

## RESEARCH

# NETest: serial liquid biopsies in gastroenteropancreatic NET surveillance

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## Abstract

**Objective:** Up to now, serial NETest measurements in individuals assessing the disease course of gastroenteropancreatic neuroendocrine tumors (GEPNETs) at long-term follow-up and treatment response were not studied.

**Design:** The study was a longitudinal validation study of serial NETest measurements – a blood-based gene expression signature – in 132 patients with GEPNETs on therapy or watch-and-wait strategy.

**Methods:** Serial samples were collected during 46 (range: 6–71) months of follow-up. NETest scores were compared with Response Evaluation Criteria in Solid Tumors version 1.1-defined treatment response (e.g. no evidence of disease (NED), stable disease (SD) or progressive disease (PD)).

**Results:** Consecutive NETest scores fluctuated substantially (range: 0–100) over time in individuals with SD ( $n = 28$ ) and NED ( $n = 30$ ). Follow-up samples were significantly higher in SD (samples 3–5) and NED subgroups (samples 2–5) compared with baseline results, without changes in imaging. In 82% of untreated patients with PD, consecutive NETest scores consistently remained high. In patients undergoing systemic treatment, the median pre-treatment NETest score in treatment-responders was 76.5 ( $n = 22$ ) vs 33 ( $n = 12$ ) in non-responders ( $P = 0.001$ ). Patients with low pre-treatment scores had 21 months reduced progression-free survival (10 vs 31 months;  $P = 0.01$ ). The accuracy of the NETest for treatment response prediction was 0.73 ( $P = 0.009$ ).

**Conclusion:** In patients not undergoing treatment, consecutive low NETest scores are associated with indolent behavior. Patients who develop PD exhibit elevated scores. Elevated results have important predictive value for treatment responsiveness and could be used for individualizing decisions on systemic therapy. The clinical value of follow-up NETest scores for patients who choose to watch and wait requires further study.

## Key Words

- ▶ gastroenteropancreatic neuroendocrine tumors
- ▶ liquid biopsy
- ▶ treatment response
- ▶ surveillance
- ▶ gene expression signature

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## Introduction

Neuroendocrine tumors (NETs) are malignant neoplasms originating from neuroendocrine cells and can occur throughout the body. Gastroenteropancreatic NETs (GEPNETs) are the most prevalent subgroup. Both incidence

and survival have increased over the past decades, most likely because of improvements in diagnostic techniques and disease awareness (1, 2, 3). GEPNETs are grouped based on their shared neuroendocrine markers and proteins,

such as chromogranin and synaptophysin, but are very diverse in differentiation, secretion, proliferation and molecular profile, leading to a wide spectrum of clinical behavior (4, 5).

As a consequence of this heterogeneity, prediction of the course of disease in an individual patient is difficult. The absence of accurate markers that identify early changes in disease status, predict efficacy of therapy or detect minimal residual disease in individual patients forces clinicians to fall back on regular, pre-defined screening intervals for all patients (6). Multiple nomograms for NETs of various origins (rectal, small intestine, gastric, enteropancreatic) have been developed to predict treatment efficacy or overall survival but a measure of the underlying tumor biology that reflects tumor development remains elusive (7, 8, 9, 10, 11). As a result, modern management in GEPNETs is far from individualized.

Currently, tumor aggressiveness is estimated based on histopathological parameters, including differentiation and tumor grade (12). Re-evaluation of tumor aggressiveness and the expected disease course is mostly based on PET/CT or anatomical imaging since repeated tumor tissue collection is invasive and harmful. As a result, histopathology and imaging, mainly 68-gallium DOTA-peptide PET/CT (DOTA-PET/CT), are used for risk stratification. Consequently, DOTA-PET/CT is currently one of the few diagnostics in NET disease with a strong impact on clinical management. Its diagnostic sensitivity is superior to anatomical imaging, leading to better disease staging (e.g. primary tumor/bone metastasis) and subsequently change of management while somatostatin receptor expression can be used to select patients for peptide receptor radionuclide therapy (PRRT) (13, 14, 15, 16). Changes in surveillance and treatment strategies in individual patients are mostly based on retrospective observations including observed tumor growth and symptom evolution. The next step toward personalized medicine is an aggregation of imaging, evaluating changes in tumor load and measures of tumor biology that can predict tumor behavior. Such an aggregation could theoretically lead to a more timely intervention.

An emerging and promising predictive biomarker, the NETest, measures gene expression of 51 target genes that are involved in NET biology (17). Circulating transcripts are quantified and gene expression is interrogated by mathematical algorithms to create a tumor activity score that indicates stable disease (SD) or progressive disease (PD) (18). In an independent, cross-sectional validation study, the NETest reliably predicted SD and was the strongest predictor of PD in a large group of GEPNETs (19).

To date, no studies on consecutive NETest measurements in individual patients at long-term follow-up have been published. Therefore, the aim of the present study was to evaluate whether serial NETest measurements reflect disease evolution over time in individual patients and to assess the predictive value of therapy outcomes.

## Methods

### Population

Patients with histological confirmation of well-differentiated sporadic GEPNETs (according to the World Health Organization 2017 grading system) were recruited between March 2014 and March 2017 at the Netherlands Cancer Institute (Amsterdam, The Netherlands), an ENETS Center of Excellence (20). The study was approved by Netherlands Cancer Institute local ethics committee, and written informed consent from all subjects was obtained. Inclusion criteria were a minimum of two samples per patient with simultaneous imaging evaluation and at least 6 months of follow-up. Patients were followed and treated according to ENETS guidelines. At inclusion and during outpatient clinic visits, samples (6 mL of whole blood in EDTA tubes) were collected in combination with radiological imaging studies and samples were stored as previously described (19, 21). Follow-up samples were collected until January 2019.

