



Recommendations for the investigation of macroanalytes

Introduction

In most subjects, the predominant circulating form of analytes in serum is monomeric.

However, in some individuals, there is an additional circulating form, referred to as macroanalyte, in which the analyte is bound to immunoglobulins, has minimal bioactivity in vivo and is detected by varying degrees by different immunoassays.

Macroanalytes have a higher molecular mass making their clearance slower and more difficult and resulting in a spurious increase in serum concentration.

The prevalence of macroanalytes is variable among different patient groups and not well studied.

A failure to detect macroanalytes by the laboratory can result in unnecessary concern for both the patient and clinician, diagnostic confusion, unnecessary further investigations, possible inappropriate treatment and waste of healthcare resources.

Therefore, investigation of persistently elevated analytes not in keeping with clinical status requires both a logical and systematic approach, in which a macroanalyte is excluded in every instance at the earliest opportunity.

It is important to note that macro forms of the analyte can be one of the causes of assay interference, but others should also be excluded.

Polyethylene glycol (PEG) precipitation has been validated as a method for detecting macroprolactin, but its use in some other macroanalytes has not been fully validated and is more used as a screen.

The commonly quoted macroanalytes are macroprolactin, macroamylase, macro TSH, macro alkaline phosphatase, macro creatine kinase, macro troponin, macro B12 and macro aspartate aminotransferase. Several others have been described, but are not mentioned in this document.

I reviewed the current literature and local laboratory information when compiling these recommendations, which should help the laboratory in their approach to the investigation of macroanalytes.

Macroprolactin

- In most subjects with hyperprolactinemia, the predominant circulating form of prolactin is the 23kDa monomer secreted by the pituitary which is bioactive in vivo.
- However, in some individuals, there are additional circulating molecular mass forms called macroprolactin (MPRL). MPRL is most frequently a complex of prolactin bound to IgG and has minimal bioactivity in vivo, but remains reactive to varying degrees in all prolactin immunoassays.
- It has been estimated that hyperprolactinaemia due to the elevated concentrations of MPRL with normal concentrations of bioactive monomeric prolactin (macroprolactinaemia) is the cause of between 5 and 15% of all cases of hyperprolactinaemia.
- Macroprolactinaemia has no pathological significance, but can confound the interpretation of the results.
- Best laboratory practice requires that macroprolactinaemia should be excluded in all samples with a prolactin concentration above the upper limit of the reference interval quoted by the laboratory. This requirement has not been universally followed, because of practical difficulties in accommodating the workload for PEG precipitation and the knowledge that modestly elevated prolactin concentrations may not be clinically significant, and these factors have resulted in higher thresholds for screening for macroprolactinaemia being widely used in the UK. However, it should be noted that macroprolactinaemia is most common in cases of modest hyperprolactinaemia. Work with one assay has shown that use of a screening threshold of 700 mU/L would miss more than 50% of cases of macroprolactinaemia and a threshold of 1000 mU/L would miss 85%. Therefore, the implementation of a higher threshold for screening must be made in conjunction with the local clinical team and GPs using the service.
- Polyethylene glycol (PEG)precipitation is the most convenient means of identifying the presence of macroprolactinaemia, i.e.: hyperprolactinaemia due to the presence of excess macroprolactin and provides a measure of the bioactive monomeric prolactin concentration (BMP). Briefly, 250 ug of the sample is mixed with an equal volume of 25% w/v of PEG 6000 solution in phosphate buffered saline pH7.4. Vortex mix for approximately 10 seconds. Incubation for 10 minutes at room temperature is recommended in order to precipitate MPRL. After centrifugation at 14000 g, the residual BMP is measured in the supernatant. However, manufacturers may provide specific guidance on this that should be verified if being followed.
- Bioactive monomeric prolactin (BMP) should be reported if macroprolactinaemia is detected and PEG precipitation repeated on all subsequent samples, so that the BMP can be monitored for patient management. A yearly assessment of macroprolactin should be sufficient.
- It is recommended that the PEG solution is made up fresh monthly and that aliquots are stored at 4°C. Each aliquot should be equilibrated to room temperature before use. Laboratories should verify the PEG stability locally, since this may vary depending on the analytical platform used.
- UK NEQAS provides a scheme for MPRL in their Peptide Hormone assay scheme. However, as there are not many distributions with MPRL present, it may be worthwhile for laboratories to participate in a locally organised sample swap scheme or to perform an additional monthly assessment using a stored anonymised patient serum with a known concentration of prolactin/post PEG prolactin.

