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TRAB Measurements: Ready for Prime Time?

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The article in this journal "Outcome of Graves' disease treated with anti-thyroid drugs and time course of anti-TSH receptor antibodies" adds to the growing body of literature on the clinical utility of TRAb. As stated in the article, the remission rate of Graves' Disease (GD) after 18 months of Anti-thyroid Drugs (ATD) treatment remains around 50%. A biomarker that allows prediction of who will remit or relapse will be a great boon in the medical management of GD. Accurate measurements of TRAb are emerging as an essential tool in clinical endocrinology.

Thyroid Receptor Antibodies (TRAb) are the diagnostic marker for Graves' disease (GD) with a sensitivity and specificity of over 98% [1]. There are three different kinds of TRAb (stimulating, blocking or neutral) and stimulating TRAbs are the most common [2]. The presence of TRAbs can be an indicator of risk of GD even in subclinical hyperthyroidism [3]. TRAb has been used in the differential diagnosis of hyperthyroidism, prediction of remission after treatment of Graves' hyperthyroidism, prediction of fetal/neonatal thyrotoxicosis, and assessment of Graves' ophthalmopathy [4]. TRAb levels decline with treatment, especially with surgery followed by drugs and radioiodine ablation. Monitoring the TRAb at presentation and cessation of therapy is essential in the follow up of patients with GD. TRAb levels can predict the likelihood of remission and prognosis of Graves' ophthalmopathy [5].

The 1st generation TRAb assays were competitive immunoassays measuring the inhibition of Thyroid Stimulating Hormone (TSH) binding to the TSH receptor (TSHR). They had high specificity but low sensitivity and so many GD patients were labelled "TRAb negative" by these assays. Second generation TRAb assays have improved clinical sensitivity. Until recently, the 2nd generation assay remained the gold standard. New 3rd generation TRAb assays have been available since 2008 [6]. In these new TRAb assays, autoantibodies inhibit the binding of human TSH monoclonal antibodies labelled with biotin to TSHR-coated capture surfaces (plates, wells or tubes). These assays are fully automated, and several studies have demonstrated that their diagnostic sensitivity and specificity are higher than 2nd generation TRAb assays. Tozzoli R, et al. [7] compared an automated 3rd generation TRAb (RAD 120; Radim, Pomezia, Italy) against a second 2nd generation TRAb immunoassay (Lumitest TRAK human; Brahms, Berlin, Germany). The ROC plot of the 3rd generation assay was excellent (AUC 0.994), with a lower optimal threshold of 1.25 U/L compared to second generation value of 1.99 U/l. The 3rd generation assay had a higher sensitivity of 97.6% *versus* 95.1% for the 2nd generation assay. The 3rd generation assay also had greater analytical precision with a faster turnaround time.

In our own laboratory, we compared the 3rd generation Roche TRAb Electrochemiluminescence Assay (ECLA) on the Cobas e601 platform with the 2nd generation Brahms TRAK radio-receptor assay [6]. The two methods were well correlated (r=0.93). In 49 cases of hyperthyroidism, the 3rd generation assay was positive in all 49 cases, but the 2nd generation assay was positive in only 47 of the cases, underscoring the higher sensitivity of the 3rd generation TRAb assay. However, most 3rd generation TRAb assays measure thyroid-binding inhibiting immunoglobulins (TBII), and thus they do not differentiate between stimulating and blocking antibodies. In some cases, this may cause a difference between TRAb levels reported by these assays and the severity/outcome of GD because the TBII assays cannot differentiate between the functional properties of the different types of TRAb measured [8]. This may also explain why the 3rd generation TRAb assays caused false-positive readings for neonatal GD [9] and was not correlated with severity of Graves' opthalmopathy (inactive cases still had high readings of TRAb) [10].

There remains a high inter-method variability between the 2nd and 3rd generation TRAb immunoassays due to a lack of harmonization despite calibration to the same reference standard WHO 90/672 [11]. In the study by Massart C, et al. [11] of negative-TRAb GD patients, one patient with borderline results (1.2 IU/L) on the 2nd generation assay Brahms hTRAK was positive on the 3rd generation assay Roche ECLA) but negative on the 2nd generation assay Beckman-Coulter pRRA as well as in the 3rd generation assay Medipan ELISA. It is recommended that the same TRAb assay be used when monitoring patients.

