NETest: A Systematic Review Focusing on the Prognostic and Predictive Role

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Keywords

NETest · Neuroendocrine neoplasms · Liquid biopsy · Predictive biomarker · Prognostic biomarker

Abstract

The NETest is a standardized and reproducible liquid biopsy for neuroendocrine tumors (NETs). It evaluates the expression of 51 NET genes by real-time polymerase chain reaction, providing an accurate molecular profile of the neoplasm. Diagnostic utility of NETest has been widely demonstrated, while its role in predicting prognosis and treatment response is less studied. This systematic review aims to collect and discuss the available evidence on the prognostic and predictive role of NETest, trying to answer 3 questions, frequently raised in clinical practice. *Is NETest able to differentiate stable from progressive disease?* Increased NETest levels (at least >40%) correlate with disease progression. *Is NETest able to predict tumor progression and tumor response to treatment?* Some studies demonstrated that the baseline NETest score >33–40% could predict tumor progression. Moreover, NETest performed after treatment (as peptide receptor radionuclide therapy) could predict treatment response also be-

fore radiological findings, since the decrease or stability of NETest score predicts tumor response to treatment. *Is NETest able to evaluate tumor recurrence risk after surgery?* NETest can predict surgical treatment outcome detecting minimal residual disease after radical surgery, which is characterized by a lower but positive NETest score (20–40%), while a higher score (>33–40%) is associated with nonradical surgery. In conclusion, in addition to its demonstrated diagnostic role, this systematic review highlights the efficacy of NETest to assess disease status at the moment of the NETest execution and to predict tumor recurrence after surgery. The efficacy for other applications should be proven by additional studies.

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Introduction

Neuroendocrine neoplasms (NENs) are heterogeneous in terms of primary sites, neuroendocrine differentiation, clinical behavior, and response to treatments. In this field, the possibility to rely on easy to execute markers for estimating the prognosis and for predicting re-

sponse to treatment could be essential for improving the clinical management of these patients [1]. Nowadays in patients affected by neuroendocrine tumors (NETs), the strongest predictors of overall survival (OS) are tumor grade and stage [2].

Circulating biomarkers have been studied for diagnosis and follow-up of patients affected by NENs. While in functional NENs it is possible to analyze secretory products or their metabolites in blood and/or in urinary sample, in nonfunctioning tumors only the so-called general tumor markers can be used [3]. Chromogranin A (CgA) is the most used general tumor marker, with both diagnostic and predictive value [4]. The prognostic role of this marker has been advocated since the demonstration of the correlation with OS [5], poorer outcome [6], and tumor burden [7]. However, there are multiple limitations of CgA use. A false-positive value can be observed in non-oncological diseases, such as atrophic gastritis, hypergastrinemia, heterophile antibodies, impaired kidney function, or use of antisecretory medications, especially proton pump inhibitors [3, 8, 9]. False-negative results can happen in case of less differentiated disease, since CgA is a secretory product of the neuroendocrine cells [10].

Neuron-specific enolase (NSE) is physiologically present in neurons and neuroendocrine cells and its circulating form is a biomarker for OS in gastroenteropancreatic (GEP) NEN patients [11, 12]. However, serum NSE is increased only in 30–50% of these patients [13]. A significant association of NSE levels with survival was also demonstrated in patients affected by small cell lung cancer [14]. As a prognostic marker of NETs, NSE is minimally correlated with tumor size but associated with grading. In fact, its levels result higher in patients with poorly differentiated neuroendocrine carcinoma (NEC) [15].

Preliminary data of the use of serum neutrophil-lymphocyte ratio (NLR) are available in patients affected by lung NECs. In these patients, an increasing preoperative NLR is associated with higher stage and inversely correlates with post-resection OS and relapse-free survival [16, 17]. The predictive value of worse survival of the pre-operative NLR was also demonstrated in patients with gastric NENs undergoing surgery [18] and in intestinal and pancreatic NETs (Ki-67 <10%) treated with lanreotide [19]. Recently, NETest has been developed and proposed mostly for the diagnosis of NENs, demonstrating in a recent meta-analysis on 10 studies a diagnostic accuracy of 95–96% [20].

The aim of this systematic review is to collect and discuss the available evidence on the role of NETest in predicting prognosis and treatment response, including both

systemic treatments, used in metastatic patients, and surgical and ablative strategies, used in localized disease, in patients affected by NENs, trying to answer the following questions: (1) Is NETest able to differentiate stable from progressive disease? (2) Is NETest able to predict tumor progression and response to therapy? (3) Is NETest able to predict tumor recurrence after surgery?

Materials and Methods

We performed this study according to the Cochrane Collaboration and PRISMA statement [21].

Data Sources and Searches

From June to November 2020, we searched for English-language articles in MEDLINE. No date restriction has been applied. Search terms used were: “NETest”; “predictive biomarker” AND “neuroendocrine”; “prognostic biomarker” AND “neuroendocrine”; “liquid biopsy” AND “neuroendocrine.” Additionally, we searched in EMBASE, Cochrane Library, and SCOPUS using “NETest” as a search term.