- Laboratories should verify sample stability for their MPRL assay if the sample storage exceeds the storage condition specified in the prolactin kit insert.
- Post PEG prolactin is first multiplied by 2 to account for the 1:1 dilution with PEG for the calculation of BMP. This BMP value is reported alongside an assay and gender-specific BMP range. If the manufacturers reformulated their prolactin reagents, one may need to establish a new BMP reference range for the method.
- A locally agreed policy should determine at what prolactin level results should be telephoned to the requesting clinician. Additionally, if the patient is symptomatic, such as having visual field disturbances, then the result should be authorised and telephoned to the requesting clinician without waiting for PEG precipitation to confirm the presence of macroprolactin.
- If the screen for macroprolactin is positive and the BMP is not within reference range, then true hyperprolactinaemia is present in addition to macroprolactin. However, if the screen is negative if true hyperprolactinaemia is present after exclusion of secondary causes such as stress, medication, renal impairment, hypothyroidism (it may be appropriate to add thyroid function and electrolytes to the request) and pregnancy, then endocrine referral is appropriate. Pituitary imaging, if positive, would indicate a hypothalamic/pituitary tumour and, if negative, an idiopathic hyperprolactinaemia.
- Clinicians need to be made aware of the physiological, pathological, but, above all, the extensive pharmacological causes of hyperprolactinaemia before investigating for macroprolactinaemia.

Macroamylase

- An element of macroamylase is found in around 4.5% of patients with hyperamylasaemia and there does not appear to be a gender bias.
- Macroamylase should be considered in any patient with elevated levels of serum amylase on more than one occasion, and whose serum lipase is normal in the face of unimpaired renal function and salivary amylase being the cause of hyperamylasaemia has been ruled out.
- A urine/serum amylase ratio of <0.02 is compatible with macroamylase.
- PEG precipitation with confirmation by gel filtration chromatography is the best approach to detect macroamylasaemia, as electrophoretic methods tend to be subjective and do not always detect macroamylase.
- A failure to identify macroamylase as the cause of unexplained but benign hyperamylasaemia correctly can lead to expensive investigations, such as ultrasonography and computerised tomography, as well as invasive and unpleasant investigations such as ERCP and laparotomy to rule out pancreatic disease, and could induce the prescription of unnecessary elemental diets and replacement therapies.

MacroTSH

- Macro TSH is a large molecular-sized TSH that is mostly a complex of TSH bound rather weakly to IgG and is in a state of equilibrium with the free TSH in the circulation.
- It has a prevalence of between 0.6 and 1.6% and is an under-recognised laboratory interference.

- Patients with macro TSH have an elevated serum TSH and normal thyroid hormone levels, and no clinical symptoms of thyroid dysfunction.
- TSH is often markedly increased, but cases with only slightly elevated TSH have been reported.
- Biochemically, this mimics subclinical hypothyroidism, which may lead to inappropriate L thyroxine therapy. However, a reduction in TSH in response to L thyroxine therapy cannot exclude the presence of macro TSH.
- Anti-human TSH autoantibodies are a major component of macro TSH, and may cause diagnostic and therapeutic difficulties.
- No routine TSH immunoassay can disclose the presence of macro TSH. For this purpose, the gold standard is gel filtration chromatography, which separates the various TSH fractions according to the molecular weight. Dilution studies and measurement of TSH after PEG precipitation might be helpful in identifying assay interference, without being definitive regarding the cause of such interference. Considering lower costs and higher accessibility than with gel filtration chromatography, such an approach may be a valid alternative for detection of macro TSH, although results must be confirmed by gel filtration chromatography.
- It has been suggested that macro TSH should be suspected in cases where the TSH is >10 IU/L while some have suggested especially screening women of childbearing age in whom L thyroxine therapy for subclinical hypothyroidism is considered. However, since the prevalence of macro TSH is low, such an approach is controversial and would significantly increase the cost of thyroid function tests. TSH levels >20 IU/L with a free T4 within the reference range should be investigated for possible interference.
- A close dialogue between the clinician and the laboratory is important in approaching such cases.

Macro Alkaline Phosphatase (Macro ALP)

- Macro ALP is a rare benign condition caused by immunoglobulin-bound ALP, thus reducing its clearance.
- This condition has been described in patients with a clinically unexplained non-progressive (>6 months) mildly increased serum ALP (<2 x the upper reference range) and a normal serum gamma glutamyl transferase (GGT), and can also be seen in cases of subclinical Paget's disease.
- ALP isoenzyme electrophoresis does not demonstrate a predominant liver or bone fraction. Instead, a broad band migrating around the position of intestinal isoenzymes, which almost entirely disappear following PEG precipitation.
- Reference laboratories do not accept requests for isoenzyme analysis if the total alkaline phosphatase is <250 IU/L due to poor resolution.
- Benign Familial Hyperphosphasaemia (BFH) presents in a similar manner and can be distinguished from macro ALP by an autosomal dominant family history and the presence of intestinal ALP on isoenzyme electrophoresis.
- Early recognition of these benign conditions can reduce unnecessary investigations.

