

Discrepancy between Haemagglutination and Radioimmunological Techniques for Measurement of Serum Thyroglobulin Autoantibodies

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Recently, it has been suggested that in some patients with autoimmune thyroid diseases the tanned red cell (TRC) method for detection of thyroglobulin autoantibodies (TgAb) is negative where TgAb measured by radioimmunoassay (RIA) show positive values. To investigate this further, patients with thyroid diseases, pernicious anaemia and a control group were studied for serum concentrations of TgAb by TRC and by quantitative RIA, calibrated against MRC Standard A65/93. Antibodies for microsomes (MAb) were measured immunofluoretically. There was in all patient groups (Hashimoto's thyroiditis ($n = 41$), Graves' disease ($n = 50$), idiopathic myxoedema ($n = 12$), euthyroid Graves' disease ($n = 7$), pernicious anaemia ($n = 81$)) a discrepancy between TgAb measured by TRC and RIA, respectively, whereas there was a reasonable correlation between the presence of TgAb by RIA and the presence of MAb. A possible interference from antinuclear antibodies and rheumatoid factors was ruled out. There was no increased frequency of TgAb measured by RIA in the control group. Fractionation of TRC negative sera revealed macromolecular TRC-activity, whereas TgAb positive sera by both methods had almost exclusively RIA and TRC activity corresponding to IgG. Based on these results and others it seems that the TRC method for measurement of serum TgAb is of limited diagnostic value. Furthermore, the TRC method is in many cases not sensitive enough for screening for TgAb prior to measurement of serum Tg, which is of importance as this method shows false values in the presence of TgAb due to methodological interference.

Key words: autoimmune thyroid diseases; pernicious anaemia; radioimmunoassay; thyroid autoantibodies.

Accepted for publication 27 August 1982

The most common method for measurement of thyroglobulin autoantibodies (TgAb) is the tanned red cell method (TRC) described by Fulthorpe (9). In the last decade, however, a number of radio (RIA)- and enzyme-immunological methods have been developed for investigative purposes (3, 12, 15, 17). In the first reports by Mori & Kriss (13) and Salabé (17) it was evident that a number of patients with

autoimmune thyroid diseases were negative by TRC and positive by RIA, explained by increased sensitivity of the RIA methods. Later, however, Pinchera et al. (15) showed that in the competitive radioassay for TgAb the discrepancy was in some cases due to elevated levels of serum thyroglobulin (Tg) giving rise to falsely elevated TgAb by RIA. The coprecipitation RIA method, however, also showed this dis-

Presented in part at the 24th International Symposium of Clinical Endocrinology, Paris, 1981.

crepancy (17), though elevated serum Tg in this method caused falsely low values (7).

In patients with Graves' disease an explanation was given by the demonstration of an inhibitor of the second order reaction of haemagglutination inhibition (TRC) (24).

The purpose of the present investigation was to compare results from TRC and coprecipitation methods for determination of TgAb in a variety of autoimmune disorders and to investigate the molecular pattern of the immunoglobulins involved in the reactions by gel-chromatographic studies.

MATERIAL

Patients with several autoimmune disorders and with high frequency of TgAb were studied.

1) 41 patients with Hashimoto's disease characterized by presence of goitre, hypothyroidism and elevated titres of TgAb by TRC and/or microsomal antibodies (MAb). All patients received L-thyroxine.

2) 50 patients with Graves' disease studied during ($n = 27$) and 10 years after successful medical treatment with propylthiouracil ($n = 23$). Eleven patients had also received ^{131}I in order to obtain euthyroidism. The diagnosis was based on presence of clinical and biochemical hyperthyroidism with or without diffuse goitre (5).

3) Seven patients with unilateral euthyroid Graves' ophthalmopathy and without previous or present thyroid disease as described elsewhere (14).

4) 12 patients with untreated idiopathic myxoedema and without goitre (atrophic autoimmune thyroiditis).

5) 80 patients with pernicious anaemia. The diagnosis was based upon megaloblastic cells in the bone-marrow, low level of serum vitamin B12 and lack of intrinsic factor. All patients were supplemented with B12 at the time of investigation.

