Endocrine Journal 2016, **63** (2), 151-157

ORIGINAL

TSH receptor antibody titers measured with a third-generation assay did not reflect the activity of Graves' ophthalmopathy in untreated Japanese Graves' disease patients

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Abstract. TSH receptor antibody (TRAb) titer has been reported to be correlated with Graves' ophthalmopathy (GO). However, the correlation between GO activity and TRAb titer assessed with a third-generation assay has not been reported. We enrolled 238 untreated Graves' disease patients who came to the outpatient clinic of Ito Hospital and 28 patients who were euthyroid. All of the patients were assessed for GO by an ophthalmologist within 3 months of their initial visit to Ito Hospital. Clinical activity score (CAS), short inversion time inversion recovery (STIR), and sum of the maximum external orbital muscle areas (SEOMA) on a frontal sectional magnetic resonance imaging (MRI). The TRAb titer was significantly higher in patients with inactive ophthalmopathy (the inactive-GO group) than in patients with active ophthalmopathy (the active-GO group) (17.7 ± 13.5 IU/L *vs* 13.0 ± 13.1 IU/L, p=0.0082). The SEOMA values were not correlated with TRAb titer. The prevalence of active-GO was higher in euthyroid patients than in hyperthyroid patients although the difference was not significant. In conclusion, TRAb titer measured with a third-generation assay dose not correlate with GO activity based on MRI findings in untreated Graves' disease patients, and the prevalence of active-GO is higher in euthyroid patients with lower TRAb titers than in hyperthyroid patients.

Key words: Graves' ophthalmopathy, TSH receptor antibody, Clinical activity score, Magnetic resonance imaging

GRAVES' OPHTHALMOPATHY (GO) is clinically associated with autoimmune thyroid disease, and it primarily affects orbital fat and the extraocular muscles. Autoantibodies to thyroidal antigens, particularly TSH receptor antibodies (TRAb), may be involved in the disease progression of GO. Although the pathogenesis of GO is still unknown, one potential explanation for the involvement of the orbital contents in Graves' disease is that the existence of target antigens common to the thyroid and the retro-ocular tissues against which the autoimmune response is directed [1-3]. The results of a number of studies support the hypothesis of TRAb involvement in GO. Although a role of TRAb in GO is now accepted by many researchers and clinicians, the use of TRAb in the management of GO has been less well studied than their use in the diagnosis and monitoring of Graves' disease.

In recent years some researchers have reported finding correlations between TRAb titer and the severity and activity of GO [4], and their findings were made possible by a second generation assay for TRAb [5]. Smith and colleague reported a human thyroid-stimulating monoclonal autoantibody to the TSH receptor [6] and developed a novel third-generation TRAb assay that has considerable advantages over earlier assays in terms of high sensitivity for the diagnosis of Graves' disease [7, 8]. However, there have been no reports of a correlation between GO activity and TRAb titer measured with a third-generation assay in untreated Graves' disease patients.

In this study we investigated whether TRAb titer measured by a third-generation assay correlate with the activity of GO in untreated Japanese Graves' disease patients.

Submitted Mar. 7, 2015; Accepted Oct. 29, 2015 as EJ15-0137
Released online in J-STAGE as advance publication Nov. 18, 2015
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Materials and Methods

Patients

We enrolled untreated 238 Graves' disease patients with Graves' ophthalmopathy (188 women and 50 men), who came to the outpatient clinic of Ito Hospital for the first time between January 1, 2010 and December 31, 2010 and were referred to Olympia Eye Hospital for evaluation of GO within 3 months of their initial visit to Ito Hospital. A diagnosis of Graves' disease was made when a patient had typical hyperthyroid symptoms (tachycardia, body weight loss, tremor, sweating), a diffuse goiter, or ophthalmic symptoms and was TRAb positive. In TRAb-negative patients, radioiodine uptake ratio was measured, and Graves' disease was diagnosed when the uptake ratio was above 30%. Euthyroid Graves' disease was diagnosed when ophthalmopathy was present with normal thyroid function irrespective of the results of the TRAb assay. Characteristics of the patients are shown in Table 1. The active-GO group was significantly older than the inactive-GO group. Thyroid hormones were significantly higher than in the inactive-GO group than the active-GO group. Twenty eight patients were euthyroid, aged 13 to 78 years and 22 patients were female. Patients with a history of prior treatment for thyroid disease, neck irradiation, or neck surgery were excluded from this study. This study was approved by the ethics committee of Ito Hospital, and informed consent was obtained from all of the patients.

