J Endocrinol Invest
DOI 10.1007/s40618-016-0478-9

ORIGINAL ARTICLE

Highly variable sensitivity of five binding and two bio-assays for TSH-receptor antibodies

T. Diana[1] · C. Wüster[2] · M. Kanitz[1] · G. J. Kahaly[1]

Received: 20 April 2016 / Accepted: 23 April 2016
© Italian Society of Endocrinology (SIE) 2016

Abstract
Purpose TSH-receptor (TSHR) antibodies (Ab) can be measured with binding or bio-assays. Sensitivity and specificity of five binding and two bio-assays were compared.
Methods TSHR-blocking (TBAb) and TSHR-stimulating (TSAb) Ab were measured with reporter bio-assays. Blocking activity was defined as percent inhibition of luciferase expression relative to induction with bTSH alone. TSAb was reported as percentage of specimen-to-reference ratio (SRR%). TSHR-binding inhibitory immunoglobulins (TBII) were measured with Kronus, Dynex, Kryptor, Cobas, and Immulite.
Results Sixty patients with Graves' disease (GD), 20 with Hashimoto's thyroiditis (HT), and 20 healthy controls (C) were included. C tested negative in all assays (specificity 100 %) while all 60 hyperthyroid GD patients tested positive in the TSAb bio-assay (sensitivity 100 %). Among these 60 GD patients, 20 had low TSAb positivity (SRR% 140–279), but were TBII positive in only 20 (100 %), 7 (35 %), 9 (45 %), 11 (55 %), and 18 (90 %) using the Kronus, Dynex, Kryptor, Cobas, and Immulite, respectively. In 20 moderate TSAb-positive (SRR% 280–420) patients, TBII tested positive in 20 (100 %), 14 (70 %), 13 (65 %), 16 (80 %), and 19 (95 %), respectively. The high (SRR% > 420) TSAb-positive patients were all TBII positive. All 20 hypothyroid HT patients tested TBAb positive (sensitivity 100 %) in the bio-assay while they tested TBII positive in 20 (100 %), 18 (90 %), 20, 20, and 18, respectively. Results obtained with two luminometers correlated for TSAb positive ($r = 0.99$, $p < 0.001$), TBAb positive ($r = 0.88$, $p < 0.001$), and C ($r = 0.86$, $p < 0.001$). None of the binding assays differentiated between TSAb and TBAb.
Conclusions Sensitivity is highly variable between binding and bio-assays for TSHR-Abs.

Keywords TSH-receptor binding inhibitory immunoglobulins · Thyroid-stimulating antibodies · Thyroid-blocking antibodies · Bio-assays · Binding assays · Sensitivity · Specificity

Abbreviations

Ab	Antibodies
AITD	Autoimmune thyroid disease
cAMP	Cyclic adenosine 3′,5′-monophosphate
FT3	Free triiodothyronine
FT4	Free thyroxine
GD	Graves' disease
LH-CG	Luteinizing and chorionic gonadotropin
TBAb	Thyroid-blocking antibodies
TBII	TSHR-binding inhibitory immunoglobulins
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TSAb	Thyroid-stimulating antibodies
TSHR	Thyroid-stimulating hormone receptor

Introduction

Autoimmune thyroid disease (AITD) represents a complex group of disorders with diverse clinical manifestations including hyperthyroidism, hypothyroidism, and goiter

✉ G. J. Kahaly
kahaly@ukmainz.de

[1] Molecular Thyroid Research Laboratory, Department of Medicine I, Johannes Gutenberg University (JGU) Medical Center, Mainz, Germany

[2] Endocrine Practice, Mainz, Germany

Published online: 19 May 2016

[1–7]. Autoantibodies (Abs) against the thyroid-stimulating hormone receptor (TSHR) induce the clinical phenotype and are so far the only known specific biomarkers of these AITDs [8–12]. TSHR-Abs act either as an agonist, stimulating unregulated thyroid growth and thyroid hormone production, or as antagonist, blocking the activity of the natural ligand thyrotropin. Graves' disease (GD) is caused by persistent, unregulated stimulation of thyroid cells by TSHR-stimulating Ab (TSAb) that activate the TSHR [13, 14]. TSAb, like TSH, bind primarily to the large amino (N)-terminal ectodomain of the TSHR. Functional TSHR-blocking autoantibodies (TBAb) occur in patients with Hashimoto's thyroiditis (HT) and induce primary autoimmune hypothyroidism.

