Clinical Application of Thyrotropin Receptor Antibodies

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ABSTRACT

Thyrotropin receptor antibodies (TRAb) are specific for Graves' disease (GD) and play a crucial role in the pathogenesis of GD. TRAb assays have recently been greatly improved. This review discusses the clinical application of TRAb in the differential diagnosis of hyperthyroidism, the prognosis of GD, GD in gestation and pediatrics, and GD related ophthalmopathy (GO). In addition to the classical competition and bioassays, a new bridging assay has emerged for TRAb assays. TRAb is the main pathogenic mechanism of hyperthyroidism in GD. Treated GD still has a high rate of recurrence and even a short-term surge of TRAb, leading to rapid deterioration of GO. Fetal goiter may be associated with elevated maternal TRAb during pregnancy, overtreatment may lead to fetal hypothyroidism. Pediatric patients with GD have high TRAb, poor remission from treatment, and insignificant manifestations of GO. TRAb is significantly correlated with GO activity and severity. Currently, TRAb assay has high specificity and sensitivity and can be used directly to identify the cause of hyperthyroidism. TRAb can be used to predict recurrence of drug treated GD or progression of GO after RAI therapy. TRAb should be measured regularly for GD in gestation to guide anti-thyroid medication to avoid thyrotoxicosis or hypothyroidism in the fetus. Monitoring TRAb in pediatric GD may help control the progression of GO. TRAb assay is an important guide for the treatment of GO.

ABBREVIATIONS

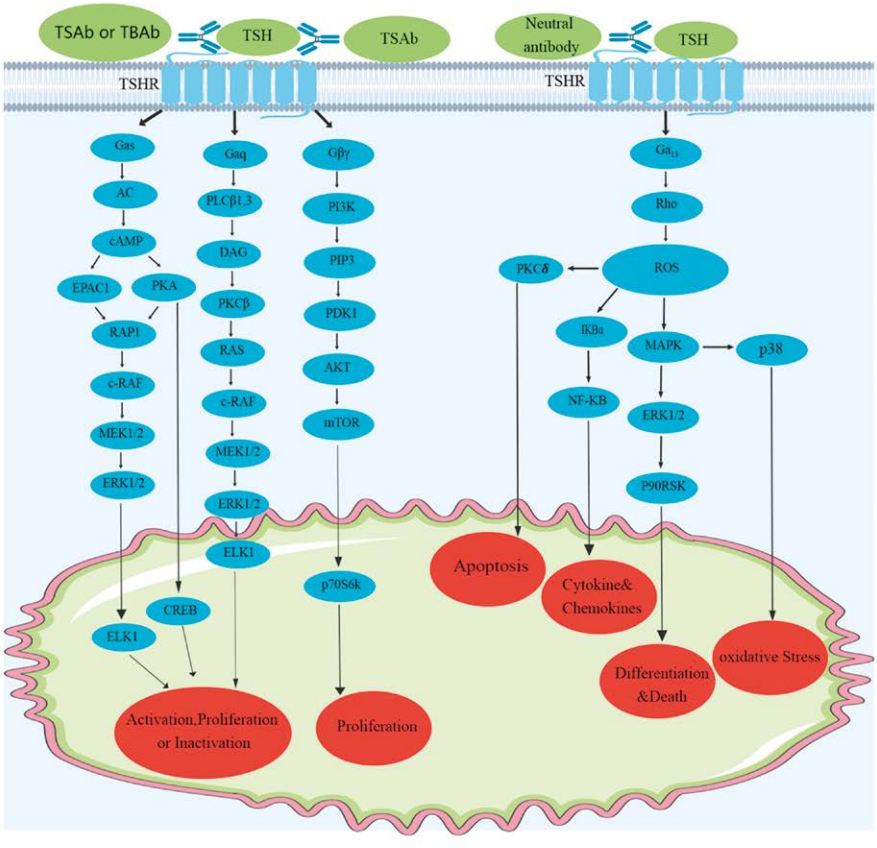
GD	Graves' disease
TRAb	Thyrotropin receptor antibodies
GO	Graves' orbitopathy
LATS	Long-acting thyroid stimulator
PTM	Pretibial myxoedema
LRD	Leucine-rich domain
TSH	Thyrotropin
TSAb	Thyrotropin-receptor stimulating antibodies
TSHR	Thyrotropin receptor
TBAb	Thyrotropin-receptor blocking antibodies
mROS	mitochondrial ROS
TBII	TSHR binding inhibitory immunoglobulin
TSI	Thyroid-stimulating immunoglobulin
ATA	American Thyroid Association
RAIU	Radioactive iodine uptake
TPO	Thyroid peroxidase
HT	Hashimoto's thyroiditis
SPT	Subacute painless thyroiditis
ATDs	Antithyroid drugs
GTT	Gestational transient thyrotoxicosis

