

# CLINICAL, HORMONAL, AND ULTRASOUND CHARACTERISTICS OF PATIENTS WITH NEWLY DIAGNOSED GRAVES' DISEASE AND DIFFERENT THYROID ANTIBODY PROFILES

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**Abstract. Objective:** Graves' disease (GD) is characterized by elevated TSH-receptor antibodies (TRAb) and less often – thyroid peroxidase (TPOAb) and thyroglobulin antibodies (TgAb). Our aim was to examine the hormonal and ultrasound characteristics of patients with newly diagnosed GD with differing positive thyroid antibodies. **Materials and Methods:** This study included 249 patients with newly diagnosed GD (191 women, 58 men). 40.2% of them had Graves' ophthalmopathy. The serum levels of TSH, free T4, free T3, TRAb, TPOAb, and TgAb were measured with third generation ECLIA assays (Roche Diagnostics, Switzerland). Thyroid ultrasound was performed with a Fukuda-Denshi 550 device (Fukuda Corp., Japan) and an Ultrasonix device (Ultrasonix Medical Corp., Canada). Statistical analyses were done using the SPSS 23.0 statistical package (Chicago, IL). **Results:** 64% of the patients were TPOAb+ and 36% – TgAb+. One third were only TRAb+, 1/3 had two positive antibodies (TRAb + second antibody) and 1/3 – all three positive antibodies. Patients with more positive antibodies tended to be younger, had higher fT4, TRAb levels, thyroid volume but rarely had nodules on US and accompanying GO. Positive TPOAb antibodies were found in younger patients, with higher fT4 and TRAb levels, higher thyroid volume and lower prevalence of nodules and GO. The same trends were found in patients with positive TgAb. **Conclusion:** The different characteristics of GD patients with varying thyroid antibody profiles may be due to a variation in the pathogenesis of the disease. An individualized clinical approach may be suitable in those cases.

**Key words:** Graves' disease, Graves' orbitopathy, thyroid antibodies, clinical/hormonal/ultrasound characteristics

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## INTRODUCTION

Graves' disease (GD) is the most common cause of thyrotoxicosis due to autoimmune thyroid disease (AITD) [1]. The main driving force of the hyperthyroidism seems to be the

presence of TSH-receptor antibodies (TRAb) [2]. High levels of TRAb in hyperthyroidism are one of the cornerstones in the diagnosis of GD, together with increased uniform radio-iodine uptake and diffuse ultrasound changes [1, 3]. In addition, AITD is marked also by elevation of anti-thyroid peroxidase

and anti-thyroglobulin antibodies (TPOAb, TgAb) [4-6]. Their positivity and significance are being better studied in the setting of autoimmune thyroiditis. However, they are often elevated in GD. Different studies reported a 50-70% prevalence of positive TPOAb and 30-50% positive TgAb in newly discovered GD [7-15]. Their clinical significance is less well understood. Some older studies have linked TPOAb positivity in GD with lower rate of accompanying Graves' ophthalmopathy (GO), higher thyroid volume as well as with lower rates of complete remission of the AITD [8, 9, 12, 16, 17]. Other studies found an association of anti-TPO positivity with GO in adolescents [18]. In the RISG study the thyroid volume was positively related to the anti-TPO antibodies [16]. Measurement of TPOAb levels 6 months after the initial diagnosis was found useful in the prediction of a possible relapse of GD [14].

The presence of TgAb in GD is even less well studied. It was associated mainly with the prevalence and pathophysiology of GO [17, 19-21]. Most of those studies were of moderate size (< 100 patients), several studies were retrospective analyses. As those two antibodies are often measured in GD with or without GO, it is possible to link their positivity and levels to several clinical, ultrasound and hormonal parameters at the presentation of GD in an attempt to predict the future course of the AITD and the possible association of GO. As far as we know, there are no published analyses of the differences in the clinical presentation of GD depending on the number of positive antibodies in addition to TRAb.

The aim of the present study was to examine the clinical characteristics of patients with newly diagnosed GD who differ in the number of positive anti-thyroid antibodies as well as to find associations of the TPOAb and TgAb levels with important clinical, hormonal and ultrasound parameters.