Numerous assays have been developed to measure TSHR-Ab. In general, these assays fall into one of two types: (1) competitive binding assays that measure the inhibition of binding of ligand to the TSHR or (2) bio-assays that assess the functional activity of these Ab directly. These assays have been referred to as TSHR-binding inhibitory immunoglobulin (TBII) or TSHR-Ab assays. Unlike competitive binding assays, bio-assays have the advantage of indicating not only the presence of Abs but their functional activity and potency. The most commonly used assays clinically are competitive radiolabelled or electrochemiluminescent binding assays based on displacement of either labeled bovine (b) TSH, or more recently, a purely anti-TSHR-stimulating monoclonal autoantibody (MAb, M22) [15–18]. In contrast, bio-assays are cell-based assays that measure induction of cyclic adenosine 3′,5′-monophosphate (cAMP) signal transduction mediated by binding of ligand to the TSHR [19, 20].

The present study aims to investigate and compare the diagnostic accuracy of two bio-assays and five different binding assays for the measurement of TSHR-Ab.

Materials and methods

Study subjects

A total of 100 well-characterized and well-defined subjects were enrolled in this study, including 60 untreated hyperthyroid patients with Graves' disease with and without thyroid-associated orbitopathy, 20 patients with Hashimoto's thyroiditis, and 20 euthyroid healthy controls, respectively. Informed consent was obtained from all participants enrolled in the study. The study protocol was approved by the Ethics Committee of the Johannes Gutenberg University Medical Center and was carried out in accordance with the ethical guidelines of the Helsinki Declaration.

Thyroid-related hormones and antibodies

Serum levels of baseline TSH, free triiodothyronine (fT3), free thyroxine (fT4), anti-thyroglobulin (Tg), and anti-thyroid peroxidase antibodies (TPO) concentrations were measured using electrochemiluminescent immunoassays (CLIA, Abbott, Wiesbaden, Germany) according to the manufacturer's instructions.

TSHR-binding inhibitory immunoglobulin assays

Serum TSHR-binding inhibitory immunoglobulin (TBII) concentrations were measured with five different binding assays according to the manufacturer's instructions: Immulite 2000 XPi (Siemens, Erlangen, Germany, reference value <0.55 IU/L), DS2 (Dynex, Tecomedical, Bünde, Germany, <2 U/L), Kronus (Star Idaho, USA, <1 U/L), Kryptor, TRACE technology (Time-Resolved Amplified Cryptate Emission, Hennigsdorf, Germany, <1.8 IU/L), and Cobas e411, Elecsys (Roche Diagnostics, Mannheim, Germany, <1.75 IU/L), respectively.

Bio-assays for TSH-receptor antibodies

Both bio-assays utilize CHO cells expressing a chimeric TSHR (Mc4) and a cAMP response element (CRE)-dependent luciferase. In the Mc4 construct, the amino acid (AA) residues 262–368 of the human TSHR are replaced with the AA residues 262–334 from the rat luteinizing and chorionic gonadotropin (LH-CG) hormone receptor. In the plasmid, the *TSHR* cDNA is driven by the SV40 promoter/enhancer (SV40 pro) and a preceding beta globin intron. The firefly luciferase reporter gene is driven by a glycoprotein hormone alpha subunit promoter, which contains two CREB (cAMP response element-binding protein) binding sites [21].

Thyroid-stimulating antibody bio-assay

Serum TSAb activity was measured with a chimeric TSHR bio-assay according to manufacturer's instructions [20, 21]. Briefly, a frozen vial of CHO-Mc4 cells (6.7×10^4 cells/well) were seeded and grown to a confluent cell monolayer in 96-well plates for 15–18 h at 37 °C, 5 % CO_2. Serum samples, positive, reference, and normal controls were diluted 1:11 in reaction buffer and added to the cell monolayers, and each plate was incubated for 3 h at 37 °C, 5 % CO_2. Subsequently, the CHO-Mc4 cells were lysed and the relative light units (RLU) were quantified in two luminometers (Tecan Infinite M200; Tecan, Crailsheim, Germany or Veritas, Turner Biosystems, Sunnyvale, CA, USA). The cutoff for the TSAb assay is specimen-to-reference ratio, SRR < 140 %.