Graves' disease (GD), also named von Basedow disease, is clinically often manifested as hyperthyroidism, causing thyrotoxicosis, involving multiple organs outside the thyroid. The most common complication is GD-associated orbitopathy (GO), in which patients usually present with orbital edema and exophthalmos. Some patients may also present with localized dermopathy, namely pretibial myxoedema (PTM). GD is the most common cause of hyperthyroidism [1], with an annual incidence of 20–40 cases per 100 000 population [2], and the incidence is increasing year by year, among which the incidence of women is 6 times higher than that of men [3]. If left untreated, GD may lead to an increased risk of cardiovascular disease, lung disease, diabetes [1, 4–6], and lead to a higher risk of death from breast cancer [7], and even lead to an increased risk of death [8]. GD is now recognized as a refractory disease.

At first, GD was thought to be caused by the pituitary gland producing excessive thyrotropin (TSH). It was not until 1956 that Adams found thyrotropin receptor (TSHR) antibody in GD patients that GD was identified as an autoimmune disease [9]. Initially, TSHR antibody (TRAb) was called long-acting thyroid stimulator-LATS, and generally believed it combining full-length receptor. It continuously stimulates thyroid follicular epithelial cells, resulting in thyroid gland overactive in GD patients. Subsequently, it was soon discovered that TRAb mainly binds to the leucine-rich domain (LRD) on TSHR [10, 11], activates TSHR signaling, and stimulates thyroid proliferation and thyroid hormone secretion and synthesis. According to its binding to different sites on LRD, different biological effects can be produced; TRAb can be divided into thyrotropin-receptor stimulating antibodies (TSAb), thyrotropin-receptor blocking antibodies (TBAb) and neutral antibodies. TSAb combined with TSHR initiates the second signal, which plays a major decisive role in the pathogenesis of GD hyperthyroidism.

Biological characteristics of TRAb

TSAb and TBAb are conformational and play different roles by combining different sites on the LRD. TSAb mimics the biological behavior of TSH, combined with the C terminus of LRD [12], mainly activates $G\alpha_s$, initiates the PKA/CREB pathway or PKA-dependent Ras family of GTP binding proteins (Rap)1-b-Raf-ERK-Ets-like transcription factor (Elk1) cascade or the PKA- independent exchange protein activated by cAMP (EPAC1) signaling pathway, induces cAMP production (▶ **Fig. 1**). At high concentrations of TSH or TSAb, TSHR activate PLC-β by coupling $G\alpha_q$, which leads to increased intracellular Ca^{2+} levels via the DAG-IP_3 pathway, resulting in gene



▶ **Fig. 1** Signaling cascade by TSHR autoantibodies: ▶ **Fig. 1** illustrates that the TSHR autoimmune antibody competes with TSH to bind TSHR, inducing TSHR to couple different signaling pathways and activate different effects of downstream factors. Both TSAb and TBAb can activate Gαs and Gαq to stimulate or inhibit cell proliferation. TSAb can also activate Gβγ to inhibit the production of reactive oxygen species and maintain normal cell proliferation. Conversely, neutral antibody activates $G\alpha_{13}$, which stimulate PKC-induced apoptosis, NF-κB-induced cytokine and chemokine expansion, and induction of oxidative stress in P38, which damages thyrocytes.

▶ **Table 1** Comparison of TBII assay, TSI assay and immulite TSI immunoassay.

TRAb Assay Methods	Types of ELISA assays	Assay indicator	Labelled substances	Sensitivity (%)	Specificity (%)
1. Competitive binding test-TBII assay					
First generation	Liquid-phase radioimmunoassay	TRAb	Bovine TSH	79.8	99.2
Second generation	Solid-phase competition ELISA		Bovine TSH	96.4	98.1
Third generation handheld			M22	97.2	99.2
Third generation automated (Roche)			M22	98.0	99.2
2. Biofunctional assay-TSI assay					
First generation	Radioimmunoassay	TSAb or TBAb	–	83.0	93.0
Second generation	Competition ELISA			85.0	92.0
Third generation				90.0	90.0
3. Immulite TSI immunoassay*					
Handheld*	Non-competitive immunoassay	TSAb*	Signaling receptor	99.8*	99.1*
Automated*				100*	99.0*

*The manufacturer claims that the immulite TSI immunoassay is available as an assay for stimulation of TRAb, and the data on sensitivity and specificity are derived from the references [27–29].

transcription (▶ **Fig. 1**). TSAb also can activate $G\beta_{\gamma}$, and inhibit the generation of proapoptotic reactive oxygen species by activating the AKT/mTOR/S6K pathway (▶ **Fig. 1**). Activation of these signaling pathways stimulates the proliferation of thyroid follicular epithelial cells to synthesize excess thyroid hormones, eventually causing hyperthyroidism.

