subject DNA with wild-type DNA. This is, however, less of a problem in autosomal dominant diseases. Although an initial capital investment in the HPLC instrument is required, the combination of low running costs and the tremendous reduction in the effort of sequencing make the DHPLC technique a suitable method for mutation detection *(26, 27)*.

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An Unusual Form of Big, Big (Macro) Prolactin in a Pregnant Patient, *Michael J. Diver,[1*] David L. Ewins,[2] Richard C. Worth,[2] Shirley Bowles,[2] James A. Ahlquist,[3] and Michael N. Fahie-Wilson[3]* ([1] Department of Clinical Chemistry, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP, United Kingdom; [2] Countess of Chester Hospital, Liverpool Road, Chester, United Kingdom; [3] Southend Hospital, Westcliffe-on-Sea, Essex SS0 0RY, United Kingdom; * author for correspondence: fax 44-151-706-5813, e-mail mjdiver@liv.ac.uk.)

It is well recognized that circulating prolactin may exist in several forms, including little (monomeric), big, and big, big (macroprolactin) prolactin with molecular masses of 23, 50, and 150–170 kDa, respectively *(1)*.

We report the case of a 30-year-old woman who initially attended her primary care physician because of the onset of painful irregular periods. Her cycle usually was regular, but she had had an 8-week interval of amenorrhea, followed by a particularly painful bleed for which she sought medical advice. Before this, and subsequently, her menstruation had been completely regular with a 28-day cycle. She had no other problems.

The patient's initial serum prolactin was recorded as 15 800 mIU/L (~530 μg/L) in a Bayer Immuno 1^{TM} assay (Bayer Corporation). Other investigations at the time were entirely normal.

When the subject was monitored 2 months later, she was symptomless and menstruating regularly; her serum prolactin, using the same assay as before, was 8440 mIU/L (~270 μg/L). Pituitary imaging by magnetic resonance was normal. She had, of choice, never been pregnant.

Because of the patient's lack of symptoms, normal pituitary imaging, and regular cycles, further analytical investigations were carried out on a sample of her serum. After polyethylene glycol (PEG) precipitation, the recovery of prolactin was low, indicating the presence of macroprolactin *(2)*, and 15% of her total prolactin was estimated to be monomeric prolactin.

The patient's serum prolactin concentration was remeasured using a Wallac Delfia^{TM} assay (EG & G Wallac) and

compared with that measured previously on the Bayer Immuno 1 analyzer. The Delfia assay recorded a prolactin concentration of 2980 mIU/L (~100 μg/L), a difference of 70%. This marked discrepancy between the results prompted measurement of prolactin in the specimen by three other commonly used automated immunoanalyzers (Table 1).

In our experience, the Delfia, Immuno 1, and Elecsys (Roche Diagnostics) assays are consistently high-reacting assays with samples containing macroprolactin, and the ACS:180 (Bayer) is, most frequently, a relatively low-reacting assay *(3)*. In 24 cases of macroprolactinemia, results from the Delfia assay always exceeded those from the ACS:180 assay *(4)*, presumably because the IgG antibody masks the epitope of prolactin with which the ACS:180 prolactin antibody reacts.

The presence of macroprolactinemia was confirmed by the Delfia assay and further investigated by the following techniques (see Table 1 for results): (*a*) PEG precipitation *(2)*; (*b*) ^{125}I binding *(5)*; (*c*) ultrafiltration *(6)*; (*d*) gel filtration chromatography *(2)*; and (*e*) protein G affinity chromatography *(5)*.

Treatment of normal serum containing monomeric prolactin with 4 mol/L urea causes some denaturation of immunoreactive prolactin detected by both the Delfia and ACS:180 assays. In our experience, treatment of serum containing macroprolactin converts this to monomeric prolactin (Fig. 1A), decreasing the amount of immunoreactive prolactin detected by the Delfia assay and increasing the amount detected by the ACS:180 assay.

