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Gross Variability in the Detection of Prolactin in Sera Containing Big Big Prolactin (Macroprolactin) by Commercial Immunoassays

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A high molecular mass form of prolactin (PRL), macroprolactin, accumulates in the sera of some subjects. Although macroprolactin exhibits limited bioactivity *in vivo*, it retains immunoreactivity. We examined the frequency of macroprolactinemia in clinical practice and the ability of immunoassay systems to distinguish between macroprolactin and monomeric PRL. Of 300 hyperprolactinemic sera identified, 71 normalized following treatment of sera with polyethylene glycol, indicating that 24% of hyperprolactinemia could be accounted for by macroprolactin. Ten of these macroprolactinemic sera were circulated to 18 clinical laboratories. Two sets of PRL measurements of the 10 untreated sera were obtained from each of the nine most commonly used immunoassay systems. Across the nine assay systems, differences in the PRL estimates ranged from 2.3- to 7.8-fold. Elecsys users reported the highest PRL levels. Somewhat lower values were reported for DELFIA systems followed by Immuno-1, AxSYM, and Architect assays. The Immulite 2000 assay generated PRL levels equivalent to approximately 50% of those reported by the high-reading methods. The lowest PRL levels were reported by Access, ACS:180, and Centaur systems. To avoid confusion caused by the frequent presence of macroprolactin accounting for hyperprolactinemia, secondary screening for the presence of macroprolactin is recommended. (*J Clin Endocrinol Metab* 87: 5410–5415, 2002)

FRACTIONATION OF HUMAN sera by gel filtration chromatography reveals that the polypeptide hormone prolactin (PRL) circulates in three discrete forms (1, 2). These include a monomer of molecular mass 23 kDa, which accounts for approximately 85% of the PRL present in normal individuals, a 50-kDa species accounting for 10–15%, and a small but variable amount of a high-molecular-mass form (~150 kDa) termed big big PRL or macroprolactin. In the case of sera from hyperprolactinemic individuals, the relative proportions of such circulating forms can be quite different (3, 4). The prevalence of macroprolactin in hyperprolactinemic sera has been reported as 15–26% (5–9). The possibility that confusion might arise from the coincidental finding of macroprolactinemia in the investigation of patients presenting with symptoms consistent with but not exclusive to hyperprolactinemia has received little attention.

The existence of macroprolactin was first reported over 25 yr ago (1). Although PRL polymers may account for a minor portion, macroprolactin is primarily a molecular complex of PRL and an IgG antibody thought to be directed against the PRL molecule (10–13). Macroprolactin is cleared more slowly than monomeric PRL and hence accumulates in the sera of affected subjects. However, because of its high molecular mass, the autoimmune complex is confined to the vasculature and hence exhibits limited bioactivity *in vivo* (3, 14–16). As a consequence, subjects whose hyperprolactinemia can be accounted for by the presence of macroprolactin, *i.e.* have macroprolactinemia, may not exhibit the classic signs or symptoms of the hyperprolactinemic syndrome such as galactorrhea, menstrual irregularities, or infertility (3, 4, 17). However, these nonspecific symptoms prompt measurement of PRL. Although not causally related, some subjects may coincidentally demonstrate galactorrhea, menstrual irregularities, or infertility together with hyperprolactinemia entirely caused by macroprolactin. It is therefore important to distinguish such individuals from those with true hyperprolactinemia to avoid unnecessary biochemical and imaging investigations and misleading diagnosis and thus prevent inappropriate drug and surgical treatment (6, 9, 18–21). It is equally important to correctly identify and address the underlying disorder responsible for the clinical presentation in these patients.

Screening for hyperprolactinemia assumes that the assays used to measure PRL will provide clinically relevant results. Moreover, the widespread use of a common international calibration standard should ensure that PRL estimations are consistent, irrespective of the methodology used. However, current PRL immunoassays in routine use exhibit variable degrees of reactivity with macroprolactin. In a limited study, the United Kingdom National External Quality Assessment Scheme (UK NEQAS) reported that PRL estimations on serum from one macroprolactinemic individual varied from 476 mU/liter to 3212 mU/liter and that the variability was dependent on the immunoassay used (22). In a somewhat more comprehensive investigation, Cavaco *et al.* (16) reported results obtained when several macroprolactinemic sera were examined with four commercially available im-

Abbreviations: PEG, Polyethylene glycol; UK NEQAS, United Kingdom National External Quality Assessment Scheme.

