Chapter 3: Biochemical markers

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Practice Points

1. Serum Chromogranin A is the most established biomarker for diagnosis, monitoring progression or treatment response in patients with neuroendocrine neoplasms (NENs) and is more sensitive than Ur 5HIAA.
2. Serum Chromogranin A levels can be falsely elevated by various other medical conditions and by commonly used medications, for example proton pump inhibitors.
3. Serum Chromogranin A levels can vary across different laboratory assays and hence the same assay should be used for the one patient.
4. Ur 5HIAA is a highly specific marker in patients with metastatic NET with carcinoid syndrome in terms of disease bulk/risk of carcinoid crisis and may be predictive for the risk of cardiac valve disease.
5. Ur 5HIAA needs to be collected with strict dietary and medical restrictions and within an acidified collection bottle.
6. In patients with pancreatic NETs, hormone/s (such as insulin, gastrin, glucagon, VIP, etc) should only be measured where a functional syndrome is suspected.
7. Patients with large tumour load, high chromogranin A levels, or high urinary 5-HIAA values are more likely to experience a carcinoid crisis during surgery.
8. Frequency of biomarker measurement varies subject to the patient having fully resected or metastatic disease.

Introduction

Neuroendocrine neoplasms (NENs) frequently demonstrate elevation of one or more biochemical markers which may be used to follow the course of disease and response to therapy. Some of these markers may be associated with a syndrome due to hormone excess. Positive immunohistochemistry for a marker may not be associated with measurable hormonal overproduction and a syndrome. Hormone production may alter over the course of disease in terms of the absolute hormone level amount and the hormone type.[1, 2]

Commonly available markers are described. Consideration should be given to referring patients with functional pancreatic NETs (those patients with clinical hormone syndrome) to an endocrinology service.

1. Serum Chromogranin A (CgA)

Serum Chromogranin A (CgA) is the most established NEN marker for diagnosis and monitoring progression or treatment response.

CgA stabilises intracellular vesicles and regulates post-translational protein processing. It is elevated in between 60% and 100% of patients with NENs, including functioning and non-functioning midgut NETs and pancreatic NETs (functioning and non-functioning) and phaeochromocytomas. Specificity may be lower in midgut NETs. It is proportional to tumour size.

CgA is more reliable than Ur 5HIAA (urine 5-hydroxyindoleacetic acid) in terms of sensitivity and for detecting small recurrences in follow-up. CgA false positives may result from decreased renal function, atrophic gastritis, liver function impairment, hepatitis/cirrhosis, cardiac failure, inflammatory bowel disease, proton pump inhibitor (PPI) therapy, histamine type-2 receptor antagonists and other non-neuroendocrine neoplasms.

CgA may be useful to estimate prognosis, for early detection of recurrence post-treatment, to monitor response to therapy and possibly for monitoring for progression. It has much less utility in the initial diagnostic setting.[3, 4]. Its reliability is greater especially where its initial values were elevated. Correlations with response and progression may be lost during SSA therapy. CgA should be used in combination with imaging to accurately measure tumour bulk and response.

Hence CgA can be used for monitoring in the following circumstances:

- In patients with completely resected disease, for relapse. For example, if CgA is trending upwards (i.e. > 50% baseline outside normal range) and there are no likely causes of a false positive, further investigation should be conducted. Consider a CT scan and functional imaging with somatostatin receptor imaging (octreotide scan or Gallium-68 Dotatate PET scan).
- In a patient who has had metastatic disease treated, for the evaluation of response or progression.

CgA is not a measure of tumour bulk for gastrinomas, therefore other hormonal markers need to be measured. It is also not a useful measure for patients on proton pump inhibitors, and if appropriate, attempts should be made to measure it where this drug therapy can be interrupted.

Chromogranin A concentration may be normal in the case of neuroendocrine tumours with low proliferative potential, including those of the appendix, as well as the majority of insulinomas. Other sites include the lung (typical carcinoids), duodenum and rectum. Rapidly proliferating, poorly differentiated neuroendocrine tumours may also not release the marker, giving false negative results.[5]

Antibody-derived assays are variable and hence patient's samples should be measured in the same lab consistently. Given the potential inaccuracy of reports of high levels (with anecdotal reports of ±20% in same sample measured on two different occasions, but usual uncertainty of assay ±17%) the laboratory(s) should be asked for confidence intervals and interpatient variation in duplicate samples.

Note: Chromogranin B (CgB)

This assay is not currently available in Australia and NZ (but can be done in the UK). If available, it is preferred to CgA in patients with renal and hepatic impairment. Compared with chromogranin A, chromogranin B may be more useful during proton pump inhibitor treatment.[6]

2. Ur 5HIAA

Ur 5HIAA (urine 5-hydroxyindoleacetic acid) is a by-product of serotonin metabolism and hence serotonin producing tumours such as the classical midgut tumours and, rarely, NETs at other sites. It may be a marker for the risk of carcinoid cardiac disease.