Serum from our patient behaved differently, with an increase in immunoreactive prolactin in the Delfia assay and a decrease with the ACS:180 after treatment with urea as well as a more complex pattern on gel filtration chromatography (Fig. 1B).

To further examine the glycoprotein content of this novel high-molecular mass form of prolactin, we analyzed serum samples after lectin affinity chromatography. Using the immobilized lectin concanavalin A linked to Sepharose™ *(5)*, we found that 36% of the immunoreactive prolactin from our patient's serum was retained on the concanavalin A-Sepharose column compared with <10% from samples containing only simple prolactin. The macroprolactin peak accounted for 91% of the total immunoreactive prolactin in the Delfia assay. Using the peaks of albumin and monomeric prolactin to define the relationship between elution volume and molecular mass, we estimated that the molecular mass of the macroprolactin from this patient was 234 kDa. In 13 other cases of macroprolactin, the mean molecular mass was estimated at 162 kDa (range, 119–237 kDa).

After these original investigations, the patient remained well, with continued regular periods, on no therapy, and with no other problems. After a 2-year interval, she became pregnant, and a serum prolactin measured at 6 months of gestation revealed (Bayer Immuno 1) a concentration of 29 949 mIU/L (~1000 μg/L), of which 1700 mIU/L (5.6%) was found to be monomeric after PEG precipitation. The pregnancy was uneventful, and the patient was delivered of a 3.6-kg boy following induction at 42 weeks of gestation. She lactated without difficulty, but decided not to breastfeed the baby.

Two weeks postpartum, her serum prolactin measured on the Bayer Immuno 1 analyzer was 42 000 mIU/L (~1400 μg/L), of which 4277 mIU/L (~140 μg/L) was monomeric. One month later, her serum prolactin (Bayer Immuno 1) was 17 660 mIU/L (~600 μg/L), similar to the concentration measured when she was first investigated.

The data presented on this patient suggest that the macroprolactin had some typical characteristics in that it is a prolactin-IgG antibody complex that is not bioactive in vivo. It is, however, unusual in being extensively glycosylated with, consequently, higher molecular mass and markedly different reactivity with commonly used immunoassay systems. A patient with extensively glycosylated high-molecular mass prolactin was reported by Hattori *(5)*, but an IgG component was not demonstrated by protein-G affinity chromatography and there was no increased binding of ^{125}I-labeled prolactin.

Hyperprolactinemia attributable to macroprolactinemia is commonly found in patients with moderate but not markedly increased serum prolactin concentrations [usually <3000 mIU/L (100 μg/L)]. Serum prolactin concentrations >6000 mIU/L (200 μg/L) are commonly taken as evidence of a prolactin-secreting pituitary adenoma *(7)*. Others investigators *(8)* have reported the presence of big (as opposed to macro-) prolactin in two men with pituitary adenomas. Big, big prolactin, or macroprolactin, has been described as the major immunoreactive prolactin

Table 1. Serum prolactin measured by five different automated analyzers and results following various treatments of index patient's serum.

Analyzer	Serum PRL,[a] mIU/L	Change with 4 mol/L urea, %	% monomeric PRL after PEG treatment (50%)[b]	% ultrafiltrable (40%)[b]	% monomeric after gel filtration	Lectin affinity, % (<10%)[b]	^{125}I binding, % (<11%)[b]
Immuno 1	8440						
Delfia	2980	194	15	16.7	9.0	36	22.5
ACS:180	12308	67					
Elecsys	5968						
AxSYM	1800						

[a] PRL, prolactin.

[b] Value in parentheses indicates percentage obtained for normoprolactinemic subjects.