### Measurements

Details on NETest (PCR methodology, mathematical analysis and validation) and selection of imaging studies have been described in our previous validation study (19). In brief, the NETest comprises a two-step protocol (RNA isolation/cDNA synthesis followed by qPCR) to determine gene expression of 51 target genes (17). Transcript levels were normalized and quantified vs a population control. Four mathematical algorithms with integration of relevant gene clusters generate a disease activity score ranging from 0 to 100 (18). The NETest was performed at Wren Laboratories (Branford, CT, USA). Baseline and follow-up samples were sent in separate anonymized batches between October 2015 and April 2020. The upper limit of normal (ULN) has previously been set at 20 (21); SD is defined as  $\leq 40$ , and PD as an activity score  $> 40$  with 41–79 as intermediate tumor activity and scores  $\geq 80$  as high tumor activity. According to our previous validation study, the highest accuracy for the NETest to predict the disease status was +12 months,

meaning a NETest sample reflected Response Evaluation Criteria in Solid Tumors (RECIST)-defined disease status best when drawn a year before radiological evaluation. Therefore, we applied this time period in our study.

The disease status was defined by RECIST version 1.1 (RECISTv1.1). All anatomical imaging procedures during patient follow-up were assessed by two independent senior radiologists, who were blinded to biomarker results. Outcomes of functional imaging (DOTA-PET/CT) were used in cases where conventional radiological imaging was not available. Anatomical imaging always followed DOTA-PET/CT so RECIST could be applied. New lesions had to be confirmed on consecutive imaging to avoid false-positive outcomes.

No evidence of disease (NED) was defined as negative consecutive imaging results (minimum two) after surgery with curative intent to avoid false-negative findings.

To evaluate the accuracy of the NETest to reflect tumor behavior over time, consecutive NETest outcomes were compared with disease status over time (PD, SD or NED) in the subgroup of patients not receiving any treatment during the study period.

Patients receiving systemic therapy (e.g. PRRT, chemotherapy) were selected for our treatment subgroup analyses. They were classified as treatment-responders if they had at least 1-year progression-free survival (PFS) (SD or partial response (PR) according to RECISTv1.1). Patients with PR were grouped together with patients with SD in all analyses (SD group). In patients who underwent multiple systemic treatments during follow-up, only one intervention was selected for evaluation of the NETest predictive value for treatment efficacy because multiple measurements within the same patient could introduce bias. In patients receiving multiple treatments, the selection of which intervention to analyze was based on the availability of NETest samples pre- and post-treatment.

### Statistical analysis

Analyses included two-tailed nonparametric tests (Mann-Whitney U test, Wilcoxon signed-rank test), chi-square test, Spearman correlation, receiver operating characteristics analysis and Kaplan-Meier curves with log-rank tests (PFS). A linear regression with a continuous autoregressive residual covariance (i.e. GEE type) matrix to correct for multiple measurements per patient was conducted in patients without treatment and ongoing SD to evaluate a possible relation between sample score and the time since diagnosis. Predictive values are described by area under the receiver operating characteristic curve (AUROC),

sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the originally described cutoffs. Statistical analyses are performed using Statistical Package for the Social Sciences (SPSS) 25. Figures were created with SPSS, Microsoft Excel and Rstudio version 4.1.1 (package ggplot2).  $P < 0.05$  was considered statistically significant. Clinical characteristics and NETest scores are presented as mean  $\pm$  s.d. or median with range according to the distribution of data, respectively (Kolmogorov-Smirnov test).

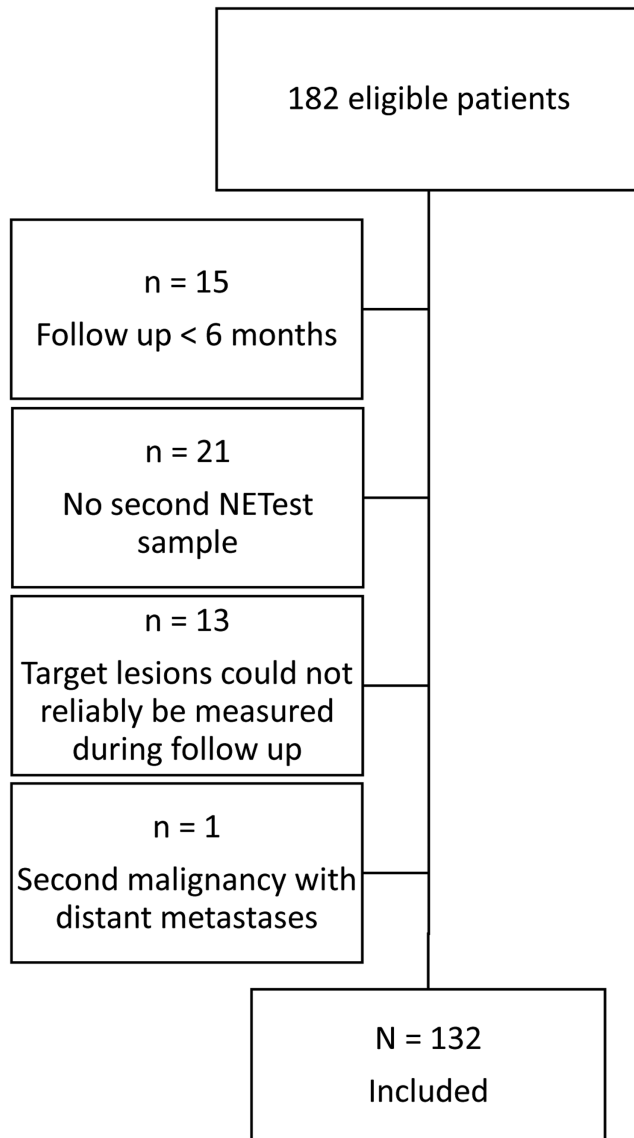
The predefined primary outcomes of the study were: (i) the ability of longitudinal NETest outcomes to predict the evolving disease status according to RECISTv1.1 and (ii) the association between serial intra-individual NETest samples and the RECISTv1.1-defined disease status. The secondary outcome was the utility of the NETest to predict the PFS of patients after treatment. PFS was calculated as the length of time between the baseline measurement and the first date patients were considered to have PD.

