Prolactin Immunoreactivity in Rheumatoid Factor-Containing Specimens: Is it Prolactin, Macroprolactin or Assay Interference?

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Abstract: Specimens containing Rheumatoid factor (RF) were examined for possible interference in Prolactin (PRL) measurement. PRL levels following polyethylene glycol (PEG) treatment were significantly lower than in untreated RF-positive samples ($p<0.001$; n=43) and spurious hyperprolactinemia was indicated in 7 percent. Gel filtration chromatography suggested that the PRL-like immunoreactivity was from assay interference rather than a macroPRL complex and that PEG treatment successfully removed the interference. It is important that laboratories consider explanations other than macroPRL when interpreting PRL results following PEG treatment.

INTRODUCTION

Polyethylene glycol (PEG) precipitation is used in clinical laboratories to remove large molecular mass substances that react in immunoassays. These include macro complexes (for example macroprolactin), heterophile antibodies and rheumatoid factors (RF) that may adversely affect some immunometric assays in a complex and unpredictable manner [1, 2]. Rheumatoid factors have been identified as the cause of false elevations in several immunoassays including thyroxine, Troponin I, sex-hormone binding globulin and carbohydrate antigen 19-9 [3-6]. There is, however, a paucity of published data and few manufacturers' immunoassay data sheets include information on RF interference, which is necessarily specific to a method, not an analyte.

Raised prolactin levels are associated with some autoimmune diseases including systemic lupus erythematous (SLE) and rheumatoid arthritis (RA) [7] and with other pathologies [8]. It is possible that apparent hyperprolactinemia detected in these patients could be due to the presence of macroPRL or to interfering factors. A high frequency of macroprolactinemia has been reported in SLE patients [9]. In patients selected for active RA, one study [10] reported hyperprolactinemia associated with increased levels of RF and detection of IgG-PRL but another [11] detected neither hyperprolactinemia nor macroPRL. In our study we have selected samples containing raised levels of Rheumatoid Factor. We used PEG treatment to investigate spurious hyperprolactinemia, and gel filtration to examine whether macroPRL was a likely cause and whether PEG removed any interfering factors.

SAMPLES AND METHODS

The RF-positive group comprised 43 samples containing raised RF levels (>100 IU/ml) selected from residual specimens previously submitted for routine diagnostic measurement of RF. The concentrations of RF ranged from 107 – 10,500 IU/ml (median 350 IU/ml). The reference interval for the RF assay is <30 IU/ml. RF was measured by immunonephelometry using the N Latex RF assay (Dade Behring, Marburg GmbH).

The reference group comprised 206 healthy volunteer specimens that were the most recently collected samples from a larger group reported previously [12]. PEG treatment and gel filtration chromatography (GFC) had shown that 4 of these samples contained macroPRL. We measured RF levels in these 4 and in a random selection of the rest. RF levels were low in all samples tested; ≤ 12 IU/ml, n= 21.

PRL immunoreactivity (irPRL) was measured using the immunoenzymatic Prolactin assay on an Access2 analyzer (Beckman-Coulter Inc, Fullerton, USA). PEG treatment was performed by mixing equal volumes of the RF-positive samples and PEG 6000 (250 g/L buffer) as described previously for the healthy volunteer samples [12]. The PRL concentration in the PEG supernatant was corrected for dilution (monomeric PRL) and also expressed as a percentage of the pre-treatment level (total PRL) to determine the percent PRL recovery. The reference intervals for total and monomeric PRL levels are 80-530 mIU/L (females) and 80-350 mIU/L (males). For linearity studies, samples were pre-diluted in the manufacturer's diluent. The intra- and inter-assay CVs for PRL assays were < 2 % and <4 % respectively at 107 mIU/L and 840 mIU/L PRL and for the PEG test were <3 % and <5 % respectively for samples with low (59%) and normal (104%) PRL recovery.

Gel filtration chromatography was used to examine whether RF-positive samples that showed low post-PEG PRL recovery contained macroPRL, defined as irPRL in an elution position corresponding to a molecular mass of approximately 150kD. The RF-positive group comprised 5 of the 9 samples with PRL recovery values of <60% that had sufficient volume remaining, including 2 of the 3 with apparent hyperprolactinemia. We performed FPLC over Superdex 75 as previously described [13] on 0.5 ml sample aliquots and measured the PRL content in the 0.3ml fractions immediately (Access PRL assay). The elution volume of the

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high molecular mass irPRL peak was divided by that for the monomeric PRL peak and this ratio compared to those calculated for a group of macroprolactinemic samples. The control "macroPRL" samples were 40 hyperprolactinemic specimens that had been examined previously by GFC [12,13] and shown evidence of a large molecular weight peak of irPRL.

Data were analyzed non-parametrically using Wilcoxon Rank tests for median comparisons, Spearman's rank test for correlation and Fisher's exact test to compare proportions.