## MATERIALS AND METHODS

### *Design*

This cross-sectional study included newly diagnosed GD patients from a tertiary hospital-based specialized endocrine unit. The study was approved by the Ethical committee of the Scientific Council at the Medical University Sofia (Research Project 13/2012, written consent) and was conducted in compliance with ethical standards and the Declaration of Helsinki. All patients signed informed consent prior to any procedure. All procedures were part of the routine clinical work-up in AITD. The inclusion criteria were age above 18 years, presence of newly diagnosed GD and willingness to participate. The exclusion criteria were glucocorticoid,

interferon or any other type of immune-modulating treatment for concomitant disease.

### *Methodology*

Complete physical examination was performed including eye symptoms, cardio-vascular signs of thyrotoxicosis and palpation of the thyroid. Smoking status, presence of concomitant autoimmune diseases and family history for thyroid disease were reviewed.

The serum levels of the thyroid hormones (TSH, free T4 and free T3) and antibodies (TRAb, TPOAb, TgAb) were measured from a fasting morning blood sample with third generation ECLIA assays (Roche Diagnostics, Switzerland) on an Elecsys 2010 analyzer. The following reference ranges were applied: TSH (0.27-4.2 mU/l); fT4 (12-22 pmol/l); fT3 (3.54-6.47 pmol/l); TPOAb (0-34 IU/ml); TgAb (0-115 IU/ml); TRAb (< 1.75 IU/l). Thyroid ultrasound was performed with a 9 MHz linear transducer on a Fukuda-Denshi 550 device (Fukuda Corp., Japan). The thyroid volume was calculated in ml (cm<sup>3</sup>) according to the formula by Brunn et al. [22].

Thyroid Volume of the studied lobe (cm<sup>3</sup>) = antero-posterior size x transverse size x length x 0.479 (all in centimeters).

The presence of nodules was also reported. Additionally, power and color Doppler ultrasound was performed on a 14 MHz Ultrasonix device (Ultrasonix Medical Corp., Burnaby, Canada) for vascularity and blood flow assessment. The diagnosis of GD was made in the presence of thyrotoxicosis (clinical or subclinical) with elevated levels of TRAb, diffuse hypoechogeneity of the thyroid parenchyma on ultrasound with increased blood flow on Doppler ultrasound [23]. Whenever available a technetium scan was reviewed and increased uniform uptake by the thyroid was used as confirmation [24]. Patients were evaluated by the clinical endocrinologists and experienced ophthalmologists for the presence of thyroid-associated ophthalmopathy (GO). The disease severity (according to the NOSPECS classification) and activity (according to the clinical activity score, CAS) were recorded [25].

Patients with elevated levels of TRAb only were marked as group 1+, those with an additional positive TPOAb or TgAb were marked as group 2+, and those who were positive for all the three antibodies – as group 3+. Patients who had elevated levels of TPOAb were considered as TPOAb + and those with elevated levels of TgAb – as TgAb +.

### *Statistical analysis*

Statistical analyses were done using the SPSS 23.0 statistical package for Windows (SPSS Inc., Chi-

cago, IL, USA). Descriptive statistics and variation analysis were first performed. A normal distribution was tested by the Shapiro-Wilk and Kolmogorov-Smirnov tests. Descriptive statistics were compared between groups via the Mann-Whitney and Kruskal-Wallis tests for continuous variables. ANOVA and correlation analyses were performed. Missing values were not included in the computations. Statistical significance was set as  $p \leq 0.05$ ; all tests were two-tailed.

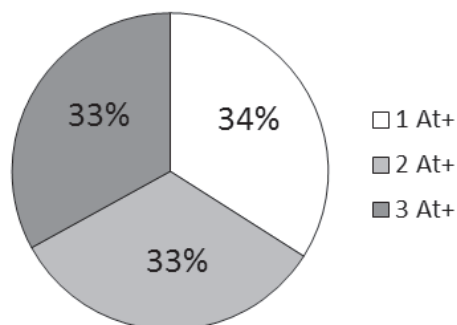
## RESULTS

249 patients with newly diagnosed GD (191 women, 58 men) agreed to participate. Their mean age was  $44.2 \pm 14.4$  years (women only –  $44.2 \pm 14.6$  years; men only –  $44.0 \pm 14.0$  years). 40.2% of them (39.2% of women and 48.2% of men) had symptoms and signs of accompanying GO. The baseline characteristics of the study population are presented in Table 1. There were no significant differences between men and women in their baseline characteristics. 28.7% of all participants were current smokers (29.1% of women and 27.5% of men), 18.7% were previous smokers (17.3% of women and 23.5% of men) and 52.6% were never smokers (53.6% of women and 49.0% of men). In 30.2% there was family history for thyroid disease was positive (32.8% of women and 21.2% of men). 8.5% of patients had a concomitant autoimmune disease (2% vitiligo, 2% type 1 diabetes mellitus, 4.5% – other autoimmune disorder).