About 95% of patients with carcinoid syndrome have hepatic metastases; in the remaining 5% retroperitoneal or ovarian metastases bypass the portal circulation, leading to systemic exposure and hence the syndrome.

Ur 5HIAA needs to be collected with strict dietary and medical restrictions and within an acidified collection bottle. It has sensitivity of 73% and specificity of 100% for midgut carcinoids. CgA is more sensitive (87%).

At present plasma 5HIAA rests within the clinical trial setting.

Substances which may falsely elevate urinary 5-HIAA

Tryptophan-rich foods: avocados, pineapples, bananas, kiwi fruit, plums, eggplants, walnuts, pecans, tomatoes, plantains.

Drugs: paracetamol (acetaminophen), phenobarbital, ephedrine, methamphetamine, nicotine, phentolamine, caffeine, fluorouracil, melphalan, phenacetin.

Substances which may falsely lower urinary 5-HIAA

Drugs: ethanol, imipramine, levodopa, monoamine oxidase inhibitors, phenothiazines, aspirin, isoniazid, streptozotocin, heparin, methyldopa.

Selected substances which may interfere with measured urinary 5-HIAA (adapted from Maton PN. The Carcinoid Syndrome. JAMA 1988;260(11):1602-5)

3. Other serum biochemical markers

- Pancreatic NETs: Subject to tumoural functional status, clinical symptoms, histological features and immunohistochemistry:[7]
    - Insulin (blood glucose, C-peptide, Pro-insulin)
    - Gastrin
    - Glucagon
    - VIP
    - Somatostatin
    - Calcitonin
    - Others rarely: ACTHomas, GRFomas, PTH-related protein tumours
      Note: Non-functional pancreatic NETs can show increased hormone levels and positive immunohistochemistry without a syndrome: hormonal screen generally is not performed in such cases.
- Pancreatic polypeptide: general tumour marker for pancreatic NETs.
- Rectal NETs: Serum acid phosphatase (in rectal carcinoids if positive on immunohistochemistry), Pancreatic polypeptide and beta-HCG.
- MEN-1 syndrome suspected: Serum Calcium, PTH and pancreatic polypeptide, pituitary hormonal screen.
- Urinary Serotonin and Platelet Serotonin can provide additional information and especially the platelet levels are not influenced by dietary factors. In some studies may be more sensitive than Ur 5HIAA.
- Serum Histamine for atypical carcinoid syndrome.
- Others especially in midgut and hindgut carcinoids include neurotensin, substance P and K, enkephalin, alpha-HCG, pancreastatin, and neurokinin: Not routinely measured.
- The NETest: A PCR-based 51 RNA transcript signature of neuroendocrine tumour (NETs) that captures tumour biology and disease activity.[8, 9]. The NETest at present does not have a role in screening for GEP NETs in healthy individuals.[10] In patients with a GEP NET, it has been found to be more accurate than serum CgA in NET diagnosis and also confirming their diagnosis when CGA levels are low.[8, 10, 11] Also for differentiating NET from adenocarcinoma.[12] It

has been shown to be more informative than CgA changes in consistently predicting disease course and response to treatments. [13-16] The NETest at present is a commercial assay not currently available in Australia and has not entered routine clinical use internationally.

4. Correlation of biochemical marker levels with disease activity and the risk of carcinoid syndrome/carcinoid crisis/carcinoid cardiac disease

Both Chromogranin A and Urinary 5HIAA are proportional to disease bulk and disease progression. However, CgA is the more sensitive of the two in all regards, and also in detecting small recurrences after radical therapy.

Ur 5HIAA levels are correlated with the risk and presence of carcinoid cardiac disease. Serum Pro-BNP has also been correlated with presence and severity of carcinoid cardiac disease.[17]

Patients with large tumour load, high chromogranin A levels, or high urinary 5-HIAA values are more likely to experience a carcinoid crisis during surgery; however, not all of these risk factors have consistently been confirmed.[18]

5. Pre-operative measurement

For an asymptomatic patient, CgA and Ur 5HIAA (in patients with midgut NETs) should be measured prior to surgery.

Measurement of other markers should be considered, depending on the tumour site. For example, for pancreatic tumours, request measurement of pancreatic polypeptide.

If the patient is asymptomatic and CgA normal, there is no need to do additional hormonal tests (e.g. insulin measurement) as everything else is likely to be normal.

