A new criteria for screening macroprolactinemia using polyethylene glycol treatment combined with different assays for prolactin

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Abstract. – **OBJECTIVE:** To establish the criteria for screening macroprolactinemia (MP) after Polyethylene glycol (PEG) treatment plus different assays of Prolactin (PRL) in a clinical setting.

PATIENTS AND METHODS: This study recruited 122 patients, and their PRL-elevated serum samples were collected. Twenty-two of the 122 serum samples were treated by gel filtration chromatography (GFC) and PEG precipitation. The PRL content in the eluent and supernatant were tested separately by two analyzers: i2000sr (Abbott Laboratories, Chicago, IL, USA) and E170 (Roche Diagnostics, Basel, Switzerland). The GFC-method (the gold standard) was applied, in order to establish the criteria of screening MP after PEG-method. The MP positive rate and PRL concentrations in 100 PRL-elevated patients were compared between the literature criteria (a PRL recovery of < 40%) and the new criteria.

RESULTS: The detected value of macroprolactin with the new criteria in the i2000sr (a PRL recovery of < 50%) was higher than in the E170 (a PRL recovery of < 60%). The E170 analyzer detected 38 cases of MP in 100 PRL-elevated samples by using new criteria, which was higher than the literature criteria (24 cases) ($p < 0.05$). Therefore, there were 15 samples could not be judged consistently using the literature criteria by the i2000sr and E170 analyzers. There was also a significant difference in PRL concentrations between the two groups ($p < 0.01$). If using the new criteria, the inconsistency between the two analyzers was reduced to 2 samples. There was a very significant difference in the inconsistency between two criteria ($p < 0.01$). The reported values of PRL from the i2000sr and E170 were 27.8 (16.2-42.6) ng/ml and 32.0 (19.6-49.9) ng/ml. There were no significant differences between two analyzers.

CONCLUSIONS: Establishment of different criteria for screening MP by using PEG-method is helpful for the accuracy of PRL determination and its comparability.

Key Words:
Polyethylene glycols/diagnostic, Hyperprolactinemia/diagnosis, Prolactin, Macroprolactin, Gel filtration chromatography.

Introduction

Three molecular forms of prolactin (PRL) exist in circulating blood: (1) unimolecular PRL (also referred to as free PRL, fPRL) with a molecular weight of about 23 kD, (2) big PRL (bPRL) with a molecular weight of 40-60 kD, and (3) macroprolactin (MPRL) with a molecular weight >100 kD[1]. Essentially MPRL, it is a complex form of PRL with its autoantibody[2-4]. MPRL cannot efficiently pass through the capillary wall due to its large size, and binding to receptors is restricted due to spatial factors. Therefore, MPRL is considered to have no physiological activity in vivo[5-6]. Additionally, MPRL accumulates in circulating blood and accounts for a large part of the prolactin in blood due to slow clearance, which results in elevated PRL levels[7,8]. Macroprolactinaemia (MP) is defined as hyperprolactinemia (HP), which is the result of elevated MPRL in circulating blood[9]. MP screenings in patients with elevated serum PRL can effectively prevent misdiagnosis that is caused by elevated MPRL[10].

MPRL levels detected using different commercial kits are inconsistent[7]. Fahie-Wilson et al and others[11-13] performed MP screenings by calculating the PRL recovery after precipitation of MPRL with polyethylene glycol (PEG). The convenient method from Fahie-Wilson is used as the criteria for MP screening in clinical laboratories with a PRL recovery of < 40%[1]. However, significant differences of part PRL results have been found in practical work with different laboratories (or using different analyzers), even when PEG precipitation is carried out for MP screenings in patients with elevated PRL. Thus, we established and applied the criteria for MP screening by using PEG treatment in combination with different PRL assays, in order to investigate the reasons for the significant differences of part PRL results and correction methods.

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Patients and methods

Patients

Sample sources: PRL-elevated serum samples were obtained from 122 patients in our hospital (PRL >25 ng/ml)[14]. The patients were aged between 11 and 47 (median: 33 years old) years old.