64% of all patients were TPOAb positive and 36% were TgAb positive. 51% of all patients had enlarged thyroids (using cutoffs of  $> 18.0$  ml for females and  $> 25.0$  ml for males). 40% of the patients had symptoms and/or signs of GO. It was active in 71% of them. 21% of the patients had thyroid nodules on ultrasound.

## Patient characteristics according to the number of positive antibodies

The patients were equally distributed according to the number of positive antibodies. In one third TRAb was the only elevated antibody, one third had additionally elevated TPOAb or TgAb (in 11% of them it was TgAb and in 89% – TPOAb. In the remaining one third all three antibodies were elevated (Figure 1).



**Fig. 1.** The distribution of the patients according to the number of positive thyroid antibodies is shown

The patients' characteristics according to the number of positive antibodies are displayed as medians in Figure 2. Patients with more positive antibodies tended to be younger (Figure 2 A). The median fT4 was lower in patients who were only TRAb positive (Figure 2 B). The fT3 levels did not differ among the groups. Patients with more positive antibodies had higher levels of TRAb (Figure 2 C), TPOAb (Figure 2 D) and TgAb (Figure 2 E). The thyroid volume was rising with the accumulation of positive antibodies (Figure 2F) and the prevalence of goiter on US was highest in the 3+ group (61%) and lowest in TRAb only positive patients (Figure 2 G). Most of the patients with enlarged thyroid volume were in group 3+ (40%), while those TRAb only positive constituted

**Table 1.** The baseline characteristics of the participants are shown as median values and range

	All subjects (N = 249)		Women (N = 191)		Men (N = 58)	
	Median	Range	Median	Range	Median	Range
Cigarettes /d (if current smoker)	12.5	5-40	10.0	5-40	17.5	5-20
TSH, mUI/L	0.005	0.001-0.410	0.005	0.001-0.169	0.005	0.001-0.410
fT4, pmol/L	45.5	14.1-145.6	45.3	14.1-45.5	45.5	15.5-100.0
fT3, pmol/L	13.2	4.2-50.1	13.2	4.3-50.0	13.2	4.2-50.1
TRAb, UI/L	9.0	1.9-40.00	8.7	2.1-40.0	11.6	1.9-40.0
TPOAb, UI/ml	91.2	5.0-2199.0	75.8	5.0-2199.0	147.2	5.0-1000.0
TgAb, UI/ml	50.5	10.0-4441.0	42.1	10.0-4000.0	80.0	10.0-4441.0
Thyroid volume, ml	18.5	5.0-112.1	17.0	5.0-112.1	22.0	9.0-77.0

