Comparison of Thyroperoxidase and Microsomal Antibody Assays in Sera from Patients with Graves Disease

Catherine Massart, Isabelle Guilhem, Jacqueline Gibassier, Hubert Allanic, and Marc Nicol

Anti-microsomal (anti-Mic Ab) and anti-thyroperoxidase antibody activities (anti-TPO Ab) were compared by using commercially available radioassay kits. Sera were collected from 52 patients with Graves disease before and after administration of carbimazole (1-methyl-2-thio-3-carbethoxyimidazole). The two antibody concentrations were significantly correlated, both before treatment (r = 0.835, P < 0.001, n = 52) and at the end of treatment (r = 0.584, P < 0.001, n = 52). Twenty-nine (Group I) of the 52 patients were in remission for two years after drug withdrawal, whereas 23 (Group II) relapsed. Within each group, the anti-Mic and anti-TPO Ab concentrations were significantly correlated (Group I: r = 0.781, P < 0.0001; Group II: r = 0.866, P < 0.0001). Relapse vs nonrelapse was linked to the antibody positivities measured before treatment: 91% vs 65% (χ² = 4.75, P < 0.02) for anti-Mic Ab and 87% vs 62% (χ² = 4.05, P < 0.02) for anti-TPO Ab. We conclude that assays of anti-Mic and anti-TPO Ab are equally reliable analytically and equally informative clinically. Because of its rapid implementation, the anti-TPO assay may advantageously replace anti-Mic Ab assay, especially for forming a prognosis of Graves disease.

Additional Keyphrases: radioimmunoassay  immunoradiometric assay  autoantibodies  effect of carbimazole treatment

Graves disease (GD) is an autoimmune disease in which antibodies (Ab) are active against thyroid microsomal antigens (1). Unfortunately, the methods currently used to measure anti-microsomal (anti-Mic) Ab concentration rely on thyroid membrane preparations contaminated with other antigens, especially thyroglobulin, which is also involved in thyroid autoimmunity. Thyroid peroxidase (TPO) has recently been identified as a thyroid microsomal antigen (2, 3). Taking advantage of highly purified TPO and of a carefully selected monoclonal anti-TPO Ab, Ruf et al. (4) developed an anti-TPO radioimmunoassay suitable for routine clinical testing. Here we compare their method for measuring anti-TPO Ab with an immunoradiometric assay.

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1 Nonstandard abbreviations: GD, Graves disease; Ab, antibodies; Mic, microsomal; TPO, thyroid peroxidase; IRMA, immunoradiometric assay; and ELISA, enzyme-linked immunosorbent assay.
(IRMA) of anti-Mic Ab concentration: analytically, by qualitative assessment of the two methods, and clinically, by correlating the results observed in GD patients before and after drug therapy. We also determined the utility of anti-Mic or anti-TPO Ab concentrations as prognostic markers of GD.

Materials and Methods

Patients

The study involved 52 GD patients diagnosed from typical clinical signs: goiter, occasional exophthalmos, and increased free thyroid hormone concentrations (free triiodothyronine >8.9 pmol/L and free thyroxin >23.4 pmol/L). Patients showing toxic nodules on computed tomographic scan were excluded. All patients were treated for six or 18 months with carbimazole (1-methyl-2-thio-3-carbethoxyimidazole), initially at a high dose (40 mg/day), then with decreasing doses that maintained a euthyroid state without hormone replacement.

Twenty sex- and age-matched healthy blood donors served as controls. Blood samples, taken before drug therapy and at the end of treatment, were collected in anticoagulant-free tubes and centrifuged at 1000 × g for 10 min at 4 °C. Sera were decanted for storage at −20 °C until assay.

Antibody Assays

Anti-Mic assay. Anti-Mic Ab activity was measured with a commercially available IRMA kit (RIA Promak; Henning Labs., Berlin, F.R.G.), by the technique described by the manufacturer: 20 μL of serum was diluted with 750 μL of buffer in tubes coated with microsomal antigens. After incubation for 2 h at 37 °C, the supernates were aspirated, the coated tubes were washed, and 1 mL of 125I-labeled Protein A was added to each tube. After another 2-h incubation at 37 °C, the supernates were discarded and the tubes washed. The bound radioactivity in each tube was counted for 2 min in a gamma counter. In the absence of international standards, antibody concentrations were expressed as units/mL with reference to Medical Research Council (MRC) Standard 66/387. The mean (SEM) anti-Mic Ab activity in 20 normal sera was 280 (60) units/mL. Values ≤400 units/mL were considered negative.

