Letter to the Editor

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Assessment of intraindividual agreement in prolactin results after post-polyethylene glycol precipitation test for the estimation of macroprolactin. Should the precipitation procedure be repeated in the same patient?

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To the Editor,

Patients with high serum prolactin are usually screened for macroprolactinemia, which is a benign condition but may lead to a potential misdiagnosis of hyperprolactinemia. Macroprolactin (MCPRL), defined as a complex of monomeric prolactin and IgG antibodies (>150 kDa), interferes with most immunoassays to different degrees. Predominant presence of macroprolactin is commonly assessed by polyethylene glycol (PEG) precipitation. PEG addition unspecifically precipitates molecules with a molecular weight >100 kDa, leaving monomeric prolactin free in the supernatant to be measured [1]. To our knowledge there are no guidelines about recommendations regarding whether the PEG-precipitation test should be repeated in different samples of the same patient. We aimed to assess the degree of intra-individual concordance in the PEG-precipitation results, in order to acquire useful knowledge to establish hyperprolactinemia retest laboratory algorithms.

We performed a retrospective study with data extracted from the laboratory database from June 2016 to January 2020, in a hospital attending requests from primary and secondary care. Patients with prolactin concentration above the upper reference value (men: 19.4 µg/L; women: 26.5 µg/L) and <150 µg/L were included in the study according to recommendations of Clinical Guidelines [2]. In our laboratory post-PEG PRL reference interval for monomeric prolactin (men: 2.7–14.9 µg/L; women: 4.0–20.4 µg/L) was calculated according to Beltran et al. [3]. All statistical analyses were performed using the IBM SPSS software package, version 24 (IBM Corp., Armonk, NY, USA).

Total and post-PEG prolactin were determined by immunoassay (Abbott Architect, Chicago, IL, USA) calibrated against the WHO Third International Standard for prolactin (84/500). Total coefficient variation was 4.7 and 3.5% at mean prolactin concentrations of 7 and 35.8 µg/L, respectively; sensitivity was 0.6 µg/L. Interassay precision of the PEG precipitation method assessed by a pool of sera with a mean total prolactin concentration of 36 µg/L and a mean post PEG recovery of 73.1% was 4.4%.

For the PEG-precipitation test, an equal volume of serum and 25% PEG solution (25 g of PEG 6000 in 100 mL of NaCl 0.9%) was mixed for 40 s with a vortex and centrifuged at 3,000 rpm for 30 min at 4 °C. Prolactin concentration was measured in the supernatant and corrected for the dilution factor (1:2). Recovery percentage was calculated using initial prolactin (PRL) and post-PEG PRL concentration (% Recovery = (post-PEG PRL/PRL) × 100). According to our internal algorithms, we considered a post-PEG recovery <50% as positive for macroprolactinemia.

In the last four years, a total of 5126 PEG-precipitation tests, corresponding to 3,931 individual patients, have been performed in our laboratory; 495 of 3,931 (12.6%) patients tested positive for MCPRL. Among those, 63
patients (12.7%) had an elevated monomeric prolactin and 432 (87.3%) had a monomeric prolactin within the normal range. Of the 3,436 patients with a post-PEG recovery >50%, 3,105 (90.4%) had elevated monomeric prolactin and 331 (9.6%) had it within normal range. The odds ratio of a high monomeric prolactin in patients with negative MCPRL was 64.3 (95% CI: 48.2–85.7; p<0.0001) with respect to those with positive MCPRL.

To assess the within-subject variability of the PEG procedure, we analyzed results obtained in the 790 patients with two or more measurements. Results thereof are shown in Figure 1. Only 30 patients showed discordant MCPRL results when using a single cutoff (<50%). Those were reclassified considering recoveries between 40 and 60% to be borderline; and truly positive and negative MCPRL those with recoveries <40 and > 60%, respectively. After reclassification 10 out of 30 patients had all determinations in the borderline zone; 10 patients had all determinations within the borderline and the negative zone (all >40%); seven patients had all determinations within the borderline and the positive zone (all <60%). The imprecision of the PEG precipitation procedure could have accounted for some of these discrepancies. Only three patients had fully discordant results (<40 and >60%). Possible causes of PEG interferences in these three patients were ruled out (such as presence of a serum IgM monoclonal protein, amyloidosis, renal failure) in these patients.

There are no uniform consensuses that indicate which patients’ samples should be screened for MCPRL. In our study we found a prevalence of positive MCPRL of 12.2% taking a cut-off of 50%. This is in line with what is published in the literature that ranges between 4 and 40% [1, 3, 4]. The prevalence of positive MCPRL depends on the immunoassay used, since antibodies from different manufacturers have different cross reactivities for MCPRL. We used the Architect Abbott Diagnostics immunoassay, which according to other authors has an intermediate cross reactivity for MCPRL [4–6].

The prevalence of positive MCPRL also depends greatly on the cut-off used. There is no general agreement that establishes a single cut-off but the most commonly used are 40 and 50. Most authors consider a positive MCPRL if recoveries <40% and a negative macroprolactin if recoveries >60 [5–7]. However, more than the fact that a subject is classified as being MCPRL positive or negative, the important thing to the clinician is whether the monomeric prolactin calculated post-PEG is within the limits of normality, since this measurement will give information about whether bioactive prolactin is within the normal range or if it is high [3, 8]. In our study the odds ratio of a high monomeric prolactin in patients with negative MCPRL is 64.3 with respect to those with positive MCPRL and both post-PEG recovery and serum prolactin concentrations were independent predictors of high monomeric prolactin.

We have 790 patients with multiple by PEG precipitation test in the four-year period studied (Figure 1). The concordance of repeated measurements of MCPRL in the same patient was very high: 96.2% of the patients had concordance (either a positive or a negative MCPRL) in all their determinations, but 30 patients (3.8%) had discordant results, in accordance with others [7, 8]. If we consider, like others, that a recovery between 40 and 60% is a gray or borderline zone instead of considering a single cut-off point of 50, only three out of 790 patients (0.38%) would have been discordant with results below 40% and above 60%.

Performing a PEG precipitation in all samples with prolactin above the upper reference range leads to a high workload in the laboratory, due to repeatedly high prolactin results in the same patient. In order to reduce the

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**Figure 1:** Patients with 2 or more post-PEG prolactin tests. Prevalence of positive, negative and discordant macroprolactin results.
total amount of macroprolactin measurements some authors have suggested rationalizing the precipitation procedure by increasing the prolactin concentration from the upper limit of normality to higher values [7]. However, with these criteria some patients with macroprolactinemia would be overlooked [4]. The persistence of significant amount of MCPRL in the same patient over time has been previously reported [9, 10]. After an initial assessment of whether the monomeric prolactin concentration is normal or elevated in a given patient (using method-specific reference ranges), successive measurements of prolactin in the follow-up of the same patient yielding concentrations equal or lower than that previously reported should not systematically generate a repeated post-PEG precipitation test owing to the high intraindividual concordance. We suggest that repeated assessment should be limited to cases in which serum prolactin concentration significantly increases in comparison with the previous value.

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Availability of data and materials: The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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