Clinical Relevance of Thyroid-Stimulating Autoantibodies in Pediatric Graves’ Disease—A Multicenter Study


Context and Objective: The incidence of TSH receptor (TSHR) stimulating autoantibodies (TSAb) in pediatric Graves’ disease (GD) is controversial. This large, multicenter study evaluated the clinical relevance of TSAb in children with GD both with Graves’ orbitopathy (GO) and without orbital disease.

Design: We conducted a cross-sectional retrospective study.

Setting: Sera were collected in seven American and European academic referral centers and evaluated in a central laboratory.

Patients and Samples: A total of 422 serum samples from 157 children with GD, 101 control individuals with other thyroid and nonthyroid autoimmune diseases, and 50 healthy children were studied.

Main Outcome Measures: TSAb were measured using a novel, chimeric TSHR bioassay and a cAMP response element-dependent luciferase. TSH binding-inhibitory Ig (TBII) and parameters of thyroid function were also determined.

Results: In 82 untreated children with GD, sensitivity, specificity, and positive and negative predictive values for TSAb and TBII were: 100 and 92.68% (P = .031), 100 and 100%, 100 and 100%, and 100 and 96.15%, respectively. TSAb and TBII were present in 147 (94%) and 138 (87.9%) of the 157 children with GD (P = .039), respectively; and in 247 (94%) and 233 (89%) of the 263 samples from this group (P < .0075), respectively. In children with GD and GO, TSAb and TBII were noted in 100 and 96% (P < .001), respectively. Hyperthyroid children with GD and GO showed markedly higher TSAb levels compared to those with thyroidal GD only (P < .0001). No significant differences were noted for TBII between the two groups. After a 3-year (median) medical treatment, the decrease of TSAb levels was 69% in GD vs 20% in GD and GO (P < .001). All 31 samples of euthyroid children with GO were TSAb positive; in contrast, only 24 were TBII positive (P = .016). All children with Hashimoto’s thyroiditis, nonautoimmune hyperthyroidism, type 1 diabetes, and juvenile arthritis and the healthy controls were TSAb and TBII negative.

Conclusions: Serum TSAb level is a sensitive, specific, and reproducible biomarker for pediatric GD and correlates well with disease severity and extrathyroidal manifestations. (J Clin Endocrinol Metab 99: 1648–1655, 2014)
 Graves’ disease (GD) is the most common cause of hyperthyroidism in the pediatric age range (1–5) but frequently needs to be distinguished from other causes of thyrotoxicosis, particularly the toxic phase of subacute or chronic lymphocytic thyroiditis, in which antithyroid drug therapy is not required. Extrathyroidal manifestations of GD, eg, Graves’ orbitopathy (GO), in children and adolescents are normally mild (6–9) and self-limited and characteristically occur during the acute hyperthyroid state (10). Like in adults, the hyperthyroidism of childhood GD is caused by Ig that bind to the TSH receptor (TSHR) and stimulate the cAMP signal transduction cascade (11). Measurement of TSHR antibodies is therefore often used as a convenient, cost-effective method to confirm the diagnosis and avoid the use of radioactive iodine uptake when the picture is not clear (12). In the last decade, increasingly sensitive, specific, and robust assays for TSHR antibodies measured by binding assay (TSH binding inhibitory Ig [TBII]) have been developed and are now routinely available (13, 14). When measured by modern methods, TBIIIs are detectable by binding assays in >90% of pediatric patients with GD (15); however, evidence that these antibodies are biologically active in stimulating cAMP production is not measured. In contrast, until recently, bioassays for TSHR antibodies (TSHR stimulating antibodies [TSAbs]) have been more time-consuming and labor-intensive and less reproducible (16), and few studies have focused on children (17, 18). Indeed, TSAbs were detectable in only 50% of children and adolescents with GD in several studies (5, 16, 17), leading some investigators to postulate that the frequency of TSAbs might be lower in children than in adults (19).

