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ORIGINAL ARTICLE

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Serum prolactin overestimation and risk of misdiagnosis

Sunita M.C. De Sousa[1,2,3,4] | **Mohamed Saleem**[3,5] | **Wayne Rankin**[1,3,5] | **David J. Torpy**[1,3]

[1]Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, South Australia, Australia

[2]Department of Genetics and Molecular Pathology, Centre for Cancer Biology, an SA Pathology and University of South Australia Alliance, Adelaide, South Australia, Australia

[3]School of Medicine, University of Adelaide, Adelaide, South Australia, Australia

[4]Adult Genetics Unit, Royal Adelaide Hospital, Adelaide, South Australia, Australia

[5]Department of Chemical Pathology, SA Pathology, Adelaide, South Australia, Australia

Correspondence
Sunita M. C. De Sousa, Endocrine & Metabolic Unit, Royal Adelaide Hospital, Adelaide, SA, Australia.
Email: Sunita.Desousa@sa.gov.au

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Summary

Background: Falsely elevated prolactin measurements risk overdiagnosis, and unnecessary imaging and treatment.

Design: We conducted a clinical audit of 18 patients who presented with hyperprolactinaemia, followed by a laboratory audit of 40 split samples across a range of serum prolactin (5-5051 mIU/L). In each case (total n = 58), serum prolactin was measured on both Roche and Siemens platforms.

Results: Serum prolactin as measured by Roche was higher than the corresponding Siemens value in every case, despite similar reference ranges. The mean discrepancy in serum prolactin by Roche vs. Siemens was +81% in the clinical audit and +50% in the laboratory audit. This led to unnecessary interventions in 7/18 patients (39%) in the clinical audit.

Conclusions: Serum prolactin is overestimated on the Roche relative to the Siemens platform. Laboratories should review Roche reference intervals for serum prolactin, and clinicians should consider repeating serum prolactin on another platform if the serum prolactin is incongruent with the clinical scenario.

KEYWORDS
hyperprolactinaemia, pituitary, prolactin

1 | INTRODUCTION

The magnitude of prolactin elevation guides the differential diagnosis of hyperprolactinaemia and typically parallels tumour diameter in prolactinomas. Severe hyperprolactinaemia (>10-fold normal) is almost always due to macroprolactinomas (diameter > 1 cm), pregnancy or breastfeeding.[1] Causes of mild hyperprolactinaemia (<4-fold normal) include microprolactinomas (diameter < 1 cm), dopamine interference (eg stalk compression/transection in the "stalk effect," antipsychotics, metoclopramide), primary hypothyroidism, polycystic ovary syndrome and prolactin co-secretion in acromegaly or Cushing's disease. Mild, transient increases in prolactin may follow stress, pain, coitus, exercise, sleep, meals or seizures.[2-5]

2 | CLINICAL AUDIT

We performed an audit of 18 patients (12 women, 6 men, age 26-79 years, mean 51 years) with consistently higher serum prolactin on the Roche compared with the Siemens platform (Table 1). The Siemens Centaur® platform and either the Roche Cobas® or Roche Modular E170® platforms were employed in each case. Macroprolactinaemia was excluded by polyethylene glycol (PEG) precipitation in 6/18 patients. PEG precipitation was not performed in the remaining 12 patients as prolactin was normal or near-normal on repeat testing on the Siemens platform (eight patients) or macroprolactinaemia had previously been excluded (four patients).

Clinical confounders were absent in all but three patients. Patient 1 commenced cabergoline after the Roche measurement

TABLE 1 Serum prolactin interassay discordance encountered in routine clinical practice

Pt	Diagnosis	Roche absolute level	Roche ULN	Roche normalised level	Siemens absolute level	Siemens ULN	Siemens normalised level	% Roche increment, absolute[a]	% Roche increment, normalised[b]
1	PRLoma	25 233	500	50.5	20 836	375	55.6	21%	−9%
2	PRLoma	2000	400	5.0	1588	375	4.2	26%	18%
3	Other pituitary mass	3341	630	5.3	2431	620	3.9	37%	35%
4	NFPA	701	500	1.4	489	375	1.3	43%	8%
5	Normal or transient idiopathic hyperPRL	222	630	0.4	148	620	0.2	50%	48%
6	PRLoma	13 051	400	32.6	8650	375	23.1	51%	41%
7	PRLoma	2475	400	6.2	1632	375	4.4	52%	42%
8	PRLoma	5065	500	10.1	3258	619	5.3	55%	92%
9	PRLoma	18 852	500	37.7	12 109	620	19.5	56%	93%
10	Idiopathic hyperPRL or escitalopram	3466	500	6.9	2060	619	3.3	68%	108%
11	Idiopathic hyperPRL	1344	500	2.7	759	620	1.2	77%	120%
12	Normal	780	500	1.6	437	619	0.7	78%	121%
13	Idiopathic hyperPRL	939	500	1.9	434	620	0.7	116%	168%
14	Flupentixol	3378	500	6.8	1538	619	2.5	120%	172%
15	Normal	598	500	1.2	225	375	0.6	166%	99%
16	NFPA	1037	500	2.1	328	619	0.5	216%	291%
17	Normal	2140	500	4.3	143	619	0.2	1397%	1753%
18	Normal or metoclopramide	3895	500	7.8	139	620	0.2	2702%	3375%

