

Bioassays for TSH Receptor Antibodies: Quo Vadis?

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Autoantibodies (Ab) to the TSH receptor (TSHR) are responsible for many of the clinical manifestations of Graves' disease (GD) and are specific biomarkers of this autoimmune thyroid disorder (AITD) [1–3]. These Ab can be measured either via competitive-binding immunoassays or with bioassays [4]. Antibody-binding assays only report the presence or absence of TSHR-Ab and their concentrations, but do not indicate their functional activity. Bioassays, in contrast, indicate whether TSHR-Ab have stimulatory or blocking activity [5]. Historically, bioassays for TSHR-Ab were research tools used to study the pathophysiology of GD. Recently, however, there are increasing data that demonstrate the clinical utility of TSHR-Ab bioassays in the diagnosis and management of patients with GD and in the characterization of AITD patients with hyperthyroidism and hypothyroidism [6].

Advances in protein and cellular bioengineering have facilitated the development of improved bioassays for measuring the biological activity of molecules and this has been specifically and successfully applied to TSHR-Ab [7]. TSHR bioassays are functional cell-based tests that directly assess the bioactive immunoglobulins having either stimulating or inhibitory input on the TSHR cAMP-dependent signaling [8]. TSHR-stimulating Ab (TSAb) evoke metabolic changes and/or cytokine responses within TSHR-expressing target cells [9]. Bioassays for TSHR-Ab measure the ability of these Ab to either stimulate or block TSHR signal transduction [10]. These functional activities of TSHR-Ab highly correlate with activity of the thyroid in patients with GD [11]. In addition, they are associated with extrathyroidal manifes-

tations of GD [12]. TSHR bioassays show outstanding features. The biological activity of specific immunoglobulins is directly assessed on a fully functional TSHR holoreceptor expressed on intact live cells, a platform that is easily adaptable and tailored to detect Ab of specific function. The TSHR protein structure can be bioengineered and stably expressed in cell lines with protocols optimized for detection of TSAb or blocking Ab (TBAb). Another feature is the autoreactivity of an individual patient is revealed with added clinical value; the bioassay of TSHR-Ab measures the Ab function that is highly correlated with GD activity [13]. Furthermore, monitoring of TSAb levels and TSAb titers adds another dimension to the assessment of GD activity with potential to predict relapse or remission of individual patient [14]. High persistent TSAb levels are associated with active and severe systemic manifestations with poor responses to therapy [15]. In contrast, low TSAb levels are associated with patients in remission. Thus, bioassays may improve the personalized management of GD patients.

In this issue of *European Thyroid Journal*, a new bioassay is introduced which uses a frozen Chinese hamster ovary cell line expressing the TSHR, cAMP-gated calcium channel and aequorin [16]. The principle of the method is that the TSHR-induced increase in intracellular cAMP leads to the direct activation of the cyclic nucleotide-gated calcium channel, the resulting intracellular calcium influx then activating an intracellular photoprotein, aequorin, which emits a blue light at relaxation, the intensity of which is therefore correlated with the degree of TSHR activation. Activated Gs-coupled adenylate cyclase in-

creases intracellular cAMP, which then binds to the cyclic nucleotide-gated calcium channel. Activation of this channel allows Ca^{2+} to enter the cell, and influx of Ca^{2+} can be measured with aequorin, which is quantified by a luminometer. With the help of the aequorin bioassay, positive TSAb results were obtained in 98.9% of untreated patients with GD, and only 2.3% of the patients with painless thyroiditis had positive TSAb. All patients with subacute thyroiditis and controls showed negative TSAb. As for chronic thyroiditis, all euthyroid patients showed negative TSAb. Conventional porcine TSAb and Elecsys TSHR-binding Ab were positive in 69.3 and 95.5% of GD, respectively. The aequorin bioassay can be conducted in a few hours without a sterilized condition and may be useful in general clinical laboratories.

Thus, the commonly held view that TSHR bioassays are cumbersome and time-consuming procedures not suitable for routine use in GD diagnostics is no longer accurate. Indeed, recently developed bioassays show requisite clinical sensitivity and high specificity with robust performance [17, 18]. Also, procedural advantages and simplicity of newly introduced bioassays (no serum starvation, no serum concentration or IgG purification, minimal handling of the cells, etc.) have markedly improved the application of such diagnostic tools in the clinical laboratory routine. However, major challenges and issues must be resolved before a new generation of TSHR bioassays become an integral part of the multidisciplinary approach to the management and care of patients with AITD. Further optimization of the bioassays for the measurement of TSHR-Ab could be reached by: (1) standardization of the quantification of the obtained results in recognized international units [19, 20] instead of the current percentage of specimen to reference ratio; (2) semiautomatization through repeated washing steps of the 96-multiwell plates; (3) marked reduction of the incubation time of the cells after thawing without losing diagnostic accuracy, sensitivity and specificity of the assay; (4) further time reduction of the target cell stimulation by added patient sera, and (5) specialization and experience of the responsible laboratory technician allowing measurement in duplicate instead of in triplicate, thus leading to a relevant increase of number of sera tested in each plate and to a larger volume of daily Ab testing.

Introduction of the bioassays into routine GD diagnostics also requires clear demonstration of clinical added value and cost-effectiveness compared with existing TSHR-binding assays. In line with this, prospective studies of TSAb levels and TSAb titers at baseline and at regular time intervals during treatment are warranted to determine if the TSAb biomarker has utility to optimize pa-

tient responses to therapy and for prediction of relapse and remission. Further, the impact of functional bioassays on reducing the need for follow-up thyroid scans and expensive imaging techniques should be evaluated. More studies are also needed on the prevalence and clinical significance of TSAb and TBAb in patients with Hashimoto's thyroiditis, pediatric patients [21] and pregnant women to help physicians better interpret their role in various clinical presentations of AITD.

In addition to practical improvements in TSHR-Ab bioassays and more clinical studies on TSHR-Ab, there are major opportunities in this field for significant translational research as more information is obtained about the function of the TSHR and the alternative signal transduction pathways [22]. The clinical importance of these pathways and their possible activation by TSHR-Ab opens the prospect for novel bioassays and studies on the clinical importance of 'neutral' TSHR-Ab [23] as well as the possible identification of new pathophysiological mechanisms in AITD. Current measurement of the presence of TSAb and/or TBAb uses the cAMP and CREB/luciferase pathway [1, 2]. The recent description of other pathways used by 'neutral' TSHR-Ab is challenging and may explain why results of bioassay testing have not reached maximal sensitivity and specificity yet. Identification of different intracellular pathways involved in TSHR binding induced signal transduction will enhance our knowledge in this field and will probably lead to the measurement of other endpoints. Also, development of novel transfected cell lines expressing modified TSHR peptides exclusively binding, stimulating or blocking Ab may better differentiate between the functional characters of the variety of TSHR-Ab.

Therefore, introduction of the bioassay into routine AITD diagnostics requires coordination between multiple needs: clear demonstration of clinical added value and cost-effectiveness compared with existing TSHR-binding assays, pricing by manufacturers and reimbursement policies of national health insurance. Recent iterations of bioassays are offered by clinical reference laboratories. Presumably and hopefully, they will become more standardized and widely available to clinicians. Thus, continued improvements in these bioassays will help facilitate their routine performance by clinical laboratories.

Disclosure Statement

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