PRL analyzers and assay reagents: The PRL analyzers were i2000sr chemiluminescence analyzer (Abbott Laboratories, Chicago, IL, USA) with accompanying PRL assay kit (lot: 35917UI00) (Abbott Ireland diagnostics division, Lisnamuck, Longford, Ireland), and E170 electrochemiluminescence analyzer (Roche Diagnostics, Basel, Switzerland) with accompanying PRL 2nd-generation assay kit (lot: 17236204), QC (Bio-Rad Laboratories, Hercules, CA, USA) (lot: 52470).

PEG solution: 25 g of PEG 6000 (Sigma, St. Louis, MO, USA) was dissolved in 0.9% sodium chloride injection, and diluted to 100ml to obtain a 25% PEG solution.

Methods

PEG treatment of serum samples and calculations of PRL recovery: An equal quantity of 25% PEG was added to 200 µl of the PRL-elevated serum sample. After full mixing, the mixture was centrifuged at 1500×g for 30 minutes, and then the supernatant was isolated for PRL analysis. The PRL recovery was calculated using the following formula: (2×PRL level following PEG treatment/ PRL level before PEG treatment) × 100%[13].

Separation and detection of PRL components: PRL components in the serum were separated on a Sephacryl S-100 HR chromatographic column (GE Healthcare, Pittsburgh, PA, USA) in connection with an Akta Explore protein purifier (GE Healthcare, Pittsburgh, PA, USA). A 0.9% sodium chloride solution was used as the eluent, and the flow rate was 1.0 ml/min. The eluents were collected using a Frac-900 collector (GE Healthcare, Pittsburgh, PA, USA) with tubes (1.2 ml per tube). PRL in eluents were separately assayed on the i2000sr and the E170 analyzers.

Criteria for the assessment of MP following GFC treatment: PRL in eluents was separately assayed on the i2000sr and the E170 analyzers after GFC treatment. MP was considered when the content of MPRL components accounted for more than 50% of the total PRL[15].

Establishment of MP screening criteria following PEG treatment and reporting of PRL: Using MP established by GFC as the gold standard, the criteria for MP screening by PRL recovery after PEG treatment were established. The PRL reporting method used was proposed by Fahie-Wilson at the Pathology Progress Conference in Birmingham in 2000. Serum PRL results in patients with MP were reported as the level after PEG treatment multiplied by 2, and the serum PRL results in patients without MP were reported as the PRL level before PEG treatment.

Statistical Analysis

Serum PRL concentrations were non-normally distributed and therefore were presented as medians (M) and quartiles (QR). Chi square (x^2) tests were used for the comparison of MP detection rates. Rank sum tests of paired data (Wilcoxon tests) were adopted for analyzing differences of PRL concentrations from different analyzers. SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) was used. The level of statistically significant differences was set at $p < 0.05$.

Results

Separation and analysis of PRL Components

PRL contents in eluents of 22 PRL-elevated serum samples were separately determined on the i2000sr analyzer and the E170 analyzer using GFC separation. The values of the PRL components (fPRL, bPRL, MPRL) were detected on the two analyzers with the fPRL values of 0.31 (0.13-0.65) ng/ml (i2000sr) and 0.35 (0.17-0.54) ng/ml (E170) (Z=-0.422, p=0.673), the bPRL values of 0.43 (0.24-0.81) ng/ml (i2000sr) and 0.43 (0.30-0.70) ng/ml (E170) (Z=-0.696, p=0.487), the MPRL values of 1.49 (0.11-2.40) ng/ml (i2000sr) and 0.98 (0.23-1.37) ng/ml (E170) (Z=-2.764, p=0.006).There was a statistical difference of MPRL with a higher value from i2000sr. The assay results of sample A were the main PRL form of MPRL, and of sample B were the main PRL forms of fPRL and bPRL on both analyzers (Figure 1).

