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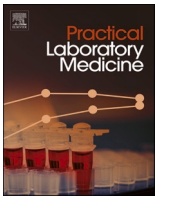

The validation of macroprolactin analysis by polyethylene glycol precipitation using Fujirebio Lumipulse

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Hyperprolactinemia is one of the most common problems in clinical endocrinology. Hyperprolactinemia is characterized by an overproduction of prolactin by the anterior pituitary lactotropic cells, often caused by a prolactinoma [1]. Prolactinomas account for 40% of all pituitary tumors [2]. Prolactin is a hormone that acts on the mammary gland to induce and maintain lactation, therefore the concentrations found in premenopausal women are higher compared to the concentrations found in men and postmenopausal women. Hyperprolactinemia in women results in the induction of lactation and irregularity or loss of the menstrual cycle. In men it causes headache and a decreased libido or sexual potency. In both men and women it could also result in vision loss and visual field defects when the tumor grows large enough to exert a mass effect [3]. Case detection is performed by measuring the prolactin serum concentration. When this concentration is out of reference range on the higher side, a MRI-scan is performed. Also, the patient could be sent to an optometrist to analyze their eyesight. Hyperprolactinemia can be treated with dopamine antagonist since dopamine is the main inhibitor for prolactin secretion [4].

Prolactin circulates through the body in different forms. Most often prolactin is seen as a monomer (23 kDa). Less often it is seen as a dimer of the monomeric form; big-prolactin (48 kDa) (<10%) or as complexes of the monomeric form bound to immunoglobulins, most often IgG's; big-big-prolactin or macroprolactin (>150 kDa). Big prolactin and big-big prolactin have very low biological activity compared to the monomeric form and do not contribute to hyperprolactinemia.

[5]. All commercially available immunoassays detect total prolactin. Patients with high concentration of macroprolactin (macroprolactemia) can be falsely diagnosed with hyperprolactinemia. The ratio macroprolactin, prolactin varies between individuals. To ascertain whether the prolactin levels are a true reflection of a hyperprolactinemic state, one must make sure to rule out macroprolactemia. Size exclusion chromatography (SEC) is recognized to be the "gold standard" for detection of macroprolactin. This technique is very time consuming and expensive and therefore polyethylene glycol (PEG) precipitation is often used to remove macroprolactin from the serum. When PEG is added to the serum it increases the chemical potential of macroprolactin and

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big-prolactin because the chemical potential exceeds the level of a saturated solution [6]. PEG precipitates the prolactin bound to immunoglobulin G but the monomeric form remains measureable in the supernatant.

The use of PEG precipitation for the detection of monomeric prolactin has been successfully validated for multiple immunoassays, e.g. Architect, Auto-Delfia, Cobas, Dimension Vista, Immulite, Kryptor [7].

Here, we present data on the use of PEG precipitation in hyperprolactinemic samples using a fully automated two-step immunoassay EIA (Fujirebio Lumipulse *G* System [Fujirebio, Inc., Tokyo, Japan]). The Lumipulse G prolactin assay was traceable to the Auto—Delfia immunoassay (Auto-Delfia, Perkinelmer, Turku, Finland). To assure the correct interpretation, size exclusion chromatography was performed in all samples (Superdex 75 10/300 GL, 30 cm × 10 mm, 13 μm average particle size, GE Healthcare). For the Lumipulse assay the serum samples were diluted 5 times with 0.9% NaCl. The diluted samples were again diluted twice with polyethylene glycol and vortexed for 10 s. Finally, the samples were centrifuged at 10800 g (14000 rpm) for 6 min. Both the supernatant and the originally diluted samples (5 × 0.9% NaCl) were measured and the recovery for prolactin was determined. For Auto-Delfia measurements, a slightly different protocol was used as the serum was not pre-diluted prior to PEG precipitation.

