UNIT NO. 3

UPDATES ON THYROID FUNCTION TESTS

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ABSTRACT

Thyroid-stimulating hormone receptor autoantibodies (TRAbs) are pathogenetic and diagnostic in Graves' Disease (GD). We briefly review the value of these antibodies in GD during diagnosis, treatment, relapse and pregnancy. Currently available methods for monitoring are immunoassays for TRAbs and bioassay and immunoassay for TSI specifically.

In the last 50 years several methods have been used to detect autoantibodies against TRAbs, based on bioassays or immunoassays. The bioassays measure functional activity of TRAb; cumbersome, time consuming and unsuitable for routine use in clinical laboratories. Immunoassays measure binding of the autoantibodies to the receptor without functional discrimination, are better standardised, much less expensive, and easily automatable for routine use in clinical laboratories. We briefly discuss the latest available immunoassay with discrimination of the autoantibody, i.e. Thyroid Stimulating Immunoglobulin (TSI).

Keywords: Graves' Disease; TSH-R-stimulating Immunoglobulins (TSI); Thyroid Receptor Autoantibodies (TRAbs); Bioassay; Immunoassay;

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UPDATES ON THYROID FUNCTION TESTS — LATEST MARKERS

The most common cause of thyroid disorders worldwide is iodine deficiency, leading to goitre formation and hypothyroidism. In iodine replete areas, most persons with thyroid disorders have autoimmune disease.

Epidemiology of Thyroid Disease

Country	Population	Hypothyroid	Hyperthyroid
UK[1]	Whickham Study (n=2779)	9.3% female 1.36% males	3.9% females 0.2% males
US[2]	NHANES III data	0.3% overt 4.3% subclinical	0.5% overt 0.7% subclinical
Netherlands[3]	Nijmegen Biomedical Study (n=5167)	0.4% overt 4.0% subclinical	0.4% overt 0.8% subclinical
China[4]	Chemical Company Ningbo (n=10,405)	0.3% overt 3.4% subclinical	0.4% overt 0.8% subclinical
Spain[5]	Subset>60 years old	6.9%	3.3%

Because the thyroid is integral to metabolic regulation, the thyroid hormone can affect every organ system. Excess can lead to a variety of symptoms, most of which are nonspecific and may be mistaken for symptoms of other illnesses.

Graves' Disease (GD) or diffuse toxic goitre, is the most common form of hyperthyroidism affecting an estimated 1.2 percent of the world's population. GD is found 7 to 8 times more commonly in females compared to males, and usually in the middle aged. However, the disorder can also be found in children, adolescents and the elderly.[6,7,8]

PATHOLOGY OF GRAVES' DISEASE

GD is an autoimmune disorder in which the immune system produces autoantibodies, namely thyroid-stimulating immunoglobulin (TSI) that will bind to thyroid-stimulating hormone (TSH) receptor sites on the thyroid follicular cells. These follicular cells will routinely activate thyroid hormone production. Hence, TSI is competing with TSH to bind on the TSH receptor and mimic the action of TSH, stimulating an excess production of triiodothronine (T3) and thyroxine (T4). The negative feedback system that normally regulates the production of thyroid hormones is hampered in the presence of TSI, resulting in excess thyroid hormones.

Hyperthyroidism, including GD, has vague and non-specific symptoms that may mimic other conditions. Symptoms include: anxiety and irritability; hand tremors; heat sensitivity and sweating; abnormal weight loss; goitre; and rapid or irregular heartbeat.

Up to 40 percent of GD patients may exhibit thyroid-associated orbitopathy or Graves' ophthalmopathy (GO). The TSH-R-stimulating immunoglobulins (TSIs) mediate the metabolic changes in the TSH-R-positive fibroblasts, targeting the cells of the orbital tissue and leading to GO.[9]

A differential diagnosis of hyperthyroidism is challenging, but critical to determining the most appropriate treatment of the patient.

First-line tests today are a sensitive TSH test and often free Thyroxine (fT4) test. In overt hyperthyroidism, TSH is typically low and fT4 increased. These results alone do not provide clarity of cause of hyperthyroidism, but are useful in the differential diagnosis.