Thyroid-blocking antibody bio-assay

Serum TBAb activity was measured as previously described [22, 23]. Briefly, a frozen vial of Chinese Hamster Ovary (CHO)-Mc4 cells (6.7×10^4 cells/well) were seeded and grown to a confluent cell monolayer in 96-well plates for 15–18 h at 37 °C, 5 % CO_2. The cells were plated in the inner 48 wells of a 96-well plate with black-wall, clear-bottom wells. For the sample preparation, 180 µl reaction buffer, 220 µl bovine (b) TSH (Sigma-Aldrich, St. Louis, MO, USA), and 40 µl of the sample were mixed. The final dilution was 1:11, and 100 µl of each sample was added to each well. Serum samples and three controls, consisting of a reference standard bTSH, normal serum, and positive TBAb control, were tested in duplicate. After 3 h induction time, the luciferase expression levels of cell lysates were measured directly in the wells following addition of substrate and lysis reagent using two luminometers (Tecan Infinite M200, Tecan GmbH, Crailsheim, Germany or Veritas, Turner Bio Systems, Sunnyvale, CA, USA). Blocking activity was defined as percent inhibition of luciferase expression relative to induction with bTSH alone ($100 \times (1 - \text{sample} + \text{bTSH/bTSH alone})$. The concentration of bTSH (reference) in the well after dilution for the chimeric CHO cells is 100 mIU/L. Prior to calculating the % inhibition, the background relative light units (RLU) were subtracted from the sample RLU and the bTSH RLU. Background RLU were determined using normal serum diluted 1:11 in reaction buffer. The reference control comprises bTSH 1:11 diluted in normal serum and reaction buffer. The cutoff for the TBAb assay is <40 % inhibition.

Statistical analysis

Data are presented both as mean ± SD and median (range), respectively. In this study, all experimental data were statistically analyzed with SPSS version 23 (SPSS, Chicago, IL, USA) and GraphPad Prism software, Inc. (version 5.04), San Diego, CA, USA. Correlations were verified with the Pearson's test. All p values are two-sided, and a result was indicated significant when $p < 0.05$.

Results

Demographic data and prevalence of TSH-receptor antibody positivity

Demographic and clinical data of all five investigated study groups are given in Table 1. Thyroid-associated orbitopathy (TAO) was present in 1/20 (5 %), 9/20 (45 %), and 20/20 (100 %) low, moderate, and high TSAb-positive patients were with GD, respectively. TAO was absent in all patients with HT and in controls.

The prevalence of TSHR-Ab positivity found in the two bio-assays for thyroid-stimulating (TSAb) and thyroid-blocking (TBAb) antibodies with two different luminometers as well as for five binding assays is summarized in Table 2.

Diagnostic accuracy

Sensitivity and specificity

The healthy controls were negative in all assays (specificity 100 %), while all 60 hyperthyroid patients with GD tested positive in the TSAb bio-assay (sensitivity 100 %). Among these 60 patients with GD, 20 had low TSAb positivity (SRR% 140–279), but were TBII positive in 100, 35, 45, 55, and 90 % using the Kronus, Dynex, Kryptor, Cobas, and Immulite, respectively. In 20 moderate TSAb-positive (SRR% 280–420) patients with GD, TBII tested positive in 100, 70, 65, 80, and 95 %, respectively. All 20 hypothyroid patients with HT tested TBAb positive (sensitivity 100 %) in the TBAb bio-assay.

