AMERICAN THYROID ASSOCIATION

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Thyroglobulin (Tg) Assays

ABOUT

The Laboratory Services Committee of the American Thyroid Association® (ATA) conducted a survey of ATA® members to identify areas of member interest for education in pathology and laboratory medicine. In response to the results of the survey, the Lab Service Committee developed a series of educational materials to share with the ATA® membership. The topics below were ranked as high educational priorities amongst the membership.

MEASUREMENT OF SERUM THYROGLOBULIN

Thyroglobulin (Tg) measurement is an integral part of the follow up and management of patients with differentiated thyroid cancer (DTC). Although, measurement of Tg for initial evaluation of suspicious thyroid nodules is not recommended, serum Tg measurements are used postoperatively to monitor residual or recurrent disease. During initial follow-up, the recommended interval for serum Tg measurement is 6–12 months; although, for high-risk patients more frequent Tg measurements may be appropriate.[1]

Current recommendations for the measurement of Tg include the use of assays with functional sensitivity of <1 ng/mL and calibrated against the certified reference material BCR®457. Ideally the same Tg assay should be used over time and anti-thyroglobulin autoantibodies (TgAb) should be measured in all samples tested for Tg in order to identify samples with potentially falsely low or falsely high Tg due to TgAb interference.

Tg assays could be grouped into two main methodologies: immunoassays (radioimmunoassays and immunometric assays) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays. Today, most Tg methods are standardized against the certified reference material BCR®457 (European Commission Institute for Reference Materials)[2,3] resulting in reduced inter-method variability, however, as high as 2-fold differences can still be observed between the Tg concentrations when the same sample is measured by different methods.[4, 5, 6] These between-method differences reflect differences in method specificities for Tg isoforms, including potential differences in glycosylation and iodination. As a result, it is important that postoperative longitudinal Tg measurements are performed using the same manufacturer Tg assay and ideally the same laboratory.[1, 7, 8]

RADIOIMMUNOASSAYS (TG-RIA)

In Tg-RIA, Tg from the patient sample competes with a radiolabelled (125I) human Tg for binding to a limited amount of a high-affinity rabbit polyclonal anti-Tg antibody.[9, 10] An anti-rabbit IgG is then used to precipitate the antibody-Tg complex. The amount of radioactivity precipitated is inversely proportional to the Tg concentration in the patient sample. Tg-RIA are not widely used and their utility in the clinical care of DTC patients is limited. In the US, a Tg-RIA with a functional sensitivity of 0.5 µg/L is available for clinical purposes.[9, 10] Although the functional sensitivity of this assay is still suboptimal, it has been extensively used as a "gold standard" in studies evaluating the effect of TgAb interference in Tg IMA.[4, 11, 12, 13] This assay is promoted as being more resistant to TgAb interferences due to the use of polyclonal antibodies that could recognize Tg epitopes even when bound to TgAb.[9] However, interferences in Tg-RIA results have been reported. Early studies reported that TgAb caused an overestimation of Tg by RIA.[14] Others have reported underestimation of Tg by RIA in the presence of TgAb.[15,16]

IMMUNOMETRIC ASSAYS

Tg-IMA are based on a two-site reaction that involves Tg capture by a solid-phase antibody followed by addition of a labeled antibody that targets different epitopes on the captured Tg. Most clinical laboratories currently use Tg-IMA as these assays are less-labor intensive than Tg-RIA and are widely available in automated high-throughput instruments with shorter turnaround times. A number of commercially available Tg-IMAs have functional sensitivities of 0.1 µg/L or less. The introduction of these Tg-IMAs in clinical practice has reduced the need for measuring TSH-stimulated Tg concentrations during the initial and long-term follow-up of some patients with DTC.[16-19]

The main limitation of Tg-IMA is their susceptibility to TgAb and HAb interferences. The presence of TgAb might cause falsely low/undetectable Tg that can mask disease; whereas HAb might cause falsely high Tg that can be mistaken for residual or recurrent disease. The effect of TgAb interference in four commonly used Tg-IMAs has been shown to be variable but all demonstrate a negative bias. One study reported negative biases of 41% for the Beckman Coulter Access, 42% for the Thermo-Brahms, 50% for the Roche Elecsys, and 86% for the Siemens-Immulite assays when Tg results of TgAb positive samples were compared to a Tg mass spectrometry assay.[6]

MASS SPECTROMETRY ASSAYS

Measurement of Tg by LC-MS/MS (Tg-MS) has been introduced as a solution for accurate Tg quantitation in the presence of TgAb. In recent years, this methodology has been adopted by several US commercial laboratories. Tg-MS assays are based on peptide quantitation after tryptic digestion and immunocapture of a Tg-specific peptide. The advantage of trypsin digestion is that all proteins are cleaved, including TgAbs and HAb, thus eliminating them as interferences.

