Comparison of Calcitonin Determinations by Polyclonal and Monoclonal IRMAs

To the Editor:
Serum calcitonin (CT) has important diagnostic and prognostic value in patients with medullary thyroid carcinoma (MTC). Although CT can be routinely quantified by a variety of 2-site immunoassays, different assays may yield very different results. This variability, particularly at low CT concentrations, led to the recommendation to use reliable and sensitive assays in the screening of MTC and C-cell hyperplasia (1).

To highlight CT variability at high concentrations, we measured 43 serum samples by use of a polyclonal IRMA and by a monoclonal IRMA. The single-step polyclonal assay used 2 different goat antibodies (as the capture and labeled antibodies), which were specific for well-defined regions of the CT molecule, with no cross-reactivity with parathyroid hormone, thyroid-stimulating hormone, CT gene–related peptide, porcine CT, or salmon CT. The reagent set was used according to the manufacturer’s instructions (Calcitonin Assay, Scantibodies Laboratory, Inc.). The concentrations of calibrators ranged from 10 to 1000 ng/L. Expected values were ≤9.9 ng/L for women and ≤17.0 ng/L for men. The single-step monoclonal assay uses 2 antibodies with no cross-reactivity with procalcitonin. The reagent set was used according to manufacturer’s instructions (CIS Biointernational). The concentrations of calibrators ranged from 10 to 1500 ng/L, with a reference interval of <10 ng/L. Both assays were calibrated against the 2nd Calcitonin International Standard 89/620.

Of the 43 serum samples, 2 demonstrated a hook effect. One CT serum sample analyzed by the polyclonal assay was 699 ng/L undiluted and 6564 ng/L after 1:100 dilution; the same sample, measured by the monoclonal assay, was >1500 ng/L undiluted and 2968 after 1:20 dilution. A 2nd sample analyzed with both assays gave polyclonal assay values of 510 ng/L undiluted and 30236 ng/L after 1:100 dilution, and monoclonal assay values of 634 ng/L undiluted and 3225 ng/L after 1:100 dilution. The hook effect seems less likely to occur in a 1-step method when 2 monoclonal antibodies are used, probably because of monospecificity of the signal antibody (2). The 2 samples with the hook effect were excluded from the subsequent analysis because they were deemed to be outliers in a nonparametric distribution (Statistica for Windows 98, Statsoft Inc.).

The remaining 41 serum samples exhibited CT concentrations of 2.87–344 ng/L (median, 46.8 ng/L) by the polyclonal assay and 1.72–148 ng/L (median, 31.6 ng/L) by the monoclonal assay. To compare the 2 methods we used the Bland-Altman representation (3), which focuses on the mean and variability of differences between pairs of measurements: a scatter plot of the difference between the measurements (y axis) against their mean (x axis) allows detection of lack of individual agreement. Near agreement appeared for CT concentrations <40 ng/L. For higher values, the monoclonal assay tended to produce lower results than the polyclonal assay. In addition, the higher the mean value, the greater the difference between the measurements (Fig. 1).

Analysis by Passing-Bablok regression showed a significant deviation from linearity (P <0.01). A possible explanation is that at higher concentrations of mature monomeric CT, polymeric forms and other immunoreactive fragments increase in the circulation. In these circumstances, polyclonal antibodies might yield higher values than those measured by the monoclonal assay.

The lack of a linear relationship between the 2 assays, particularly at higher concentrations, precludes interchangeability of results between the 2 methods. Furthermore, a strikingly discordant value (Fig. 1, circled sample) was obtained in a patient with CT concentrations of 157 and 1.5 ng/L by the polyclonal and the monoclonal assays, respectively. The patient was later diagnosed with cholangioadenocarcinoma. Some monoclonal immunoassays cross-react with high concentrations of procalcitonin (4). The sample was
analyzed for procalcitonin (Liaison Brahms PCT), yielding a result of 0.88 μg/L, slightly higher than the cutoff value (0.58 μg/L). The cross-reactivity with procalcitonin might be more likely in our sample measured with a polyclonal assay. Other interferences cannot be excluded.

The 2 CT assays give markedly different results. The introduction of an internationally agreed upon standard would contribute to optimizing the CT assay, thereby providing a more reliable tool for the treatment of patients with MTC.

References

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DOI: 10.1373/clinchem.2006.083733

To the Editor:
We evaluated hemoglobin (Hb) interference in the total bilirubin (TBIL) and neonatal bilirubin (NBIL) [the sum of unconjugated (Bu) and conjugated (Bc) bilirubins] methods on the Vitros 5,1 FS analyzer and assessed the reliability of the analyzer’s hemolysis and icterus index values by comparing them to measured concentrations of bilirubin and Hb in test specimens. We also compared TBIL with NBIL values in specimens from neonates 1–14 days old and obtained estimates of the extent of hemolysis in these specimens.

We prepared a hemolysate from EDTA blood drawn from volunteers. After centrifugation, the cells were washed 5 times with physiologic saline, diluted with deionized water, and stored at −20 °C overnight. After thawing, the hemolysate was centrifuged to remove the stroma. The Hb concentration was measured with the CELL-DYN 4000 analyzer. We prepared a stock solution of Bu (NIST SRM 916) in pooled human serum samples from healthy volunteers as described previously (1); dilutions were made with the same pooled sera.

A solution of Bc was prepared by adding to pooled human sera a mixture of bilirubin mono- and diglucuronide isolated from human bile (2); the solution was dispensed into vials, lyophilized, and stored at −70 °C. Vials containing the pooled human sera were also lyophilized. The com-