MACROPROLACTIN
Current Processes and Recommendations

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HYPERPROLACTINAEMIA

Most common endocrine disorder of the hypothalamic-pituitary axis

amenorrhoea / oligomenorrhoea, galactorrhoea, infertility, decreased libido, decreased bone mass (reduced E2), gynecomastia (male)

(Pituitary mass- headache, visual field defects)

HIGH Prl  ➡️ suppression hypothalamic GNRH
           ➡️ reduction in gonadotrophins
           ➡️ hypogonadism
Known causes of hyperprolactinemia

- **Pituitary**
  - Prolactinoma
  - Nonfunctioning adenoma
  - Hypophysitis
  - Stalk section
  - Infiltrative disease

- **Hypothalamic**
  - Tumors
  - Infiltrative disease

- **Secondary**
  - Renal failure
  - Primary hypothyroidism
  - Adrenal insufficiency
  - Polycystic ovary syndrome

- **Physiological**
  - Pregnancy
  - Breast stimulation
  - Stress

- **Medication**
  - Antipsychotics
  - Antiemetics
  - Antihypertensives
  - Estrogen

- **Analytical**
  - Macroprolactin
  - Heterophilic antibodies

Smith TP et al. (2007) Technology Insight: measuring prolactin in clinical samples
*Nat Clin Pract Endocrinol Metab* 3: 279–289 doi:10.1038/ncpendmet0447
**Prolactin forms**

**LITTLE PROLACTIN** 65-85%

**MONOMER/FREE/ACTIVE Prl**
- single chain, 199aa, 3 disulphide bonds
- Non-glycosylated - 23 kDa
  - most biologically active
- Glycosylated - 25 kDa (25% total Prl)
  - reduced biological activity
  - variable immunoreactivity

**BIG PROLACTIN** 10-20%

**DIMER** of monomer Prl 40-60 kDa or breakdown product of macroPrl?
- reduced biological activity
- variable immunoreactivity

**BIG-BIG PROLACTIN** variable%

**MACROPROLACTIN** > 100kDa
- (150-170kDa) - complex Prl with IgG antibody
- also aggregated glycosylated forms (200-669KDa)

Prl bound to receptor/binding protein

**OTHERS**
- Prl mRNA splice variants
- Proteolytically cleaved forms (14, 16 kDa)
- Phosphorylated, sulphated, deamidated forms
**Macroprolactin**

- **Prl bound to anti-Prl Ab mainly IgG (also IgA and IgM)** - also some PRL non-specifically bound to Ig
  - Low affinity, high capacity,
  - Bind to N and C terminal epitopes-conformational, near receptor binding site 1
  - Mainly IgG4- continued antigenic stimulation
  - Develop before middle age – chronic condition
  - No specific association with autoimmune disorders

Antigen - post translational modifications to Prl
  - Phosphorylated pituitary Prl may be the antigen
  - In RA matrix metalloproteinase-3 may result in formation new epitopes on Prl

- **Initially though to be biologically active in-vitro** *(Nb2 rat lymphoma cell bioassay)* so able to bind to Prl receptor. Activity due to dissociation of macroprolactin forming monomer Prl in the assay
Minimal biological activity in-vivo

- Macroprolactin originally identified in patients with high Prl but no clinical symptoms associated with hyperprolactinaemia
- Increase in PRL in response to TRH or dopamine receptor agonist blunted in high PRI due to prolactinoma due to Prl feedback. Normal response in macroPrl indicates that macroPrl not able to feedback
- Monomeric Prl concentration similar in normal subjects with and without macroPrl indicating that macroPRL is not able to feedback and modulate Prl levels
- Gonadotrophins and E2 higher in women with macroPrl compared to true hyperprolactinaemia indicating macroPrl not able to inhibit GNRH

MacroPrl not able to bind to receptor- Prl ab binds close to receptor binging site on Prl causing steric hindrance

High mol wt so confined to vascular space
Macroprolactin

- Longer $\frac{1}{2}$ life - levels increase in blood
- Incidence 1-5%, Hyperprolactinaemia up to 35% (depends on assay and definition of macroprolactinaemia)

  - 3.7% macro, 1.1% high Prl due macro
  - 3.3% high Prl (34% macro)

  - 0.6% macro, 0.5% high Prl due to macro
  - 4.1% high Prl (12.5% macro)
- Presence of symptoms suggestive of high Prl are common
- Presence of symptoms not useful in distinguishing true hyperPrl from macroprolactinaemia

  7.4% high Prl (20% macro, 80% true hyperPrl)

