Neuroendocrinology

Research Article

Neuroendocrinology
DOI: 10.1159/000508106

Received: March 1, 2020
Accepted: April 21, 2020
Published online: April 24, 2020

# Prospective Evaluation of the NETest as a Liquid Biopsy for Gastroenteropancreatic and Bronchopulmonary Neuroendocrine Tumors: An ENETS Center of Excellence Experience

Anna Malczewska[a] Magdalena Witkowska[a] Monika Wójcik-Giertuga[a] Katarzyna Kuśnierz[b] Agnes Bocian[a] Agata Walter[a] Mateusz Rydel[c] Amanda Robek[d] Sylwia Pierzchała[a] Magdalena Malczewska[e] Izabela Leś-Zielińska[f] Damian Czyżewski[c] Dariusz Ziora[f] Joanna Pilch-Kowalczyk[g] Wojciech Zajęcki[h] Beata Kos-Kudła[a]

[a]Department of Endocrinology and Neuroendocrine Tumors, Medical University of Silesia, Katowice, Poland; [b]Department of Gastrointestinal Surgery, Medical University of Silesia, Katowice, Poland; [c]Department of Thoracic Surgery, Medical University of Silesia, Zabrze, Poland; [d]Department of Oncology, Medical University of Silesia, Katowice, Poland; [e]Department of Cardiology and Structural Heart Diseases, Medical University of Silesia, Katowice, Poland; [f]Department of Pulmonology, Medical University of Silesia, Zabrze, Poland; [g]Department of Radiology and Nuclear Medicine, Medical University of Silesia, Katowice, Poland; [h]Department of Pathology, Medical University of Silesia, Zabrze, Poland

**Keywords**
NETest · Liquid biopsy · mRNA · Molecular genomics · Neuroendocrine · NET · Carcinoid · Biomarker · Chromogranin A · 68Ga-SSA PET/CT

**Abstract**

***Background:*** There is a substantial unmet clinical need for an accurate and effective blood biomarker for neuroendocrine neoplasms (NEN). We therefore evaluated, under real-world conditions in an ENETS Center of Excellence (CoE), the clinical utility of the NETest as a liquid biopsy and compared its utility with chromogranin A (CgA) measurement. ***Methods:*** The cohorts were: gastroenteropancreatic NEN (GEP-NEN; *n* = 253), bronchopulmonary NEN (BPNEN; *n* = 64), thymic NEN (*n* = 1), colon cancer (*n* = 37), non-small-cell lung cancer (NSCLC; *n* = 63), benign lung disease (*n* = 59), and controls (*n* = 86). In the GEPNEN group, 164 (65%) had image-positive disease (IPD, *n* = 135) or were image-negative but resection-margin/biopsy-positive (*n* = 29), and were graded as G1 (*n* = 106), G2 (*n* = 49), G3 (*n* = 7), or no data (*n* = 2). The remainder (*n* = 71) had no evidence of disease (NED). In the BPNEN group, 43/64 (67%) had IPD. Histology revealed typical carcinoids (TC, *n* = 14), atypical carcinoids (AC, *n* = 14), small-cell lung cancer (SCLC, *n* = 11), and large-cell neuroendocrine carcinoma (LCNEC, *n* = 4). Disease status (stable or progressive) was evaluated according to RECIST v1.1. Blood sampling involved NETest (*n* = 563) and NETest/CgA analysis matched samples (*n* = 178). NETest was performed by PCR (on a scale of 0–100), with a score ≥20 reflecting a disease-positive status and >40 reflecting progressive disease. CgA positivity was determined by ELISA. Samples were deidentified and measurements blinded. The Kruskal-Wallis, Mann-Whitney U, and McNemar tests, and the area under the curve (AUC) of the receiver-operating characteristics (ROC) were used in the statistical analysis. ***Results:*** In the

karger@karger.com
www.karger.com/nen



Anna Malczewska, MD, PhD
Department of Endocrinology and Neuroendocrine Tumors
Medical University of Silesia, ul. Ceglana 35
PL–40-514 Katowice (Poland)
malczewska.an@gmail.com

GEPNEN group, NETest was significantly higher (34.4 ± 1.8, $p < 0.0001$) in disease-positive patients than in patients with NED (10.5 ± 1, $p < 0.0001$), colon cancer patients (18 ± 4, $p < 0.0004$), and controls (7 ± 0.5, $p < 0.0001$). Sensitivity for detecting disease compared to controls was 89% and specificity was 94%. NETest levels were increased in G2 vs. G1 (39 ± 3 vs. 32 ± 2, $p = 0.02$) and correlated with stage (localized: 26 ± 2 vs. regional/distant: 40 ± 3, $p = 0.0002$) and progression (55 ± 5 vs. 34 ± 2 in stable disease, $p = 0.0005$). In the BPNEN group, diagnostic sensitivity was 100% and levels were significantly higher in patients with bronchopulmonary carcinoids (BPC; 30 ± 1.3) who had IPD than in controls (7 ± 0.5, $p < 0.0001$), patients with NED (24.1 ± 1.3, $p < 0.005$), and NSCLC patients (17 ± 3, $p = 0.0001$). NETest levels were higher in patients with poorly differentiated BPNEN (LCNEC + SCLC; 59 ± 7) than in those with BPC (30 ± 1.3, $p = 0.0005$) or progressive disease (57.8 ± 7), compared to those with stable disease (29.4 ± 1, $p < 0.0001$). The AUC for differentiating disease from controls was 0.87 in the GEPNEN group and 0.99 in BPC patients ($p < 0.0001$). Matched CgA analysis was performed in 178 patients. In the GEPNEN group ($n = 135$), NETest was significantly more accurate for detecting disease (99%) than CgA positivity (53%; McNemar test $\chi^2 = 87$, $p < 0.0001$). In the BPNEN group ($n = 43$), NETest was significantly more accurate for disease detection (100%) than CgA positivity (26%; McNemar's test $\chi^2 = 30$, $p < 0.0001$). ***Conclusions:*** The NETest is an accurate diagnostic for GEPNEN and BPNEN. It exhibits tumor biology correlation with grading, staging, and progression. CgA as a biomarker is significantly less accurate than NETest. The NETest has substantial clinical utility that can facilitate patient management.



## Introduction

It is an unfortunate reality that neuroendocrine tumor (NET) disease is an underserved division of oncology due to its relatively low incidence and the limited number of therapeutic strategies that have been developed for its treatment [1]. In general, NETs are difficult to manage since they are usually identified late in their natural history and thus present the challenges associated with treating metastatic disease [2, 3]. This reflects the fact that most symptomatology is only evident once disease spread has occurred and the common complaints are often dismissed as being nonneoplastic in origin [4].

Given the fact that advanced disease is the most common scenario for NET clinical management, the need for a tool to better define the biology of the disease and the precise status of the tumor and its metastases is an important unmet need [5]. Similarly, the identification of effective therapy preadministration and the accurate monitoring of disease response and status are critical goals in assuring judicious patient management [5]. Broadly speaking, there are 3 strategies that can be used to accomplish this. First is clinical assessment which is useful, but inefficient given the subtlety and sporadic nature of the symptoms and the subjective nature of the art [6]. Second is imaging which can be highly effective for localizing disease, but has limitations for identifying progress due to the often indolent nature of the disease and the reality that sophisticated technology is expensive and not widely available [7, 8]. Third is the detection of sophisticated and sensitive blood biomarkers that can provide tumor-related information in real-time, aid in assessing numerous biological aspects of the disease, and are precise enough to define alterations at a molecular level [5].

In the last decade, there have been substantial advances in imaging techniques and NET disease can now be assessed effectively both anatomically, i.e., with computed tomography (CT) or magnetic resonance imaging (MRI), and functionally, i.e., with $^{68}$Ga-SSA positron emission tomography combined with CT (PET/CT) or $^{18}$F-FDG PET/CT [9]. Despite such innovations, there are nevertheless still limitations in the identification of early disease progress and in-depth assessment of the biological status of a tumor. Advances in the development of metabolically specific isotopes, sophisticated mathematical analysis of voxel counts, and the use of techniques such as deep neural learning will, in the future, amplify image-derived information [10]. Currently, imaging studies cannot be serially undertaken to monitor a disease due to health economic costs, radiation exposure, and the relative unavailability of sophisticated technology [11].

A viable alternative strategy is the development of genomic tumor biomarkers in the blood, which provide a real-time, infinitely repeatable surrogate of tumor status and response to therapy. The classical method in the past was tissue biopsy and direct tumor sampling, which has significant limitations including random and heterogeneous sampling, invasiveness and morbidity, and restrictions in terms of repetition [12]. The alternative, i.e., sampling blood to evaluate tumor biomarkers, although attractive, has been somewhat ineffective because the monoanalyte biomarkers measured (insulin, gastrin, serotonin, etc.,) are secretory markers (monoanalytes) that do not provide information regarding the molecular biology of the tumor [13, 14]. Such markers do not assess the so-called "hallmarks of neoplasia" proposed by Hanahan and Weinberg [15]. Similarly, the

use of the general secretory amine, chromogranin A (CgA), as a biomarker, has proved to have limited efficacy in the diagnosis and management of NETs [16–19]. Measuring the secretory parameter of a tumor cell provides little information about tumor proliferation, growth factor regulation, metabolic function, treatment efficacy, and the identification of druggable targets [15].

In the field of oncology, recognition of the limitations of monoanalyte biomarkers and the complexities of imaging has resulted in a shift of focus to the development of "liquid biopsies." This technique, to assess multianalyte genomic biomarkers in the blood, can be used to provide a detailed assessment of tumor status in real-time [12]. In breast, lung, colon, and prostate cancer, such tools have proven to be of substantial clinical utility [20–24]. This strategy has recently been utilized in NET disease with the development of a multigene mRNA test (NETest) of the blood. This strategy involves the mathematical algorithmic analysis of a series of specific "omic clusters" that capture the biology of a NET [25, 26]. Numerous clinical studies and an independent meta-analysis have demonstrated that the NETest has an overall accuracy of >90% [25, 27–33] and provides real-time information that identifies residual tumor and progression or response to therapy [27, 31–39]. Direct comparison studies indicate that it is significantly more accurate than CgA positivity and that it detects lesions prior to their identification on imaging [26, 39].

At a NET Center of Excellence (CoE), we undertook to prospectively evaluate the clinical utility of the NETest under real-world conditions. Our goals were to assess accuracy as an in vitro diagnostic and compare it with our usual CoE-based assessment strategies which include image-derived information and the biomarker CgA. Overall, our purpose was to determine if a multianalyte genomic assessment of NET disease provided reliable information useful for clinical management.

## Material and Methods

*Cohorts*

The study overall assessed 563 individuals with one of the following conditions (Table 1):

- Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs, $n$ = 253): pancreatic (PNEN, $n$ = 83), ileal/jejunal, i.e., small intestinal (SINEN, $n$ = 54), duodenal (DNEN, $n$ = 12), gastric (GNEN, $n$ = 46), rectal (RNEN, $n$ = 46), or appendiceal (ANEN, $n$ = 12)
- Lung or bronchopulmonary neuroendocrine neoplasms (BP-NENs, $n$ = 64): bronchopulmonary carcinoids (BPC, $n$ = 49; either typical carcinoids [TC], $n$ = 30 or atypical carcinoids [AC], $n$ = 19), large-cell neuroendocrine carcinoma (LCNEC, $n$ = 4), small-cell lung cancer (SCLC, $n$ = 11); and single thymic NET ($n$ = 1).

