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Macroprolactinemia

The Consequences of a Laboratory Pitfall

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The objective of this study was to assess the prevalence of macroprolactin, a macromolecule with reduced bioactivity, in hyperprolactinemic patients. Prolactin was measured before and after precipitation of macroprolactin by polyethylene glycol in 306 patients. Only patients with prolactin values >700 mIU/L (n = 270) entered the study. In 23% of the patients, macroprolactinemia was found. In women, the occurrence of macroprolactinemia increased with advancing age (< 30 yr: 16%; 30–45 yr: 28%; > 45 yr: 42%; $p < 0.05$). *A priori* clinical signs of hyperprolactinemia (morphological abnormalities in pituitary imaging, galactorrhea infertility) occurred significantly less frequently in macroprolactinemia than in true hyperprolactinemia. In eight females macroprolactinemia and true hyperprolactinemia appeared simultaneously. To avoid diagnostic and therapeutic pitfalls, the screening for macroprolactinemia of all patients with prolactin levels of > 700 mIU/L is recommended.

Key Words: Macroprolactinemia; true hyperprolactinemia; clinical consequence; screening.

Introduction

The molecular mass of the predominant forms of circulating prolactin (PRL) is either 23 or 50–60 kDa, or both *(1,2)*. In a much lower concentration, macroprolactin (big–big PRL; bbPRL molecular mass of 150–170 kDa) is also present in the circulation. In the bbPRL molecule the hormone is likely to be bound to IgG-type antibodies and aggregates of PRL may also be present. The bioactivity of bbPRL is largely reduced or absent *(3–5)*. The relative proportion of the circulating forms (bbPRL vs monomeric PRL) may be quite different from patient to patient *(6)*. The routinely available immunoassay methods usually measure all circulating fractions of PRL together. Depending on the assay design—mainly on the characteristics of the antibody used—bbPRL is picked up to varying degrees *(5–14)*. Owing to these facts, a selective analytical procedure of the various forms of PRL is of diagnostic and clinical importance. The "gold standard" of the determination of PRL fractions is gel filtration chromatography *(14–17)*. However, the routine application of this method is cumbersome and time-consuming. Faster, cheaper, and simpler methods such as ultrafiltration, immunoseparation, and PEG-precipitation are satisfactory for routine purposes *(5,7,18,19)*. The PEG-precipitation method has recently been described, and various investigators *(5–9,14,15,20–28)* have repeatedly reconfirmed its diagnostic applicability. However, the age-dependency of the occurrence of bbPRL and the significance of the simultaneous presence of high levels of biologically active PRL together with high bbPRL levels have not been studied in detail so far. The number of patients involved in comparative investigation of circulating bbPRL to various MRI findings is also limited in the literature *(15,21,22,27)*. The present retrospective study on 306 patients with high immunoreactive PRL levels (hyperprolactinemia; HPRL) was performed to elucidate these questions, by measuring circulating PRL with an electrochemiluminescence assay (ECLIA) before and after PEG-precipitation. The ECLIA method is one of the most widely used routine techniques for PRL measurement; it is known to be a high reading method for bbPRL *(5–9)*. Using the PEG-precipitation method, samples with HPRL were divided into a subgroup with bbPRL as the predominant form of PRL (macroprolactinemia, MPRL) and into a subgroup with predominantly high biologically active PRL (true hyperprolactinemia: tHPRL). Clinical validity of the PEG-precipitation method has been investigated by comparing the frequency of the leading clinical symptoms of tHPRL (occurrence of galactorrhea, infertility, menstrual abnormality), symptoms of hyperandrogenism, headache, findings of pituitary MRI in the two patient groups (MPRL and tHPRL). The relationship between the occurrence of MPRL and age of the patient has been analysed as well.

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Table 1
Effect of PEG-Precipitation on PRL Values

Patients	PRL in native serum (mIU/L)	PRL after PEG-precipitation (mIU/L)	Number of cases with MPRL	PRL recovery percentage+	
				MPRL[a]	tHPRL[b]
Children (n = 9)	1268 ± 747¯	802 ± 587*	2	21 ± 12	77 ± 9***
Men (n = 7)	1629 ± 1559	1240 ± 1450	1	10 ± 0	96 ± 19***
Women (n = 254)	1439 ± 1300	874 ± 990**	59	22 ± 11	80 ± 21***

[a] MPRL: macroprolactinemia (PRL recovery < 40%).
[b] tHPRL: true hyperprolactinemia (PRL recovery > 40%).
¯Mean ± standard deviation.
*$p < 0.05$ vs native serum (t-test).
**$p < 0.001$ vs native serum (t-test).
***$p < 0.001$ vs MPRL (t-test).
+Recovery percentage of PRL was calculated according to the formula: (mIU/L PRL after PEG precipitation in the supernatant/ mIU/L PRL in the native serum) * 100.

