the confusion matrix at each fold of the 5-fold cross-validation. Descriptive statistics, that is, mean, standard deviation, and minimum and maximum values, were calculated for each of the performance metrics. Misclassification rate, the rate for which the response with the highest fitted probability is not the observed category, is also provided. Receiver operating characteristic curves and area under the curve were calculated with the software Python 3.7.0 (package: *sklearn*). The top proteins were selected after identifying most frequent biomarkers, in 2 or more folds, in the 5-fold cross-validation in the first random forest analysis.

Results

Baseline Characteristics

Of the 175 screened patients, the final SI-NET cohort that fulfilled the inclusion and exclusion criteria had complete biomarker data and was included in the data analyses that consisted of 135 patients. Clinical characteristics of these 135 patients with SI-NETs are given in Table 1.

Median age of all patients was 66 years (range 38–89) and 79 (59%) were males. Median age of the control group was 67 years (range 36–84) and 83 (58%) were males. In patients (n = 30) with CgA concentrations equal or ≤ULN, median age was 66 years (range 44–84) and 20 (66%) were males.

NET-related symptoms registered at diagnosis in patients with SI-NETs were as follows: 20 (15%) had >3 daily bowel movements, 10 (7%) had >3 daily flushes, 13 (10%) had >3 daily episodes of abdominal pain, and 35 (26%) had at least 1 of the 3 symptoms described above. In comparison, in the patient population with plasma CgA ≤ ULN (n = 30), symptoms were as follows: 3 (10%) had >3 daily bowel movements, 1 (3%) had >3 daily flushes, 2 (7%) had >3 daily episodes of abdominal pain, and 6 (20%) had at least 1 of the 3 symptoms described above.

Plasma Chromogranin A and 5-HIAA

Median circulating plasma CgA concentration in the control group was 3.3 nmol/L (range 2–43). The control group provided a 97th percentile value at 5.3 nmol/L. A