TBAb binds to the N-terminus of LRD [13], activates $G\alpha_s$ and $G\alpha_q$, initiates PKA and PKC by increasing CREB, c-Raf, and ERK, producing mild thyroid proliferative effects [14] and inducing low-level cAMP production (▶ **Fig. 1**). However, it mainly blocks the binding of TSH to TSHR, while also blocking the constitutive activity of TSHR, which may regulate the degree of receptor activation or completely block all downstream signaling. It has been suggested that this regulation may lead to changes from overactive thyroid to underactive thyroid, known as Graves' Alternans [15], which may be the cause of GD complicated by hypothyroidism.

Neutral antibody is a linear antibody, which is thought to neither excite stimulatory activity nor block the binding of TSH to TSHR. It can bind to the hinge region epitope including C terminus, activates $G\alpha_{13}$, which leads to MAPK, NF-κB and PKC_{δ} signaling via Rho-GTPase activation (▶ **Fig. 1**). Caspase, annexin V, and mitochondrial ROS may be highly induced by these signaling cascades, which confirms that apoptosis and oxidative stress may be the main mechanisms by which neutral antibodies induce thyroid cell damage and death [16, 17]. Neutral antibodies may induce cell death and initiate inflammation by participating in the apoptosis and stress mechanism of thyroid cells, further sustaining the vicious cycle of chronic inflammation. This may explain recent studies reporting the discovery of partial apoptotic zones in thyroid tissue in GD patients. Simultaneously this mechanism can also be used to explain recent studies reported the discovery of partial apoptotic areas in the thyroid tissue of GD patients [18].

All three antibodies may be present in Intrasubject at the same time and may lead to changes in thyroid function when the overall activity of TRAb is altered. It has been reported that the presence of TRAb can be detected in 100 % of untreated GD patients, so TRAb plays a crucial role in confirming the diagnosis of hyperthyroidism in GD and has important implications for the treatment and prognosis of GD [19]. This requires that the TRAb assay should be highly sensitive and specific.

Assay methods of TRAb

TRAb has 3 primary pathogenic mechanisms: 1) TRAb competitively bound to TSHR; 2) TRAb stimulates cAMP production; 3) TSAb binds mainly to specific sites in the ECD region of TSHR. Hence, three assays for TRAb have emerged: 1) testing the ability of TRAb in patient serum to compete with TSH for binding TSHR; 2) measuring the ability of patient serum to stimulate cAMP production in CHO cells stably transfected with human TSHR; 3) detecting serum TSAb in GD patients according to the bridging technique used by TSAb to primarily bind TSHR ex 261aa. While the previous two assays are classical and have been used in clinical applications, the latter assay is emerging and has not yet been clinically validated on a large scale.

(1) **Competitive binding test-TBII assay**

TRAb is usually evaluated in competitive binding assays, hence TRAb is also known as TSHR binding inhibitory immunoglobulin (TBII). This assay, also known as the TBII assay, is the most common method used in clinical practice to detect serum TRAb in patients and focuses on the presence of this antibody that inhibits the binding of TSHR ligands (TSH or monoclonal anti-TSHR antibodies) to TSHR in the patient's serum. It has undergone three generations of updates from