munoassays. The PRL levels varied widely from assay to assay. Moreover, the relative sensitivity of the assays to macroprolactin was variable for the sera examined, *i.e.* the ranking of PRL values was not consistent among assays. The variable behavior of different macroprolactinemic sera in any given assay and the extent of reactivity of macroprolactin in several widely used immunoassay systems may lead to misdiagnosis in clinical practice (6, 9, 18–21). To address this problem, polyethylene glycol (PEG) immunoprecipitation has been advocated as one means whereby macroprolactin can be removed from sera before measurement of PRL (5–7, 23). Although results generated using PEG correlate well with those of gel filtration, numerically they differ significantly, a finding that can be explained by the nonspecific way PEG reduces protein solubility (24). Moreover, PEG interferes with a considerable number of commonly used immunoassay methodologies and as such its use is not always applicable.

In the present study, we examined the frequency with which macroprolactin accounted for hyperprolactinemia in our practice. To examine the extent to which macroprolactinemia is related to the assay system, we submitted 10 hyperprolactinemic serum samples known to contain predominantly macroprolactin to nine of the most commonly used immunoassay systems. The content of monomeric PRL in the sera was determined following gel filtration chromatography. In addition, these serum samples were assayed for PRL following treatment with PEG. We also wished to determine whether the relative sensitivity of the PRL immunoassays for different macroprolactinemic samples was the same or variable and recognizing that the absolute values achieved were likely to differ. This examination was undertaken to provide insight as to whether the autoantibody giving rise to macroprolactin was directed against one or several discrete epitopes on the PRL molecule.

Subjects and Methods

Subjects

We identified 300 sera with PRL levels in excess of 700 mU/liter using the PRL DELFIA immunoassay routinely used in our laboratory. These were derived from a general endocrinology service in a university-affiliated teaching hospital and tertiary referral center. In addition, the levels of PRL in the pre- and post-PEG-treated sera from 62 healthy normoprolactinemic control subjects were established. For a diagnosis of macroprolactinemia to be made in this study, it was necessary for PEG treatment to correct hyperprolactinemia to levels obtained in normoprolactinemic sera following PEG treatment.

A study was undertaken to evaluate the clinical management of hyperprolactinemic patients when it was not known that macroprolactin was the cause (21). Ten patients identified as having macroprolactin as the major circulating form of PRL in their sera agreed to participate in the immunoassay cross-reactivity portion of the study. No other selection criteria were applied. There were eight women, mean age 42 ± 12 yr, and two men, mean age 42 ± 2 yr. Whole blood, approximately 600 ml, was collected by venepuncture and allowed to clot. Serum recovered by centrifugation was aliquoted and stored at −20 C before dispatch to participating laboratories for analysis. The clinical characteristics and PRL levels before and after treatment with PEG of the 10 patients studied are outlined in Table 1. Of the eight female patients studied, three presented with both oligomenorrhea and galactorrhea. Three additional patients were identified during routine investigations for breast disease. The two male patients in the study complained either of infertility or loss of libido. Serum total PRL levels measured by the DELFIA analyzer system (Wallac, Inc., Turku, Finland) in the 10 patients ranged from 750-3975 mU/liter. Following treatment with PEG and removal of the macroprolactin complex, PRL levels fell to less than 380 mU/liter in each case with recoveries of PRL ranging from 6–33%.

Methods

The study was conducted in association with the Irish External Quality Assessment Scheme and involved 13 clinical laboratories in the Republic of Ireland that routinely measure PRL. In addition, five laboratories in the United Kingdom were invited to participate. The analytical methods used by the participants were all automated and included two of each of the following instruments: Architect and AxSYM (Abbott, Abbott Park, IL); Immuno-1, ACS 180, and Centaur (Bayer Corp., Pittsburgh, PA); Access (Beckman, Brea, CA); Immulite 2000 (Diagnostic Products, Los Angeles, CA); Elecsys (Roche, Indianapolis, IN); and DELFIA (Wallac, Inc.). All the above immunoassays are calibrated to the World Health Organization international reference preparation for PRL 84/500.