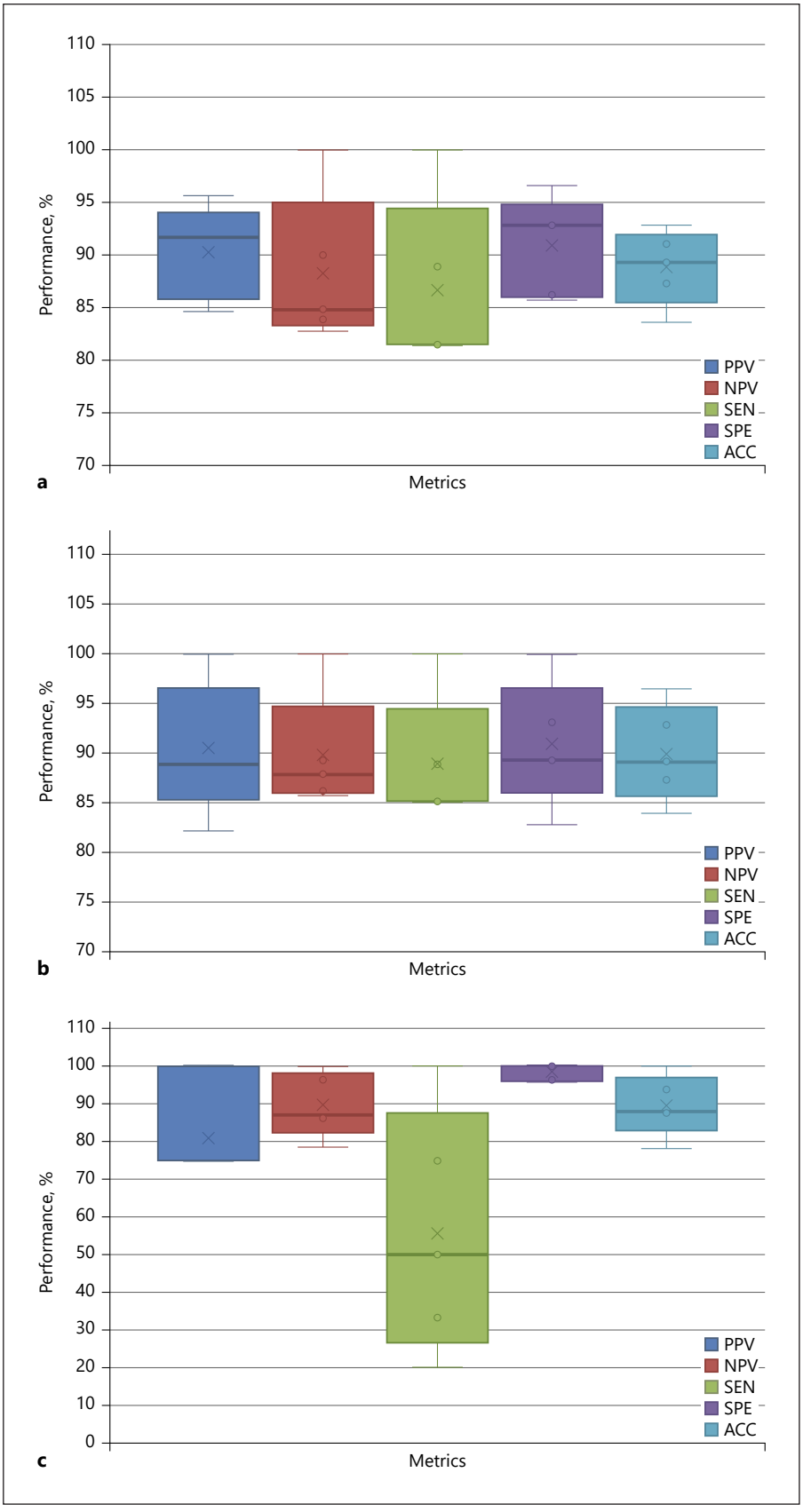

Fig. 1. a–c Box plots of the random forest performances (PPV, NPV, SEN, SPE, and ACC) in a 5-fold cross-validation including all patients, 92 plasma protein biomarkers, and CgA-log (model 1, [**a**]), including all patients and the 12 most frequently identified biomarkers (**b**) and including the control group and patients with SI-NETs and low CgA (≤ULN), 92 biomarkers, and excluding the CgA-log (model 2, [**c**]). PPV, positive predictive value; NPV, negative predictive value; SEN, sensitivity; SPE, specificity; ACC, accuracy; CgA, chromogranin A; SI-NET, small intestinal neuroendocrine tumor.

cutoff value of 5.3 nmol/L results in an SEN and SPE for CgA alone similar to what has been shown in other studies (see below).

Using the 97th percentile (i.e., upper limit of normal, ≤ULN) as the limit for normal CgA, 22% of patients (n = 30) had CgA concentrations below or equal to this value at the time of diagnosis. Patients with CgA ≤ ULN also showed a lower number of metastasis (median 2, range 1–13) compared with the whole SI-NET patient population (median 7, range 1–36). Of the patients with CgA ≤ ULN, 9 had 1 metastasis (1 patient had 1 liver metastasis and 8 patients had 1 lymph node metastasis only). Plasma CgA concentrations in all patients with SI-NETs and those with CgA ≤ ULN are presented in Table 1.

In this study, the mean (SD) diagnostic SEN was 79% (±7), SPE was 87% (±5), PPV was 85% (±5), and NPV was 81% (±5) for CgA alone. At the time of diagnosis, 12 (8%) were in the control group, 21 (16%) of the patients with SI-NETs, and 3 (10%) of the patients with CgA ≤ ULN were treated with PPIs. Urine/serum 5-HIAA levels were increased (>100% ULN) in 59 (71%) of the 83 patients providing samples within 3 months after the first visit.

Machine Learning Analysis

RF performances (model 1) evaluated as NPV, PPV, SEN, and SPE in a 5-fold cross-validation (mean [SD]) were 88 (±7), 90 (±4), 87 (±8), and 91% (±5), respectively. Table 2 shows all the descriptive statistics for the performance metrics evaluated in model 1. Figure 1a summarizes the 5-fold cross-validation results for model 1 expressed as PPV, NPV, SEN, SEN, and ACC. The graph aims to show the variability of the model's metrics across the different folds. Using all 92 biomarkers and CgA, the model did not discriminate between grade 1 and 2 at the time of diagnosis.

An additional RF analysis was performed including only the biomarkers with the highest contribution to model 1. Thus, a total of 12 biomarkers, that is, most frequently identified in the 5-fold cross-validation (in 2 or more folds) and having the highest contribution to the classification model, were selected (Table 3).