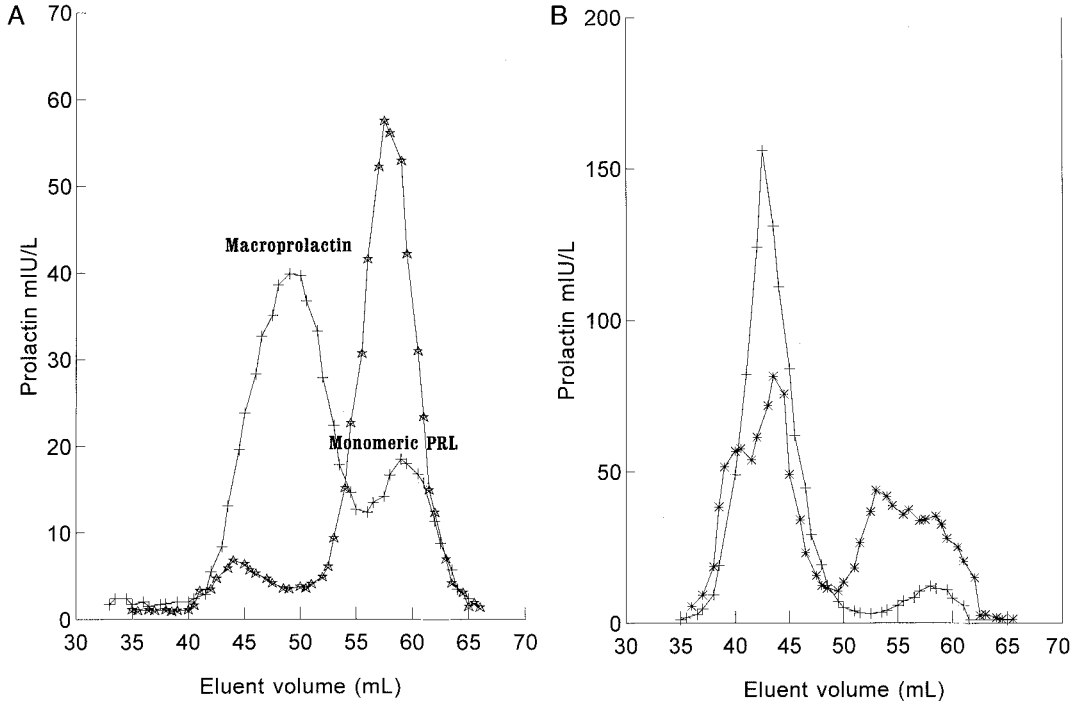

Fig. 1. Gel filtration chromatography of serum prolactin.

(*A*), gel filtration chromatography of serum prolactin in a case of macroprolactinemia before (+) and after (☆) treatment with 4 mol/L urea. (*B*), gel filtration chromatography of serum prolactin in the index case before (+) and after (∗) treatment with 4 mol/L urea.

species in the serum of several subjects before and during pregnancy *(5, 9–11)*.

In the case we report, monomeric prolactin increased, but macroprolactin remained the major immunoreactive prolactin component in serum during pregnancy. Given the medical history and subsequent progress of this present patient, it is unlikely that the vast majority of her circulating prolactin (up to 90% macroprolactin) was bioactive.

The data presented on this patient confirm that, depending on the assay system used, widely varying estimates of serum prolactin concentrations may be encountered in serum containing macroprolactin. It seems probable that the varying response of assay systems reflects the variable structure of macroprolactin and the availability of epitopes on the prolactin component to react with assay antibodies.

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Detection of *Mbo*II Polymorphism at the 5′ Promoter Region of *CYP3A4*, *Selma A. Cavalli,* Mario H. Hirata, and Rosario D.C. Hirata* (Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences of the Sao Paulo University, Av. Lineu Prestes 580, B17, CEP 05508-900, Sao Paulo, SP, Brazil; * author for correspondence: fax 55-11-3813-2197, e-mail scavalli@usp.br)

The P450 cytochromes are a superfamily of hemeproteins that catalyze the metabolism of a large number of xenobiotics and endobiotics. CYP3A4 is the major form of P450 in human liver, metabolizing >50% of all drugs *(1)*. Differences in drug metabolism rates can lead to severe