

Positive TgAbs in patients with Graves' orbitopathy are associated with lower risk of its active form – preliminary study

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Abstract

INTRODUCTION: In this retrospective, single-centre cohort study, we report our 10-year experience concerning clinical manifestation, diagnosis and treatment of Graves' orbitopathy (GO), with particular regard to antithyroid antibodies in iodine sufficient Polish population.

MATERIALS AND METHODS: Data on thyrometabolic and immunological status of 71 patients diagnosed with GO, were collected. Also, we gathered information on the selection of the applied treatment in these cases of Graves' disease (GD): pharmacological, radioiodine or surgery. The chi-square test, correlation coefficient and univariate logistic regression analysis were used to determine the influence of clinical parameters on activity of GO.

RESULTS: Significantly lower frequency of active GO in positive anti-thyroglobulin antibodies (TgAbs) patients was documented ($p < 0.05$, chi-square analysis). Expectedly, concentration of antibodies against thyrotropin receptor (TRAbs) did constitute linear factor positively associated with clinical activity score (CAS) ($p < 0.05$). Moreover, the pretreatment with radioiodine and smoking were significantly associated with the increased GO activity ($p < 0.05$ and $p < 0.05$, respectively).

CONCLUSION: Our data suggest some kind of "protective" effect of TgAbs presence against the active form of GO.

INTRODUCTION

Graves' disease (GD) is a relatively common organ-specific autoimmune disease characterized by the presence of antibodies against thyrotropin receptor (TRAbs). Graves' orbitopathy (GO) is the most common extra-thyroidal manifestation of GD, occurring in 25% of patients with GD (Tanda *et al.* 2013). GO may occur during or after the onset of

hyperthyroidism and less frequently also, in euthyroid or hypothyroid patients. The prevalence of GO seems to be declining in the last decades (Perros *et al.* 2017). Nowadays, GO is usually mild and rarely progresses to more severe forms (Pianntanida *et al.* 2013). The possible explanation of this fact could be modification of risk factors, such as decrease in tobacco smoking prevalence and more effective restoration of euthyroidism.

Nevertheless, it is not completely clear which factors lead to the development of GO in the individual patient with GD. While TRAbs appear to be useful in predicting the course of GO and response to therapy, its predictive value regarding onset of GO has not been well-documented; only some patients with positive TRAbs develop GO which suggests that other antibodies may be of importance. The contribution of anti-thyroglobulin antibodies (TgAbs) and thyroperoxidase antibodies (TPOAbs) to GO development is mostly unclear. TgAbs and TPOAbs can be detected in peripheral blood in about 10% of healthy subjects, however their prevalence among GD patients is higher, i.e. about 50–60%. Furthermore, the relationship between TgAbs or TPOAbs levels and course of GO may differ depending on the iodine supply. Therefore, we decided to evaluate the association between TgAbs or TPOAbs and the course of GO in patients suffering from GD and living in iodine sufficient area.

As it was mentioned above, iodine supply has an impact on thyroid autoimmunity. A mandatory program of salt iodization for households has been introduced in Poland in 1997 (Zygmunt *et al.* 2014) and currently Poland is considered to be iodine sufficient country. Differences in endemic iodine intake are very important for the occurrence of GD. In areas with high to normal iodine intake, the incidence of new cases of GD is higher when compared to areas with low iodine intake. The earlier study revealed that salt iodization was not associated with a change in the incidence of GO in Danish population (Lauberg *et al.* 2012).

In the present retrospective, single-centre cohort study, we report our 10-year experience concerning clinical manifestations, diagnosis and treatment of GO, with particular regard to serum concentrations of antithyroid antibodies in iodine sufficient Polish population.

METHODS

The medical records of 71 patients hospitalized and further treated for GO in the last 10 years in the Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, were retrospectively analyzed. The diagnosis of GO was based on standard criteria, including positive tests for TRAb and ophthalmic assessment. GO was classified as clinically active or inactive and as mild, moderate to severe, as recommended by the European Group on Graves' Orbitopathy (EUGOGO) (Bartanela *et al.* 2016). To exclude other reasons for proptosis, orbital imaging [magnetic resonance imaging (MRI) or computed tomography (CT)] was performed.

The primary outcome measures were thyrometabolic and immunologic conditions of patients and secondary outcome measures - the clinical phenotype of GO, as well as quality of life (QoL). Quality of life was assessed with the disease-specific Graves' orbitopathy

quality of life questionnaire (GO-QoL). It is a validated Polish version of the GO-QoL, which consists of 15 questions summarized in two subscales. The first one refers to visual functioning and the second one refers to psychosocial consequences of changed appearance. Also, we gathered information on the selection of the applied treatment in these cases of GD: pharmacological, radioiodine or surgery.