The amount of macroprolactin present in the sera under investigation was quantified by DELFIA following gel filtration over Sephacryl S-300 (50 × 1.5 cm; 30 ml/h; Pharmacia, Uppsala, Sweden) equilibrated in 10 mmol/liter Tris, 140 mmol/liter NaCl, 1.25 mmol/liter $CaCl_2$, and 0.5 mmol/liter Mg Cl_2 (pH 7.4), as described previously (5). In addition, all specimens were subjected to treatment with an equal volume of 25% (wt/vol) PEG in PBS (pH 7.4) for 10 min at room temperature before centrifugation (1800 × *g*, 30 min) and reanalysis of the supernatant using the DELFIA system. Recovery of monomeric PRL (DELFIA recombinant standard) following PEG treatment was 97 ± 2%. To examine the reproducibility of PEG precipitation applied to sera containing significant

TABLE 1. Clinical characteristics and serum PRL levels in the patients under investigation

Patient	Age (yr)	Sex	Serum PRL (mU/liter)[a]		Clinical presentation	CT/MRI scan
			Untreated	PEG-treated		
1	33	F	757	204	Breast lump, galactorrhea	Normal
2	46	F	750	88	Breast lump, mastalgia	Not performed
3	27	F	983	191	Oligomenorrhea, galactorrhea	Possible microadenoma[b]
4	35	F	933	310	Infertility	Normal
5	40	M	1203	120	Infertility	Not performed
6	43	F	1234	216	Breast lump, mastalgia	Normal
7	38	F	1858	103	Oligomenorrhea, galactorrhea	Not performed
8	43	M	2425	195	Loss of libido, hemochromatosis	Not performed
9	64	F	2959	202	Fatigue[c]	Normal
10	53	F	3975	380	Oligomenorrhea, galactorrhea	Normal

Specimens referred to in subsequent tables and figures were obtained from patients with corresponding numbers. CT, Computed tomography; MRI, magnetic resonance imaging.

[a] Results obtained using the Wallac Delfia.

[b] Ten percent of all scans detect pituitary anomalies consistent with a microadenoma (33).

[c] Referred from primary care where hyperprolactinemia was detected on screening.

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but different absolute amounts of macroprolactin, we repeated the examination on a number of occasions. The interassay coefficients of variation for this procedure were 5.3% for serum with a mean total PRL of 297 mU/liter and a mean level of 128 mU/liter following PEG treatment (n = 43), 5.6% for sera with a mean total PRL of 627 mU/liter and a mean level of 291 mU/liter following PEG treatment (n = 34), and 4.9% for serum with mean total PRL of 1229 mU/liter and a mean level of 139 mU/liter following PEG treatment (n = 22).

Statistical analysis

Pearson correlation coefficients and *t* tests were determined using the statistical package Analyze It [Microsoft Corp. Excel (25)]. A *P* value less than 0.05 was accepted as statistically significant. Data were expressed as means and SD.

The study was approved by the Research Ethics Committee, St. Vincent's University Hospital, and all participants provided informed consent.

Results

Prevalence of macroprolactinemia to account for hyperprolactinemia

Three hundred randomly selected hyperprolactinemic serum samples, *i.e.* PRL levels in excess of 700 mU/liter as determined by the DELFIA (Wallac, Inc.), were submitted to PEG treatment. As a control group, sera from 62 normal healthy women were examined and found to have total PRL levels ranging from 78–466 mU/liter. PEG treatment of the 62 normoprolactinemic control sera yielded PRL levels of 197 ± 146 mU/liter (mean ± 2 SD). The absolute levels ranged from 70–390 mU/liter, and this was used as a reference or normal range for serum treated in this way.

Seventy-one of the 300 hyperprolactinemic samples yielded PRL levels of less than 390 mU/liter following treatment with PEG. Therefore, 24% of hyperprolactinemic sera could be accounted for by the presence of macroprolactin. The percentage of sera with PRL levels in excess of 1000 mU/liter whose PRL levels corrected following treatment with PEG was 19%, and the percentage of patients with PRL levels in excess of 2000 mU/liter, which corrected to less than 390 mU/liter, was 8%. In our patients, therefore, the higher the PRL level, the less frequently macroprolactin accounts for hyperprolactinemia.

