Introducing a chromogranin A assay within Glasgow

J. M. Wadsworth[1], P. Maxwell[1], N. Reed[2] and K. Smith[1]

1. Department of Clinical Biochemisty, Glasgow Royal Infirmary, Castle Street, Glasgow, G4 0SF

2. Beatson Oncology Centre, Gartnavel General Hospital, Great Western Road, Glasgow, G12 0YN

Introduction

Chromogranin A is an acidic 48 kDa glycoprotein originating from the chromaffin granules of most neuroendocrine cell types. In health, chromogranin A is released as a pro-hormone together with other peptide hormones in response to stimulation. In disease, larger quantities of Chromogranin A are produced by neuroendocrine derived tumours thus allowing its use as a tumour marker. Due to the different clinical scenarios for measuring Chromogranin A requesting practices within Scotland vary considerably. Currently, laboratories in Scotland either send a combined gut hormone profile to London or they send samples for a single chromogranin A measurement to London, Manchester or Sheffield.

In the broadest sense neuroendocrine tumours (NETs) can be defined as neoplasms that arise from cells of the endocrine and nervous systems. NETs can be located anywhere in the body but are most commonly found in the locations shown in figure 1.

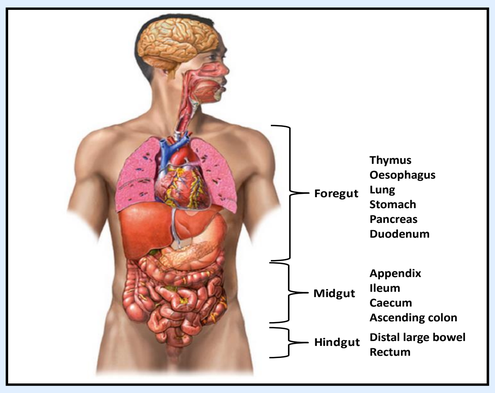

Figure 1: Common locations of NETs.

Analytical Verification

Parameter	Manufacturer's claim	Method	Our Result
Sensitivity	11 µg/L	n=10 replicates of low patient pool, across 4 kits	15 µg/L, CV 5%
Dilution Protocol	90-130% recovery upon dilution	5 samples diluted x2, x5, x10, x15 and x20	Correct diluent is imperative. Mean CV 6%, range 3-11%. 94-109% recovery
Linearity	7-800 µg/L	n=12 replicates of calibrators. Range 55-800 µg/L	Linear, mean R^2 >0.99
Imprecision	Mean inter-assay CV 7%	n=10 replicates of low and high 3rd party QC and patient pools across 4 assays	3rd party QC CV 10% Patient pools CV 9%
Stability (RT)		n=5, samples stored for 5 days	Deviation from initial concentration <10%
Stability (4 ^{O}C)		n=5, samples stored for 3 days	Deviation from initial concentration <10%
Stability (Freeze/Thaw)		n=3, 3 freeze thaw cycles	Deviation from initial concentration <10%
Accuracy		Analysis of n=15 EQA samples.	

Clinical Implementation

Following the successful analytical validation a comparison study was undertaken. Duplicate samples from 100 patients were collected and analysed using the existing Charing Cross assay as well as our new CisBio assay. A summary of the patient comparison is shown in figure 2a. Graphical analysis of the comparison is shown in figure 2b. Clinically the most concerning group were patients who had detectable chromogranin A results using the old assay but who were now classed as non-chromogranin A secretors using the new assay. More information on these 9 patients is shown in figure 2c.

