A MULTIANALYTE ALGORITHM PCR-BASED BLOOD TEST OUTPERFORMS SINGLE ANALYTE ELISA-BASED BLOOD TESTS FOR NEUROENDOCRINE TUMOR DETECTION

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ABSTRACT

A key issue in management of neuroendocrine tumors (NETs) is the specific and sensitive biomarkers. Measurements of single analytes in blood are widely utilized but have significant limitations. We developed a 51 transcript blood net signature and compared it with standard approaches. The multianalyte signature was evaluated in prospectively collected NETs (n=41; 61 small intestinal, 50 metastatic, 44% under treatment). These were age (NETs: mean 56.9 years, range: 31-76; controls: mean 56.4, range: 33-75) matched (MF:10:31) with controls. Samples were analyzed by 2-step PCR protocol and ELISAs: (DAKOD-CAg, pancreastatin (Cusabio), and neurokinin A (RayBiotech-NKA). Sensitivity comparisons included chi-square, non-parametric measurements and ROC analyses. The NETest identified thirty eight of 41 NETs with equivalent performance metrics: sensitivity/specificity 93% and an AUC of 0.96. For the single analyte ELISAs, metrics ranged from 0.53-0.95 and AUCs from 0.55-0.67. The multianalyte NETest significantly outperformed single analyzed transcripts (2-Statistic=c=4.85-6.58, p<0.0001).

CONCLUSIONS

1. The multi-transcript molecular signature is both sensitive and specific (>90%) for the detection of neuroendocrine tumor disease.
2. The PCR test is robust and significantly more sensitive and specific (accurate) (p<0.0001) than currently used single analytes including Chromogranin A, pancreastatin and neurokinin A.

CRITICAL ISSUE

A key issue in management of neuroendocrine tumors (NETs) is specific and sensitive biomarkers. Measurements of single analytes in blood are widely utilized but have significant limitations.

HYPOTHESIS

A multianalyte test will provide increased sensitivity and specificity for the detection of neuroendocrine tumors.

BACKGROUND

•Gastroenteropancreatic neuroendocrine tumors (GF-NEPNT) are common (incidence: 2/100,000), occurring as frequently as testicular tumors, Hodgkin’s disease, glomas and multiple myeloma and are estimated to have a prevalence of 35/100,000 [3].
•They represent a heterogeneous group of cancers both in terms of tumor biology and the variety of bioactive products they synthesize and secrete, and exhibit a range of different behaviors (paraneoplastic and/or metastatic) which reflects the diverse cells (and sites) of origin.
•There is a paucity of effective therapies as well as accurate tools to assess response and metastasis.

METHODS

All peripheral blood samples (5ml, EDTA tube) were collected and analyzed according an IRB protocol (Yale University School of Medicine). The protocol was specifically approved for this study. Matched cases and controls: We prospectively collected NET patients (Sept-Dec 2013) and controls, matching 41 cases with a control (1:1) by sex and age to within 2 years. The ethnicity was exclusively Caucasian. There were no differences in sex distribution: M = 10:13, both groups) or age between the two groups (NET: mean 56.9, range: 31-76; controls: mean 56.4, range: 33-75) confirming appropriateness of matching. Multianalyte Assay (Whole blood samples) We prospectively collected NET patients (Sept-Dec 2013) and controls, matching 41 cases with a control (1:1) by sex and age to within 2 years. The ethnicity was exclusively Caucasian. There were no differences in sex distribution: M = 10:13, both groups) or age between the two groups (NET: mean 56.9, range: 31-76; controls: mean 56.4, range: 33-75) confirming appropriateness of matching. Multianalyte Assay (Whole blood samples) We prospectively collected NET patients (Sept-Dec 2013) and controls, matching 41 cases with a control (1:1) by sex and age to within 2 years. The ethnicity was exclusively Caucasian. There were no differences in sex distribution: M = 10:13, both groups) or age between the two groups (NET: mean 56.9, range: 31-76; controls: mean 56.4, range: 33-75) confirming appropriateness of matching.

RESULTS

NETEST PERFORMANCE METRICS

Sensitivity = 93% Specificity = 93%

PPV = 93% NPV = 93%

ACKNOWLEDGEMENTS

This study was funded by Clifton Life Sciences

REFERENCE


Table 2: Pairwise comparison of ROC curves

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MAA</th>
<th>NET</th>
<th>Controls</th>
<th>p-value</th>
<th>AUC 95% CI</th>
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<tr>
<td>CgA</td>
<td>0.00</td>
<td>0.01</td>
<td>0.02</td>
<td>0.0002</td>
<td>0.63 [0.51, 0.74]</td>
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<tr>
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<td>0.63 [0.51, 0.74]</td>
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Table 1: Performance Metrics

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