Results

The effect of PEG-precipitation on the PRL levels is illustrated in Table 1. PEG precipitation significantly decreased PRL levels in women and children. The low number of observations in men and children makes it problematic to draw conclusions. However, the same tendency as in women is quite obvious in these patients as well. In total, MPRL was detected in 62 cases, representing 23% of total observations. The mean PRL recovery values were significantly different in the MPRL and the tHPRL groups, thus the 40% cutoff value resulted in a well-defined (fourfold to ninefold) difference between the recovery percentages of the MPRL and the tHPRL patient groups.

The recovery values of PRL do not represent a normal distribution. The two peak values are 21–40% (MPRL) and 61–80% (tHPRL). In 13% (n = 27) of the tHPRL patients, the rate of recovery was between 40% and 60% [the "grey zone" *(18,19)*], whereas in the majority (87%; n = 181) recovery appeared to be higher than 60% (Fig. 1).

Pituitary MRI was performed in 123 patients, most of the cases after the measurement of high PRL shortly following the beginning of bromocriptine therapy. Normal pituitary imaging was found in 88 cases (representing 72%), but it was significantly more frequent in the MPRL group than in the tHPRL group. Pituitary macroadenoma or other large-sized MRI abnormalities (meningeoma, craniopharyngeoma, or Rathke's pouch cysta) did not occur in the MPRL group, whereas these abnormalities were diagnosed in 8% of the tHPRL group. Pituitary microadenoma also occurred in a significantly (threefold) higher percentage in the tHPRL than in the MPRL group. Taken together, morphological abnormalities in pituitary imaging were found fourfold more frequently in tHPRL than in MPRL (Table 2).

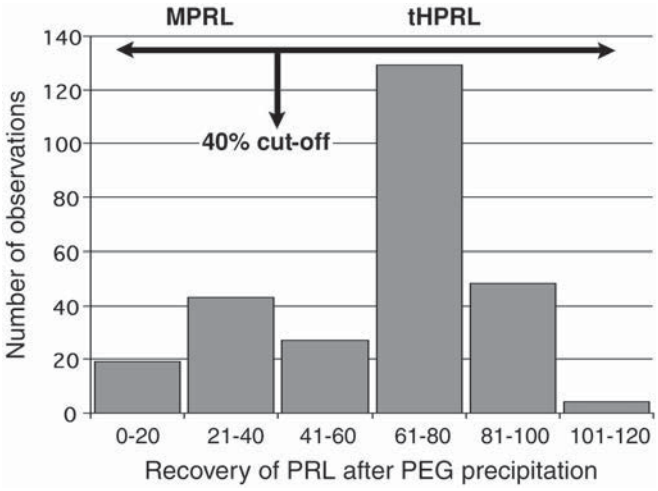

Fig. 1. Distribution of PRL recovery values in sera with high immunoreactive PRL. The vertical arrow delineates the cut-off value of 40%. Recovery percentage of PRL was calculated according to the formula: (mIU/L PRL after PEG precipitation in the supernatant/ mIU/L PRL in the native serum) * 100; MPRL: macroprolactinemia (PRL recovery < 40%); tHPRL: true hyperprolactinemia (PRL recovery > 40%).

The native PRL levels in the MPRL group were not different from those in the tHPRL group. On the other hand, following PEG-precipitation, PRL showed a significant (p < 0.001) drop in the MPRL but not in the tHPRL group.

Age-related differences in the occurrence of MPRL were analyzed in the 254 female patients studied. PEG-precipitation resulted in a significant decrease in the PRL levels in all age groups. The frequency of the occurrence of MPRL in the female patients was gradually and significantly increasing ($p < 0.05$) with advancing age (Table 3).