Eligibility criteria for study selection included studies on humans with any of the following designs: randomized clinical trials, prospective non-randomized trials, retrospective studies, and case series. We selected: (1) articles on NETest; (2) data on prognostic value or treatment response prediction or treatment response assessment of NETest; and (3) patients affected by any subtypes of NENs. Exclusion criteria were: (1) non-original articles or case reports; and (2) articles reporting only data on the diagnostic value of NETest. A final update of the search was conducted in May 2021 and one additional study was included.

Article Selection

Each study was screened by abstract and title and potentially eligible studies were further assessed in detail by retrieving full-length articles. Each full-length article was independently reviewed by two separate authors (G.P. and V.D.V.) following inclusion criteria. Two authors independently extracted data from the articles that met the inclusion criteria. A standardized form was used to extract the following information: year of publication, type of study, number of included patients, age at diagnosis, sex, histopathological examination, staging and outcomes, treatment strategy (surgery, medical treatment, and radiotherapy), time of execution and values of NETest, correlation of NETest with disease status, prognosis, and treatment response. Quality of studies has been assessed by MINORS score [22].

Results

From the original number of 244 articles, 26 have been selected by title and abstract. After full-text evaluation, a total of 20 articles were included in the systematic review (Fig. 1).

Fig. 1. Flow diagram of included and excluded studies. *Respectively on PubMed, Embase, Cochrane Library, and Scopus. No time restriction has been applied in the search strategy; articles included in the systematic review have been published from 2015 to 2021.

Liquid Biopsy and NETest

In recent years, liquid biopsy has received growing attention. It is a molecular biology technique that allows to identify and to characterize neoplasms, through molecular analyses performed on a venous blood sample. Liquid biopsy is performed analyzing the following elements: circulating cancer cells, free nucleic acids, exosomes, and tumor-educated platelets (TEPs); the name of the latter originates from their ability to engulf the circulating RNA released by the tumor, with therefore a correlation between TEPs and tumor growth and dissemination in various kinds of neoplasms [23, 24]. One of the advantages of liquid biopsy analysis is that it can be serially repeated, allowing to get real-time information from the lesion so that we can promptly make changes in therapy [25]; moreover, given the characteristic heterogeneity of NENs, this diagnostic strategy could probably be more effective in representing the totality of the disease compared to a biopsy that could only provide a partial view [26, 27].

In this field of liquid biopsy applied to NENs, NETest has been developed in recent years. This is a standardized and reproducible clinical laboratory measurement for the diagnosis of NENs, whose clinical utility has been documented in GEP and bronchopulmonary NENs and in paragangliomas and pheochromocytomas (PPGLs) [28–31]. The efficacy of this biomarker for diagnostic purpose originates also from its independence from patient's characteristics, such as age, sex or ethnicity, and treatments. As a result, it is advantageous compared to CgA [31, 32].

After mRNA isolation and cDNA production, NETest uses real-time polymerase chain reaction (RT-PCR) in order to quantify circulating transcriptional products of neoplastic origin. In particular, NETest evaluates the expression of 51 genes, 30 of which can be categorized into 9 clusters, related to cell proliferation and apoptosis (Proliferome, Growth factor signalome, and Apoptoma), peptide secretion (Secretome I, general and Secretome II, progressive), epigenomic changes (Epigenome), and somatostatin receptor (SSTRome), providing tumor molecular profile [33, 34]. After RT-PCR, results are analyzed through algorithms, designed to differentiate healthy controls from NENs patients, determining a 0–8 score: samples scored 0–2 are classified as normal, while levels 3–8 are categorized as NENs [35]. To expand the utility of the NETest from a diagnostic tool to an instrument able to capture the biology of NENs, a second algorithm-based analysis quantifies the expression of 6 of the above-mentioned clusters (SSTRome, Proliferome, Metabolome, Secretome, Epigenome, and Plurome) and, incorporated also the machine-learning derived 0–8 score, generates a clinical activity score scaled from 0 to 100% (the NETest score). While the first score is able to diagnose NENs from controls, the second score is related to tumor activity, being more able to differentiate stable (SD) from progressive disease (PD) [31]. Thus, the NETest is able to capture the biology of a specific NEN defining its molecular status.

Table 1. Articles evaluating the role of NETest in differentiating stable disease from progressive disease, assessed by morphological imaging, at the time of blood collection