Suliman et al (2003) Patients with Prl >1000 (reviewed records)
  retrospective unselected
  24% macroprolactinaemia (n=21)
  57% oiligo/amenhorroea
  23% galactorrhoea
  29% infertility
Interference in assays

Smith, T. P. et al. J Clin Endocrinol Metab 2002;87:5410-5415
Two-site assay

Macroprolactin Detected

Not Detected
Interference in assays

- Epitopes recognised by anti-Prl Ab and reagent antibodies (epitope masking)
- Incubation time - macroPrl reacts more slowly
- Other factors - solid phase/ signal generation system eg Enzymum and Elecsys - same abs different recognition
- Dissociation of macroPrl in assay system (pH, salts, volume, time) so actually measuring monomer Prl
Macroprolactin

- Biologically inactive form of Prl
- Accumulates in blood
- Falsely elevated Prl
- Common cause of hyperprolactinaemia

MISDIAGNOSIS of HYPERPROLACTINAEMIA

Anxiety and inconvenience
Unnecessary expensive investigation (MRI, CT)
Inappropriate treatment (drugs, pituitary surgery)

Suliman et al 2003 - retrospectively identified macroPrl pituitary imaging 93%, dopamine agonist 87%
Clinically useful measurement of Prl in the presence of macroprolactin

(ie is biologically active prolactin normal or elevated)

- Check for presence of macroprolactin
- Estimate the free/monomer prolactin level
  (high free prolactin in addition to macroprolactin)
Investigation of MacroPrl

Screen all samples with high Prl
Remove macroPrl
Measure monomer

- Measure total Prl
- Prl elevated - remove macroPrl
- Assay monomer - correct for any dilution
- Calculate % recovery monomer Prl \( \frac{\text{monomer}}{\text{total}} \times 100 \)

**High recovery**
- mostly monomer
- true high Prl - report total

**Low recovery**
- predominately macroPrl
- high Prl due to the presence of macroPrl

Report % recovery or comment eg falsely high Prl due to the presence of macroprolactin
Investigation of MacroPrl

Patient may have elevated monomer Prl (true hyperprolactinaemia) in addition to macroPrl

eg Total Prl – 3000, Monomer -1000
33 % recovery – high Prl due to macroprolactin monomer also elevated

Report monomer level with appropriate RI

Methods to separate MacroPrl and Prl

- Gel Filtration Chromatography (GFC) (size exclusion) — molecular weight
- Polyethylene Glycol (PEG)
- Protein A or G Sepharose/Agarose
  Binds Ig complexes
- Anti-human IgG Agarose
  Binds IgG complexes
- Centrifugal Ultrafiltration - size
Gel Filtration Chromatography (GFC)

- Gold standard
- Separate by molecular weight
- Assay fractions - Need to use same assay or one that has higher detection of macroPrl than screening assay
- Allows detection all Prl forms - depending on assay and resolution of column (Monomer peak may include big Prl)

- Special equipment
- Technical expertise
- Time consuming
- Expensive
- Used for confirmation or investigation

PEG Precipitation

• Most used routine screening method
• Precipitates high MW molecules - Ig including macroPrl and big Prl- assay supernatant (monomer Prl).
• PEG remove H2O of hydration of protein - increased concentration - precipitation
• Add equal vol 25% PEG 6000, mix, centrifuge, assay supernatant, correct for ½ dilution, calculate %recovery
• Quick, easy, cheap
• Manual – not suited to assay on demand
• Validated against GFC
• Correlates best with GFC
PEG vs GFC

n=171 high Prl, Delfia, Sephacryl S200

Poor monomer Prl recovery?
Macro?

n=195 high Prl, Delfia

Variable recovery
Non-specific precipitation
Monomer Prl with PEG?

PEG Precipitation-limitations

- High IgG – False positive macro (poor Prl recovery) eg HIV
- Partial precipitation IgA – may miss IgA-macro
- PEG interference eg Immulite and Access - over recovery
- What % Prl recovery to indicate macroPrl presence?
  < 40%, 50%, 60%, 70%?
- Variable monomer Prl recovery with PEG <100% - monomer Prl underestimated, matrix effects - can’t use normal RI

- **Correction of Prl level by average recovery of monomer, use normal RI.**
Current Best Practice

- screen all samples with high Prl for macroprolactin
- Using PEG
- determine % macroPrl
- report monomer Prl level with appropriate reference intervals
- GFC for unusual cases

Macroprolactinaemia- hyperprolactinaemia due to presence of excess macroprolactin with a normal level of monomeric Prl

Fahie-Wilson & Smith. Best practice and research clinical endocrinology and metabolism 201327;725-742
Mc Kenna. Clin Endocrinol200971 466-469
Macroprolactin, to seek or to ignore: a trans-Atlantic division

