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The utility of prolactin serial sampling and the best prolactin cut-offs associated with persistent hyperprolactinemia

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Abstract

Background: A single prolactin sampling is recommended for the diagnosis of hyperprolactinemia. We aimed to study the utility of the prolactin serial sampling and to determine the best cut-offs associated with persistent hyperprolactinemia.

Methods: Retrospective study of hyperprolactinemic patients [referral prolactin (rPRL)] that underwent prolactin serial samplings. Prolactin at 0 minutes (PRL0′), 20 to 30, and 40 to 60 minutes. The lowest of these last 2 was defined as nadir prolactin (nPRL). Persistent hyperprolactinemia was defined as nPRL above normal. We excluded patients under dopamine receptor agonists. Receiver-operating characteristic (ROC) curves were used to determine the best rPRL and PRL0′ cut-offs predicting persistent hyperprolactinemia.

Results: We studied 53 patients (3 males). Median rPRL 48.0 ng/mL (39.5–72.5), PRL0′ 34.3 ng/mL (18.0–50.8) and nPRL 29.5 ng/mL (11.4–44.4). PRL0′ was elevated in 35 (66.0%) patients and in 7 of them a normal nPRL was reached; therefore 28 (52.8%) had persistent hyperprolactinemia. The area under curve (AUC) for the association between rPRL and persistent hyperprolactinemia was 0.70 (95%CI: 0.56–0.84); best cut-off: 53.4 ng/mL [sensitivity 53.6%, specificity 80.0%, positive predictive value (PPV) 75.0%, and negative predictive value (NPV) 60.6%]. In the 35 patients with elevated PRL0′, the AUC was 0.92 (95%CI: 0.81–1.00); best cut-off: 35.2 ng/mL (sensitivity 85.7%, specificity 85.7%, PPV 60.0%, and NPV 96.0%).

Conclusions: Approximately 1/3 of the patients reached a normal PRL0′. In an additional 20%, prolactin normalized after serial samplings. Patients with rPRL >53.4 ng/mL had 75% probability of having persistent hyperprolactinemia and those with PRL0′ <35.2 ng/mL had a 96% probability of not having persistent hyperprolactinemia.

Abbreviations: rPRL = referral prolactin; PRL0' = prolactin at 0 minutes; nPRL = nadir prolactin; ROC = receiver-operating characteristic; AUC = area under curve.

Keywords: hyperprolactinemia, prolactin pool, prolactin serial sampling, stress hyperprolactinemia

Introduction

Hyperprolactinemia is a common endocrine disorder, occurring more frequently in women.[1] Although its estimated prevalence is less than 1% in the general population, it can occur in up to 5% to 14% of women with secondary amenorrhea.[2] Prolactin is a 23 kDa polypeptide hormone produced in the lactotroph cells of the anterior pituitary gland and secreted, not only in a pulsatile manner but also with a circadian variation.[3] The hypothalamic control of prolactin secretion is mostly inhibitory through dopamine's action on type 2 dopamine (D2) receptor located on lactotrophs.

Causes of hyperprolactinemia can be categorized as physiological or pathological, the latter includes pharmacological causes.[4,5] Some physiological factors known to increase prolactin secretion are stress, sleep, exercise, food ingestion, pregnancy, or breastfeeding. Pathological causes include several systemic disorders (such as chronic renal failure, liver cirrhosis or primary hypothyroidism) and hypothalamic or pituitary diseases.[6] Medications and sellar/parasellar masses are the most common pathological causes of hyperprolactinemia.[7] Among the most common drugs interfering with the production, transport and/or action of dopamine are antipsychotics, antidepressants and oestrogen therapy.[8]

Current guidelines recommend a single prolactin sampling for the diagnosis of hyperprolactinemia, as long as the sample is withdrawn without excessive venipuncture stress,[8] which may be difficult to accomplish. Therefore, in some patients, prolactin levels may normalize in a subsequent sampling or if prolactin is collected through a venous catheter sometime after puncture. Considering that circulating prolactin has a half-life of 20 to 50 minutes,[9] a pool based analysis can be a useful method to distinguish real hyperprolactinemias from those caused by

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venipuncture stress, in patients initially presenting elevated levels of prolactin. In this way, it would be possible to avoid overdiagnosis of hyperprolactinemia. Scarcely any data has been published to this date about prolactin pool sampling and the few that have been showed controversial results.[10–12]

Our objective was to evaluate the proportion of patients in which prolactin remained elevated after the prolactin serial sampling and to determine the best prolactin cut-offs associated with persistent hyperprolactinemia.

