All Wales Clinical Biochemistry Audit Group

Standards for the Investigation of Macroprolactinaemia

INTRODUCTION

In most subjects the predominant circulating form of prolactin in serum is monomeric. However, in some individuals there is an additional circulating form, usually called macroprolactin, in which prolactin is bound to IgG. This high molecular weight complex has a longer half-life than monomeric prolactin, is unable to cross capillary membranes to stimulate prolactin receptors and is therefore biologically inactive. Immunoassays detect monomeric prolactin and react variably to prolactin bound to IgG, so that apparent hyperprolactinaemia can be obtained when the circulating concentration of monomeric prolactin is normal. Unless detected by the laboratory, this can lead to diagnostic confusion, unnecessary further testing and possibly inappropriate treatment. It is therefore essential that all laboratories can identify macroprolactin as a cause of hyperprolactinaemia. A few patients may have a true increase in monomeric prolactin together with the presence of macroprolactin. A recent survey of laboratories in Wales, presented at an audit meeting in June 2011, showed variations in practice for investigating suspected macroprolactinaemia. The following standards are recommended in the light of the survey findings, discussion at this meeting and previous publications.

STANDARDS

1. All laboratories should report prolactin results in mU/L.

2. All laboratories should use manufacturer-specific, gender-related reference ranges.

3. Each laboratory performing prolactin assays should ensure that appropriate internal quality control (IQC) and external quality assessment (EQA) procedures are in place.

4. All laboratories should be aware that macroprolactin is a cause of hyperprolactinaemia and is essentially biologically inactive. All current assays detect macroprolactin to a variable extent.

5. All laboratories should screen hyperprolactinaemic samples for the presence of macroprolactin, using ≥700 mU/L as a cut-off. Rarely it may be necessary to consider screening in patients with confirmed and persistent prolactin concentrations between the reference range and 700 mU/L, in whom other causes have been excluded.

6. The preferred screening method is polyethylene glycol precipitation. Briefly, 250 µL sample is mixed thoroughly with an equal volume of 25% (w/v) PEG 6000 solution (25 g in 100 mL PBS), incubated for 10 minutes at room temperature then centrifuged at 3,000 rpm for 30 minutes. The supernatant is then re-assayed for prolactin.

7. It is recommended that the PEG solution is made fresh fortnightly and stored at 4°C. It should be equilibrated to room temperature before use.

8. Interpretation of the result should be as described by Beltran *et al*, 2008, Clin Chem; 54: 1673-1681. Post-PEG prolactin concentration is first multiplied by 2 to account for the 1:1 dilution with PEG. This value is reported alongside a **method- and gender-specific post-PEG reference range,** see table 1.

	Male reference range (mU/L)	Female reference range (mU/L)
Siemens Centaur	61 – 196	66 – 278
Roche Elecsys	63 – 245	75 – 381
Beckman Access	70 – 301	92 – 469
Abbott Architect	72 – 229	79 – 347

Table 1: Gender-related manufacturer-specific post-PEG reference ranges from Beltran *et al*, 2008.

Advantages of this approach:

- Ensures that **monomeric prolactin** is reported, as cases of monomeric hyperprolactinaemia coinciding with significant macroprolactin are not uncommon. These could be missed if only "% recovery" was reported.
- Less labour-intensive (no requirement to analyse sample diluted 1:1 with PBS alongside PEG-precipitated sample).
- Interpretation is much simpler.
- No longer have an "equivocal" range of 40-60% recovery.
- Negates the need for Beckman users to dilute the post-PEG supernatant 1 in 5 before analysis. Post-PEG reference range takes into account positive interference by PEG in this assay.

Disadvantages of this approach:

- If manufacturers reformulated their prolactin reagents we may need to establish a new post-PEG reference range for that method. The manufacturers may be willing to do this themselves.

9. Reports for macroprolactin-positive samples should be transparent and not include the term "macroprolactin", as there is potential for confusion with "macroprolactinoma".

10. Turnaround times for reporting prolactin should be 24 hours Monday to Friday, or 4 working days if requiring PEG precipitation.

11. Prolactin results ≥10,000 mU/L should be telephoned immediately without waiting for PEG precipitation.

12. Laboratories should be aware of their assay's susceptibility to the hook effect. Assaying neat and at a 1:10 dilution may be beneficial in known macroprolactinoma patients.

13. Endocrine referral should be recommended if prolactin concentration is ≥800 mU/L following exclusion of secondary causes (e.g. stress, medication, renal impairment, hypothyroidism and pregnancy) – see appendix A and B. It may be appropriate to add TFTs and/or U&Es onto the request if no recent results are available. Endocrine referral may also be appropriate when prolactin is persistently raised but is below this cut-off, *if patient is symptomatic* with secondary causes excluded.