Evaluation of Graves' ophthalmopathy and grouping of the patients

All of the patients were referred to Olympia Eye Hospital within 3 months after their initial visit to Ito Hospital and received a routine ophthalmologic examination and a magnetic resonance imaging (MRI) examination. Clinical activity score (CAS) was graded according to the criteria developed by modified CAS system by EUGOGO [9]. Graves' ophthalmopathy was diagnosed with symptoms (eyelid swelling, eyelid retraction, proptosis, eye muscle restriction, and redness of conjunctiva) and MRI findings (increased orbital fat volume, orbital muscle enlargement, and prolonged short inversion time inversion recovery (STIR)) by expert ophthalmologists.

The patients were divided according to age, into an older group 40 years of age and over and a younger group under 40 years of age; and according to their CAS, into a low CAS group whose CAS was 0 and a high CAS group whose CAS was more than 1.

Blood tests and MRI measurements

All patients received a physical examination that included thyroid palpation, blood tests, and measurement of their serum free triiodothyronine (FT3), free thyroxine (FT4), TSH, and TRAb levels. TSH, FT3, and FT4 were measured by chemiluminescence assays (ECLusis TSH, FT3, and FT4, Roche Diagnostics Ltd., Basel, Switzerland), and their normal ranges at Ito Hospital are TSH, 0.2-4.5 μIU/L; FT3, 3.37-6.58 pmol/L; and FT4, 10.3-20.6 pmol/L. TRAb was also measured by chemiluminescence (ECLusis TRAb, Roche Diagnostics Ltd., Basel, Switzerland), and a serum concentration greater than 2.0 IU/L was considered positive. Graves' ophthalmopathy was considered active when the short inversion time inversion recovery (STIR) of the external orbital muscles in an orbital

Table 1 Characteristics of the patients and GO activity

		All n=238	GO activity according to STIR		*p* value
			Inactive n=195	Active n=43	
Age (yrs)	Median (range)	36 (7-74)	35 (7-71)	47 (16-74)	*<0.0001*
Female	n (%)	188 (79)	154 (79)	34 (79)	NS
History of smoking	n (%)	80 (34)	66 (34)	14 (33)	NS
Clinical activity score (n)	0	76	70	6	
	1	112	85	27	
	2	40	31	9	
	≥3	10	9	1	
FT3 (pmol/L)	Median (range)	23 (4-50)	27 (4-50)	13 (4-48)	*0.0024*
FT4 (pmol/L)	Median (range)	59 (9-100)	65 (9-100)	43 (12-100)	*0.0002*
Exophthalmos (mm)	Median (range)	16.5 (11-22)	17 (9.5-24)	16.5 (11-22)	NS

Analysis by Wilcoxson test for values expressed as median with range.

MRI examination was above 1. The external orbital muscle area was calculated as the maximum muscle area of each muscle, and the sum of the external orbital muscle areas (SEOMA) was calculated bilaterally on frontal section MRI scans.

Statistical analysis

Age, FT3, FT4 and TRAb titers are reported as the median value and range. The chi-square test were used for comparing frequencies and Wilcoxon's test were used for comparing means of two groups. A p-value less than 0.05 was considered statistically significant. The statistical analyses were performed by using JMP 11.0 programs for Windows (SAS Institute, Cary, NC, USA).

Results

Characteristics of the patients

The characteristics of the patients are shown in Table 1. Most of the patients had a low CAS, and 79% of the patients had a CAS of 0 or 1 point. FT3 and FT4 were significantly higher in the inactive-GO group than in the active-GO group.

Correlations between GO activity, CAS, and sum of external orbital muscle areas (SEOMA)

The correlation between GO activity and SEOMA is shown in Fig. 1(a). SEOMA was significantly larger in the active-GO group than in the inactive-GO group (3.37 ± 0.95 cm^2 *vs* 2.78 ± 0.39 cm^2, $p < 0.0001$). There was no significant correlation between CAS and SEOMA (Fig. 1(b)).