Diagnostic predictive values

In the TSAb and TBAb bio-assays, the positive predictive and the negative predictive values (PPV and NPV) are both 100 % when measuring low, moderate, and high TSAb-positive patients with GD. In all five binding assays the PPV values is 100 %. However, the NPV values are in low positive TSAb patients with GD for Kronus, Dynex, Kryptor, Cobas, and Immulite, 100, 60.6, 64.5, 68.9, and 90.9 %, respectively. In moderate TSAb-positive patients with GD, the NPV values are 100, 76.9, 74.1, 83.3, and 95.2 % respectively. In high TSAb-positive patients with GD, the NPV values are 100 % for all five binding assays.

Serum TSH-receptor antibody concentrations

TSAb and TBAb concentrations measured in both bio-assays for all investigated study groups are listed in Table 3 while TBII concentrations for five binding assays are represented for all groups in Table 4.

Correlations

Highly significant correlations of the results were obtained with the two luminometers for TSAb-positive patients with GD ($r = 0.99$, $p < 0.001$), for the TBAb-positive patients with HT ($r = 0.88$, $p < 0.001$), and for the healthy controls ($r = 0.86$, $p < 0.001$). None of the five binding assays differentiated between stimulating and blocking Ab.

Table 1 Demographic and serological data of all investigated study groups (euthyroid healthy controls; thyroid-blocking antibody (TBAb)-positive patients with Hashimoto's thyroiditis (HT); and low, moderate, and high thyroid-stimulating antibody (TSAb)-positive patients with Graves' disease (GD), respectively)

	Healthy controls	Patients with HT	Patients with GD		
		TBAb+	Low TSAb+	Moderate TSAb+	High TSAb+
Gender (F/M)	10/10	20/0	15/5	12/8	17/3
Age (years)					
Mean ± SD	30 ± 11	37 ± 7	41 ± 9	45 ± 10	54 ± 11
Median (min–max)	24 (22–57)	35 (31–59)	42 (23–57)	40 (33–68)	52 (31–73)
TSH (0.4–4.9 mU/L)					
Mean ± SD	1.9 ± 0.7	10 ± 0.9	0.1 ± 0.2	0.01 ± 0.1	0.01 ± 0.01
Median (min–max)	1.8 (0.8–3.5)	8.5 (5–21)	0.1 (0.01–0.2)	0.01 (0.01–0.1)	0.01 (0.01–0.01)
fT3 (2–4.4 pmol/L)					
Mean ± SD	3.5 ± 0.5	1 ± 0.4	4.9 ± 0.4	5.8 ± 0.8	6.8 ± 2.1
Median (min–max)	3.45 (2.6–3.4)	0.9 (0.5–2)	5 (4.5–9.1)	5.7 (4.8–12.2)	6.2 (5.4–17.2)
fT4 (0.9–1.7 ng/dl)					
Mean ± SD	1.4 ± 0.1	0.6 ± 0.2	2.4 ± 0.5	4.5 ± 0.3	6 ± 1.3
Median (min–max)	1.3 (1.2–1.5)	0.5 (0.1–0.7)	2.1 (1.7–3)	3 (1.9–13.5)	4.8 (2.4–13.6)
Tg-Ab (<4.1 IU/ml)					
Mean ± SD	1 ± 0.7	38.4 ± 20.2	251.7 ± 357.7	65.7 ± 145.6	69.6 ± 160.3
Median (min–max)	1.2 (0–3.5)	33.3 (20–67.1)	95 (1.3–1000)	13.2 (1–417.6)	8.7 (1.5–432.8)
TPO-Ab (<6 IU/ml)					
Mean ± SD	2 ± 0.9	395.5 ± 404.3	371.2 ± 416.3	404.1 ± 362.1	321.4 ± 358.7
Median (min–max)	1.8 (0–4.6)	215 (152–1000)	223 (3–1000)	292 (8–1000)	140 (28–1000)

The patients with GD were divided in low (SRR% 140–279), moderate (SRR% 280–420, two cutoffs), and high (SRR% > 420, three cutoffs) positive TSAb levels based on the measurements in the TSAb bio-assay. Thyroid-associated orbitopathy (TAO) was present in 1/20 (5 %), 9/20 (45 %), and 20/20 (100 %) low, moderate, and high TSAb-positive patients with GD, respectively. TAO was absent in all patients with HT and in controls