The purpose of our multicenter collaborative approach was to assess the sensitivity, specificity, reproducibility, and clinical relevance of TSAbs in a large international pediatric collective of children and adolescents with GD with and without orbital involvement (GO), as well as in children with nontoxic autoimmune diseases or nonautoimmune thyroid diseases using a novel, commercially available bioassay; results were compared with a widely used automated TBII assay.

Subjects and Methods

Subjects

The study was approved by the Ethical Committee of the Johannes Gutenberg University (JGU) Medical Center (Mainz, Germany) and the Committee on Clinical Investigation, Boston Children’s Hospital, Harvard Medical School (Boston, MA). A total of 422 serum samples were obtained from 308 pediatric patients and healthy control children recruited from seven American and European academic referral centers. Sera were available from all hyperthyroid patients with suspected GD seen in the European centers during the time period indicated below; in Boston, blood was drawn consecutively in all children seen with suspected hyperthyroidism, but sera were not available in a few patients. Serum samples from 31, 72, 26, 9, 11, and 8 pediatric patients with GD were obtained from Mainz (time period, 2003–2013), Boston (2003–2006), Bialystok (two centers, 2006–2012), Rome (2002–2012), Poznan (2008–2012), and Katowice (2010–2011). In several pediatric patients with GD, numerous samples were available, and all were tested. In the European centers, the diagnosis of GD was suspected on the basis of a typical clinical presentation, the presence of a diffusely enlarged thyroid gland, and evidence of extrathyroidal involvement, if present, and was confirmed by the typical ultrasound image including markedly enhanced perfusion of the thyroid gland on Doppler examination. In Boston, the diagnosis of GD was based on the above-mentioned characteristic clinical picture and the presence of TSHR antibodies (binding assay); radionuclide thyroid uptake and scan or a thyroid ultrasound was performed when the diagnosis was unclear. All pediatric patients with GD were screened for signs and symptoms of orbital involvement. GO was diagnosed according to the recommendations of the European Group on Graves’ Orbitopathy (EUGOGO) (20–22). According to EUGOGO, severity classification was done as follows: patients with mild GO have only minor eyelid signs, soft tissue involvement, and proptosis, whereas these changes are more severe in moderate-to-severe GO. Hyperthyroid subjects without GO and/or children with GD and with mild GO were seen by the endocrinologists. Children with moderate-to-severe GO had an ophthalmological examination. At the JGU Medical Center, children with GD are managed within a joint thyroid-eye clinic.

In all pediatric patients, a complete endocrine, serological, and immunological investigation was performed by the individual hospital laboratory to confirm the diagnosis of GD with or without ophthalmopathy. Control groups included Hashimoto’s thyroiditis (HT), nonautoimmune hyperthyroidism (NAH; eg, toxic nodules, subacute thyroiditis), type 1 diabetes (T1D), and juvenile idiopathic arthritis (JA). Fifty consecutive healthy children with negative personal and family history of autoimmune endocrine and nonendocrine diseases and with normal serum values for thyroid-related hormones and autoantibodies were selected as controls. HT was defined as the presence of at least a 5-fold increased serum level of thyroid peroxidase (TPO) autoantibodies, a typical hypoechogenic ultrasound pattern, and euthyroid hyperthyroidism. Subacute thyroiditis was defined as clinically painful enlargement of the thyroid gland accompanied by suppressed serum baseline TSH, increased inflammatory signs, eg, C-reactive protein and sedimentation rate, and/or a nodular structure on thyroid ultrasound. Toxic nodules were detected via ultrasound.
thryroid scintigraphy. T1D was defined as complete insulin deficiency with the presence of β-cell autoantibodies against the islet cell antigens, tyrosine phosphatase IA-2, insulin, and/or glutamic acid decarboxylase-65. Children with JA showed a typical clinical phenotype with classical serological pattern.