Prevalence of active-GO patients in each age group

The prevalence of active-GO in each age group is shown in Fig. 2. The prevalence of active GO in the older group was significantly higher than in the younger group (Fig. 2 (a), 33.3% *vs* 9.3%, $p < 0.0001$). However, the difference between the age groups in prevalence of high CAS was not significantly different (Fig. 2 (b)).

TRAb titers and thyroid hormone levels according to GO activity and CAS

The TRAb titers in each GO activity group and CAS group are shown in Fig. 3(a). The TRAb titers were significantly higher in the inactive-GO group than in the active-GO group (17.7 ± 13.5 IU/L *vs*. 13.1 ± 13.1 IU/L, p=0.0082). There was no significant correlation between CAS and TRAb titers (Fig. 3(b)). Both FT3 and FT4 levels were significantly lower in the active-GO group than in the inactive-GO group (data not shown). In contrast, there was no significant correlation between thyroid hormone levels and CAS.

GO in euthyroid and hyperthyroid Graves' disease patients

In 28 patients with GO thyroid function was normal

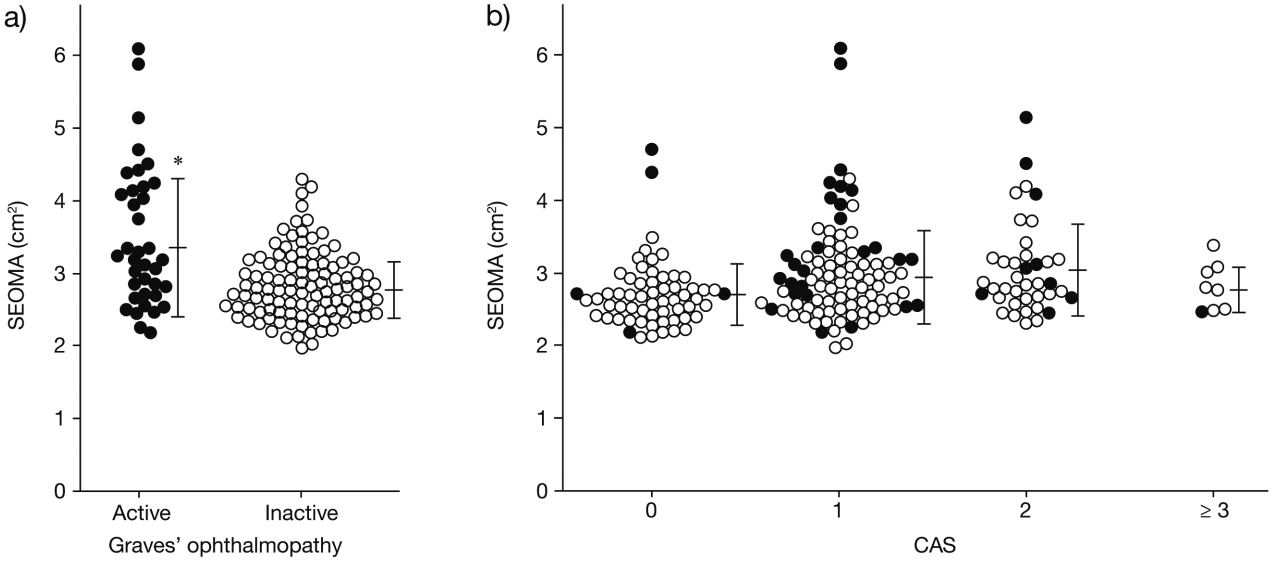

Fig. 1 Correlations between GO activity (a), CAS (b), and the sum of maximum external orbital muscles areas (SEOMA). Active GO subjects with STIR are indicated in black dots. * p<0.0001 *versus* inactive GO.

