Hyperprolactinaemia (hyperPRL) is the presence of too much prolactin (PRL) in the blood and is the most common endocrine disorder of the hypothalamic-pituitary axis. The presenting symptoms depend on the sex of the patient; in women there is typically oligomenorrhoea/amenorrhoea and galactorrhoea, sexual disorders and infertility, and in men decreased libido and gynaecomastia. Fast and accurate diagnosis of hyperPRL is essential to enable the disorder to be treated promptly and to restore the patient’s quality of life. Pregnancy and certain neuroleptic drugs that reduce dopaminergic effects on the pituitary can be responsible for hyperPRL and these causes can be ruled out through comprehensive history taking and simple tests. Where neither is implicated, a prolactin-secreting pituitary adenoma (prolactinoma) is usually suspected. Diagnostic imaging techniques can be used to confirm some adenomas, but small adenomas will not be detected. However, imaging is not specific, so cannot be relied upon as indicating true hyperPRL, rather than macroprolactinaemia. In addition 15-26% of patients who appear hyperprolactinaemic according to quantitative serum PRL immunoassay. The symptoms of hyperPRL can occur. Their presence cannot therefore be relied upon as indicating true hyperPRL, rather than macroprolactinaemia. In addition 15-26% of patients who appear hyperprolactinaemic (PRL >700 mIU/L) actually have raised levels of macroprolactin rather than the bioactive PRL monomer in the macroprolactin complex than in true hyperPRL, but they can occur. Their presence cannot therefore be relied upon as indicating true hyperPRL, rather than macroprolactinaemia. In addition 15-26% of patients who appear hyperprolactinaemic (PRL >700 mIU/L) actually have raised levels of macroprolactin rather than the bioactive PRL monomer [1]. If routine screening for macroprolactin and estimation of monomeric PRL levels became the norm in European laboratories, this would help ensure that further investigations and treatments for hyperPRL were only given to the appropriate patients [1].

Several forms of prolactin

In serum, PRL exists in several forms, as a bioactive monomer with a molecular mass of 23 kDa, and in two higher molecular mass forms - big prolactin (40-60 kDa) and big-big prolactin (80-100 kDa). In healthy individuals, approximately 85% of circulating PRL is in the bioactive monomer form. However in hyperPRL, the level of any or all of these forms can be raised and their relative proportions can vary considerably.

Detection of hyperprolactinaemia

Dopamine agonists (bromocriptine, pergolide, cabergoline) are very effective in restoring menses/gonadal function in patients with hyperPRL and, in the case of prolactinoma, reducing tumour size. With prolactinoma, pituitary surgery is also an option. Macroprolactinaemia (hyperprolactinaemia due to excess of macroprolactin) is responsible for hyperPRL in up to 25% of the cases, with no need for further investigation or treatment. HyperPRL is typically identified through a combination of clinical symptoms and a quantitative serum PRL immunoassay. The symptoms of hyperPRL are less common in macroprolactinaemia than in true hyperPRL. HyperPRL is usually suspected. Diagnostic imaging techniques can be used to confirm some adenomas, but small adenomas will not be detected. However, imaging is not specific, so cannot be relied upon alone for diagnosis; lesions indicative of adenoma can be detected in approximately 10% of the normal population.

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are more likely to react to macroprolactin than others. As macroprolactin has a longer half life and is therefore cleared from the blood circulation more slowly than monomeric PRL, those immunoassays that react strongly with the macroprolactin complex may report high levels of PRL. It is advisable that laboratories test samples with elevated immunoreactive PRL for macroprolactin and to determine the monomeric PRL component that is active in vivo.

**PRL immunoassays and macroprolactin**

Some manufacturers have developed PRL assays that react less strongly with macroprolactin. This is confirmed by several studies, which have measured the variance in this reactivity to macroprolactin. In one study by Smith et al, 10 macroprolactinaemic sera were circulated to 18 laboratories and the samples tested for PRL using the nine most widely available immunoassay systems [1]. The serum PRL levels reported for the immunoassays varied by 2.3 to 7.8-fold between the highest and lowest estimations [Figure 2]. The highest serum PRL levels were reported with the Elecsys assay (Roche) (mean range, 828-4604 mIU/L) Lower values were reported by DELFIA (Wallac, Inc) (range, 743-4133 mIU/L), Immuno-1 (Bayer Corp, 640-3690 mIU/L), AxSYM (Abbott, 452-2982 mIU/L) and Architect (Abbott, 290-1189 mIU/L), Centaur (Bayer Corp, 243-947 mIU/L) and Access (Beckman Coulter, 228-940 mIU/L) systems [1].