As comparators, we evaluated:

- Colon cancers: adenocarcinoma (ACC, $n$ = 37)
- Non-small-cell lung cancers (NSCLCs, $n$ = 63): ACC ($n$ = 37), squamous-cell carcinoma (SCC, $n$ = 20), large-cell carcinoma (LCC, $n$ = 2), or not otherwise specified (NOS, $n$ = 4)
- Idiopathic pulmonary fibrosis (IPF, $n$ = 50)
- Chronic obstructive pulmonary disease (COPD, $n$ = 9).

The control group ($n$ = 86) comprised family members of the hospital personnel, and nonaffected family members of the patients attending the Endocrinology Department. All controls indicated they were asymptomatic and in good health and none exhibited or identified any known malignancy at the time of blood draw (Table 1).

The NETest was evaluated in the entire cohort ($n$ = 563) and CgA measurement was undertaken in 496 individuals. For matched analyses, we used samples taken from patients with image-positive disease (IPD) ($n$ = 178, including 135 GEPNENs and 43 BPNENs).

*Methods*

Strategy

We examined circulating NETest levels and CgA positivity in the NET cohort and compared these to controls and nonneuroendocrine disease of the bowel (colon cancer) or lung (NSCLC, IPF, and COPD). The diagnostic accuracy and metrics, i.e., the area under the curve (AUC) of the receiver-operating characteristics (ROC), i.e. AUROC, and the sensitivity and specificity for the NETest and CgA measurement were calculated. Separately, we assessed the agreement of the NETest with imaging (a correlation between NETest positivity and image-detectable disease) or histology when image-negative but with evidence of microscopic disease (a positive resection margin or biopsy). Disease-positive (DP) refers to image-positive, or histology-positive even if image-negative; disease-negative (i.e., no evidence of disease [NED]) refers to when there is no disease detectable on imaging and no evidence of histology-positive resection margins or biopsy.

Sample Collection

*Blood for NETest Measurement*. Peripheral blood samples (3 mL) were collected in EDTA tubes, mixed, and stored on ice. Tubes were deidentified and anonymously coded and stored at –80 °C within 2 h of collection [40]. Deidentified blood samples were sent to a central laboratory (Wren Laboratories, CT, USA). Test analysis data were provided in numeric coded form to the Medical University of Silesia and the blinded data were independently evaluated by the study authors.

*Plasma for CgA Measurement*. Plasma samples were collected as per standard protocol for CgA measurement using the NEOLISA TM kit (EuroDiagnostica), ULN: 108 μg/L. Samples were sent deidentified (as above) to Wren Laboratories for measurement. Blinded data were provided to the study authors for independent evaluation.

Radiological Evaluation of NET Disease

*Disease Extent Was Determined by:* anatomical imaging, CT or MRI, and/or functional 68Ga-DOTA-TATE PET/CT were performed in well-differentiated NETs or 18F-FDG PET/CT in G2/G3 NENs. Image assessment was undertaken by specialized radi-

Neuroendocrinology
DOI: 10.1159/000508106



**Table 1.** Demographic and clinicopathological characteristics of the study cohort

| | GEPNENS (*n* = 253) | | | | | | | BPNENs (*n* = 64) | | | Other NENs | Comparators (*n* = 245) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PNEN (*n* = 83) | SINEN (*n* = 54) | DNEN (*n* = 12) | GNEN T1 (*n* = 42) | GNEN T3 (*n* = 4) | RNEN (*n* = 46) | ANEN (*n* = 12) | BPC (*n* = 49) | LCNEC (*n* = 4) | SCLC (*n* = 11) | thymic NET (*n* = 1) | NSCLC (*n* = 63) | | | | benign lung disease (*n* = 59) | | colon cancer (*n* = 37) | controls (*n* = 86) |
| | | | | | | | | | | | | ACC (*n* = 37) | SCC (*n* = 20) | LCC (*n* = 2) | NOS (*n* = 4) | COPD (*n* = 9) | IPF (*n* = 50) | | |
| Age, years | | | | | | | | | | | | | | | | | | | |
| Mean | 55 | 60 | 51 | 55 | 55 | 57 | 40 | 60 | 72 | 69 | 38 | 64 | 69 | 77 | 69 | 63 | 66 | 65 | 47 |
| Range | 19–87 | 27–77 | 29–67 | 28–87 | 40–84 | 37–79 | 18–65 | 32–78 | 65–76 | 59–81 | n.a. | 37–80 | 57–79 | 72–83 | 62–78 | 32–85 | 43–87 | 38–78 | 23–78 |
| Gender | | | | | | | | | | | | | | | | | | | |
| Male:female | 31:52 | 31:23 | 7:5 | 10:32 | 3:1 | 24:22 | 5:7 | 15:34 | 2:2 | 9:2 | 1:0 | 19:18 | 18:2 | 1:1 | 3:1 | 7:2 | 40:10 | 24:13 | 40:46 |
| Functional status | | | | | | | | | | | | | | | | | | | |
| NF:F | 73:10 | 33:21 | 12:0 | 42:0 | 4:0 | 46:0 | 12:0 | 46:3 | 4:0 | 11:0 | 0 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Grade | | | | | | | | | | | | | | | | | | | |
| G1*[a] | 39 | 40 | 9 | 32 | 0 | 42 | 11 | 30 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 3 | n.a. |
| G2[b] | 36 | 13 | 2 | 10 | 0 | 4 | 1 | 19 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | | | 33 | |
| G3 NET | 3 | 1 | 0 | 0 | 1 | 0 | 0 | n.a. | n.a. | n.a. | 1 | n.a. | n.a. | n.a. | n.a. | | | – | |
| G3 NEC[c] | 3 | 0 | 1 | 0 | 3 | 0 | 0 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | | | 1 | |
| No data | 2 | – | – | – | – | – | – | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | | | – | |
| TNM stage | | | | | | | | | | | | | | | | | | | |
| Localized | | | | | | | | | | | | | | | | | | | |
| IND | 23 | 2 | 2 | 25 | 2 | 37 | 10 | 19 | 0 | 0 | – | – | – | 0 | – | n.a. | n.a. | – | n.a. |
| IPD | 17 | 0 | 5 | 15 | 1 | 1 | 0 | 10 | 1 | 2 | – | 26 | 13 | 2 | 1 | | | 19 | |
| Regional metastatic | | | | | | | | | | | | | | | | | | | |
| IND | 2 | 8 | 0 | 2 | 1 | 1 | 2 | 2 | 0 | 0 | 1 | – | – | – | – | | | – | |
| IPD | 11 | 11 | 5 | 0 | 0 | 1 | 0 | 10 | 1 | 3 | – | 7 | 4 | – | 2 | | | 9 | |
| Distant metastatic | | | | | | | | | | | | | | | | | | | |
| IND | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – | – | – | – | | | – | |
| IPD | 30 | 32 | 0 | 0 | 0 | 6 | 0 | 8 | 2 | 6 | – | 2 | 3 | – | 1 | | | 9 | |
| No data | – | – | – | – | – | – | – | – | – | – | – | 2 | – | – | – | | | – | |
| Disease status (RECIST v1.1) | | | | | | | | | | | | | | | | | | | |
| Progressive | 16 | 4 | 2 | 0 | 0 | 4 | n.a. | 4 | 3 | 9 | – | 8 | 6 | 0 | 2 | n.a. | n.a. | [d] | n.a. |
| Stable | 42 | 39 | 8 | 15 | 1 | 4 | n.a. | 24 | 1 | 2 | – | 29 | 13 | 2 | 2 | | | [d] | |
| No data | – | – | – | – | – | – | – | – | – | – | – | – | 1 | – | – | | | – | |
| Therapy at blood draw | | | | | | | | | | | | | | | | | | | |
| SSA | 33 | 32 | 0 | 0 | 0 | 5 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | n.a. | n.a. | 0 | n.a. |
| MTT | 2 | 1[e] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 0 | |
| Cx | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 11 | 0 | 5 | 2 | 0 | 1 | | | 0 | |
| Resection | 16 | 10 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 29 | 13 | 2 | 2 | | | 35 | |
| Previous therapy | | | | | | | | | | | | | | | | | | | |
| Resection/LRT | 45 | 40 | 3 | 34 | 3 | 45 | 12 | 41 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | n.a. | n.a. | 0 | n.a. |
| PRRT | 13 | 12 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 0 | |
| Cx | 7 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 5 | 1 | 1 | 2 | | | 2 | |
| Rx | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | | | 5 | |

ACC, adenocarcinoma; Cx, chemotherapy; IPF, idiopathic pulmonary fibrosis; LCC, large-cell carcinoma; LRT, locoregional therapy; MTT, molecular targeted therapy; n.a., not applicable; NF, nonfunctioning; F, functioning; NOS, not otherwise specified; PRRT, peptide receptor radionuclide therapy; Rx, radiotherapy; SCC, squamous-cell cancer; SSA, somatostatin analogs.

[a] Typical carcinoid for BPC; [b] Atypical carcinoid for BPC; [c] G3 for colon cancer; [d] partial response in 1/6 patients after neoadjuvant Rx or Rx+Cx, in 31 diagnosed de novo; [e] two previously treated with MTT.

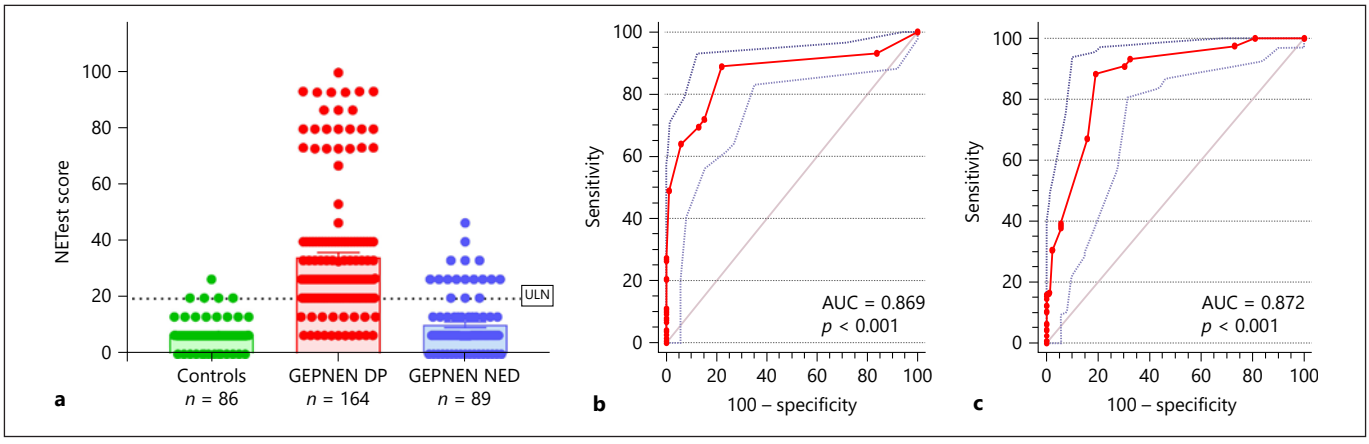


**Fig. 1.** NETest levels in GEPNENs and controls. **a** NETest in disease-positive (DP) GEPNENs, GEPNENs with no evidence of disease (NED), and controls. Kruskal-Wallis (KW) statistic 190.7, $p < 0.0001$. NETest ULN: 20%. Mean ± SEM. **b** The AUROC for NETest in GEPNENs and controls: AUC 0.87 (95% CI 0.83–0.9, $p < 0.0001$). **c** The AUROC for GEPNEN DP and GEPNEN NED: AUC 0.87 (95% CI 0.82–0.91, $p < 0.0001$).

ologists who were part of the ENETS CoE. IPD was defined as positive on CT/MRI (anatomical) and/or $^{68}$Ga-DOTA-TATE/$^{18}$F-FDG PET/CT (functional). Image-negative disease (IND) was defined as negative on anatomical (CT/MRI) and/or functional $^{68}$Ga-DOTA-TATE PET/CT in well-differentiated tumors.