First author [ref.]	Study design	Patients, N	NENs localization (n)	Histology and grading	Mean age (range min-max)	Sex	Functionality status (NF/F)	Treatment before study entry	NETest values in SD versus PD (p value) Cut-off (sensitivity and specificity)	Quality assessment (MINORS)
Kidd et al. [34]	Prospective cohort study	222	Test set (63) GEP (60)	G1 30; G2 18; G3 2; N/A 13	56 (18-80)	M 33 F 30	N/A	Surgery; interferon; CHT; SSA	SD: 34.1±27% PD: 83.7±24.4% ($p < 0.0001$) Cut-off: 45% (sensitivity 91.1%, specificity 85.3%)	10
			Lung (3)							
			Independent set (159) GEP (141)	G1 87; G2 29; G3 5; N/A 38	57.1 (27-83)	M 86 F 73	N/A			
			Lung (5)							
			N/A (12)							
Cwikla et al. [36]	Prospective cohort study	63	Test set, basal (35) GEP (35)	NET (grade NA)	58 (33-82)	M 12 F 23	N/A		SD: 32±19% PD: 82%±12% ($p < 0.0001$) Cut-off: 80% (sensitivity >80%, specificity >95%)	9
Pavel et al. [37]	Prospective cohort study	34	Gut (25) Pancreas (9)	G1 17; G2 14; G3 1; N/A 2	60.2 (43-83)	M 17 F 17	N/A	None 14; SSA 16; + everolimus 1; streptozotocin/5-fu 3 (no cut-off proposed)	SD: 41.6±5.8% PD: 67.2%±7.1% ($p < 0.05$)	12
Filosso et al. [38]	Retrospective and prospective cohort study	226	Lung (set 1, diagnostic cohort: 131)	Typical carcinoids; BP carcinoids; atypical carcinoids; 58.2 (21-79) LCNEC; SCLC	63 (47-73)	BP carcinoids: M; 73 F Other lung NENs: 7 M; 6 F	N/A	N/A	SD: 36±19% PD: 73%±22% ($p < 0.001$) (no cut-off proposed)	12
Kidd et al. [30]	Pilot cohort study	25	Lung (25)	TC 18; AC 7	62 (46-77)	M 4 F 21	N/A	SSA 5; CHT 1	SD: 32±7% PD: 85%±11% ($p < 0.0001$) cut-off proposed)	7
Peczowska et al. [29]	Prospective cohort study	32	PPGLs	Localized 18; multicentric 7; metastatic 4	34 (12-62)	M 17 F 15	NF 17 F 15	Surgery 25; embolization 5; PRRT 5; EBRT 3; brachytherapy 1	SD: 41±5% PD: 86%±2% ($p < 0.0001$) Cut-off: 53% (sensitivity 100%, specificity 85.7%)	5
Liu et al. [39]	Prospective cohort study	100	GEP (68)	G1 34; G2 13; G3 2	61.5 (14-83)	M 34 F 66	N/A	Surgery: 69	NETest <40%: SD in 54 pts (87%) NETest >80%: PD in 21 pts (81%) Overall concordance: 75/88 (88%)	8
			Lung (20) Other sites (12) ¹	TC 11; AC 6; SCLC 1; NA: 2						
Malczewska et al. [40]	Prospective cohort study	111	Pancreas (67)	G1 33; G2 27; NET G3 2; NEC G3 3; N/A 2	56 (19-87)	M 24 F 43	NF 58 F 9	Surgery 44; PRRT 11; CHT 6; everolimus 1; loco-regional (liver) 6; RT: 3	SD: 29±14% PD: 61%±26% ($p = 0.0001$) Cut-off: 40% (accuracy 95%)	14
			Gut (44)	G1 33; G2 10; NET G3 1	59 (29-77)	M 26 F 18	NF 37 F 7	Surgery 39; PRRT 12; CHT 2; everolimus: N/A; loco-regional (liver) 3; RT: N/A		

Table 1 (continued)

First author [ref.]	Study design	Patients, N	NENs localization (n)	Histology and grading	Mean age (range min-max)	Sex	Functionality status (NF/F)	Functionality status (NF/F)	NETest values in SD versus PD (p value) Cut-off (sensitivity and specificity)	Quality assessment (MINORS)
Malczewska et al. [41]	Prospective cohort study	122	Lung (99)	TC 62; AC 37	59 (22–83)	M 37 F 62			SD: 35.5±18% PD: 61%±26% ($p < 0.0001$) (no cut-off proposed)	15
Malczewska et al. [42]	Prospective cohort study	318	GEP (253)	G1 173; G2 66; G3 NET 5; G3 NEC 7; N/A 2	53.3 (19–87)	M 111 F 142	NF 222 F 31	Resection/LRT 182; PRRT 26; CHT 9; RT 3	SD: 34±2% PD: 55%±5% ($p = 0.0005$) (no cut-off proposed)	15
			Lung (64)	G1 30; G2 19; N/A 15	67 (32–81)	M 26 F 38	NF 61 F 3	Resection/LRT 44; PRRT 2; CHT 6; RT 5	SD: 29.4±1% PD: 57.8%±7% ($p < 0.0001$) (no cut-off proposed)	
			Thymic (1)	G3	38	M 1	NF 0 G 0	Resection/LRT 1; RT 1		

GEP, gastroenteropancreatic; N/A, not available; CHT, chemotherapy; SSA, somatostatin analogues; SD, stable disease; PD, progressive disease; NF, nonfunctioning; F, functioning; PRRT, peptide receptor radionuclide therapy; RT, radiotherapy; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; BP, bronchopulmonary; NEN, neuroendocrine neoplasia; TC, typical carcinoid; AC, atypical carcinoid; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; LRT, locoregional therapy; PTS, patients; PGLs, pheochromocytomas/paragangliomas. ¹Other sites include cancer of unknown primary origin ($n = 7$), ovary, vulva, vagina, and pituitary ($n = 2$).

Prognostic Role of NETest

Is NETest Able to Differentiate Stable from Progressive Disease?

Ten studies have demonstrated that NETest is able to differentiate between SD and PD, in many subtypes of NENs (Table 1). Using the initial linear score, ranging for 0–8, Kidd et al. [34] demonstrated that a score between 0 and 5 was associated with SD in over 90% of patients, while a score of 8 correctly identified PD in over 90% of patients. The study was performed on an overall population of 111 patients with SD and 48 with PD, mostly grade 1–2 GEP-NET [34]. In the same study, using the NETest score, the authors demonstrated that SD samples had significantly lower activity scores than PD samples ($34.1 \pm 27\%$ vs. $83.7 \pm 24.4\%$, $p < 0.0001$), proposing 45% as a cutoff (upper 95% confidence interval for SD) [34].