### Results

Of the 182 eligible patients, 132 were enrolled in the study (Fig. 1). Clinical characteristics of the study population are illustrated in Table 1. A total of 632 samples were collected in 132 patients with a median of 4 samples per patient (range: 2–12) in an average of 46 months of follow-up (6–71 months). CT was the most common imaging modality used ( $n=533$ ), followed by DOTA-PET/CT ( $n=118$ ) and MRI ( $n=71$ ). During follow-up, 70 patients (53%) developed PD, 39 patients (30%) had SD of whom 11 (28%) received new treatment during this study and 23 patients had NED. The distribution of the disease status in our population at the end of the study is illustrated in Table 2.

### Reproducibility of value to predict disease status after 12 months

The median NETest level for our total study population ( $n=132$ ) at baseline was 33 (range: 7–100). The ability of the baseline NETest to predict SD and PD up to 1 year of follow-up was good. In contrast, the serial NETest measurements taken during follow-up did not predict disease status at 1 year after the sample was drawn. Specifically, the NETest AUROC of the baseline sample was 0.74 (95% CI 0.64–0.83;  $P < 0.001$ ), while the AUROC of the first follow-up sample was 0.55 (95% CI 0.43–0.67;  $P = 0.39$ ;  $n=132$ ) and the AUROC of the second follow-up sample was 0.45 (95% CI 0.34–0.56;  $P = 0.38$ ;  $n=108$ ).



**Figure 1**

Study enrolment schematic. Of 182 eligible patients, a total of 132 were enrolled in the study for evaluation of serial NETest measurements. Fifteen patients did not have more than 6 months of follow-up with at least 2 consecutive imaging procedures. Twenty-one patients did not have a second NETest measurement during follow-up. Thirteen additional patients were excluded because tumor lesions could not reliably be measured during follow-up (according to RECIST v1.1). One patient developed a second malignancy with distant metastasis and was therefore excluded.

### Reflection of disease status over time in patients not receiving any treatment

In the 28 patients with SD during total follow-up (median 59 (20-68) months), the median NETest score at baseline was 27. A total of 75% had NETest scores  $\leq 40$ . The median NETest scores of follow-up samples 2-6 were: 33 ( $n=28$ ;

**Table 1** Baseline characteristics.

Patients	132
Age (range)	62 (25-81)
Mortality (%)	23 (17%)
Primary tumor	
Small bowel	91 (69%)
Pancreatic	19 (14%)
Unknown origin	9 (7%)
Appendiceal	5 (4%)
Colorectal	4 (3%)
Gastric/duodenal	4 (3%)
Tumor grade	
Grade 1	92 (70%)
Grade 2	37 (28%)
Grade 3	2 (2%)
Missing	1 (1%)
Tumor stage	
No evidence of disease	30 (23%)
Stage IV	102 (77%)
Samples per patient	4 (2-12)
NETest samples < 6 months before treatment	34
NETest samples < 6 months after treatment	40

$P=0.27$ ), 47 ( $n=26$ ;  $P=0.03$ ), 80 ( $n=23$ ;  $P=0.003$ ), 83.5 ( $n=12$ ;  $P=0.006$ ) and 27 ( $n=6$ ;  $P=0.6$ ), respectively. No significant correlation was found between the serial NETest scores. The linear regression showed no relevant influence of follow-up time (time between diagnosis and sample collection) in test outcomes (estimate: 0.08 increase in score per month; 95% CI  $-0.054$  to  $0.219$   $P=0.17$ ). In all but one of the patients, at least one NETest score suggested the presence of high tumor activity ( $\geq 80$ ). A total of 71% had multiple NETest scores above the ULN for SD.

Imaging confirming SD at least 12 months after the last NETest sample was available in 25 of 28 (89%) patients. **Figure 2** illustrates the serial NETest scores in each of those 25 individuals.

Sixteen patients developed PD during follow-up but did not receive any treatment because of limited tumor load or as a result of shared decision-making. The median

**Table 2** The disease status during follow-up. If no progression was observed during the entire surveillance, a patient is classified as SD. Thirty patients had no evidence of disease at baseline, 7 had a recurrence of disease and thus were classified as PD and 23 had no evidence of disease at final follow-up.

Disease status	Treatment	No treatment	Total
PD	53	17	70
SD	11	28	39
NED	0	23	23
Total	64	68	132

NED, no evidence of disease; PD, progressive disease; SD, stable disease.

