TITLE: Thyroglobulin and Thyroglobulin Antibody

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Title slide

Slide 2:
Thyroid cancer is the most common malignant tumor of the endocrine system. The National Cancer Institute estimated that in 2010, 45000 new cases of thyroid cancer were diagnosed in the US. Thyroid cancer could be divided into poorly differentiated and differentiated thyroid carcinoma. The poorly differentiated medullary and anaplastic thyroid cancers account for ~3% of thyroid cancer cases and tend to be very aggressive and usually have a poor prognosis. Papillary and follicular thyroid cancers are differentiated tumors derived from the follicular cells of the thyroid and account for ~97% of thyroid cancer cases. These are highly treatable and curable cancers. In these cancers, measurement of thyroglobulin levels is considered a standard of practice for patient follow-up.

The treatment of differentiated thyroid cancer consists of total removal of the thyroid gland followed by radioactive iodine (131I) treatment to destroy any remaining healthy thyroid tissue, as well as microscopic areas of thyroid cancer that were not removed during surgery. Because of the good prognosis of the majority of patients with differentiated thyroid cancer, measurement of thyroglobulin has emerged as a noninvasive and cost-effective follow-up tool to monitor recurrent or persistent disease.

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Thyroglobulin (Tg) is a 660,000 molecular weight glycoprotein produced exclusively by the follicular cells of the thyroid. It is secreted into the follicular lumen, where it serves as the precursor of, and storage reservoir for the thyroid hormones, thyroxine (T4) and triiodothyronine (T3). T4 and T3 are released after Tg is endocytosed and proteolytically degraded in the thyrocyte. Small amounts of intact Tg are secreted alongside T4 and T3 and are detectable in the serum of normal individuals with levels roughly paralleling thyroid size. The concentration of serum thyroglobulin increases substantially due to follicular destruction through inflammation (such as in the cases of thyroiditis and autoimmune hypothyroidism), or rapid disordered growth of thyroid tissue, as may be observed in Graves disease or follicular cell-derived thyroid neoplasms.

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Thyroglobulin measurement is not recommended for the screening or the initial diagnosis of thyroid cancer due to the significant overlap found between the levels observed in benign thyroid diseases and those in thyroid cancer patients. In addition, in patients with small cancers, Tg levels might overlap with the levels seen in normal individuals.

The primary use of serum Tg measurements is in the follow-up of patient with differentiated thyroid cancer following total thyroidectomy and radioactive iodine ablation. The American Thyroid Association guidelines for the management of differentiated thyroid cancer suggest that athyrotic thyroid cancer patients (total thyroidectomy and radioiodine remnant ablation) should have unstimulated (on T4) and stimulated (thyroid hormone withdrawal or recombinant human TSH stimulation) serum Tg concentrations ≤2 ng/mL. Patients with higher levels should be investigated for persistent or recurrent disease. Furthermore, athyrotic thyroid cancer patients with unstimulated or stimulated serum Tg concentrations >10 ng/mL are likely to have evidence of persistent or recurrent disease.

For patients with small thyroid remnants, there are currently no universally accepted cut-off levels for Tg. It has been suggested that Tg levels should not exceed ~0.5 ng/mL per gram of remnant tissue in patients with a suppressed TSH <0.1 mIU/L, or ~1 ng/mL per gram of remnant tissue if TSH is ≥ 0.1 mIU/L.

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Most laboratories perform Tg measurements by automated noncompetitive immunometric assays (IMA). The clinical utility of thyroglobulin testing can be negatively affected by various analytical issues. Interference caused by TgAb remains the most serious problem limiting the clinical utility of thyroglobulin testing. TgAbs are detected in up to 30% of patients with differentiated thyroid cancer, compared with the 10% incidence reported for the general population. TgAb interference is characterized by undetectable or low thyroglobulin levels using immunometric assay. Due to this problem various practice guidelines, including those from the NACB and ATA, stressed that anti-Tg autoantibodies should be measured on all samples tested for Tg. Failure to detect TgAb interference in the presence of an undetectable Tg value could greatly impact patient management as disease recurrence might go undiagnosed. Screening for TgAb should be performed by immunoassay method and not by a recovery test. It is now widely recognized that recovery tests are an unreliable means for detecting interfering TgAb.

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TgAb and Tg show mutual interference in their immunoassays. As shown in the panel on the left, in a typical immunometric Tg assay, the Tg present in the patient serum is sandwiched between a capture and detection antibody. In the presence of TgAb, binding of thyroglobulin can be prevented by blocking the access of the capture and/or binding antibody to their respective epitopes on Tg. This will result in falsely low levels of Tg.

The right panel shows the effect of high amounts of Tg in the measurement of TgAb. In a TgAb immunometric assay, the TgAbs present in the patient's serum will bind to immobilized Tg and are detected by antihuman IgG detection antibodies. Tg in the patient's serum might sequester the TgAb, making them unavailable to bind the immobilized Tg. The detection Abs will still bind the TgAb but

complexes will not be captured, resulting in a falsely low TgAb value. In contrast, if a competitive assay is used for TgAb detection, high concentrations of Tg could result in falsely elevated TgAb levels.

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Another type of analytical interference is caused by the presence of heterophile antibodies, which are human antibodies that can bind to animal derived antibodies used in immunoassay. Heterophile antibodies are a potential problem for all immunometric assays. These antibodies will most frequently interact with the capture and/or labeled antibodies simulating the presence of analyte (in this case thyroglobulin) and can create a falsely elevated result, even when thyroglobulin is absent in the sample.

