Difficulties in Analysis of CA 125 in Diluted Samples

To the Editor:

Several analytical phenomena have been reported with CA 125 assays, including the hook effect (1) and dilutional nonlinearity (2). On the basis of our experience with a patient whose undiluted result did not match a diluted result, we examined linearity upon dilution of samples analyzed in our laboratory.

We used a commercial, first-generation, homologous, double-determinant CA 125 RIA (Abbott CA 125 RIA Diagnostic Kit, catalog no. 3984-24; Abbott Labs., Diagnostic Division, Abbott Park, IL). Performance of the test includes running a pool of the patients' serum samples to exclude the possibility of a hook effect. If pools were >50 kU/L, the individual patients' serum samples were rerun at a 10-fold dilution. Within these serum samples, undiluted values <500 kU/L and >130 kU/L were chosen for further examination. A value of 130 kU/L was used as the lower limit for the study because a 10-fold dilution results in a value of 13 kU/L, the lower limit of linearity. The stated upper limit of the assay is 500 kU/L.

Overall, the study comprised 98 of 2206 (4.4%) samples from 57 of 508 (18.5%) patients over a 22-month period at our institution.

Shown in Fig. 1 are 95 undiluted and diluted determinations done on 57 patients' samples from the present study. Not shown are data on three samples in which a hook effect was seen. Of 57 patients, 36 had only one undiluted and diluted determination; 21 patients had more than one diluted and undiluted determination. Of these 21, 15 had two determinations, 3 had three determinations, 2 had four determinations, and 1 patient had 14 determinations. As can been seen in Fig. 1 beyond 425 kU/L (undiluted), corrected diluted values greater than twofold greater than undiluted values become especially prevalent. The table within the figure shows the percent of patients' samples between 130 and 225 kU/L, 226 and 321 kU/L, 322 and 424 kU/L, and 425 and 500 kU/L that are beyond a certain percent difference between undiluted and corrected diluted values. The percent change reflects the absolute percent difference between the diluted and undiluted value divided by the undiluted value. Of the samples, >90% were higher upon dilution after correction. Among 11 of 18 (57.8%) patients in which the sampling was done for >1 week, this dilutional problem was not consistently >20%. Linear regression analysis for the 130–500 kU/L range shows S_{xy} = 153.1, y = 1.87x – 136.2. If one examines the 130–424 kU/L range, S_{xy} = 89.1, y = 1.51x – 51.3. Serial dilutions on 69 specimens with CA 125 values >500 kU/L were performed. All samples were higher upon dilution, with 13 samples displaying the hook effect. Of the remaining 56 samples, the percent difference between 10-fold diluted (corrected for dilution) and undiluted samples ranged from 2.4% to 494.2%, with 57.1% having 100% or greater difference. A control experiment with the 500 kU/L kit calibrator showed a difference of ~9.4% between 10-fold diluted (corrected) and undiluted calibrator, suggesting that the difference was not systematic in nature. From analysis of the data above it appears that the actual range of linearity should be ~400 kU/L instead of 500 kU/L.

One possibility for this discrepancy between undiluted and diluted samples is the presence of peritoneal CA 125 isoforms in serum. Barbati et al. (3), using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting with the OC 125 antibody, showed marked differences in the molecular mass of proteins detected in serum and peritoneal fluid from a patient with ovarian carcinoma. However, in a patient chart review, there was no difference in the presence or amount of ascites, age, the type of tumor, or stage between patients who diluted linearly and those who did not (percent difference ≥20%). In addition, none of these patients was exposed to mouse anti-CA 125 antibodies for imaging studies before obtaining a CA 125 serum concentration, making unlikely the presence of human anti-mouse antibodies that might cause a false increase of CA 125 upon dilution. Another theoretical possibility for the increased CA 125 values is that more epitopes are being revealed upon dilution, a reflection of the source of CA 125. Recently a second-generation CA 125 IRMA has been developed, which demonstrates far better dilutional linearity (4). This is presumably attributable to different epitopes being recognized by the different antibodies used in this assay. Purification of the actual molecule or molecules and identification of the actual epitope recognized by the CA 125 antibody would be of substantial benefit in identifying the source of the dilutional nonlinearity in present and future CA 125 assays.