hyperPRL, hyperprolactinaemia; NFPA, nonfunctioning pituitary adenoma; PRLoma, prolactinoma; Pt, patient number; ULN, upper limit of normal; %, percentage increase comparing Roche against Siemens.

[a]Calculated as (Roche absolute level − Siemens absolute level)/Siemens absolute level.

[b]Calculated as (Roche normalised level − Siemens normalised level)/Siemens normalised level.

but possibly one day prior to the Siemens measurement. Patient 17 ceased low-dose sertraline in the interval between testing on the Roche and Siemens platforms. Patient 18 took 20 mg metoclopramide the day prior to both the Roche and Siemens measurements but cumulative metoclopramide use may have differed in the preceding weeks. In the remaining 15 patients, absolute prolactin level by Roche was 81% higher (range 26%-216%), and normalised prolactin level (absolute level/upper limit of normal) was 97% higher (range 8%-291%) compared with Siemens. The normalised prolactin increment by Roche was more pronounced in women (Roche 125% higher) than men (Roche 42% higher), and in patients with prolactinomas (Roche 117% higher) than patients with no final diagnosis of prolactinoma (Roche 57% higher).

The interassay discordance was often clinically significant. For example, baseline prolactin by Roche was 10-fold normal in Patient 8, suggesting a macroprolactinoma, whereas the Siemens result of 5-fold normal was more consistent with the 7 mm pituitary tumour subsequently detected on MRI. If this patient had a macroadenoma, the mixed findings of mild and severe hyperprolactinaemia would have made it difficult to distinguish between macroprolactinoma and nonfunctioning pituitary adenoma with stalk effect hyperprolactinaemia. In another patient with schizophrenia, hyperprolactinaemia at 7-fold normal by Roche prompted investigation for a concomitant prolactinoma. MRI showed a normal pituitary and repeat prolactin by Siemens was only 2.5-fold elevated, in keeping with known antipsychotic use. Overall, 7/18 patients had unnecessary endocrine reviews and/or MRI, with incidental findings in 3/6 MRI reports.

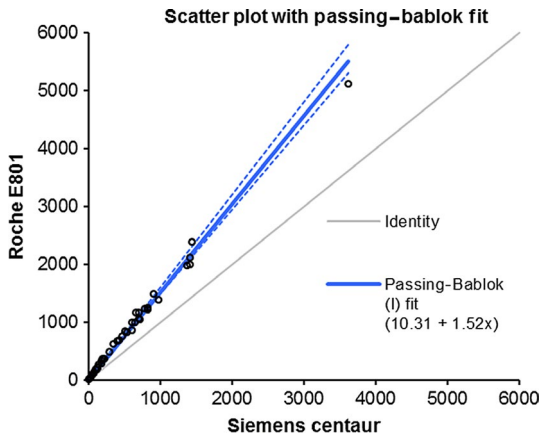

FIGURE 1 Comparative performance of prolactin by Roche Cobas vs Siemens Centaur (n = 40); Passing-Bablok fit

TABLE 2 Potential implications of serum prolactin overestimation

True result	Overestimated result	True diagnosis	False diagnosis	Potential implications
Normal PRL	Mild hyperPRL	Normal	MicroPRLoma or other pituitary mass with stalk effect hyperPRL	• Unnecessary pituitary MRI • Unnecessary endocrine review • Incidental findings • Unnecessary DA therapy with risk of side effects
		Adequately controlled PRLoma on DA therapy	DA resistance or escape	• Unnecessary increase in DA dose with increased risk of side effects • Unnecessary referral for surgery/radiotherapy
		Other cause of infertility or menstrual disturbance	Occult microPRLoma	• Unnecessary pituitary MRI • Incidental findings • Unnecessary/ineffective DA therapy • Inappropriate deferral of investigations for other reproductive pathology
Mild hyperPRL	Severe hyperPRL	Drug-induced hyperPRL	PRLoma or other pituitary mass with stalk effect hyperPRL	• Unnecessary pituitary MRI • Unnecessary endocrine review • Incidental findings
		Pituitary mass with stalk effect hyperPRL	MacroPRLoma	• Unnecessary/ineffective DA therapy • Inappropriate delay in surgical intervention

DA, dopamine agonist; hyperPRL, hyperprolactinaemia; macroPRLoma, macroprolactinoma; microPRLoma, microprolactinoma; PRLoma, prolactinoma.