Table 2 Prevalence of TSH-receptor antibody (Ab) positivity in controls and patients with autoimmune thyroid disease measured with two bio-assays for thyroid-stimulating (TSAb) and thyroid-blocking (TBAb) antibodies and five binding assays

	Bio-assays				Binding assays				
	TSAb+	TSAb-	TBAb+	TBAb-	Elisa (Kronus)	Elisa DS2 Dynex (Tecomedical)	Kryptor (Thermo Fisher)	Cobas e411 (Roche)	Immulite 2000 Xpi (Siemens)
Healthy controls ($n = 20$)	0	0	0	0	0	0	0	0	0
Patients with GD low TSAb + ($n = 20$)	20	0	0	0	20	7	9	11	18
Patients with GD moderate TSAb + ($n = 20$)	20	0	0	0	20	14	13	16	19
Patients with GD high TSAb + ($n = 20$)	20	0	0	0	20	20	20	20	20
Patients with HT TBAb + ($n = 20$)	0	0	20	0	20	18	20	20	18

Table 3 Serum TSAb and TBAb levels measured in both bio-assays with two different luminometers (Infinite and Glomax) in all study groups

Study group	Infinite M200 (Tecan)	Glomax (Promega)
Controls		
TSAb assay		
Mean ± SD	35 ± 4	34 ± 4
Median (min–max)	36 (28–42)	33 (26–41)
TBAb assay		
Mean ± SD	16 ± 9	19 ± 12
Median (min–max)	17 (−1 to 31)	20 (0–32)
Patients with GD		
Low TSAb+		
Mean ± SD	223 ± 39	207 ± 41
Median (min–max)	235 (145–278)	217 (142–271)
Moderate TSAb+		
Mean ± SD	334 ± 38	312 ± 29
Median (min–max)	332 (283–416)	305 (281–386)
High TSAb+		
Mean ± SD	457 ± 29	464 ± 24
Median (min–max)	447 (421–523)	458 (429–498)
Patients with HT		
TBAb+		
Mean ± SD	93 ± 5	96 ± 5
Median (min–max)	94 (80–100)	96 (84–103)

The TSAb assay cutoff is at 140 SRR% and for the TBAb assay at 40 % inhibition, respectively

Discussion

This is the first comparative study prospectively evaluating the diagnostic accuracy, sensitivity, and specificity of seven widely distributed assays for the measurement of TSHR-Abs in well-characterized samples of well-defined patients with autoimmune thyroid disease as well as healthy controls devoid of thyroid, autoimmune, and tumor pathologies. This study demonstrates the very high sensitivity of the cell-based bio-assays for the diagnosis of autoimmune induced hyper- and hypothyroidism and clearly shows the detection of all samples at very low antibody activity with the reporter bio-assays. This paper also demonstrates the sole capability of the bio-assays to differentiate between stimulatory and blocking activity of the circulating TSHR-Abs in contrast to two Elisa and three recently introduced semi-automated binding assays.

In detail, the present study compares the variability of the diagnostic accuracy of two cell-based bio-assays and five binding assays for the measurement of TSHR-Ab. All applied binding assays detected the titers for TSHR-Ab in all 20 patients with GD with very high TSAb activity (>420 SRR%). However, the measurement of positive TSHR-Ab results in low positive patients with GD was markedly variable in the different binding assays used. In contrast, the Kronus Elisa exclusively was very sensitive in detecting low, moderate, and high levels of TSAb compared to the other binding assays. This assay uses the stimulating MAb

Table 4 Thyroid-binding inhibitory immunoglobulins (TBII) titers measured with the five binding assays in all study groups