Mean (SD) SEN and NPV improved slightly by including only the 12 most highly ranked plasma protein biomarkers, from 87 (±8) to 89% (±6) and from 88 (±7) to 90% (±6), respectively. All the metrics performances are presented in Table 2 and Figure 1b. The area under the receiver operator characteristic curve (AUROC) analysis including only 12 biomarkers identified a mean (±SD) AUROC of 99% (±0.4) (Fig. 2). Model 2 included the control group and patients with SI-NETs and CgA ≤ ULN (n = 30). CgA-log was excluded from the data analysis since the marker has normal values within the groups of patients. The 5-fold cross-validation yielded the following performances (mean [SD]): NPV 90% (±9), PPV 90% (±14), SEN 56% (±32), and SPE 98% (±2). Additional performance metrics are presented in Table 2. Figure 1c summarizes the 5-fold cross-validation results for model 2. Table 3 shows the 12 biomarkers most frequently identified in the 5-fold cross-validation with the highest contribution to the classification model between the control group and patients with SI-NETs and CgA ≤ ULN.

Table 3. Most frequently identified biomarkers in the 5-fold cross-validation (in 2 or more folds)

Biomarker	Frequency in 5-fold cross-validation	
	model 1	model 2
CgA-log	5	NA
LYN	5	5
ITGAV	4	4
CDKN1A	4	NI
CAIX	4	NI
ABL1	3	3
5′-NT	3	NI
EGF	3	3
PVRL4	2	NI
TRAIL	2	2
VIM	2	4
MUC-16	2	NI
CRNN	NI	3
VEGFA	NI	3
CD70	NI	3
hK14	NI	2
S100A11	NI	2

Information on each protein can be found at https://www.uniprot.org. NA, not applicable; NI, not identified; model 1, all patients with SI-NETs (n = 135); model 2, patients with SI-NETs, and CgA ≤ ULN (≤5.3 nmol/L) (n = 30).

Discussion

To our knowledge, this is the first study that analyzes the relative levels of close to 100 putative cancer-related plasma proteins at the time of diagnosis in a large SI-NET patient population in a real-life clinical setting. In this interim analysis, the main finding is that PEA and combined machine learning techniques improve diagnostic PPV and NPV in patients with SI-NETs at the time of diagnosis, including those with CgA concentrations