*Imaging Modalities.* For radiological assessment, anatomical and functional imaging modalities were utilized. Anatomical imaging comprised multiphase CT with administration of iodine contrast with a 16-slice LightSpeed CT scanner, or multiphase MRI with a 1.5-T MRI scanner (both from General Electrics, USA) with injection of gadolinium contrast (slice thickness 4–6 mm) and T1/T2 sequences being obtained. Functional imaging was performed with hybrid PET/CT scanners with administration of $^{68}$Ga-DOTA-TATE in well-differentiated NETs or $^{18}$F-FDG in higher-grade (poorly and well-differentiated) tumors.

*Endoscopy.* Assessment of GNENs and RNENs (gastroscopy or colonoscopy, respectively) was performed by endoscopy.

*Disease Status.* Progressive disease was defined based on anatomical imaging and the RECIST v1.1 criteria. Parameters were an at least 20% increase in the sum of diameters of the target lesions (min 5 mm) measured on anatomical imaging (CT) or the detection of new lesions by imaging of the same modality when subsequently performed [41].

Histological Diagnosis

All NEN patients had histologically confirmed NEN disease, reported by an independent expert NEN pathologist (W.Z.) in accordance with the WHO 2017 and TNM 8th edition classification of NENs [42–45]. All biopsy specimens were evaluated (by H&E staining or immunohistochemistry) and reviewed by the same pathologist.

NETest

Details of the PCR methodology, mathematical analysis, and validation have been published in detail. In brief, they comprise a 2-step protocol (RNA isolation/cDNA production and qPCR) from EDTA-collected whole blood [34, 37, 40]. Assays are undertaken using deidentified samples in a central USA clinically and federally certified laboratory (Wren Laboratories CL-0704, CLIA 07D2081388). Transcripts (mRNA) are isolated from the samples (Blood Mini Kit, Qiagen, Valencia, CA, USA) and real-time PCR performed on prespotted plates [46]. Target transcript levels are normalized and quantified versus a population control, and final results are expressed as an activity index (NETest score) on a scale of 0–100 (normal score cut-off: 20) [34, 37, 40, 46].

Statistical Analysis

The required total sample size (NETs and controls, power 0.8 and $\alpha = 0.05$) to attain significant differences in NETest scores (from previously published means ± SD) was calculated to be a minimum of 11 individuals in each group. Intergroup analyses were undertaken using 2-tailed nonparametric tests (the Mann-Whitney U test), or Kruskal-Wallis multiple testing with Dunn's correction or $\chi^2$ (with Yates correction). AUROC analysis was used to determine the diagnostic accuracy of the NETest [47–49]. Metrics calculated included sensitivity and specificity. Prism v7.0 for Windows (GraphPad Software, La Jolla, CA, USA) and MedCalc statistical software v16.2.1 (Ostend, Belgium) were utilized. Statistical significance was defined as a $p$ value <0.05 and data expressed as means ± SEM.

## Results

Cohort demographics and clinical information are shown in Table 1. The majority of the NEN cohort was well-differentiated, consisting of 244/253 GEPNENs (96%) and 49/64 BPNENs (77%).

*NETest*

Diagnostic Utility in GEPNENs

NETest levels in all (i.e., IPD and IND as a single cohort) GEPNENs (26 ± 1.4) were significantly higher than

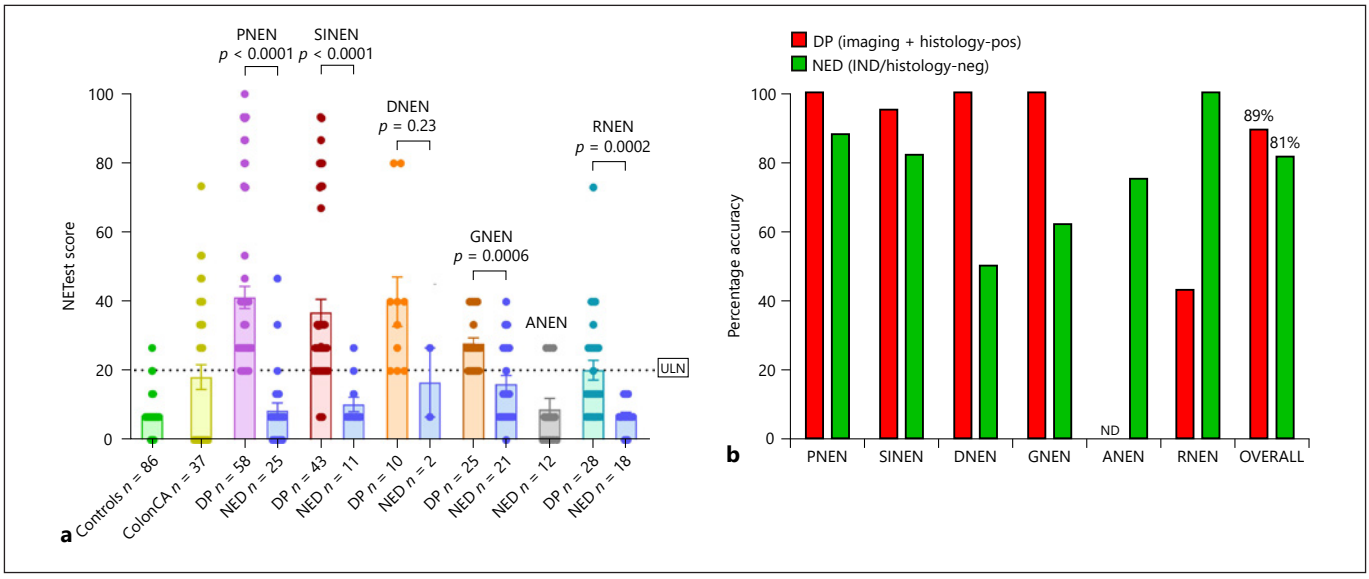


**Fig. 2.** Comparison and accuracy for disease detection using NETest levels by GEPNEN site and disease extent identified by imaging and histology. **a** NETest levels in disease-positive (DP) cases versus those with no evidence of disease (NED) were significantly higher in PNENs ($p < 0.0001$), SINENs ($p < 0.0001$), GNENs ($p = 0.0006$), and RNENs ($p = 0.0002$), but not DNENs ($p = 0.23$). ANENs all had NED. **b** Accuracy for detecting PNEN, DNEN, and GNEN was 100%, but 42% for RNEN. Disease accuracy for non-detectable disease ranged from 50% (DNEN) to 100% (RNEN). Accuracy of NETest in IPD was 89% (146/164) and for IND it was 81% (72/89). NETest overall accuracy of disease identification was 86%. NETest ULN: 20%. Mean ± SEM. ColonCA, colon cancer; GEPNEN, gastroenteropancreatic NEN; PNEN, pancreatic NEN; SINEN, small-intestine NEN; DNEN, duodenal NEN; GNEN, gastric NEN; ANEN, appendiceal NEN; RNEN, rectal NEN; ND, no data.

in controls (7 ± 0.5, $p < 0.0001$) (Fig. 1a). NETest levels in DP GEPNENs ($n = 164$), as defined by image-positive ($n = 135$) and image-negative but resection margin/biopsy-positive (microscopic disease) GEPNENs ($n = 29$), were significantly higher (34.4 ± 1.8) than in NED cases on either imaging or histology (10.5 ± 1, $p < 0.0001$), or in controls (7 ± 0.5, $p < 0.0001$; Fig. 1a). NETest was positive in significantly more DP GEPNENs (146/164) than in controls (5/86)( Fisher's test: $p < 0.0001$, $\chi^2 = 160$). The AUROC for differentiating GEPNENs from controls was 0.87 (95% CI 0.83–0.9; $p < 0.0001$; Fig. 1b). Accuracy for detecting DP cases compared to controls was 91%, sensitivity was 89%, and specificity was 94%. The AUROC for differentiating DP from NED cases was 0.87 (95% CI 0.82–0.91; $p < 0.0001$; Fig. 1c).

## Relationship between NETest and Evidence of Disease on Imaging and Histology

IPD was present in 135 GEPNENs, i.e., in 58/83 PNENs, 43/54 SINENs, 10/12 DNENs, 16/46 GNENs, and 8/46 RNENs; all ANENs ($n = 12$) were image-negative.

First, we evaluated the NETest levels in DP and NED GEPNENs (Fig. 2a). NETest levels were significantly higher in DP cases than in NED cases in PNENs (DP [$n = 58$] 41 ± 3.2 vs. NED [$n = 25$] 8.5 ± 2.2, $p < 0.0001$), SINENs (DP [$n = 43$] 37 ± 3.8 vs. NED [$n = 11$] 10 ± 2, $p < 0.0001$), GNENs (DP [$n = 25$] 28 ± 1.5 vs. NED [$n = 21$] 16 ± 2.5, $p = 0.0006$), and RNENs (DP [$n = 28$] 20.2 ± 2.9 vs. NED [$n = 18$] 7 ± 1, $p = 0.0002$). We did not, however, identify a difference in DNENs (DP [$n = 10$] 40 ± 7.2 vs. NED [$n = 2$] 16.7 ± 10, $p = 0.23$). All ANENs were NED cases.

Second, we evaluated the agreement between the NETest and disease status based on imaging and histology results. An examination of the relationship between a positive NETest score and disease detection (by imaging and/or histology) was 100% for PNENs, DNENs, and GNENs, and 95% for SINENs (41/43). Agreement was 43% for RNENs (12/28; Fig. 2b; online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000508106).