	Elisa (Kronus, <1 U/L)	Elisa DS2 Dynex (Tecomedical, <2 U/L)	Kryptor (Thermo Fisher, <1.8 IU/L)	Cobas e411 (Roche, <1.75 IU/L)	Immulite 2000 Xpi (Siemens, <0.55 IU/L)
Healthy controls	0.5	0.6	<0.27	0.3	<0.10
Patients with GD					
Low TSAb+					
Mean ± SD	4 ± 3	2 ± 2	3 ± 3	3 ± 2	2 ± 1
Median (min–max)	2 (1–12)	2 (1–9)	2 (0.27–11)	5 (1–18)	1 (0.3–4)
Moderate TSAb+					
Mean ± SD	10 ± 9	9 ± 9	7 ± 6	7 ± 6	5 ± 5
Median (min–max)	4 (1–26)	4 (1–28)	4 (1–17)	2 (1–8)	3 (0.5–17)
High TSAb+					
Mean ± SD	20 ± 15	22 ± 19	27 ± 26	20 ± 15	12 ± 11
Median (min–max)	17 (3–54)	16 (2–61)	20 (1–20)	15 (2–40)	9 (0.8–40)
Patients with HT					
TBAb+					
Mean ± SD	47.9 ± 0.6	44.8 ± 19.6	20 ± 0	32.6 ± 13.4	31.5 ± 13.5
Median (min–max)	48.1 (46.3–48.5)	54.6 (0.2–61.1)	20 (20–20)	40 (0.3–40)	40 (0.1–40)

in form of M22 peroxidase as tracer. As previously shown [24], the analytical sensitivity is very high for cell-based bio-assays in comparison to the binding assays, because bio-assays are capable to detect very low amounts of M22 MAb, i.e., at 0.02 ng/ml. Further improvement in the TSAb bio-assay was shown by the feasibility of calibrating TSAb serum levels against the second international standard (08/204) from the National Institute for Biological Standards and Control, Hertfordshire, UK [20].

In comparison, the recently introduced automated quantitative Immulite assay for the measurement of TSHR-Ab uses a Bridge technology. Two TSHR chimeras were used: one capture receptor with an intact N-terminus and having AA residues 261–370 near the carboxy (C)-terminus of the extracellular domain (ECD) substituted with the corresponding residues from the LH-CG receptor, and second, the signal receptor consisting AA 21–261 of the ECD of the TSHR fused with secretory alkaline phosphatase [25]. However, nearly all samples of the hypothyroid patients with HT and measured high levels of TSHR-blocking Ab in the bio-assay were positive in the Immulite assay. Thus, this novel Immulite TSHR-Ab assay cannot differentiate between the Ab functionality and can be viewed as a classical binding assay, only.

Based on the data presented, the commonly held view that bio-assays for TSHR-Ab are cumbersome and time-consuming procedures not suitable for routine use in GD diagnostics is no longer accurate. Recently developed bio-assays show requisite clinical sensitivity and high specificity with robust performance [26]. However, major challenges and issues must be resolved before a new generation of TSHR-Ab bio-assays becomes an integral part of the multidisciplinary approach to the management and care of patients with AITD. Introduction of the bio-assays into routine GD diagnostics requires clear demonstration of clinical added value and cost-effectiveness compared with existing TBII assays. In line with this, prospective studies of TSAb levels and TSAb titers at baseline and at regular time intervals during treatment are in progress to determine whether the TSAb biomarker has utility to optimize patient responses to therapy and for prediction of relapse and remission. Further, the impact of functional bio-assays on reducing the need for follow-up thyroid scans and expensive imaging techniques is currently under evaluation. More studies are also needed on the prevalence and clinical significance of TSAb and TBAb in HT, in pediatric patients, and pregnant women, to help physicians better interpret their role in various clinical presentations of AITD. In line with this, recently published guidelines of the American Thyroid Association for both the management of hyperthyroidism and thyroid diseases during pregnancy recommend the measurement of TSHR-Ab in all patients with a positive history of AITD during the first weeks of pregnancy and, if positive, during the second and third trimester of gestation [27–29]. Since the functionality of maternal antibody may be relevant for the offspring, differentiation between stimulatory and blocking antibody activity is most recommended. Further, if a patient is hyperthyroid due to Graves' disease, is it truly important to establish whether the confirmatory TSHR-Ab test does not distinguish between stimulating and blocking antibodies? Finally, in clinical practice, measurement of TSHR stimulatory Ab in patients with HT is useful for those patients who, being euthyroid or hypothyroid (and TPOAb and/or TgAb positive), are also affected with TAO [11].

Acknowledgments We thank Elisa Kolbe (study nurse, JGU Thyroid Lab) for blood specimen collection. We are also most grateful to Susanne Lütkemeier and Lena Thummerer (both lab technicians in the endocrine practice of Prof. C. Wüster) for TSHR-Ab measurements.

