Comparison of LC-MS/MS to Immunoassay for Measurement of Thyroglobulin in Fine-Needle Aspiration Samples

To the Editor:

The measurement of thyroglobulin (Tg) by immunoassay (IA) is affected by the presence of anti-Tg autoantibodies (Tg-AAbs), which may cause false-negative or falsely low results (1). It has been shown that Tg quantification by LC-MS/MS overcomes interference of Tg-AAbs in measurement of Tg (2). We previously observed that >20% of Tg-AAbs–positive serum samples that tested negative for the presence of Tg by IA(<0.1 ng/mL) had Tg concentrations ≥0.5 ng/mL when tested by LC-MS/MS (2). Here we discuss the potential utility of applying this LC-MS/MS method to testing Tg in fine-needle aspiration (FNA) samples.

Ultrasound-guided FNA cytology and measurement of Tg concentrations in FNA samples are commonly used in the evaluation of suspicious lymph nodes in the diagnosis of thyroid carcinoma. Tg measurement in needle washout fluids (FNA-Tg) allows improved diagnostic accuracy of cytology (3) and is useful in patients with aggressive tumors, low serum thyroglobulin concentrations, or circulating Tg-AAbs. FNA-Tg concentrations >10 ng/mL are considered abnormal (4). Jeon et al. (5) proposed that Tg-AAbs could interfere with FNA-Tg measurements and cause falsely low FNA-Tg concentrations. Thus, we evaluated whether our LC-MS/MS method intended for serum Tg (2) could be used for measurement of FNA-Tg, and whether any of the FNA samples that tested negative by IA would have measurable Tg when analyzed by LC-MS/MS, suggesting Tg-AAb interference with IA. Samples used in the study were deidentified FNA patient samples submitted to ARUP Laboratories (Salt Lake City, UT) for routine testing. The study was approved by the University of Utah Institutional Review Board.

We adapted the LC-MS/MS method for measuring serum Tg (2) for analysis of FNA-Tg. In brief, the samples were diluted with Tg-negative serum (ratio of 1 part to 9 parts, respectively), and Tg was enriched using rabbit polyclonal anti-Tg antibody and protein precipitation. The enriched proteins were then denatured and reduced and, after addition of an internal standard, digested with trypsin. A Tg-specific tryptic peptide was purified by immunoaffinity extraction and analyzed by LC-MS/MS (AB Sciex 5500) operated in positive ion, electrospray ionization mode. Two mass transitions were monitored for the targeted peptide (m/z 636.9/1060.5; 636.9/913.5) and the internal standard (m/z 639.9/1066.5; 639.9/919.5). Instrument cycle time was 6.5 min per sample. Imprecision of triplicate analysis of FNA samples containing 30, 2030, and 80 500 ng/mL was 15.2%, 11.2%, and 2.5%, respectively; between-run imprecision at 2 ng/mL (analyzed once per day for 15 days) was 13.9%. We evaluated performance of the methods in the vicinity of cutoff required for Tg-FNA samples by analysis of Tg-negative FNA samples (n = 4) spiked with Tg to 15 ng/mL using IA and LC-MS/MS. The mean (CV) measured concentrations for IA and LC-MS/MS were 14.6 ng/mL (2.2%) and 18.4 ng/mL (16.0%), respectively.

Using the LC-MS/MS method, we analyzed 73 FNA samples previously analyzed for Tg with the Beckman Coulter Access™ Tg-IA. FNA samples (1 part diluted with 9 parts Tg-AAb negative serum) were tested for the presence of Tg-AAb using the Thyroglobulin Antibody II™ assay (Beckman Coulter). Serum samples of 7 individuals whose FNA samples were analyzed in this study were positive for Tg-AAb.

None of the analyzed FNA samples had detectable Tg-AAb (cutoff 4 IU/mL). The range of Tg concentrations in the samples was 0–160 000 ng/mL; 18 samples tested negative for Tg (cutoff 5 ng/mL) by both methods. The median concentrations of Tg by LC-MS/MS and immunoassay were 58 and 63 ng/mL, respectively. The Deming regression for the entire set was IA = 1.14 * LC-MS/MS + 473, r = 0.994, S_y|x = 2220; for samples with Tg <70 ng/mL, it was IA = 1.63 * LC-MS/MS – 1.2, r = 0.879, S_y|x = 4.8 (Fig. 1). For the 7 FNA samples with corresponding Tg-AAb–positive serum samples, the Deming regression equation for Tg was IA = 1.10 * LC-MS/MS – 34.3, r = 0.976, S_y|x = 162; the FNA-Tg concentration range was 0–2400 ng/mL. In 1 sample, the Tg concentration was substantially underestimated by the IA, and in 2 samples concentrations measured by IA were 2.6 and 2.9 times greater than by LC-MS/MS.

The data showed reasonably good agreement between LC-MS/MS and Beckman IA at Tg concentrations >70 ng/mL; the concentrations determined by LC-MS/MS were lower than by IA in samples with Tg <70 ng/mL. The lower observed concentrations could be related to Tg degradation during storage of the FNA samples; degradation could be related to the use of saline as a solvent for dilution of the needle biopsy samples. Alternatives to the saline diluents used for FNA samples should be evaluated to find a matrix that allows for improved stability of Tg. Future studies in which clinical information on the partici-
pants is available will be needed to assess the diagnostic utility of FNA-Tg testing using LC-MS/MS and immunoassays.

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References


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To the Editor:
The detection and monitoring of preanalytical bias in the clinical laboratory can be challenging, as bias can be introduced at any point from specimen collection to sample processing, and those biases stemming from sample collection or handling before delivery to the laboratory can be difficult to measure and eliminate. In particular, variations in potassium concentrations due to preanalytical sources of error are a pervasive and clinically significant problem (1–3).

Initial concern regarding spuriously increased potassium concentrations (>5.2 mmol/L) occurring in the laboratory of the Dana Farber Cancer Institute was raised by clinicians reporting patients displaying increased potassium without any apparent clinical justification. Review of the medical records of the patients involved demonstrated that the majority had potassium values within the reference interval on the draw before the in-