In IND cases, where there was no evidence of positive resection margins (NED), the NETest agreement was 88% for PNENs (22/25), 82% (9/11) for SINENs, 100% for RNENs (18/18), 75% for ANENs (9/12), and 62% for GNENs (13/21). One of the 2 DNENs had a positive NETest. In IPD cases, the NETest was positive in 146/164 (89%) DP GEPNENs and negative in 72/89 (81%) NED GEPNENs

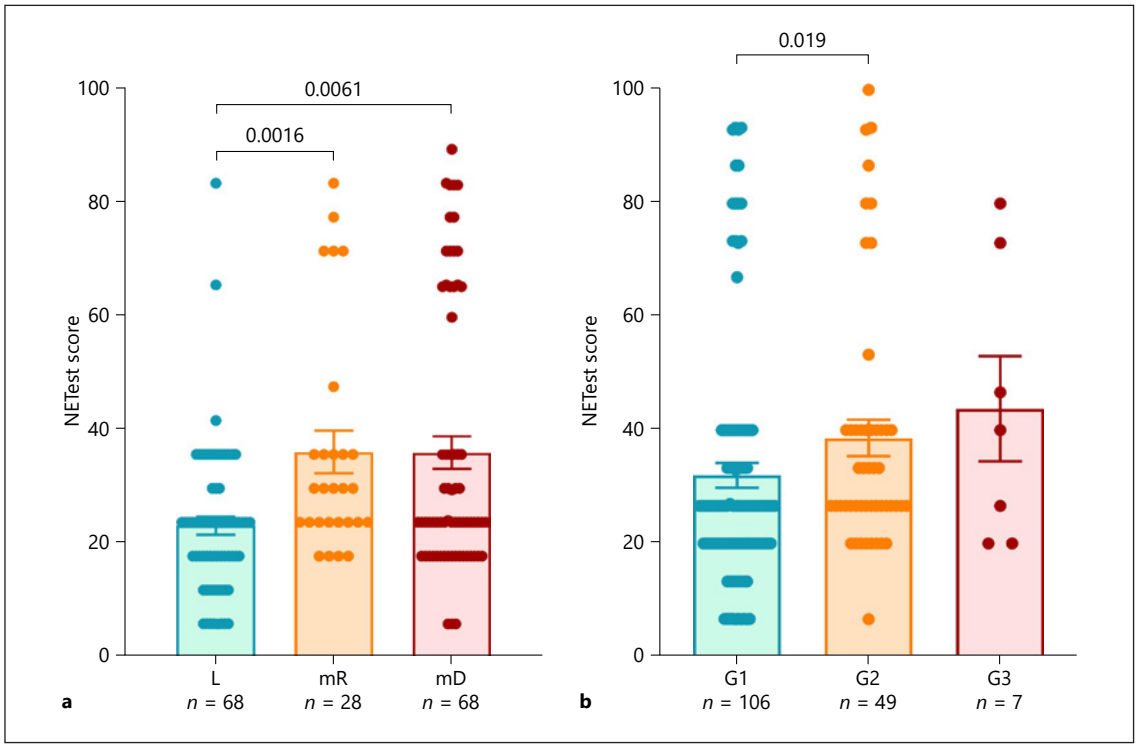


**Fig. 3.** Comparison of NETest levels in GEPNENs between stage and grade. **a** GEPNENs: NETest levels were significantly different between L, mR, and mD disease (KW statistic 14.6, $p = 0.0007$). **b** Significant differences were identified in NETest levels based on grade (KW statistic 8.7, $p = 0.013$) and especially between grade 1 (G1) and G2 tumors ($p < 0.02$). NETest ULN: 20%. Mean ± SEM. G3 ($n = 7$): 4 well-differentiated (3 PNENs and 1 GNET type 3), and 3 poorly differentiated PNENs. GNET, gastric neuroendocrine tumor; L, localized; mR, regional metastatic; mD, distant metastatic.

($\chi^2 = 120$, $p < 0.0001$). The overall agreement of the NETest with any detectable disease (imaging or histology) was 86% (218/253). The diagnostic metrics for the NETest were accuracy 86%, sensitivity 89%, and specificity 81%.

In the control group, 5/86 individuals were NETest positive (94% agreement, 7 ± 0.5); in colon cancer ($n = 37$), 17 tested positive for the NETest (54% agreement, 18 ± 3.5; online suppl. Table 1). All colon cancer patients had disease at the time of blood collection for the NETest (Table 1).

*Analysis by Disease Stage and Grade*

We next evaluated whether NETest levels correlated with disease stage and grade in DP subjects ($n = 164$).

Stage

We first evaluated the cohort as a group ($n = 164$). Disease was localized in 68 patients, 28 had regional metastases, and 68 exhibited distant metastases. The Kruskal-Wallis analysis with Dunn's correction identified significant differences in NETest levels across these 3 stages (Kruskal-Wallis statistic 14.6, $p = 0.0007$). Specifically, regional disease (40.5 ± 4.2, $p = 0.0016$) and distant metastatic (40.4 ± 3.2, $p = 0.0061$) disease had significantly higher NETest scores than localized disease (26 ± 1.8; Fig. 3a). Comparing localized disease with any metastatic disease revealed that the NETest level was significantly elevated in patients with metastases (40 ± 2.6, $p = 0.0002$).

We next evaluated individual sites. In the case of PNENs, 17 were localized, 11 had regional metastases, and 30 exhibited distant metastases. NETest levels were similar in these 3 stages (36.9 ± 4.8, 39.4 ± 6, and 44.4 ± 5, respectively, $p = 0.84$). In SINENs, NETest levels in regional ($n = 11$, 36 ± 7) and distant metastatic ($n = 32$, 37 ± 4.6) disease were similar ($p = 0.8$). In DNENs, NETest levels were significantly higher in regional ($n = 5$, 54.7 ± 10.4) than localized ($n = 5$, 25 ± 4, $p = 0.032$) disease. In RNENs, NETest levels in distant metastases were higher ($n = 6$, 37.7 ± 7.8) than in localized disease ($n = 21$, 14.9 ± 2.1, $p = 0.0015$), and there was only 1 patient with regional metastasis (NETest 26.7%).

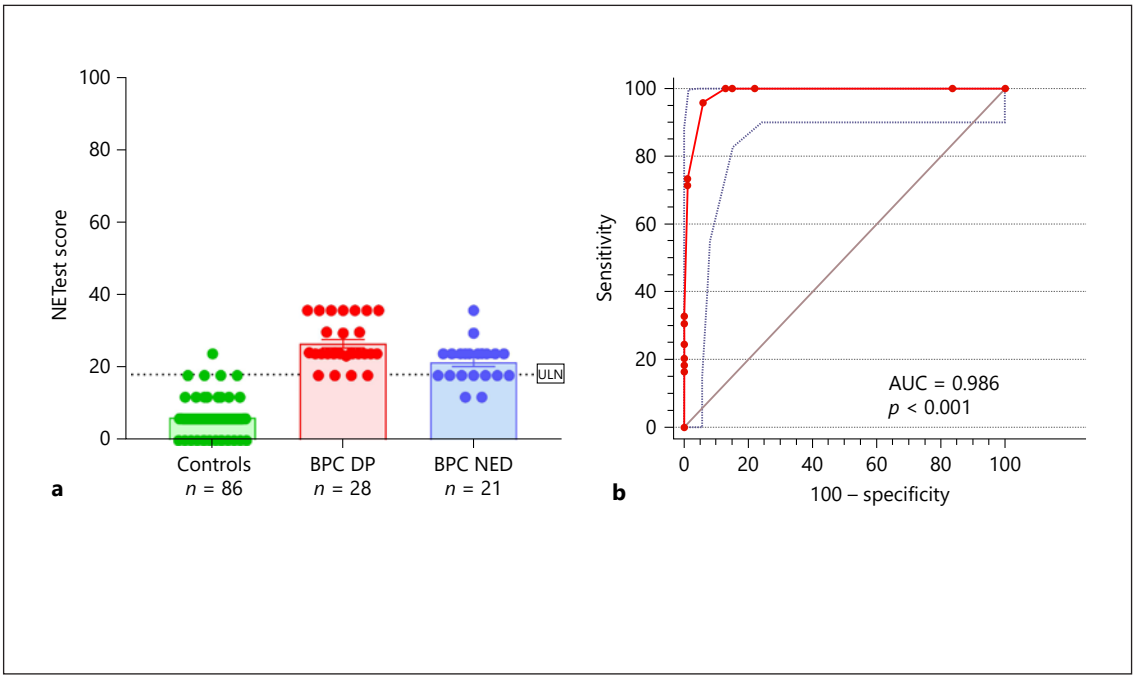


**Fig. 4.** NETest levels in BPC. **a** NETest levels in disease-positive (DP) BPC ($n$ = 28) vs. BPC with no evidence of disease (NED) and controls. KW statistic 90.3, $p < 0.0001$. NETest ULN: 20%. Mean ± SEM. **b** The AUROC for NETest in BPC and controls: AUC 0.99 (95% CI 0.95–0.99, $p < 0001$). BPC, bronchopulmonary carcinoids.

Grade

We first evaluated the cohort as a group ($n$ = 164), assigning patients a disease grade (G) of G1 ($n$ = 106), G2 ($n$ = 49), G3 ($n$ = 7; 4 well-differentiated NETs [3 PNENs and 1 GNEN type 3] and 3 poorly differentiated PNENs), or no data ($n$ = 2; one received diagnosis in 1997 and the other had tumor biopsy material that was nonevaluable for grading). The Kruskal-Wallis analysis with Dunn's correction identified significant differences in NETest levels between the grades (Kruskal-Wallis statistic 8.7, $p$ = 0.013). Specifically, G2 disease (39 ± 3.2, $p$ = 0.019) had significantly higher NETest scores than G1 disease (32 ± 2.2; Fig. 3b). There were too few G3 samples for an accurate statistical analysis, but levels were the highest (44 ± 9).

We next evaluated individual sites. For PNENs, NETest levels in G1 (40 ± 6) and G2 (42 ± 4.4) disease were similar ($p$ = 0.39). This may have reflected the aggressive biology of well-differentiated PNENs irrespective of grade. For SINENs, there was no significant difference between G1 (39.8 ± 4.8) and G2 (28.5 ± 5, $p$ = 0.35), but numbers were low. There were also insufficient numbers in the other subcohorts (DNENs, GNENs, and RNENs) for formal statistical analyses.

*Diagnostic Utility in BPC and Poorly Differentiated Lung NENs*

NETest levels in all BPC (IPD and IND; 27 ± 1) were significantly higher than in controls (7 ± 0.5, $p < 0.0001$). Twenty-eight (14 TC and 14 AC) BPC were identified as IPD and 21 (16 TC and 5 AC) as IND. NETest levels in IPD (30 ± 1.3) were significantly higher than in NED cases (24.1 ± 1.3, $p$ = 0.0049) and controls (7 ± 0.5, $p < 0.0001$; Fig. 4a, online suppl. Table 1).

NETest was positive in significantly more DP BPC (28/28) than in controls (5/86; $\chi^2$ = 87, $p < 0.0001$). The AUROC for differentiating DP BPC from controls was 0.99 (95% CI 0.95–0.99; $p < 0.0001$; Fig. 4b). The sensitivity for detecting IPD compared to controls was 100% and the specificity was 94%. In the IND BPC group, 19/21 were NETest-positive (online suppl. Table 1). The AUROC for differentiating these patients from DP patients was 0.72 (95% CI 0.6–0.84; $p$ = 0.0012).

In the poorly differentiated lung tumor group, all 15 poorly differentiated NENs (SCLC, $n$ = 11; LCNEC, $n$ = 4), were NETest-positive. Levels were significantly higher (59 ± 7) than in controls (7 ± 0.5, $p < 0.0001$). NETest accuracy for the poorly differentiated NEN group was 95%, with a sensitivity of 100% and a specificity of 94%. The AUROC was 0.99 ($p < 0.0001$).