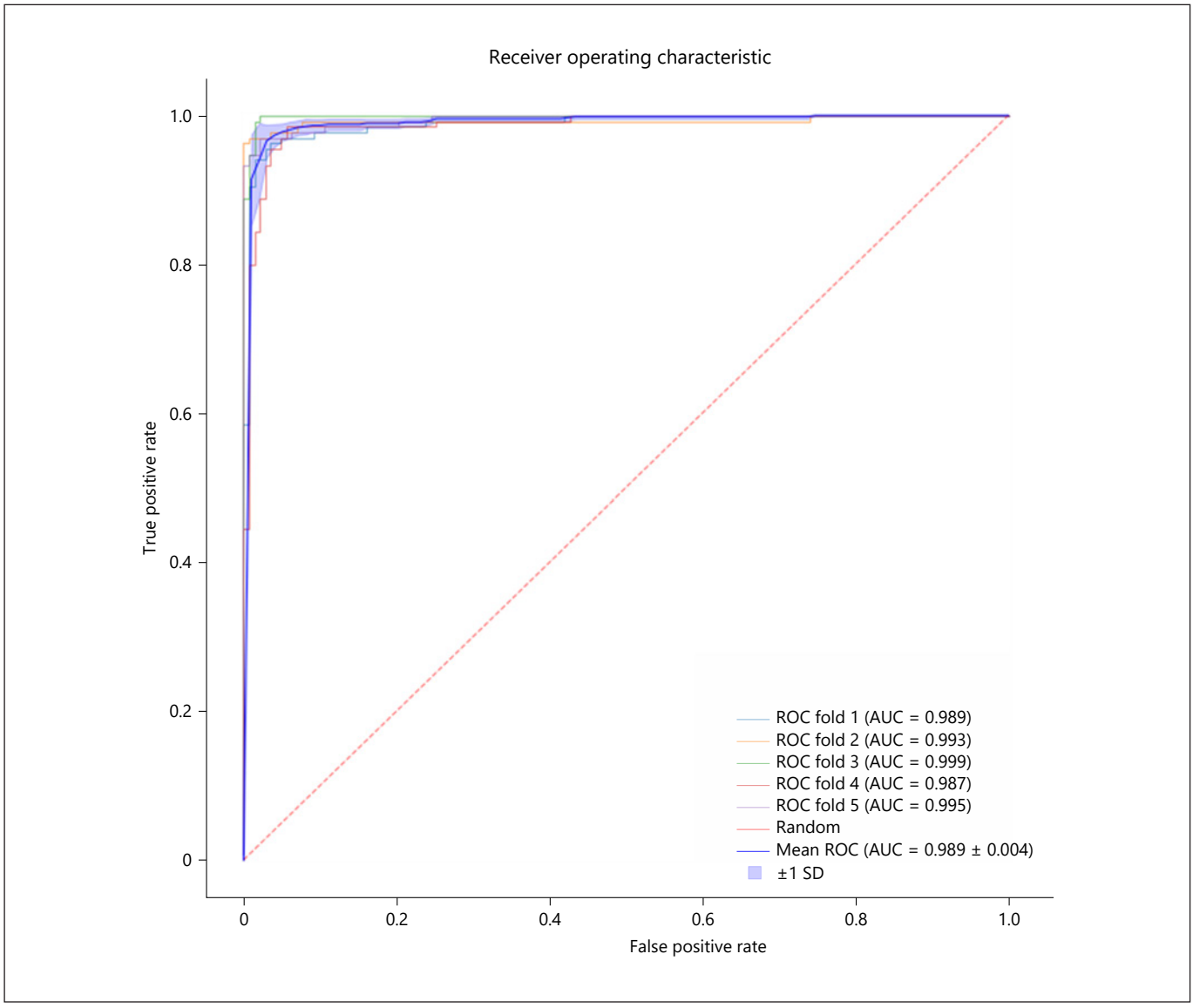

Fig. 2. ROC curves and the corresponding AUC values generated from a random forest model analysis including the control group and patients with SI-NETs and the top 12 ranked identified plasma protein biomarkers. ROC, receiver operating characteristic; AUC, area under the curve.

≤ULN, providing both a PPV and NPV of 90%. The results imply that this multi-biomarker strategy may be useful for early detection of SI-NETs, both at presentation and to possibly detect recurrence after radical resection of the primary tumor. Methods with better SEN and SPE to detect SI-NETs at an early and curable stage are warranted as blood biomarkers such as CgA, neuron-specific enolase, pancreastatin, and circulating tumor cells so far lack the SEN, SPE, and predictive capacity needed in routine clinical practice [13].

The PEA technology used for measuring plasma proteins in the present study is not yet adapted to and validated for routine clinical practice. However, it is valuable in the discovery phase of research since it generates large amounts of biomarker data. In order to better analyze our data, we used supervised machine learning techniques. This approach clearly out-performs analysis of plasma CgA alone. Our finding suggests that plasma CgA does not provide sufficient diagnostic ACC as a mono-biomarker in accordance with previous studies [12, 14]. Of

note, several factors are known to influence CgA concentrations, for example, intake of PPIs, renal failure, atrophic gastritis, and hepatic disease [35–37]. Zatelli et al. [38] demonstrated that CgA as a mono-biomarker had an SEN around 0.8 at its best, similar to the findings of the present study.

Importantly, a significant finding of the present study is that our biomarker test improved detection of SI-NETs in patients with regional or metastatic disease who had normal CgA concentrations. This patient subgroup was further characterized by lower concentrations of 5-HIAA, a low Ki-67 index, and low number of metastases compared with patients with verified metastatic disease and elevated CgA concentrations (Table 1). This patient subgroup displays fewer NET-specific symptoms and is the most difficult to diagnose. In our study, mean NPV and PPV for this patient subgroup were as high as 90%, with an AUROC of 97%. Consequently, this method could aid in accurate and timely primary diagnosis. In addition, it could be used to identify early recurrences in the follow-up of patients after radical surgical resection and eventually also in screening of members in families with increased incidence of SI-NETs [39]. This multi-biomarker strategy could thus decrease the need for expensive and burdensome radiologic examinations.

Our study has several strengths including the "real-life clinical setting" design, the large number of patients with SI-NETs with matched controls, as well as centralized analysis of CgA and biomarkers. However, this is a first exploratory study and the model has not been tested to discriminate between patients with SI-NETs and non-SI-NETs with similar symptomatology, such as diarrhea, abdominal pain, and flushing. Future validation studies are needed to clarify these aspects including other primary tumors, for example, pancreatic NET. Furthermore, even if the primary tumor was removed prior to inclusion in 35 patients (as a result of small intestinal surgery), the patients still had advanced disease when they were included. It can, however, be speculated that in some patients this may have impaired the SEN and SPE of the biomarker profile.