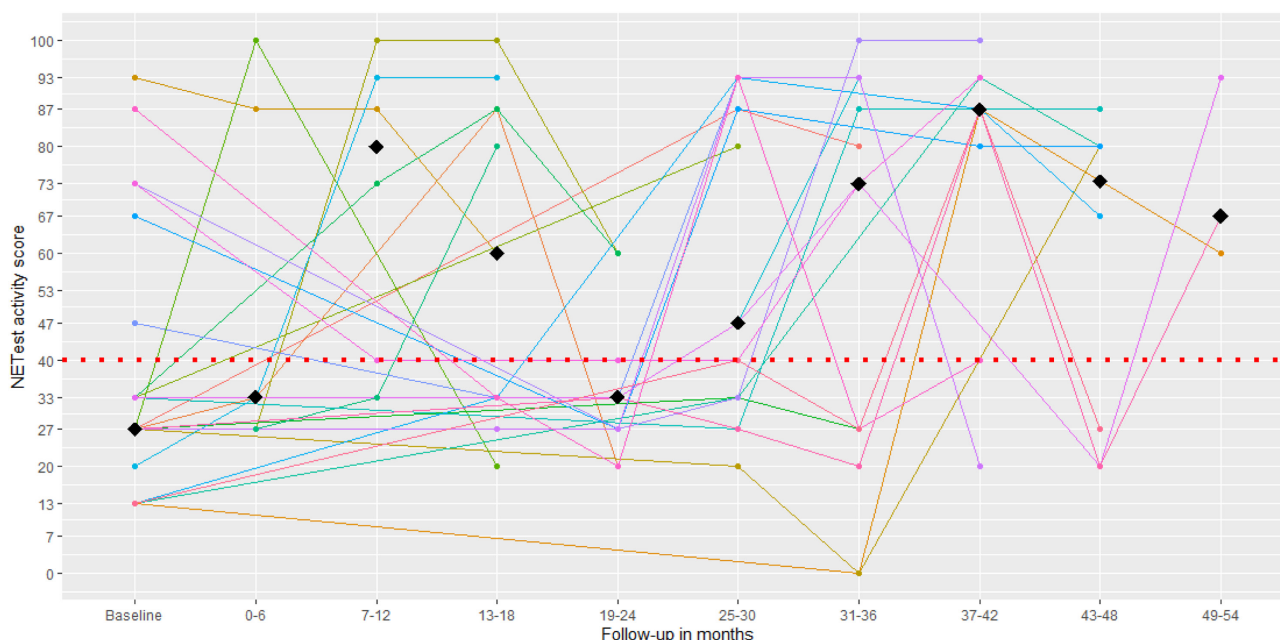
follow-up in this group was 47.5 (range: 15–66) months. The median time to progression was 24.5 (range: 0–62) months. In 12 patients, a NETest score in the year prior to PD (median time between sample and progression: 1.5 months) was available. The median NETest score was 80 (range: 13–93) with 10/12 (83%) NETest outcomes showing elevated tumor activity (1/10 intermediate tumor activity and 9/10 with high tumor activity). After PD on imaging was concluded, 17 samples were collected in absence of any treatment and 14/17 NETest outcomes (82%) showed intermediate- to high tumor activity scores reflecting ongoing progression (Fig. 3).

Thirty patients had NED on imaging at baseline after surgery with curative intent. The median study follow-up was 47 (range: 19–68) months. The median time between surgery and the last study visit was 79 (42–148) months. The average scan frequency was 5 (range: 2–7) times. In the majority of patients (83%), NED was confirmed with <sup>68</sup>Ga-DOTA PET-CT in addition to anatomical imaging. A total of 7 (23%) patients had a recurrence of the disease during follow-up. All 30 patients showed NETest outcomes above the threshold of 20 at some moment during follow-up, suggesting residual disease. Ninety-one percent of all patients with NED at final follow-up had NETest scores indicating intermediate (2/23) or high (19/23) tumor activity. Follow-up samples showed significantly higher