**TSAb bioassay**

Serum TSAb levels were measured at the JGU Thyroid Laboratory with a TSAb reporter cell-based bioassay (Thyretatin; Quidel). The manufacturer’s instructions were followed as previously described (23). Briefly, a frozen vial of CHO-MC4 cells supplied by the manufacturer were seeded and grown to a confluent cell monolayer in 96-well plates for 15 to 18 hours. Pediatric 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 hours at 37°C in 5% CO2. Subsequently, the CHO-MC4 cells were lysed, and the relative light units were quantified in a luminometer (Tecan Infinite M200; Tecan). All samples were measured in triplicate and reported as the percentage of specimen-to-reference ratio (SRR%). A TSAb value ≥140% is considered to be positive.

**TBII assay, TPO and thyroglobulin (Tg) assays, and thyroid-related hormones**

For the purposes of the study, TBII, serum anti-TPO, and anti-Tg antibodies, as well as serum TSH, free T3, and free T4 were remeasured in all pediatric subjects in a central laboratory (JGU Medical Center) using commercially available kits (electrochemiluminescence, ECLIA immunoassay, Elecsys, Cobas e411; Roche Diagnostics) according to the manufacturer’s instructions. In five patients for whom insufficient sera were available, the laboratory data obtained by the hospital laboratory at which the patients were seen were used.

**Statistical analysis**

Statistical data analysis was carried out using the statistics software program SPSS version 20.0 (SPSS Inc) and SAS version 9.3 (SAS Institute). All P values are two-sided and are, descriptively, referred to as statistically significant if they do not exceed .05. Proportions were compared by χ2 tests for 2-by-2 tables or Fisher’s exact test if the expected cell frequencies did not exceed 5. Positivity of TSAb and TBII was compared, in various subgroups, by the exact McNemar test when considering only the baseline sample of each child. Comparison on a blood sample level was performed by comparing the number of positive counts per subject by means of the exact sign test. Diagnostic indices (specificity, sensitivity, negative and positive predictive values) were defined by formally setting up the diagnostic task to detect GD in the first available and baseline blood samples of 82 untreated children. The exact McNemar test was used to compare TSAb with TBII with respect to specificity and sensitivity. For both diagnostic tasks, receiver operating characteristic (ROC) curves were set up, and TSAb and TBII were compared with respect to the area under the curve (AUC) by means of standard asymptotic tests. Statistical comparison between two groups of children with respect to serum levels of TSAb and TBII were done by the exact Mann-Whitney U test or by the Kruskal-Wallis test for comparing more than two groups (Figure 1, which was done with GraphPad Prism version 5). Association between age and serum levels was assessed by the Spearman’s rank correlation coefficient and the corresponding test for zero correlation.

**Results**

**Diagnostic accuracy**

Demographic data of all six study groups are shown in Table 1. In the 82 untreated children with GD and both GD and GO, sensitivity, specificity, and positive and negative predictive values for TSAb and TBII were 100 and 92.68% (P = .031), 100 and 100%, 100 and 100%, and 100 and 96.15%, respectively. When comparing the American (n = 31) and European (n = 51) samples of untreated children, sensitivity (100%), specificity (100%), and positive (100%) and negative (100%) predictive values for TSAb were identical in both collectives. Of all 157 children with