Materials and methods

We retrospectively studied all patients referred to the endocrinology clinic of the Centro Hospitalar do Tâmega e Sousa E.P.E. from 2006 to 2019 due to hyperprolactinemia that underwent prolactin serial sampling (also sometimes referred to as prolactin pool or hyperprolactinemia rest test).[10]

Hyperprolactinemic patients were mainly referred by primary care physicians and, less often by other outpatient departments within our hospital. This prolactin value was defined as the referral prolactin (rPRL). The common practice in our clinic is to collect a new single prolactin sample after potential hyperprolactinemia-inducing drugs are stopped and pregnancy is ruled out. Less frequently, a prolactin serial sampling is performed if deem useful by the patient attending endocrinologist. Only those patients that underwent the prolactin serial sampling were included in this study. We excluded patients with missing data on rPRL and those treated with dopamine receptor agonists. From a total of 92 patients submitted to a pooled prolactin collection, 23 were excluded because they had missing rPRL data and 16 because they were treated with dopamine agonists at the time of the test. Eight patients performed the prolactin serial sampling under possible hyperprolactinemia inducing drugs since its discontinuation could exacerbate their psychiatric condition.

The prolactin serial sampling starts with the introduction of an indwelling catheter to a patient at rest. Prolactin at 0 minutes (PRL0′), 20 to 30, and 40 to 60 minutes. The lowest value of these last 2 samples was defined as the nadir prolactin (nPRL). Persistent hyperprolactinemia was considered present if the nPRL was above the normal reference range for sex and menopausal status. The prolactin assay used in our laboratory and the one used for the repeated prolactin samples was *Access Prolactin from Beckman and Coulter*. Its normal references range is 2.6 to 13.1 ng/mL for men, 3.3 to 26.7 ng/mL for premenopausal women and 2.7 to 19.6 ng/mL for postmenopausal women. Since rPRL was mainly sampled in external laboratories we do not know which assays were used.

Demographic, clinical, analytical, and radiologic data was collected by reviewing the patients' medical records. Drugs that have been associated with elevated prolactin levels[13] were documented.

The endpoint under analysis was persistent hyperprolactinemia.

Statistical analysis

The proportion of patients with normal PRL0′ and nPRL was calculated.

Receiver-operating characteristic (ROC) curves were used to determine the best rPRL and PRL0′ cut-offs (Youden index) for the association with persistent hyperprolactinemia. The area under curve (AUC) was determined.

A logistic regression analysis was used to test the association between the rPRL cut-offs and persistent hyperprolactinemia.

Table 1

Patients' characteristics and laboratory results

Variable	All patients (n = 53)
Age (yr), mean (SD)	34 ± 3
Male sex, n (%)	3 (5.7)
Type 2 diabetes mellitus, n (%)	1 (1.9)
Hypothyroidism, n (%)	1 (1.9)
Psychiatric disease, n (%)	8 (15.1)
Hyperprolactinemic drugs, n (%)	25 (47.2)
Prolactinoma, n (%)	15 (28.6)
rPRL (ng/mL), median (IQR)	48.0 (39.5–72.5)
PRL0′ (ng/mL), median (IQR)	34.3 (18.0–50.8)
PRL20–30′ (ng/mL), median (IQR)	33.4 (13.7–46.8)
PRL40–60′ (ng/mL), median (IQR)	21.0 (10.2–35.2)
nPRL (ng/mL), median (IQR)	29.5 (11.4–44.4)
Elevated PRL0′, n (%)	35 (66.0)
Persistent hyperprolactinemia, n (%)	28 (52.8)

IQR = interquartile range; nPRL = nadir prolactin; PRL20–30′ = prolactin value at 20–30 min; PRL40–60′ = prolactin value at 40–60 min; PRL0′ = prolactin at 0 minutes; rPRL = referral prolactin; SD = standard deviation.

We stored and analysed data using IBM SPSS Statistics, version 22.0.

