Thyroid stimulating antibody: an index of thyroid stimulation in Graves' disease?

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Abstract. Early (20 min) thyroid radio-iodine uptake (ERU) and thyroid-stimulating antibodies (TSab) were determined in 27 untreated unselected patients with Graves' disease at the time of diagnosis. In 21 subjects the same tests were further performed in parallel during combined carbimazole-L-T₃ therapy (mean duration of follow-up: 10.8 ± 5.8 months; mean ± sd). TSab was determined by a cAMP-human thyrocyte culture stimulation assay and expressed in μl-equivalent of a TSab standard/ml (μl-equiv/ml). Before treatment, ERU, ranging from 15 to 54% of the injected dose (normal ≤ 8% dose) correlated with serum T₃ (r: 0.54; P < 0.01); TSab, ranging from 6 to 85 μl-equiv/ml was detected in 21/27 patients. There was a significant correlation between ERU and TSab (Spearman rank test: r: 0.57; P < 0.01). During the first months of treatment, 5 of the 21 patients sequentially studied had undetectable TSab levels throughout the study and in these patients ERU decreased by 57% of its initial value; the remaining 16 subjects were divided into two groups according to ERU changes: in group A (9 patients), initial ERU decreased by 50% or more or the absolute value became less than 20% of the dose and TSab decreased from 10.9 ± 4.8 μl-equiv/ml to 5.3 ± 1.6 μl-equiv/ml (P < 0.01); in group B (7 patients), the fall of ERU was less than 50% or the absolute value remained greater than 20% of the dose and TSab values remained unchanged. Furthermore, the values of ERU and TSab serially obtained during treatment varied in parallel in the 9 patients of group A, whereas in group B, TSab and ERU evolutions were discordant. These two patterns of TSab and ERU changes could not be related to any clinical or biological findings. We conclude that 1) ERU is more closely related to thyroid overstimulation than TSab in untreated patients with Graves' disease, but that there is a significant relationship between ERU and TSab values; 2) during antithyroid therapy, the TSab decrement was significantly greater in patients in whom initial ERU decreased by 50% or more or the absolute value became less than 20% of the dose than in patients with persistent high ERU levels; 3) the follow-up of these patients during antithyroid-L-T₃ treatment showed two patterns of changes for ERU and TSab, parallel in some patients, discordant in others, suggesting an heterogeneity in the in vivo TSab effect from one patient to another.

Thyroid-stimulating immunoglobulins are involved in the pathogenesis of Graves' disease. Evaluation of the relationship between circulating TSab levels and the degree of thyroid hyperfunction has practical as well as pathophysiological importance. Thyroid uptake, determined early after radio-iodine injection, measures the iodide trapping mechanism which closely reflects thyroid activity (Higgins 1955). Despite the findings of Ibbertson et al. (1970), this function is generally considered unaffected by the administration of carbimazole (Higgins 1955; Alexander et al. 1967). Therefore, early radio-iodine uptake may reflect the degree of thyroid stimulation in vivo, even during antithyroid drug treatment. Doctor
et al. (1980) and Gossage et al. (1983 a) evaluated the correlation between thyroid uptake of radio-iodine or pertechnetate and thyrotropin receptor antibody levels determined by the inhibition of the binding of [\( ^{125}\text{I} \)]-bTSH to human thyroid cell membranes (thyrotropin-binding inhibitory antibodies-TBIab) and found several examples of unexplained inconsistencies. However, TBIab may not closely reflect the stimulatory action of the IgG in Graves' disease. It was therefore of interest to assess the relationship between early radio-iodine uptake and TSab. In the present work, we studied in parallel the early radio-iodine thyroid uptake and the TSab levels determined by their stimulating effect on human thyroid cell adenylate cyclase in patients with Graves' disease before and during carbimazole therapy.