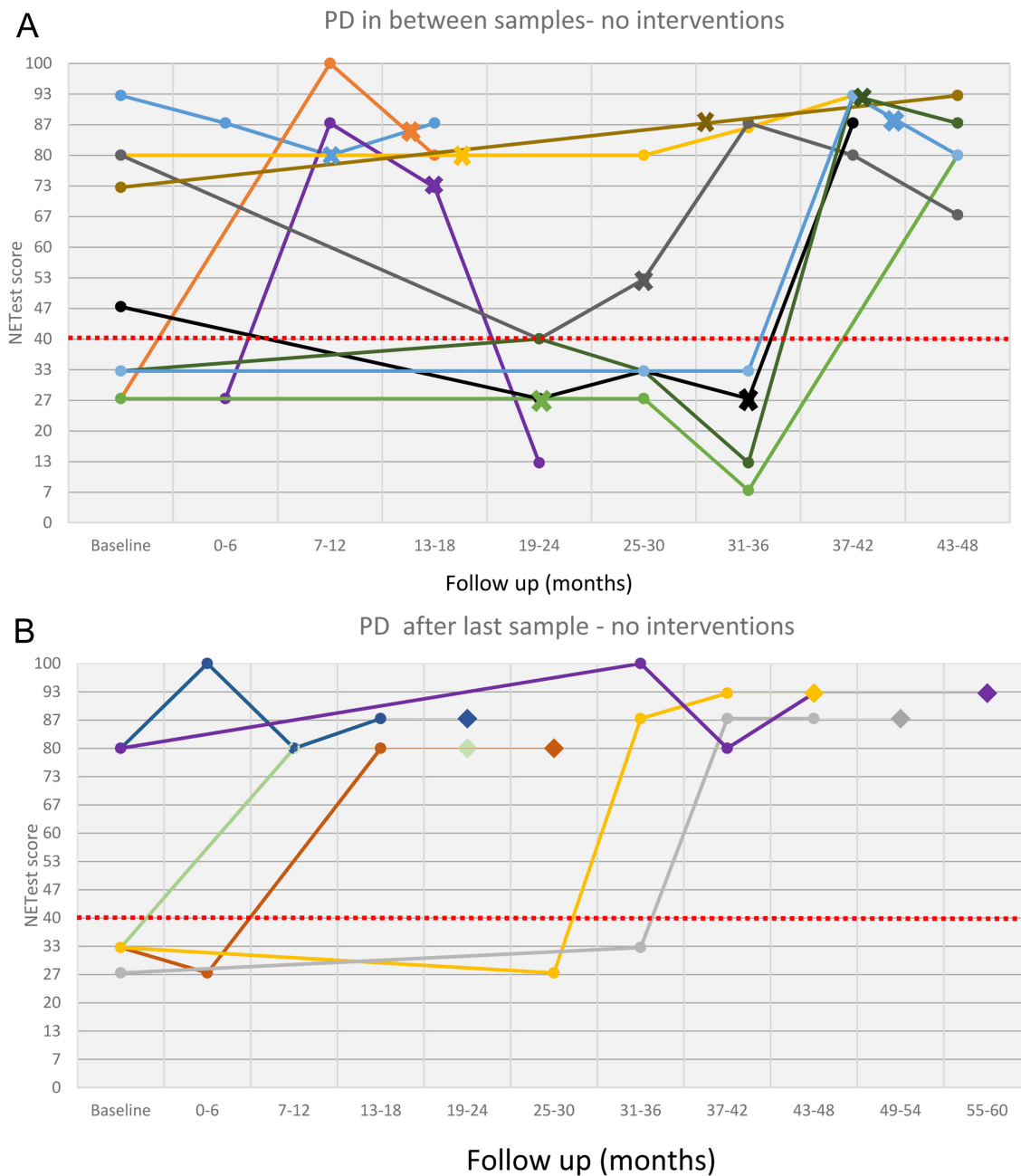
scores compared with baseline in the no recurrence group (sample 1: 27 vs median 80 ( $P=0.004$ ), 80 ( $P=0.002$ ), 80 ( $P=0.008$ ) and 76.5 ( $P=0.027$ ) for sample 2–5, respectively). No significant differences were found in consecutive NETest outcomes between patients with recurrence or continuous NED during the study period (Fig. 4).

### Prediction and reflection of treatment efficacy

A total of 49 patients had NETest samples that were suitable for the evaluation of systemic treatment efficacy. Nine patients had only a single NETest sample collected <6 months before the start of treatment and no post-treatment sample. A total of 15 patients had only a single sample <6 months after the start of treatment and in 25 patients both pre- and post-treatment samples were available. In 42 out of 49 (86%) patients, a new therapy was initiated because of PD according to RECIST v1.1, and in the remaining 7 patients (14%), new treatment was initiated because of refractory symptoms or clinical progression. A total of 25 patients received PRRT (51%); 16 started somatostatin analogs (33%), 4 started chemotherapy with capecitabine and temozolomide (8%), 3 patients received everolimus (6%) and 1 patient received sunitinib (2%). Treatment response was defined as PFS  $\geq$  12 months after treatment initiation. A total of 34 patients showed response to treatment (69%).



**Figure 2** Consecutive NETest scores in patients with stable disease. Each line represents the fluctuations in NETest activity score over time in patients with stable disease, defined by RECIST v1.1. All patients illustrated had at least 12 months of follow-up with imaging after the last NETest sample to confirm stable disease. The black diamonds represent the median score for each time period.

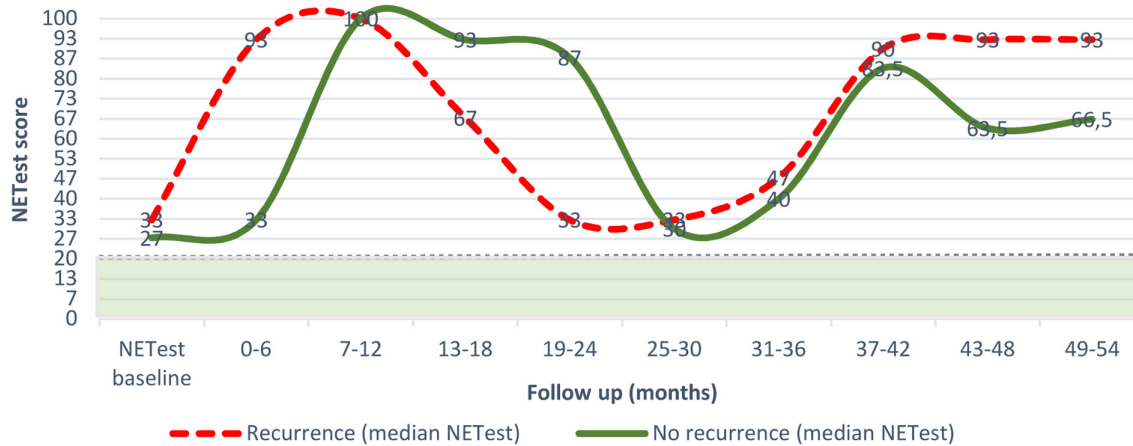


**Figure 3** Consecutive NETest scores in patients with PD and no intervention. Each line represents the serial outcomes of the NETest over time in patients with PD at a certain time point during this study. (A) NETest results of patients in whom PD was concluded in between samples PD is indicated by a cross. (B) Patients who had PD after the last sample was drawn (dashed line with diamond).