Anti-TPO Ab assay. Anti-TPO Ab were measured with a competitive IRMA (Dyntest RIA; Henning Labs.) based on a principle described by Ruf et al. (4). Briefly, 50 μL of serum was diluted with 1 mL of buffer and mixed with 100 μL of 125I-labeled TPO distributed in tubes coated with monoclonal anti-TPO Ab. After incubating the samples for 2 h on a horizontal shaker at room temperature, we aspirated the supernates, washed the tubes, and counted the radioactivity remaining in the tubes for 2 min with a gamma counter. Anti-TPO Ab concentrations also were expressed as units/mL with reference to MRC Standard 66/387. The mean anti-TPO Ab activity in 20 normal sera was 69 (SEM 15) units/mL. Values >99 units/mL were considered negative.

Analytical Variables

The repeatability of anti-Mic and anti-TPO Ab assays was evaluated in terms of the within-run CV for 12 or 10 assays of three patients’ sera.

The reproducibility was determined for 20 or 12 series of assays. Between-run CVs were computed from assays of two samples (one patient’s serum and one commercial control sample). The between-run data were generated with 20 or six batches of kits for anti-Mic and anti-TPO Ab assays, respectively.

Statistics. Quantitative analysis was performed with the Mann–Whitney U test and qualitative analysis with the χ² test.

Results

Analytical Variables

The repeatability and reproducibility of anti-Mic and anti-TPO Ab assays are reported in Table 1. Between-run and within-run precision was satisfactory for both assays, with CVs <15%.

We serially diluted with antibody-free serum patients’ sera rich in anti-Mic and anti-TPO Ab. The dilution curves (Figure 1) were parallel to the standard curves for each antibody.

Assays of Samples from GD Patients (Figure 2)

Before treatment. Positive anti-Mic values (>400 units/mL) were observed in 40 of the 52 GD patients (77%), the median value being 8105 units/mL (range 117–49 805 units/mL). Although anti-TPO Ab results were positive in only 38 of these sera (73%);—median value 2079 units/mL, range 40–12 728 units/mL—the anti-Mic and anti-TPO Ab activities were significantly correlated (r = 0.835; P <0.0001). Thirty-seven of the 52 sera gave positive results by both assays. Results differed for only three sera: anti-TPO Ab was negative and anti-Mic Ab was positive for two sera, and one serum was negative for anti-Mic Ab and positive for anti-TPO Ab.

Twenty-nine of the 52 patients (Group I) were still in remission two years after drug withdrawal, whereas 23 had relapsed (Group II). Nineteen of the 29 Group I patients (65%) were anti-Mic Ab-positive (median 6035 units/mL, range 117–35 266), and 18 of these (62%) were anti-TPO Ab-positive (median 1435 units/mL,

Table 1. Precision of the Anti-Mic and Anti-TPO Assays

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<th>Within-run</th>
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The anti-Mic and anti-TPO Ab values in Group I correlated significantly \( (r = 0.781, P < 0.0001) \). Of the 23 Group II patients, 21 (91%) were anti-Mic Ab-positive (median 10 715 units/mL, range 172–49 805); 20 (87%) were anti-TPO Ab-positive (median 2689 units/mL, range 73–12 728). Anti-Mic and anti-TPO Ab concentrations were also strongly correlated in Group II \( (r = 0.866, P < 0.0001) \). The relapse rate was anti-Mic Ab-positive-dependent (91% in Group II vs 65% in Group I, \( \chi^2 = 4.76, P < 0.02 \), or anti-TPO Ab-positive-dependent (87% in Group II vs 62% in Group I, \( \chi^2 = 4.05, P < 0.02 \)). These data indicate that, in patients with high concentrations of anti-Mic or anti-TPO Ab, remission as a result of treatment is less likely. There was no significant difference between Group I and Group II values for anti-Mic Ab \( (z = -1.971, P > 0.05) \) and anti-TPO Ab \( (z = -2.008, P > 0.05) \).