RESULTS

Frequency distributions for the percent PRL recovery in the RF-containing and healthy volunteer samples were markedly different as shown in Fig. (**1**).

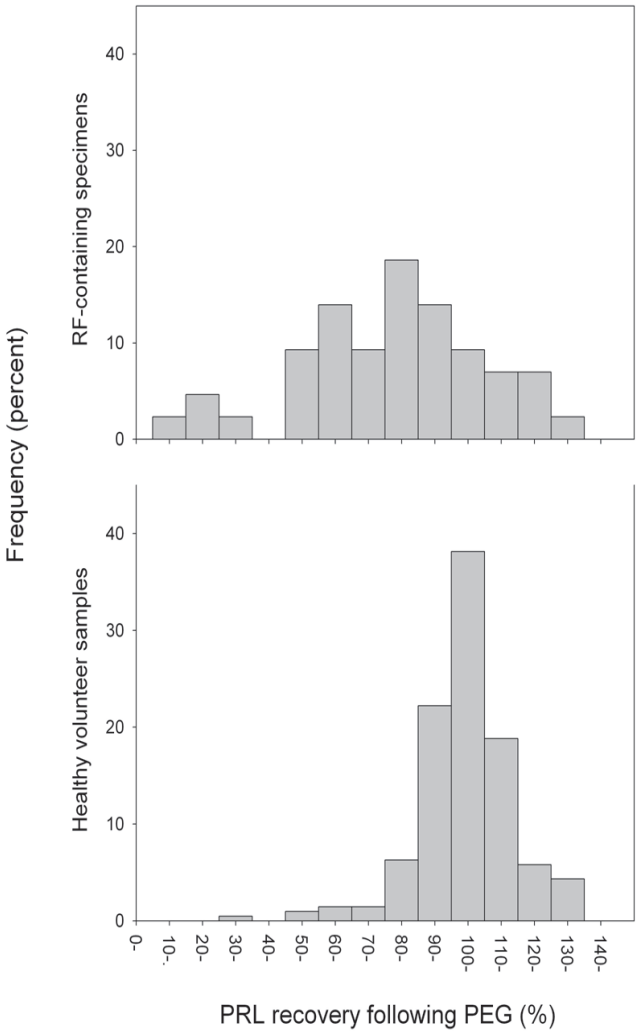

Fig. (1). Distribution of PRL recovery values in Rheumatoid factor-containing specimens (n=43; upper panel) and reference samples from healthy volunteers (n=206, lower panel).

The median PRL recovery was 81% in the RF-containing specimens (range 10-131%; inter-quartile range 39.4%) compared to 104% (range 31-136%; inter-quartile range 14.5%) in the reference group ($p<0.001$). There was a tendency for the percent PRL recovery to correlate with the concentration of RF ($r = -0.276$, $p=0.07$). Using a previously defined cut-off value (<60% PRL recovery) for macroPRL detection [12], less than 2 percent of the reference specimens met this criterion compared to 21 percent in the RF-positive group ($p<0.001$).

The concentrations of total PRL in the 43 RF-positive samples ranged from 90 to 1444 mIU/L (median; 213 mIU/L) and following PEG treatment the values were lower ($p<0.001$) at 72–896 mIU/L (median 148 mIU/L). One sample had raised levels before (1444 mIU/L) and after treatment (896 mIU/L) suggesting a moderately elevated PRL augmented by macroPRL or assay interference. In 3 specimens (7 percent), however, there was apparent hyperprolactinemia, with total PRL levels of 1240, 1380 and 468 mIU/L (the latter a male) falling after PEG treatment to 286, 136 and 96 mIU/L respectively. Serial dilution of the first two of these samples (there was insufficient to test the third) resulted in decreasing total PRL concentrations, reaching levels of 340 and 420 mIU/L respectively after a 1 in 20 dilution. Such non-linearity on sample dilution is a common pattern in positive assay interference.

Following GFC, for all 5 RF-positive samples irPRL was detected in the column void volume and not in the elution position expected for macroPRL. The median elution volume ratio was 0.66; with a range of 0.65 – 0.66. This was significantly lower ($p<0.001$) than the ratio for the putatively macroPRL-positive group (0.73; 0.66-0.75). There was one extreme outlier in this latter group, as shown in Fig. (**2**). The elution volume ratio for this specimen was 0.66 and this subject was found subsequently to have elevated levels of rheumatoid factor (>140 IU/ml).