REFERENCES

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Appendix A: Causes of raised prolactin

Causes	Mechanism	Prolactin concentration
Prolactinoma		
Pituitary stalk interruption Trauma Surgery Pituitary/hypothalamic tumours Infiltrative disorders of hypothalamus	Interference in dopamine inhibition of prolactin secretion	Usually up to 1000 mU/L Rarely above 2100 mU/L
Drugs	Effects on delivery of dopamine or antagonising dopamine receptors	Generally up to 2500 mU/L. Ideally baseline PRL should be measured prior to commencement of therapy. If possible can also repeat PRL after withdrawal of drug (if safe to do so) for at least 72 hours
Primary hypothyroidism	TRH stimulates prolactin release	Usually up to 1000 mU/L
Renal failure	Reduced clearance of prolactin	Usually up to 1000 mU/L Can reach 3000 mU/L in ESRD
Chest wall stimulation	Mimics suckling	600 – 1000 mU/L
Pregnancy/breastfeeding		Up to 6000 mU/L in third trimester
Physical/psychological stress		Rarely exceeds 800 mU/L
Seizures		
PCOS		
Liver cirrhosis		
Anorexia nervosa		
Marijuana use		

Appendix B: Medications that may cause hyperprolactinaemia

Type of medication	**Examples**	
Antipsychotics	Phenothiazines	Prochlorperazine, Fluhenazine, Perphenazine, Trifluoroperazine
	Thioxanthenes	
	Butyrophenones	Benperidol, Haloperidol
	Atypical	Risperidone, Molindone, Olanzapine
Antidepressants	Tricyclic/tetracyclic	Clomipramine, Desipramine, Amitriptyline
	SSRIs	Citalopram, Fluoxetine, Paroxetine
Opiates and cocaine		
Antihypertensives		Verapamil, Methyldopa, Reserpine
Gastrointestinal	Histamine 2 receptor blockers	Cimetidine, Famotidine, Nizatidine, Ranitidine
	Others	Metoclopramide, Domperidone
Oestrogens		

Appendix C: Calendar of Audit Process for Standards for Investigation of Macroprolactinaemia

March 2001 Survey of Welsh laboratories' strategies for investigating macroprolactinaemia (13/13 laboratories replied) undertaken by Dr.R.John (Consultant Biochemist, University Hospital of Wales, Cardiff). Findings presented at an All Wales Clinical Biochemistry Audit Group meeting at the Vale of Glamorgan Hotel, Hensol on 30th March 2001.

Summer 2001 Initial draft standards prepared by Dr.R.John and considered at an All Wales Clinical Biochemistry Audit Group committee meeting on 24th May 2001.

Autumn 2001 Draft standards sent for consultation to consultant biochemists and endocrinologists in Wales to seek their views. Final draft of standards presented at the All Wales Clinical Welsh Biochemistry Audit Group meeting on 18th October 2001 and at the Welsh Endocrine and Diabetes Society business meeting on 1st November 2001.

Nov. 2001 Standards agreed in principle and ratified at an All Wales Clinical Biochemistry Audit Group committee meeting (on 22nd November 2001) by Dr.K.Griffiths (chairman).

March 2003 Standards finalised after further comments received from Mr.M.Fahie-Wilson.

2005 Proposed date of re-audit and review of standards

Summer 2011 Survey of Welsh laboratories' strategies for investigating macroprolactinaemia (12/12 laboratories replied) undertaken by Dr. C. Searell (Trainee Clinical Biochemist, University Hospital of Wales, Cardiff). Findings presented at an All Wales Clinical Biochemistry Audit Group meeting at Nevill Hall Hospital, Abergavenny on 28th June 2011.

Autumn 2011 Initial draft standards prepared by Dr. C. Searell and Dr. C. Evans discussed at the Wales Region ACB Autumn meeting, 17th November 2011.

Spring 2012 Findings of audit and draft guidelines presented and discussed at the Welsh Endocrine and Diabetes Society meeting on 17th May 2012.

Summer 2012 Draft standards sent for consultation to consultant biochemists and endocrinologists in Wales to seek their views. Final draft of standards accepted by the Welsh Endocrine and Diabetes Society

Autumn 2012 Standards agreed and ratified at an All Wales Clinical Biochemistry Audit Group committee meeting on 19th November 2012 by Mrs. A. Thomas (chair).

2017 Proposed date of re-audit and review of standards.