The median TTP after the start of treatment was 20 months (2–66 months).

Significant differences were observed in pre-treatment NETest categories between responders and non-responders ( $P=0.02$ ). Most responders had an elevated pre-treatment NETest score (18% had an intermediate (40–80) and 55% high (>80) activity score). Only 27% of the treatment responders had a low tumor activity score (<40) in contrast

to 92% of all non-responders. The median pre-treatment NETest score in responders to systemic therapy was 76.5 (13–100;  $n=22$ ) vs 33 (27–47;  $n=12$ ) in non-responders ( $P=0.001$ ). The accuracy (AUROC) to predict treatment response by pre-treatment samples was 0.73 ( $P=0.009$ ). Elevated NETest scores (>40) predicted tumor response in 94% (PPV), and low NETest scores ( $\leq 40$ ) predicted treatment failure in 65% (NPV).



**Figure 4**

NETest scores in patients with NED over time. The changes in median NETest activity scores in patients with no evidence of disease at baseline. The solid line represents patients with no evidence of disease at the final visit. Seven patients had recurrence (dashed line).

There was no difference between post-treatment NETest scores in responders compared with non-responders to systemic treatment (median 80 in both groups;  $P=0.634$ ). No other notable differences were observed between the two groups in post-treatment NETest scores. A median increase of 46 (range: 0 to +73) in NETest score was observed in non-responders compared with a median 3.5 score difference in pre- and post-treatment scores in responders (range: -27 to +66;  $P=0.04$ ). Changes in NETest outcomes are plotted against the response to treatment in each individual in Fig. 5.

Patients with NETest scores indicating low tumor activity (0–40) prior to treatment had a reduced median PFS compared to those with intermediate (40–80) or high (>80) NETest scores (10 vs 31 months; log rank 0.01; Fig. 6). No differences were observed between post-treatment scores.

### Co-morbidity

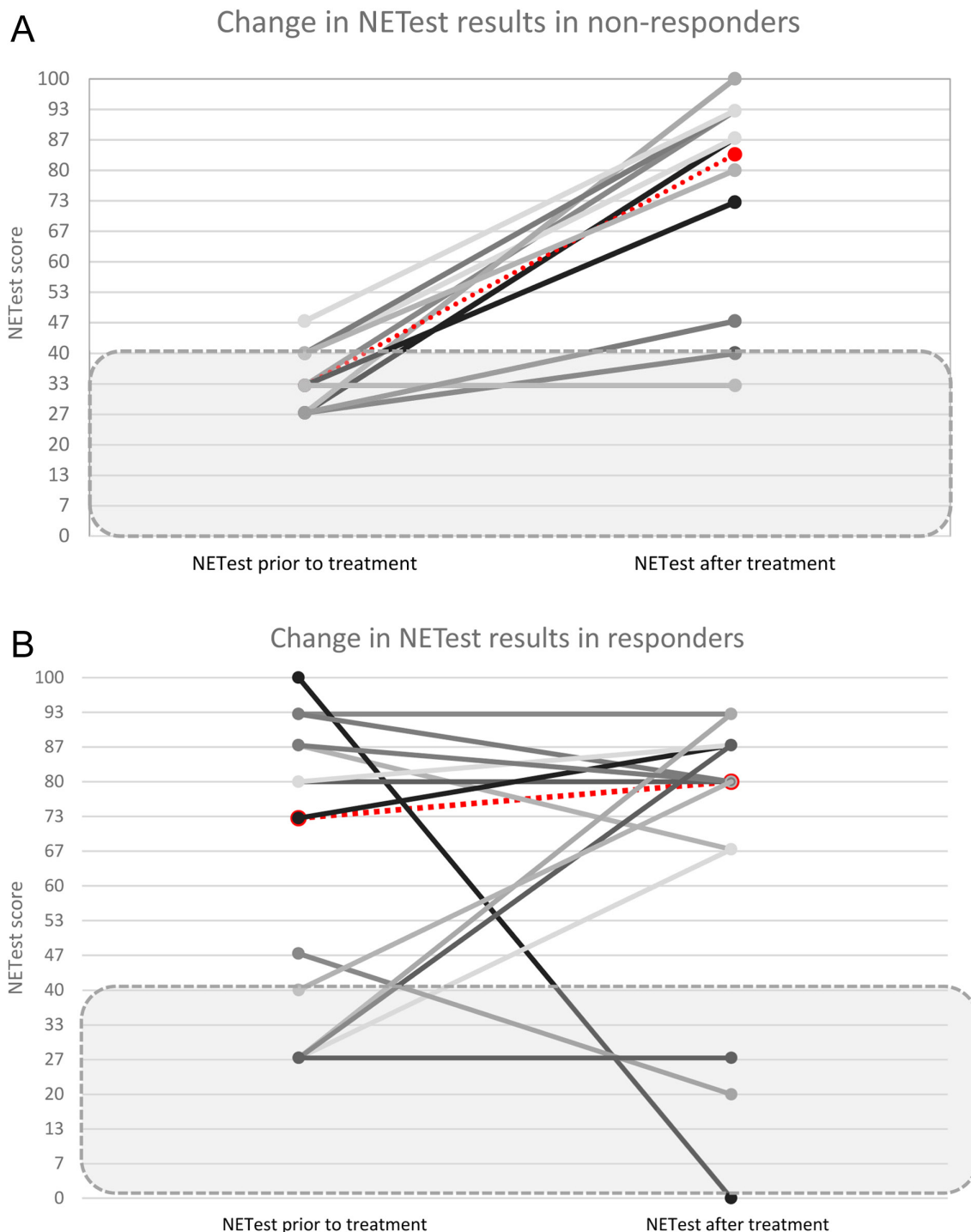
Seventy-six patients had no other disease besides a NET, whereas 56 had a comorbidity: cardiovascular disease ( $n=15$ ), diabetes ( $n=11$ ), secondary malignancy ( $n=6$ ) and OSAS ( $n=6$ ) were the most frequent. No significant differences were observed between patients with comorbidity and patients with no other disease than GEPNET.