Results

We studied a total of 53 patients with a mean age of 34 ± 3 years and 3 (5.7%) of which were males. The medians (interquartile range) rPRL, PRL0′ and nPRL were 48.0 (39.5–72.5) ng/mL, 34.3 (18.0–50.8) ng/mL and 29.5 (11.4–44.4) ng/mL, respectively. PRL0′ remained elevated in 35 (66.0%) patients (Table 1). Additionally, 7 patients (20%) with elevated PRL0′ reached a normal nPRL value in the pooled measurement. Therefore, 28 (52.8%) patients presented persistent hyperprolactinemia.

Twenty-five (47.2%) patients were treated with possible hyperprolactinemic drugs at the time of the rPRL sampling. The more frequent drugs used were oral oestrogens (56.0%) followed by selective serotonin reuptake inhibitors (12%). The remainder were amitriptyline, amisulpride, and metoclopramide. Among those patients with normalized prolactin, hyperprolactinemia was considered drug-induced in 7 (28%) and stress-related in 18 (72%). In the persistent hyperprolactinemia group, 15 (53.6%) patients were diagnosed with a prolactinoma, 8 (28.6%) with drug-induced hyperprolactinemia and 5 (17.9%) with idiopathic hyperprolactinemia.

The area under the ROC curve for the association between rPRL and persistent hyperprolactinemia was 0.70 (95%CI: 0.56–0.84), $P = .01$ (Fig. 1). The best rPRL cut-off for persistent hyperprolactinemia prediction was 53.4 ng/mL (Youden index of 1.336). The results would have been similar if we had excluded the 8 patients treated with possible hyperprolactinemia-inducing drugs that were not discontinued. Using this cut-off, a persistently elevated nPRL could be found with a sensitivity of 53.6% and a specificity of 80.0%. The positive predictive value (PPV) was 75.0% and the negative predictive value (NPV) was 60.6%.

In the 35 patients with elevated PRL0′, the area under the ROC curve for the association between PRL0′ and persistent hyperprolactinemia was 0.92 (95%CI: 0.81–1.00), $P = .001$ (Fig. 2). The best PRL0′ cut-off was 35.2 ng/mL with a sensitivity and specificity of 85.7%, a PPV of 60.0% and a NPV of 96.0%.

In the logistic regression analysis, patients with a rPRL above 53.4 ng/mL had an odds ratio of elevated nPRL of 3.65 (95%CI: 1.12–11.90, $P = .03$).

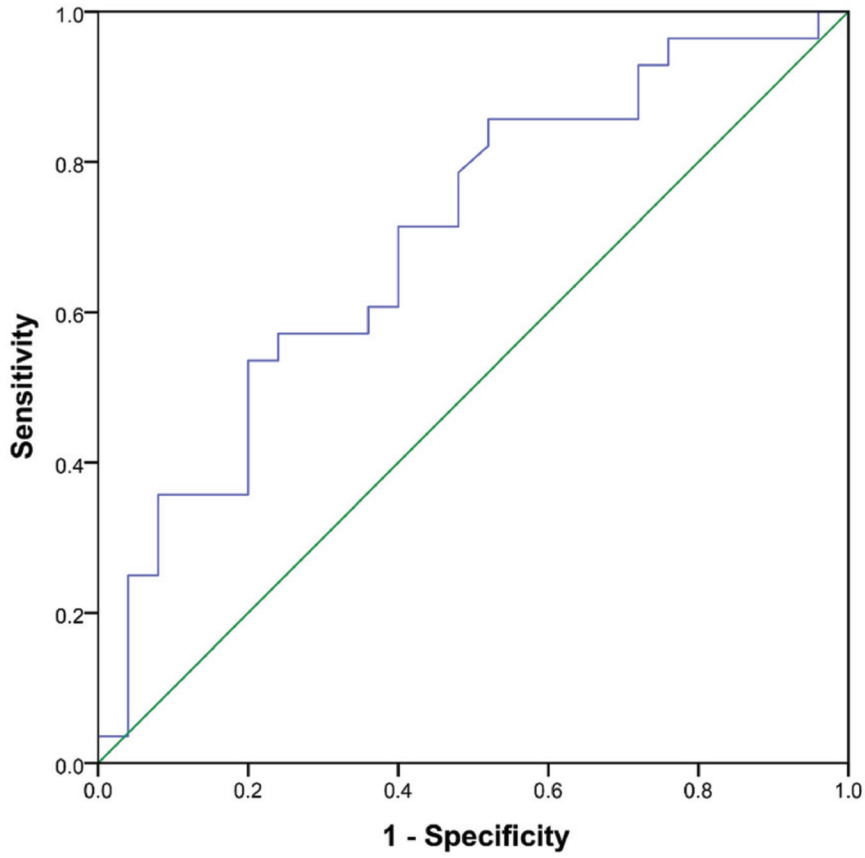

Figure 1. The area under the receiver-operating characteristic (ROC) curve for the association between referral prolactin (rPRL) and persistent hyperprolactinemia.