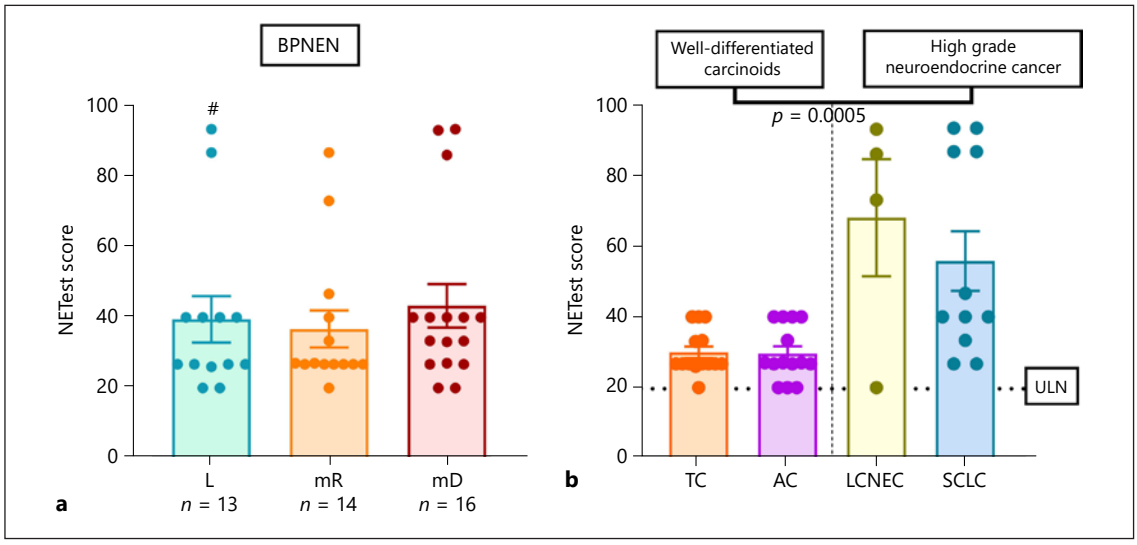


**Fig. 5.** Comparison of NETest levels in lung NENs by disease stage and grade. **a** NETest levels were not different between L, mR, and mD disease ($p < 0.05$). # In the localized group, the 2 highest NETest scores (93 and 87) were in progressive SCLCs. **b** NETest levels were significantly ($p = 0.0005$) higher in poorly differentiated BPNENs (LCNEC, $n = 4$, and SCLC, $n = 11$, 59 ± 7) than in well-differentiated BPC (TC, $n = 14$, and AC, $n = 14$; 30 ± 1). NETest ULN: 20%. Mean ± SEM. TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung cancer; L, localized; mR, regional metastatic; mD, distant metastatic.

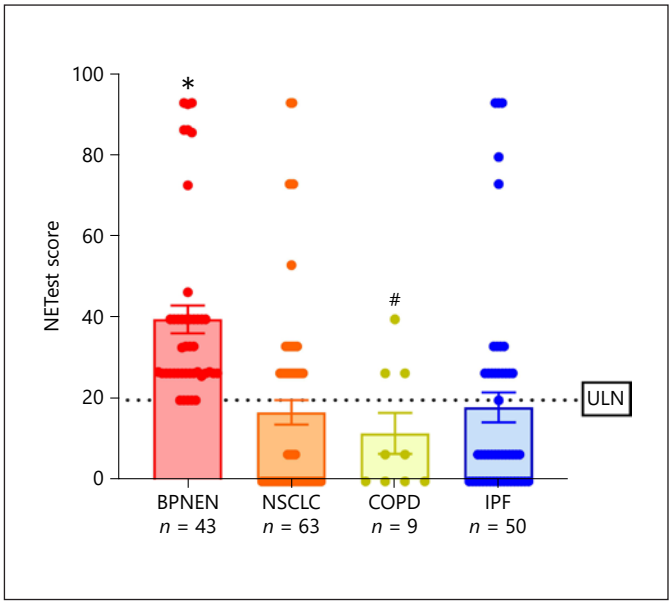


**Fig. 6.** Comparison of NETest levels in lung NENs versus malignant and nonmalignant lung diseases. NETest levels in NEN were significantly elevated compared to in NSCLC, COPD, and IPF. KW statistic 38, $p < 0.0001$. NETest ULN: 20%. Mean ± SEM. * $p < 0.0001$ versus other malignant and nonmalignant diseases. # $p = 0.0004$ vs. other lung diseases. BPNEN, bronchopulmonary neuroendocrine neoplasm; NSCLC, non-small-cell lung cancer; COPD, chronic obstructive pulmonary disease; IPF, interstitial pulmonary fibrosis.

*Analysis of Lung Neuroendocrine Neoplasms by Stage and Grade*

Stage

There were 43 BPNENs with IPD (28 BPC, 4 LCNECs, and 11 SCLCs). In this group, levels between stages were not different ($p = 0.5$; Fig. 5a; localized 39 ± 6.5 [$n = 13$: 10 BPC, 1 LCNECs, and 2 SCLCs]; regional metastatic 37 ± 5 [$n = 14$: 10 BPC, 1 LCNEC, and 3 SCLCs]; distant metastatic 43 ± 6 [$n = 16$: 8 BPC, 2 LCNECs, and 6 SCLCs]). In the localized group, the 2 highest NETest scores of 93 and 87 were in progressive SCLCs. A subanalysis identified no differences between stages for either TC ($p = 0.25$) or AC ($p = 0.86$).

Grade

We compared the 28 BPC with IPD (TC/AC) and the 15 poorly differentiated/high-grade neoplasms (4 LCNECs and 11 SCLCs). A significant difference in NETest levels was noted between 14 TC cases (30 ± 1.7), 14 AC cases (30 ± 2), 11 SCLC cases (56 ± 8), and 4 LCNEC cases (68 ± 16.5) (Kruskal-Wallis statistic 11.5, $p = 0.009$; Fig. 5b). Moreover, comparing BPC as a group (30 ± 1.3; $n = 28$) with LCNEC and SCLC revealed significantly elevated NETest levels in the poorly differentiated cohort (59 ± 7; $p = 0.0005$, Mann-Whitney U test; $n = 15$).

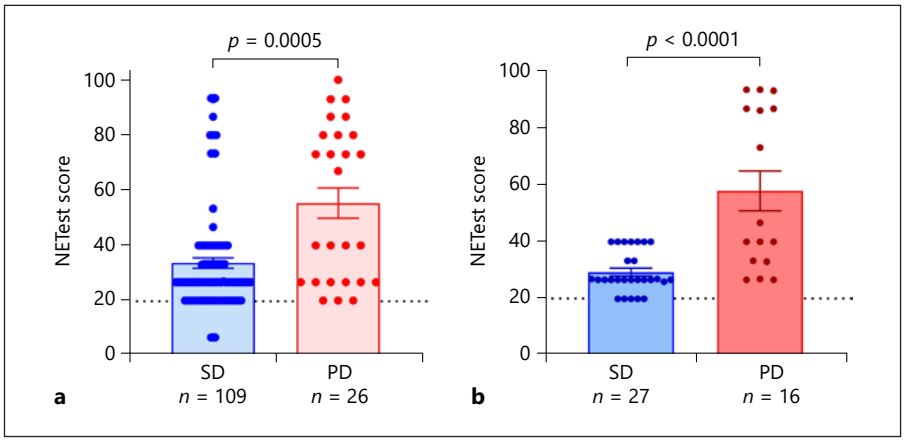


**Fig. 7.** NETest levels in stable versus progressive disease as radiologically (RECIST v1.1) assessed. **a** In GEPNENs ($n$ = 135) NETest levels were significantly higher ($p$ = 0.0005) in subjects with progressive disease (PD) than in those with stable disease (SD). **b** In BPNENs ($n$ = 43), NETest levels were significantly higher ($p$ < 0.0001) in PD than SD. NETest ULN: 20%. Mean ± SEM.

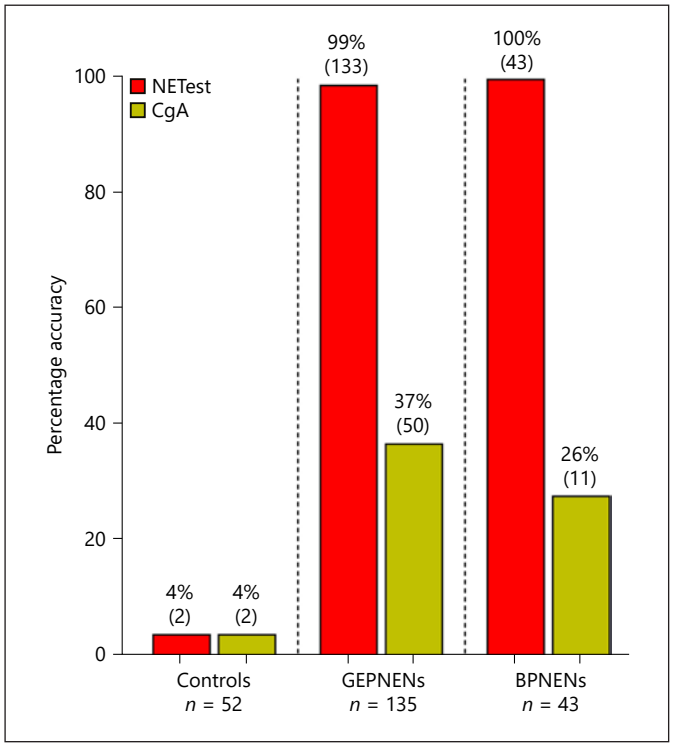


**Fig. 8.** Accuracy of NETest versus CgA in controls and in NENs with IPD (GEPNENs and BPNENs). NETest was significantly more accurate than CgA measurement ($p$ < 0.0001, McNemar analysis) in both GEPNENs ($n$ = 135) and BPNENs ($n$ = 43).

*NENs versus NSCLCs and Benign Diseases*

We then compared NETest levels in BPNENs with IPD ($n$ = 43; 28 BPC, 11 SCLC, 4 LCNEC) and other non-neuroendocrine lung cancers (NSCLC, $n$ = 63), and benign lung diseases (COPD [$n$ = 9] and IPF [$n$ = 50]). BPNEN NETest levels (40 ± 3) were significantly higher than in NSCLCs (17 ± 3, $p$ < 0.0001) and benign lung diseases (COPD: 12 ± 5, $p$ = 0.0004; IPF: 18 ± 4, $p$ <0.0001; Fig. 6). All BPNENs were NETest-positive (100%) compared to 41% of NSCLCs (26/63), 33% of COPD cases (3/9), and 36% of IPF cases (18/50). The NETest was significantly more accurate for BPNEN than other benign and malignant lung pathologies ($\chi^2$ = 28.5–42; $p$ < 0.0001).

*NETest Levels in Stable versus Progressive Disease*

In the GEPNENs with IPD ($n$ = 135), 109 were stable and 26 were progressive (RECIST v1.1). NETest levels for progressive disease (55 ± 5.5) were higher than in stable disease (33.6 ± 2, $p$ = 0.0005; Fig. 7a). In the BPNEN cohort, 27 were stable while 16 were progressive (4 BPC, 3 LCNECs, and 9 SCLCs). Levels were elevated in progressive (57.8 ± 7) compared to stable (29.4 ± 1, $p$ < 0.0001) disease (Fig. 7b).

*Comparing the Diagnostic Accuracy of NETest and CgA Measurement in GEPNENs and BPNENs with IPD*

GEPNENs

NETest and CgA data are included in online supplementary Tables 1 and 2 and in Figure 8. The NETest accurately identified 99% of GEPNENs with IPD (133/135), while CgA was only positive in 37% (50/135). NETest diagnostic accuracy for detecting IPD compared to controls was 97%, sensitivity was 99%, and specificity was 94%. In the CgA analysis, the diagnostic accuracy, sensitivity and specificity were 53, 37, and 96%, respectively. The McNemar analysis identified significantly better performance by the NETest than CgA measurement ($\chi^2$ = 87, $p$ < 0.0001; online suppl. Table 3).