Control group comprised 97 healthy non-hospitalized persons, who had not had any endocrinological disease previously and had no family history of thyroid diseases. They received no

medication at the time of investigation (including hormonal contraception), and had not been subjected to X-ray investigations within the past 6 months. Body weights were within normal limits related to their heights. Serum concentrations of TSH, triiodothyronine and thyroxine were all within reference limits (5).

METHODS

Serum TgAb were measured quantitatively by a radioimmunological method (3) standardized according to the Medical Research Council's research standard A 69/93, which by definition contained 1 Mega Unit per l (MU/l). TgAb were also measured by the TRC method (Wellcome, Thymune-T[®]). MAb were detected by immunofluorescence technique (23). In some sera antinuclear antibodies (ANA) were detected by immunofluorescence, as well as IgG and IgM rheumatoid factors (IgG- and IgM-RF) by radioimmunoassay (13). IgM-RF was also evaluated by the latex fixation test (18) and by the method of Waaler-Rose (16, 22). HLA-A, B and C typings of the patients with Hashimoto's thyroiditis were performed by the NIH technique (19) and HLA-D typing was performed as described by Thomsen et al. (21) by the use of HLA-D homozygous typing cells defining the specificities Dw 1-8 and the local specificity DH.

Table 1

Concentration of TgAb in sera used for the study of inhibiting influence of TRC negative (1-3) on TRC positive serum (4-6) in patients with Hashimoto's thyroiditis. All negative sera were tested against all positive sera (nine set-ups)

Patient No.	Tg Ab	
	RIA (MU/l)	TRC (reverse titre)
1	0.448	neg
2	0.185	neg
3	0.073	neg
4	9.02	600
5	67.4	3200
6	2.84	6400

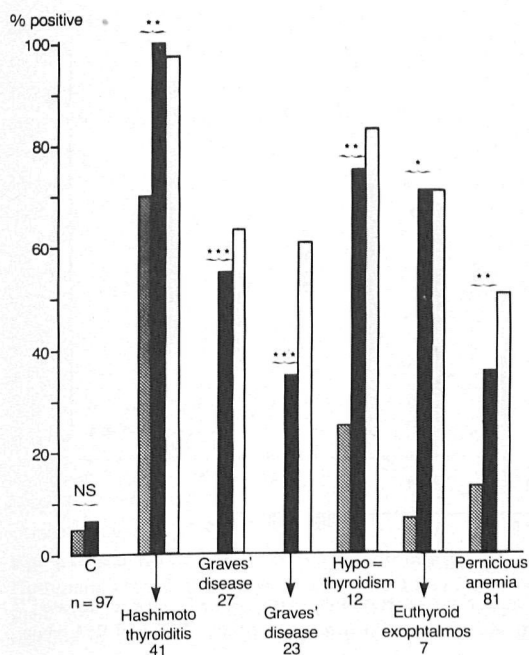


Fig. 1. Percentage of patients positive for thyroglobulin antibodies by TRC (▨) and RIA (■) as well as for microsomal antibodies (□). NS = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Table 2

Relation between thyroglobulin antibodies (TgAb-RIA) and microsomal antibodies (MAB) in serum from patients with Hashimoto's thyroiditis ($n = 41$), Graves' disease ($n = 50$), hypothyroidism ($n = 12$), euthyroid exophthalmos ($n = 7$) and pernicious anemia ($n = 81$). There was good agreement between the two methods ($P < 0.001$)

	MAB		Total
	No. positive	No. negative	
TgAb-RIA			
No. positive	97	8	105
No. negative	28*	58	86
Total	125	66	191

* The majority of these sera were found among patients with Graves' disease (12) and pernicious anemia (15).

All specimens were stored at -20°C avoiding repeated freezing and thawing. Fractionation of serum specimens was done largely as previously described (8) using a K 9/60[®] column (Pharmacia Fine Chemicals, Sweden), and Sepharose CL 6B. The fractions were tested for TgAb by RIA and TRC, for IgG and IgM by immunoelectrophoresis and for immune complex (IC) activity by a rheumatoid factor dependent particle counting assay (PACIA) (2). Investigation of the inhibiting influence of TRC negative serum (RIA-positive) ($n = 3$) on TRC positive serum ($n = 3$) was performed in patients with Hashimoto's thyroiditis in a total of nine set-ups as described by Wilkin et al. (24). The concentration of TgAb measured by RIA in the serum specimens is seen in Table 1. In brief, serial dilutions of a TRC negative serum were mixed with serial dilutions of a TRC positive serum and the mixture was tested for haemagglutinating activity in the TRC method for TgAb.

