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Review Article

Autoantibodies as Biomarkers in Thyroid Diseases

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ABSTRACT

Thyroid diseases are common pathologies in primary care settings, and many patients with such diseases are treated by physicians who are not specialized in thyroid conditions. To diagnose such diseases earlier, some biological biomarkers have been identified. Anti-TSH receptor antibodies, which are considered the cause of Graves' disease, are autoantibodies that bind to TSH receptors and activate the thyroid gland. The measurement of anti-TSH receptor antibodies is crucial for diagnosing, managing, and monitoring the treatment of Graves' disease. This test has high sensitivity and specificity as a diagnostic tool. Conversely, antibodies against the TSH receptor do not have just one property; thus, the anti-TSH receptor antibody assay system could be improved to distinguish the different functions of antibodies. Patients diagnosed with thyroid disorders can generate TSH receptor autoantibodies that can either impede or encourage thyroid hormone production. Additionally, the levels of thyroid-stimulating antibodies present in the bloodstream have a positive correlation with the severity of Graves' orbitopathy. To clearly reflect these pathologies in the clinical laboratory results, more detailed tests should be promoted. The molecular characteristics of thyroid-related antibodies are being elucidated and are expected to have clinical applications not only in testing but also in treatment.

Keywords: Autoantibodies; Thyroidal diseases; Autoimmunity; Graves' disease

INTRODUCTION

Thyroid diseases are common pathologies in primary care settings therefore, the importance of thyroid function tests in primary care cannot be overemphasized [1,2]. Although many patients with thyroid diseases are treated by general physicians (who are not specialized in thyroid pathologies), some of the complications of thyroid diseases severely threaten the patient's quality of life. The early identification of complications results in a good prognosis for patient's quality of life. To diagnose the condition earlier, some biomarkers have been reported. Clinical laboratory medicine plays a significant role in the management of thyroid diseases. Blood test results, which can directly impact the diagnosis of the condition, can fluctuate greatly. Furthermore, treatment decisions can be made based on test results, and treatment methods can be adjusted based on test results throughout the treatment process. Ultrasonography is a highly specific test that not only contributes to the diagnosis of neoplastic diseases but also provides information about functional abnormalities such as Graves' disease (GD). Additionally, a conclusive diagnosis of neoplastic ailments can be established through the execution of fine needle aspiration guided by ultrasound.

LITERATURE REVIEW

Thyroid dysfunction in clinical laboratory medicine

Thyroid dysfunction can be objectively detected by measuring FT_3 and FT_4 levels [3]. Elevated titers of these markers indicate hyperthyroidism, while low titers indicate hypothyroidism. Thyroid Stimulating Hormone (TSH), a hormone secreted by the pituitary gland, binds to TSH receptors on the surface of thyroid cells, promoting the secretion of thyroid hormones. The

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secretion of TSH is influenced by negative feedback from FT_3 and FT_4 levels. During hyperthyroidism, TSH levels are low; conversely, TSH levels are high during hypothyroidism. However, during secondary hypothyroidism due to pituitary dysfunction, both TSH and thyroid hormone levels could be low.