Serum PRL estimations using nine different immunoassay systems

Two operators of nine different assay systems each received 10 hyperprolactinemic sera samples known to contain a variable amount of macroprolactin that when processed to remove macroprolactin and remeasured yielded PRL levels within the normal range. Thus, these sera samples were deemed to have mild to severe hyperprolactinemia, which was corrected when macroprolactin was removed. Table 2 illustrates the mean PRL levels in the 10 serum specimens examined as reported by nine different immunoanalyzer systems. We have arranged the PRL levels in the sera samples with increasing PRL levels tabulated from left to right against the assay systems, generally giving the highest to lowest values running from top to bottom. In addition, the monomeric PRL content of each sample isolated by gel filtration chromatography is also provided for reference purposes. These data are presented graphically in Fig. 1. Serum PRL levels reported by the commercially available systems for any given specimen varied from 2.3- to 7.8-fold between the highest and lowest estimations, depending on the serum examined. In all cases Elecsys (Roche) users reported the highest serum PRL levels (mean range, 828-4604 mU/liter). Somewhat lower values were reported by DELFIA (Wallac, Inc.) users (range, 743-4133 mU/liter) followed in general by users of the Immuno-1 (Bayer Corp.) (range, 640-3690 mU/liter), AxSYM (Abbott) (448–3021 mU/liter) and Architect (Abbott) (range, 452-2982 mU/liter). Users of Immulite 2000 (DPC) reported PRL levels ranging from 393-1837 mU/liter, approximately 50% of the values as measured by the high reading methods, Elecsys (Roche), DELFIA (Wallac, Inc.), Immuno-1 (Bayer Corp.), and AxSYM and Architect (Abbott). The lowest PRL levels were reported by ACS:180 (Bayer Corp.) (range, 290-1189 mU/liter), Centaur (Bayer Corp.) (range, 243–947 mU/liter), and Access (Beckman) (range, 228–940 mU/liter) users.

Nearly consistent stratification of PRL measurements across different assay systems was observed in the 10 sera. In other words, when the individual sera PRL values were ranked from lowest to highest, the hierarchy obtained tended to be reproducible for each of the nine methods examined with only minor deviations (Table 2). The Access (Beckman) and Centaur (Bayer Corp.) systems each gave a reading in excess of 700 mU/liter for only 1 of the 10 macroprolactinemic samples assayed. The ACS:180 system (Bayer Corp.) yielded values in excess of 700 mU/liter in 2 of the 10 macroprolactinemic samples. Using the Immulite 2000 (DPC), 4 of the 10 samples crossed the 700 mU/liter threshold, 7 of 10 samples using the Architect (Abbott), 8 of 10 samples using the AxSYM (Abbott) and the Immuno-1 (Bayer Corp.), and all 10 samples had measured PRL in excess of 700 mU/liter using the DELFIA (Wallac, Inc.) and Elecsys (Roche). In 9 of the 10 samples examined, PRL levels measured by DELFIA (Wallac, Inc.) following the removal of macroprolactin by gel filtration were considerably lower than those reported by any of the immunoanalyzer systems using sera that had not been fractionated (Table 2 and Fig. 1).

Measurement of serum PRL following gel filtration chromatography or pretreatment with PEG

Gel filtration chromatography provides the reference method for isolation of monomeric PRL. The mean PRL value obtained following PEG precipitation in the 10 macroprolactinemic sera distributed, 201 ± 90 mU/liter, were consistently lower than those obtained following chromatographic separation and quantitation of monomeric PRL, 303 ± 133 mU/liter ($P < 0.001$, Table 2). When the PRL values obtained following treatment of serum with PEG were correlated against those obtained following chromatographic isolation of monomeric PRL, the correlation coefficient obtained was 0.92 ($P < 0.001$).

Discussion

Measurement of PRL is one of the most commonly undertaken hormonal investigations in evaluating patients with reproductive disorders. Hyperprolactinemia accounts for menstrual disorders in approximately one fifth of affected

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TABLE 2A. Mean serum PRL levels (mU/liter) in 10 sera assayed by 9 analytical methods