**Table 1. Demographic Data of All Study Subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of Subjects</th>
<th>No. of Samples</th>
<th>Gender (F/M)</th>
<th>Age, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>157</td>
<td>263</td>
<td>125/32</td>
<td>13.6 ± 4.4</td>
</tr>
<tr>
<td>HT</td>
<td>37</td>
<td>43</td>
<td>29/8</td>
<td>12.2 ± 3.5</td>
</tr>
<tr>
<td>Nonautoimmune</td>
<td>9</td>
<td>11</td>
<td>7/2</td>
<td>10.6 ± 7.7</td>
</tr>
<tr>
<td>hyperthyroidism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1D</td>
<td>50</td>
<td>50</td>
<td>23/27</td>
<td>13.6 ± 3.7</td>
</tr>
<tr>
<td>JA</td>
<td>5</td>
<td>5</td>
<td>2/3</td>
<td>14.3 ± 3.4</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>50</td>
<td>50</td>
<td>27/23</td>
<td>12.4 ± 4.3</td>
</tr>
<tr>
<td>Total</td>
<td>308</td>
<td>422</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male. Age is expressed as mean ± SD.
GD and both GD and GO, TSAb and TBII were present in 147 (94%) and 138 (87.89%) patients (P < .039), as well as in 247 (94%) and 233 (89%) of 263 samples (P < .0075), respectively. Compared to children with GD, all control subjects and children with HT, NAH, T1D, and JA were TSAb and TBII negative (Figure 1). In all 157 children with GD, TSAb and TBII serum levels positively correlated (R = 0.65; P < .001), whereas a correlation of R = 0.55 (P < .001) was noted in the 75 patients with GD and GO. The TSAb values of 20 pediatric patients with GD measured with the same TSAb bioassay in two centers (Boston and Mainz) showed a strong correlation (R = 0.88; P < .001).

Figure 2A illustrates the ROC curve and the AUC when evaluating the 82 untreated children with GD and with GD and GO vs the 151 patients with other thyroidal and nonthyroidal autoimmune diseases, as well as euthyroid healthy controls, whereas Figure 2B illustrates the ROC curve for all 308 enrolled children. When comparing the 76 untreated hyperthyroid children with all other non-GD groups, the AUC was 100 for TSAb and 0.999 for TBII (P = .21). Furthermore, the AUC for TSAb and TBII was 0.678 and 0.651 (P = .48), respectively, when comparing the 75 children with GD and GO to the 82 children with GD only.

**TSHR antibodies in GD and GO**

Forty-seven (30%) of the 157 children with GD were younger than the average pubertal age of 12 years (Table 2). Nearly all children with GD and GO younger than 12 years were female, in contrast to the older group. However, the prevalence of GO in both groups was not significantly different. Mild GO was noted in the vast majority of prepubertal children younger than 12 years. In 75 children with GD and GO, TSAb and TBII were present in 75 (100%) and 69 (92%) patients (P < .031), respectively, as well as in 163 (100%) and 152 (93%) of 163 samples (P < .004), respectively. All 31 samples of untreated euthyroid children with GO were TSAb positive; in contrast, only 24 were TBII positive (P = .016).

When separately analyzing GD children aged 6 or less (P = .004), age 8 or less (P = .004), or age 12 or less (P < .0002), serum TSAb levels were always markedly higher in the GD and GO collective in contrast to those with thyroidal disease only. Hyperthyroid children with GD and GO (n = 54) showed significantly higher TSAb levels compared to those with thyroidal GO only (n = 62) (median SRR%, 481 vs 395; range, 150–651 vs 160–671; P < .001). In contrast, serum levels of TBII were not significantly different between both groups (P = .125). The same holds true for treated euthyroid children with GD and GO (n = 13; SRR%, 384; range, 142–585) vs those with only GD (n = 12; SRR%, 123; range, 41–452; P = .02).

The documentation of symptoms and signs recommended by EUGOGO to classify the patients as active and/or moderate-to-severe GO was fulfilled in 25 children. Within this subgroup analysis, 10 patients classified as inactive and mild were all adolescents, whereas of 15 documented as active and/or moderate-to-severe, only two were younger than 12 years old. Sight-threatening GO was not observed. Median (range) values for visual acuity were 0.95 (0.9–1.0) and 0.8 (0.6–1.0) in mild and moderate-to-severe GO, respectively. For proptosis median was 18 mm and range was 14–22 mm in mild GO, and the median was 22 mm (range 20–27 mm) in moderate to severe GO. Lid aperture median was 10 mm (range 9–12 mm) in mild GO and 13 mm (10–17 mm) in moderate-to-severe GO. Median clinical activity score was 1 point (0–2) vs 4 points (3–6) in mild vs moderate-to-severe GO.
Lagophthalmos, chemosis, and disturbances of eye muscle motility were present in five, five, and six of 15 patients with active and/or moderate-to-severe GO, respectively, but were not present in those with mild GO. Serum TSAb levels were SRR% 536.0 (402–735) and 259 (142–384) (P < 0.001) in moderate-to-severe and mild GO, respectively, whereas for TBII, the values were 17.2 IU/L (2.3–40.0) and 2.58 IU/L (0.5–13) (P < 0.001).