### Discussion

This is the first study assessing the predictive and prognostic value of serial measurements of an emerging molecular biomarker – the NETest – in a large population

of patients with GEPNETs. To evaluate whether consecutive scores reflected tumor behavior over years of follow-up, NETest outcomes were studied only in patients not undergoing any treatment. Fluctuations in NETest outcomes were observed in patients with SD according to RECIST v1.1; these fluctuations were likewise observed in patients with NED. In contrast, in patients with PD, the vast majority had corresponding elevated test outcomes and only 5/29 samples (17%) were in the low range. These results collectively point toward a high NPV but a low PPV. In other words, the discriminative value of elevated NETest results confirms disease presence, but there is limited data to support future disease progress. Samples that exhibit lower tumor activity strongly indicate indolent tumor behavior. These metrics are in line with our and other previous cross-sectional studies, but the reproducibility of NETest scores in individuals has not been studied before since long-term data on repeated NETest samples (>1 year) were missing (19, 22, 23, 24). AUROC analysis of consecutive samples showed a shift in test accuracy during years of disease evolution and a decrease in predictive value, putting the NETest role as follow-up marker into a new perspective.

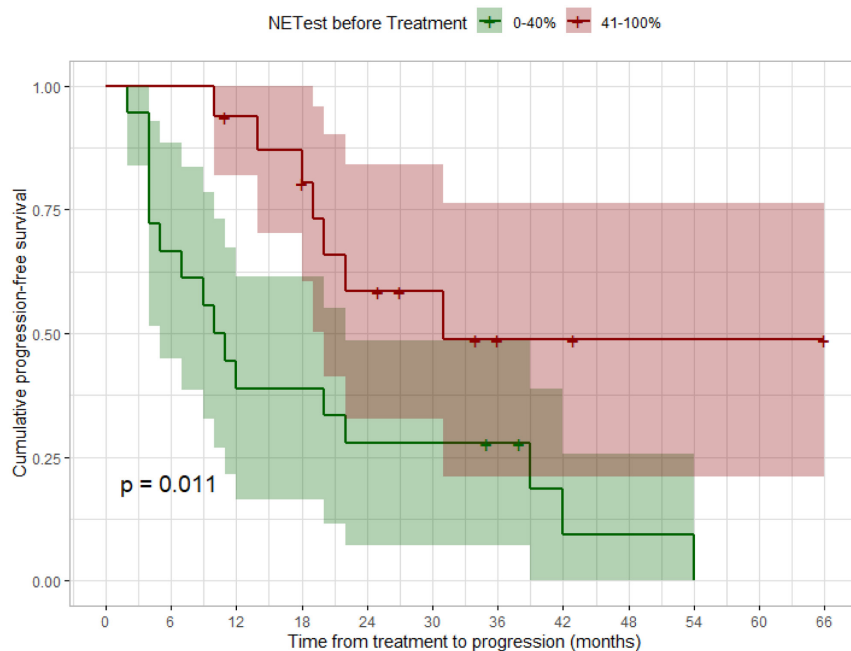
Fluctuations in NETest scores played a role in decreased test performance, and these changes in individuals could be the result of test characteristics or tumor characteristics. The NETest output is based on circulating mRNA, but there is conflicting data on the utility of mRNA as the basis for liquid biopsy techniques. mRNA in peripheral blood is considered less reliable because of instability due to RNases, the low abundance of RNA and the ‘contamination’ of RNA from normal tissue cells in the peripheral blood (25), all factors which can influence test outcomes.



**Figure 5**

Treatment response and changes in NETest scores: Changes in NETest results in each individual vs treatment initiation. The gray box indicates low tumor activity scores (0–40%). The red line illustrates the difference in median pre- and post-treatment scores. (A) Patients with progression within 12 months after treatment initiation (non-responders) showed a significant rise in NETest score ( $P = 0.005$ ). (B) Patients with a minimum of 12 months progression-free survival (responders) illustrated comparable pre- and post-treatment NETest outcome ( $P = 0.37$ ).



**Figure 6**

Progression-free survival after treatment initiation. Median PFS was significantly reduced in patients with low NETest scores prior to treatment initiation compared with patients with intermediate/high NETest scores (10 vs 31 months;  $P = 0.01$ ).