Compliance with ethical standards

Conflict of interest TD, CW, MK have nothing to disclose; GJK consults for Quidel, CA, USA.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Bartalena L, Macchia PE, Marcocci C, Salvi M, Vermiglio F (2015) Effects of treatment modalities for Graves' hyperthyroidism on Graves' orbitopathy: a 2015 Italian Society of Endocrinology Consensus Statement. J Endocrinol Invest 38(4):481–487. doi:10.1007/s40618-015-0257-z
2. Bartalena L, Fatourechi V (2014) Extrathyroidal manifestations of Graves' disease: a 2014 update. J Endocrinol Invest 37(8):691–700. doi:10.1007/s40618-014-0097-2
3. Bartalena L, Chiovato L (2014) Graves'-like orbitopathy: do not forget IgG4-related disease. J Endocrinol Invest 37(12):1233–1235. doi:10.1007/s40618-014-0171-9
4. Bartalena L, Burch HB, Burman KD, Kahaly GJ (2016) A 2013 European survey of clinical practice patterns in the management of Graves' disease. Clin Endocrinol (Oxf) 84(1):115–120. doi:10.1111/cen.12688
5. Bartalena L (2013) Diagnosis and management of Graves disease: a global overview. Nat Rev Endocrinol 9(12):724–734. doi:10.1038/nrendo.2013.193
6. Caturegli P, De Remigis A, Rose NR (2014) Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev 13(4–5):391–397. doi:10.1016/j.autrev.2014.01.007
7. Effraimidis G, Wiersinga WM (2014) Mechanisms in endocrinology: autoimmune thyroid disease: old and new players. Eur J Endocrinol 170(6):R241–R252. doi:10.1530/EJE-14-0047
8. Ponto KA, Diana T, Binder H, Matheis N, Pitz S, Pfeiffer N, Kahaly GJ (2015) Thyroid-stimulating immunoglobulins