Establishment of criteria for MP Screening After PEG Precipitation in Combination with Different PRL Assays

The PRL contents in eluents of 22 PRL-elevated serum samples were separately determined on the i2000sr analyzer and the E170 analyzer after GFC treatment, and the percentages of MPRL components were calculated. Plotting was performed with the percentages of MPRL components obtained on the i2000sr analyzer after GFC as the ordinate (MPRL component of >50% was the gold standard for MP assessment by GFC), and

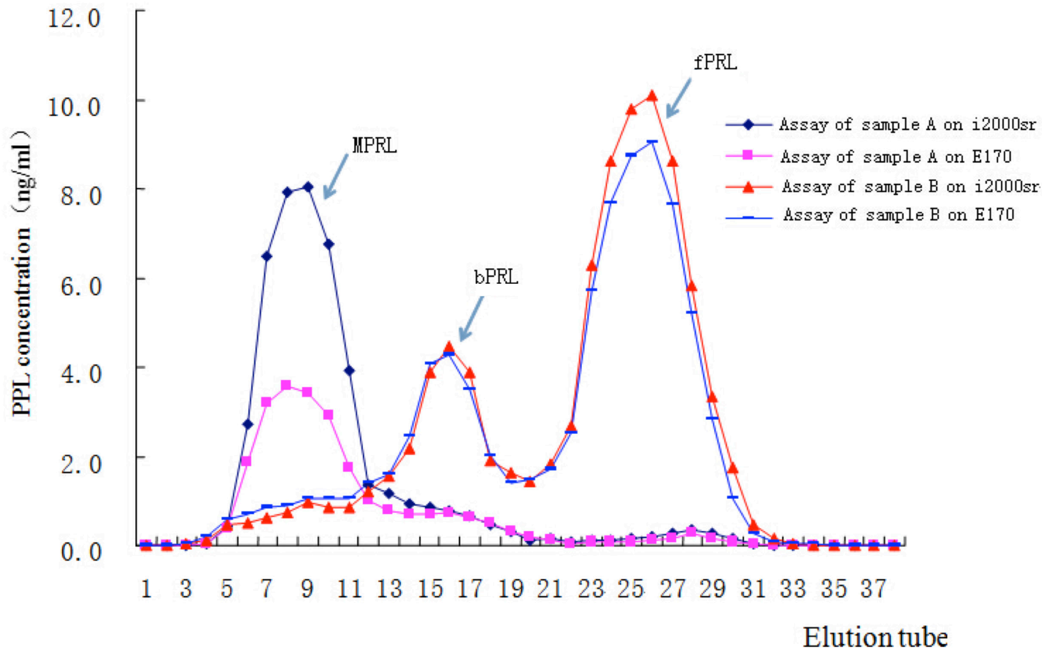

Figure 1. Assay of PRL components on i2000sr and E170 analyzers.

PRL recovery obtained on the same analyzer after PEG treatment as the abscissa (Figure 2).

The criteria for MP screening after PEG treatment in combination with i2000sr chemiluminescence assay were established: the PRL recovery after PEG treatment of <50%. With the criteria, the sensitivity, specificity and accuracy were 100%, 80% and 90.9%. Plotting was performed

