Beyond Chromogranin A: Molecular Surveillance with NETest2.0® Improves Detection of Neuroendocrine Tumor Progression

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Abstract

Background: Reliable biomarkers for longitudinal surveillance of neuroendocrine tumors (NETs) remain an unmet clinical need. Chromogranin A (CgA), historically the most widely used circulating biomarker, has limited sensitivity and often correlates poorly with disease progression. We evaluated whether serial molecular assessment using NETest2.0®, a blood-based multigene transcriptomic liquid biopsy, more accurately identifies progression during routine NET surveillance.
Methods: Patients enrolled in RegisterNET (NCT02270567) with paired blood samples and contemporaneous imaging assessments were analyzed. NETest2.0® scores were generated from a 51-gene RT-PCR transcript signature and compared with serial serum CgA measurements. Disease status at follow-up was determined by CT, MRI, and/or SSTR-PET imaging with RECIST 1.1 criteria applied when appropriate. Longitudinal changes between sequential samples were assessed using predefined NETest2.0® thresholds (>0% and >5%) and compared with the conventional CgA surveillance threshold (>50%).
Results: A total of 191 patients were evaluated, with a median surveillance interval of 6 months. Exploratory analysis demonstrated markedly superior discrimination for progression using serial NETest2.0® changes compared with CgA changes (AUC 0.893 vs. 0.538; p<0.0001). In the primary surveillance analysis, NETest2.0® >0% achieved an AUC of 0.860 and NETest2.0® >5% an AUC of 0.822, both significantly superior to CgA (AUC 0.553; p<0.0001 for both comparisons). NETest2.0® >0% demonstrated the highest sensitivity (86.4%), while NETest2.0® >5% provided the optimal balance of performance with 70.5% sensitivity, 93.9% specificity, 77.5% positive predictive value, 91.4% negative predictive value, and 88.5% overall accuracy. In contrast, CgA demonstrated only 18.2% sensitivity and 75.4% overall accuracy. Multivariable and logistic regression analyses identified changes in NETest2.0® as the strongest independent predictor of progression (all p<0.0001; OR 52.99–61.26), whereas serial CgA changes were not independently associated with progression.
Conclusions: Serial NETest2.0® assessment significantly outperformed Chromogranin A for identifying progression during NET surveillance. Molecular changes measured by NETest2.0® were strongly associated with clinical outcome and represented the dominant independent predictor of progression. These findings support NETest2.0® as a molecular surveillance biomarker that may facilitate biologically informed follow-up and risk-adapted imaging strategies in patients with NETs.

Background

Monitoring strategies for GEN-NEN rely principally on cross-sectional or functional imaging which are indispensable but have recognized limitations and on blood biomarkers such as chromogranin A (CgA).
The clinical utility of CgA is, however, constrained by poor sensitivity, substantial intra- and inter-individual variability, non-standardized assay platforms, and multiple false-positive causes including proton-pump inhibitor therapy, renal dysfunction, and inflammatory states. Importantly, CgA often correlates poorly with tumor burden or treatment response.
NETest2.0® is a blood-based, machine-learning-enhanced multigene transcript assay that measures a 51-gene neuroendocrine-specific expression signature in whole blood. The assay is designed as a liquid biopsy, capturing circulating tumor-associated transcripts and generating a standardized 0–100 score with a validated positivity threshold of 50.

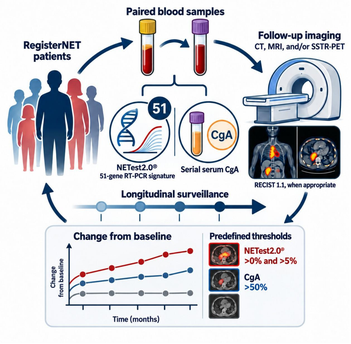
Hypothesis

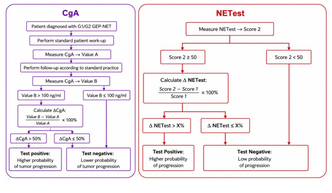
NETest2.0® may perform better than CgA for tumor monitoring as it measures disease-associated transcriptomic activity rather than levels of a secreted protein.

Study Design and Approach

One hundred and ninety-one patients enrolled in RegisterNET (NCT02270567) with paired blood samples and contemporaneous imaging assessments were analyzed (**Figure 1**).

NETest2.0® scores were generated from a 51-gene RT-PCR transcript signature and compared with serial serum CgA measurements. Disease status at follow-up was determined by CT, MRI, and/or SSTR-PET imaging with RECIST 1.1 criteria applied when appropriate.





Longitudinal changes between sequential samples were assessed using predefined NETest2.0® thresholds (>0% and >5%) and compared with the conventional CgA surveillance threshold (>50%) (**Figure 2**).