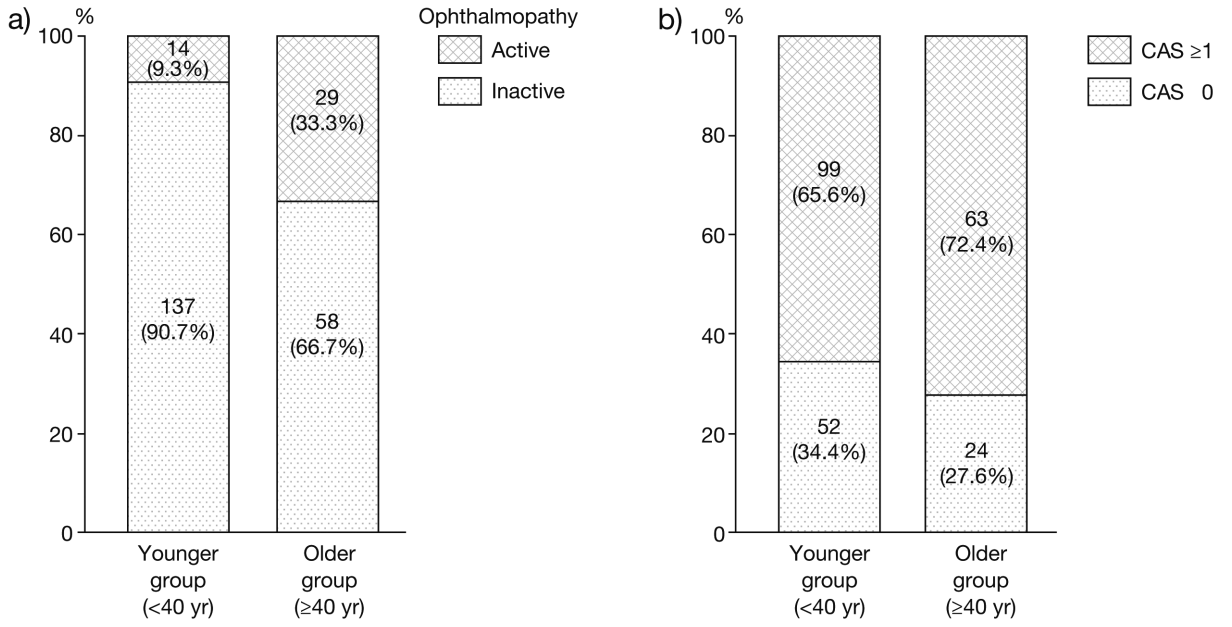

Fig. 2 Prevalence of active GO in each age group (GO activity evaluated on the basis of STIR (a) and CAS (b)).

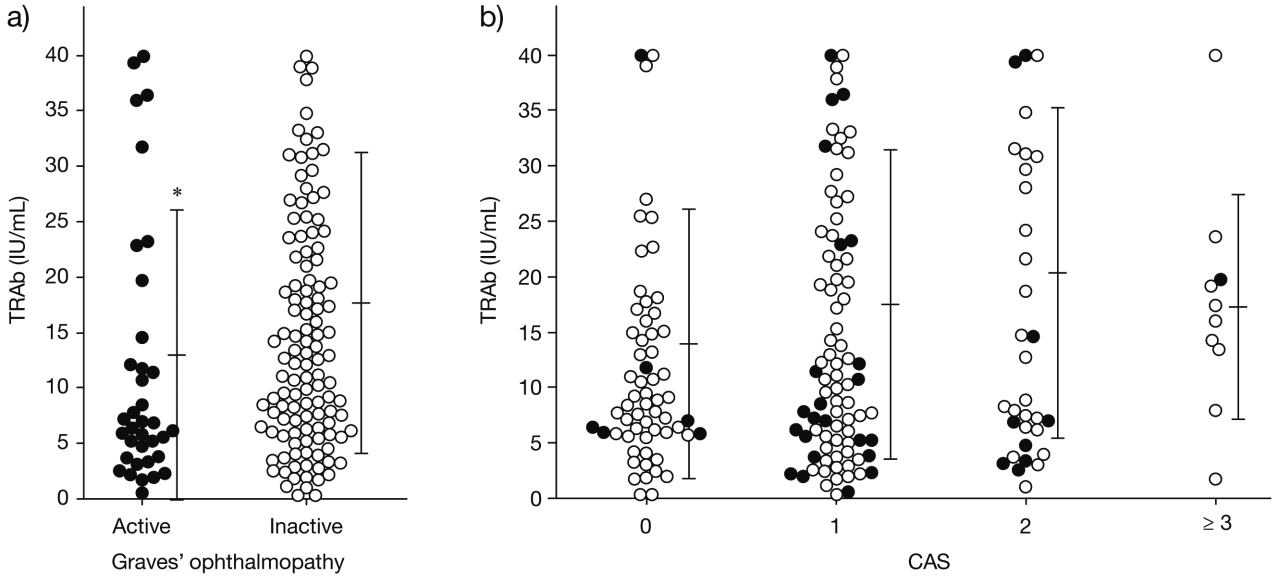

Fig. 3 TRAb titer in each GO activity group and CAS group. GO activity evaluated on the basis of STIR (a) and CAS (b)). Active GO subjects diagnosed with STIR are indicated in black dots. * p=0.0082 *versus* inactive GO.