Autoimmunity in the thyroid gland

GD and Hashimoto's disease are caused by autoimmune reactions triggered by thyroid autoantibodies. Autoantibodies are antibodies that are produced against one's own cells or tissues, and these autoantibodies include anti-TSH Receptor Antibodies (TRAb), anti-Thyroglobulin Antibodies (TgAb), and anti-Thyroid Peroxidase Antibodies (TPOAb). The titers of these three autoantibodies are useful in identifying the underlying cause of the disease. GD is a condition characterized by hyperthyroidism. The binding of TRAb (an autoantibody directed against the TSH receptors on thyroid follicular cells) to TSH receptors stimulates the thyroid gland and is considered the causative substance for GD. Graves' orbitopathy, a complication of GD, poses a significant threat to the patient's quality of life. In an animal model, it was reported that the TSH receptor acts as the antigen responsible for Graves' orbitopathy [4]. Thyroid-stimulating antibodies not only enhance thyroid hormone secretion but can also trigger symptoms linked to thyroid eye disease. Reducing the number of cells that produce pathogenic autoantibodies has been shown to be a possible treatment for autoimmune diseases. Studies have demonstrated that anti-human CD20 antibodies effectively decrease the severity of thyroid eye disease [5]. MHC class II molecule genetic types are the most strongly associated with GD susceptibility, and The MHC class II molecules are predominantly expressed on immunological cells. Despite the nonexistence of MHC class II molecules in typical thyroid tissues, these molecules are expressed in the thyroid tissues of individuals affected by GD. Upon the binding of TSH receptors to MHC class II molecules, their antigenicity changes; this change in antigenicity disrupts immune tolerance, leading to the production of autoantibodies, as seen in GD [6]. TPOAb is an autoantibody directed against thyroid peroxidase, an enzyme in the thyroid microsomal fraction [7]. This autoantibody is frequently detected in GD and Hashimoto's disease [8]. It is not clear how anti-TPO antibodies contribute to the pathogenesis of these diseases; however, they have been reported to regulate autoreactive T cells [9,10]. Interactions between B cells and T cells are vital in immune regulatory mechanisms [11]. A previous study conducted on a mouse model reported that B cells are required for the development of autoimmune thyroiditis and that both T and B cells infiltrate thyroid tissues [12]; however, we still await the more detailed elucidation of regulatory mechanisms. TgAb is an autoantibody that targets thyroglobulin, the primary constituent of the colloid within thyroid follicular cells. These antibodies are particularly highly positive in Hashimoto's disease. They can also be positive in GD [13]; however, their levels, in this case, tend to be lower than those in Hashimoto's disease. Previous studies have reported that TgAb alters Tg processing and participates in epitope spreading [14]. Thyroid dysfunction was also reported *via* programmed cell death-1 (PD-1 blockade therapy, an immune checkpoint treatment [15]. Autoimmune thyroid dysfunction could also be instigated by the generation of autoantibodies resulting from immune tolerance failure; however, the specific mechanism remains unclear.

Biomarkers in thyroid diseases

The measurement of TRAb is essential for the diagnosis, management, and treatment follow-up of GD. TRAb titers are often quantified by a competitive method against M22 antibodies The ROC analysis revealed 99.1% specificity and 97.0% sensitivity at a decision threshold of 1.86 IU/L when compared to untreated GD and destructive thyroiditis. A fully automated immunoassay for TRAb detection is in practical use, with a reported 97% sensitivity and 99% specificity when the cutoff value for TRAb is 1.75 IU/L [16-18]. On the other hand, to use as a biomarker for treatment efficacy, the test value must change per the activity of the disease. TRAb titers in GD decrease with treatment; however, they persist in some patients [19]. TRAb titers cannot completely predict the presence or absence of recurrence in postdrug therapy [20,21]. Further evidence of the relationship between antibody titer fluctuations and therapeutic efficacy needs to be accumulated. Antibodies directed against the TSH receptor exhibit a range of properties, with certain antibodies yielding affirmative signals and others hindering the binding of TSH to the TSH receptor [22-25]. The TRAb assay system would benefit from improvement, as the system gauges a composite of antibodies with these disparate functions in a consolidated manner. The Thyroid-Stimulating Antibodies (TSAb) assay system, like the TRAb system, measures anti-TSH receptor antibodies; however, the measurement method differs [26]. TRAb measurement systems measure all types of antibodies that stimulate, inhibit, or neutralize the TSH receptor, whereas TSAb is quantified using a bioassay that reflects and quantifies the pure stimulation activity. TSAb is commonly found in severe/active TAO, with serum TSAb levels have demonstrated a positive correlation with the clinical severity of the disease [27-29]. Our previous findings suggest that the proportion of TSAb to TRAb serves as a valuable biomarker of Graves' orbitopathy [30]. Patients with thyroid diseases can simultaneously produce blocking and stimulating anti-TSH receptor autoantibodies [31]. Furthermore, switching from antibodies that stimulate the thyroid gland, causing GD, to blocking antibodies that cause hypothyroidism has been reported. The opposite switch can occur, and these switches are caused by differences in the concentration, affinity, and titer of TSAb versus TSH receptor-blocking antibodies (TBAb) in individual patients [32]. It has been reported that some patients with TBAb-positive hypothyroidism developed TSAb-positive Graves' hyperthyroidism, and some patients with TSAb-positive Graves' hyperthyroidism developed TBAb-positive hypothyroidism, suggesting that TBAb-positive hypothyroidism and TSAb-positive hyperthyroidism could be two aspects of a single disease, "TRAb disease" [33]. Another TSH receptor antibody, neutral Abs, is an antibody that neither inhibits TSH binding nor induces the intracellular second messenger, cyclic Adenosine Monophosphate (cAMP). Neutral Abs were frequently