Malczewska et al. [40], in a study on 72 patients affected by radiologically detectable GEP-NENs (42 pancreatic NETs, 33 small-intestinal NETs), 11 progressive and 64 stable, confirmed that the NETest score was significantly increased in PD (mean $\pm$ standard deviation: $61 \pm 26\%$) compared to SD ($29 \pm 14\%$); the cutoff score of 40% showed an accuracy of 95%. Similar results were also confirmed by Pavel et al. [37] (PD group vs. SD group $67.2 \pm 7.1\%$ vs. $41.6 \pm 5.8\%$, $p < 0.05$).

The ability of NETest to differentiated PD from SD was confirmed also in studies enrolling only pulmonary NENs. In a study on 112 patients, the NETest score was significantly lower in SD ($36 \pm 19\%$) than in PD ($73 \pm 22\%$, $p < 0.001$) [38]. In a cohort of 25 typical and atypical lung carcinoids, NETest was confirmed to differentiate tumor progression ($85 \pm 11\%$ vs. $32 \pm 7\%$) [30]. Despite the good performance, as demonstrated by high AUC, in both cases the authors did not propose a specific cutoff [30, 38]. Another study confirmed that NETest accurately distinguished PD from SD (61 ± 26 vs. 35.5 ± 18 , $p < 0.0001$); moreover, in pulmonary carcinoids (data on 99 patients), NETest levels were elevated in metastatic disease irrespective of histology (typical, 78 patients, and atypical carcinoids, 21 patients), both in SD cohort and in PD cohort [41]. A recent study by Malczewska et al. [42] confirmed, on a large series of patients (135 GEP and 64 pulmonary), that NETest was able to differentiate PD from SD both in GEP-NENs (55 ± 5 vs. 34 ± 2 , $p = 0.0005$) and in pulmonary carcinoids (57.8 ± 7 vs. 29.4 ± 1 , $p < 0.001$) and PPGLs [29].

However, other articles identified a cutoff of 80% for PD [36]. This higher cutoff (80%) has been identified also in the study by Liu et al. [39]. The study demonstrated the high concordance between low NETest (<40%) and SD

(83% of patients with SD) and high NETest (>80%) and PD (60% of patients with PD), with an overall concordance of 88%. Other studies demonstrated that a significant difference in NETest score is also found between localized and metastatic disease, both in GEP and in pulmonary NENs [42, 43].

A study on patients affected by PPGLs identified a cut-off of 53% for differentiating PD (11 patients) and SD (19 patients) at the moment of the blood collection (0.93, confidence interval: 0.84–1.03, $p < 0.0001$); moreover, the NETest score was significantly higher in multicentric and metastatic disease than in localized disease [29].

In summary, the NETest score >40% demonstrated a high concordance with progression evidenced by radiological imaging. This finding does not change also considering prospective studies only. This is in accordance with the cutoff of 40% reported in the meta-analysis by Oberg et al. [20].

Is NETest Able to Predict Tumor Progression and Tumor Response to Treatment?

Some studies have evaluated the possibility for NETest to predict tumor progression in the months following the execution of the NETest (Table 2). An interesting study by Pavel et al. [37], on 34 patients affected by stable GEP-NETs, demonstrated that, in patients with SD at baseline, the basal value of NETest >40% can predict subsequent tumor progression despite multimodal treatment strategies, while values <40% predict stability over 5 years. Moreover, the basal NETest score was associated with progression-free survival (PFS) (hazard ratio = 1.022, 95% confidence interval = 1.005–1.04, $p < 0.012$) and Kaplan-Meier analyses demonstrated that baseline NETest <40% predicted longer PFS (median PFS 2.78 years in case of basal NETest <40% and 0.68 years in case of basal NETest >80%) [37].

The correlation of basal NETest score with PFS was confirmed also in a cohort of 100 patients (68 GEP, 20 bronchopulmonary, and 12 mixed NENs). Mean PFS of patients with NETest scores <40% without treatment was 12 months, while mean PFS was only 3 months in the case of basal NETest scores >80%, despite treatment [39]. In the same study, Liu et al. [39] evaluated the utility of the NETest in a watch-and-wait program (45 patients). Patients with a basal low score ($\leq 40\%$; $n = 27$) maintained SD, while all patients with a high NETest ($\geq 80\%$; $n = 14$) required treatment intervention and/or developed PD [39].

A recent interesting prospective study has enrolled 152 patients with sporadic GEP-NENs, followed for 36

months (range 4–56); 119 had measurable disease and 33 had no evidence of disease at enrolling. Basal NETest categories (low tumor activity <33%; intermediate tumor activity 34–79%; and high tumor activity $\geq 80\%$) predict median PFS, which was, respectively, 55, 18, and 11 months. Patients with NETest >33% had an overall 9 times higher risk of developing PD than those with NETest $\leq 33\%$ (with a reported odds ratio of 8.6). Of the overall number of 152 patients, 55 with measurable disease were enrolled in the watchful waiting group (no treatment) and 32% of these patients developed PD within 1 year. Only 16% of patients with low tumor activity had PD, compared to 50% and 54% of intermediate and high activity categories. In parallel, 64 patients were treated since baseline and PD within 12 months of follow-up were observed in 45%. Once again, basal NETest predicted the risk of PD at 12 months: progression was observed in 17% of patients with low activity scores, in 61% with intermediate, and in 74% with high tumor activity. As confirmation, 70% of patients with low tumor activity in the watchful waiting group and 64% of patients with low tumor activity in the treatment group had SD after 24 months. Finally, considering the 33 patients without evidence of disease at baseline, no patients with negative NETest (<20%) developed recurrence; moreover, the median value of NETest in patients who remain free of disease at follow-up was 27% compared to 53% in patients with recurrence [45].