Specimen	1	2	3	4	5	6	7	8	9	10
Analytical methods used twice[a]										
Roche Elecsys	828 (1)	892 (1)	1180 (1)	1219 (1)	1295 (1)	1444 (1)	2622 (1)	2728 (1)	3408 (1)	4604 (1)
Wallac DELFIA	743 (2)	750 (2)	971 (2)	961 (2)	1182 (2)	1217 (2)	1964 (2)	2498 (2)	3000 (2)	4133 (2)
Bayer Immuno-1	640 (3)	652 (3)	824 (4)	914 (3)	940 (3)	1025 (4)	1855 (3)	2205 (3)	2605 (3)	3690 (3)
Abbott AxSYM	596 (4)	448 (6)	855 (3)	884 (4)	729 (4)	1082 (3)	1528 (4)	1781 (4)	2102 (4)	3021 (4)
Abbott Architect	460 (5)	452 (5)	717 (5)	798 (6)	669 (5)	908 (5)	1224 (5)	1757 (5)	2040 (5)	2982 (5)
DPC Immulite 2000	393 (6)	459 (4)	519 (6)	573 (8)	600 (6)	647 (6)	1001 (6)	1262 (6)	1145 (6)	1837 (6)
Bayer ACS:180	327 (8)	290 (7)	356 (8)	830 (5)	507 (7)	610 (7)	535 (7)	584 (7)	532 (7)	1189 (7)
Bayer Centaur	307 (9)	243 (8)	347 (9)	644 (7)	431 (8)	497 (8)	454 (9)	496 (9)	460 (8)	947 (8)
Beckman Access	363 (7)	228 (9)	379 (7)	466 (9)	273 (9)	460 (9)	517 (8)	516 (8)	436 (9)	940 (9)
Analytical methods used once										
Gel filtration	339	126	277	383	216	379	221	193	293	599
PEG	204	88	191	310	120	216	103	195	202	380

The monomeric PRL content of the sera, determined after gel filtration chromatography, is shown, as is the PRL level after PEG treatment. Numbers in parentheses represent ranked mean PRL levels reported from highest (1) to lowest (9) for each serum according to analytical method.
[a] See Table 2B for PRL levels reported by individual laboratories.

TABLE 2B. PRL levels (mU/liter) reported by the individual laboratories participating in the study for each of the 10 macroprolactinemic sera

Specimen	1	2	3	4	5	6	7	8	9	10
Roche Elecsys	819	871	1149	1184	1299	1373	2546	2653	3403	4487
	836	912	1210	1253	1291	1515	2697	2803	3413	4721
Wallac DELFIA	757	750	983	933	1203	1234	1858	2425	2959	3975
	728	749	959	989	1160	1200	2070	2570	3040	4290
Bayer Immuno-1	610	600	770	870	870	940	1740	2020	2410	3400
	670	703	878	958	1010	1110	1970	2390	2800	3980
Abbott AxSYM	554	373	768	898	811	1066	1449	1638	1946	2954
	638	522	942	870	646	1098	1607	1923	2257	3088
Abbott Architect	536	456	799	855	689	911	1320	1939	2407	3164
	383	447	635	740	648	905	1127	1574	1673	2800
DPC Immulite 2000	371	429	492	532	560	625	945	1158	1118	1777
	415	488	545	614	640	669	1056	1366	1171	1896
Bayer ACS:180	353	293	354	839	448	599	547	589	531	1067
	301	286	358	820	565	620	522	579	533	1310
Bayer Centaur	284	241	348	622	472	499	449	486	438	924
	329	244	345	666	390	494	458	505	482	969
Beckman Access	348	222	367	443	264	447	505	506	422	920
	377	233	391	488	282	473	529	526	450	960

women (26). Recently reports have suggested that hyperprolactinemia may be accounted for by the presence of macroprolactin in 15–26% of cases (5–9). In general, macroprolactin is regarded as biologically inactive. The diagnosis of hyperprolactinemia when it is entirely due to the presence of macroprolactin gives rise to mismanagement of patients whose symptoms may arise from another disorder and in whom macroprolactinemia is coincidental. A small number of reports have suggested that the extent to which the presence of macroprolactin is recognized in sera is assay system dependent (16, 22, 27, 28). It is important therefore that those performing assays and reporting results and those responsible for their interpretation and the management of patients are aware of the extent to which the assay system used in the measurement of PRL may detect macroprolactin. When it is recognized that the assay system used is highly sensitive to the presence of macroprolactin, it is prudent that an alternative procedure is introduced to ensure that macroprolactin levels are reported. The present study was undertaken to examine the extent to which widely used PRL assays systems failed to distinguish between macroprolactin and monomeric PRL.