Discussion and conclusions

In our population of hyperprolactinemic patients who underwent repeated prolactin samplings, we found that the prolactin levels remained above the normal reference range in the first sample collected right after puncture in two-thirds of them and only 20% of them reached a normal prolactin value in subsequent collections. Additionally, we suggest a prolactin value of 53.4 ng/mL as the best prolactin cut-off to detect persistent hyperprolactinemia, therefore diminishing the need for prolactin resampling. Patients with a prolactin value above 53.4 ng/mL (2 times the upper reference level) had more than 75% probability of having persistent hyperprolactinemia. In addition, patients with a PRL0′ below 35.2 ng/mL had a 96% probability of normal prolactin value after repeated samplings from an indwelling catheter, but these results should be analysed cautiously due to the very small sample size.

Previous studies have focused on the utility of prolactin serial sampling in the diagnosis of true hyperprolactinemia. Their results revealed that 27.2% to 68.1% of patients with initially elevated prolactin would normalize during serial samplings.[10,12,14] Around 36% to 73% of patients whose values normalized did so in the first, or in the zero-minute sample.[10,12,14] Therefore, 11.8% to 55.0% with elevated PRL0′ normalized during additional sampling.[10,12,15] We found similar results, with 47.2% of patients reaching a normal prolactin value, 72% of these at PRL0′ and from those with elevated PRL0′ 20% normalized in the subsequent samples. This has important clinical implications since current guidelines[8] recommend a single prolactin sampling under optimal conditions for the diagnosis of hyperprolactinemia and thus it could misdiagnose about one-third of patients,[10,12,15] possibly leading to unnecessary subsequent testing and eventually unwarranted treatment.

Nevertheless, different methodologies were used in these studies and therefore they are not easily comparable. Francés et al obtained a sample at baseline and at 30 minutes. Given the half-life of prolactin, some elevated results at the 30 minutes sampling could still normalize further in time. Whyte et al collected prolactin samples at baseline and at 120 minutes and, surprisingly, had a lower rate of prolactin normalization after serial samplings. As prolactin is expected to decrease with time and the authors collected the second sample at a later time, other factors might have influenced the results. Differences in the patients' characteristics and in the hyperprolactinemia aetiologies might have played a role. A former study conducted by Muneyyirci-Delale et al,[10] who collected samples at 15 minutes intervals up to 90 minutes, found that in all patients who reached euprolactinemia did so by the 60 minutes mark. Since we did not collect samples beyond 60 minutes, there is the possibility that some patients might have reached normal prolactin values. This finding by Muneyyirci-Delale et al reassures our strategy.

As far as we are aware, only one other study suggested a prolactin cut-off for persistent hyperprolactinemia. Whyte et al advocated a value of 96.2 ng/mL (4 times the upper reference level) as a cut-off point for rPRL measurement, with 97% specificity to detect true hyperprolactinemia, although only in women.[12] The authors chose this cut-off in order to minimize