The data were analysed for statistical significance by Fisher's exact test and parametric correlation.

RESULTS

The percentage of patients positive for TgAb by RIA and TRC, respectively, as well as for MAb is seen in Fig. 1. A significant discrepancy between the frequency of TgAb measured by RIA or TRC was seen in all patient groups, whereas the frequency of patients positive by RIA correlated with the presence of MAb (Table 2). From Fig. 2 can be seen that patients with autoimmune thyroid disease who were positive by TRC were also positive by RIA with a significant correlation ($r = 0.9$, $P < 0.01$) between their positive quantitative measurements. There were, however, a number of patients ($n = 42$; 38%) who were negative for TgAb by measured TRC and positive measured by RIA, some with considerable elevations ($P < 0.01$). In patients with pernicious anaemia (Fig. 3) there was no correlation, and the number of patients positive by RIA and negative by TRC was in this case 18 (22%).

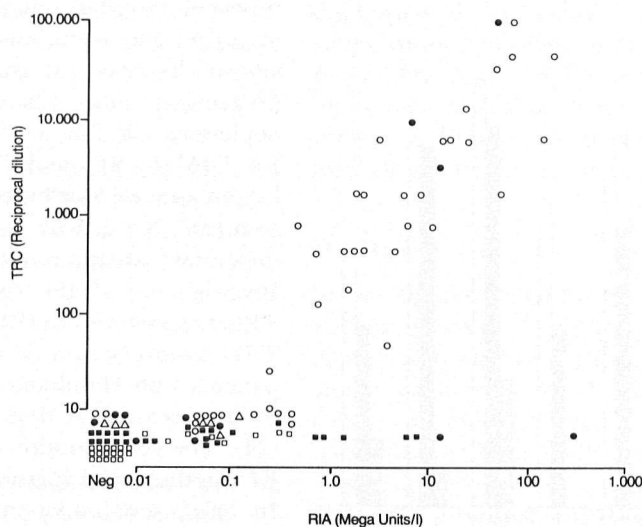


Fig. 2. Levels of TgAb by TRC and RIA in various thyroid autoimmune diseases (Hashimoto's thyroiditis $\circ$, Graves' disease $\square$ and $\blacksquare$, primary myxoedema $\bullet$, euthyroid exophthalmos $\triangle$). There was a significant correlation between TRC and RIA when testing concomitantly positive values ($r = 0.9$, $P < 0.01$).

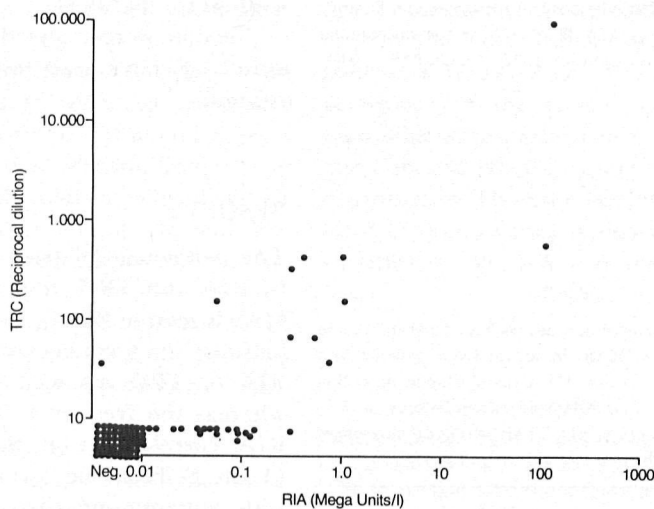


Fig. 3. Correlation between levels of TgAb by TRC and RIA in patients with pernicious anaemia. There was no significant correlation.

Fractionation of serum was performed in two patients with Hashimoto's thyroiditis with positive TgAb-RIA (0.068 MU/l and 0.204 MU/l) and negative TgAb by TRC. By separation of serum TRC positivity was revealed in the high molecular range (IgM/Tg: approx. 600,000 MW), whereas RIA positivity was mainly located

in fractions belonging to the molecular weight of IgG (Fig. 4). There was no evidence of IC-activity in this area of high MW. Fractionation of four sera with high concentration of TgAb by both TRC and RIA showed, for both methods, main location of activity corresponding to IgG (example from one patient shown in Fig. 5).