Compared to TBII-positive patients, children with negative serum TBII levels had lower serum free T3 (4.14 vs 7.17 pg/mL) and free T4 (1.45 vs 3 ng/dL) as well as a lower rate of GO (31.5 vs 50%). All six TBII-negative children had mild GO, whereas 15 of 69 (22%) TBII-positive children had moderate-to-severe GO. One hundred of the 138 (72.5%) TBII-positive children were older than 12 years, and 111 (80.5%) were female.

### TSHR antibodies and medical treatment

Serum levels of TSAb and TBII during medical therapy are shown in Table 3. Approximately half of the patients (n = 82) were untreated and hyperthyroid, whereas another 35 (25.5%) pediatric subjects with GD were still hyperthyroid during or after treatment, making a total of 116 (74%) hyperthyroid children evaluated in Table 2.

### Table 2

Demographic and Serological Data of 75 Children With Graves’ Thyroidal and Orbital Disease (GD and GO)

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt;12 y</th>
<th>≥12 y</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of children with GD</td>
<td>47</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>GD and GO, n (%)</td>
<td>20 (43)</td>
<td>55 (50)</td>
<td></td>
</tr>
<tr>
<td>Median age (range), y</td>
<td>9.26 (3.7–12)</td>
<td>15.8 (12.4–20.9)</td>
<td></td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>19 (95)</td>
<td>41 (74.5)</td>
<td>0.057</td>
</tr>
<tr>
<td>Male</td>
<td>1 (5)</td>
<td>14 (25.5)</td>
<td></td>
</tr>
<tr>
<td>Thyroid function, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>15</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Subclinical hyperthyroid</td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Euthyroid</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Subclinical hypothyroid</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Prevalence, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSAb positive</td>
<td>20 (100)</td>
<td>55 (100)</td>
<td>1</td>
</tr>
<tr>
<td>TBII positive</td>
<td>18 (90)</td>
<td>51 (93)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Thyroid hormones and antibodies (median values and range)

