Biomarkers in Neuroendocrine tumors

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Overview

- Circulating biomarkers
  - Site specific markers
  - General tumor markers

- Tissue markers
  - mTOR pathway
  - Angiogenic factors
  - SSTR profiles

- New developments
  - microRNAs
  - circulating gene transcripts
  - circulating tumor cells
Clinical application of Neuroendocrine Biomarkers

- Tumor Screening
- Diagnosis
- Prognostication
- Tumor follow-up
Markers of Neuroendocrine Cells

- **Synaptophysin**: Small synaptic vesicles
- **Chromogranin A**: Membrane protein of neurosecretory granules
- **Peptide hormone**: In neurosecretory granule

Secreted into the circulation
Circulating biomarkers in NET

Specific tumor markers

General tumor markers

5-HIAA = 5-hydroxy-3-indoleacetic acid
5-HT = serotonin
GHRH = gonadotropin hormone release hormone
hCG = human chorionic gonadotropin
ANP/BNP = atrial natriuretic peptide and brain/ventricular natriuretic peptide
NSE = neuron-specific enolase
PYY = peptide YY

Biomarkers in NET

**General Tumor markers:**
- Chromogranin A
- NSE
- Pancreatic Polypeptide
- α-Subunit, β-hCG

**Specific NET Tumor markers:**
- Serotonin, 5-HIAA
- Gastrin
- Insulin
- Glucagon, VIP, Somatostatin
- Catecholamines
- Calcitonin
- PTHrP, ACTH, GHRH…..
Circulating Chromogranin A

- Diagnostic value
- Prognostic value
- Predictive value
- Methodological aspects
Chromogranin Family

Granins
Chromogranin A, B; Secretogranin II-VI
acidic, soluble secretory proteins
co-secreted with hormones /amines

Gene structures of the Granin-Family
Chromogranin A
Gene and Peptide structure

Chromosome 14
431-445 Amino acids
MW 49 kd

Proteolytic Fragments:
- Chromacin (bacteriolytic, antifungal)
- Catestatin (inhibits catecholamine secretion)
- Vasostatin I,II (inhibits vasoconstriction & PTH-Secretion, stimulates fibroblast adhesion)
- Pancreastatin (inhibits Insulin secretion)
- Parastatin (inhibits PTH secretion)

Taupenot et al NEJM 2003 348:1134
Chromogranin A Diagnostic value

- **Sensitivity:**
  - **Gastrinoma** (100%)
  - **Gastric NET (type I, II, and III)**; > 95%
  - **Ileal NET** (~ 80%)
  - **Non-functional pNET** (~ 70%)

Chromogranin A and Chromogranin B for diagnosis of NET

Chromogranins A and B levels in patients with different neuroendocrine tumours compared with healthy individuals.

Öberg et al, Endocr Rel Cancer 2011
CgA in relation to tumor mass

Öberg et al, Endocr Rel Cancer 2011
Chromogranin A in relation to tumor grade

Sensitivity and Specificity of CgA in the detection of NET: Comparison to other biomarkers

<table>
<thead>
<tr>
<th>Study</th>
<th>NET Group</th>
<th>Comparison Group</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSE</td>
<td>33</td>
<td>100</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Urine 5HIAA</td>
<td>35</td>
<td>100</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CEA</td>
<td>15</td>
<td>91</td>
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<tr>
<td>Campana et al[^152]</td>
<td>n = 238 GEP-NETs</td>
<td>N = 42 CAG N = 48 disease-free patients</td>
<td>CgA vs disease free</td>
<td>85</td>
<td>96</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CgA vs CAG and disease free</td>
<td>75</td>
<td>84</td>
</tr>
<tr>
<td>Nobels et al[^107]</td>
<td>n = 211 NETs</td>
<td>n = 180 non-NET cancers</td>
<td>CgA[^b]</td>
<td>53</td>
<td>93</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>NSE</td>
<td>46</td>
<td>65</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>α-SU</td>
<td>26</td>
<td>85</td>
</tr>
<tr>
<td>Cimitan et al[^69]</td>
<td>N = 63 Lung and GEP-NETs</td>
<td>No control</td>
<td>CgA[^a]</td>
<td>55</td>
<td>94</td>
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<td></td>
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<td>SRS[^c]</td>
<td>77</td>
<td></td>
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<tr>
<td>Namwongprom et al[^106]</td>
<td>N = 125 NET</td>
<td>No control</td>
<td>CgA[^d]</td>
<td>62</td>
<td>84</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>SRS[^e]</td>
<td>83</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CgA and SRS</td>
<td>93</td>
<td>81</td>
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</tbody>
</table>