Table 2
Morphological Abnormalities in Pituitary MRI in 123 Patients with High PRL

Findings of pituitary MRI	MPRL[a] group (41 patients)	tHPRL[b] group (82 patients)
Negative	37 (90%)**	51 (62%)
Microadenoma[1]	4 (10%)*	25 (31%)
Macroadenoma[2]	0 (0%)	3 (4%)
Other diagnosis (meningeoma, craniopharyngeoma, Rathke's pouch cysta)[1]	0 (0%)	3 (4%)
Total number (percent) of MRI abnormalities	4 (10%)**	31 (38%)
PRL levels in the native sera (mIU/L)	1445 ± 1180[–]	1271 ± 778
PRL levels after PEG precipitation (mIU/L)	260 ± 185***	1019 ± 603

[a]MPRL: macroprolactinemia (PRL recovery < 40%).
[b]tHPRL: true hyperprolactinemia (PRL recovery > 40%).
[–]Mean ± SD (standard deviation).
*$p < 0.01$ (χ^2-test: significant differences between tHPRL and MPRL groups).
**$p < 0.001$ (χ^2-test: significant differences between tHPRL and MPRL groups).
***$p < 0.001$ (t-test: significant differences vs MPRL group).
[1]All cases are women; two patients have MPRL and tHPRL simultaneously.
[2]All cases are men.

Table 3
Age-Related Differences in the Occurrence of MPRL in Women

Age-groups (mean ± SD)	PRL in native serum (mIU/L)		PRL after PEG-precipitation (mIU/L)		Recovery percentage[c]		Frequency of MPRL in hyperprolactinemia[d]
	MPRL[a]	tHPRL[b]	MPRL	tHPRL	MPRL	tHPRL	
< 30 yr (25 ± 4)	1693 ± 1105[e]	1343 ± 1543	390 ± 211	1048 ± 1260	25 ± 9	80 ± 21	16% (24/146)
30–45 yr (36 ± 5)	2090 ± 1000	1264 ± 876	388 ± 141	1022 ± 714	22 ± 10	83 ± 23	28%* (20/72)
> 45 yr (52 ± 6)	1852 ± 1444	1140 ± 596	264 ± 214	951 ± 613	18 ± 13	79 ± 18	42%* (15/36)

[a]MPRL: macroprolactinemia (PRL recovery < 40%).
[b]tHPRL: true hyperprolactinemia (PRL recovery > 40%).
[c]Recovery percentage of PRL was calculated according to the formula: (mIU/L PRL after PEG precipitation in the supernatant/ mIU/L PRL in the native serum) * 100.
[d](MPRL/MPRL+tHPRL)*100 (in brackets: number of observations).
[e]Mean ± SD (standard deviation).
*$p < 0.05$ vs the age group of < 30 yr (χ^2-test).

Some leading clinical symptoms of hyperprolactinemia (galactorrhea, infertility) occurred significantly more frequently in patients with tHPRL than in patients with MPRL only. Amenorrhea, on the other hand, occurred only slightly more frequently in tHPRL (6%) than in MPRL (3%). Other, less characteristic symptoms related to hyperprolactinemia (menstrual disorders, headache, acne, hirsutism, polycystic ovaries syndrome, alopecia) also appeared slightly—albeit statistically not significantly—more frequently in tHPRL than in MPRL (data not shown). Significant differences were revealed in PRL levels of the tHPRL patients exhibiting symptoms of galactorrhea ($p < 0.01$) and amenorrhea ($p < 0.001$), as compared to tHPRL patients without these clinical symptoms. PRL values of tHPRL patients with galactorrhea and amenorrhea were significantly higher in the native serum as well as after PEG precipitation (Table 4).

In 8 out of 59 sera (14%) of female patients with MPRL, both MPRL and tHPRL appeared simultaneously. Thus, PRL levels in these patients remained in the pathologically high range after PEG-precipitation in spite of the fact that the recovery was lower than 40%, indicating the simultaneous presence of MPRL and the monomeric form of PRL

Table 4
Clinical Symptoms of Hyperprolactinemia in Women[a]

	n^b = 208 Galactorrhea		n = 128 Infertility		n = 173 Amenorrhea	
Patient's group	Yes	No	Yes	No	Yes	No
MPRL[c]						
n (%)	2 (4%)	44 (96%)	4 (17%)	19 (83%)	1 (3%)	31 (97%)
PRL in native serum (mIU/L)	1304 ± 722	1789 ± 947	1452 ± 557	1821 ± 1134	1145 ± 0	2020 ± 1315
PRL after PEG precipitation (mIU/L)	264 ± 46	292 ± 116	342 ± 53	320 ± 99	340 ± 0	299 ± 81
tHPRL[d]						
n (%)	31 (19%)*	131 (81%)*	46 (44%)*	59 (56%)*	8 (6%)	133 (94%)
PRL in native serum (mIU/L)	1975 ± 2824**	1205 ± 747[e]	1620 ± 2357	1318 ± 883	6571 ± 6477***	1285 ± 745
PRL after PEG-precipitation (mIU/L)	1538 ± 2296**	968 ± 655	1322 ± 1961	987 ± 680	5592 ± 5270***	984 ± 540