McKenna Endocrine Abstracts 2014 34 SE1.3

Diagnosis and treatment of hyperprolactinemia: An Endocrine Society Clinical practice guideline  JCEM 2011 96;273-288

Suggest screening for macroprolactin in investigation of asymptomatic hyperprolactinemia

(Definition macroPrl- high proportion macroPrl)


Whether macroprolactin should be measured in patients with ‘classic symptoms’ is controversial
Final Paragraph –

‘It is remarkable that the problem of macroprolactinemia, first recognized over 35 years ago, is still misunderstood. A simple laboratory procedure to detect macroprolactinemia is available and has been advocated, evaluated, validated, and refined since 1997 (19-22). Best clinical and laboratory practice requires that macroprolactinemia be excluded in all cases of hyperprolactinemia and this should be correctly reflected in the guidelines issued by The Endocrine Society.’
‘However, we respectfully disagree that "if macroprolactinemia is not recognized it will result in misdiagnosis and mistreatment, cause unnecessary concern for patient and physician, and waste precious healthcare resources" The literature is not that clear-cut, and does not support this statement.’

Concluding sentence
‘In our experience, many patients with macroprolactinemia also have "normal" hyperprolactinemia, are symptomatic and respond well to therapy. Accordingly, there is no evidence that macroprolactinemia should be excluded in symptomatic patients, since they should be treated regardless, and most assays will in fact detect macroprolactinemia.’
What does the rest of the world do?

Google snap shot 2012-2015
27 papers

% recovery or monomer Prl
9 - %recovery
15 - monomer Prl

Screen all high Prl or Asymptomatics
10 – all high Prl
10 – asymptomatic

Brazil, Bulgaria, Canada, India, Japan, Malaysia, Mexico, Pakistan, Poland, Portugal, Spain, Thailand, Turkey, UK, USA
MACROPROLACTIN SURVEY 2012

Lab details Lab size, instrumentation, public/private

Macroprolactin screening Criteria for testing, testing frequency and location

PEG method Method source, PEG preparation and storage, details of procedure

Reporting % recovery for +ve macroPrl, how reported

Macroprolactin QC What is used, interest in macroPrl QC

Circulated to RCPA QAP Endocrine and Tumour marker QAP participants
Screen for macroPrl: 54/55 (44/54 on site)

Prl level for screening: 74% above a limit (20% if suspicion of macroPrl)
- 64% above upper ref interval or rounded figure near RI,
- 30% >200 above upper RI - ranged up to 2000mIU/L

On request only: 24% (31% only if suspicion of macroPrl)

Screening method: All labs used PEG (4 confirmatory testing gel filtration)
**PEG PROCEDURE**

**PEG** - 77% PEG 6000, 9% PEG 8000

**Diluent** - 59% water, 25% buffer, 5% saline
Tris, PBS, phosphate +/- EDTA, Triton, azide

%PEG – 25% all labs

**Vortex time** – varied from no fixed time (64%) to 3 minutes

**Incubation time**

**PEG Storage**

**Centrifugation Conditions**

%PEG – 25% all labs
**PROLACTIN REPORTING**

**% Recovery for MacroPrl +ve**

![Bar graph showing recovery percentages for MacroPrl positive samples.]

**Borderline positive**
- <40%; 40-60 (n=6), 40-50 (n=2), 40-80 (n=1)
- <35%; 35-45
- <60%; 60-80 (n=3), 60-90 (n=1)

**Reporting**

- 71% report numerical value and comment
- 25% comment only

**Monomeric Prolactin 43%**
- 8/19 – post PEG reference interval
- 9/19 – normal reference interval after correction for PEG recovery
Variability in: Prl assay, Prl ref range, Prl level for macro screen, PEG procedure, % recovery for macro+ve, how result reported

Potential for significant differences in Prl result reported by different labs for samples containing macroPrl.

AACB Endocrine WP survey 2012

MacroPrl QAP may help determine the extent of the problem

MacroPrl QC/QAP material being developed – including reporting

Develop method guidelines to promote harmonisation
Summary

- Prl frequently measured in the investigation of infertility and menstrual problems
- MacroPrl is a continuing problem that could lead to misdiagnosis of hyperprolactinaemia and inappropriate investigation and treatment
- Screening all high sample for macroPrl and estimating monomer level should provide clinician with relevant clinical information (is biologically active Prl high or normal)
- Clinicians should be informed of the macroPrl problem
- We need to address quality issues associated with testing and reporting Prl in the presence of macroPrl to ensure we are not adding to the problem.