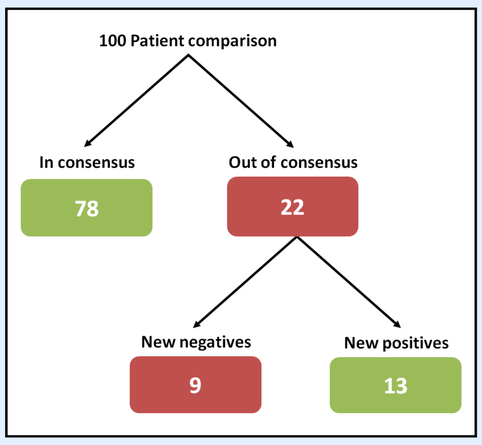

Figure 2a: Diagram of patient comparison results

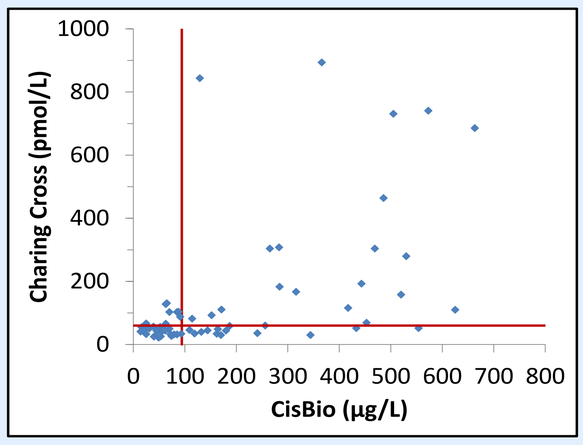

Figure 2b: Comparison of new CisBio results with Charing Cross assay

Patient	CisBio (µg/L)	Charing X (pmol/L)	Clinical Info
1	85	103	Did not fit clinically, discounted as a flyer/incorrect and a limitation of the old method.
2	91	88	No radiological evidence of malignancy
3	25	67	Confirmed metastatic NET. Chromo A was only ever 105 falling to 67 post octerotide therapy. Awaiting surgery.
4	63	66	pT1a carcinoid tumour. Totally resected. No nodal involvement. Monitor only
5	65	131	Known carcinoid patient. METs in nodes that are visible on octerotide scan. Very slow growing i.e. nodes doubles in size over 5 years. Watch and wait.
6	63	128	A somatostatin and Chromo B secretor
7	70	103	Previously NET tumour (surgery in 2015) stable Chromo A since but always above 100. No evidence of recurrence on octreotide scan.
8	88	104	Likely NET in tail of pancreas. Small.
9	41	77	Full resected NET of the bowel but with multiple liver mets.

Figure 2c: Detailed comparison and clinical history of the 9 patients who would no longer be classed as chromogranin A secretors

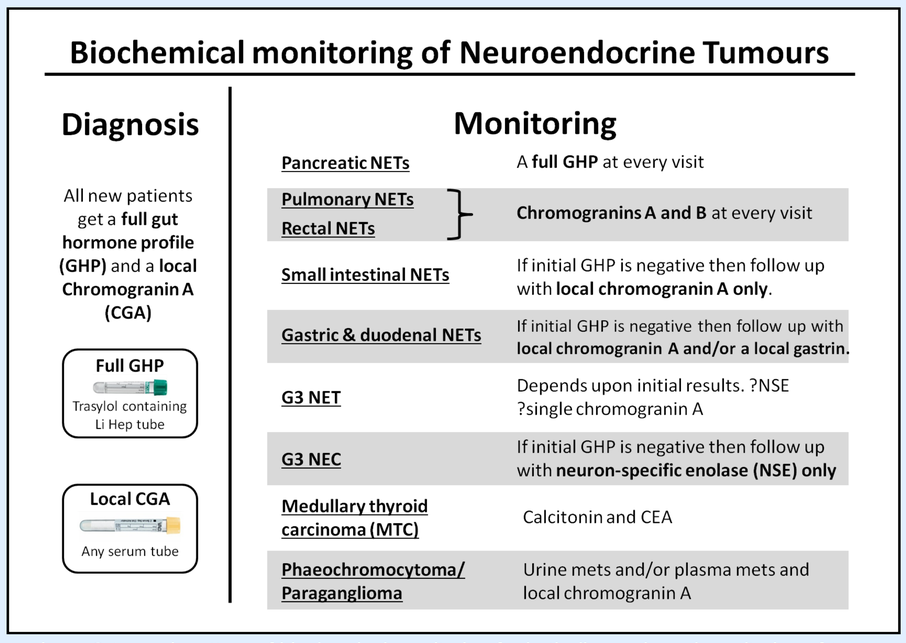

Figure 3 Agreed pattern of biochemical monitoring for both diagnosis and monitoring of NETs

Chromogranin A assays show considerable variation as evidenced by the published scientific literature and their performance within the National External Quality Assessment Services (UKNEQAS) external quality assessment (EQA) scheme. A certain degree of variation is to be expected from immunoassays designed to detect a large protein containing numerous post-translational modifications. There is also no chromogranin A reference material available or a published reference method. The patient comparison data must be considered in this context.

Following acceptance of the method an agreed testing methodology was agreed with the relevant oncology and endocrinology clinicians. At diagnosis it was agreed that both a full gut hormone and local chromogranin A should be request to assess the best marker, check for functional tumours and to get a baseline chromograin A result. As pancreatic NETs are more likely to produce other gut hormones it was felt that a full gut hormone screen was required at every surveillance visit. Local experience suggests that pulmonary and rectal NETs are more likely to secrete chromogranin A and B and should therefore be monitored using both assays. All other surveillance should only require a single chromogranin A measurement, as per the guide shown in figure 3.

Conclusion

Despite the numerous challenges of moving a large cohort of patients from one tumour marker to another this new referral service offers considerable benefits for both patient care and cost effectiveness. These benefits include:

- Improved TAT – from 6 weeks down to 3 weeks.
- Cost savings – in-house assay costs £25.00 per sample compared to £98.00 for the old send-away test.
- Increased standardisation within Scotland – before this assay health boards within Scotland were using four different chromogranin A assays.

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Neoplasia, cancer and late effects

John Wadsworth

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