Statistical analysis

We summarized the main demographic and clinical features, using means and standard deviations, for continuous and categorical variables, respectively. Descriptive statistics were estimated in the overall patient population. Further statistical analyses were performed using the Statistica 13.1. The chi-square test and correlation coefficient were used to determine the influence of clinical features on the activity of GO. Univariate logistic regression analysis was used to determine which continuous variable might have been associated with active GO assessed by clinical activity score (CAS) and MRI. P value less than 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the characteristics of the patients enrolled into the study. To define the activity of GO we assigned a CAS according to EUGOGO recommendations. A CAS ≥ 3 , indicating active GO, was observed in 74.6% of patients. Orbital MRI revealed active GO in 47% patients, increased signal intensity in T2 in 31.5% patients, increased fat volume in 36.8% patient, and increased muscle volume in 31.5% patients. The association between GO activity in MRI and CAS value was confirmed ($p < 0.05$) by chi-square test. Moreover, variables such as age, TSH, FT3, FT4, TRAb, TPOAb, TgAb levels were subjected to a univariate regression model. The purpose of this model was to determine which of those continuous variables might have been independently associated with GO activity, increased intensity of T2 signal, increased volume of fat and muscle in MRI; the age did constitute linear factor positively associated with GO activity and increased intensity of T2 signal in MRI with statistical significance ($p < 0.05$ and $p < 0.05$, respectively) (Table 2 and Table 3).

Among 23 patients with active GO, we dispensed GO-QoL questionnaire; 19% of answers confirmed that patients' visual functioning and appearance are seriously limited/impaired, 33% of answers confirmed that their visual functioning and appearance are a little limited/impaired and 48% of answers confirmed that there is no disturbance in visual function and appearance. The most frequently limited activities were reading (30% of answers "seriously limited"), doing hobbies or pastimes (22% of answers "seriously limited"), watching TV (13% of answers "seriously limited") and driving (also 13% of answers "seriously limited"). Most partici-

pants perceived the changed appearance (52%), showed a weakened self-confidence (27%), avoided appearing in the pictures (27%) and sought to hide changes in appearance due to disease (27%).

TRAb test was positive in the majority of subjects (91.8%), TPOAb test was positive in more than a half (60%), and TgAb test was positive in about one third (35.9%) (Table 1). Among measured linear variables, concentration of TRAb did constitute linear factor positively associated with CAS in patients with active GO ($p < 0.05$) and after radioiodine treatment ($p < 0.05$). There was significantly lower frequency of active GO in TgAb-positive patients in comparison to TgAb-negative patients ($p < 0.05$, chi-square analysis) (Figure 1). In contrast, such a relation was not observed for TPOAbs, which presence did not influence GO activity in any significant way ($p = 0.613$).

Moreover, we noted a negative correlation between the concentration of TgAbs and CAS value in a group of smokers ($p < 0.05$) and in a group of patients not treated with radioiodine ($p < 0.05$).

There was a large preponderance of female (81.7%) and the mean age of patients was 48.0 ± 13.8 years. At diagnosis, 71.8% patients were hyperthyroid and 28.2% patients were euthyroid or hypothyroid. Furthermore, 62% patients were on antithyroid drug (ATD) treatment, while about 5% were on substitution therapy with L-thyroxine. When radical treatment was considered, almost one third of patients (28.2%) underwent radioiodine treatment and 10% underwent thyroid surgery before diagnosis of GO (Table 1). Accordingly to the results of chi-square test, the pretreatment with radioiodine was significantly associated with increased GO activity ($p < 0.05$), in contrast to surgery ($p = 0.49$). The prevalence of current smokers was around 39% and significant relation was found between smoking habits and activity of GO assessed by CAS ($p < 0.05$).

DISCUSSION

The most important finding of the present study is the negative relation between levels of TgAb and GO activity, which - in a certain sense - can be treated as the "protective" role of TgAbs in developing of the active form of GO, especially in smoking patients. Formerly, authors have demonstrated that patients with GO had significantly higher TRAb but lower TPOAb and TgAb levels (Goh *et al.* 2004). Antibodies against TPO have also been observed to correlate negatively with the onset of orbitopathy in GD patients (Lantz *et al.* 2014), however we have not confirmed this in our study. Recently published data shows that 87% of patients who developed GO, had TRAbs above 6.3 IU/L and TPOAbs below 20 IU/ml (Lantz *et al.* 2014). In concordance with our results, authors of the earlier study revealed that combination of TPOAb and TRAb does not exert any other effect on the activity and severity of GO than the effect of TRAb alone (Eckstein *et al.* 2004). Our data