present in the sera of GD patients (found in 16 of 27 patients, 59%). Neutral antibodies mediate a different signaling pathway than TSH and induce apoptosis under certain conditions [34]. To use neutral antibodies as biomarkers, it is expected that information on the association between clinical findings and neutral antibody titers will be accumulated. A new biomarker in thyroid disease is anti-pendrin antibodies. The corresponding antigen, pendrin, is a protein antigen that is present in the inner membranes of thyroid follicles and is responsible for the release of iodine into them [35,36]. This antibody seems to be common in Hashimoto's disease, and further knowledge of the relationship between the titers of this antibody and the disease activity or course of treatment is expected to be accumulated.

DISCUSSION

Technical issues in thyroid testing

To compare test values across facilities, reference materials are required. TRAb titers are expressed in international units per liter against the World Health Organization (WHO) reference preparation; the M22 antibody is used as the WHO international reference material for thyroid-stimulating antibodies. The results of thyroid function tests can vary greatly depending on the measurement method, and caution is necessary when comparing results between different facilities. The standardization of testing is currently being promoted [37,38]. While a standard measurement method has been established for FT_4 , TSH is difficult to standardize because it is not a single molecule to be measured [39,40]. Harmonization, which is becoming increasingly popular, effectively makes it possible to compare TSH values across facilities [41]. Immunoassay techniques are advantageous as they can detect minute concentrations with high precision, without the requirement of utilizing radioisotopes. However, it is essential to take into account the potential impact of interfering substances. The excessive consumption of nonphysiological biotin has the potential to disrupt certain immunoassay evaluations, including those for thyroid hormones, TSH, thyroglobulin, and TSH receptor-binding inhibitory antibodies, resulting in inaccurate diagnoses. Patients who undergo thyroid tests should be asked about their biotin consumption [42].

Molecular characteristics and clinical applicability of thyroid-associated antibodies

J Sanders, et al. isolated M22, an antibody that possesses thyroid-stimulating activity, from lymphocytes of patients diagnosed with GD [43]. This antibody can compete with the majority of human TSAb or TBAb, rendering it clinically instrumental. M22 has already been implemented in a TRAb assay kit. Additionally, antibodies that inhibit the TSH receptor have been extracted from individuals with hypothyroidism and the potential clinical application of measurement systems utilizing these antibodies is also being explored [22,31]. Stimulatory activity of TSH receptor antibodies in patients with GD is reproduced by a combination of stimulatory and inhibitory monoclonal antibodies. The concentration of TRAbs as proteins and the antibody titer in the M22 antibody inhibition assay have a linear relationship, with small differences between the stimulatory and inhibitory types.

On the other hand, small amounts of TSAbs can stimulate the TSH receptor; however, much larger amounts of TBAbs are needed to inhibit this receptor [44]. These findings provide important clues in the pursuit of the antibody pathogenesis of autoimmune thyroid diseases. The development of antibody-based therapy using monoclonal antibodies is underway. The therapeutic potential of TBAb has been reported in vivo [45,46]. Furthermore, there have been case reports of TBAb being an effective treatment for Graves' orbitopathy [47]. These studies need to be conducted to increase the number of available treatment options for autoimmune diseases. The expansion of the corresponding antigens of autoantibodies is explained by epitope spreading. During TSH receptor immunization for mice, both inhibitory and neutral monoclonal antibodies were isolated [48]. It has been reported that antigenic "hot spots" exist on the hinge region of the TSH receptor protein [49]. The epitopes could potentially spread to TSAb and TBAb by means of antibodies targeting these hot spots as an initial point, ultimately contributing toward the clarification of the pathogenesis involved in autoimmune thyroid diseases. While the IgG isotype remains the predominant type of TSH receptor antibodies, studies have also documented the occurrence of IgA and IgE isotypes of TSH receptor antibodies and more knowledge is expected to be accumulated regarding the relationship between these isotypes and patients' symptoms [50].

CONCLUSION

Whereas TRAb is a useful biomarker in thyroid diseases, the separate interpretation of TSAb and TBAb may provide more detailed laboratory values reflecting disease variability. Thyroid function testing has a significant technical influence by clinical examination; thus, dialog between physicians and laboratory personnel is crucial in interpreting clinical examinations of patients with thyroid disorders [51]. The molecular characteristics of thyroid-related antibodies are being elucidated, and they are expected to have clinical applications not only in testing but also in treatment.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the research.

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