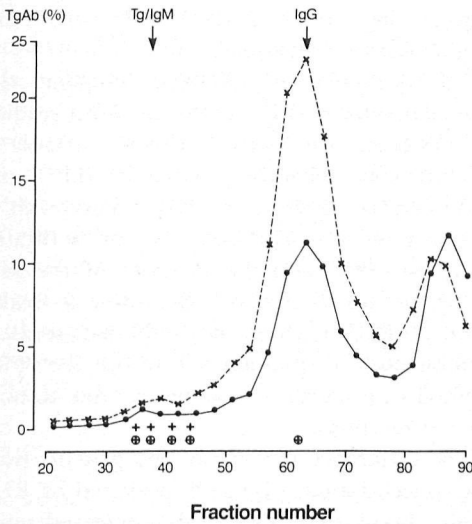


Fig. 4. Fractionation profile of two sera from patients with Hashimoto's thyroiditis both with positive TgAb-RIA one of them with negative TRC and one with a weakly positive reaction in undiluted serum. TRC activity (+, $\oplus$) is revealed in both sera in the high molecular weight region (IgM/Tg), whereas TgAb-RIA activity is located as IgG ($\times$ — $\times$, $\bullet$ — $\bullet$).

None of the patients with Hashimoto's thyroiditis had IgG (0/26) and two showed a weak reaction for IgM-RF (2/26). Seven of 41 patients had ANA. Neither the presence of ANA nor the HLA-type correlated with the combination of negative TgAb by TRC and positive by RIA.

Investigations of the inhibiting influence of TRC negative serum on TRC positive serum in Hashimoto's thyroiditis showed no inhibition, not even from undiluted sera (final dilution 1:2). A retest in the TRC method of some of the TgAb-RIA positive and TRC negative sera was thereafter performed in undiluted sera, showing a weakly positive reaction in 7/7 patients with Hashimoto's thyroiditis, in 15/17 with pernicious anaemia, and in 6/8 with Graves' disease.

DISCUSSION

The discrepancy between two methods for measurement of TgAb in serum (RIA and TRC) has previously been reported in patients with Graves' and Hashimoto's diseases (12, 15, 17, 24). This was confirmed in the present investigation and was also shown in patients with idiopathic myxoedema, euthyroid exophthalmos and pernicious anaemia.

Previously, an inhibitor of the haemagglutination reaction has been demonstrated in serum from patients with Graves' disease, with fluctuating levels during treatment as a possible explanation for the discrepancy. However, in the present study no evidence of such inhibition was found in patients with Hashimoto's thyroiditis. Therefore, non-antigen specific in-

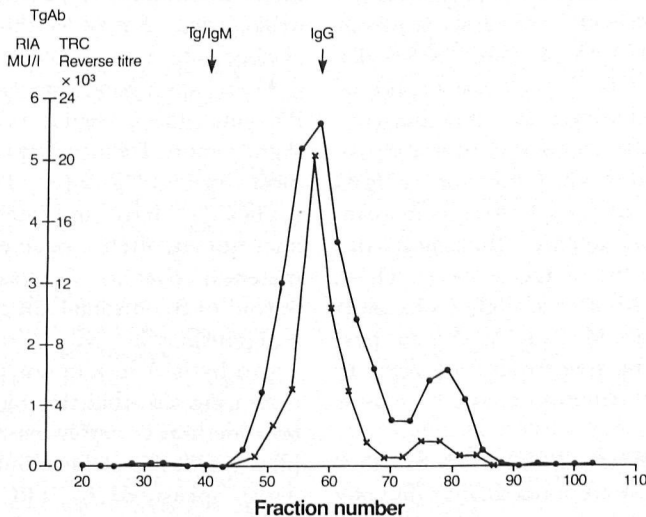


Fig. 5. Fractionation profile of one serum specimen (Hashimoto's thyroiditis) concomitantly positive by TRC ($\times$) and RIA ($\bullet$) for TgAb.

hibition could not be a general explanation for the demonstrated discrepancy between the methods.