A study in 2003 by Preissner et al. [J Clin Endocrinol Metab. 2003;88(7):3069-74] detected heterophile interference in approximately 3% of specimens tested for thyroglobulin. Despite the manufacturers' efforts to overcome this problem, some patients' specimens still exhibit heterophile interference in Tg immunoassays. In situations where an elevated Tg level does not fit the clinical picture, the laboratory should be contacted for evaluation of possible heterophile interference.

Hook-effect in immunometric Tg assay occurs when very high concentrations of Tg saturate both the capture and detection antibody preventing the formation of the antibody complex and resulting in falsely low Tg levels.

The fourth issue concerning Tg measurement is assay standardization. A thyroglobulin reference preparation (CRM 457) was introduced in 1996 and current Tg methods claimed to be standardized to this preparation. However, a study of six currently available CRM-457-standardized methods found an approximate twofold difference in mean Tg levels in a cohort of 68 euthyroid, TgAb-negative control individuals. This highlights the fact that Tg values should not be compared amongst different assays for thyroid cancer follow-up. Moreover the ATA guidelines emphasized that the same Tg assay should be employed over time in individual patients. If a change in the methods is necessary, re-baselining of the individual patients should be performed.

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More recently, the measurement of Tg in FNAB needle-washings from lymph nodes is becoming a common practice to assist in the diagnosis of thyroid cancer metastasis. The introduction of this practice has been driven by the fact that depending on the institution, ~10-20% of FNAB cytology are nondiagnostic. Measurement of Tg in FNAB needle washes has been shown to have comparable diagnostic performance than cytology and it is also diagnostic in most cases with a nondiagnostic cytology. Another advantage of measuring Tg in the FNAB washes is that it is unaffected by the presence of TgAb.

The advantage of performing Tg measurement in FNAB washes is that the cytological examination and measurement of Tg can be performed on the same specimen. To measure Tg, the FNA needle is rinsed with a small volume of saline solution immediately after the sample for cytological examination has been expelled from the needle for a smear preparation. Tg levels are measured in the needle wash. Interpretation of the result should be based in the laboratory established clinical decision limits.

In our institution, we use a Tg value of 1ng/mL based on a collection volume of 1.0 to 1.5 mls. Values above 1ng/mL are considered positive for the presence of Thyroid cancer in the biopsied lymph node. The Tg cutoff would be dependent on the collection volume and laboratories should provide guidance on how the specimen should be collected based on their assay validation.

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In clinical practice, TgAb are used in the diagnosis of thyroid autoimmune diseases and to assess the reliability of the Tg measurement in thyroid cancer follow-up. In patients with autoimmune hypothyroidism, ~70% of patient will have detectable anti-Tg autoantibodies, while up to 90% will have detectable anti-TPO autoantibodies. In Graves' disease, 30% of patients will have detectable TgAb antibodies. In both of these situations, measurement of TgAb is useful as an aid in diagnosis of thyroid autoimmune disease. In thyroid cancer, as discussed earlier, TgAbs interfere in Tg immunometric assays, resulting in falsely low levels of Tg. In patients that are TgAb positive, serial measurements of TgAb may be useful as a prognostic indicator. TgAb concentrations gradually decline in cured athyrotic patients. Patients that do not show a gradual decline in serum TgAb concentrations following treatment have a significant likelihood of residual or recurrent disease.

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All FDA approved TgAb assays are aimed for the aid in the diagnosis of thyroid autoimmune diseases and as such, the majority of them can only detect relatively high TgAb concentrations and could miss significant TgAb interference in the Tg assay. Furthermore, because of the poor numerical agreement between the TgAb assays in any given sample, it is very difficult to determine the minimal level of TgAb that is likely to cause interference in the Tg assay. In addition, the manufacturer-provided diagnostic cut-offs are not optimized for the detection of interference in Tg assays and values within the normal range could still cause significant interference. Ideally, each laboratory should establish the value of TgAb that interfere with their respective Tg assay.

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This figure shows a method comparison between 4 different TgAb assays using 100 samples. In the x-axis, the Beckman Coulter Access assay was used as the reference method and the status of the TgAb, whether they were positive or negative, was based on this method. Unfortunately this method has recently become unavailable. The other assays tested were the Siemens Immulite TgAb assay, the Roche Elecsys TgAb assay and the Siemens Centaur assay. The poor correlation between the assays is clear from the regression analysis with correlation coefficients ranging from 0.05 to 0.30 and slopes ranging from 1.7 to 12.1. This means that patients will have very discordant results and a patient considered TgAb negative in one assay might be considered TgAb positive with another assay. This could potentially affect patient management as clinicians might be confused if there is a significant difference in the TgAb values when patients are being followed up over time. Furthermore, laboratories will have to determine the level of TgAb that causes interference with each of the different assays.

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To summarize, Tg is a thyroid specific marker that has become a standard of practice in the follow-up of patients with differentiated thyroid cancer after total thyroidectomy. As a tumor marker, Tg lacks specificity since it could be up-regulated in benign thyroid diseases, and as such should not be used in the initial diagnosis of thyroid cancer.

The presence of the TgAb in up to 30% of thyroid cancer patients is problematic as they interfere in Tg immunometric assays. It is very important that the each Tg measurement has a TgAb value associated with it to determine the accuracy of the result. If TgAb are present, then the clinician should be alerted to recognize that the Tg result might be unreliable especially if the value is undetectable.

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References