Lung NENs

The NETest accurately identified NENs with IPD in 100% of the patients, while CgA was positive in only 26%

(11/43; Fig. 8). NETest diagnostic accuracy, sensitivity, and specificity for detecting lung NENs compared to controls was 96, 100, and 94%, respectively. For CgA, the diagnostic accuracy, sensitivity, and specificity were 64, 25, and 96%, respectively. The McNemar analysis identified a significantly better performance for the NETest than for CgA measurement ($\chi^2 = 30$, $p < 0.0001$; online suppl. Table 3).

## Discussion

Over 2 years, we undertook a large-scale evaluation of the NETest in 563 subjects including 318 with either GEPNEN or BPNEN, 100 with other cancers, 86 controls, and 59 subjects with nonmalignant diseases. Our data demonstrate that the NETest is an effective diagnostic for both GEPNEN and BPNEN with an accuracy of >90%. Furthermore, we identified that a positive NETest score (≥20) was significantly associated (86–100%) with the detection of disease using either standard imaging modalities or positive histology. It was evident that NETest score correlated with grade and disease extent in GEPNENs, and with grade in BPNENs. Of particular note was the observation that NETest levels correlated with disease progression (RECIST v1.1) as identified by imaging. Finally, in a matched sample analysis of 178 GEPNEN ($n$ = 135) and BPNEN ($n$ = 43) patients with IPD, we determined that the NETest was significantly more accurate than CgA measurement (99 vs. 34%, respectively) in diagnosing NENs.

The strengths of our study include the large number of patients enrolled at a single center, and the availability of imaging and surgical histology data for all patients. In addition, we were able to correlate clinical data with blood biomarker data. Furthermore, the study included other cancers and nonmalignant diseases as comparators for the substantial cohorts of GEP- and BP-derived tumors.

Our study does have some limitations. These include the relatively low numbers of DNENs, ANENs, and LCNECs, the incomplete functional imaging data on all patients, the absence of a centralized review of histology, and a paucity of G3 neoplasia. Nevertheless, these groups are rare even within an uncommon tumor type and did not influence the conclusions for the 2 main groups, GEPNEN and BPC, while the histological diagnosis was reviewed by an expert pathologist in every NEN patient. As we undertook a "real-world", single-center study, centralized histological review and functional imaging at every blood collection were not feasible. An ENETS CoE is, we feel, able to provide a fair assessment of "real-life" patient management. The majority of tumors were G1/G2, and we plan to evaluate the NETest in G3 NENs in a separate study.

*GEPNENs*

Our evaluation of the NETest in GEPNENs ($n$ = 253) found the assay to be accurate (91%) and have a high sensitivity (89%) and specificity (94%) for detecting NETs compared to 86 controls. This is consistent with previous observations in a similarly large case-control study by van Treijen et al. [29]. In their study of 140 GEPNETs and 113 controls, they identified an overall accuracy of 81%, with a sensitivity and specificity of 89 and 72%, respectively. The 2 studies have similar accuracy and sensitivity, consistent with the NETest accurately diagnosing GEPNENs. These data were confirmed in a recently published meta-analysis by Oberg et al. [33] that reported an overall diagnostic accuracy of 95–96%.

We also evaluated the utility of the NETest in individuals with and without macroscopic and microscopic evidence of disease. Our findings demonstrated that the NETest had high accuracy (86%, AUC 0.87) for differentiating disease from NED. Separating tumors according to the organ of origin identified elevated NETest levels in DP pancreatic, small bowel, gastric, and rectal NENs. Seventeen samples were considered NED but had positive NETest scores. Three of 25 (12%) PNENs with NED were NETest-positive. These were all untreated after surgery; 1 was a G2, 2 were G1, and 2 of the 3 were large tumors (T3 and T4, respectively), while 1 had a history of lymph node metastases. Two SINENs with NED were NETest-positive; both were untreated after surgery and 1 was a T3 tumor. Eight GNENs with NED were NETest-positive. This may have reflected the fact that 5 were G2, 1 was G3, and 1 had a history of lymph node metastasis. Of note, no RNENs with NED were NETest-positive. We identified that 3 ANENs were NETest-positive; 2 patients had G2 tumors and only 1 had had a right hemicolectomy. In addition, 1 duodenal NEN was NED but was NETest-positive. This was a T3 tumor with a Ki-67 level of 60% and currently remains untreated. Overall, we consider that the most likely explanation for the positive scores in these 17 individuals was residual disease not yet identifiable on imaging. Previous reports have documented that image identification of disease may lag 1–2 years behind NETest identification [34, 40]. We are currently closely following up these patients to facilitate assessment of the disease recurrence.

The lower overall accuracy for NED (80%) reflects the preponderance of positive NETest scores in GNENs.

These lesions are well known to exhibit widespread gastric mucosal neuroendocrine cell transformation before manifesting as focal nodularity or tumors [50]. Subjects would therefore be anticipated to be NETest-positive, irrespective of tumor removal, given the fact that type I/II gastric carcinoids represent an enterochromaffin-like (ECL) cell transformation field defect in the parietal cell mucosa [50]. The NETest therefore detects molecular evidence of ECL cell neoplastic transformation not identifiable on endoscopy, imaging, or random biopsy. Indeed, this recapitulates what is known about the natural history of this disease.

We separately examined NETest scores in colon cancer. Typically, scores were low (18 ± 4, below the upper limit of normal of 20) but we did identify that 17 (46%) were NETest-positive. This probably represents the fact that neuroendocrine differentiation in colon cancer is not uncommon. For example, Ogimi et al. [51] histologically evaluated 354 curatively resected cases of stage II/III colon cancer and 36 cases of rectal cancer for NET markers and identified CgA immunopositivity in 72 cases (18.4%). In this study, well-differentiated and moderately differentiated tumors were CgA-positive (20 and 18.7%, respectively). This suggests that neuroendocrine differentiation is a common feature of colon cancer and is consistent with our detection of a neuroendocrine signature in the blood. The higher level in our study presumably reflected the greater sensitivity of molecular detection of neuroendocrine transcripts than that achievable with immunohistochemistry. Moreover, recent evidence from sequencing data indicates that, although NETs can arise de novo, tumors with neuroendocrine features can also develop as a result of lineage plasticity in response to the alteration in mechanistic molecular-dependencies induced by targeted therapies [52]. This has been described in detail in prostate neoplasia [53]. Two of the 17 cases of NETest-positive colon cancer had undergone chemotherapy. Five (29%) of the 17 NETest-positive cases exhibited a score >40 which is consistent with progressive neuroendocrine disease [39, 33]. While we were unable to reevaluate the histology of these samples for neuroendocrine features, we consider that these tumors may have a neuroendocrine phenotype and will closely evaluate their response to standard therapy. We anticipate that those with a high NETest level will likely experience treatment failure.

In other studies [29, 39] no relationship was identified between NETest scores and disease extent (localized vs. metastatic) in GEPNENs. In the study by van Treijen et al. [29], 94% of subjects had distant metastases; 96% had stage IV disease in the study by Liu et al. [39]. These reports led to concerns as to whether the NETest identified metastatic spread [54]. In our GEPNEN cohort, 68 had localized tumors, 28 had regional metastases, and 68 exhibited distant metastases. This allowed us to examine in more detail whether a NETest score might correlate with disease extent. We noted that the NETest level was significantly elevated ($p = 0.0002$) in those with any metastatic disease compared to localized disease, and thus conclude that our study has confirmed that there is indeed a relationship with metastatic disease. We note that there was no difference in scores between regional and distant metastatic disease. This suggests to us that the NETest is detecting aggressive (malignant and metastatic) disease, or that histopathology may not detect disease at sites not evident on imaging and therefore not sampled. We predict that, given its sensitivity, a blood-based molecular test would identify such covert metastasis [11, 55]. We identified that 25/96 (26%) subjects with metastases had progressive disease whereas only 2/39 (5%) subjects with localized disease were clinically regarded as having progressive disease (Fisher's test, $p < 0.0045$). In addition, we noted that PNENs, irrespective of disease extent, exhibited elevated scores. We interpret this to reflect the previously well-described more malignant nature of pancreatic NEN disease [56]. The elevated scores may therefore be consistent with disease progression but also the fact that molecular measurements of the various omic clusters (that constitute the hallmarks of neoplasia) identify the mechanistic oncogenic drivers that provide the basis for the increased intrinsic malignancy of pancreatic NEN disease.

One other measure of malignancy is grade. Previous studies [29, 39], have not identified a relationship between NETest scores and grade in GEPNENs and this has been of concern when using the liquid biopsy approach [54]. In van Treijen et al. [29], 99% of subjects were low/intermediate grade; Liu et al. [39] had a similar finding of 96%. We specifically evaluated the relationship between grade and NETest score in our cohort. There were 106 G1 subjects (65%), 49 G2 subjects (30%) and 7 G3 subjects (4%) (4 NETs and 3 poorly differentiated PNENs). The NETest was significantly higher in G2 (39 ± 3.2) than G1 (32 ± 2.2, $p < 0.02$) disease, providing a basis for the consideration that the NETest is linked to disease grade. The correlation with grade in our study likely reflects the relative homogeneity of these 2 groups; 87% (68/78) of G1 tumors and 73% (35/48) of G2 tumors were stable. In the 7 patients with high-grade disease, the numbers were inadequate for viable statistical analysis. We did, however,

note that the highest NETest was detected in the high-grade cohort (44 ± 9).

A relationship with disease "activity" was further confirmed by the identification that NETest scores were significantly elevated ($p = 0.0005$) in those with clinically significant or progressive disease. These observations confirm those in smaller-scale studies that identified that the NETest is a marker of disease status [33, 39, 40]. They also extend the value of the proposal that the NETest is a marker of grade and metastatic spread. We anticipate that larger-scale studies addressing these specific questions will likely shed more light on this issue. It does, however, seem likely that a multigene analysis to evaluate the different omic clusters that constitute malignancy would identify specific tumors that have a propensity for rapid or slow growth. This is likely the basis for previous retrospective clinical observations that some tumors are indolent and others are aggressive [56, 57].

Some centers (including ours) continue to use CgA for NET assessment, although diminished enthusiasm for this practice has been expressed in both the ENETS and NANETS guidelines [58, 59]. We therefore compared its diagnostic utility with the NETest. In a head-to-head study of 135 matched blood samples, we identified that the NETest was significantly more accurate ($p < 0.0001$) than CgA measurement for the diagnosis of a GEPNEN. In patients with any disease (macroscopic or microscopic), both the NETest and CgA were positive in 51 (31%) and negative in 15 (9%), the latter including 14 RNENs with R1 disease. The NETest was also positive in 95 (58%; all CgA-negative) while CgA was only positive in 2 (1%). We have interpreted these data to explicitly demonstrate that the NETest functions significantly better than CgA positivity for detecting GEPNEN disease. Of interest was the low utility of both markers in the identification of image-negative but R1 RNENs. This may reflect that there are insufficient tumor cells or that they have a very low activity and cannot be determined by the assays. This hypothesis is consistent with the indolent biology common in low-grade, localized RNENs. It seems likely that RNENs comprise more than one type of disease, much like gastric carcinoids. In many cases, they may be the equivalent of type I gastric carcinoids and do not exhibit the behavior of classic neoplasia or the field transformation in the ECL cells evident in the adjacent gastric mucosa [60].