Although there was no assay system that succeeded in providing normoprolactinemic values in all the sera known to contain macroprolactin, the performance of the Centaur (Bayer Corp.) and the Access (Beckman) and to a lesser degree the ACS:180 (Bayer Corp.) was superior to that of the other assay systems examined. It is therefore particularly important that those using the Immulite 2000 (DPC), AxSYM (Abbott), Architect (Abbott), Immuno-1 (Bayer Corp.), DELFIA (Wallac, Inc.), and Elecsys (Roche) should undertake specific steps to identify the presence of macroprolactin when hyperprolactinemia is detected. Treatment of hyperprolactinemic sera with PEG and remeasurement of PRL is one strategy that could be adopted. However, this will mean a more labor-intensive and costly procedure requiring two PRL measurements. In addition, PEG interference with a number of immunoassay formats [AxSYM (Abbott), ACS:180 (Bayer Corp.), Immuno-1 (Bayer Corp.)] has limited its general applicability. Furthermore, the procedure is prone to underrecovery of monomeric PRL in sera, compared with the reference procedure gel filtration (5, 7).

Assessment of the number of users using the various assay systems examined to generate PRL results revealed that in total they accounted for 91% of UK NEQAS and 85% of College of American Pathologist proficiency testing program

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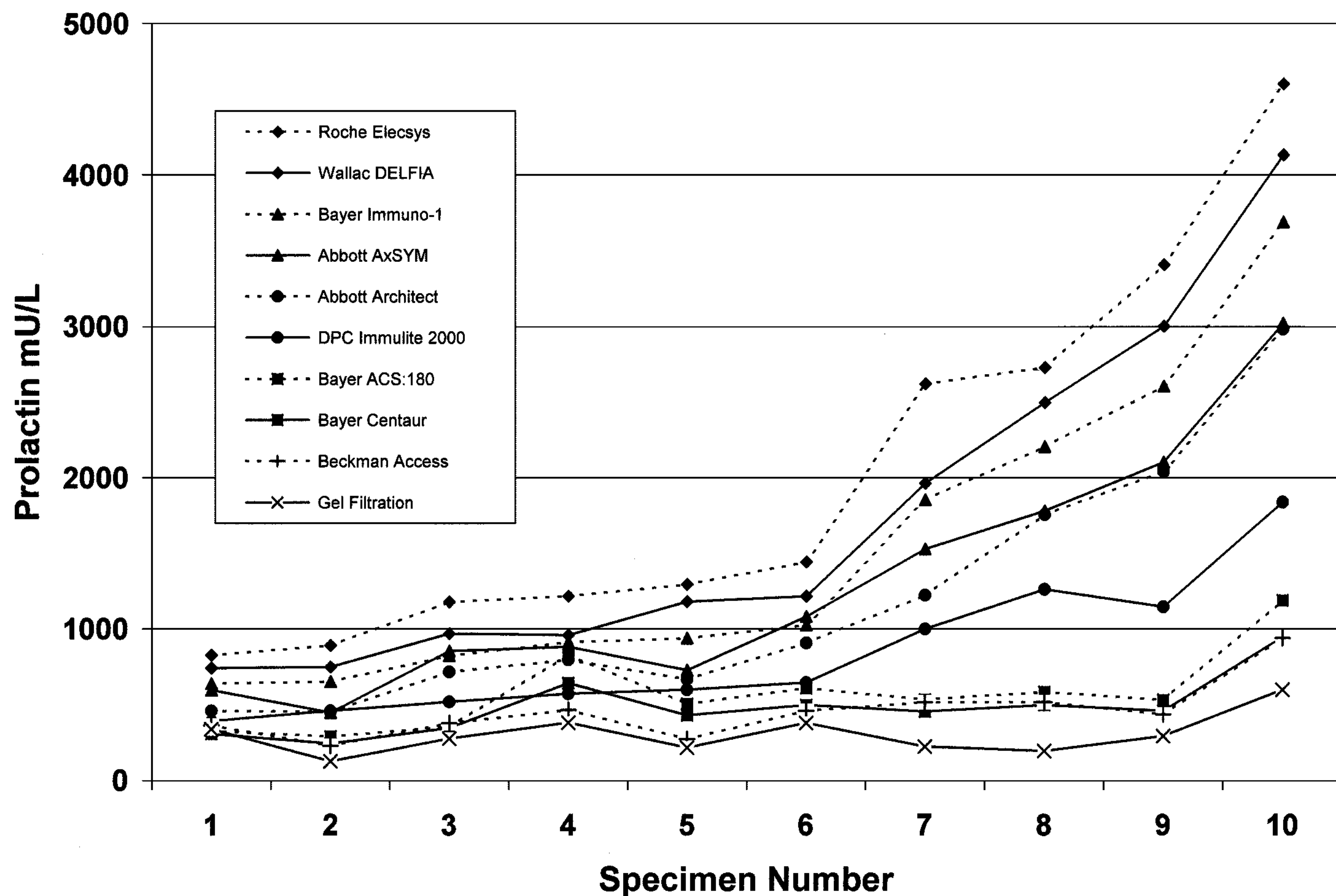