<table>
<thead>
<tr>
<th></th>
<th>TSH (0.27–4.2 mIU/L)</th>
<th>Free T3 (2.02–4.43 pg/mL)</th>
<th>Free T4 (0.93–1.71 ng/dL)</th>
<th>TSAb (140 SRR%)</th>
<th>TBII (1.75 IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated children (n = 82)</td>
<td>0.01 (0.005–8.24)</td>
<td>9.9 (3.4–32.6)</td>
<td>3.5 (0.8–7.77)</td>
<td>452 (205–371)</td>
<td>19.85 (1.18–40)</td>
</tr>
<tr>
<td>Hyperthyroid (n = 76)</td>
<td>0.008 (0.005–0.06)</td>
<td>3.8 (0.8–7.77)</td>
<td>1.27 (0.92–1.64)</td>
<td>245 (8–600)</td>
<td>60 (10–1000)</td>
</tr>
<tr>
<td>Euthyroid (n = 6, all with GO)</td>
<td>2.07 (1.18–3.5)</td>
<td>1.27 (0.92–1.64)</td>
<td>3.6 (2.97–4.51)</td>
<td>512 (340–680)</td>
<td>13.6 (0.6–40)</td>
</tr>
<tr>
<td>Treated children (n = 75)</td>
<td>0.02 (0.005–0.21)</td>
<td>3.2 (1.8–7.77)</td>
<td>1.4 (1–1.64)</td>
<td>491 (165–602)</td>
<td>13.6 (0.6–40)</td>
</tr>
<tr>
<td>Hyperthyroid (n = 35)</td>
<td>0.17 (0.03–0.22)</td>
<td>3.2 (1.8–7.77)</td>
<td>1.4 (1–1.64)</td>
<td>512 (165–602)</td>
<td>13.6 (0.6–40)</td>
</tr>
<tr>
<td>Subclinical hyperthyroid (n = 5)</td>
<td>0.008 (0.005–0.06)</td>
<td>1.27 (0.92–1.64)</td>
<td>2.3 (1.8–3.5)</td>
<td>13.6 (0.6–40)</td>
<td>13.6 (0.6–40)</td>
</tr>
<tr>
<td>Euthyroid (n = 19)</td>
<td>2.4 (0.5–4)</td>
<td>1.2 (1.19–1.44)</td>
<td>2.3 (2.3–4.6)</td>
<td>13.6 (0.6–40)</td>
<td>13.6 (0.6–40)</td>
</tr>
<tr>
<td>Subclinical hypothyroid (n = 4)</td>
<td>5.15 (4.8–6.4)</td>
<td>1.2 (1.19–1.44)</td>
<td>2.3 (2.3–4.6)</td>
<td>13.6 (0.6–40)</td>
<td>13.6 (0.6–40)</td>
</tr>
<tr>
<td>Hypothyroid (n = 12)</td>
<td>8.05 (4.3–73.35)</td>
<td>1 (0.2–1.43)</td>
<td>3.15 (0.7–4.4)</td>
<td>13.6 (0.6–40)</td>
<td>13.6 (0.6–40)</td>
</tr>
</tbody>
</table>

Data are expressed as median (range). The antithyroid drug MMI was administered on average for 3 years (range, 2 to 7 y). The starting initial dose was 0.5 mg MMI/kg/d (0.3–0.6 mg), and the median maintenance dose was 0.2 mg MMI/kg/d (0.1–0.3 mg).
this study. Taken together, TSAb and TBII were present in 116 (100%) and 111 (96%) hyperthyroid (untreated and treated) children (P < .063), respectively. After a 3-year median duration of antithyroid drug treatment (methimazole [MMI]; range, 2–7 y), a starting initial dose of 0.5 mg MMI/kg/d (range, 0.3–0.6 mg) and a median maintenance dose of 0.2 mg MMI/kg/d (range, 0.1–0.3 mg), the decrease of TSAb levels was 69% in GD vs 20% in GD and GO (P < .001). In contrast, no differences were noted for TBII (90 and 89% in GD and GO vs GD only; P = not significant).

**Discussion**

This international, multicenter, cross-sectional study is the largest evaluation of TSHR antibodies in children and adolescents with GD done to date and demonstrates the excellent sensitivity, specificity, and reproducibility of this novel TSHR antibody bioassay. In addition to being a useful biomarker for disease activity, TSAb also correlated with the presence of GO independent of thyroid function, suggesting a possible causal role of TSAb in the immunopathogenesis of GO and in the development of the clinical phenotype of thyroid eye disease with proptosis. Similar findings have been reported recently in adults with GD and GO (24, 25) and in a case report of an 11-year-old girl in whom GO and positive TSAbs preceded by 3 months the development of hyperthyroidism (26). In addition, the present TSAb bioassay proved to be more sensitive in the diagnosis of untreated pediatric GD than a commonly used automated third-generation binding assay.