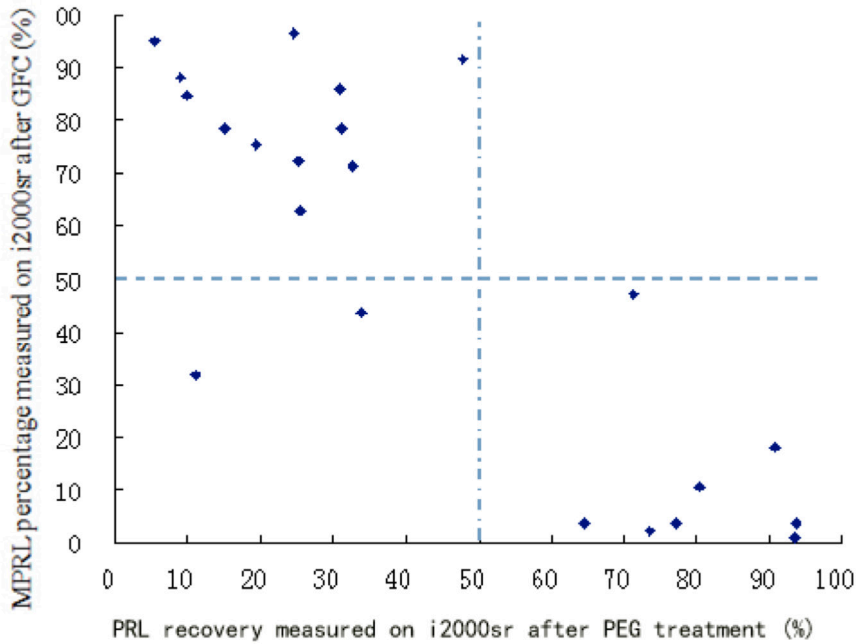

Figure 2. Criteria for MP screening by using PEG precipitation in combination with i2000sr chemiluminescence.

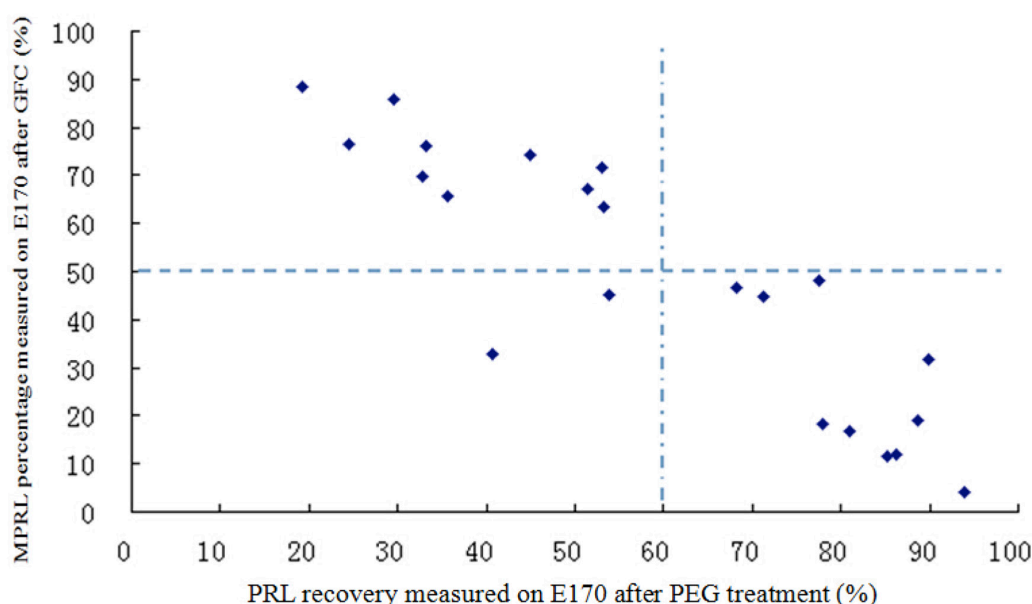

Figure 3. Criteria for MP screening by using PEG precipitation in combination with E170 chemiluminescence.

with the percentages of MPRL components obtained on the E170 analyzer after GFC as the ordinate and PRL recovery obtained on the same analyzer after PEG treatment as the abscissa (Figure 3). The criteria for MP screening after PEG treatment in combination with E170 chemiluminescence assay were established: the PRL recovery after PEG treatment of <60%. With the criteria, the sensitivity and specificity were 100% and 83.3%, and the accuracy was the same as the criteria of PEG in combination with i2000sr assay.