**Patients and Methods**

**Patients**

Oral informed consent was obtained from each patient before inclusion into the study. Twenty-seven unselected patients were studied, 25 women and 2 men, age range 14—47 years (mean ± SD = 28.8 ± 16.1). Graves' disease was confirmed by clinical history and usual biological investigations. Goitre was present in 24 subjects: small (<30 g) in 13, moderate (<50 g) in 8, and important (>50 g) in 3 patients with symptoms of hypervascularization in 9/24 patients. Ocular changes were observed in all the patients except one: brightness stare only in 16, associated with lid retraction in 8, and exophtalmos in 2. Thyroid hormone levels were: total T\(_3\) (TT\(_3\)) = 7.8 ± 2.8 nmol/l (normal range = 1.2—3.4 nmol/l); total T\(_4\) (TT\(_4\)) = 238 ± 37 nmol/l (normal range = 60—140 nmol/l); plasma TSH was undetectable. All patients were treated with a constant dose of carbimazole (10 mg every 8 h) and L-T\(_3\) (25 µg two or three times a day); pindolol (two or three doses of 5 mg/day) was administered when necessary during the first 3—5 weeks of treatment. TSab and early radio-iodine uptake (ERU) were determined in each patient before treatment. The same measurements were repeated after 2—4 (20 patients), 5—8 (15 patients), and 10—12 months (6 patients) of treatment. Duration of follow-up on antithyroid drug therapy ranged between 3 and 21 months and averaged 10.8 ± 5.8 months.

**Methods**

1. **ERU.** The 20 min \( ^{131}\text{I} \) thyroid uptake was measured according to Wilkin et al. (1981). \( ^{131}\text{I} \) was injected iv. Thyroid uptake was calculated and expressed as follows:

\[
\text{ERU} = \frac{\text{cervical radioactivity - thigh radioactivity}}{\text{injected radioactivity}}
\]

The error of the determination was less than 0.5% of the injected dose. In normal controls, ERU was less than 8%.

2. **TSab assay.** Thyroid stimulating antibodies (TSab) were detected as previously reported by Madec et al. (1986) using an assay system based on stimulation of the production of cAMP by cultured human thyroid cells. Determinations were performed in triplicate in 96-well plates on 40 µl samples of whole serum incubated for 2 h with \( 10^5 \) thyrocytes per well in a final volume of 0.2 ml. Thyroid cells which had been stored in liquid nitrogen were routinely used.

With this assay system, activity of 0.010 U/l of bTSH was always significantly detectable, and stimulation by 0.3 U/l averaged 698 ± 81% (mean ± SEM of 12 assays). Each assay contained TSab standards of 0.5, 1.5 and 20 µl from a pool of ten sera with potent TSab activity. Sera in the presence of which the amount of cAMP was significantly greater than in control wells were considered as TSab-positive. TSab activity was expressed as microtiter-equivalent of standard TSab per ml (TSab µg/ml). Activity of 10 µg/ml corresponded usually to a stimulation level of cAMP production of approximately 300—350% over basal. In the assay, 0.15 µl of standard TSab was usually non-stimulatory, therefore, in the calculation, negative TSab was taken as 4 µg/ml. Based on 12 different assays, intra-assay reproducibility was 10%; inter-assay reproducibility was 17% for 0.1 U/l bTSH, and 15% for 1.5 µl of standard TSab. Sera were usually tested at a single dilution. However, occasionally sera with very potent TSab activity were assayed at a lower concentration. Negative sera were also assayed at a lower concentration and tested for the presence of blocking antibodies in co-incubation experiments with a standard dose of bTSH or TSab. No serum was found to be inhibitory. Serial samples from each patient were tested in the same assay.

3. **Hormone and antithyroid antibody determination.** TT\(_3\), TT\(_4\) and plasma TSH were measured using commercial radioimmunoassay kits (TT\(_3\) and TT\(_4\); Clinical Assays-Travenol Laboratories Paris; TSH: BioMérieux Laboratory Lyon). Antithyroglobulin antibodies (Tgab) were determined by haemagglutination (Wellcome Reagents Ltd Paris; significant titre \( \geq 1/100 \)) and antimicrosomal antibodies (Mab) by immunofluorescence (significant titre \( \geq 1/10 \)).