The most commonly used laboratory tests for the diagnosis of GD is TSH-R antibodies (TRAb), including TSIs. Assessing the presence of TSI can help verify the presence of GD, since TSIs are present in almost all untreated GD patients. Thyroid radioactive iodine uptake (RUIS) can also be used for differential diagnoses of GD. However, TSI offers a less expensive and shorter time to GD diagnosis and an improved performance in identifying subclinical patients. TSI has been reported to reduce

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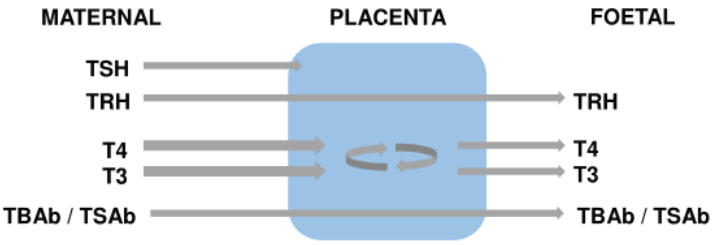
the GD misdiagnosis rate by up to 85 percent.[10]

Hyperthyroidism can occur with approximately 80 percent of the cases with GO but not necessarily presenting at the same time. GO may present long before the onset of thyroid dysfunction or during a euthyroid phase after therapy.[11] Hence, we have a clinical picture of GO and a euthyroid status, which may be confusing. TSI are found to be present in 98 percent of GO patients and correlate with both activity and severity.[12]

Ponto et al[11] presented findings of all patients with active GO testing positive for TSI, but only 84 percent for TRAb assay. It was also found that the higher the initial result, the greater the risk of a more severe disease outcome. Lytton et al obtained a clinical sensitivity of 97 percent versus 77 percent and a specificity of 89 percent versus 43 percent for the TSI compared to the TRAb assay in GO.

Figure 1: Maternal Thyroid Hormones that Cross over to the Foetus



GRAVES' DISEASE IN PREGNANCY

Disease activity will persist through pregnancy, and in some patients they may develop the disease during pregnancy. Maternal thyroid hormones, both endogenous and exogenous will cross the placenta in limited amounts. However, both TSI and anti-thyroid drugs readily cross the placenta and affect foetal thyroid function.[13]

The stimulation of thyroid hormone production may be increased in the first weeks and months of the newborn's life due to slow clearance of TSI. Hence, the increased risk of delayed and significant neonatal hyperthyroidism. When a mother on anti-thyroid drugs becomes euthyroid, the foetus has a tendency to become over treated, with resulting hypothyroidism. Hence, the monitoring of thyroid control during pregnancy is essential for both mother and neonate.

With thyroid receptor antibodies freely crossing the placenta and able to stimulate the foetal thyroid, maternal antibodies should be measured in high-risk cases.

The suggested criteria for TRAb measurement in pregnancy are:[14,15]

- Mothers with current GD;
- Mothers with a history of GD;
- Mothers previously treated with I131 or a thyroidectomy;
- Previous neonate with GD; or
- Previously elevated TRAb result.

In addition to these recommendations, any pregnant GD cases on anti-thyroid drugs should be measured for thyroid receptor antibodies in the last trimester. In the event high levels of antibody are detected, the neonate should be screened to exclude hyperthyroidism.[13] In Singapore, we routinely screen newborn cord blood TSH for thyroid status at birth.

MONITORING ANTI-THYROID DRUG TREATMENT

Anti-thyroid drugs are an effective treatment to obtain a euthyroid status in GD, with periodic monitoring of treatment required to assess remission or relapse of disease. TSI titres are closely associated with the active phase or relapse of GD. Measurement of the TSI titre at the time of drug withdrawal is a useful predictive indicator of long-term remission or relapse. Liu et al reported 60 out of 73 patients (82%) achieved remission with the disappearance of TSI.[16]

Thyroid-blocking antibodies (TBAb) may also be detected in patients with GD. With treatment, the concentrations of both blocking and stimulating antibodies may change over time. Our routinely used TRAb assays are unable to show the changes in the blocking and stimulating antibodies during the course of treatment. The newly available TSI immunoassays have the ability to specifically measure the TSI changes over time that the TRAb assays may not reflect.