[a]Patients with simultaneous occurrence MPRL and tHPRL were NOT included either into the MPRL, or into the tHPRL group.
[b]n, number of investigated cases.
[c]MPRL: macroprolactinemia (PRL recovery < 40%).
[d]tHPRL: true hyperprolactinemia (PRL recovery > 40%).
[e]Mean ± SD (standard deviation).
*Significantly ($p < 0.02$) different from the MPRL patients (χ^2-test).
**Significantly ($p < 0.01$) different from tHPRL patients without galactorrhea (ANOVA followed by the post-hoc LSD-test).
***Significantly ($p < 0.001$) different in the tHPRL group from patients without amenorrhea (ANOVA followed by the post-hoc LSD-test).

Table 5
Clinical Data of Women with Simultaneous Occurrence of MPRL[a] and tHPRL[b,c]

Patient	Age (yr)	Initial clinical findings, complaints	PRL in native serum (mIU/L)	PRL after PEG precipitation (mIU/L)	PRL Recovery (%)	Pituitary MRI	Recovery Drug treatment
1	22	Raromenorrhea, transient breast discomfort	2838	1002	35	microadenoma	bromocriptine
2	43	Regular menses, Headache	2194	570	26	microadenoma	bromocriptine
3	54	Headache, uterus extirpation 8 yr before	6153	898	15	normal	bromocriptine
4	28	Alopecia, hirsutismus, raromenorrhea, infertility	1652	662	40	normal	bromocriptine
5	28	Oligomenorrhea, infertility	6006	706	12	normal	estrogen
6	24	Acne, alopecia raromenorrhea	2228	780	35	normal	estrogen
7	31	Raromenorrhea, galactorrhea	2000	800	40	normal	estrogen
8	25	PCOS,* oligomenorrhea	1546	550	38	normal	nothing

*PCOS polycystic ovary syndrome.
[a]MPRL: macroprolactinemia (PRL recovery < 40%).
[b]tHPRL: true hyperprolactinemia (PRL recovery > 40%).
[c]PRL measurements were performed several month after the initiation of bromocriptine or contraceptive (estrogen) therapy.

(tHPRL). Clinical data of these patients are summarized in Table 5. In five out of the eight patients estrogen therapy (contraceptive pills) or hyperandrogenism (also causing clinical symptoms of acne, alopecia, hirsutism, polycystic ovaries syndrome) justify the high biologically active PRL values. These PRL values, however, remained in the modestly elevated range (706–800 mIU/L). Four patients were treated with bromocriptine and two of them had pituitary microadenoma. PRL measurements were performed several months after the initiation of bromocriptine or contraceptive (estrogen) therapy.

Discussion

Serum PRL is considered to be elevated if its value exceeds 520 mIU/L with the ECLIA method. Therefore, at the

initial phase of the present study all sera with PRL levels higher than this reference range were screened for bbPRL. However, bbPRL could not be detected unless the immunoreactive PRL level exceeded 700 mIU/L and thus screening was limited later to these samples. In the present study, 270 sera were found to have a PRL level > 700 mIU/L. This represents 15% of the total of 1747 consecutive PRL measurements of the ambulatory samples of a tertiary-care general hospital. PRL estimation is generally initiated by clinicians due to various indications, including patients taking medication known to affect PRL levels. In a similar study performed recently in Ireland *(22)*, 7% of the sera were found to contain higher than 700 mIU/L PRL.

In both studies, however, the incidence of bbPRL was found to be 23–26% of the samples with high PRL *(6,8,18, 21,22)*. Based on these findings, screening for bbPRL can only be recommended if immunoreactive PRL exceeds 700 mIU/L and approximately one out of four or five samples with high PRL is to be expected to contain bbPRL. A cutoff value of < 40% recovery was established for the diagnosis of bbPRL, based on earlier reports of other investigators *(6,11,12,22,23)*. The fact that some leading clinical symptoms of hyperprolactinemia (e.g., galactorrhea or infertility) significantly less frequently appear in the presence of bbPRL (at a cutoff of < 40%) than in the presence of tHPRL, clearly indicates that this cutoff is suitable to differentiate the biologically active PRL from the inactive fractions. Clinical symptoms of hyperprolactinemia in the MPRL patient group does not necessarily mean that these symptoms were produced by MRPL per se, as a variety of other clinical conditions may also result in the appearance of these symptoms.