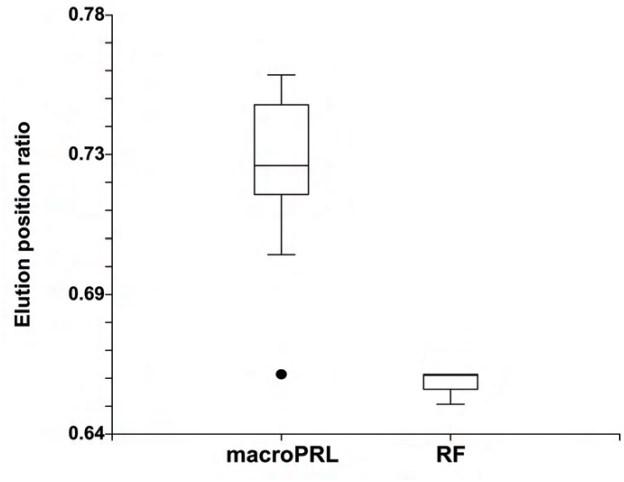

Fig. (2). Box plot (inner fence 1.5; outer fence 3) for 5 RF-positive specimens (RF) compared to 40 samples investigated for macroPRL content (macroPRL). The elution position ratio is the elution volume of the high molecular mass GFC peak divided by that for the monomericPRL peak.

The amount of irPRL recovered after chromatography of the RF-positive specimens was low (26-60 percent) compared to the macroPRL-containing samples (>90 percent). Furthermore, the PRL-like immunoreactivity in the void volume was unstable because it was not detectable on repeat PRL assay following storage of the column aliquots (data not shown). The concentrations of monomeric PRL calculated

from the irPRL in the areas under the curves (AUC) for the monoPRL peaks isolated following GFC, were similar to the PRL concentrations in the PEG supernatants for the 5 samples tested (Table **1**). These results support the efficacy of the PEG test for the reporting of monomeric prolactin levels in RF-containing specimens, in association with method-specific reference intervals.

Table 1. Prolactin Concentrations (Total and Monomeric) in 5 Specimens that Showed Evidence of RF Interference

Total PRL mIU/L	PEG monoPRL mIU/L	GFC monoPRL mIU/L
1240	286	208
1380	136	132
247	146	106
338	102	60
278	170	153

Total PRL is the PRL measured in untreated specimens. The concentrations of monomeric PRL were calculated from both the irPRL in the PEG supernatant, after correction for dilution and from the irPRL recovered in the AUC of the monoPRL peak and the volume of sample gel filtered (Superdex 75).

DISCUSSION

Most laboratories use the non-specific PEG test as a routine screen for macroPRL but it is important to consider alternative interpretations of the result. Low post-PEG recovery may also be caused by endogenous interfering antibodies. We report here that the pattern of PRL recovery following PEG treatment is very different in samples containing RF than in those from healthy volunteers, consistent with an increased incidence of a high molecular mass substance that may be a PRL complex (macroPRL or bigPRL) or an assay interfering factor. Gel filtration of RF-positive samples with low PRL recovery (<60%) indicated that there was no 150 kD-sized macroPRL present in the samples tested. The findings were consistent with an assay interference that is unlikely to be a complex with prolactin.

The RF assay interference in the Access PRL assay appeared relatively common; however interferences do not necessarily cause abnormal results. In our study diagnoses would have been affected in few cases because the total PRL concentrations were within assay reference intervals in 91 percent of samples. The value of the PEG test lies not only in identifying spurious results but in allowing a "true" estimate of analyte concentration, contingent on applicable reference intervals for that method being available. The PEG test itself, however, may be subject to interferences or sample effects. Increasing concentrations of gamma globulins were shown [14] to cause co-precipitation of monomeric PRL, resulting in low PRL recovery and possibly false estimates of monomeric PRL. Rheumatoid factors may affect the PEG test similarly but for the 5 of 9 samples with low PRL recovery that we investigated by GFC, the results were consistent with a large Mol Wt factor able to cause a positive signal in the assay. Furthermore, the monomeric PRL concentrations calculated from the GFC profiles were similar to the PEG supernatant levels.

Awareness of the non-specificity of the PEG and other interference tests is important because there may be underlying pathology. For example, during follow-up of testosterone assay interference, a polyclonal gammopathy and acute myelogenous leukemia was subsequently identified [15]. In a report subsequent to our study [14], an IgG myeloma and a polygammaglobulinemia due to HIV infection, have been identified as a reason for low PRL recovery following PEG treatment, rather than macroPRL presence. From the results we present, it appears that about 1 in 40 samples reported as macroPRL-positive could instead have underlying interference from rheumatoid factors. The prevalence of interference in a PRL assay by RF will be influenced by the source of clinical PRL specimens, for example autoimmune patients, and by the susceptibility of an individual PRL assay to interference from RF. Experienced immunoassayists may be aware of potential RF interference but there is little documentation in the literature and we suggest that manufacturers should be proactive in testing and reporting in their assay data sheets the extent of RF interference.

In conclusion, our results suggest that interference by large molecular mass rheumatoid factors can occur in the Access Prolactin assay and may result in spurious hyperprolactinemia. We suggest that the PEG test can be used to detect the interference and that the resulting concentration is likely to reflect monomeric PRL. The interference may, however, be misinterpreted as macroPRL.

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