4. **Statistical analysis.** Data are expressed as mean ± SD. Comparison between values obtained initially and after treatment or between two groups were made by the Mann-Whitney ranking test. Correlation analysis was made by non-parametric statistics (Spearman’s coefficient).
Results

1. Before treatment

In the 27 patients studied before treatment, ERU ranged between 15 and 54% and averaged 33.5 ± 12.8%. ERU was significantly correlated with TT₃ (r: 0.54; P < 0.01) but not with TT₄. In this series of patients, TSab was undetectable in 6; in 20 patients, TSab ranged between 6.0 and 30.0 µ-eq/ml with a mean of 12.3 ± 5.9 µ-eq/ml; in the remaining patient TSab was 85 µ-eq/ml. TSab values were not correlated with either TT₄ or TT₃. A significant correlation was found between ERU and TSab (r = 0.57; P < 0.01). Fig. 1.

2. During carbimazole treatment

Twenty one patients were sequentially studied during carbimazole-L-T₃ treatment. In each patient, serial TSH values were always < 2.5 mU/l.

Fig. 1.
Initial values of ERU and TSab in 26 patients with untreated Graves' disease. (Spearman Rank test: r = 0.57; P < 0.01). ——- Detection limit in TSab assay.

Fig. 2.
ERU and TSab values before treatment (I) and at 3–7 months of follow-up under therapy (T) in patients in whom (panel a) ERU decreased by 50% or more of its initial value or the absolute value became less than 20% of the dose (group A, N = 9) or ERU fell by less than 50% or the absolute value remained greater than 20% of the dose (group B, N = 7), and (panel b) patients in whom TSab were initially undetectable.

—- Mean of the group. ——- Detection limit in TSab assay.
The relationship between ERU and TSab was evaluated during the first months of carbimazole-L-T₃ treatment. Since the purpose of this study was to compare changes in ERU and TSab levels, the 5 patients in whom TSab remained undetectable throughout the study were considered as a separate group (Fig. 2, panel b). The remaining 16 patients were sub-grouped according to evolution of ERU after 3–7 months of treatment as compared with pre-treatment levels. Group A consisted of 9 patients in whom initial ERU decreased by 50% or more, or the ERU absolute value became less than 20% of the dose. Group B consisted of 7 patients in whom the fall of ERU was less than 50% or the absolute value remained greater than 20% of the dose. Among these 16 patients, 12 were tested at 6 months of treatment, one at 7 months, 2 at 4 months and one at 3 months. Mean duration of treatment at the time of study was 5.6 ± 1.2 and 5.7 ± 0.8 months, in groups A and B, respectively. Pre-treatment ERU values were 36.0 ± 13.5 and 38.1 ± 7.9% in group A and B, respectively. During treatment, ERU values were 17.8 ± 5.5 and 34.4 ± 15.3%, in group A and B, respectively (P < 0.01). Initial TSab levels were not different in group A and B (10.9 ± 4.8 vs 10.8 ± 3.6 μl-equiv/ml). However, TSab values had significantly decreased to 5.3 ± 1.6 μl-equiv/ml in group A (P < 0.01, initial values vs values during treatment), but were unchanged (10.7 ± 3.5 μl-equiv/ml) in group B (mean ± SD of 6 patients, one patient’s TSab value of 162 μl-equiv/ml was not included in this calculation). Interestingly enough, in the patients with no detectable initial TSab, ERU levels were 23.4 ± 7.4 and 10.0 ± 7.5% before and after 5 ± 1.9 months of treatment, respectively, and this 57% decrease in ERU is similar to the 58% figure which defined group A patients. Fig. 2, summarizes these data. Assuming that the subdivision into groups A and B is justified, it appears that the patterns of ERU and TSab evolution differ between group A and B during the first months of treatment with carbimazole-L-T₃ combination. To analyse these data, serial TSab and ERU individual values obtained during the first year of follow-up were studied for correlation. For each patient in groups A and B, the series of corresponding TSab and ERU values were studied by the Spearman rank test. Each group’s individual TSab-ERU correlation parameters were then pooled in group A and B which differed (P < 0.05): a significant relationship was observed between TSab and ERU during treatment in group A, but not in group B.