Effects of Screening Criteria on the MP Detection Rate

Among 100 PRL-elevated serum samples after PEG treatment: MP was detected by E170 from 24 samples with a PRL recovery of <40%. When the criteria reported in the literature was adopted as the MP screening criteria, 38 samples were detected and the newly established PRL recovery was < 60%. The detection rate was significantly increased ($x^2 = 4.582$, $p < 0.05$). With the newly established screening criteria (i2000sr <50%, E170 <60%), MP was confirmed in 38 and non-MP in 60 by both analytical systems. The assessment results were consistent in 98 (98%) patients. Namely, a significant increase was demonstrated as compared to the consistency rate against the screening criteria of recovery < 40% [85 (85%)] ($x^2 = 10.865$, $p < 0.01$). The number of samples giving inconsistent results decreased from 15 to 2, which also showed a significant difference ($x^2 = 10.865$, $p < 0.01$) (Table I).

Table I. Effects of different screening criteria on the MP detection rate.

Screening criteria	Total number	Number of patients with MP detected		Number of patients with consistent results		Number of patients with inconsistent results
		i2000sr	E170	MP	Non-MP	
Screening criteria in literature*	100	39	24	24	61	15
New screening criteria*	100	40	38[a]	38[a]	60	2[b]

Note: "*" screening criteria in literature: PRL recovery of <40%; new screening criteria: PRL recovery of <50% for i2000sr, PRL of <60% for E170. "a": p<0.05 as compared to screening criteria in literature; "b": p<0.01 as compared to screening criteria in literature.

Table II. Effects of different serum treatment methods on PRL concentration reports.

Treatment	PRL concentration report (ng/ml)		
	i2000sr	E170	*p*-value
Without MP screening	124.0 (103.9~137.7)	54.4 (39.8~84.7)[a]	<0.01
Screening criteria in literature*	27.8 (16.2~42.6)	54.4 (39.8~84.7)[a]	<0.01
New screening criteria*	27.8 (16.2~42.6)	32.0 (19.6~49.9)[b]	>0.05

Note: "*" screening criteria in literature: PRL recovery of <40%; new screening criteria: PRL recovery of <50% for i2000sr, PRL of <60% for E170. "a": p<0.01 as compared to results on i2000sr; "b": *p*>0.05 as compared to results on i2000sr.

Effects of Different Serum Treatment Methods on the Reported Values of PRL

After PEG treatment, MP was detected by i2000sr, but non-MP by E170 in 15 out of the 100 PRL-elevated samples using the PRL recovery of < 40% reported in the literature as the screening criteria. In these 15 samples, MP screening was performed without PEG precipitation. The concentration of PRL reported by i2000sr was 124.0 (103.9-137.7) ng/ml, which was significantly higher than the PRL reported by E170 [54.4 (39.8-84.7) ng/ml] (Z=-3.408, $p < 0.01$). The recovery of <40% was adopted as the MP screening criteria after precipitation treatment. The concentration reported by i2000sr was 27.8 (16.2~42.6) ng/ml, which was significantly lower than the PRL reported by E170 [54.4 (39.8~84.7) ng/ml] (Z=-3.408 = p<0.01). However, PRL concentrations reported by i2000sr and E170 after precipitation treatment were 27.8 (16.2~42.6) ng/ml and 32.0 (19.6~49.9) ng/ml, when the newly established MP screening criteria were used. Thus, no significant difference were found (Z=-1.079 = p<0.05) (Table II). Differences between PRL concentrations reported by i2000sr and E170 were plotted against different treatment methods (Fig. 4). It was found that the difference between PRL values reported by the two analyzers was close to zero when the newly established criteria were adopted.

Figure 4. Effects of different treatment methods on differences between PRL reported values of i2000sr and E170.