its discovery to the present. 1) The first generation assay was performed in liquid phase assay using porcine thyroid membrane extracts to detect the ability of IgG to inhibit the binding of ^{125}I-labeled bovine TSH to TSHR in sera from patients with GD. The assay was subsequently improved by some investigators using CHO cells stably expressing a recombinant protein of the successfully cloned human TSHR gene, but its sensitivity was still only 79.8 % [20] (▶ **Table 1**); 2) To improve the sensitivity of TRAb assays, second generation assays using solid phase Enzyme-linked Immunosorbent Assay (ELISA) assays have been developed that use recombinant human or purified porcine TSHR coated ELISA plates to detect the ability of TRAb in patient serum to inhibit its binding to ^{125}I-labeled bovine TSH, acridin ester, or biotin streptavidin peroxida. Second generation commercial tests using purified porcine TSHR or recombinant human TSHR respectively, but their sensitivity is rather low and even many false positive results occur [21] (▶ **Table 1**); 3) Smith developed a third generation assay in 2004, which used human derived stimulated TSHR monoclonal antibody (M22) instead of bovine TSH as a ligand, which greatly improved the positivity of the cut-off value of TRAb at lower levels (0.4 IU/l) in the second generation assay [22], and improved the sensitivity of the assay, but this method is prone to artificial error; 4) Roche Diagnostics introduced a fully automated third-generation assay in 2007, which Schott believes has a sensitivity and specificity of 99 % [23] (▶ **Table 1**). This provides a reliable test for the diagnostic identification of the cause of hyperthyroidism in clinical practice. However, TBII assay only indicates the presence of antibodies in the serum that specifically bind TSHR and does not detect the biological activity of the antibody.

(2) **Biofunctional assay-TSI assay**

This method mainly detects the excess cAMP produced by TSAb binding to TSHR in pathological states. They only test for TSAb or thyroid-stimulating immunoglobulin (TSI), and are therefore also known as TSI assays. This is a cell-based assay that detects the amount of cAMP induced by ligand binding to TSHR, primarily by detecting the effect of the antibody on cAMP production by cells stably transfected with this receptor. 1) The first generation TSI assay uses human thyroid monolayer cells, porcine thyroid cells or rat thyroid cell line (FRTL-5) incubated with patient serum to detect cAMP. Despite improvements in this method using ammonium sulphate, polyethylene glycol and hypotonic media, the sensitivity of TSI was only 83 % [24] (▶ **Table 1**); 2) Following the successful cloning of the human TSHR gene, a second generation TSI assay has been established using CHO cells stably transfected with human TSHR, which increased the sensitivity of TSI to 85–90 % [21] (▶ **Table 1**). However, this method is not widely used because of its multiple steps and time-consuming operation; 3) In the third generation TSAb bioassay, genetic modification and optimization of CHO cells transfected with TSHR by incorporating cAMP-dependent reporter genes and multiple cAMP-responsive elements in their promoter regions shortened the time for such assays to one day; 4) The fourth generation bioassay was established using residues 261–329 of the rat lutropin/ choriogonadotropin receptor stably expressed in CHO cells in place of residues 261–370 of the human thyroid hormone receptor and Mc4 receptor chimeras, named thyretain, which was the first FDA approved in-vitro assay for diagnosing TSAb [25]. Despite the relative increase in sensitivity and specificity, these traditional bioassay methods mentioned above require a high laboratory environment and long turnaround times; 5) The recent development of a new method incorporating human TSHR expression, cAMP-gated calcium channels and aequorin for the detection of TSAb has been followed by studies using luciferase to further optimize this novel biosensor method, allowing TSAb results to be obtained within 1 hour [26]. These successively developed biological assays are of great significance for the bioactive typing of TRAb, especially the recently developed assays are clinically highly sensitive and specific and are of great value to the clinician in determining the presence of TSI in a patient without the presence of hyperthyroidism.

(3) **Immulite TSI* immunoassay system**

Recently, Frank has proposed a new TRAb assay, the Immulite TSI* immunoassay system, which uses a bridging technique to directly detect TSAb concentrations in patient serum [27]. The technique is based on the principle that TRAb has 2 antigen binding sites that form a bridge between 2 different recombinant TSHR molecules, while removing the recombinant TSHR that binds the TBAb binding site. Residues 261–329 of the rat lutropin/choriogonadotropin receptor are used to capture the receptor in place of residues 261–370 of the human TSHR, fixed to microtiter plates, and bound to one arm of the autoantibody. The density of the capture receptors is also controlled so that the spacing allows only a single arm of the antibody molecule to interact with the capture receptor. The signaling receptor uses residue 21–261 of human TSHR chimerized with residues 1–519 of secretory alkaline phosphatase, which binds to the other single arm of the antibody, and the intensity of the detected chemiluminescent signal increases when the level of TSAb in the serum is higher. While studies have reported sensitivity and specificity of handheld and automated TSI* methods to be more than 99 % [28] (▶ **Table 1**), their specificity remains controversial. Kim [29] found that the results of the bridging assay were more consistent with the clinical diagnosis of the patient than the third generation TBI assay. However, it has also been found [27, 30] that the bridging assay has been positive in patients with a negative TSI* bioassay or a clinical diagnosis of hypothyroidism. Therefore, it is obvious that there is no more data to substantiate the Immulite TSI* immunoassay as a stimulatory TRAb assay, and the specificity and sensitivity of this assay still needs further research and observation.