Cwikla et al. [36], in the prospective part of their study, evaluated NETest score before starting somatostatin analog (SSA) treatment for GEP-NETs (G1 and G2). A basal level of 80% predicts the development of disease progression in 100% of patients (14 patients). Even in case of basal NETest <80%, the NETest score was higher in patients ($57.5 \pm 6\%$ vs. $41 \pm 2\%$, $p = 0.02$) that subsequently developed PD [36].

Some studies, reporting multiple NETest determinations, evaluated if NETest score variation could assess treatment efficacy. These studies focused mainly on peptide receptor radionuclide therapy (PRRT) and SSA.

Bodei et al. [44] evaluated the changing in NETest score according to PRRT efficacy. In this study, responders to PRRT were defined as patients showing complete response, partial response, or SD evaluated by computed tomography according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1, while non-responders showed tumor progression. After 6 months from treatment starting, a reduction in NETest was seen in 88% of responder patients (no change in 12% of responders), while an increase in NETest was seen in 90% of non-responders, showing high agreement between ra-

diological findings and NETest (89% of concordance) [44]. These preliminary results have been subsequently confirmed by another study on 122 patients affected by lung and GEP-NETs. In case of objective response to PRRT, NETest decreased (mean change: $-47 \pm 3\%$), while NETest remains high in the majority of non-responders. Authors proposed a post-treatment cutoff of 40% for differentiating SD from PD and therefore for determining PRRT response [46].

In a study on repeated NETest measurements on 9 patients affected by PPGLs, 4 patients with SD at baseline and a good response to treatment with SSA demonstrated a fall or a stability in the NETest score at follow-up, while 2 SD at baseline with following PD showed an increase in NETest score. Considering patients with PD at baseline, the NETest score increases in 1 patient with further progression, decreases in 1 patient who underwent surgery, and remains stable in 1 patient without further progression on SSA treatment [29]. Cwikla et al. [36] demonstrated that elevated NETest (80–100%) during SSA treatment for GEP-NETs (G1 and G2) was significantly associated with therapy failure (sensitivity 100%; specificity 57%; positive predictive value 70%; and negative predictive value 100%).

Liu et al. [39], in a cohort of 55 NEN patients treated mainly with SSA (84%), confirmed that NETest was clinically useful in treatment monitoring. In fact, 100% of patients with a low score exhibited SD at 6–12 months, while an increase in NETest during treatment required further therapy modifications, as an increase in SSA dosage or addition of an alternative agent [39].

Overall, high NETest values (>40%) in patients without contemporary radiological progression could predict subsequent progression. All the available studies are prospective, but since study treatments and end-points vary between studies, no definitive conclusions on the application of NETest in this clinical context can be deduced.

Is NETest Able to Evaluate Tumor Recurrence Risk after Surgery?

In a study on 35 GEP-NENs (only 1 G3), in which 27 patients underwent surgical resection and 8 embolization [35], resection was associated to a decrease in the NETest score (from $80 \pm 5\%$ to $29 \pm 5\%$, $p < 0.0001$). The study included NETest evaluation 1 month after treatment and radiological evaluation 3 and 6 months after treatment. Interestingly, considering the surgical cohort, authors compared 15 R0 patients with 12 R1 patients. In the group with radical surgical resection, the reduction in NETest score was more marked (R0: from $80 \pm 6.3\%$ to $28.9 \pm$

5.5% ; R1: $79.5 \pm 8.5\%$ to $47.2 \pm 9.9\%$). Moreover, the same authors reported that 12 patients with R0 and no clinical or radiologic evidence of disease recurrence after 5 years showed a maximum post-operative NETest score of 14% (negative score), while in other cases the NETest score showed a moderate disease activity (>20%). This is consistent with the finding that 4 of 11 R0 patients with increased NETest at 1 month developed subsequent positive imaging (sensitivity 100%, specificity 20%) [35].

In a study on 13 small intestine NETs, surgery determined a significant lowering of NETest score. However, a significant reduction was associated to curative surgery (NETest 20–40%, classified as low), while, in case of resection in the setting of metastatic disease, the reduction was lower; in particular in case of PD after surgery, the NETest score was above the levels of 40% (over 60% in 3/5 cases) [47]. Similarly, in a study reporting NETest score before and 6 months after a combined treatment with both surgical intervention and PRRT in 9 patients affected by metastatic small intestinal NETs, authors reported a reduction in NETest score after treatment (from $83 \pm 12\%$ to $34 \pm 15\%$) and identified 40% as post-treatment cut-off for detecting SD [48].