In the absence of TgAb, Tg-MS demonstrates excellent agreement and correlation with commonly used Tg-IMA. For most TgAb positive samples, Tg concentrations are significantly higher in Tg-MS assays than those from Tg-IMA. More importantly, the use of Tg-MS assays allows

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for the identification of those patients with an undetectable Tg by immunoassay due to the presence of TgAb. Approximately 20% of TgAb-positive samples, with an undetectable Tg concentrations by immunoassay (<0.1 µg/L), had detectable Tg concentrations by Tg-MS.[20]

A limited number of retrospective studies have been published to evaluate the clinical performance of Tg-MS assays. In TgAb positive patients, Tg was undetectable by Tg-MS methods in approximately 23-44% of cases with persistent disease.[6, 11, 21] Despite the analytical advantages of Tg-MS assays, clinical studies have failed to show superior clinical sensitivity for detection of recurrent or residual thyroid cancer when compared to Tg-IMA with a functional sensitivity of 0.1 ng/mL or less. One explanation for this is the suboptimal functional sensitivity (0.5-1.0 ng/mL) of Tg-MS assays. Attempts to improve the functional sensitivity of Tg-MS assays are ongoing and should allow for validation if improved clinical performance is observed with improved assay sensitivity.

TGAB INTERFERENCE

TgAb are present in approximately 10% of the general population and in up to 30% of patients with DTC.[22-24] TgAb can interfere with Tg measurements resulting in false low results or falsely high results as described above. This problem is compounded by the fact that TgAb interference is variable between patients and Tg immunoassays. Moreover, the degree of interference does not correlate with the TgAb concentrations.[23, 25, 26]

Therefore, TgAb measurements should be performed in conjunction with serum Tg when monitoring DTC patients to assess the reliability of the Tg result.[1, 27, 28] Professional guidelines recommend that all samples preferably be prescreened for TgAb by sensitive immunoassay methods prior to Tg testing.[1, 8, 29] Commercially available TgAb immunoassays show poor between assays concordance[26, 30] resulting in difference in TgAb classification (negative or positive) and different TgAb concentration.

This is further complicated by the fact that the manufacturers recommended TgAb cutoffs are for the diagnosis of autoimmune thyroid disease and often too high for detecting TgAb concentrations that interfere in Tg immunoassays. The use of the assays' limit of quantification (LoQ) is recommended for the evaluation of TgAb interference.[9] Similar to Tg, the same TgAb assay and ideally, the same laboratory should be used for the longitudinal monitoring of DTC patients.[1]

TG ASSAYS IMPLEMENTATION IN CLINICAL PRACTICE

Most clinical laboratories in the United States offer a reflex strategy for the quantitation of Tg. Testing begins with the measurement of TgAb and then based on the TgAb status (negative or positive), Tg testing is directed to an IMA for TgAb-negative samples; or a RIA or LC-MS/MS for TgAb positive samples.

The reflex strategy allows for optimal use of the various Tg assays. In TgAb negative patients, Tg-IMA with functional sensitivities of 0.1 ng/mL should remain the prefer assay. The use of a Tg-RIA or Tg-MS method is unlikely to provide any additional benefit in the clinical management of these patients. In TgAb positive patients, the use of Tg-MS or Tg-RIA will identify additional Tg positive cases.

A disadvantage of the reflex strategy is that a change in TgAb status (positive to negative or vice versa) could result in a change in the assay used for longitudinal Tg measurements and will likely required rebaseline of Tg concentrations.

CONCLUSION

The follow-up of DTC patients relies on the measurement of Tg to detect the presence of residual or recurrent disease. The improvement of Tg immunoassays over the years as well as the introduction of newer assays such as Tg-MS has allowed for better understanding of the limitations and advantages of the different Tg methodologies. Understanding of these limitations and advantages by laboratorians and clinicians is recommended in order to select the best assay for patient care.

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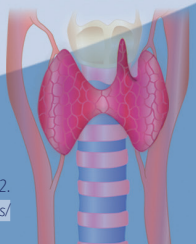

FURTHER INFORMATION

For more information on the survey findings, see *Thyroid, Vol. 27, No. 12.* https://www.thyroid.org/professionals/ata-publications/

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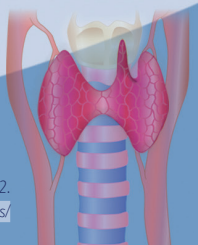

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