Clinical application of TRAb

The diagnostic value of TRAb

TRAb is an IgG antibody produced oligoclonically in GD patients. It competes with TSH for binding to TSHR in pathological states leading

to excessive proliferation of thyroid follicular epithelial cells, which is the main pathogenic mechanism leading to hyperthyroidism in GD patients. TRAb is generally not present in normal individuals. Thus, measurement of serum TRAb levels is a valuable laboratory assay that can be used to clarify whether GD is the cause of hyperthyroidism. As early as 2011, clinicians in Europe included TRAb testing as one of the diagnostic criteria for hyperthyroidism in GD, and they have advocated the usage of TRAb as a primary test in the initial screening for hyperthyroidism [31]. However, it has only been in 2016 that the American Thyroid Association (ATA) proposed the testing of TRAb as one of the criteria for the diagnosis of GD [32]. This may be related to the longer waiting period for early TRAb reports compared to other tests (including T3, T4, TSH and thyroid scans); another significant reason may be that early TRAb assays have been unsatisfactory and the cost of early TRAb assay is relatively high. In recently years, the specificity of the second-generation TBI method test for the diagnosis of untreated GD hyperthyroidism is close to 100 % [33], especially with the update of the third-generation automated TRAb assay technology, which not only circumvents the errors caused by human manipulation, but also makes the cost of TRAb testing relatively lower, making it not much different from the cost of testing using conventional radioactive iodine uptake (RAIU) + thyroid scan [20]. The TRAb assay has a higher sensitivity for confirming the diagnosis of GD and a higher specificity for discriminating GD from other thyroid diseases. Some scholars believe that its assay is even more accurate than the anti-glutamine transaminase antibody test, which is currently commonly used to diagnose celiac disease, and the anti-citrullinated peptide antibody test, which is used to diagnose rheumatoid arthritis, and even suggest using the TRAb test instead of the RAIU for all patients to avoid unnecessary medical radiation exposure [20].

TRAb positive patients are 1367–3420-fold more likely to develop GD than negative patients [20], thus the TRAb assay may be considered for initial evaluation and early avoidance prevention in patients suspected of having GD. Both thyroglobulin antibodies and thyroid peroxidase (TPO) are elevated in patients with Hashimoto's thyroiditis (HT) and GD hyperthyroidism, but the current combined test for TBI and TSI make it possible to clarify the etiology of hyperthyroidism. For clinically undefined diseases with thyrotoxicosis, such as Subacute painless thyroiditis (SPT), Unilateral exophthalmos, normal thyroid function GO, subclinical hyperthyroidism and thyrotoxicosis associated with pregnancy vomiting, testing the patient's serum TRAb level can be used for diagnosis and differential diagnosis. These disorders can manifest clinically with symptoms of hyperthyroidism similar to those of GD, and almost all patients with SPT test positive for elevated TPO. However, the manifestations of these diseases are mainly caused by the destruction of the autoimmune system or stress response, rather than by the presence of TSHR autoantibodies that continuously stimulate the thyroid gland to synthesize hormones, so the TRAb test is clearly specific for distinguishing these diseases from GD. Also, TRAb titer testing can be used to differentiate between autoimmune and amiodarone induced thyrotoxicosis. Therefore, TRAb assay is currently a cost-effective and practical tool for diagnosing hyperthyroidism due to GD in most people. However, the sensitivity of TRAb assay in its application to diagnose elderly patients with GD seems to be somewhat unsatisfactory. In the reported study, it has been found that some of the elderly patients with GD who presented clinically with subclinical hyperthyroidism and diffuse lesions on thyroid scan had negative TRAb test results [34]. This presentation differs from the current academic research on the pathogenic mechanism of GD, which is known to be atypical in elderly GD patients with mainly indifferent hyperthyroidism, and therefore the pathogenic mechanism of geriatric GD still needs further in-depth study and exploration.

The prognostic value of TRAb

TRAb titer levels change with the course of GD disease, and varying treatment modalities can also affect TRAb titer levels. Antithyroid drugs (ATDs) can alleviate clinical symptoms and lower TRAb titers in GD patients in the short term, but TRAb levels can rapidly rise, leading to relapse, which are more pronounced in those with high TRAb [35]. Therefore, the previously used discontinuation test to determine whether patients with GD relapse is not feasible, and this prognostic indicator has been found to make the risk of relapse significantly higher [36]. Recent studies have found that detecting TRAb before treatment and at the time of treatment-withdrawal, and establishing a cut-off value for TRAb, is effective in predicting the time to relapse in GD [37]. However, negative TRAb results have been shown to predict only short-term relapse rates after discontinuation, and long-term relapse rates remain unpredictable.