*BPNENs*

Separately, we evaluated the NETest in BPNENs and in other neoplastic and benign disorders of the lung. We separated the BPNEN cohort into BPC and poorly differentiated NENs (SCLC and LCNEC). First, we identified that the assay was accurate (96%) and had a high sensitivity (100%) and specificity (94%) for detecting BPC compared to controls. This is consistent with previous observations by Filosso et al. [37]. In their study of 118 BPC and 90 controls, they identified an overall accuracy of 95%, a sensitivity of 93%, and a specificity of 97%. In our study, levels in BPC were elevated in patients with proven disease (30 ± 1.3). We also evaluated 15 other lung NENs (11 SCLCs and 4 LCNECs) and identified that all were NETest-positive with a score of 59 ± 7. Filosso et al. [37] identified the NETest to be positive in 10 (77%) of 13 SCLC/LCNECs. In our study, the NETest accurately differentiated SCLC/LCNECs from controls (accuracy 95%, sensitivity 100%, and specificity 94%). This indicates that the NETest could be used not only to diagnose BPC but also SCLCs and LCNECs. Overall, we identified the NETest to be positive in 43/43 lung NENs (BPC/SCLCs/LCNECs), consistent with it accurately detecting lung neuroendocrine disease.

Separately, we examined NETest scores in other lung diseases. Our data identified that the NETest was positive in 33–36% of benign lung diseases (IPF and COPD) but had a low expression (12 ± 5 and 18 ± 4, respectively). This is consistent with the report by Filosso et al. [37] and confirms that low-level neuroendocrine gene upregulation may be a feature of lung diseases. Indeed, an increase in neuroendocrine cell proliferation has been reported in DIPNECH, which, as an entity, is now considered a subset of peripheral carcinoid tumors with a low malignant potential [61].

Our finding that 41% of NSCLCs were NETest-positive provide support for the supposition that lung neoplasia is associated with neuroendocrine cell proliferation or the presence of a neuroendocrine genotype in the evolution of certain kinds of cancer, as has been noted for a number of other cancers (of the prostate, lung, breast, etc.) [62]. Earlier reports identified NETest positivity in 25/68 (37%) of NSCLCs evaluated [37], consistent with data from a large multicenter NIH study of 10,224 tumors undertaken by Chen et al. [63]. They demonstrated that 31% of all lung ACCs were NETest-positive, i.e., the NETest levels and expression of other neuroendocrine mRNAs (e.g., *CgA* and *TPH)* were high in tumor tissue samples. This was confirmed in a separate genomic study by the same authors in which they reported that 22% of lung ACCs and SCCs shared molecular features with NETs and co-clustered with LCNECs [64]. This particular class of NSCLC shared histological features with

LCNECs and was the most undifferentiated of the lung cancers evaluated [64]. Six of the NSCLC patients had a high NETest level (i.e., >40). We presume that this reflected either a conversion to a neuroendocrine phenotype (2 had undergone chemotherapy which has recently been identified as being associated with therapeutic resistance in NSCLC [62, 65]), or reflected the underlying biology of these particular lesions.

We separately examined the relationship between NETest levels and grade and were able to identify a correlation. Specifically, levels were significantly higher ($p$ = 0.0005) in SCLC/LCNECs than in well-differentiated BPC (no differences between AC and TC, as previously determined [37]). When we evaluated disease status, we identified that progressive disease was indeed associated with a higher NETest score ($p$ = 0.0005) for BPNENs. We interpreted this to reflect that the NETest functions as a marker of clinical status. This confirms earlier observations [37]. We next evaluated the relationship between the NETest and disease stage (localized vs. regional metastatic and distant metastatic disease) in a combined cohort of well-differentiated carcinoids and highly aggressive LCNECs and SCLCs. No differences were identified in the subgroups ($p$ = 0.5), presumably reflecting the heterogeneity in the small groups which contained patients with poorly differentiated tumors (3/13, 4/14, and 8/16, respectively) as well as individuals with progressive disease (2/13, 4/14, 10/16, respectively). Overall, the lung NETest data are broadly comparable to the clinical utility evident in GEPNENs and provide evidence that the NETest is a marker of grade and clinical status for BPNENs.

Finally, although CgA is considered ineffective as a biomarker in BPNENs [5] and recent studies and meta-analyses [17] have confirmed its lack of utility, we had the opportunity to evaluate whether it was as effective as the NETest. Of the 43 BPNEN patients, all were NETest-positive; however, only 11 (26%) were CgA-positive. Our results confirm previous observations that CgA measurements are ineffective in lung NENs [5, 17] and that the NETest is significantly more effective than CgA positivity as a diagnostic for lung NENs [37].

## Conclusions

Our work validates previous reports that the NETest is a useful clinical biomarker for GEPNENs and BPNENs (diagnosis and disease status identification). This is consistent with added accuracy provided by a multigene biomarker assay compared to a monoanalyte such as CgA. The data support the utility of the NETest as a tool to facilitate clinical management [33]. As a diagnostic, the NETest exhibits metrics consistent with the criteria proposed by the NIH for being an optimal biomarker [66]. NETest levels correlate with anatomical and functional imaging detection of disease and the assay is also effective in the detection of microscopic disease. The NETest scores correlated with grade, disease extent, and clinically progressive disease. In contrast, CgA measurement had no diagnostic or management utility.

Overall, our study demonstrated that this multigene liquid biopsy for neuroendocrine disease has excellent metrics, provides real-time noninvasive assessment, and exhibits multilevel correlation with tumor biology. We conclude that the NETest biomarker is an effective diagnostic, will facilitate clinical management, and be of significant clinical utility. In contrast, our analysis found CgA to not be effective as a biomarker, consistent with other reports available in the literature. Based on our experience, we believe that the NETest will play an important role in NET management and be included in management strategies for these neoplasms. We anticipate that the assay will be used initially at diagnosis to establish the level of aggression of disease and provide the baseline for further comparisons, and thereafter at standard oncological intervals or at follow-up assessment (based on the individualized patient program) to evaluate disease progress and treatment efficacy. This will allow for further accumulation of considerable experience with the NETest, facilitate the timely detection of disease progression/recurrence, the development of early intervention protocols, and the overall optimization of NET management.

## Acknowledgement

We thank Wren Laboratories for providing sample measurement pro bono.

## Statement of Ethics

The study was approved by the Institutional (Medical University of Silesia) Ethics Committee. Informed written consent was obtained from all study participants.

## Disclosure Statement

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

## Funding Sources

No financial support was received in relation to this study. Wren Laboratories provided sample measurement pro bono.

## Author Contributions

A.M. and B.K.-K.: study concept and design. All authors contributed to drafting and critical revision of the manuscript, and the acquisition, analysis, and interpretation of data.

## References

1 Capdevila J, Bodei L, Davies P, Gorbounova V, Jensen RT, Knigge UP, et al.; ENETS 2016 Munich Advisory Board Participants; ENETS 2016 Munich Advisory Board Participants. Unmet Medical Needs in Metastatic Lung and Digestive Neuroendocrine Neoplasms. Neuroendocrinology. 2019;108(1):18–25.
2 Pavel M, O'Toole D, Costa F, Capdevila J, Gross D, Kianmanesh R, et al.; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for the Management of Distant Metastatic Disease of Intestinal, Pancreatic, Bronchial Neuroendocrine Neoplasms (NEN) and NEN of Unknown Primary Site. Neuroendocrinology. 2016;103(2):172–85.
3 Gibson WE, Gonzalez RS, Cates JMM, Liu E, Shi C. Hepatic micrometastases are associated with poor prognosis in patients with liver metastases from neuroendocrine tumors of the digestive tract. Hum Pathol. 2018 Sep;79:109–15.
4 Cives M, Strosberg JR. Gastroenteropancreatic Neuroendocrine Tumors. CA Cancer J Clin. 2018 Nov;68(6):471–87.
5 Oberg K, Modlin I, DeHerder W, Pavel M, Klimstra D, Frilling A, et al. Biomarkers for Neuroendocrine Tumor Disease: A Delphic Consensus assessment of Multianalytes, Genomics, Circulating Cells and Monoanalytes. Lancet Oncol. 2015;16:e435046.
6 Niederle B, Pape UF, Costa F, Gross D, Kelestimur F, Knigge U, et al.; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for Neuroendocrine Neoplasms of the Jejunum and Ileum. Neuroendocrinology. 2016;103(2):125–38.
7 Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol. 2007 May;25(13):1753–9.
8 Bodei L, Sundin A, Kidd M, Prasad V, Modlin IM. The status of neuroendocrine tumor imaging: from darkness to light? Neuroendocrinology. 2015;101(1):1–17.
9 Toumpanakis C, Kim MK, Rinke A, Bergestuen DS, Thirlwell C, Khan MS, et al. Combination of cross-sectional and molecular imaging studies in the localization of gastroenteropancreatic neuroendocrine tumors. Neuroendocrinology. 2014;99(2):63–74.
10 Modlin IM, Kidd M, Drozdov IA, Bodei L. The use of Deep Learning and Neural Networks in Imaging - Welcome to the new Mathematical Milieu of Medicine. Neuroendocrinology. 2020;110(5):322-327.
11 Modlin IM, Kidd M, Malczewska A, Drozdov I, Bodei L, Matar S, et al. The NETest: The Clinical Utility of Multigene Blood Analysis in the Diagnosis and Management of Neuroendocrine Tumors. Endocrinol Metab Clin North Am. 2018 Sep;47(3):485–504.
12 Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol. 2017 Sep;14(9):531–48.
13 Modlin IM, Oberg K, Taylor A, Drozdov I, Bodei L, Kidd M. Neuroendocrine tumor biomarkers: current status and perspectives. Neuroendocrinology. 2014;100(4):265–77.
14 Modlin IM, Bodei L, Kidd M. Neuroendocrine tumor biomarkers: from monoanalytes to transcripts and algorithms. Best Pract Res Clin Endocrinol Metab. 2016 Jan;30(1):59–77.
15 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar;144(5):646–74.
16 Marotta V, Nuzzo V, Ferrara T, Zuccoli A, Masone M, Nocerino L, et al. Limitations of Chromogranin A in clinical practice. Biomarkers. 2012 Mar;17(2):186–91.
17 Malczewska A, Kidd M, Matar S, Kos-Kudła B, Bodei L, Oberg K, et al. An Assessment of Circulating Chromogranin A as a Biomarker of Bronchopulmonary Neuroendocrine Neoplasia: A Systematic Review and Meta-Analysis. Neuroendocrinology. 2020;110(3-4):198–216.
18 Matar S, Malczewska A, Oberg K, Bodei L, Aslanian H, Lewczuk-Myslicka A, et al. Blood Chromogranin A is Not Effective as a Biomarker for Diagnosis or Management of Bronchopulmonary NET/NET. Neuroendocrinology. 2020;110(3-4):185–97.
19 Pulvirenti A, Rao D, McIntyre CA, Gonen M, Tang LH, Klimstra DS, et al. Limited role of Chromogranin A as a clinical biomarker for pancreatic neuroendocrine tumors. HPB (Oxford). 2019 May;21(5):612–18.
20 Chabon JJ, Simmons AD, Lovejoy AF, Esfahani MS, Newman AM, Haringsma HJ, et al. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun. 2016 Jun;7(1):11815.
21 Wills B, Gorse E, Lee V. Role of liquid biopsies in colorectal cancer. Curr Probl Cancer. 2018 Nov;42(6):593–600.
22 Alimirzaie S, Bagherzadeh M, Akbari MR. Liquid biopsy in breast cancer: A comprehensive review. Clin Genet. 2019 Jun;95(6):643–60.
23 Hodara E, Morrison G, Cunha A, Zainfeld D, Xu T, Xu Y, et al. Multiparametric liquid biopsy analysis in metastatic prostate cancer. JCI Insight. 2019 Mar 7;4(5).125529.
24 McDonald BR, Contente-Cuomo T, Sammut SJ, Odenheimer-Bergman A, Ernst B, Perdigones N, et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. Sci Transl Med. 2019 Aug;11(504):eaax7392.
25 Modlin IM, Drozdov I, Kidd M. The identification of gut neuroendocrine tumor disease by multiple synchronous transcript analysis in blood. PLoS One. 2013 May;8(5):e63364.
26 Modlin IM, Drozdov I, Alaimo D, Callahan S, Teixiera N, Bodei L, et al. A multianalyte PCR blood test outperforms single analyte ELISAs (chromogranin A, pancreastatin, neurokinin A) for neuroendocrine tumor detection. Endocr Relat Cancer. 2014 Aug;21(4):615–28.
27 Pavel M. Translation of molecular pathways into clinical trials of neuroendocrine tumors. Neuroendocrinology. 2013;97(1):99–112.
28 Pęczkowska M, Cwikla J, Kidd M, Lewczuk A, Kolasinska-Ćwikła A, Niec D, et al. The clinical utility of circulating neuroendocrine gene transcript analysis in well-differentiated paragangliomas and pheochromocytomas. Eur J Endocrinol. 2017 Feb;176(2):143–57.
29 van Treijen MJC, Korse CM, van Leeuwaarde RS, Saveur LJ, Vriens MR, Verbeek WHM, et al. Blood Transcript Profiling for the Detection of Neuroendocrine Tumors: Results of a Large Independent Validation Study. Front Endocrinol (Lausanne). 2018 Dec 4;9:740.
30 Al-Toubah TE, Cives M, Valone T, Blue K, Strosberg JR. Sensitivity and specificity of the NETest: A validation study. J Clin Oncol. 2019;37(4_suppl):222.
31 Malczewska A, Oberg K, Bodei L, Aslanian H, Lewczuk A, Filosso PL, et al. NETest Liquid Biopsy Is Diagnostic of Lung Neuroendocrine Tumors and Identifies Progressive Disease. Neuroendocrinology. 2019;108(3):219–31.
32 Malczewska A, Witkowska M, Makulik K, Bocian A, Walter A, Pilch-Kowalczyk J, et al. NETest liquid biopsy is diagnostic of small intestine and pancreatic neuroendocrine tumors and correlates with imaging. Endocr Connect. 2019 Mar;1(4):19–0030.