indicate the onset of dysthyroid optic neuropathy. J Endocrinol Invest 38(7):769–777. doi:10.1007/s40618-015-0254-2
9. Kampmann E, Diana T, Kanitz M, Hoppe D, Kahaly GJ (2015) Thyroid stimulating but not blocking autoantibodies are highly prevalent in severe and active thyroid-associated orbitopathy: a prospective study. Int J Endocrinol 2015:678194. doi:10.1155/2015/678194
10. Ponto KA, Schuppan D, Zwiener I, Binder H, Mirshahi A, Diana T, Pitz S, Pfeiffer N, Kahaly GJ (2014) Thyroid-associated orbitopathy is linked to gastrointestinal autoimmunity. Clin Exp Immunol 178(1):57–64. doi:10.1111/cei.12395
11. Kahaly GJ, Diana T, Glang J, Kanitz M, Pitz S, Konig J (2016) Thyroid stimulating antibodies are highly prevalent in Hashimoto's thyroiditis and associated orbitopathy. J Clin Endocrinol Metab. doi:10.1210/jc.2016-1220
12. Jacobson DL, Gange SJ, Rose NR, Graham NM (1997) Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin Immunol Immunopathol 84(3):223–243
13. Weetman AP (2000) Graves' disease. N Engl J Med 343(17):1236–1248. doi:10.1056/NEJM200010263431707
14. Diana T, Brown RS, Bossowski A, Segni M, Niedziela M, Konig J, Bossowska A, Ziora K, Hale A, Smith J, Pitz S, Kanitz M, Kahaly GJ (2014) Clinical relevance of thyroid-stimulating autoantibodies in pediatric graves' disease-a multicenter study. J Clin Endocrinol Metab 99(5):1648–1655. doi:10.1210/jc.2013-4026
15. Shewring G, Smith BR (1982) An improved radioreceptor assay for TSH receptor antibodies. Clin Endocrinol (Oxf) 17(4):409–417
16. Costagliola S, Morgenthaler NG, Hoermann R, Badenhoop K, Struck J, Freitag D, Poertl S, Weglohner W, Hollidt JM, Quadbeck B, Dumont JE, Schumm-Draeger PM, Bergmann A, Mann K, Vassart G, Usadel KH (1999) Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. J Clin Endocrinol Metab 84(1):90–97. doi:10.1210/jcem.84.1.5415
17. Gassner D, Stock W, Golla R, Roth HJ (2009) First automated assay for thyrotropin receptor autoantibodies. Clin Chem Lab Med 47(9):1091–1095. doi:10.1515/CCLM.2009.245
18. Hermsen D, Broecker-Preuss M, Casati M, Mas JC, Eckstein A, Gassner D, van Helden J, Inomata K, Jarausch J, Kratzsch J, Mann K, Miyazaki N, Navarro Moreno MA, Murakami T, Roth HJ, Noh JY, Scherbaum WA, Schott M (2009) Technical evaluation of the first fully automated assay for the detection of TSH receptor autoantibodies. Clin Chim Acta 401(1–2):84–89. doi:10.1016/j.cca.2008.11.025
19. Lytton SD, Kahaly GJ (2010) Bioassays for TSH-receptor autoantibodies: an update. Autoimmun Rev 10(2):116–122. doi:10.1016/j.autrev.2010.08.018
20. Diana T, Kanitz M, Lehmann M, Li Y, Olivo PD, Kahaly GJ (2015) Standardization of a bioassay for thyrotropin receptor stimulating autoantibodies. Thyroid 25(2):169–175. doi:10.1089/thy.2014.0346
21. Lytton SD, Li Y, Olivo PD, Kohn LD, Kahaly GJ (2010) Novel chimeric thyroid-stimulating hormone-receptor bioassay for thyroid-stimulating immunoglobulins. Clin Exp Immunol 162(3):438–446. doi:10.1111/j.1365-2249.2010.04266.x
22. Li Y, Kim J, Diana T, Klasen R, Olivo PD, Kahaly GJ (2013) A novel bioassay for anti-thyrotrophin receptor autoantibodies detects both thyroid-blocking and stimulating activity. Clin Exp Immunol 173(3):390–397. doi:10.1111/cei.12129
23. Diana T, Li Y, Olivo P, Lackner K, Kim H, Kanitz M, Kahaly GJ (2016) Analytical performance and validation of a bioassay for thyroid-blocking antibodies. Thyroid. doi:10.1089/thy.2015.0447
24. Leschik JJ, Diana T, Olivo PD, Konig J, Krahn U, Li Y, Kanitz M, Kahaly GJ (2013) Analytical performance and clinical utility of a bioassay for thyroid-stimulating immunoglobulins. Am J Clin Pathol 139(2):192–200. doi:10.1309/AJCPZUT7CNUEU7OP
25. Frank CU, Braeth S, Dietrich JW, Wanjura D, Loos U (2015) Bridge technology with TSH receptor chimera for sensitive direct detection of TSH receptor antibodies causing Graves' Disease: analytical and clinical evaluation. Horm Metab Res 47(12):880–888. doi:10.1055/s-0035-1554662
26. Lytton SD, Ponto KA, Kanitz M, Matheis N, Kohn LD, Kahaly GJ (2010) A novel thyroid stimulating immunoglobulin bioassay is a functional indicator of activity and severity of Graves' orbitopathy. J Clin Endocrinol Metab 95(5):2123–2131. doi:10.1210/jc.2009-2470
27. Bahn Chair RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, Laurberg P, McDougall IR, Montori VM, Rivkees SA, Ross DS, Sosa JA, Stan MN, American Thyroid A, American Association of Clinical E (2011) Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. Thyroid 21(6):593–646. doi:10.1089/thy.2010.0417
28. Kahaly GJ, Bartalena L, Hegedus L (2011) The American Thyroid Association/American Association of Clinical Endocrinologists guidelines for hyperthyroidism and other causes of thyrotoxicosis: a European perspective. Thyroid 21(6):585–591. doi:10.1089/thy.2011.2106.ed3
29. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S, Wiersinga W, American Thyroid Association Taskforce on Thyroid Disease During P, Postpartum (2011) Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid 21(10):1081–1125. doi:10.1089/thy.2011.0087