Patients whose TRAb-positive GD relapses or fails to resolve after treatment with antithyroid drugs are usually recommended for radioiodine therapy (RAI) or thyroidectomy. Patients whose TRAb-negative GD with significant symptoms of hyperthyroidism are also recommended for both treatments. RAI treatment may lead to a surge in TRAb [38] and exacerbate the course of GO [39], whereas thyroidectomy only puts the patient into temporary autoimmune remission [38] and does not alter the course of GO. Through the observation of the prognosis of different GD treatment modalities, it is not difficult to find that the changes of TRAb in patients have a great influence on the development of the whole course of GD, and it can even be considered that TRAb may be the root cause of GD relapse. It has been proposed that age, gender, thyroid volume, thyroid vascular hyperplasia, and degree of thyroiditis in patients with GD can be used as predictors of recurrence risk in treated patients [40]. Comprehensive tools based on clinical, biochemical (including TRAb) and genetic variants, such as the GREAT and GREAT + scores, have been introduced to predict recurrence of GD [37]. However, the predictive index is still lower than that of TRAb as a single indicator, despite recent attempts to incorporate three generations of TRAb assays into the GREAT score. These predictor variables are susceptible to exogenous factors, and the GREAT + score requires genetic analysis, which limits its routine use. Therefore, the use of TRAb as a predictor is a simpler, more cost-effective and reproducible tool for predicting clinical course and guiding therapeutic decisions.

The initial studies used relapse after 0–3 years of stopping ATDs treatment as a criterion for GD relapse. Since autoimmune disease like GD itself has a remission and relapse period, TRAb may be present in the patient for a prolonged period of time and may show different titer levels or switch from TSAb-dominated to TBAb-dominated during the remission period, which leads to a high probability

of relapse of GD hyperthyroidism soon after remission, which means that the original relapse criteria may be too long. It has been found that using 8 weeks or 6 months after drug discontinuation as the time to detect TRAb levels in patients has a good predictive value for assessing GD relapse [21], while the threshold value of TRAb in relapsers is higher than the threshold value for the initial diagnosis of GD. This may be due to the fact that patients with GD are chronically hyperthyroid and the organism's inherent TSHR stimulation threshold is higher than normal. Therefore, ATA suggests that TRAb titers should be monitored after discontinuation of ATDs therapy to predict recurrence of GD, so that clinical treatment can be adjusted at any time. In addition, the ATA recommends that CHO cells transfected with TSHR be used to monitor levels of TSAb during treatment of patients with GD [32]. Patients treated with RAI or surgery may experience elevated TRAb and uncontrolled GO progression, which may even lead to rapid GO progression in a short period of time, suggesting the need to closely monitor patients' TRAb to prevent GD recurrence and to intervene in a timely manner in GO progression. Consequently, TRAb assays for patients with GD, whether on treatment or after treatment, are of great interest in identifying active disease and recurrence of GD and monitoring the development of GO.