[^e]: CgA performs better than other biochemical markers in NET.
Limitations
Causes of Chromogranin A elevation

Chronic atrophic Gastritis
- Pheochromocytoma
- Hyperparathyroidism
- Pituitary Tumors
- Medullary Thyroid Carcinoma
- Hyperthyroidism
- Small cell lung cancer
- Prostate cancer
- Breast cancer
- Ovary Cancer
- Arterial Hypertension
- Cardiac insufficiency
- Acute coronary syndrome
- Giant cell arteritis

ENDOCRINE DISEASE
- Chronic atrophic gastritis
- Pancreatitis
- Inflammatory bowel disease
- Irritable bowel syndrome
- Liver cirrhosis
- Chronic hepatitis
- Colon cancer
- HCC
- Pancreatic Adenocarcinoma

NON-GI CANCER

GASTRO-INTESTINAL DISORDERS

CARDIOVASCULAR DISEASE

RENAI DISORDERS

INFLAMMATORY DISEASES

DRUGS
- PPI
- H2RAs

Modlin et al, Ann Surg Oncol 2010
CgA: Sensitivity and specificity

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
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<tbody>
<tr>
<td>CIS RIA</td>
<td>67</td>
<td>96</td>
<td>97</td>
<td>63</td>
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<tr>
<td>DAKO ELISA</td>
<td>85</td>
<td>85</td>
<td>91</td>
<td>75</td>
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<tr>
<td>ED RIA</td>
<td>93</td>
<td>88</td>
<td>93</td>
<td>88</td>
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</tbody>
</table>

CgA Assays are not comparable

Reference range in serum:

secretory protein
High concentrations in serum
Normal reference range depending on Assay and Controls
Significant differences for specificity and determination of the peptide or fragments

<table>
<thead>
<tr>
<th>Table 1 assessed</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>s.d.</td>
</tr>
<tr>
<td>No. Mean</td>
<td>31.2</td>
<td>10.1</td>
</tr>
<tr>
<td>Median</td>
<td>40.0</td>
<td>10.4</td>
</tr>
<tr>
<td>Range</td>
<td>17–269</td>
<td>5–106.5</td>
</tr>
<tr>
<td>Percentile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5th</td>
<td>21.0</td>
<td>6.2</td>
</tr>
<tr>
<td>25th</td>
<td>31.0</td>
<td>8.0</td>
</tr>
<tr>
<td>50th</td>
<td>40.0</td>
<td>10.4</td>
</tr>
<tr>
<td>75th</td>
<td>54.0</td>
<td>13.7</td>
</tr>
<tr>
<td>95th</td>
<td>86.0</td>
<td>19.3</td>
</tr>
</tbody>
</table>

Standardization is needed!
Need for prognostic markers
Prognostic value of CgA

CgA correlates with hepatic tumor load
Higher CgA levels indicate lower survival

Predictor of disease progression and survival

RADIANT-1 study, n=115 with progressive pancreatic NET

A

PFS by baseline CgA

Censoring Times

- CgA > 2× ULN (n/N = 41/65)
- CgA ≤ 2× ULN (n/N = 20/49)

HR, 0.55
95% CI, 0.32-0.95
Kaplan-Meier medians
CgA > 2× ULN: 8.34 mo
CgA ≤ 2× ULN: 15.64 mo
2-sided log rank $P = 0.03064$