Tab. 1. Characteristics of patients included in the study

Characteristics	All patients (n=71)
Age at study enrollment Mean \pm SD	47.99 \pm 13.77
Gender	
Female	58 (81.7%)
Male	13 (18.3%)
TSH[mIU/L]	2.51 \pm 5.68
FreeT3 [pg/ml]	4.33 \pm 3.78
Free T4[ng/dl]	1.43 \pm 0.89
^a Euthyroid/Hypothyroid	21 (29.6%)
Hyperthyroid	50 (70.4%)
^b TRAb[IU/L]	15.41 \pm 13.87
Positive	63 (91.8%)
Negative	6 (8.2%)
No data	2
^c TPOAb [IU/ml]	159.61 \pm 128.22
Positive	39 (60%)
Negative	26 (40%)
No data	6
^d TgAb [IU/ml]	211.36 \pm 317.32
Positive	22 (35.9%)
Negative	41 (64.1%)
No data	7
CAS value	
≥ 3	53 (74.6%)
< 3	18 (25.4%)
Antithyroid drug treatment	
Yes	44 (62%)
No	27 (38%)
L-thyroxine administration	
Yes	4 (5.6%)
No	67 (94.4%)
¹³¹ I	
Yes	20 (28.2%)
No	51 (71.8%)
Surgery	
Yes	7 (9.9%)
No	64 (90.1%)
Smoking	
Yes	28 (39.4%)
No	43 (60.6%)
No data	0
MRI	
Active	18 (47%)
Inactive	20 (53%)
No data	33

^a Patients were considered euthyroid when TSH 0.27–4.2 mIU/L; FT3 2.6–4.4 pg/ml and FT4 0.93–1.7 ng/dl.

^b TRAb - negative < 1.75 IU/L

^c TPOAb - negative < 34 IU/ml

^d TgAb - negative < 115 IU/ml

suggest "protective" role of TgAbs in GO, thus indicates the need to determine TgAbs concentrations in patients with GO. Antibodies against Tg could be another prognostic factor of GO that could facilitate the prognosis

Tab. 2. Univariate logistic regression analysis of the clinical and laboratory parameters (variables), performed in patients with active and inactive GO in MRI. OR - odds ratio; CI - confidence interval; the level of statistical significance is given in italic; * $p < 0.05$.

Variable	Univariate regression		
	OR	95% CI	<i>p</i>
Age [years]	1.09	1.01-1.02	<i>0.01*</i>
TSH [mIU/L]	0.99	0.92-1.07	<i>0.87</i>
FT4 [ng/dl]	0.88	0.44-1.78	<i>0.72</i>
FT3 [pg/ml]	0.99	0.84-1.15	<i>0.91</i>
TPOAb [IU/ml]	1.00	0.99-1.00	<i>0.32</i>
TgAb [IU/ml]	1.00	0.99-1.00	<i>0.22</i>
TRAb [IU/L]	1.02	0.97-1.07	<i>0.35</i>

and therapeutic process of the disease. Our findings may also shed some more light upon the role of TgAbs in GO etiopathogenesis.

In our study, the active GO was constituted 74.6% of all GO cases examined. To the contrary, Savku and Gunduz (2014) demonstrated that GO was in active phase in 32.6% of patients with GO. The explanation of this discrepancy is the fact that the patients are most often referred to our Department for administration of glucocorticoid therapy, thus with a moderate or severe active form of GO.

Furthermore, we have also confirmed that GO activity in MRI is related to CAS. Using the logistic regression analysis we have proved that age is positively associated with GO activity and with increased signal in T2 images in MRI. The results published so far point at association between high signal intensity in T2 images and good response to methyloprednisolone therapy (Müller-Forell & Kahaly, 2012). In the light of aforementioned observations, we could hypothesize that older people have more often active GO but also might respond better to immunosuppressive treatment, however this hypothesis requires further research. In addition, we have demonstrated that GO-QoL is the best tool to measure how deeply ophthalmic manifestations of the disease impair psychological and social areas of

patients' life. In case of our patients, the problem with the acceptance of the appearance was the most often reported. Therefore, GO-QoL and MRI could be helpful when making therapeutic decisions or measuring the efficacy of treatment.

Many studies have reported the relationship between TRAb levels and GO activity (Savku and Gunduz 2014). In the current study, TRAb tests were positive in the majority of subjects (88.7%) and, similarly, we found higher TRAb levels in patients with active GO assessed by CAS. Our results confirm previous findings that TRAbs concentration is a direct and strong factor correlated with the GO activity (Gerding *et al.* 2000), thus TRAb levels are used to predict or to prognose the response to immunosuppressive therapy.