The PEG-precipitation method appears to be an adequate screening method for routine purposes; however, it has been noted that nonspecific precipitation (with the monomeric PRL molecule) may occur as well *(5,11)*. It might be a matter of discussion whether the laboratory should automatically screen for bbPRL in every instance of high PRL exceeding 700 mIU/L, or screening should be performed upon clinical consultation only (when high, the PRL finding does not seem to fit into the clinical signs). Our practical experience is that clinicians rather easily accept high PRL findings, mainly because of a preconception of hyperprolactinemia. Menstrual disorders, hirsutism, or infertility are rather common clinical problems, frequently combined with high PRL. In agreement with the opinion put forward by other investigators *(6,14,16,19–28)* screening of bbPRL, initiated by the laboratory itself without any further clinical consultation, might be recommended. This might be a more cost-effective approach as well because since it may prevent redundant pituitary MRI or unnecessary treatment (e.g., bromocriptine therapy).

A special category is represented by pituitary microadenoma incidentally discovered following the MRI scan *(29, 30)*. It is well known that about 10% of the asymptomatic, normal, adult population might have pituitary abnormalities detectable by MRI scan that may not have any functional or diagnostic significance *(29,31)*. According to Leslie et al. *(22)*, pituitary microadenoma was present in approx 10% of MPRL patients, similarly to the data obtained from our study. As far as the occurrence of the negative pituitary MRI finding is concerned, some literary data principally differ from our findings. Hauache et al. *(21)* and Vallette-Kasic et al. *(27)* found negative pituitary image (with CT or MRI) in 78% in MPRL, which was not different from the 69% negative finding rate in tHPRL patients. In our present investigation, the negative MRI finding was significantly more frequent in the MPRL group than in the tHPRL group (90% vs 62% occurrence, respectively). The reason for this discrepancy may be related to differences in the diagnostic and clinical sensitivity of the method used. The low prevalence of positive MRI findings found in our patients with MPRL seems be a reliable sign of the independence of MPRL formation from the pituitary pathology. It is questionable whether neuroimaging is necessary when significant macroprolactinemia is identified and the level of PRL following PEG-precipitation is in the normal range *(31)*. This disorder—together with a high immunoreactive PRL level and an undisclosed bbPRL—initiates long-term, unnecessary treatment often with side effects and resulting in an iatrogenic psychological stress for the patient as well.

Of practical interest is the finding that a tHPRL and bbPRL occurred simultaneously in 14% of the MPRL group. Very recently, several investigators *(6,28,31)* also noted that some patients with bbPRL have elevated levels of monomeric PRL. Thus the presence of bbPRL does not exclude the diagnosis of clinically relevant hyperprolactinemia. In three patients, estrogen-containing contraceptives may explain the appearance of tHPRL. The use of an appropriate reference interval for the PEG precipitation procedure may be of particular importance in those patients who have an excess of both the macroprolactin and the monomeric form *(28)*. The consultation between laboratory specialists and clinicians is increasingly important in these cases. Screening for bbPRL may also be useful in the course of the long-term follow-up of patients with previously verified, pharmacologically treated prolactinoma (Table 5). Increasing HPRL values during bromocriptine treatment may indicate the necessity of a higher drug dose, a change in drug therapy, or the MRI control of the patient. If, however, bbPRL—rather than tHPRL—is increasing in these patients, the clinical consequence is limited, if any. One might even hypothesize that unnecessary bromocriptine therapy was initiated in some patients.

A major novel conclusion of this article is that bbPRL is more frequent as age advances. As a result of this, bbPRL occurs in women over 45 yr three times more frequently than in women under 30 yr. The exact reason of age-related accumulation of MPRL is not known. It might be supposed

that this observation may be linked to the age-related increase in autoimmune susceptibility *(32)*. It is well known that the immune system is subject to hormonal modulations. A role for gonadal function as a modulator of the immune processes has been suspected because of the gender differences in the prevalence of the autoimmune diseases. Estradiol potentiates mitogen-induced B-cell stimulation and T-lymphocyte functions fluctuate during the menstrual cycle *(33)*. Thus, theoretically, age-related accumulation of MPRL may be in correlation with age-related changes in estrogen levels. This hypothesis, however, needs further experimental support.