Pinchera et al. (15) have shown that patients with high levels of serum thyroglobulin had falsely elevated concentrations of TgAb measured by competitive RIA. This method is, however, methodologically completely different from the method used by us and by Wilkin et al. (24) in which the principle is coprecipitation with radiolabelled thyroglobulin. In this method elevated serum Tg caused falsely low concentrations of TgAb (6), and could therefore not account for the disagreement. Further, the discrepancy was also seen in patients with idiopathic myxoedema who had low concentration of Tg (4).

Our experiments with serum fractionation revealed that sera positive by both methods showed activity corresponding to the molecular weight of IgG. However, sera negative by TRC and positive by RIA showed a TRC reaction in the region of high molecular weight, indicating that this activity might be accounted for either by IgM antibodies (which would not be detected in the TgAb-RIA method) or by IC consisting of Tg and TgAb (TgIC). It has previously been shown that IC are frequently present in patients with Hashimoto's disease (10) in some studies shown to be TgIC (11). We were able to demonstrate antigen unspecific IC activity by PACIA technique in the fractions from these patients but with a lower molecular weight than TgIC (10). The characteristics of these IC, however, indicate that they may contain Tg antigen (10), although more studies are needed for the final proof. Formation of TgIC lowers the concentration of TgAb quantitatively corresponding to complex formation with the antigen binding site of IgG FAb (7). Theoretically, one free FAb on each IgG molecule is sufficient to show reaction in RIA, whereas possibly both have to be free to give appropriate TRC reaction and furthermore, IgG is a poor agglutinator.

The possible interference from RF or ANA was ruled out, and we were not able to find any relation to the HLA tissue types indicating that genetic differences in immune response pattern

between the patients probably did not account for the observed disagreement.

The possibility of different antigenic determinants involved in the two methods studied has not been investigated but seems unlikely as patients concomitantly positive by TRC and RIA had comparable concentrations with a strong positive correlation, as also indicated previously (24), and the majority of the sera giving rise to discrepancy did show a weakly positive TRC reaction in undiluted sera. A general use of undiluted sera in the TRC method is, however, not applicable due to non-specific reactions.

The difference in some of the patients (with low concentrations of TgAb measured by RIA) might therefore be due to the increased sensitivity of the RIA method. However, a number of sera had high TgAb by RIA and negative TRC as demonstrated by Wilkin et al. (24) and no definite conclusions concerning the reason for the discrepancy can be drawn.

The large difference in frequency of TgAb positive sera in the patients with Graves' disease was most probably due to two factors: 1) presence of a fluctuating TRC inhibitor (24) and 2) increased sensitivity of TgAb RIA (Fig. 1). In previous studies a number of these patients have been positive by TRC (12, 17), indicating an autoimmune reaction of the thyroid. This reaction has, however, always been shown to be weaker than that of Hashimoto's thyroiditis as evidenced by lower concentrations of TgAb.

There was a good correlation between TgAb-RIA and the presence of MAb, which were slightly more frequently present than TgAb in most patient groups. Previous absorption studies (1) have indicated that the thyroid microsomes often contained also Tg antigenic material, whereas Tg was more likely to be devoid of microsomal antigen, and hence more antigen-specific. No increased frequency of TgAb by RIA in a donor population was seen, indicating also that the TgAb-RIA coprecipitation method is highly specific. The clinical implication of the present findings are that serum TgAb measured by TRC have limited diagnostic value in thyroid diseases. Furthermore, TgAb-RIA gives a quantitative measure, which

offers an advantage in respect to follow-up studies and investigative purposes (4, 7). Finally, it is sufficiently sensitive to serve as screening for the presence of TgAb in relation to measurement of serum Tg, which has been shown to be an important adjunct in the detection of metastases or relapse in patients treated for differentiated thyroid carcinoma. As the RIA method for serum Tg is liable to false reactions (negative or positive) in the presence of TgAb it is of utmost importance to have access to a sensitive and reliable method for measurement of TgAb (6).

ACKNOWLEDGEMENTS

The technical assistance from Dorte Hvidtved, is gratefully acknowledged and Anne Lise Fougst is thanked for typing the manuscript. Drs. Mølholm Hansen and Siersbæk Nielsen are thanked for aid in classification of the patients.

This work was supported by grants from the Danish Medical Research Council and P. Carl Petersen's Foundation.

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