Clinical Chemistry

Measurement of Thyroglobulin by Liquid Chromatography–Tandem Mass Spectrometry in Serum and Plasma in the Presence of Antithyroglobulin Autoantibodies

Article:
M.M. Kushnir, A.L. Rockwood, W.L. Roberts, D. Abraham, A.N. Hoofnagle, and A.W. Meikle.
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Guest:
Dr. Mark Kushnir is a Senior Scientist at the ARUP Institute for Clinical and Experimental Pathology.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett. Measurement of serum thyroglobulin may be complicated by the presence of endogenous anti-thyroglobulin auto-antibodies which have the potential to interfere with immunoassays and cause false negative results.

A paper in the June 2013 issue of *Clinical Chemistry* describes the measurement of thyroglobulin by liquid chromatography tandem mass spectrometry that overcomes these problems. The lead author of that study was Dr. Mark Kushnir, a Senior Scientist at the ARUP Institute for Clinical and Experimental Pathology. Dr. Kushnir joins us in this podcast.

Doctor, why does thyroglobulin need to be measured, and who should be tested for this biomarker?

Dr. Mark Kushnir: Thyroid cancer is the ninth most common type of cancer in humans. The most commonly used treatment for patients diagnosed with thyroid cancer consists of surgical removal of thyroid gland followed by radioactive iodine oblation of the residual thyroid tissue.

Thyroglobulin is produced in human body only in thyroid gland. And because of this, after total thyroidectomy, thyroglobulin should not be present in the patient's blood. Rising concentrations of thyroglobulin in patients followed-up after treatment were shown to be indicative of the recurrence of thyroid cancer.

Considering this, patients treated for differentiated thyroid carcinoma should be tested for thyroglobulin to monitor recurrence of thyroid cancer.

Approximately 25% of patients treated for thyroid cancer have circulating endogenous thyroglobulin auto-antibodies, which interfere with measurement of thyroglobulin using immunoassays.

Bob Barrett: How common are thyroglobulin auto-antibodies, and how do they interfere with usual measurements of thyroglobulin?

Dr. Mark Kushnir: Immunoassays are currently the most common type of methodology that is used for measuring thyroglobulin. Immunoassays typically work well in patients who don't have thyroglobulin auto-antibodies in their blood. But, if someone has circulating autoantibodies, immunoassay may produce false negative results, and this means that the recurrence of thyroid cancer in this patient will not be timely detected.

Bob Barrett: Doctor, what's the principle of this method that you've just published, and why is it novel?

Dr. Mark Kushnir: Proof of principle that mass spectrometry-based test may overcome interference of thyroglobulin auto-antibodies was shown by Dr. Andrew Hoofnagle in an article published in 2008 in *Clinical Chemistry*. In this proposed method, the authors used proteolytic digestion that cleaves proteins to peptides. During the digestion, auto-antibodies get destroyed and thyroglobulin gets digested to peptides. Quantitative measurement of thyroglobulin in this method is based on quantitative analysis of thyroglobulin-specific peptide produced during the digestion.

The article published by Dr. Hoofnagle, served as a proof of principle that mass spectrometry-based test can overcome the interference of auto-antibodies, but this published method was insufficiently sensitive for quantitation of thyroglobulin at concentrations characteristic of the early stages of the reocurrence of thyroid cancer; and also it was not sufficiently robust for routine diagnostic use.

The main breakthrough that allowed us to develop sensitive and robust method is in the strategy that we developed for enrichment of thyroglobulin from serum and plasma samples. In our method we were able to efficiently enrich thyroglobulin from samples and reduce total content of proteins in the enriched fraction. This allowed us to increase initial sample volume, reduce sample complexity, and reduce use of trypsin.

The method that we developed consist the following steps: We start with the enrichment of thyroglobulin from serum and plasma samples. This is followed by denaturation and reduction of proteins in the enriched fraction. Digestion of proteins using trypsin, enrichment of thyroglobulin-specific peptide using anti-peptide antibody conjugated to magnetic beads, followed by analysis of the prepared samples using liquid chromatography tandem mass spectrometry. Total

time required for analysis of up to 90 samples is approximately 24 hours.

Bob Barrett: Are there other methods for measurement of thyroglobulin available that can overcome this interference of thyroglobulin auto-antibodies?

Dr. Mark Kushnir: Different approaches were evaluated for overcoming interference of thyroglobulin auto-antibodies with measurement of thyroglobulin, but the only technique that was shown to resolve the interference is mass spectrometry.

Bob Barrett: Well, how do concentrations of thyroglobulin determined by liquid chromatography tandem mass spectrometry method compare to the results determined with commercial immunoassays?

Dr. Mark Kushnir: As part of the method validation, we compared our method to tandem mass spectrometry-based method of the University of Washington, using thyroglobulin auto-antibody negative and auto-antibody positive samples. In both sets of samples, we observed a good agreement between these two methods.

We also compared our method to a commercial immunoassay from Beckman Coulter using thyroglobulin auto-antibody negative and auto-antibody positive samples. We observed good agreement between the methods for auto-antibody negative samples, while there was disagreement between methods for auto-antibody positive samples, especially in the samples containing less than 2 ng/mL of thyroglobulin.

As part of method validation, we established reference intervals of thyroglobulin in children and adults who did not have circulating thyroglobulin auto-antibodies in their blood. The reference intervals were in agreement between the mass spectrometry-based method and immunoassay Access™ from Beckman Coulter.

Bob Barrett: Well, finally doctor, should liquid chromatography tandem mass spectrometry methods be used for testing for thyroglobulin in all patients?

Dr. Mark Kushnir: Considering that good agreement was observed between our mass spectrometry-based method and Beckman Coulter immunoassay in auto-antibody negative samples, and poor agreement observed for auto-antibody positive samples, one possible strategy is to analyze auto-antibody negative samples with immunoassay and analyze auto-antibody positive samples by mass spectrometry-based method. An alternative to this strategy is to use mass spectrometry-

based method for testing of all samples, but this approach will be more costly.

Bob Barrett: Dr. Mark Kushnir is a Senior Scientist at the ARUP Institute for Clinical and Experimental Pathology. He has been our guest in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thanks for listening!