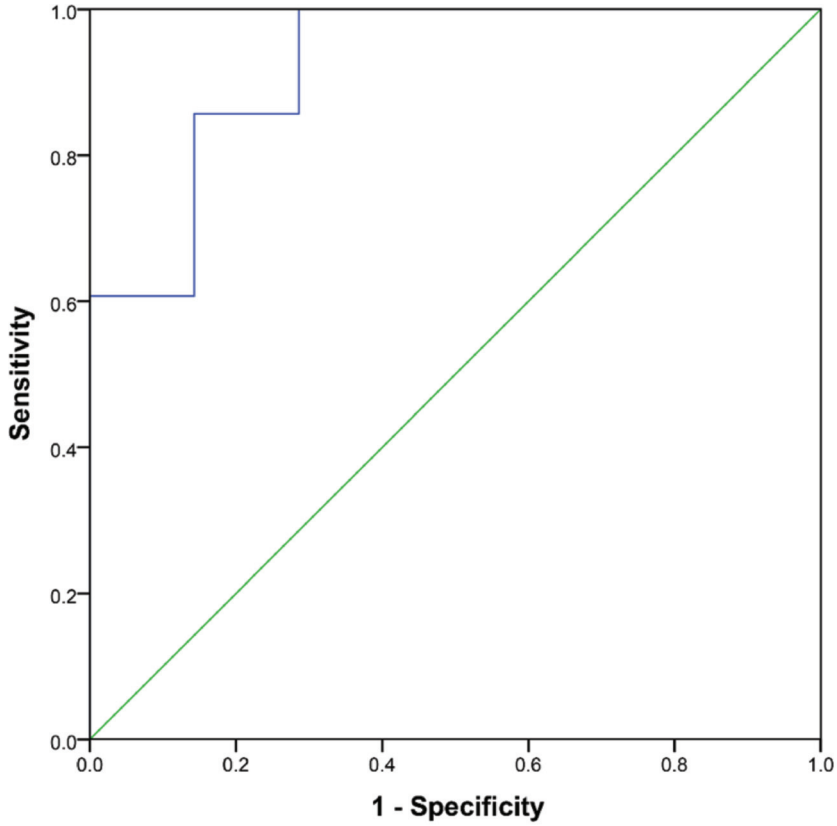

Figure 2. The area under the receiver-operating characteristic (ROC) curve for the association between PRL0′ and persistent hyperprolactinemia.

false positive results. We established a 53.4 ng/mL as the best prolactin cut-off (Youden method) to detect persistent hyperprolactinemia regardless of the gender. If we had chosen to improve the cut-off specificity in our patient population to match that proposed by Whyte et al, we would have obtained a more identical result: a prolactin value of 124.8 ng/mL with a specificity of 96.0%, sensitivity of 25.0%, a PPV of 87.5% and a NPV of 53.4%. Francés et al were not able to recommend a cut-off level since prolactin values obtained in patients with and without confirmed hyperprolactinemia overlapped. In fact, very high values (for instance 277.6 ng/mL) were reported in the group that did not have confirmed hyperprolactinemia after serial sampling.[14]

In our study, we demonstrated that 20% of patients with elevated prolactin value at baseline reached a normal value in the subsequent measurements. The possibility of normalization of prolactin values suggests that this test could be a useful tool to avoid misdiagnosis as well as to prevent further unnecessary testing and overtreating of many patients, all of which are associated with a significant burden of costs. It is important to notice that by preventing unnecessary testing, not only do we avert the exposure to unnecessary radiation, but also incidental diagnosis of pituitary adenomas, since up to 20% of the population may present this finding.[16] On the other hand, the prolactin repeated sampling could be onerous. It is more costly as it requires more samples, it consumes more healthcare resources and takes more of the patients' time. Nevertheless, in selected patients, for instance those with lower prolactin levels, it can be helpful in the exclusion of stress hyperprolactinemia and subsequent higher costs from further medical tests and treatments.

Our study has limitations beyond its retrospective design. The small sample size increases the likelihood of type II errors. We did not systematically test for the presence of macroprolactinemia and it could interfere with the proportion of patients who remained hyperprolactinemic. We also did not have any information regarding the assay used or the collection methods for the rPRL, consequently making a solid conclusion regarding rPRL cut-off even more difficult. However, the results obtained reflect the challenge confronted by endocrinologists in their daily clinical practice and the cut-off suggested is derived from these common non-ideal conditions in every-day routine. Additionally, almost half of our patient population was taking drugs known to possibly induce prolactin secretion, mostly oestrogens and selective serotonin reuptake inhibitors, possibly contributing as a confounding factor for the values obtained. The decision to submit the patient to a prolactin repeated sampling was made by the attending physician. The rationale behind the decision cannot be inferred from clinical records and therefore, the possibility of a selection bias cannot be ruled out. Prolactin sampling would more likely be proposed to patients with higher clinical suspicion of stress hyperprolactinemia.

Conclusions

Approximately one-third of the patients with hyperprolactinemia reached a normal PRL0′ level in a new prolactin collection when possible interferents were accounted for. Of those, an additional

20% normalized their prolactin levels in the serial prolactin samplings. Patients with a rPRL above 53.4 ng/mL (about 2 times the upper reference level) have 75% probability of having persistent hyperprolactinemia.

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Conflicts of interest

The authors declare no competing interests.

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