ture with gentle shaking, staining was performed in 150 mL of a 1 g/L silver nitrate solution for 10 min at room temperature with gentle shaking. The gel was rinsed twice with distilled water to remove excess silver solution and was developed in a solution containing, per liter, 15 g of NaOH, 0.1 g of NaBH₄, and 0.48 g of formaldehyde. The gel was first washed in 150 mL of this solution (for ~30 s) to precipitate the excess silver, and then it was incubated in 150 mL of the same solution, with gentle shaking, until the bands were visualized (~20 min). The stain was fixed with an aqueous solution of 7.5 g/L NaCO₃ for 10 min at room temperature with gentle shaking. The gel was laid on a bench top and covered with Whatman 3MM paper. The Whatman paper was picked up and turned over, so that the gel was peeled off the bench top. The gel was covered with Saran Cling Wrap and stored at 4 °C (up to 2 weeks) or dried under reduced pressure for 1.5 h at 80 °C.

We believe this procedure is useful both for its economical cost and for its sensitivity.

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References


Macroprolactin Reactivities in Prolactin Assays: An Issue for Clinical Laboratories and Equipment Manufacturers

To the Editor:

Macroprolactin is a complex of prolactin with immunoglobulin (IgG) that in vivo appears to have limited or no biological activity, possibly because of the failure of the high-molecular weight complex to cross capillary walls (1). The predominant form of prolactin in serum in the general population is monomeric prolactin, but the prevalence of macroprolactin in serum is not known. However, when samples with increased prolactin are further investigated, it has been reported that up to 26% of these samples contain macroprolactin as the predominant form (2, 3). Immunoassays for prolactin have variable reactivity with macroprolactin; therefore, its presence should be considered in the differential diagnosis of hyperprolactinemia. This clinical problem is illustrated by the following two patients who were referred, from the same local hospital, to our endocrine center for the investigation of hyperprolactinemia.

Clinical case 1. A 38-year-old female volunteer in a clinical trial had serum prolactin concentrations of 1168–1748 mIU/L (reference interval <420 mIU/L) on three occasions at her local hospital. She had occasional headaches, but regular menstrual cycles and no galactorrhea or other clinical symptoms of the hyperprolactinemic syndrome. On referral to our endocrine center, imaging of her pituitary revealed no evidence of a prolactinoma, although a small incidental tentorial meningioma was seen. Testing with the dopamine antagonist, domperidone, (10 mg, administered intravenously) produced a normal increase in serum prolactin 30 min after administration (basal prolactin, 663 mIU/L, increasing to 5220 mIU/L; prolactin measured at our endocrine center). No treatment was given, but serum prolactin measured 1 year later at her local hospital was still increased at 2220 mIU/L.

Clinical case 2. A 43-year-old female presented with a history of irregular menstrual periods, recent weight gain, dryness of skin, and facial hair, but no galactorrhea or visual problems. Domperidone testing gave a normal response, and a computed tomography scan revealed no abnormality. However, it was noticed that her serum prolactin was increased at her local hospital but not significantly increased on referral to our center. In July 1995, her serum prolactin at our endocrine center was 510 mIU/L, whereas in August and September 1996, her serum prolactin at her local hospital was 1729 and 2134 mIU/L. A sample collected in October 1996 and measured in each laboratory was 733 mIU/L at our endocrine center and 2322 mIU/L at her local hospital. Serum prolactin, in this and clinical case 1, was measured at her local hospital by a radioimmunoassay procedure and at our endocrine center by a two-site immunnochemiluminometric assay on the Bayer Chiron ACS:180.

This discrepancy between the two assays prompted an investigation into the response of other assays to this patient’s sample, utilizing the United Kingdom External Quality Assessment Scheme (UKNEQAS). An aliquot of serum from the second clinical case was distributed by UKNEQAS to 70 laboratories in the United Kingdom, which use 21 different methods for prolactin measurement. The method mean prolactin concentrations varied from 556 mIU/L to 2055 mIU/L, depending on the method used. The mean prolactin concentrations by the main methods are shown in Fig. 1 for the patient specimen and for two hyperprolactinemic pools, one with exogenous prolactin and one endogenous. Gel filtration studies of samples from both patients showed that macroprolactin was the predominant form of prolactin in their sera.

All methods for measuring prolactin will show some cross-reactivity with macroprolactin, and the extent to which it contributes to the

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measured prolactin concentration depends on the choice of reagent antibody. Because the presence of macroprolactin does not appear to contribute to the hyperprolactinemic syndrome in the majority of patients, it is important to raise awareness of the problem in clinical chemistry laboratories and with physicians. We recommend that manufacturers of prolactin reagents state in their product leaflets to what extent macroprolactin interferes in their prolactin assay and have available a validated method to confirm the presence of macroprolactin. It is also important that manufacturers address the problem with prolactin assays to minimize reaction with macroprolactin. In the meantime, polyethylene glycol (PEG) precipitation has been shown to be a valid screening test for macroprolactin when used with the Wallac DELFIA assay. It cannot, however, be used for all methods because of interference from PEG, and it needs to be validated with other assay systems before being brought into use.

Confirmation of the presence of macroprolactin can be made by gel filtration chromatography, but this is time-consuming, costly, and beyond the scope of most clinical laboratories. Laboratories in the United Kingdom have been using mainly PEG precipitation techniques to identify macroprolactinemia, but we urge equipment manufacturers to address this problem. As highlighted in these two clinical cases, macroprolactin needs to be identified early in a patient’s work-up to avoid unnecessary, costly, and invasive procedures.

References


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Plasma Ferritin in Acute Hepatocellular Damage

To the Editor:

Plasma ferritin concentrations are increased in iron overload, liver diseases, infections, inflammatory conditions, and malignancy. Very high concentrations (>10 000 µg/L) have been described in Still disease (1) and less commonly in hemochromatosis. There are very few reports of ferritin concentrations in patients with acute hepatocellular damage (AHD). A review of extreme increases in ferritin found that liver disease was the cause of 20% of cases but did not specify actual concentrations seen and did not differentiate acute and chronic liver disease (2). We have determined ferritin concentrations in AHD patients.

Plasma ferritins were measured in eight patients who had plasma alanine aminotransferase (ALT) values <100 U/L pre-AHD and >1000 U/L post-AHD. ALT, iron, and transferrin were measured on a Hitachi 917 (Roche Diagnostics), and ferritin was measured on the ACS:180 (Bayer Diagnostics).

The results for individual patients are shown in Table 1. In one patient, serum ferritin did not exceed the upper limit of the reference interval during AHD. This patient was iron-deficient with a basal ferritin of 22 µg/L.

The plasma ALT increased from a basal mean (SD) of 45 (29) U/L to 2970 (1540) U/L during AHD, and ferritin increased from 200 (130) µg/L to 18 260 (17 860) µg/L. The mean plasma iron increased from 12 (11) µmol/L to 16 (19) µmol/L, transferrin decreased from 27 (11) µmol/L to 22 (7) µmol/L, and transferrin saturation increased from 18% (7%) to 31% (30%).

The rapid increase in ferritin in AHD suggests that ferritin is present in the cytosol of the hepatocytes. There was significant (P <0.01) correlation between the increase in ALT and ferritin in AHD. Despite the marked increase in ferritin in five of six patients in whom iron and transferrin were measured, the transferrin saturation increased above the reference interval in only one of these patients.

In conclusion, ferritin concentrations were markedly increased after AHD and are of no diagnostic value during this time. These data imply that ferritin and ALT are present in the cytosol of the hepatocytes.