The value of TRAb in combined GD during pregnancy

The most common cause of hyperthyroidism in pregnancy is GD and gestational transient thyrotoxicosis (GTT) [41]. Although both GD and GTT exhibit decreased TSH and elevated T3 and T4, the causative mechanisms and treatment of these two disorders are drastically distinct. GTT is caused by a transient elevation of human chorionic gonadotropin (hCG), which activates TSHR on thyroid follicular epithelial cells and stimulates elevated thyroxine [42]. As the peak hCG falls back in mid-pregnancy, GTT also falls and does not cause serious pregnancy complications. Therefore, for GTT-induced hyperthyroidism, ATDs are not clinically intervened and only symptomatic supportive treatment is given when the patient develops severe symptoms. However, pregnant women with GD exhibit elevated TRAb levels and maternal TRAb can cross the placenta to stimulate the fetal thyroid and induce thyroid dysfunction. In the absence of clinical intervention, this can even lead to serious risks such as preeclampsia, miscarriage, congestive heart failure, thyroid storm, preterm delivery, intrauterine growth retardation, low birth weight fetuses and neonatal respiratory distress [43]. Although TRAb decreases in pregnant patients with combined GD in mid to late gestation, a small percentage of newborns or fetuses will still exhibit thyrotoxicosis, probably because the decline in maternal TRAb is simply the patient entering a period of immune tolerance. ATDs are now mostly used clinically to treat patients with GD in combination with pregnancy. However, since both methimazole (MMI) and propylthiouracil (PTU), which are currently commonly used, have a risk of hepatic impairment and congenital malformations in the fetus [44–46], the dosage of ATDs in women with combined GD during pregnancy is extremely important. It has been found that timely monitoring of changes in TRAb levels during treatment with ATDs can help clinicians to adjust the medication to the patient's condition [47]. This is mainly because fetal thyroid function may return to normal by mid-gestation and ATDs can cross the placenta and affect fetal thyroid function. Normal thyroid function may be restored after maternal treatment with an overdose of ATD, whereas the fetus may develop hypothyroidism [43] with a corresponding increase in TSH, inducing fetal goiter, IUGR, delayed bone age, and bradycardia. Therefore, TRAb monitoring is crucial for accurate clinical judgment and effective and rational intervention in patients with GD in pregnancy. In addition, TRAb testing can effectively predict fetal or neonatal hyperthyroidism in pregnant women with GD during pregnancy. In pregnant women with a previous history of GD, thyroidectomy or radioiodine treatment for GD, TRAb, especially TSI levels, should be checked early in pregnancy, and TSI levels should be reviewed in mid-pregnancy and fetal thyroid levels monitored by fetal ultrasound [48]. This is because TRAb may persist in patients even after surgical removal of the thyroid gland, and a surge of TRAb may occur in patients after radioactive iodine treatment. In addition, ATA has long suggested in 2011 that maternal TRAb levels should be measured in the middle of pregnancy and the fetus should be closely monitored for high TRAb levels [49]. TBI and TSI levels should be performed simultaneously in those with persistently elevated TRAb in mid-pregnancy, mainly because it is possible that the cause of GD remission in pregnant women is related to the conversion of TRAb from TSAb to TBAb and the presence of blocking TRAb during pregnancy may lead to congenital hypothyroidism in some newborns.

The value of TRAb in pediatric GD

The incidence of pediatric GD has been on the rise in recent years [50]. The presentation of GD hyperthyroidism in neonates is diverse, mostly presenting as goiter with tracheal compression, feeding difficulties, low weight, irritability, periorbital edema, retraction of the eyelid and even heart failure [51–53]. These symptoms are not specific, which leads to easy neglect of neonatal hyperthyroidism, resulting in a mortality rate of up to 20 % [51]. Patients with comorbid GD in pregnancy, whether or not GD is active, are likely to have transient hyperthyroidism in 10 % of their newborns, and it is therefore believed that all pregnant women at risk should be carefully monitored [54]. Studies suggest that maternal or cord blood should be monitored for TRAb levels. If maternal or cord blood is positive for TRAb, the newborn is considered to be at risk for secondary hyperthyroidism which should be monitored closely and thyroid function of the newborn should be tested regularly [51]. It has also been reported that fetal ultrasonography in pregnant women with high TRAb levels showed much higher fetal goiter than those with lower TRAb levels or TRAb negative, and neonatal TRAb levels were also higher in those with high TRAb levels, and the sensitivity of TRAb in diagnosing neonatal thyroid dysfunction was as high as 100 % and the specificity was as high as 92–94 % [55]. The symptoms of hyperthyroidism in older children are also not obvious, and most of them only show behavioral changes, emotional instability, and decreased academic performance. Therefore, childhood GD is very easily confused with attention deficit and hyperactivity disorder, which makes it extremely easy to miss the diagnosis of childhood GD. The diagnosis of hyperthyroidism is determined by measuring the patient's thyroid function. Patients usually present with decreased thyroid stimulating hormone and increased T3 and T4 levels. The main causes of hyperthyroidism are GD and HT. TRAb is a specific antibody for patients with GD, the determination of TRAb in the patient's serum is important for the