Newer methods of TRAb evaluation have been developed, with some that measure the stimulating TRAb (TSI) component directly [12]. Although the sensitivity was 100%, the correlation between the new TSI assays and current TRAb assays was just acceptable, which may be due to the new assays measuring some level of blocking antibodies as well. In addition, possible false positive results appeared in patients with hypothyroidism testing positive for TSI. Kemble DJ, et al. [13] also compared a TSI assay (Siemens Medical Solutions) with a TRAb assay (Roche Diagnostics) and a Thyretain assay (Diagnostic Hybrids, Athens). All assays performed equally well in patients with GD, but 21 out of 81 patient samples showed discordant results between the three assays. As such, further studies and refinement are needed before the new TSI assays can be fully adopted.

Some caution also needs to be exercised when evaluating biotin-based TRAb assays (e.g. Roche) in patients taking biotin supplementation, particularly if they have end-stage renal failure since biotin is excreted by the kidney [14]. Excess biotin in serum prevents the biotinylated antigen/antibody from interacting with the streptavidin-coated solid phase resulting in a low immunoassay detection signal in TRAb assays, leading to a falsely high TRAb value. However, the level of biotin required for assay interference is different across platforms and depends on many other factors such as amount of endogenous biotin and metabolites present, the type of biotin supplement, and the timing of blood draw after biotin intake [15]. TRAb assays are more sensitive to biotin than other thyroid assays, in studies where serum is spiked to a concentration of 15.6 ng/mL [16], TRAb had a more than two-fold positive bias on Roche platforms whereas there was little effect on the FT4 and TSH. Thus, in cases where patients are taking biotin or have end stage renal failure, if would be prudent to review TSH and FT4 results together with TRAb.

The higher the TRAb at diagnosis, the greater the likelihood of relapse of GD. However, recent evidence also indicates that the TRAb level at cessation of therapy is also important. Liu L, et al. [17] followed up a large group of GD patients (n=306) being treated with methimazole. Relapse after ATD cessation was associated with younger age of onset, larger thyroid glands, more notable thyroid-associated ophthalmopathy and increased FT3 levels, higher FT3/FT4 ratio and higher TRAb levels (11 ± 5 IU/L) versus those not associated with relapse (9 ± 3 U/L). In a study by Tun NN, et al. [18] of 260 GD patients treated with thionamide, it was found that a higher level of TRAb (TRAb >12 IU/L) at diagnosis was associated with 84% chance of relapse, and TRAb >1.5 IU/L at cessation of therapy was associated with a 47% chance of relapse. In another study [19] Kwon used immunoassay methods to measure stimulating TRAB and radioimmunoassay methods to measure blocking TRAB in separate groups at ATD withdrawal. There were significantly more cases of relapse in patients positive for stimulating TRAb at ATD withdrawal (median TRAb titre of 203.5%) than those negative for stimulating TRAb (median TRAb titre of 61.1%). There was no significant difference in relapse between blocking TRAb titre positive and negative patients at ATD withdrawal.

The current article (Outcome of Graves' disease treated with anti-thyroid drugs and time courses of anti-TSH receptor antibodies) supports the established literature, with remission-unlikely patients having generally higher levels of TRAb before therapy (some as high as 547 IU/L). This is also supported by the time courses shown in the article in this journal. Those with a lower initial TRAb were associated with remission (patterns 1 to 4). Remission was less likely in those with an initial low TRAb but frequent elevations of TRAb during treatment (pattern 5) and those with a high initial TRAb which persisted despite treatment (pattern 6). This article adds to our knowledge base of the utility of TRAb with the additional caveat that despite a low initial TRAb level, patients with frequent TRAb elevations during treatment can still relapse. Thus, time course analysis improves prediction of remission in addition to initial TRAb levels at diagnosis. This will help refine the decision for withdrawal of anti-thyroid drugs or to convert to ablative therapies. However, the assay used for TRAb measurements in this study is a 2nd generation immunoassay (Brahms, TRAK), and in our own laboratory studies, this assay had higher imprecision (inter assay CV of 5-10%) compared to the Roche 3rd generation assays (CV of 5.25% at TRAb of 5.1 IU/L). This may lead to patient misclassification, especially in those with low TRAb levels.

This article supports the notion that TRAb measurements should form part of the standard of care for GD. The higher the TRAb level at diagnosis, the less likely a patient will experience GD remission and would require closer monitoring. In addition, when deciding to stop ATD therapy, we must factor in the initial TRAb levels at diagnosis as well as the time course of TRAb thereafter. In those with a less favourable TRAb profiles a more prolonged period of treatment may be necessary than otherwise practiced or ablative therapy considered. The fully automated 3rd generation TRAb assays are preferred as they provide convenience, faster turnaround time and more accurate results.

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