In summary, despite the package insert stating that the linearity of the assay is from 13 to 500 kU/L, one has to be careful in diluting CA 125 values at the higher end of the assay because of higher values obtained
upon dilution. Undiluted samples between 130 and 500 KU/L are not necessarily equivalent to diluted samples using a commercial, first-generation, homologous, double-determinant CA 125 RIA kit. This dilutional nonlinearity could be of clinical significance, especially in surveillance for ovarian cancer recurrence in patients with previously known ovarian CA and increased CA 125 values.

[Author's note: Recently, the laboratory was notified that this CA 125 RIA kit would no longer be available. Clearly from this study caution should be warranted in examining other CA 125 methods.]

References

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Modification of Malondialdehyde Concentration by Administration of Protamine Sulfate

To the Editor:

The determination of malondialdehyde (MDA) is widely performed for detection of free oxygen radicals in various pathological conditions (1, 2). Methodological problems may limit the value of MDA determination for quantitative estimation of oxygen-derived radicals (3–5). Various drugs seem to influence MDA concentration (3). We describe here the effect of protamine sulfate on measured MDA.

MDA was determined peripera-

tively in patients undergoing femoropopliteal bypass surgery for limb ischemia. One group (n = 4) was treated with intravenous administration of prostaglandin E1 (Schwarz-Pharma, Mohnheim, Germany); controls received placebo (n = 4). The concentration of serum MDA (in EDTA-containing tubes) was determined as described (4).

MDA concentration was not affected by the administration of heparin. However, MDA increased significantly between 15 and 60 min after reperfusion of the extremity (from 1.07 ± 0.1 μmol/L to 1.54 ± 0.18 μmol/L, P <0.05, Mann–Whitney test) in the placebo group. The only intervention in this phase was administration of protamine sulfate to antagonize the anticoagulatory effect of systemic heparinization.

We speculated that the measured MDA concentration was affected by the administration of protamine sulfate. MDA concentration was then assessed in patients undergoing castrated endarterectomy (n = 5). Because no reperfusion syndrome occurs in these patients, factors influencing MDA concentration due to limb reperfusion could be eliminated. Again, a significant increase in MDA concentration was observed after administration of protamine sulfate (from 1.21 ± 0.11 μmol/L to 1.73 ± 0.22 μmol/L, P <0.02). The effect was not methodological, because addition of protamine sulfate, 1 or 3 units/mL, to each of five samples produced no effect on measured MDA (0.90 ± 0.23 μmol/L without protamine vs 0.94 ± 0.16 and 0.85 ± 0.24 μmol/L for 1 and 3 units/mL, respectively).

Since oxygen-derived radicals are of clinical significance in vascular and cardiac surgery, many groups study tissue reperfusion variables, including MDA. In all these procedures, protamine sulfate has to be administered. Thus it is important to consider changes in MDA concentration induced by protamine sulfate.

References


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Increased Urine Chromium Excretion in Normal Pregnancy

To the Editor:

The trace element chromium (Cr) may have a role in the action of insulin. Plasma concentrations of Cr in diabetic patients are significantly lower and urinary excretion of Cr higher than in age- and sex-matched controls (1). In healthy individuals, plasma concentrations of Cr are inversely related to those of insulin over a 24-h period following an oral glucose load (2) and during hyperinsulinemic euglycemic clamping (3). Cr may act as an essential trace element in insulin-dependent glucose uptake (4), as a reduction in Cr produces insulin resistance in the C5C12 cell line, which can be restored by returning Cr concentrations to physiological concentrations.

Reduced insulin-mediated uptake of glucose (insulin resistance) is a metabolic defect characteristic of patients with non-insulin-dependent diabetes and is seen also in nondiabetic subjects with impaired glucose tolerance (IGT). A third group that may be insulin-resistant are pregnant women, as gestational diabetes mellitus occurs frequently, characterized by the onset of diabetes or IGT during pregnancy.

Since Cr excretion is abnormally high in diabetic patients, our aim in this pilot study was to investigate whether Cr excretion is also altered in pregnancy.

Approval for a pilot random trial was obtained from the Northern General Hospital Ethics Committee. Random urine samples were collected from 120 anonymous pregnant wo-