6. Frequency of measurement

For patients whose disease has been radically resected:

- Tumour markers should be measured at 6 months and 12 months and then yearly, for at least 10 years. Where there are poor prognostic factors (e.g. high grade tumours, etc), tumour markers should be measured every 6 months, for at least 10 years. [19]

For patients with metastatic disease, where asymptomatic and being observed (low Ki-67 histology):

- Measure tumour markers every 3 months initially and then less frequently if stable; similarly if on somatostatin analogue therapy.

Ur 5HIAA should always be measured at baseline.

- If it is elevated, do annual Ur 5HIAA estimations to look at longer term patterns of secretory activity. Consider repeating during active therapy where secretory symptoms have possibly increased in severity.
- If Ur 5HIAA is negative at baseline there is no need to measure again.

References

1. NCCN, *National Comprehensive Cancer Network. Nueroendocrine and adrenal tumors. Version 1.2020- July 10,2020.* . 2020.
2. Kaltsas, G., et al., *ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Pre- and Perioperative Therapy in Patients with Neuroendocrine Tumors.* Neuroendocrinology, 2017. **105**(3): p. 245-254.
3. Rossi, R.E., et al., *Chromogranin A in the Follow-up of Gastroenteropancreatic Neuroendocrine Neoplasms: Is It Really Game Over? A Systematic Review and Meta-analysis.* Pancreas, 2018. **47**(10): p. 1249-1255.
4. Rehfeld, J.F., et al., *True Chromogranin A concentrations in plasma from patients with small intestinal neuroendocrine tumours.* Scand J Gastroenterol, 2020. **55**(5): p. 565-573.
5. Gut, P., et al., *Chromogranin A - unspecific neuroendocrine marker. Clinical utility and potential diagnostic pitfalls.* Arch Med Sci, 2016. **12**(1): p. 1-9.
6. Miki, M., et al., *Utility of chromogranin B compared with chromogranin A as a biomarker in Japanese patients with pancreatic neuroendocrine tumors.* Jpn J Clin Oncol, 2017. **47**(6): p. 520-528.
7. Bocchini, M., et al., *Biomarkers for Pancreatic Neuroendocrine Neoplasms (PanNENs) Management-An Updated Review.* Front Oncol, 2020. **10**: p. 831.
8. Modlin, I.M., I. Drozdov, and M. Kidd, *The identification of gut neuroendocrine tumor disease by multiple synchronous transcript analysis in blood.* PLoS One, 2013. **8**(5): p. e63364.
9. Kidd, M., I. Drozdov, and I. Modlin, *Blood and tissue neuroendocrine tumor gene cluster analysis correlate, define hallmarks and predict disease status.* Endocr Relat Cancer, 2015. **22**(4): p. 561-75.
10. van Treijen, M.J.C., et al., *Blood Transcript Profiling for the Detection of Neuroendocrine Tumors: Results of a Large Independent Validation Study.* Front Endocrinol (Lausanne), 2018. **9**: p. 740.
11. Modlin, I.M., et al., *A multianalyte PCR blood test outperforms single analyte ELISAs (chromogranin A, pancreastatin, neurokinin A) for neuroendocrine tumor detection.* Endocr Relat Cancer, 2014. **21**(4): p. 615-28.
12. Modlin, I.M., et al., *The clinical utility of a novel blood-based multi-transcriptome assay for the diagnosis of neuroendocrine tumors of the gastrointestinal tract.* Am J Gastroenterol, 2015. **110**(8): p. 1223-32.
13. van Treijen, M.J.C., et al., *Blood Molecular Genomic analysis predicts the disease course of GEP NET patients: a validation study of the predictive value of the NETest(R).* Neuroendocrinology, 2020.
14. Pavel, M., et al., *NET Blood Transcript Analysis Defines the Crossing of the Clinical Rubicon: When Stable Disease Becomes Progressive.* Neuroendocrinology, 2017. **104**(2): p. 170-182.
15. Oberg, K., et al., *A meta-analysis of the accuracy of a neuroendocrine tumor mRNA genomic biomarker (NETest) in blood.* Ann Oncol, 2020. **31**(2): p. 202-212.
16. Bodei, L., et al., *PRRT neuroendocrine tumor response monitored using circulating transcript analysis: the NETest.* Eur J Nucl Med Mol Imaging, 2020. **47**(4): p. 895-906.
17. Dobson, R., et al., *The association of a panel of biomarkers with the presence and severity of carcinoid heart disease: a cross-sectional study.* PLoS One, 2013. **8**(9): p. e73679.
18. Mancuso, K., et al., *Carcinoid syndrome and perioperative anesthetic considerations.* J Clin Anesth, 2011. **23**(4): p. 329-41.
19. Singh, S., et al., *Follow-up Recommendations for Completely Resected Gastroenteropancreatic Neuroendocrine Tumors.* JAMA Oncol, 2018. **4**(11): p. 1597-1604.