B

Overall survival by baseline CgA

Censoring Times

- CgA > 2× ULN (n/N = 37/65)
- CgA ≤ 2× ULN (n/N = 10/49)

HR, 0.30
95% CI, 0.15-0.61
Kaplan-Meier medians
CgA > 2× ULN: 16.95 mo
CgA ≤ 2× ULN: NA mo
2-sided log rank $P = 0.00043$

Yao et al, JCEM 2011
Response prediction
Predictive value of Chromogranin A

Response to Everolimus in pNET by early decrease of CgA

- Median PFS
  - Early response = 13.3 mos.
  - No early response = 7.5 mos.

- HR = 0.25
- 95% CI: 0.13-0.51
- p = 0.00004

Overall survival of patients by decrease of CgA to Octreotide

- > 30% decrease of CgA after octreotide test has a prognostic meaning → identification of subgroups most likely to be responsive to SSA therapy

Yao et al, J Clin Oncol 2010; Yao et al, JCEM 2011

Massironi et al, Am J Gastroenterol 2010
Indication of recurrence?
Chromogranin A indicates first disease recurrence in radically operated Midgut Carcinoid Tumors

Retrospective study in 56 patients

<table>
<thead>
<tr>
<th>First method that indicated recurrence</th>
<th>Patients</th>
<th>Median time until first indication of a recurrence</th>
<th>Median time until radiologically confirmed recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-CgA</td>
<td>22</td>
<td>6 months (2–148)</td>
<td>37 months (6–217)</td>
</tr>
<tr>
<td>P-CgA+radiology</td>
<td>2</td>
<td>53 months (10–96)</td>
<td>53 months (10–96)</td>
</tr>
<tr>
<td>P-CgA+U-5HIAA</td>
<td>3</td>
<td>5 months (3–8)</td>
<td>18 months (10–33)</td>
</tr>
<tr>
<td>P-CgA+radiology+U-5HIAA</td>
<td>1</td>
<td>21 months</td>
<td>21 months</td>
</tr>
<tr>
<td>U-5HIAA</td>
<td>2</td>
<td>37 months (2–72)</td>
<td>58 months (25–91)</td>
</tr>
<tr>
<td>Radiology</td>
<td>3</td>
<td>20 months (8–76)</td>
<td>35 months (20–80)</td>
</tr>
</tbody>
</table>

In the follow-up of patients Plasma CgA measurement should be performed first (+ ultrasonography) and unnecessary and costly examinations in asymptomatic patients avoided
Summary:
Advantages to measure CgA...

1) High sensitivity
2) Accessible & easy to measure
3) Identification of high risk patients
4) Data support responses
5) But needs to be standardized and not used as a screening marker
6) Useful in comparison to imaging
7) Helps in diagnostic decision making \(\rightarrow\) supports/avoids extended imaging
Diagnostic Value of NSE

- NSE associated with poorly differentiated NEC

- Sensitivity 63% in LCNEC (large cell NEC) and 62% in SCNEC (small cell NEC) respectively.

- Sensitivity: 19% in G1NET and 54% in G2NET

→ NSE might be of additional value to chromogranin A

Neuroendocrine Neoplasms (NEN)

- **Stage**
  - T1
  - T2
  - T3
  - T4
  - N1
  - M1

- **Grade**
  - G1
  - G2
  - G3

- **Histological Classification**
  - Well-differentiated Neuroendocrine Tumor (NET)
  - Moderately differentiated Neuroendocrine Tumor
  - Poorly differentiated Neuroendocrine Tumor

- **CgA**
  - Well and moderately differentiated Neuroendocrine Tumor
  - CgA elevated

- **NSE**
  - Poorly differentiated Neuroendocrine Carcinoma (NEC)
  - NSE elevated

- **Localized**
  - Lymph nodes involved

- **Regional**
  - Lymph nodes involved (with metastases)

- **Distant**
  - Metastases
Progastrin-Releasing Peptide (proGRP)-a novel marker in NEC G3

- In small cell NEC: Sensitivity of 73% at 95% specificity

- proGRP: complementary tumor marker for prognosis and treatment monitoring in patients with NEC, and eventually NET G2

- proGRP level of more than twice the upper reference value in patients with WDNETs is a strong indicator for a primary tumor in the lung.