At the end of treatment. Thirty-one of the 52 patients (60%) who had received drug therapy were anti-Mic Ab-positive. Twenty-nine patients (56%) were anti-TPO Ab-positive. Concentrations of anti-Mic Ab (median 2792 units/mL, range 99–23 516) and anti-TPO Ab (median 647 units/mL, range 38–5002) were lower than those observed before treatment, and were significantly correlated \( (r = 0.584, P < 0.001) \).

Fourteen of the 29 Group I patients (48%) were anti-Mic Ab-positive (median 2075 units/mL, range 196–23 516) and 15 (52%) were anti-TPO Ab-positive (median 375 units/mL, range 38–3189)—these two Ab concentrations being significantly correlated \( (r = 0.511, P < 0.005) \). Seventeen of the 23 Group II patients (73%) were anti-Mic Ab-positive and 15 (65%) were anti-TPO Ab-positive, with respective median values of 3696 (range 99–16 208) and 1005 (range 49–5003) units/mL.

The relapse rate was independent of antibody positivity (73% vs 48%, \( \chi^2 = 1.93, P > 0.05 \)) for anti-Mic Ab. The relapse rate was also independent of anti-TPO Ab positivity (65% vs 52%, \( \chi^2 = 1.42, P > 0.05 \)). There was no significant difference in antibody concentrations between Group I and Group II: \( z = 1.575, P > 0.05 \) for anti-Mic Ab results, and \( z = 1.531, P > 0.05 \) for anti-TPO Ab results.

Discussion

In this study, we measured circulating anti-Mic and anti-TPO Ab in GD patients' sera. The analytical characteristics of the methods used for Ab assay (repeatability and reproducibility, parallel test) appeared quite satisfactory both for anti-Mic and anti-TPO Ab.

Comparative studies reported in the literature mainly focus on the anti-Mic Ab assay by positive hemagglutination and the anti-TPO immunoassay (RIA or ELISA). These concentrations are well correlated \( (4–7) \), although discrepancies with anti-Mic-poor or negative sera have been observed, possibly because of the insensitivity of hemagglutination methods \( (4) \).

When comparing Anti-Mic Ab and anti-TPO Ab concentrations measured with immunoassays (IRMA, RIA, or ELISA), a moderate but significant correlation was obtained with ELISA results \( (8, 9) \). In contrast, a very close correlation was observed when comparing the two RIA results \( (5, 10) \).

The present results are fully consistent with such observations, the assays used revealing a strong corre-
lation between anti-Mic Ab and anti-TPO Ab in GD patients, either untreated, treated, in remission, or in relapse. Anti-Mic and anti-TPO Ab prevalence in patients before treatment—77% and 73%, respectively—was decreased in treated patients—60% anti-Mic and 56% anti-TPO—results that compare well with those reported in the literature: 72–88% anti-Mic Ab-positive (11–13) and 62–87% anti-TPO Ab-positive (7, 11, 14, 15) in untreated GD patients, which sharply decrease during antithyroid drug treatment (7, 15, 16).

We also determined in the present study that the relapse rate in GD patients was correlated to the anti-Mic and anti-TPO positivity values measured before treatment. Thus, patients with high Ab concentrations stand little chance of remission under drug treatment. To our knowledge, no study has focused on the utility of anti-TPO Ab assay as a prognostic marker of Graves disease, although the relapse rate has been shown to be high in GD patients with high serum anti-Mic Ab titers (17, 18). The present results on pre-treatment anti-Mic Ab titration are in accord with these studies.

In summary, this study showed that anti-Mic and anti-TPO antibody assays are comparable in analytical reliability and parallelism. Clinically, they produce correlated results in nontreated, treated, treated, and relapsed GD patients. Because the anti-TPO Ab assay takes half as long to perform, its implementation before treatment may advantageously replace the anti-Mic Ab assay as an indicator of the progression of Graves disease.

References