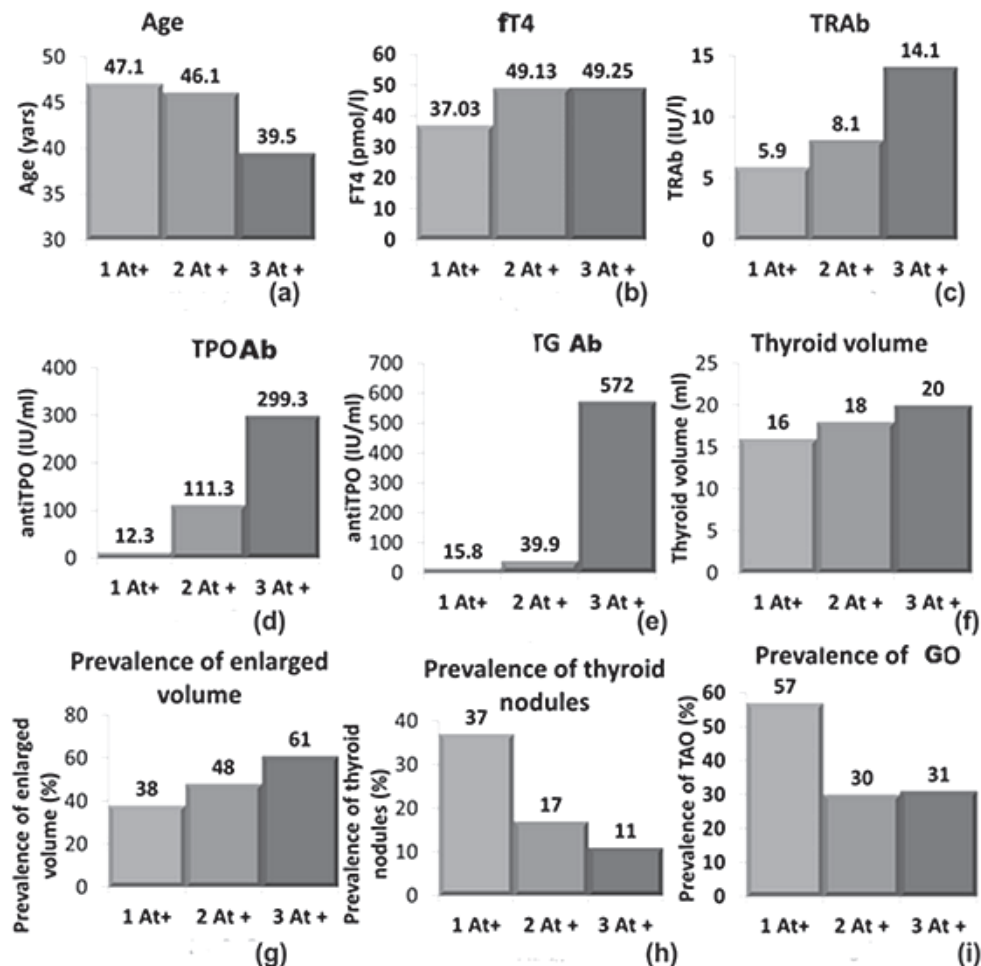
27% of the subgroup with goiter. The presence of thyroid nodules was negatively correlated with the number of positive antibodies (Figure 2 H). Nodules were seen more often in TRAb only positive patients. 63% of all nodules were found in this subgroup, while 20 % of the nodules were found in group 2+ and 17% – in group 3+. The presence of GO was negatively associated with the number of positive antibodies (Figure 2 I). Almost 2/3 of patients in group 1+ had GO vs. less than 1/3 of the patients in the other two groups. 51% of the patients with accompanying GO were in the TRAb only group (1+), while 24% and 25% of them were in group 2+ and 3+ respectively. The severity of GO didn't differ among the groups.

*Patient characteristics according to the presence of TPOAb (see Figure 3)*

64% of the patients were TPOAb+ (n = 160) with a median TPOAb level of 232.9 IU/ml (range 40.0-

2199.0 IU/ml). TPOAb levels were negatively correlated with the patients' age ( $r = 0.182$ ,  $p = 0.004$ ), and those who were TPOAb+ were younger (40 vs. 46.5 years,  $p = 0.004$ ; Figure 3 A). TPOAb+ patients had higher fT4 levels – 46.2 vs. 36.3 mU/l ( $p = 0.018$ , Figure 3 B). There was no correlation between the TPOAb levels and fT3. TPOAb levels were positively correlated with the TgAb (linear  $r = 0.614$ ,  $p < 0.005$ ) and TRAb levels ( $r = 0.204$ ,  $p = 0.003$ ). TPOAb+ patients had higher TgAb and TRAb levels (Figure 3 C and 3 D). Furthermore, the highest levels of each thyroid antibody were observed in the group where all three antibodies were positive ( $p < 0.005$ ).