Genc et al. [49] analyzed the role of NETest, performed after surgery, in predicting recurrence of pancreatic NETs (G1 and G2), identifying a cut-off of 40% (false-positive or false-negative patients were 18%). Interestingly, NETest was higher in patients with recurrence (R0 at histology) than in patients diagnosed as R1, but without clinical recurrence [49].

Partelli et al. [50] evaluated NETest before and after surgery on 30 patients affected by pancreatic NENs. Beyond the efficacy of NETest in diagnosing NETs before surgery, after surgery all patients demonstrated a decrease in NETest levels without differences in each time evaluated (post-operative day, POD 1, 5, and 30). Interestingly, among 3 patients showing levels of NETest >40%, 2 of them had R1 resection, and 1 had potentially nodal involvement. Among the remaining 15 patients, 12 exhibited a mean NETest level of 27% after resection (POD30), which is consistent with the presence of residual disease; even if the follow-up is not enough for identify recurrence, it is possible to hypothesize that the NETest score is able to identify patients at risk for recurrence [50]. In another study on gastric NENs, 5 patients underwent total gastrectomy and 8 subjects underwent partial gastrectomy. Despite all patients were disease free at endoscopic, radiological, and functional imaging, the NETest score was elevated in 6 patients, suggesting that these patients could have minimal residual disease. According

Table 2. Articles evaluating the role of NETest in predicting tumor disease progression and tumor response to treatment

First author [ref]	Study designPatients, N	NENS localization (n)	Histology and grading	Mean age (range min-max)	Sex	Functionality status (NF/F)	Treatment before study entry	Time of NETest	Role of NETest as predictor of progression and treatment response	Quality assessment (MINORS)
Cwikla et al. [36]	Prospective cohort study	63	G1 12; G2 16	60 (36–81)	M 10 F 18	N/A	Surgery 25; SSA 28; PRRT 11; CHT 5; TACE 1	Basal; T1: every 4 weeks during SSA treatment	Subsequent SD → baseline NETest 41 ± 2% Subsequent PD → baseline NETest 57.5 ± 2% (if > 80%: subsequent PD in 100% of patients) Mean NETest during SSA treatment > 80% → non-responders to SSA	9
Bodei et al. [44]	Prospective cohort study	54	G1 6; G2 20; G3 3; N/A 6	66 (43–83)	M 37 F 17	NF 33 F 21	Surgery 32; SSA 44; CHT 21; everolimus 5; sunitinib 1; interferon-α 1; PRRT 16; RT 6; TACE 4	Basal; pre PRRT; T1: NETest (basal-T1): negative in 11 + 6 months' follow-up	Basal: pre PRRT; T1: NETest (basal-T1): negative in 11 + 6 months' follow-up 88% of responders; null or positive in 90% of non-responders	11
Pavel et al. [37]	Prospective cohort study	34	G1 17; G2 14; G3 1; N/A 2	60.2 (43–83)	M 17 F 17	N/A	None 14; SSA 16; SSA + everolimus 1; streptozotocin/5-fu 3	Basal	Basal NETest < 40% → PFS 2.78 years Basal NETest > 80% → PFS 0.68 years Basal NETest > 40% predicts subsequent PD in 7/7 pts (100%)	12
Peczowska et al. [29]	Prospective cohort study	32	Localized 18; multicentric 7; metastatic 4	34 (12–62)	M 17 F 15	NF 17 F 15	Surgery 25; embolization 5; PRRT 5; EBRT 3; brachytherapy 1	Basal; T1: +2/12 months' follow-up (in 9 pts)	Basal NETest > 53% was associated to disease progression NETest score variations during follow-up were associated to disease status	5
Liu et al. [39]	Prospective cohort study	100	N/A				Basal		Basal NETest < 40% → PFS 12 months Basal NETest > 80% → PFS 3 months < 40% → responders to SSA > 80% → non-responders to SSA	8

Table 2 (continued)

First author [ref]	Study design	Patients, N	NENS localization (n)	Histology and grading	Mean age (range min-max)	Sex	Functionality status (NF/F)	Treatment before study	Time of NETest	Role of NETest as predictor of progression and treatment response	Quality assessment (MINORS)
van Treijen et al. [45]	Prospective cohort study	152	GEP	G1 105; G2 44; G3 2; N/A 1	53 (25–81)	M 82 F 70	N/A	N/A	Basal: pretherapy	All patients: basal NETest <33% → PFS 55 months Basal NETest 34–79% → PFS 18 months Basal NETest >80% → PFS 11 months Watchful waiting group (88) Basal NETest <33% → PFS 54 months Basal NETest >80% → PFS 12 months Treatment group (64) Basal NETest <33% → PFS not reached Basal NETest >80% → PFS 11 months Free of disease at baseline (33) NETest <20%: 0% recurrence	13
Bodei et al. [46]	Prospective cohort study	122	GEP (N/A) Lung (N/A)	N/A N/A	N/A	N/A	N/A	PRRT: 122 (83 responders + 39 nonresponders)	Basal; T1: follow-up (9–22 months after PRRT)	Post-treatment NETest <40% → responders to PRRT Post-treatment NETest >40% → non-responders to PRRT	12

N/A, not available; SSA, somatostatin analogues; PFS, progression-free survival; PD, progressive disease; GEP, gastroenteropancreatic; PRRT, peptide receptor radionuclide therapy; CHT, chemotherapy; TACE, transcatheter arterial chemoembolization; SD, stable disease; PPGLs, pheochromocytomas/paragangliomas; NF, nonfunctioning; F, functioning; EBRT, external beam radiation therapy; RT, radiotherapy; TC, typical carcinoid; AC, atypical carcinoid.