3 | ASSAY COMPARISON

Based on our clinical observations, we measured serum prolactin by the Siemens Centaur® and Roche Cobas ® platforms using split samples (n = 40) across a range of serum prolactin (5-5051mIU/L). Passing-Bablok regression returned an intercept of 10.31 and a gradient of 1.52 (95% CI 1.46-1.60), representing a consistent increase in serum prolactin of approximately 50% by Roche compared with Siemens (Figure 1). Reference intervals for the two assays were similar. Our review of the original Roche data revealed no technical error in reference interval calculation.

4 | DISCUSSION

Our clinical audit of 18 patients and assay comparison of 40 split samples showed that serum prolactin is consistently overestimated by Roche compared with Siemens, in both absolute values (mIU/L) and in relative values, that is compared with the upper limit of normal. This is relevant to laboratories and to clinicians typically measuring prolactin to investigate menstrual disturbances, low testosterone (in males), infertility or pituitary masses. The potential diagnostic and therapeutic implications of prolactin overestimation are outlined in Table 2. It is worth also noting the costs of further investigation due to misleadingly high serum prolactin levels, including, but not limited to, pituitary MRI scans which cost approximately AUD 600.

The cause of prolactin overestimation is unclear. We excluded errors in reference interval calculation; however, progressive positive bias with successive reagent lot numbers and antibody variability over time remain possible. Several other factors could contribute to interassay discordance. When tested on different days, the commencement or cessation of drugs that interrupt the tonic inhibition of prolactin secretion by dopamine could respectively lead to higher or lower prolactin levels on the second test. Heterophile antibodies with varying assay interactions are also possible. This was suspected in two patients in the clinical audit who had markedly higher serum prolactin on the Roche versus Siemens assays with absolute increases of 1397% in Patient 17 and 2702% in Patient 18. However, both patients were intermittently taking dopamine interfering medications and were already excluded from the final analysis. We also found no consistent relationship between the prolactin increment by Roche and age, inter-testing interval, and whether the Roche or Siemens test was performed earlier in the day (data not shown). Transient stimuli of prolactin secretion for example stress or coitus cannot be excluded but the consistency of higher Roche prolactin levels in all 58 cases, including split samples, argues against this.

Whether prolactin is overestimated by the Roche platform or underestimated by the Siemens platform could not be distinguished in the 40 split samples of the assay comparison. In the clinical audit, 7/15 cases favoured the Siemens prolactin result being correct. For example, a perimenopausal patient had a robust gonadotrophin response which was consistent with her normal serum prolactin by Siemens as opposed to her 2-fold elevation in prolactin by Roche. Another two patients were diagnosed with drug-induced hyperprolactinaemia where serum prolactin is typically 2-fold to 3-fold elevated as found by Siemens, rather than 6-fold to 7-fold elevated as found by Roche. Two women only had slight menstrual irregularity and normal pituitary MRI studies that favoured their serum prolactin values near the upper limit of normal by Siemens compared with 2-fold to 3-fold

elevations by Roche. The last two patients were being serially followed after surgery for a prolactinoma in one patient and cessation of prolactinoma dopamine agonist treatment in the other patient who had developed disruptive hypersexuality on treatment. These two patients both had gradually increasing serum prolactin levels on the Siemens assay as expected due to their known tumour remnants but their latest prolactin result by Roche created sharp inflections in their trajectories. The sharp inflections were discordant with clinical findings in both cases as both tumour remnants were stable on serial imaging and cabergoline had been restarted in the postoperative patient in the lead up to the latest test. Overall, these informative cases indicated serum prolactin overestimation by Roche.

Our findings of prolactin interassay discordance may be overcome by a higher Roche reference interval as prolactin should always be interpreted relative to the upper limit of normal rather than as an absolute value. Determining new reference intervals will require large numbers of healthy controls and patients with varying degrees of hyperprolactinaemia. In the meantime, clinicians should be aware of the potential for prolactin overestimation and the utility of repeat testing on different platforms. In mild hyperprolactinaemia by the Roche platform with normoprolactinaemia by other platforms, patients may be spared from unnecessary endocrine reviews and MRI studies. In true hyperprolactinaemia, separating patients with mild versus severe hyperprolactinaemia will narrow the diagnostic possibilities.

ETHICS STATEMENT

Ethical review was not required for audit data as per the National Health and Medical Research Council statement on "Ethical Considerations in Quality Assurance and Evaluation Activities (March 2014)."

DATA ACCESSIBILITY STATEMENT

Raw data are provided in the manuscript.

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CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and execution of the study and to the final manuscript.

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