The thyroid volume was positively correlated with TPOAb (linear  $r = 0.193$ ,  $p = 0.004$ ). TPOAb+ patients had higher thyroid volumes than TPOAb- (Figure 3 E). Patients with increased thyroid volume had higher TPOAb levels – 219.3 IU/ml versus 173.6 IU/ml in



(A) mean age ( $p < 0.005$ ); (B) median fT4 ( $p = 0.014$ ); (C) median TRAb ( $p = 0.018$ ); (D) median TPOAb ( $p < 0.005$ ); (E) median TgAb ( $p < 0.005$ ); (F) median thyroid volume ( $p = 0.015$ ); (G) prevalence of goiter on US ( $p = 0.013$ ); (H) prevalence of thyroid nodules on US ( $p < 0.005$ ); (I) prevalence of GO ( $p = 0.002$ )

**Fig. 2.** The distribution of patients with different number of positive antibodies is shown below according to their main characteristics (the significance of inter-group differences is shown in brackets)



those with normal volumes, respectively ( $p=0.046$ ). 73% of the patients with goiter on US were TPOAb+, while only 56% of those with normal volume were TPOAb+ ( $p = 0.011$ ) (Figure 3 F).

The prevalence of thyroid nodules was lower in TPOAb+ patients (Figure 3 G,  $p < 0.001$ ) and it was negatively correlated with TPOAb levels ( $p < 0.005$ ,  $r = 0.233$ ). Only 35.5% of patients with nodules were TPOAb+, while in those without nodules 71.4% were antiTPO+.

The prevalence of GO was lower in TPOAb+ patients (Figure 3 H,  $p = 0.004$ ) and its severity showed a negative association with the TPOAb levels ( $r = 0.165$ ,  $p = 0.009$ ). Half of the patients with GO were TPOAb+ (53.5%), while in those without GO TPOAb were positive in 70.8%. There was no association between the disease severity and activity and the TPOAb presence or levels.

#### *Patient characteristics according to the presence of TgAb*

TgAb were positive in 36.4% of all GD patients (in 34.4% of the women and in 43.1% of men). Regression analyses (curve estimation procedure) showed correlations of TgAb levels with fT4 (quadratic model,  $R^2 = 0.733$ ,  $p = 0.019$ ), TPOAb levels (linear model,  $R^2 = 0.987$ ,  $p < 0.001$ ), the thyroid volume in ml (linear model,  $R^2 = 0.698$ ,  $p = 0.005$ ), the severity of GO (quadratic model,  $R^2 = 0.657$ ,  $p = 0.041$ ) and the number of cigarettes per day in smokers (linear model,  $R^2 = 0.836$ ,  $p = 0.005$ ). TgAb titers were not correlated with TSH, fT3, levels of TRAb or activity of GO.

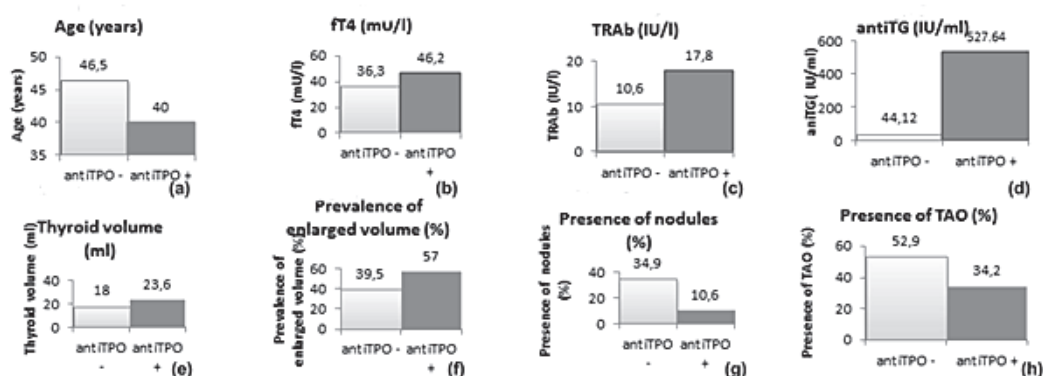
There were significant differences in the clinical characteristics between TgAb positive and TgAb negative patients. TgAb positive patients were younger (42.3 versus 46.0 years,  $p < 0.001$ ), had lower TSH (0.027 versus 0.089 mUI/l,  $p = 0.006$ ) and fT4 levels (56.7

versus 58.8 pmol/l,  $p < 0.001$ ). In addition they had higher levels of TPOAb (281.4 versus 28.48 UI/mL,  $p < 0.001$ ), TRAb (12.82 vs. 7.21 UI/mL,  $p < 0.001$ ), thyroid volume (19 versus 17 ml,  $p < 0.001$ ).