at the initial visit. The prevalence of active-GO with euthyroid was not significantly different comparing GO patients with hyperthyroid (Fig.4 (a)). The group of GO patients with euthyroid had significantly lower TRAb titer in the active-GO group (4.0 ± 2.4 IU/L *vs*. 15.1 ± 13.7 IU/L, p=0.0066) (Fig. 4 (b)). The patients in the inactive-GO group followed a similar pattern.

Discussion

Many researchers reported that TRAb correlated to GO severity and activity [4, 10, 11]. However, Thyrotropin binding inhibiting immunoglobulin (TBII) and TSAb with bioassay were used in most of these reports and our study is the first report evaluating the correlation between GO and TRAb using the third-gen-

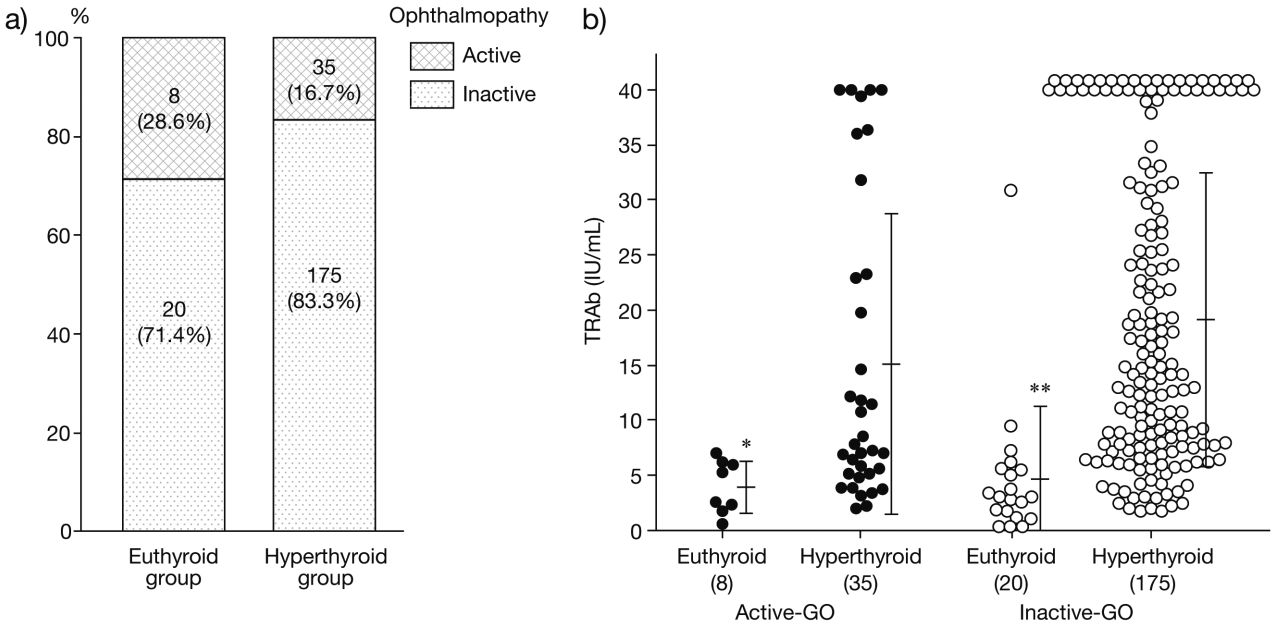

Fig. 4 Prevalence of active GO in euthyroid patients and hyperthyroid patients (a) and TRAb titers in active-GO and inactive-GO patients (b). * p=0.0066 *versus* hyperthyroid with active-GO. ** p<0.0001 *versus* hyperthyroid with inactive GO.

eration TRAb assay. Previous TRAb assays require a rather long assay time (more than 3 hours) and performance by skilled technicians to obtain accurate results because of the manual procedures. The third-generation TRAb assay is fast and fully automated, and it will be very helpful in the rapid diagnosis of Graves' disease and adjusting dosage of antithyroid drug. Moreover, the performance of the third-generation TRAb assay is clinically equivalent or superior to that of the previous manual commercial TRAb assays [12].