In the present study, the female preponderance in GO patients was confirmed and the mean age was around 48 years. Similarly, Tanda *et al.* (2013) showed that the large majority of patients with GO were females and that mean age of patients with GO in Italian population was 45.7 years. In our study, the majority of GO patients suffered from hyperthyroidism, whereas about one third were euthyroid or hypothyroid. The study performed in a Danish population showed that 87% patients were hyperthyroid, whereas 13% were euthyroid or hypothyroid (Laurberg *et al.* 2012).

Tab. 3. Univariate logistic regression analysis of the clinical and laboratory parameters (variables), performed in patients with increased signal intensity in T2 in MRI. OR - odds ratio; CI - confidence interval; the level of statistical significance is given in italic; * $p < 0.05$.

Variable	Univariate regression		
	OR	95% CI	<i>p</i>
Age [years]	1.09	1.00-1.18	<i>0.02*</i>
TSH [mIU/L]	0.93	0.79-1.08	<i>0.34</i>
FT4 [ng/dl]	0.90	0.76-1.15	<i>0.79</i>
FT3 [pg/ml]	0.94	0.42-1.94	<i>0.54</i>
TPOAb [IU/ml]	1.00	0.99-1.00	<i>0.17</i>
TgAb [IU/ml]	1.00	0.99-1.00	<i>0.15</i>
TRAb [IU/L]	1.00	0.95-1.06	<i>0.84</i>

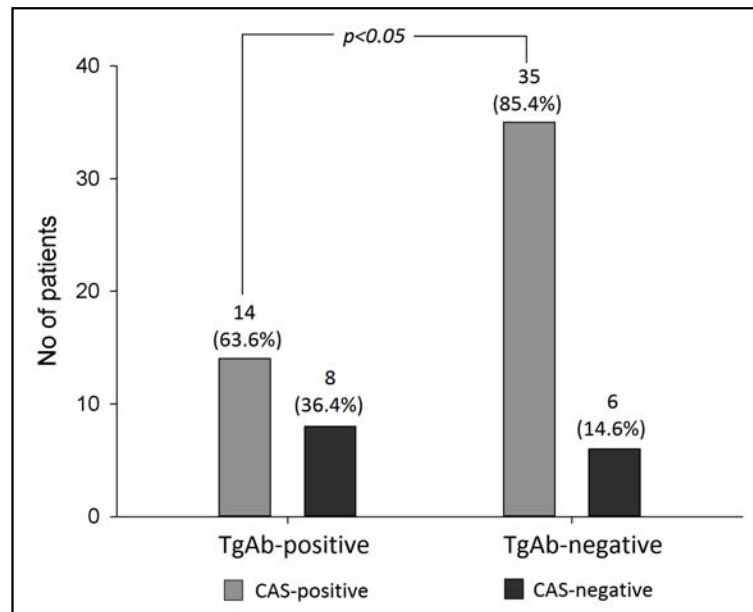


Fig. 1. The number of patients (expressed also as percentage) with active and inactive form of GO in two evaluated groups: TgAbs-positive and TgAbs-negative; as shown there is a significant difference between TgAb-positive, CAS-positive patients and TgAb-negative, CAS-positive ones ($p < 0.05$, chi-square analysis).

There is strong evidence on relation between smoking with the development of GO and its deterioration (Stan and Bahn, 2010). Results of our study showed that the prevalence of current smokers was around 39% and significant association was found between smoking habits and activity of GO. Similar results were obtained by Tanda *et al.* (2013), in their study, 35.3% patients were current smokers.

We have proved association between pretreatment with radioiodine and GO activity. Radioiodine has been shown to be a significant risk factor for the development or worsening of GO (Roos *et al.* 2018). The majority of patients developing GO after radioiodine therapy had no overt signs of GO before treatment (Sisson *et al.* 2008). The authors of the recent study have shown that smoking might be an additional risk factor (beside radioiodine) for GO development (Traisk *et al.* 2009), therefore, qualification for this treatment in the group of smoking GD patients should be very cautious.

To conclude, our results are consistent with the reports of other authors regarding clinical manifestation of GO. Furthermore, our data suggest some kind of “protective” effect of TgAbs presence against development of the active form of GO and support opinion that GO is likely to be multi-antigenic disease with the important role of TRAbs and TgAbs

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COMPETING INTERESTS

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study.

AUTHORS' CONTRIBUTIONS

KW-D designed and coordinated the study, as well as wrote draft of a manuscript.

AZ performed statistics.

AM participated in coordination of the study.

MK-L participated in designing and writing a manuscript

AL senior author, approved the study and wrote the final version of the manuscript.

All authors have read and approved the final manuscript.

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