However, evidence supports the counterargument that the majority of mRNA is protected in extracellular vesicles, and changes in RNA abundance in plasma extracellular vesicles are proven to be cancer-related (26). Whether analytical problems are a factor in the fluctuations we observed could not be evaluated in our independent validation study. ‘Time’ seemed not a contributing factor. Our linear regression estimated an increase of 1 point per year in NETest scores. Although the power for this mixed procedure is possibly too low because of the small sample size, the clinical relevance of 1 point increase in NETest score per year is negligible. Theoretically, gene expression levels among individuals might be influenced (increased) by non-cancer and patient-specific factors, which is substantiated by our results in patients with NED. When compared with patients with NED, NET-specific characteristics probably play a larger role in the NETest variability in individuals with SD. The tumor micro-environment is a complex ecosystem in which cancer cells interact with a diverse range of immune, stromal and endothelial cells, constantly shaping and changing the molecular biology of a tumor (27). Inter-tumoral and intra-tumoral heterogeneity is recognized in many solid tumors, creating obstacles in the identification and development of new biomarkers (28, 29). A recent paper by Childs and coworkers demonstrated significant intra- and inter-patient genomic heterogeneity in circulating tumor cells from NETs (29). Their findings provide evidence on a molecular level for the heterogeneous clinical entity that is already well-recognized. Small active clones within a

tumor can possibly drive NETest scores while tumor load on imaging remains stable.

A question that remains unanswered is: Do we need to change clinical management when a patient presents with high NETest scores while subgroup data illustrate that NETest tumor activity does not necessarily lead to an increase in tumor load as assessed by radiological follow-up? Guidance based on tumor load may be an outdated way of thinking, but we need further research to demonstrate that change in management based on elevated tumor activity will ultimately lead to patient benefit. Collectively, our findings illustrate that the high variation in NETest levels among individuals without treatment or with NED needs further study before the clinical utility of blood-based gene expression signatures – as marker for disease activity in tumor surveillance – can be advised.

An important limitation of this study was that imaging was used as reference standard for disease presence. Most patients had at least 1  $^{68}\text{Ga}$ -DOTA PET-CT with a reported sensitivity of 91–95% (30), but the sensitivity of CT-imaging – our most frequent used modality – varies between 58 and 92% (31). It is debatable whether 6 years of follow-up is enough to assure that our NED patients were truly cured from such indolent tumors. It is therefore important to further follow up with these patients given that imaging is not 100% reliable in excluding residual disease. Imaging is the only non-invasive tool available to detect residual disease and prove the NETest predictive accuracy. Time is therefore the only other ‘diagnostic’ to confirm these outcomes. Although CT and MRI are proven

to be accurate and interchangeable imaging techniques in predicting pathological tumor size for pancreatic NETs (32, 33), biological tumor activity does not necessarily lead to a significant (20%) increase in tumor size in short term. Other outcome measures possibly better reflecting tumor activity, like  $SUV_{MAX}$  on PET/CT, were not taken into account. This means that the NETest activity scores are only related to an increase in tumor size or new lesions on imaging in this study. With that being the case, the absence of other widely accepted criteria for disease activity or treatment response for GEPNETs makes RECIST the best accepted outcome currently available.

The high accuracy of the NETest for predicting treatment response is the second remarkable finding. Elevated NETest scores were associated with a good response to treatment in the majority of patients. Patients with low NETest scores before the start of systemic treatment had 21 months reduced (10 vs 31 months) overall PFS when compared with patients with an elevated pre-treatment NETest score. These data suggest that the NETest comprises a genomic signature that – measured in advance – predicts response to systemic therapy. Currently, no other biomarker has this predictive value in GEPNETs (34). We hypothesize that an elevated NETest score prior to treatment indicates increased expression levels of genes involved in these neoplastic processes, making these tumors more vulnerable to treatment. In other malignancies, like breast cancer, gene expression signatures derived from tumor tissue already guide individualization of treatment and have proven valuable (35). Blood-based gene signatures are now emerging in different malignancies and non-malignant diseases and some have proven to predict treatment resistance as well (36, 37, 38, 39, 40, 41), although extensive validation in daily practice is required.

The new insights from this study illustrate the necessity to use imaging studies in patients on a watch-and-wait strategy. However, the absence of a reliable biomarker that can predict treatment outcomes in GEPNETs is a gap in current surveillance strategies that could be filled by the NETest. Based on our results, pre-treatment samples could guide the timing of the next line of treatment. This means that integration of genomic data within our imaging-based surveillance programs could help to individualize management. Nevertheless, our results regarding the predictive value need to be confirmed in future studies since they are not completely in line with previous studies that evaluated the correlation between the NETest and treatment response (42, 43). Although Bodei and coworkers found significant changes in NETest scores that reflected treatment response, changes in NETest scores were mostly driven by a decline in scores post-treatment. In the present study,

significant changes in NETest results were also observed (+46 in non-responders vs +3.5 in responders), but this outcome is largely based on the differences in pre-treatment samples between responders and non-responders.

To conclude, during years of follow-up, the present study found fluctuating consecutive scores in individuals with RECIST defined SD, which is consistent with the concept of inter- and intra-tumoral genomic variability. Samples demonstrating low tumor activity indicate indolent tumor behavior. Patients who develop PD exhibit elevated scores. Elevated results have important predictive value for treatment responsiveness and could be used for individualizing decisions on systemic therapy, but validation is needed. The clinical value of elevated scores in the watch-and-wait group needs further study, elevated levels presumably cannot be disregarded in terms of their clinical implications.

The NETest remains a promising molecular tool with important values, novel to NET surveillance. Implementation of the molecular assay within our regular imaging-based screening intervals can be a useful addition to the clinical armamentarium for a large subgroup of GEPNET patients.

#### Declaration of interest

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

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#### Author contribution statement

M Tesselaar and G D Valk: authors share senior authorship.

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