A similar approach to that of the present study used an mRNA-based blood multi-biomarker model (multiple transcriptional analyses) and reported data which improved diagnostic ACC, treatment response prediction, as well as prediction of progression [40–42]. The diagnostic ACC (AUROC of 0.95–0.98) of this mRNA-based multi-biomarker model is in the same range as in the present study. A recent Delphi assessment of currently available NET biomarkers concluded that there is an imminent unmet medical need to develop more informative biomarker tools [13].

Patients who have undergone possible radical resection are 1 group of patients in whom this test could improve diagnostic ACC. The biomarker test could also aid in discriminating those patients that truly can be considered disease-free from those having microscopic remnant disease. This study provides a method for improving early diagnosis in patients with normal plasma CgA, 5-HIAA, and low tumor burden and should be validated in larger SI-NET population in the future. In addition, the SPE of the current biomarker test needs to be investigated in other NET subtypes such as pancreatic and lung NETs.

In conclusion, this is the first pre-planned interim analysis of the Nordic EXPLAIN biomarker study in an SI-NET patient population. The multi-plasma protein analysis strategy improved diagnostic SPE and SEN. Importantly, the model discriminated between healthy individuals and patients with SI-NETs characterized by normal CgA concentrations, moderate disease burden, and mild symptomatology. These biomarkers should be further studied as a possible test for early diagnosis, recurrence, and potentially treatment response in patients with NET.

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Statement of Ethics

This study was approved by the local Ethics Committees of the Nordic countries, and written informed consent was obtained from all participants.

Conflict of Interest Statement

M.K.: grants from IPSEN, consulting fees from IPSEN and Novartis Healthcare. U.K.: grants from IPSEN and Novartis Healthcare, consulting fees from IPSEN. E.T.E.: research grants from No-

vartis, consulting fees from IPSEN, and speaker honoraria from Novartis, Ipsen, and Pfizer. H.G.: research grants from the Novo Nordisk Foundation, Intercept, Abbvie, and consulting fees from IPSEN and Novartis. C.S.J.:. grant from IPSEN, consulting fees from IPSEN, Kyowa Kirin, and Novartis. H.S.: received research support from Novartis, Amgen, Ipsen and honoraria from Novartis, Ipsen, Pfizer, Keocyt, AstraZeneca, Roche, Amgen, BMS, Hutchinson, Merck, Shire, and Celgene. H.W.: no conflicts of interest. V.J.: no conflicts of interest. M.T.J.: no conflicts of interest. S.W.: consulting fees from IPSEN and Novartis Healthcare. J.A.S.: consulting fee from Novartis. S.M.: speaker honoraria from Amgen, Ipsen, Novartis, Novo Nordisk, MSD, and Sanofi. T.E.: speaker honoraria from Ipsen, AstraZeneca, and Novo Nordisk Pharma, consulting fee from Amgen, educational grants by Novartis, Ipsen, and MSD Finland to the employer institution for international symposia. F.L.: speaker honorarium from Novartis. K.L:. no conflicts of interest. F.S.: no conflicts of interest. G.W.: no conflicts of interest. M.d.P.S.: IPSEN employee. R.B.: IPSEN employee.

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Author Contributions

Design of the study: M.K., U.K., E.T.E., H.G., C.S.J., H.S., H.W., V.J., M.T.J., S.W., J.A.S., S.M., T.E., F.L., K.L., G.W., F.S., and R.B. Preparing the study protocol: R.B., M.K., U.K., E.T.E., H.G., and C.S.J. Patients inclusion: M.K., U.K., E.T.E., H.G., C.S.J., H.S., H.W., V.J., M.T.J., S.W., J.A.S., S.M., T.E., F.L., K.L., G.W., and F.S. Data management: Pharma Consulting Group, Uppsala, Sweden, and R.B. Statistical analysis: M.d.P.S. Preparation of manuscript: R.B., M.d.P.S., M.K., U.K., E.T.E., H.G., and C.S.J. All authors have reviewed/edited the manuscript and approved the final version for submission.

Data Sharing

Where patient data can be anonymized, Ipsen will share all individual participant data that underlie the results reported in this article with qualified researchers who provide a valid research question. Study documents, such as the study protocol and clinical study report, are not always available. Proposals should be submitted to DataSharing@Ipsen.com and will be assessed by a scientific review board. Data are available beginning 6 months and ending 5 years after publication; after this time, only raw data may be available.

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