Discussion

The main point of this work was to study whether circulating levels of TSab parallel the degree of thyroid hyperactivity, i.e. whether circulating TSab might reflect the intensity of the abnormal thyroid stimulation. ERU is accepted as one of the most accurate parameters of thyroid activity; the correlation between initial ERU and blood levels of T₃ in this series of untreated patients with Graves’ disease is in keeping with this statement. Moreover, ERU is generally considered as unaffected by antithyroid drug therapy; consequently, it can be used as an indicator of the level of stimulation during carbimazole administration. Pre-treatment ERU and circulating TSab values were concordant, which strongly suggests that peripheral TSab is a meaningful index of thyroidal stimulation. During antithyroid drug therapy, the individual change of ERU and TSab was unpredictable. We found, however, that in patients in whom the fall of initial ERU is 50% or more or the absolute value is less than 20% of the dose, TSab decreased; TSab remained unchanged in patients with persistent high ERU levels. Moreover, in about 50% of the patients studied, e.g. in patients in group A, ERU and TSab values changed in parallel. This observation suggests also that peripheral TSab has a functional significance. The search for such a relationship has led to variable conclusions in the literature. Recently, contrarily to Clague et al. (1976), Gossage et al. (1983 b) did not find any correlation between ERU and TIab levels in untreated patients. On the contrary, Docter et al. (1980) found TIab levels to be correlated with the 24-h thyroid iodine uptake but not with ERU. It should be noted that assays based on inhibition of TSH binding might not detect stimulatory activity of the antibodies; however, in a large number of patients with Graves’ disease, recent data have showed a significant correlation between TIab and TSab tested by the stimulation of cAMP release in a porcine bioassay (Creagh et al. 1985).

Obviously, even with an assay based on thyroid stimulation, there is no simple relationship between ERU and TSab. Among the patients with
ERU greater than 30%, TSab was undetectable in one and widely scattered in the others. Similarly, in the seven patients studied sequentially during treatment (group B), variations of ERU and TSab were discordant. In our patients, these discrepancies were not related to any clinical or biological peculiarity as it was described by Carneiro et al. (1966). Iodine contamination having been excluded by PBI determinations, ERU levels which appeared inappropriately low as compared with TSab or which decreased or remained stable despite increasing TSab levels might suggest low thyroid responsiveness to stimulation. Although the occurrence of concomitant lymphocytic thyroiditis could account for this finding, this explanation is unlikely since Tgab and Mab showed no greater differences in these patients than in those with concordant ERU and TSab. Alternatively, it is theoretically possible that, in some cases, the thyroid stimulating activity measured in vitro is artefactually overestimated; we have not been able to document this point. As to the patients with apparently inappropriately low TSab, the results might suggest that intrathyroidal TSab could more closely reflect the degree of follicular stimulation than peripheral TSab (Kendall-Taylor et al. 1984).

Finally, it was striking to notice that changes in ERU and TSab correlated only in patients in whom ERU decreased significantly during treatment. In the patients in whom the change of these two parameters apparently was quantitatively independent, TSab remained detectable during the change and ERU values fluctuated highly. The significance of this observation is unclear. Additional and more prolonged studies are necessary to know whether these different patterns of change during carbimazole therapy have pathophysiological and/or prognostic significance.

In conclusion, this study indicates that ERU was more closely related than circulating TSab activity to the degree of thyroid hyperfunction in patients with untreated Graves' disease. During the first months of antithyroid drug therapy, the decrease of TSab was more marked in patients in whom initial ERU decreased by 50% or more or the absolute value became less than 20% of the dose. The changes in ERU and TSab values were parallel in approximately only half of the patients. These data suggest that circulating TSab can be a meaningful index of the degree of thyroid overstimulation; however, discrepancies were observed which remain unexplained and which may suggest an heterogeneity in TSab affinity for thyroid cells or that the major site of TSab synthesis is the thyroid in some patients and a non-thyroid site in others.

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