Reactivity of assays

Immunoassays that react only with the bioactive monomeric PRL component are most desirable because they will reduce misdiagnosis of apparent hyperPRL related to macroprolactin, which is not bioactive in vivo. The reactivity of immunoassays to macroprolactin varies markedly. Although the Access (Beckman Coulter) and Centaur (Bayer Corp) immunoassays did not give normal PRL levels for all of the 10 macroprolactinaemic samples tested in the Smith et al study, they gave a reading above 700 mIU/L for only one sample [1]. In contrast, the ACS:180 system (Bayer Corp) gave values in excess of 700 mIU/L for two of the macroprolactinaemic samples, the Immulite 2000 (DPC) for four, the Architect (Abbott) for seven, and the AxSYM (Abbott) and the Immuno-1 (Bayer Corp) for eight. The DELFIA (Wallac, Inc) and Elecsys (Roche) assays gave readings in excess of 700 mIU/L for all ten samples. It is therefore important that laboratories are aware of the reactivity of their assays with macroprolactin and take precautionary steps to identify macroprolactin in samples with elevated PRL levels.

The variable immunoreactivity of assays is due to a number of factors. The assay antibodies clearly have a major influence on immunoreactivity, probably related to the different epitopes on PRL with which they react and the availability of these epitopes on the macroprolactin complex [1]. However, other factors are also involved because the same pair of antibodies coupled to different solid phases and signal generating systems show different reactivity with macroprolactin [2], and incubation time has also been shown to be directly related to reactivity with macroprolactin [3]. Although some assays react with macroprolactin to a lesser extent, all assays react with it to some degree, so it is sound practice to screen all hyperPRL samples for macroprolactin using other techniques. If the site required for recognition is occupied by the endogenous IgG in the macroprolactin complex the assay will not recognise macroPRL even when it is present in large amounts [1].

**Additional tests to detect and quantify macroprolactin**

There are several methods for detecting and quantifying macroprolactin in hyperPRL samples. The most widely applied techniques are gel filtration chromatography and polyethylene glycol (PEG) precipitation.
Gel filtration chromatography (GFC)

GFC is the reference method for the detection of high molecular mass forms of PRL and the quantification of monomeric PRL, but it is a time-consuming and expensive technique that requires a considerable amount of technical skill.

Precipitation with PEG

Even if it is neither perfectly specific nor sensitive, this is a simple and reliable screening test in which macrolactin present in the sample is precipitated by high concentrations of PEG. The recovery of PRL from serum after PEG precipitation is low when macrolactin is present, and the percentage recovery is proportional to the relative amount of macrolactin present. It has been recommended that the PRL concentration after PEG precipitation be reported as a measure of the bioactive serum PRL which can be compared with a reference range determined by PEG precipitation in a normal population [4]. The PEG precipitation technique has been successfully applied to a number of commercial immunoassays for PRL and many manufacturers have issued guidance notes on the use of PEG precipitation with their assay. In some systems PEG precipitation enhances or contributes to the PRL signal, resulting in PRL recoveries >100%. In most of these instances the problem can be overcome by simply adjusting the decision limits for the percentage recovery of PRL, or by diluting the supernatant after PEG precipitation to reduce the interference [5].

It is critical that laboratories are aware of the reactivity of their individual assay with macrolactin because some react more strongly than others and a 2.3 to 7.8-fold variance has been reported, between the highest and lowest total PRL estimations of different assays [1].

While there are no commercial quality assurance materials containing known proportions of macrolactin and monomeric PRL [5], hyperPRL samples should be screened for macrolactin [5, 6]. A technique that detects the presence of macrolactin and also estimates the concentration of monomeric PRL should be used, because some macrolactinaemic patients may also have elevated monomeric PRL of pathological origin. PEG precipitation or GFC fulfil these criteria.

New screening protocols

The routine use of PEG precipitation to differentiate macrolactinaemia in hyperprolactinaemic samples is cost-effective and can alter management in up to 20% of hyperprolactinaemic patients. This is shown by an audit of hyperPRL samples routinely screened for macrolactin using PEG precipitation over a period of five years [6]. The audit found that macrolactin was entirely responsible for hyperPRL in 22% (435) of the 2,089 samples screened by one centre. Further analysis revealed that the percentage of hyperPRL explained by macrolactin was similar across all levels of total PRL >700mIU/L.
Before routine screening for macroprolactin, most macroprolactinaemic patients had undergone pituitary imaging and were treated with dopamine agonists. Even when the costs of routine screening for macroprolactin were taken into account, screening resulted in substantial savings through a 15-17% reduction in imaging requests and prescription of dopamine agonists [6].

Improving healthcare resources

Manufacturers of PRL immunoassays are beginning to formulate assays which react less strongly with macroprolactin, but because the structure of macroprolactin is so variable it is unlikely that there will be immunoassays that do not react with any form of macroprolactin. A number of simple screening tests are available for the detection of macroprolactin and estimation of monomeric PRL, and although further work is required to improve their sensitivity and specificity even more, it is advisable to use them to routinely screen for macroprolactin in hyperPRL samples.

It is now recommended that clinical biochemistry laboratories providing a routine service for the measurement of serum PRL follow the procedure in Table 1 [5]. There is no doubt that by introducing such screening protocols to detect hyperPRL due to macroprolactin, clinical laboratories could contribute to improving patient care and the best use of health service resources.

References


The author

Dr Véronique Jarrige
European Scientific Manager, Immunodiagnostics, Beckman Coulter Europe, Nyon, Switzerland.