Table 3. Articles evaluating the role of NETest in predicting tumor recurrence after surgery

First author [ref.]	Study design	Patients, N	NENs localization (n)	Histology and grading	Mean age (range, min-max)	Sex	Functionality status (NF/F)	Type of surgery/resection	Time of NETest	Role of NETest as predictor of recurrence after surgery (cut-off for minimal residual disease)	Quality assessment (MINORS)
Filosso et al. [38]	Retrospective and prospective cohort study	226	Lung (set 2, surgical cohort: 19)	TC; AC; LCNEC	62 (34–82)	M 8 F 11	N/A	Lung surgery 19	Basal: before surgery; T1: +30 days surgery	Mean Δ NETest: –59% (95% RO at histological examination)	12
Modlin et al. [35]	Prospective cohort study	35	GEP (35)	G1 27; G2 7; G3 1	55.7 (33–80)	M 14 F 21	N/A	Surgery R0 15; surgery R1 12; ablative resection 8	Basal: pre-surgery; T1: +1 month surgery	Cut-off for minimal residual disease 14%	9
Laskaratos et al. [47]	Prospective cohort study	13	Small intestine (13)	G1 8; G2 5	64 (48–79)	M 10 F 3	N/A	Surgery 13	Basal: pre-surgery; T1: follow up post-surgery (median 22 months)	Cut-off for minimal residual disease 40%	8
Genc et al. [49]	Prospective cohort study	35	R0 with no recurrence pancreas (11) R0 with recurrence pancreas (12) R1 with no recurrence pancreas (12)	G1 8; G2 3 G1 5; G2 7 G1 12	63 (59–65) 62.5 (61–64) 57 (50–59.5)	M 4 F 7 M 6 F 6 M 6 F 7 F 5	NF 11 F 0 NF 11 F 1	Surgery 35	After surgery	Cut-off for minimal residual disease 40%	10
Partelli et al. [50]	Prospective cohort study	30	Pancreas (30)	G1 12; G2 17; G3 1	54 (40.6–67.4)	M 11 F 19	NF 22 F 8	Surgery 30	Basal: pre-surgery; T1: POD30	Cutoff for minimal residual disease 27%	9
Malczewska et al. [51]	Retrospective cohort study	46	Stomach	G1 32; G2 10; G3 1; NEC 3	55 (28–84)	M 13 F 33	NF 46 F 0	Polypectomy 24; partial gastrectomy 8; total gastrectomy 5; none 1	Partial T1: post-surgery	Cut-off for minimal residual disease 20% NETest in image negative R1 patients: 28±9% (100% >20%) NETest in image negative R0 patients: 16±11% ($p = 0.02$)	12
Modlin et al. [52]	Prospective cohort study	153	Pancreas (57) Small intestine (62) Lung (27) Duodenum (4) Stomach (3)	G1:29; G2: 27; NET G3:1 G1: 40; G2: 22 TC: 17; AC: 10 G1: 3; G2: 1 G1:2; NET G3: 1	58 (19–84)	M 74 F 79	NA	Surgery 153	T0: pre-surgery; T1: POD1; T2: POD30	Cut-off for minimal residual disease 20% (sensitivity: 100%; specificity: 92%, AUC: 0.97±0.02, $p > 0.0001$) R0 cohort (102): 25/31 with POD30 NETest >20% developed recurrence	14
Frilling et al. [48]	Prospectively cohort study	39	Small intestine (39)	G1:30; G2: 9	58.8 (32–78)	M 24 F 15	NF 14 F 25	Segmental resection(s) 32; right hemicolectomy 7 + PRRT	T0: pre-surgery; T1: after 6 months from PRRT	T0: NETest 83±12 T1: NETest 34±15 Cut-off for stable disease: 40% (after treatment)	10

LCNEC, large cell neuroendocrine carcinoma; N/A, not available; GEP, gastroenteropancreatic; NF, nonfunctioning; F, functioning; POD, postoperative day; SSA, somatostatin analogues; CHT, chemotherapy; PRRT, peptide receptor radionuclide therapy; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; TC, typical carcinoid; AC, atypical carcinoid.

to this hypothesis, the same study demonstrated that in case of microscopical residual disease evidenced at histological examinations (5 patients with positive polypectomy margin and 4 patients with random biopsies diagnostic for microscopic tumor), NETest was elevated ($28 \pm 9\%$), demonstrating the accuracy of this diagnostic instrument in detecting minimal residual disease before morpho-functional imaging [51].

In a recent study, Modlin et al. [52] evaluated NETest pre-operatively, on POD1 and POD30 in a cohort of 153 patients affected by surgically treated pulmonary and GEP NETs. In the R0 cohort, POD30 NETest levels decreased but remained elevated ($<20\%$) in 31 patients, and 25/31 patients with a POD30 NETest $>20\%$ developed image-identifiable recurrence by the following 18 months. However, all the patients with POD30 NETest values $<20\%$ were free from recurrence during follow-up. The authors demonstrated that a NETest value $>20\%$ on POD30 predicted residual disease with 94% accuracy and 100% sensitivity [52].