Further studies are required to elucidate whether the bbPRL-positive patients over 45 yr also have an increased occurrence of other autoimmune markers, e.g., rheuma factor, antinuclear antibodies, and thyroid antibodies *(34–36)*. Nevertheless, autoimmunity itself does not seem to be involved in the pathogenesis of hyperprolactinemia *(37)*.

Another important factor in age-related accumulation of bbPRL might be pregnancy. A self-limiting release characteristic has been found *(38)* and a pregnancy-induced normalization of hyperprolactinemia has been postulated in about one third of pregnant women with high monomeric PRL. In accordance with these findings, a lower (approx 4%) frequency of bbPRL has been found during pregnancy *(19,39)*. The question as to whether the age-related changes in bbPRL positivity are also related to the number of prior pregnancies, length of breast-feeding, or oral contraceptive therapy, remains to be answered.

Taken together, bbPRL is a relatively frequent laboratory finding advancing with age, affecting clinical symptoms of the patient and the decision-making of the clinicians. Its discovery helps prevent unnecessary clinical investigation and reduce costs. The simple screening for bbPRL, therefore, should be integrated into the routine palette of laboratories.

Materials and Methods

Sample Collection and Patients

Over a period of 8 mo, 1747 requests for serum PRL were received. Of these, in 306 (18%) pathologically elevated PRL (> 520 mIU/L) was found. The total population of the 306 sera with high PRL was used to establish the cutoff value of bbPRL. Because bbPRL did not occur in the PRL range between 520 and 700 mIU/L, only the 270 sera with PRL value >700 mIU/L were included in further studies. Sera for the detailed bbPRL study were received from 254 adult women (age: 32 ± 11 yr), 7 adult men (37 ± 15 yr), and 9 children of both sexes (age: 9 ± 6 yr). All adult patients as well as the parents of the investigated children provided us with a written consent to the clinical and laboratory investigations.

Venous blood was taken between 8 AM and 10 AM and centrifuged (1600*g*, 15 min, +4°C). Sera were stored at –20°C until assaying, but not longer than 1 wk.

The most relevant clinical and diagnostic data (prior pharmacological treatment, occurrence of galactorrhea, infertility, menstrual abnormality, and headache, symptoms of hyperandrogenism and findings of pituitary MRI scan) were gained from the patient files and from a personal consultation with the physician treating the patient (primary care physician or clinical endocrinologist). Owing to the retrospective study design, however, some clinical and diagnostic results for some patients were not always available. Information on drug treatment at the time of blood sampling was available in 247 patients. Of these, 110 patients did not receive any kind of medication. The most common pharmacological treatments were bromocriptine (63 patients); oral contraceptives and other estrogen containing drugs (65 patients); L-thyroxin (4 patients); antipsychotic drugs (5 patients).

Laboratory Methods

PRL was measured with an ECLIA assay (Elecsys 2010, Roche). The within-assay precision of PRL measurement was 2.8–2.5–3.4% CV, the total precision was 3.6–4.1–4.4% CV (in low, normal, and high range, respectively). bbPRL was measured with the PEG-precipitation test *(19)*. Sera were divided into two aliquots. In one of the aliquots, equal volumes (200 µL) of patients' sera and of a 25% solution of PEG (MW: 6000 kDa, Fluka AG, Switzerland) were mixed and centrifuged at 1500*g* for 30 min at +4°C. Immunoreactive PRL was measured from the supernatant. In the other aliquot treated in the same way, the assay diluent (Diluent Universal, Roche) was added instead of the PEG solution. Recovery percentage of PRL was calculated according to the formula: (mIU/L PRL after PEG precipitation in the supernatant/mIU/L PRL in the native serum) * 100.

In agreement with previous literature data *(10,19)* and with our previous clinical experience, a cutoff value of 40% recovery of PRL was taken as an indicator of clinically relevant macroprolactinemia (bbPRL). The within-assay CV for PRL recoveries—as measured on 10 patient samples—was 3.1%.

Statistical Methods

Data are expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by the post-hoc least significant difference (LSD) test, the χ^2 test, and the Student's *t*-test for dependent samples were used, where pertinent. A probability level of < 5% was accepted as indicating significant differences.

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