GO is an inflammatory change, and assuming that TRAb is the cause of GO, the higher TRAb titer means severer inflammation, and the TRAb titer should be higher in the active-GO phase than in the inactive-GO phase. However, correlation between TRAb titer and GO activity was not indicated in this study. TRAb may be one of the initiation factors of GO onset, but other factors besides TRAb may be involved in the etiology of GO.

Noh *et al*. reported that thyrotropin-binding inhibitor immunoglobulin (TBII) titer was not correlated with GO activity evaluated clinically [13] and similar data was reported from other part of Asia recently [14]. Although these findings were consistent with the results of our own study, there are some possibilities which can be accountable for these paradoxical results. TSAb was assayed using porcine thyroid cells and only cAMP formation was measured in this assay. TBII should include various kind of TRAb, because TBII was measured by the inhibition activity of TSH binding to TSH receptor. In contrast, the third-generation TRAb assay uses monoclonal antibody, and has high affinity for TSH receptor, and stimulates not only cAMP production but also Akt and PKC pathways [15].

Higher levels of expression of TSH receptor have been observed in orbital tissue from GO as compared to controls and gross correlation between the presence of TRAb and severity of GO [11]. However, it is unknown whether TRAb produced from the lymphocytes in thyroid tissue or in orbital tissue is one of the pathogenesis of GO. TSH receptor expression and Th1-derived cytokines is largely present only during the active stage of GO [16], and the pathogenesis of GO might be multifactorial.

Although the CAS is widely used to evaluate GO activity, there was a discrepancy between CAS and GO activity evaluated by MRI in Japanese GO patients. Hiromatsu *et al*. reported that the MRI data did not always correlate with GO activity evaluated according to the ATA classification [17], and their findings are consistent with the results of our own study. Because CAS is a subjective values assigned by ophthalmologists and MRI data, for example, STIR data, are objective, it is not surprising that sometimes there are dis-

crepancies between CAS and MRI data. Moreover, There are racial differences in the normal range of proptosis [18]. Europeans have been found to have a substantially greater risk of developing Graves' ophthalmopathy than Asians [19], and that may be one of the reason for the high prevalence of mild GO activity in our patients.

Although CT scans and echography may show ocular muscle enlargement, it is difficult to measure changes in the extraocular muscles precisely. MRI makes it possible to noninvasively assess qualitative changes as well as morphologic changes. Some researchers reported finding a correlation between the severity of the clinical picture and relative increases in muscle volume in the retrobulbar space on MRI scans [20, 21].

Since fat tissue also increases T2 times, it is difficult to measure water content in fat tissue on T2-weighted images. STIR imaging, on the other hand, reduces the fat component of the signal, making it possible to assess water content in fat tissue [17] and a STIR images provide better contrast for evaluating ophthalmopathy than T2-weighted images.

TRAb titer often decrease during treatment with antithyroid drugs, and it seems difficult to evaluate the correlation between GO activity and TRAb titer after starting antithyroid drug therapy. To minimize the changes in TRAb titer that occur during the treatment of Graves' disease, we selected untreated Graves' disease patients with GO as the subjects of our study.

Euthyroid GO is defined as ophthalmopathy in a patient without hyperthyroidism or a past history of hyperthyroidism, and the prevalence of euthyroid GO among GO patients is reported to be 0.7% [22]. The TRAb titer of the euthyroid patients with active GO in our study was lower than in the hyperthyroid patients, and another study in Japan reported that the TRAb titer was significantly lower in a euthyroid GO group than in a hyperthyroid GO group [23], although GO activity of the patients was not clearly mentioned in the report. The pathophysiology of euthyroid Graves' disease is still a matter of debate, and further study is needed.

In this study we assessed the GO activity of untreated Japanese Graves' disease patients and measured their TRAb titer with a third-generation assay, and we found that their TRAb titer at the initial visit were not correlated with GO activity. TRAb fraction has several antibody types (ex. TSAb and immunoglobulin G subclasses etc.). To detect the correlation between TRAb and GO, it is important to evaluate the variety of TRAb fraction and to check the correlation between each fraction and GO. It needs further evaluation whether TRAb titer with a third-generation assay become a prediction marker for GO development or not.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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