Macro Creatine Kinase (Macro CK)

- Macro CK, a neglected cause of a raised serum CK is a complex formed between the creatine kinase dimer CK-BB and IgG, and is present in 1-2% of the normal healthy population.
- Normal CK activity does not exclude the presence of macro CK, but has no clinical significance. No further investigations are needed.
- Clinically, the absence of symptoms or an isolated and persistently raised serum CK favours the presence of macro CK.
- Electrophoresis is the preferred technique for the detection of macro CK, as it permits the separation of isoenzymes MM (skeletal muscle), MB (myocardium) and BB (brain).

Macro troponin

- Cardiac troponin is the preferred biomarker of myocardial injury and high sensitivity troponin assays allow measurement of troponin with excellent precision.
- Macro troponin is a high molecular weight complex containing immunoreactive troponin I and IgG (macro troponin I) and is found in approximately 5% of patients with an elevated troponin I.
- Macro troponin should be considered as a differential diagnosis where a normal ECG, physical examination, radiological imaging and routine laboratory investigations do not provide an explanation for the elevated serum cardiac troponin I concentration, and should raise the suspicion of analytical interference.
- Published laboratory methods for detecting macro troponin are limited and none have yet been considered as a practical gold standard. Potential methods include protein A binding or immunoglobulin depletion, size exclusion chromatography, sucrose gradient separation, PEG precipitation, unusually large clinically inconsistent discrepancies between different cardiac troponin assays, mixture of EDTA or altering specimen types and serology-based assays, e.g.: directly measuring cardiac troponin autoantibodies. These methods differ in their complexity, precision, cost and turnaround time, and many carry differing analytical specificity for macro troponin. Except for the direct measurement of cardiac troponin autoantibodies, all other methods to detect macro troponin require repeat troponin testing by an alternative method.
- It is important for laboratories and clinicians to be aware of and develop processes to identify and manage specimens with elevated results due to macro troponin.
- Some publications have suggested that in some individuals, macro troponin (or negative interference in troponin immunoassays) may be associated with cardiomyopathy.

Macro B12

- The measurement of serum total B12 (cobalamin) is a mainstay of clinical diagnosis, primarily for the identification of deficient levels.
- However, B12 elevations are a frequent and underestimated anomaly and reported more commonly than deficient levels.

- In clinical practice, the finding of an elevated serum B12 concentration is often the consequence of supplementation with B12, either in oral form or injections. Elevated serum B12 levels may be associated with underlying disorders such as liver disease, haematological disorders, kidney or intestinal disease.
- Macro B12 is a consequence of complex formation of B12 vitamin binding proteins with immunoglobulins.
- Total serum B12 is measured by immunoassay and interference due to macro B12 may result in erroneously reported high B12 levels.
- The prevalence of macro B12 has been reported to be as high as 18%, yet this area seems to be understudied and, in the absence of routine reflex testing procedures, it may only be investigated following clinical uncertainty/discordance and liaison with the laboratory. Therefore, the correct identification of the macro B12 interference is paramount to avoid potential erroneous clinical decisions.
- Although PEG precipitation may be considered a comparatively crude method for removing interfering macromolecules, it appears effective in this capacity for removing potentially interfering macro B12 molecules, and shows potential for mitigating against misdiagnosis and mismanagement of patients with unexplained or clinically discordant elevated total B12 levels.

Macro aspartate aminotransferase (Macro AST)

- Macro AST is a complex between AST and immunoglobulins.
- Persistent elevation of AST activity in the serum due to the presence of the macro enzyme form (macro AST) may lead to diagnostic confusion in many clinical situations, in particular those associated with chronic liver disease.
- Macro AST is typically a diagnosis of exclusion.
- It is a benign and rare condition and because of its rarity, macroenzyme testing is not routinely performed in clinical practice.
- Therefore, the diagnosis is often delayed with a prolonged work-up and may include invasive evaluations such as a liver biopsy. A thorough patient history and routine laboratory tests are important to ensure that no associated or coexisting condition explains the elevated AST levels.
- The use of PEG is a simple and effective method for its detection.
- Macro AST has also been detected by assaying the serum after refrigeration at 4°C for between 3 and 5 days and showing a progressive loss of AST activity. This cold storage method has been validated and has the advantages of low cost, reliability and practicality.

Useful additional reading

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Notes

These recommendations were produced following a recent audit on macroanalytes with an emphasis on macroprolactin carried out by Dr Danni Fan, Principal Clinical Scientist, East Kent Hospitals University NHS Foundation Trust on behalf of the Thames Biochemistry Audit Group.

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The findings of the audit should be available on the audit section of the ACB website.

Peter West FRCPath

Fellow of the ACB

Retired Consultant Clinical Scientist

Chair of the Thames Biochemistry Audit Group

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