Discussion

In 1980, Whittaker et al[9] reported a HP patient without common clinical symptoms such as amenorrhea, galactorrhea and infertility. Using GFC, it was found that MPRL with giant molecules accounted for the majority of PRL in this patient. In 2000, the United Kingdom National External Quality Assessment Service (UK NEQAS) distributed a MP patient's sample to 70 laboratories, which was analyzed using 21 different assay reagents (methods). The mean PRL values of these methods ranged from 556 to 2055 mIU/L, indicating that measured values of PRL of MP patients varied substantially across laboratories when measured with different methods[15]. Schneider et al[16] also indicated that PRL immunoassay reagents of i2000sr and E170 can still bind to different PRL components, so the interference of MPRL with active PRL cannot be effectively excluded. Additionally, MPRL assay results obtained by i2000sr were significantly higher than those obtained by E170. Therefore, performing MP screening in patients with elevated PRL, and using accurate MP screening criteria can result in great differences between reported values of PRL, whether MP screening is performed in patients with elevated PRL and whether MP screening criteria are accurate are both responsible for the great differences between reported values of PRL.

Establishing MP to separate PRL components by GFC prior to analysis can be defined as a good method. However, this method has several shortcomings including complicated to operate, large time consumption and high cost, which restrict the use of this technique to clinical laboratories. The PEG treatment is used as a routine method to remove MPRL when it reaches a certain concentration, and then the PRL recovery is determined for MP screening. In 1997, Fahie-Wilson et al[17] performed MP screening by PEG in combination with time-resolved fluorescence on WALLAC analyzers and reported that when the PRL recovery of <40%, MPRL accounted for the majority of PRL in blood. Also on WALLAC analyzers, Vieria et al[18] proposed that the MP screening criteria of PRL recovery of <65%. In 2010, McCudden et al[19] proposed PRL recovery of <30% as the criteria for MP screening with PEG treatment, and reported that change of the criteria from PRL recovery between <40% to <30% could increase the specificity from 57% to 95% without reducing the sensitivity. These data indicated that PRL recovery after PEG treatment of <50% should be the benchmark for MP screening by PEG treatment in combination with i2000sr chemiluminescence assay, and PRL recovery after PEG treatment of <60% for MP screening by PEG treatment in combination with E170 chemiluminescence assay. Against the new criteria, the accuracy rates of the two different combinations both reached 90.9%, and no statistically significant differences were found between the reported PRL values.

The main function of PEG treatment is to remove macromolecular MPRL by precipitation. With the international reference preparation (IRP WHO 84/500) available for micromolecular PRL in the supernatant after precipitation, assay results of micromolecular PRL obtained by different analytical systems are consistent. The reported concentrations of PRL with the two analytical systems were 27.8 (16.2-42.6) ng/ml and 32.0 (19.6-49.9) ng/ml using the newly established screening criteria, and no statistically significant difference was found to verify the aforementioned point (p>0.05). Different analytical systems are differently capable of detecting MPRL components in serum before precipitation treatment [7]. Fahie-Wilson et al[17] pointed out that Roche 2nd-generation PRL reagent was less reactive than the 1st-generation PRL reagent. We used Roche 2nd-generation PRL reagent and Abbott PRL reagent to analyze eluents obtained after GFC and found that the former was less reactive to MPRL than the latter (Figure 1). This is the reason why the measured value of PRL by E170 before precipitation treatment was markedly lower than that by i2000sr. According to the recovery calculation formula [PRL recovery following PEG treatment = (2×PRL level after PEG treatment/PRL level before PEG treatment) × 100%], it can be determined that the numerator is constant, and the denominator value obtained by i2000sr is greater than that by E170.

As a result, the MP screening standards for PEG precipitation in combination with i2000sr chemiluminescence are lower than that for PEG precipitation in combination with E170 electrochemiluminescence. With the new criteria, the detection consistency rate of 100 clinical samples on the two analytical systems was significantly increased, and the inconsistency rate was significantly decreased. The reported concentrations of the same samples on different analytical systems were also more consistent. Therefore, physicians and patients can benefit from the new criteria in reducing rates of misdiagnosis, unnecessary treatments, and the cost of treatments.

Conclusions

Establishment of suitable criteria for MP screening by using PEG precipitation depends on the analytical system. Suitable criteria are also essential in getting accurate and comparable PRL assay results, and providing a basis for mutual recognition of PRL results among different laboratories.

Conflicts of interest

The authors declare no conflicts of interest.

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