Anti-TSH Receptor Antibody Assays

Thyroid-stimulating autoantibodies (TSAb), or now referred to as thyroid-stimulating immunoglobulins (TSI) are responsible for Graves' Disease, whereas thyroid-blocking autoantibodies (TBAb), which inhibit TSH binding to the thyroid receptor result in hypothyroidism. Today, laboratories have many assays available that detect the anti-TSH receptor antibodies and detect both the TSI and TBAb. They can be found in many assay formats, for example electrochemiluminescence, ELISA, and RIA. However, there is a lack of inter-assay comparability, even with standardised reference material being available. It is recommended that patient monitoring be performed by one method in one laboratory.

History of TRAb and TSI in Singapore

TRAbs

In the 1990s, laboratories were using a radioreceptor assay (RIA) for the quantitative determination of TSH receptor antibodies in human serum. This assay was standardised to TSAb WHO standard 90/672.

In the middle of the 21st Century's first decade, laboratories changed to the more easily performed ELISA assay. In the first reaction step, patient samples are incubated in the wells. Samples

that are positive will bind to the TSH receptors. Bound antibodies are able to inhibit the binding of biotin-labelled TSH, which is added in a second incubation step. To detect the bound TSH-biotin, a third incubation is carried out using enzyme-labelled avidin, which promotes a colour reaction. The intensity of the colour formed is inversely proportional to the concentration of antibodies against the TSH-receptor.

Later in that first decade, some laboratories changed to the electrochemiluminescence assay.

TSIs

In 2009, the FDA approved a qualitative TSI bioassay, Thyretain®, which uses genetically engineered Chinese hamster ovary cells expressing a chimeric form of the human TSH receptor and a cyclic AMP-induced luciferase reporter gene to determine functional signalling.

In March 2016, the FDA approved a semi-quantitative chemiluminescent immunoassay, Immulite 2000 TSI assay, which captures TSI using chimeric hTSHR and signals with a second alkaline phosphatase labelled chimeric receptor. This assay is traceable to WHO's 2nd IRP of TSI 08/204.

TSI in our hands

We reviewed the performance of the new Immulite TSI assay (Siemens Healthcare, NY, USA), an automated chemiluminescent immunoassay for the detection of TRAbs in routine clinical use.

The assay is constructed with a pair of recombinant hTSHRs in a bridging format: the capture and the signal receptors. The TRAb molecule in the serum will attach to the capture receptor with one arm and to the signal receptor with the other. The amount of TRAb bound is determined by the intensity of the chemiluminescent signal.

A 73-patient concordance and bias study was performed (Immulite versus Euroimmun ELISA). With this data we obtained a sensitivity for TSI of 100 percent and a specificity of 95 percent. One sample that was TRAb positive and TSI negative was from a pregnant subject and may be a result of TRAb without a TSI presence. The correlation obtained was TSI = 0.645 (TRAb) - 0.626, r = 0.841.

The reference range/cut-off for the assay of 0.55 IU/L was validated with a cohort of 56 healthy blood donors who all obtained negative results.

Reproducibility was determined with pooled serum samples of concentrations across the measuring range. They were tested in duplicate over a period of 10 days. A coefficient of variation (CV) range of 3.4 – 6.6 percent was obtained over a concentration range 0.23 – 28.3 IU/L for TSI. The TRAb assay obtained a CV of 5.9 – 7.1 percent at the high concentrations, however the concentrations around the cut-off obtained a CV >20 percent.

No interference was detected in haemolysed, lipaemic, or icteric samples.

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LEARNING POINTS

- **Thyroid-stimulating autoantibodies (TSAb), or now referred to as thyroid-stimulating immunoglobulins (TSI) are responsible for Graves' Disease.**
- **Both TSI and anti-thyroid drugs readily cross the placenta and affect foetal thyroid function. A pregnant GD patient should be regularly monitored throughout pregnancy. The newborn should be assessed based on thyroid results at birth and maternal thyroid history.**
- **Clinical laboratories routinely offer a TSAb immunoassay while TSI is more of a research tool in the bioassay format. A new TSI immunoassay has now become available to the clinical laboratories.**