33 Oberg K, Califano A, Strosberg J, Ma S, Pape U, Bodei L, et al. A Meta-Analysis of the Accuracy of a Neuroendocrine Tumor mRNA Genomic Biomarker (NETest) in Blood. Ann Oncol. 2020 Feb;31(2):202-12.
34 Ćwikła JB, Bodei L, Kolasinska-Ćwikła A, Sankowski A, Modlin IM, Kidd M. Circulating transcript analysis (NETest) in GEP-NETs treated with Somatostatin Analogs defines Therapy. J Clin Endocrinol Metab. 2015 Nov;100(11):E1437–45.
35 Bodei L, Kidd M, Modlin IM, Severi S, Drozdov I, Nicolini S, et al. 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. 2016 May;43(5):839–51.
36 Modlin IM, Frilling A, Salem RR, Alaimo D, Drymousis P, Wasan HS, et al. Blood measurement of neuroendocrine gene transcripts defines the effectiveness of operative resection and ablation strategies. Surgery. 2016 Jan;159(1):336–47.
37 Filosso PL, Kidd M, Roffinella M, Lewczuk A, Chung KM, Kolasinska-Cwikla A, et al. The utility of blood neuroendocrine gene transcript measurement in the diagnosis of bronchopulmonary neuroendocrine tumours and as a tool to evaluate surgical resection and disease progression. Eur J Cardiothorac Surg. 2018 Mar;53(3):631–9.
38 Genç CG, Jilesen AP, Nieveen van Dijkum EJ, Klümpen HJ, van Eijck CH, Drozdov I, et al. Measurement of circulating transcript levels (NETest) to detect disease recurrence and improve follow-up after curative surgical resection of well-differentiated pancreatic neuroendocrine tumors. J Surg Oncol. 2018 Jul; 118(1):37–48.
39 Liu E, Paulson S, Gulati A, Freudman J, Grosh W, Kafer S, et al. Assessment of NETest Clinical Utility in a U.S. Registry-Based Study. Oncologist. 2019 Jun;24(6):783–90.
40 Pavel M, Jann H, Prasad V, Drozdov I, Modlin IM, Kidd M. NET Blood Transcript Analysis Defines the Crossing of the Clinical Rubicon: When Stable Disease Becomes Progressive. Neuroendocrinology. 2017;104(2):170–82.
41 de Mestier L, Dromain C, d'Assignies G, Scoazec JY, Lassau N, Lebtahi R, et al. Evaluating neuroendocrine tumors progression and therapeutic response: state of the art. Endocr Relat Cancer. 2013 Dec;18:18.
42 Delle Fave G, O'Toole D, Sundin A, Taal B, Ferolla P, Ramage JK, et al.; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for Gastroduodenal Neuroendocrine Neoplasms. Neuroendocrinology. 2016;103(2):119–24.
43 Brierley JD, Wittekind C, editors. International Union Against Cancer (UICC). TNM Classification of Malignant Tumours. 8th ed. Oxford, UK: John Wiley & Sons, Ltd; 2017.
44 Kos-Kudła B, Blicharz-Dorniak J, Strzelczyk J, Bałdys-Waligórska A, Bednarczuk T, Bolanowski M, et al. Diagnostic and therapeutic guidelines for gastro-entero-pancreatic neuroendocrine neoplasms (recommended by the Polish Network of Neuroendocrine Tumours). Endokrynol Pol. 2017;68(2):79–110.
45 Lipiński M, Rydzewska G, Foltyn W, Andrysiak-Mamos E, Bałdys-Waligórska A, Bednarczuk T, et al. Gastroduodenal neuroendocrine neoplasms, including gastrinoma - management guidelines (recommended by the Polish Network of Neuroendocrine Tumours). Endokrynol Pol. 2017;68(2):138–53.
46 Kidd M, Drozdov IA, Matar S, Gurunlian N, Ferranti NJ, Malczewska A, et al. Utility of a ready-to-use PCR system for neuroendocrine tumor diagnosis. PLoS One. 2019 Jun; 14(6):e0218592.
47 Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology. 1983 Sep;148(3):839–43.
48 Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993 Apr;39(4):561–77.
49 Modlin IM, Aslanian H, Bodei L, Drozdov I, Kidd M. A PCR blood test outperforms chromogranin A in carcinoid detection and is unaffected by PPIs. Endocr Connect. 2014 Oct; 14:14–0100.
50 Grozinsky-Glasberg S, Alexandraki KI, Angelousi A, Chatzellis E, Sougioultzis S, Kaltsas G. Gastric Carcinoids. Endocrinol Metab Clin North Am. 2018 Sep;47(3):645–60.
51 Ogimi T, Sadahiro S, Kamei Y, Chan LF, Miyakita H, Saito G, et al. Distribution of Neuroendocrine Marker-Positive Cells in Colorectal Cancer Tissue and Normal Mucosal Tissue: Consideration of Histogenesis of Neuroendocrine Cancer. Oncology. 2019; 97(5):294–300.
52 Rickman DS, Beltran H, Demichelis F, Rubin MA. Biology and evolution of poorly differentiated neuroendocrine tumors. Nat Med. 2017 Jun;23(6):1–10.
53 Puca L, Vlachostergios PJ, Beltran H. Neuroendocrine Differentiation in Prostate Cancer: Emerging Biology, Models, and Therapies. Cold Spring Harb Perspect Med. 2019 Feb; 9(2):a030593.
54 Rindi G, Wiedenmann B. Neuroendocrine neoplasia goes molecular - time for a change. Nat Rev Clin Oncol. 2019 Mar;16(3):149–50.
55 Malczewska A, Bodei L, Kidd M, Modlin IM. Blood mRNA Measurement (NETest) for Neuroendocrine Tumor Diagnosis of Image-Negative Liver Metastatic Disease. J Clin Endocrinol Metab. 2019 Mar;104(3):867–72.
56 Pasricha G, Padhi P, Daboul N, Monga DK. Management of Well-differentiated Gastroenteropancreatic Neuroendocrine Tumors (GEPNETs): A Review. Clin Ther. 2017 Nov; 39(11):2146–57.
57 Zandee WT, de Herder WW. The Evolution of Neuroendocrine Tumor Treatment Reflected by ENETS Guidelines. Neuroendocrinology. 2018;106(4):357–65.
58 Vinik AI, Woltering EA, Warner RR, Caplin M, O'Dorisio TM, Wiseman GA, et al.; North American Neuroendocrine Tumor Society (NANETS). NANETS consensus guidelines for the diagnosis of neuroendocrine tumor. Pancreas. 2010 Aug;39(6):713–34.
59 Hofland J, Zandee WT, de Herder WW. Role of biomarker tests for diagnosis of neuroendocrine tumours. Nat Rev Endocrinol. 2018 Nov;14(11):656–69.
60 Spampatti MP, Massironi S, Rossi RE, Conte D, Sciola V, Ciafardini C, et al. Unusually aggressive type 1 gastric carcinoid: a case report with a review of the literature. Eur J Gastroenterol Hepatol. 2012 May;24(5):589–93.
61 Rossi G, Bertero L, Marchiò C, Papotti M. Molecular alterations of neuroendocrine tumours of the lung. Histopathology. 2018 Jan; 72(1):142–52.
62 Park JW, Lee JK, Sheu KM, Wang L, Balanis NG, Nguyen K, et al. Reprogramming normal human epithelial tissues to a common, lethal neuroendocrine cancer lineage. Science. 2018 Oct;362(6410):91–5.
63 Chen F, Zhang Y, Gibbons DL, Deneen B, Kwiatkowski DJ, Ittmann M, et al. Pan-Cancer Molecular Classes Transcending Tumor Lineage Across 32 Cancer Types, Multiple Data Platforms, and over 10,000 Cases. Clin Cancer Res. 2018 May;24(9):2182–93.
64 Chen F, Zhang Y, Parra E, Rodriguez J, Behrens C, Akbani R, et al. Multiplatform-based molecular subtypes of non-small-cell lung cancer. Oncogene. 2017 Mar;36(10):1384–93.
65 Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011 Mar;3(75):75ra26.
66 McShane LM, Hayes DF. Publication of tumor marker research results: the necessity for complete and transparent reporting. J Clin Oncol. 2012 Dec;30(34):4223–32.