Alkaline Phosphatase Predicts Survival in Patients with Metastatic Neuroendocrine Tumors

Retrospective analysis of 137 patients with metastatic neuroendocrine tumors

Alkaline phosphatase levels above normal were predictive of shorter survival in both univariate and multivariate analysis.
Novel approaches

- MicroRNAs
- Multiple Transcript Analysis in blood
- Circulating tumor cells
MicroRNA Testing in Neuroendocrine Tumors of the Gastrointestinal Tract

- family of small non-coding highly conserved single-stranded RNAs
- they are involved in the regulation of cell proliferation, differentiation, survival, and apoptosis
- dysregulation of microRNAs is a hallmark of cancer

Vincentini et al, Molecules 2014, 19, 2458-2468;
miR-21 is significantly overexpressed in pancreatic NET and it is detectable in patients’ plasma samples

By repressing pro-apoptotic genes (e.g. *PTEN* or *PDCD4*) miR21 stimulates proliferation and tumor initiation

Vincentini et al, Molecules 2014, 19, 2458-2468
Upregulated MiR-96,-182,-183,-196a & -200a in serum samples

miR-96, miR-182, miR-183, miR-196a, and miR-200a are shown to be upregulated in serum samples from different conditions: Healthy Donors (HD), Primary Tumors (PT), Lymph Node Metastases (LNM), and Liver Metastases (LM). The graphs illustrate the relative expression levels, with significance indicated by asterisks: * for p < 0.05, ** for p < 0.01, and *** for p < 0.001.

The table below lists the potential targets and functions associated with miR-196a:

- **miR-196a** targets HOXB7, LRP4, and RSPO2.
- Functions include regulation of transcription, DNA-dependent, Wnt signaling pathway, and Wnt receptor signaling pathway.

Courtesy Valeria Giandomenico, Uppsala
Downregulation of MiR-31,-129-5p,-133a & -215 in serum samples

*Courtesy Valeria Giandomenico, Uppsala*
Visualization of the GEP-NEN gene co-expression network (2545 genes)

Each node represents a gene, a link represents a GEP-NEN-specific co-expression.

Heatmap visualizing enrichment for over-represented Gene Ontology (GO) Biological Process (BP) terms assigned to the 10 largest clusters (20 genes).

Modlin et al ASCO 2013; PIOS One 2013
Gene signature:
Sensitivity und Specificity compared to CgA

Figure 5. Utility of the 51 marker gene signature for detecting P-NENs, metastases and in patients with low Chromogranin A (CgA).
A) The sensitivity and specificity of the test to detect GI-NENs (90%, 94%) and P-NENs (80%, 94%) was similar. B) The PCR-based approach could detect patients with no metastases as well as patients with metastases. C) The PCR-based test could accurately identify GEP-NENs even when plasma CgA were low (<19U/L). Overall, the PCR blood test was significantly more accurate than measurement of CgA levels to detect GEP-NENs (*p<10^{-13}, \chi^2>50).
Percentage increase in lesions on imaging grouped by the presence of CTCs

Each bar represents an individual case
Summary

- Site specific markers are measured depending on symptoms
- Early detection of NEN remains challenging
- CgA is the best available biomarker for diagnosis of NET
- In patients with G1/G2 NET and large cell NEC (LCNEC) the use of CgA is recommended
- CgA has prognostic and predictive value and is useful in monitoring of NET; CgB is an alternative biomarker
- In patients with small cell NEC (SCNEC) NSE and proGRP are of additional value and seem superior to CgA.
THANK YOU!