In total, 56 residual patient samples were analyzed for macroprolactin prior to and after PEG precipitation. The samples were exchanged between the participating centers in multiple batches and analysis were performed on multiple days. Passing-Bablok regression analysis was used. For non-PEG treated samples the mean proportional bias found in 3 different runs between the Lumipulse assay and the Auto-Delfia assay was +29% (95% CI 21%–42%) for the Lumipulse assay. No absolute bias was found, Fig. 1a. No proportional bias or absolute bias was found for PEG-treated samples in 3 different runs, Fig. 1b.

Monomeric prolactin, after PEG treatment, measured on the Lumipulse G as well as on the Auto-Delfia system correlated with the levels determined by SEC. No proportional or absolute bias was found, see Fig. 2. Also, the percentage of recovery after PEG treatment determined on the Lumipulse G system correlated with the fraction of macroprolactin measured with SEC ($R^2 = 0.53$) and even better with the sum of macroprolactin and big-prolactin ($R^2 = 0.61$).

Two samples behaved differently after PEG precipitation for both the Lumipulse assay and the Auto-Delfia assay compared to the SEC Auto-Delfia assay. These two samples had monomeric prolactin levels ≤52 U/L when measured with the Lumipulse but these levels were elevated when measured after SEC, ≥73 U/L. See Fig. 2a. Both of these measurements are outside of the 95% confidence interval. A similar pattern was found in both samples when measured after PEG precipitation with Auto-Delfia, Fig. 2b.

One might expect that these two divergent samples had very high pre-PEG concentrations of (macro)prolactin, resulting in either suboptimal or saturated precipitation. This would have resulted in a higher prolactin concentration since it still contains macroprolactin and big prolactin. This was not the case as none of these two samples were within the top 10 op highest pre-PEG prolactin concentrations. Also, these samples were not characterized by the highest fraction of big-big or big prolactin, nor had outstanding recovery fractions. For now we have no clear explanation why these samples behave differently in PEG precipitation size exclusion chromatography but it may be the fact that the macroprolactin forms in these samples were of IgA type rather than IgG as IgA forms are only partially participated [8,9]. Also, polymeric aggregates of highly glycosylated monomers only partially precipitate by use of PEG [9].

Both in the Erasmus Medical Centre and Maastricht University Medical Centre a prolactin threshold of 80% after recovery is used. If the prolactin recovery is >80%, the macroprolactin result is reported as 'negative' and the prolactin result will not be corrected. Sample A and B have a prolactin recovery of <80% for both the Lumipulse assay and the Auto-Delfia assay. Also a recovery <80% is found after SEC, see Table 1. Thus, both samples would have been reported as 'positive' for macroprolactin in all cases.

In a healthy population the macroprolactin concentration is expected to be low and to be distinguished in the background noise of the assay [10]. To confirm this we determined the prolactin recovery in 34 residual patients samples from patients who did not have a