The clinical relevance of TSHR autoantibodies in pediatric GD (27) and their predictive value at the time of diagnosis (28, 29) have been demonstrated. However, the incidence of TSHR autoantibodies when measured by cell-based bioassays is still a matter of debate. Because TBII binding methods do not provide any information about the biological activity of these antibodies and therefore do not distinguish stimulating from blocking or neutral antibodies, many investigators continue to prefer measurement of TSHR antibodies by bioassay. Unfortunately, in the past, bioassays for TSAb were not standardized, were less reproducible, and involved complex procedures such as pre-extraction of IgG from serum (30). As noted above, even in several recent studies in children with GD (5, 16, 19), a prevalence of only 50% was reported when TSAbs were measured by commercial laboratories, leading to the conclusion by some investigators (19) that TSAbs are frequently negative in children without consideration of assay performance. In contrast, the present study was performed using a Food and Drug Administration-cleared TSAb bioassay, the analytical and clinical performance of which has been well-documented (23, 31). Our finding that the present TSAb assay was more sensitive than a commonly used automated third-generation TBII method is consistent with recent results in a large collective of untreated hyperthyroid adult patients (32–34). We have recently extended the bioassay in our laboratory to measure blocking as well as stimulating TSHR antibodies (35).

Given the above information, the clinician is left to wonder which assay for TSHR antibodies should be used in his or her patients. The advantages of the third-generation TBII assay are that it is now automated, relatively sensitive, and specific with a broad concentration range. It can therefore be regarded as a useful tool for routine TSHR antibody measurement in GD. The bioassay, on the other hand, requires training of laboratory technicians and the availability of cell culture facilities, but there has been a significant simplification and improvement in assay sensitivity and reproducibility such that current TSHR bioassays can be performed in < 24 hours with excellent reproducibility, as we and others have demonstrated (23, 24, 32–34, 36–38). The unique advantage of bioassays over binding assays is their ability to measure the functional activity of the Ig and therefore to distinguish predominantly stimulating or blocking (or neutral) TSHR antibodies (35). Because of its increased sensitivity, the bioassay might be recommended in cases where a low autoantibody level is expected (31), eg, mild and/or subclinical GD or in TBII-negative patients in whom GD is suspected clinically. The bioassay might also be preferable to the binding assay in mothers in whom GD is suspected or in babies at risk for neonatal GD (39). Finally, and as previously described in adults, the close correlation of the TSAb levels with the clinical activity and severity of GO (24, 25) suggests that the bioassay might better identify patients at risk for GO.

Our study had several limitations. A prospective investigation with defined inclusion criteria and standardization of procedures in all involved centers would have been optimal rather than the retrospective approach used here. Not all sera from Boston were available for evaluation, leading to the possibility that there might have been some inherent bias in the results obtained. Although this cannot be ruled out with certainty, this is unlikely to have altered our conclusions significantly because results from the Boston cohort analyzed separately were similar to those obtained with the other collectives. Similarly, reliance in part on the presence of TSHR antibodies for the diagnosis of GD in the Boston cohort might have falsely elevated the estimate of the relative prevalence of TBII in pediatric GD. If anything, this would have been expected to have falsely decreased the difference in sensitivity between assay meth-
ods. Yet despite this potential source of bias, four samples from untreated patients in the Boston cohort who were negative in the TBII assay had unequivocally elevated TSAb, strongly supporting our conclusion that the present TSAb assay is a more sensitive and specific method. It is also possible that the assessment and classification of GO differed between cohorts. Although the identification of patients with mild GO might have differed between groups, patients with moderate findings were routinely referred to ophthalmologists at all the study sites, and the relation between TSAb and GO was sustained irrespective of the origin of the patient cohort. Finally, whereas, the low prevalence of hyperthyroidism due to GD in children necessitated a long-term period of enrollment, the large number of patients we have been able to study provides unique value in assessing the true prevalence of TSAb in this patient population.

In conclusion, serum TSAb levels are sensitive and specific biomarkers for the diagnosis of GD in childhood as well as in adults and reflect disease severity and activity. There is also an association between TSAb levels and the presence of ophthalmopathy. Whether TSAb levels can be used to predict GO in the future requires further evaluation. We are currently conducting a prospective study at our institution that will hopefully answer this question.

Acknowledgments

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