Finally, the role of NETest in evaluating the efficacy of surgical excision has been confirmed also in pulmonary NEN. In the study by Filosso et al. [38], in a subgroup of 19 patients with pulmonary NEN (12 typical carcinoids, 4 atypical carcinoids, and 3 large-cell NECs), the NETest score significantly decreased 30 days after surgery (from $69 \pm 28\%$ to $29 \pm 9\%$; mean reduction from baseline -59%). This reduction was present only in the cohort of NENs (differently from what observed in patients affected by other non-neuroendocrine pulmonary cancers, also enrolled in the study); 95% of surgically treated patients were R0 at histological examination, but no data on longer follow-up or radiological examinations were provided [38].

The 6 prospective and 2 retrospective studies reporting data on NETest after surgical intervention are summarized in Table 3. In conclusion, NETest values between 20 and 40% can identify minimal residual disease in patients after apparently radical surgery. In this view, patients with negative NETest could need less close follow-up, reducing the number of total body imaging, nuclear or conventional, with clear advantages not only from an economic point of view but also for the lower exposure to radiation [52], in a personalized medicine perspective [53].

Future: Beyond NETest

In addition to the diagnostic and prognostic role of NETest, the evaluation of tumor transcription may provide additional information. In a study on 20 patients affected by small intestine NETs, a specific subgroup of profibrotic circulating transcripts, the “Fibrosome” of the

NETest was able to predict mesenteric fibrosis in 100% of cases, even when conventional radiology was negative, providing important information to the surgeon [54]. In this sense, NETest, as other liquid biopsies, may be considered a window on the tumor, providing different types of information. Moreover, the research on NETest is continuing to develop. In 2020, Kidd et al. [55] evaluated the expression of NET-omes and their combinations in a cohort of 88 patients affected by G1 and G2 GEP-NETs, with the aim to identify pathologically relevant -omes for defining of disease status, and to investigate if these elements could provide added prognostic information to the “classical” NETest score. Scores were assessed at baseline and after a median follow-up of 9 months. Four NET-omes among those analyzed showed a prognostic value, defined as the correlation between basal levels and outcome (Prognosome: Metastasome, Epigenome, Fibrosome, and NEDome). Then, the authors further investigated the prognostic role of NETest integrated with Prognosome levels. They found that the association at baseline between a low NETest score (<40) and Prognosome levels below the upper limit of normal was an accurate prognostic factor for SD during follow-up (90%); on the contrary, a high NETest score ($>40\%$) associated with Prognosome levels above the upper limit of normal predicted PD within 3 months (100%). Integrating the 4 -omes with the NETest score (using 40% as a cut-off) generated an overall prognostic accuracy of 93%, significantly better than the prognostic value of either the NETest alone (70.5%) or the -omic analysis as a separate approach (69%) [55].

Conclusions

Prognostic Assessment by NETest: Ready for Clinical Application?

Prognostic markers in the field of NENs are lacking and necessary. NETest is the most characterized and validated application of the liquid biopsy to the field of NENs, reported as a key diagnostic advantage, and a promising tool for clinical practice [56]. Beyond its diagnostic value it is also a prognostic tool. In fact, a recent meta-analysis considering data from 6 studies estimated the accuracy of NETest of 84.5–85.5% in differentiating SD from PD [20]. By this systematic review, we tried to answer specific questions, which can be useful for clinical practice, as summarized in Figure 2. The 2 fields with more available data on the prognostic value of NETest are: the ability of NETest in differentiating SD from PD at baseline and the capacity to predict surgical treatment

Fig. 2. Possible clinical applications of NETest. Summary of questions and answers debated in the systematic review.

outcome. In particular, high levels of NETest (at least >40%) identify PD. Consequently, NETest could be useful in all situations in which it is important to identify progression, as, for example, in case of new diagnosis of NEN, when it is not possible to establish disease course because previous imaging is clearly unavailable. High baseline NETest levels predict also a subsequent progression, even in case of SSA treatment. These patients should be probably evaluated frequently and treated in a more aggressive way, even if data seem not enough strength for applying this indication to clinical practice.

Finally, given the possibility to assist to a recurrence after an apparently radical surgical procedure, another application of NETest can be the identification of minimal residual disease. Probably, only negative value of NETest (<20%), defined as values found in healthy controls [31], correctly identified radical surgical treatment. In this sense, NETest can evaluate patients at high risk of recurrence who could benefit from a more frequent radiological examination or an adjuvant approach. In the authors' opinion, the introduction of NETest in clinical practice should derive from this last application: to identify pa-

tients at high risk of recurrence despite the radical surgery, confirmed by no tumor infiltration in the resection margins at histological examination. In this setting, NETest seems not only informative but also able to reduce medical costs through the possibility to reduce the number of morphological and functional imaging during follow-up.

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Statement of Ethics

An ethics statement is not applicable because this study is based exclusively on published literature.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

G.P. and V.D.V. wrote the original draft and performed data collection, data analysis, and interpretation; T.F., F.S., R.C., C.P., and M.G.T. performed data analysis and interpretation; M.V., A.L., and A.M.I. provided the critical revision of the article; E.G. supervised the findings of this work and provided the critical revision of the article; A.F. provided conceptualization and the critical revision of the article.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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