Results

Characteristic	Value
Registry / design	RegisterNET (NCT02270567); registry-based surveillance study
Cohort	191 patients with paired blood samples
Sampling	Two serial blood draws; imaging performed at second timepoint
Reference standard	Imaging and clinical adjudication (RECIST 1.1 where applicable)
Index / comparator	NETest2.0® 2.0 versus serum chromogranin A
NETest2.0® threshold	Score ≥50 at second timepoint
Thresholds	NETest2.0® >0%, >5% versus CgA >50%
Primary endpoint	Progressive versus non-progressive disease

A total of 191 patients contributed paired blood samples and paired clinical assessments. The median (range) age was 60 years (19-88 years) with a gender ratio of 109:82 (Male:Female). One hundred (52.5%) were G1, 79 (41.4%) were G2 and 12 (6.1%) were G3. One hundred and thirty (68.1%) were lymph node positive; and 84 (44%) had liver disease.

All analyses were performed on matched sampling intervals with contemporaneous imaging at the second timepoint. The median timepoint between samples was 6 months (range: 1-12 months).

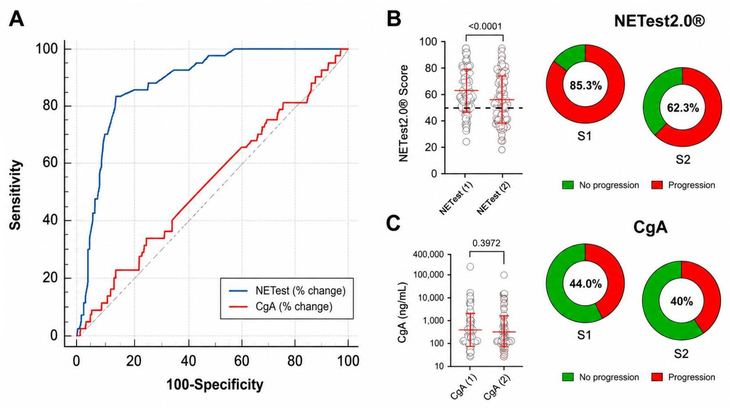
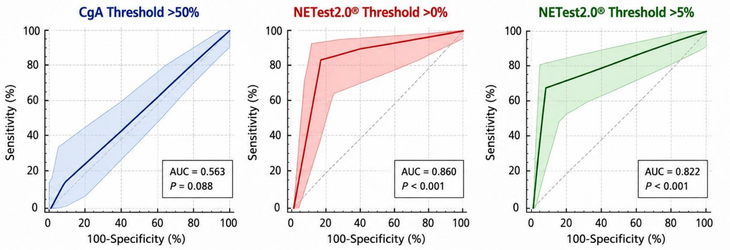


Figure 3. AUC for changes in results between the two sample times. The percentage positive is included. Mean±SD. Dotted black line is upper limit of normal for NETest2.0®..



Threshold	PD	SD	Total
>50%	8	11	19
≤50%	36	136	172
Total	44	147	191

Threshold	PD	SD	Total
>0%	38	21	59
≤0%	6	126	132
Total	44	147	191

Threshold	PD	SD	Total
>5%	31	9	40
≤5%	13	138	151
Total	44	147	191

PD, progressive disease; SD, stable disease; AUC, area under the ROC curve.

Figure 4. Head-to-head comparisons for CgA threshold vs. two different NETest thresholds using 3-point AUC analysis. 2x2 tables for each of the approaches/thresholds are included below.

ROC performance of CgA and NETest2.0® monitoring strategies

Analysis	AUC	SE	95% CI	Δ AUC vs. CgA (95% CI)	P value
ΔCgA (exploratory)*	0.538	0.0503	0.465-0.610	Reference	—
ΔNETest2.0® (exploratory)*	0.893	0.0249	0.840-0.933	0.355 (0.245-0.464)	<0.0001
CgA T>50% (primary)**	0.553	0.0314	0.480-0.625	Reference	—
NETest2.0® T>0%**	0.860	0.0299	0.803-0.906	0.307 (0.217 to 0.397)	<0.0001
NETest2.0® T>5%**	0.822	0.0362	0.760-0.873	0.268 (0.176 to 0.360)	<0.0001

Operating characteristics of CgA and NETest2.0® thresholds

Threshold	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
CgA TΔ: >50%	18.18	92.52	42.11	79.07	75.39
NETest2.0® TΔ: >0%	86.36	85.71	64.41	95.45	85.86
NETest2.0® TΔ >5%	70.45	93.88	77.50	91.39	88.48

Serial NETest2.0® assessment significantly outperformed Chromogranin A for identifying progression during NET surveillance. Molecular changes measured by NETest2.0® were strongly associated with clinical outcome and was an independent predictor of progression.

CONCLUSION

NETest2.0® is a molecular surveillance biomarker that may facilitate biologically informed follow-up and risk-adapted imaging strategies in NETs.

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