## DISCUSSION

In this cross-sectional study we examined the prevalence and clinical correlations of TPOAb and TgAb positivity in Graves' disease. We found that approximately  $\frac{2}{3}$  of the patients had positive TPOAb, while TgAb were positive in approximately  $\frac{1}{3}$  only. The antibody positivity followed a pattern of thirds: one  $\frac{1}{3}$  of the patients were only TRAb-positive, while  $\frac{1}{3}$  had two positive antibodies (the one being invariably TRAb), and  $\frac{1}{3}$  – all three positive antibodies. Positive TPOAb antibodies were found in younger patients, with higher fT4 and TRAb levels, higher thyroid volume and lower prevalence of nodules and GO. The positive TgAb were also found in patients at younger age, higher thyroid volumes, fT4, TRAb and TPOAb levels, as well as with lower TSH levels and severity of GO. The same trend was seen if these clinical parameters were correlated with the number of positive antibodies.

Elevated levels of TPOAb and TgAb are hallmarks of the underlying autoimmune disease and are more often screened for in autoimmune thyroiditis. The prevalence of positive TPOAb and TgAb antibodies in the setting of GD has been studied in the last 20 years. However, advances in immunology have modified the laboratory techniques for antibody detection and refined the search for different epitopes [13, 26-28]. This might affect the prevalence of antibody positivity as well as their predictive power. After all, very little is known about the associations of TPOAb and TgAb-positivity and different characteristics of GD.



(A) patients' age ( $p = 0.004$ ); (B) fT4 levels ( $p = 0.018$ ); (C) TRAb levels ( $p = 0.001$ ); (D) TgAb levels ( $p = 0.001$ ); (E) thyroid volume ( $p = 0.01$ ); (F) prevalence of enlarged volume ( $p = 0.01$ ); (G) presence of thyroid nodules ( $p < 0.001$ ); and (H) presence of GO ( $p = 0.004$ )

**Fig. 3.** The differences in the hormonal and ultrasound patients' characteristics according to the TPOAb positivity or negativity are shown (the significance of inter-group differences is shown in brackets)

Publications around the millenium have reported different numbers for the prevalence of TPOAb and TgAb positivity. S. Goh et al. compared thyroid antibody profiles in patients referred to Thyroid Clinics and Eye Clinics: TPOAb was positive in 79.6% and 43.7%, while TgAb – in 73.2% and 36.7% respectively [8]. D. Khoo et al. found 91.2% TPOAb positivity in GD and 62.8 % – in GO, while TgAb positivity was 77.2% and 65.1% [9]. Both author groups suggested higher risks for GO in TRAb only positive patients. Testing new quantitative measurements of autoantibodies J. Gilmour et al. registered 87% TPOAb and 73% TgAb positivity in typical GD patients [4]. In a national study TPOAb were positive in 86.3% and TgAb – in 43.1% of all GD patients [7]. All these studies were of moderate size (with a mean of around 100 participants). More recent studies have focused mainly on TPOAb positivity, which was found in 76.4% of 802 French patients with GD 15; in 7% of 111 Omani GD patients [11] and in 56.8% of 44 patients with subclinical hyperthyroidism due to GD [29]. Our data of 64% TPOAb-positivity and 36% TgAb positivity show a lower prevalence than those in the listed publications. The proportions of patients with two or three positive thyroid antibodies were not reported separately in the literature. Y. Hu et al. studied 49 GD patients and found all three positive antibodies in 48.9% which is higher than our result of only 33% [30].

A more important question is whether finding positive TPOAb and/or TgAb in GD can add practical information for the clinician. GD patients may be stratified according to the number of positive antibodies at disease presentation. Those groups would differ in age, levels of FT4 and TRAb, thyroid volume and the presence of GO or thyroid nodules. Younger age of patients with higher numbers of positive antibodies would suggest a more complex immune intolerance. Levels of FT4 and thyroid volume could affect the dose and schedule of antithyroid drug treatment. The presence of nodules is crucial for performing FNA and screening for possible thyroid malignancies, which are more often encountered in nodules in the setting of GD [31, 32]. The two most important predictions would concern the possible future manifestation of GO as well as the probability for remission of the GD itself.