FIG. 1. Mean serum PRL levels reported by nine different immunoanalyzer user groups in specimens collected from 10 macroprolactinemic subjects. For comparative purposes, the PRL level in each specimen following removal of macroprolactin by gel filtration is shown.

participants (29, 30). Twelve percent of United Kingdom laboratories and 5% of United States laboratories used either of the two assay systems that gave PRL values in excess of 700 mU/liter in 10 of 10 macroprolactinemic samples. Forty-six percent of United Kingdom laboratories and 44% of United States laboratories use assay systems that identified PRL levels of greater than 700 mU/liter in 7 or more of the 10 macroprolactinemic samples. Eighteen percent of United Kingdom participants and 30% of United States PRL quality assurance scheme participants use either the Access (Beckman) or the Centaur (Bayer Corp.) that identified a serum PRL value in excess of 700 mU/liter in only 1 of the 10 macroprolactinemic samples. Although it is recognized that the number of participants using a particular system may not reflect the relative number of samples analyzed in the assay systems because of variability of numbers processed in different centers, the information derived from analysis of the United Kingdom and United States PRL assay quality assurance programs is useful. A conservative estimate suggests that 5–10% of the total hyperprolactinemic samples reported in the United Kingdom and United States may be accounted for by the presence of macroprolactin. When a macroprolactinemic sample was circulated to UK NEQAS participants and feedback sought for the likely explanation for the finding of hyperprolactinemia, only 22% of responders suggested the possibility of macroprolactin (22). It is likely that the phenomenon of macroprolactinemia is considerably underestimated and underrecognized.

The diagnosis of macroprolactinemia previously was based on the observation that more than 60% of PRL was in the macroprolactin form as indicated by gel filtration chromatography or less than 40% of the PRL measured in untreated serum was recovered following treatment of the serum with PEG (5–7). This convention does not acknowledge that although macroprolactin could account for more than 60% of total PRL, the level of biologically active monomeric PRL could still be present in significant excess. Thus patients have been reported to have macroprolactinemia who also had monomeric PRL levels well in excess of normal values, *e.g.* greater than 1200 mU/liter (6). It appears prudent that more demanding criteria for the diagnosis of macroprolactinemia be introduced. When PEG treatment of hyperprolactinemic sera is used, the diagnosis of macroprolactinemia might be confined to those whose serum PRL level falls to that seen in sera from normoprolactinemic subjects treated with PEG, *i.e.* less than 390 mU/liter using the Delfia (Wallac, Inc.) immunoassay. This would avoid any confusion as to whether biologically active PRL is also in excess when excess macroprolactin is present.

The variability of immunoassays to detect macroprolactin

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has been noted previously (5, 12, 16). This probably relates to the extent to which the epitope on the PRL molecule that attracts the endogenous autoantibody reacts with the site toward which the antibodies used in the assay system are directed. Thus, if the site required for recognition in an assay is occupied by the endogenous antibody, the assay will not recognize PRL even when present in large amounts as occurs in patients with macroprolactinemia. The absolute PRL levels measured in any serum demonstrated a wide range, depending on the assay system used. In contrast, the ranking of the 10 samples within the various assay systems from highest to lowest level is nearly constant. It is therefore likely that the endogenous antibody present in the 10 sera examined is directed against a single epitope on PRL. However, not all sera behave in this predictable manner, suggesting that autoantibodies directed against different epitopes also exist (16, 27, 28).

Macroprolactinemia is a largely unrecognized phenomena and has not been discussed in recent comprehensive endocrinology texts (31, 32), although it has been recently reported to account for up to 26% of hyperprolactinemic samples (9). The present study has confirmed that the extent to which macroprolactin contributes to the total PRL reported is assayed system dependent. Assay systems examined, encompassing 91% of United Kingdom and 85% of United States routine methods for measuring PRL, identified hyperprolactinemia in at least 1 of the 10 samples in which hyperprolactinemia could be accounted for entirely by the presence of macroprolactin. The extent to which macroprolactin was identified varied greatly among assay systems examined. Those responsible for providing PRL results and those responsible for the interpretation and management of patients reported to have hyperprolactinemia should be aware of the extent to which macroprolactin may contribute to hyperprolactinemia in assay systems used locally.

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