differential diagnosis of hyperthyroidism in childhood. Currently, pharmacological treatment with ATDs is preferred for pediatric patients with GD, but the treatment outcome in children is lower than that in adults, mainly because children tend to have higher TRAb levels than adult patients. In particular, among children with a first diagnosis of GD, those with high TRAb titer levels have lower remission of hyperthyroidism after treatment with ATDs [56]. In addition, RAI treatment is considered to be effective in the treatment of pediatric GD [32], but not all patients can be treated with RAI treatment for hyperthyroidism. First of all, RAI treatment is not recommended for children younger than 5 years of age. In addition, the remission rate after treatment with RIA in children with high TRAb titer levels is much lower than in those with lower TRAb levels, especially at TRAb > 40 U/I, the treatment failure rate of RIA can be as high as 42.2 % [51]. This suggests that we should pay close attention to the changes of TRAb levels in the diagnosis and treatment of pediatric GD and adjust the treatment plan appropriately according to the level of TRAb in the children. Most patients with GD will have a combination of GO, mostly manifesting as protrusion of the exophthalmos and eyelid retraction, while up to 40 % of pediatric GD will exhibit ophthalmologic changes, but their symptoms are milder than those of adults, which may lead to more negligible ophthalmopathy in children with GD. For this reason, it has been suggested that even in pediatric patients with GD with mild symptoms of GO, if high TRAb levels are detected, it is advisable to use a concomitant glucocorticoid combined with an ophthalmologist treatment regimen to control the progression of GO [51].

The value of TRAb in GO

GO is the most serious complication extrathyroidal disease of GD. GO mainly manifests as eyelid swelling, ocular discomfort, conjunctivitis, dry eye, increased tearing and exophthalmos, which can lead to orbital disfigurement, double vision and even visual loss in severe cases. TRAb is a key risk factor for GO pathogenesis [57]. Orbital fibroblasts express TSHR [58], TRAb induces adipogenesis in fibroblasts by binding to target targets, activating TSHR expressing T cells, upregulating cyclooxygenase to produce peroxisome proliferator activator γ ligands [58, 59]. In addition, the resulting macrophages, monocytes and mast cells induce proliferation of orbital tissues and excessive production of glycosaminoglycans such as cytochondroitin and hyaluronic acid (HAS) [60]. After activation of TSHR by TRAb, signaling via cAMP-PKA to the hyaluronate synthase promoter binds to the cAMP response element protein site to produce more hyaluronate and further activate orbital fibroblasts [61]. In addition, TSHR can be expressed in orbital adipose tissue [58], TSHR activated by TRAb can induce GO orbital lesions through PKA, IGF1R-PI3K-Akt and mTORC1 signaling pathways acting together on adipose tissue [58, 61–64]. Serum levels of TRAb are higher in patients with GO, and the activity and severity of GO increases with higher TRAb concentrations [65]. Even in GO patients without obvious hyperthyroidism symptoms, the positive detection rate for TRAb can be as high as 90 %. Currently, the treatment of GO is still dominated by glucocorticoid therapy. However, new therapies targeting different loci have emerged for GO in recent years, and one of the most notable is the TRAb-blocking monoclonal antibody k1-70. This antibody has been used in the treatment of GD patients with high TRAb titer levels and found that the blocking TRAb activity was elevated in patients. While some patients had relief from GO symptoms, some patients in the high-dose group progressed to hypothyroidism [66, 67]. The pharmacological effects of k1-70 in GO patients remain to be further validated given the limited data reported so far, but we can assume that k1-70 may be an effective targeted drug agent for the treatment of GD or GO. In addition, it is of particular concern that long-standing studies have reported a surge in TRAb levels in patients with GD treated with radioiodine, leading to an accelerated progression of GO. Therefore, the author suggests that TRAb levels should be monitored before radioactive iodine treatment and a comprehensive assessment of GD patients should be performed to prevent further progression of GO.

Conclusion

In summary, the biological behavior and pathology of TRAb can help us to better understand the pathogenic mechanism of GD, and also enable us to understand the principles of the competitive, biological and bridging methods that have emerged for the detection of TRAb on this basis. It is with the increasing awareness of TRAb that significant technological advances have been made in the detection of TRAb, making clinical diagnosis and treatment of GD more reliable and convenient than before. Those with high TRAb titers at initial diagnosis can reasonably choose the best treatment plan based on comprehensive assessment to avoid causing worsening of GO. GD patients who choose ATDs drug therapy should monitor TRAb titers regularly to determine the best time to stop the drug to avoid patient relapse. Patients with combined GD during pregnancy should also be monitored regularly for maternal TRAb levels, and medication should be adjusted in a timely manner to avoid hyperthyroidism or hypothyroidism in the newborn. In patients with childhood GD, TRAb levels should be more actively monitored to avoid lifelong effects of GO deterioration. In addition, TRAb assays may be effective in helping us to focus on the course of GO patients and adjust clinicians' treatment mindset in a timely manner; early intervention may be able to significantly improve the course of the disease and improve long-term outcomes. The current emergence of k1-70 targeting TRAb is expected to be a potential therapeutic agent for the treatment and improvement of GD and GO.

Conflict of Interest

The authors declare that they have no conflict of interest.

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