Considered separately TPOAb positivity in our newly diagnosed GD patients was, associated with higher thyroid volume, FT4 levels and lower prevalence of GO and thyroid nodules. Two author groups found that absent TPOAb antibodies and high TRAb levels identified a group at markedly increased risk of ophthalmopathy [8, 33, 34]. In a previous work we came to a similar conclusion: TPOAb positive GD patients

rarely developed GO, while TgAb positivity was associated with an increased risk [17]. A recent study in 399 GD patients confirmed the protective role of TPOAb positivity in relation to GO [12]. In this study TPOAb < 20 kIU/L and/or TRAb > 6.3 IE/L at the time of GD diagnosis were associated with an increased risk of developing GO after the diagnosis of GD was made [12]. Although the causative effect of TPOAb in GO might be questionable, there are studies detecting thyroid peroxidase mRNA and protein in the orbital tissue [35]. Our finding of a positive association of TPOAb levels with serum FT4 is intriguing, but it could be also due to a lower type II deiodinase enzyme activity [36]. In a study addressing the natural history of subclinical hyperthyroidism in GD, positive TPOAb were associated with risk of progression to overt hyperthyroidism – with hazard ratios from 1.06 to 10.15 per year [29]. In a histological study higher TPOAb levels were correlated with intra-thyroidal germinal center development, suggesting a more aggressive immunological attack [37]. The associations of TPOAb with higher thyroid volumes and a lower prevalence of nodules still need an explanation. We found practically no previous studies addressing these issues. A great contribution of TPOAb positivity would be the prediction of the probability for remission (or risk of relapse) after successful treatment with ATD. There are studies confirming improved prediction by using the combination of TRAb and TPOAb antibodies, especially when TRAb levels remain in the non-diagnostic “grey” zone [14]. Others found no use of TPOAb levels at GD presentation in predicting the risk of future relapses [7, 10]. A few authors found non-significant trends for higher TPOAb in the presence of GD relapse [2, 38]. Anyhow, our study performed a cross-section at study presentation and is therefore not suited to answer the question about future relapses.

Considering TgAb positivity separately, our observations were not quite different from those about isolated TPOAb positivity. Both antibodies were positively correlated ( $r = 0.614$ ;  $p < 0.005$ ). From a clinician's perspective TgAb are less prevalent and may be less useful than TPOAb for prediction of thyroid dysfunction and the disease course. The role of TgAb has been examined with special focus on GO. Thyroglobulin was identified in orbital tissues of patients with GO [39]. This is in line with the original hypothesis of J.P. Kriss et al. for an ethiological role of TgAb in GD presentation and progression [40], which was endorsed by other investigators, as well as recently reviewed [20]. Immunization with thyroglobulin induced Graves-like disease in mice [41]. Absent TgAb were associated with higher prevalence of GO in the hall-

mark studies by S. Goh et al., and D. Khoo et al. [9, 33, 34]. In a previous study we came to the opposite conclusion – TgAb positivity was associated with an increased risk for the presence of GO [17]. Some authors reported a limited usefulness of anti-peroxidase and anti-thyroglobulin assays in GD patients and recommended testing for TPOAb without TgAb as a cost-effective approach [10]. Others found a place of TgAb assays in the diagnosis of GD only if TPOAb were negative [13]. TgAb might also be used for the prediction of future or expected relapses (respectively at disease presentation and at treatment end). The data on that topic are very scarce. A national study found that TgAb negativity was predictive for better outcomes (no relapse) both at GD presentation and treatment end [7]. Therefore, the place of TgAb in GD seems to need future clarification.

Our study has some limitations. First we weren't able to determine the biological effects of TRAb (stimulating, blocking or cleavage antibodies) and to assess if the type was relevant for the clinical presentation, especially the presence of GO as previously discussed [42]. Finally, our results might be affected by the fact that the study was performed in a specialized tertiary clinic, which might have introduced a bias towards more resistant or severe cases.

## CONCLUSIONS

The different clinical characteristics of GD patients with different thyroid antibody profiles may be due to a variation in the pathogenesis of the disease itself. It seems appropriate to follow the progression of the disease, treatment response and complications in patients with different antibody profiles. A different approach based on the antibody profiles might be suitable.

## Ethical considerations

Ethical clearance for this study was obtained from the Medical Ethics Committee of the Medical University of Sofia.

## Disclosure

This study was partly supported by the PhD program 2016 at the Medical University Sofia.

## Conflicts of interest

The authors declare no conflicts of interest.

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