Analytical Quality of Calcitonin Determination and Its Effect on the Adequacy of Screening for Medullary Carcinoma of the Thyroid

To the Editor:

Calcitonin, a 32–amino acid calcium-lowering peptide secreted by the C cells (parafollicular cells) of the thyroid, is used as a marker for medullary carcinoma of the thyroid (MCT). However, calcitonin is not specific for MCT, because it is also secreted by other neoplasms, including breast cancer and small cell lung cancer.

Secretion of calcitonin is regulated primarily by the concentration of extracellular calcium, but can also be stimulated by gastrin. Although calcitonin concentrations are higher in men than in women and tend to decline with age, many laboratories use a cutoff value of 10 ng/L instead of a population-based reference interval. Prior studies have shown that basal calcitonin concentrations are below this threshold in a normal population (1, 2) and in 90% of patients suffering from other nodular thyroid diseases (3). In clinical practice, a patient with a calcitonin concentration higher than 10 ng/L should undergo a pentagastrin test to exclude MCT.

It is important to note, however, that these interpretive guidelines have been developed using the Cisbio international reagent set for calcitonin measurement. This immunoradiometric assay uses 2 antibodies, one directed against the 11–17 and the other against the 24–32 sequence of the peptide. IRMA reagent sets, and particularly the Cisbio international kit, have been recommended for calcitonin measurement in the past and are still recommended by some scientific societies. However, in recent years, most medical laboratories have moved from radioisotopic methods to fully automated methods, and such a change may pose problems, because some chemiluminescent methods lack adequate sensitivity and specificity (4).

In our laboratory, we planned to move from an immunoradiometric method (Cisbio) to an automated method (Liaison, Diasorin). When both reagent sets were calibrated against the 2nd International Standard 89/620, the Cisbio reagent set clearly indicated that 1 μIU of the international standard corresponded to 3.6 pg of Cisbio calcitonin. Because this information was not provided by Diasorin, we created our own standard curve for Liaison. For that purpose, we used a sample with a very low calcitonin concentration, to which we added different amounts of the international standard; then we performed calcitonin determination on Liaison. We thus observed that 1 μIU of the international standard corresponded to 3.2 pg of Liaison calcitonin. These results demonstrate that 10 pg of Cisbio calcitonin corresponds approximately to 8 pg of Liaison calcitonin.

During the analytical validation of the Liaison reagent set, we established the limit of quantification (LOQ), defined as the lowest concentration giving a CV smaller than 20% for interassay variation (5). Our results showed the LOQ was approximately 12 ng/L. This observation was confirmed on a second set of samples (Table 1). With the Cisbio reagent set, we obtained a much lower LOQ (7 ng/L).

Basing the lowest reportable limit for a test on the determined LOQ (5), we reported any results obtained with the Liaison reagent set below 12 ng/L as “<12 ng/L.” However, with the application of a 10 ng/L threshold for pentagastrin testing as described above, the LOQ of 12 ng/L meant that patients with calcitonin concentrations between 9 and 12 ng/L (2% of women and 10% of men, in our experience) would potentially be missed with assays performed using the Liaison reagent set.

Such an outcome is problematic for a screening test. We suggest that the “old” 10 ng/L cutoff obtained with the Cisbio reagent set should be confirmed by a multicenter study involving several different calcitonin assays.

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<th>Table 1. Limit of quantification (smallest value giving an interassay CV lower than 20%) observed with the Diasorin Liaison assay.</th>
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Stabilization of Glucose in Blood Specimens: Mechanism of Delay in Fluoride Inhibition of Glycolysis

To the Editor:

The recent report by Gambino (1) brought attention to the often overlooked fact that fluoride does not prevent loss of plasma glucose during the first 30–90 min (or longer) after blood collection (2). Although fluoride is effective in preventing later loss of glucose (1, 2), the mechanism of the delay in its action is a matter of some interest.

Fluoride acts primarily by inhibiting enolase in the glycolytic pathway. Fluoride strongly inhibits the enzyme in the presence of inorganic phosphate. The inhibitory species is the fluorophosphate ion, which when bound to magnesium forms a complex with enolase and inactivates the enzyme. The delay in fluoride’s prevention of glucose loss in blood samples is sometimes attributed to a postulated delay in the entry of fluoride ion into the blood cells in which the glycolytic enzymes reside. Several observations cast doubt on this explanation, however.

As part of a project for quality improvement of sample-handling requirements, we collected blood from a volunteer into four 5-mL Vacutainer (BD) tubes, 2 containing sodium fluoride/potassium oxalate, 10 mg/8 mg, and 2 with lithium heparin, 51 U, and plasma separating tube gel. The tubes were not centrifuged. After sample collection, 1 tube of each type was kept at room temperature, and another was put immediately into an ice water bath. The tubes were kept upright and mixed immediately before removing aliquots at 0, 15, 30, 45, 60, and 90 min. Each aliquot was centrifuged immediately for 1 min at 5585g in a microcentrifuge. The plasma was removed and lactate and glucose were measured on an Architect Analyzer (Abbott). The study was repeated on blood samples obtained from a second volunteer on a different day. The results for tubes at room temperature are summarized in Fig. 1. In the tubes of blood stored in ice water, with or without fluoride, decreases in glucose and increases in lactate were ≤0.1 mmol/L even at 90 min (data omitted for clarity).

If fluoride does not enter blood cells rapidly, then it cannot rapidly inhibit the production of lactate, which is produced from pyruvate, the final product of glycolysis. By contrast, as shown in Fig. 1, fluoride completely blocked the production of lactate for >30 min during a time when consumption of glucose was brisk in the presence of fluoride. In portions of the blood that were incubated without fluoride (Fig. 1), the quantity of lactate produced was as expected, that is, almost 2 mol of lactate were produced for each 1 mol of glucose consumed (e.g., the concentration of lactate increased at 90 min by 1.1 mmol/L in samples from each volunteer, whereas the concentration of glucose decreased by 0.6 mmol/L, Fig. 1). These data are similar to those reported by Feig et al. (3) for washed human erythrocytes, which showed consumption of glucose at 10 min after addition of fluoride, but negligible production of lactate at that time point. Moreover, Astles et al. (4), although focusing on somewhat later time points after blood collection, also reported that fluoride rapidly inhibited production of lactate in whole blood.

A parsimonious explanation of these findings is that after fluoride is mixed with blood it rapidly blocks enolase (within <5 min), and that enzymes upstream of enolase in the glycolytic pathway remain active. Thus glucose continues to be metabolized to glucose 6-phosphate, which is further me-

References


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