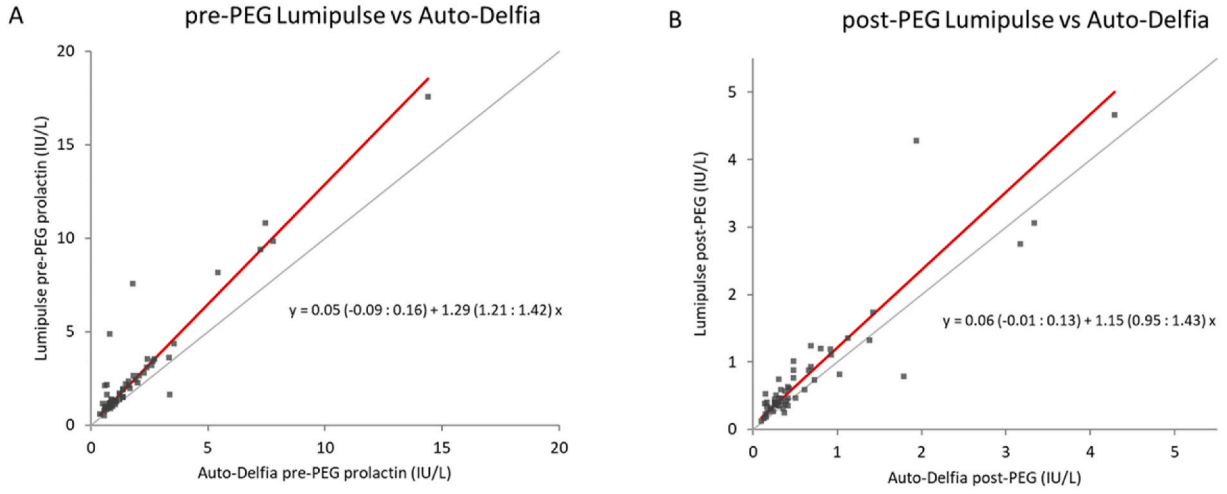

Fig. 1. Passing-Bablok method comparison between the Lumipulse G system Fujirebio prolactin assay and the Auto-Delfia prolactin assay prior to PEG precipitation (a) and post PEG precipitation (b).

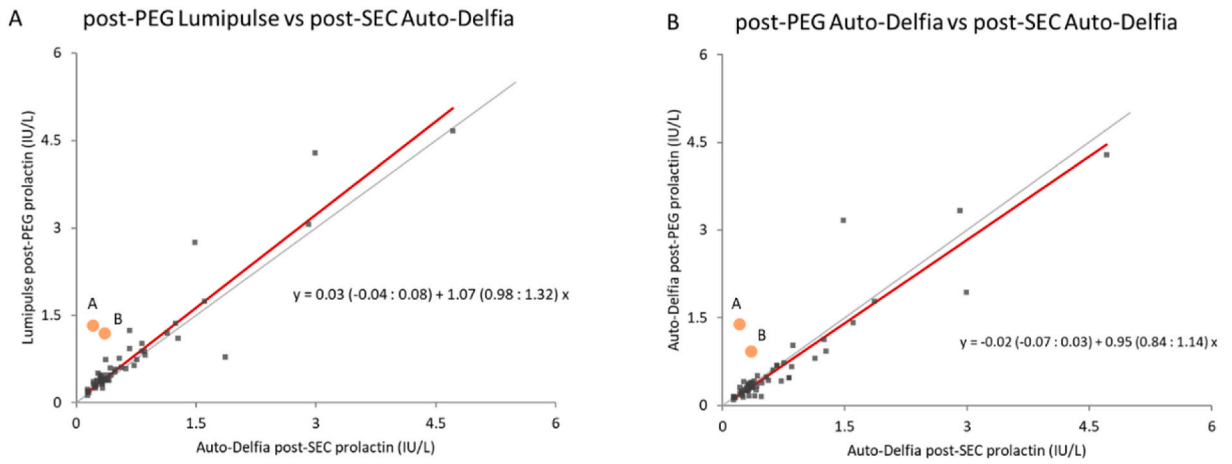

Fig. 2. Passing-Bablok method comparison between the post-PEG prolactin levels measured on the Lumipulse (a) and the Auto-Delfia (b) and post size exclusion chromatography measured on the Auto-Delfia.

Table 1
The recovery of sample A and B for the post-PEG Lumipulse and Auto-Delfia assay and the percentage of monomeric prolactin compared to total prolactin measured after size exclusion chromatography by Auto-Delfia.

Sample	Prolactin recovery Post- PEG Lumipulse	Prolactin recovery Post-PEG Auto-Delfia	Monomeric prolactin Post-SEC Auto-Delfia
A	52%	39%	8%
B	38%	34%	15%

diagnosis for hyperprolactinemia. The average recovery found (85% $^{\pm}$6%) confirmed our expectations and the threshold of 80% currently used.

Our results show that the Fujirebio Lumipulse G System Prolactin assay can be used prior and post-PEG precipitation for measurement of monomeric prolactin for case detection of hyperprolactinemia. The samples measured post-PEG with the Lumipulse assay correlated with the gold standard, size exclusion chromatography. Although these assays did correlate, care must be taken because two samples behaved differently and size exclusion chromatography should be considered upon non-normalization.

Declaration of competing interest

There is no conflict of interest.

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