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The correlation of the neutrophil–lymphocyte ratio to clinical and imaging parameters in patients with thyroid eye disease

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Abstract

Thyroid eye disease (TED) is the major extrathyroidal manifestation of Graves' disease (GD). Treatment choice is based on clinical activity and severity of TED, as evaluated with clinical activity score (CAS) and magnetic resonance (MR) imaging. We aimed to determine the relationship between neutrophil-to-lymphocyte ratio (NLR), a readily available indicator of systemic inflammation, and clinical and MR imaging parameters in TED patients. Eighty-seven consecutive TED patients were included. The average signal intensity ratio (SIR), average extraocular muscle (EOM) diameter, and proptosis of the study eye were extracted from MR images. A baseline NLR ≥ 2.0 was recorded in 37 (42.5%) patients and NLR < 2.0 in 50 (57.5%) patients. TED patients with NLR ≥ 2.0 were older, had a higher CAS, average SIR, average EOM diameter and proptosis, and a lower serum thyrotrophin receptor antibody level than patients with NLR < 2.0 (all $P < 0.05$). All MR parameters showed significant correlation with CAS ($P < 0.05$). NLR correlated significantly with CAS ($P = 0.001$), average SIR ($P = 0.004$), average EOM diameter ($P = 0.007$), and proptosis ($P = 0.007$). Multiple regression revealed a significant correlation between NLR and CAS ($P = 0.001$), average SIR ($P = 0.029$), and proptosis ($P = 0.037$). Cox regression analysis showed that a high NLR at baseline was associated with a worse clinical outcome of TED (hazard ratio 3.7, 95% CI 1.22–11.2, $P = 0.02$), at a median follow-up of 25 months. In conclusion, NLR was correlated with CAS and MR imaging parameters and was associated with a worse clinical outcome of TED at follow-up in patients with TED. Additional prospective studies are needed to validate our findings.

Key Words

- neutrophil–lymphocyte ratio
- Graves' disease
- thyroid eye disease
- clinical activity
- magnetic resonance imaging

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Introduction

Thyroid eye disease (TED), although relatively rare, is the major extrathyroidal manifestation of Graves' disease (GD). TED is an inflammatory condition affecting the orbit around the eye, with more cases mild and nonprogressive (1, 2). Treatment choices for TED are based on the assessment of clinical activity and severity. Clinical activity score (CAS) is widely used to assess TED activity. However, there are limitations with this categorical CAS system (2, 3). CAS is based on clinical evaluation of the anterior visible part of the orbit but might ignore the acute inflammatory involvement of deep orbital structures, such as extraocular muscle (EOM) (4). Meanwhile, an identical weight is given to different clinical symptoms or signs in CAS system, which might overlook their heterogeneous importance.

Magnetic resonance (MR) imaging has a high contrast resolution for soft tissues and provides a remarkable advantage for investigating EOMs and deep structures in the orbit. MR is important in the differential diagnosis of TED and also contributes to guide the management of TED patients (5). CAS has been shown to correlate with MR parameters in previous studies. Also, MR parameters have been reported to predict clinical outcomes following orbital irradiation and immunosuppressive therapy in TED patients (6, 7). These evidence suggest that MR measures could serve as an objective and quantitative indicator of TED activity and severity (4). However, the costs and availability limit routine MR application in daily practice.

Neutrophil-to-lymphocyte ratio (NLR), calculated by dividing the absolute count of neutrophils by that of lymphocytes in the peripheral blood, is easy to obtain. NLR proves to be a good indicator of systemic inflammation. NLR has been reported to be associated with clinical prognosis in multiple disease contexts, including thyroid diseases (8, 9). NLR predicts relapse in GD patients following antithyroid drug therapy (10) and disease progression in patients with anaplastic thyroid carcinoma (11). It is reasonable to speculate the role of NLR in indicating TED activity or severity, given the close relationship between TED and inflammation. Indeed, one preliminary study with 58 TED patients suggested a potential association between NLR and CAS (12), but no study has yet evaluated a direct relationship between the NLR and TED activity or severity, particularly with orbital MR measures.

In this study, we performed a retrospective analysis of 87 TED patients, all with orbital MR data, to evaluate the relationship between the NLR and TED activity or severity, presented with both clinical and MR parameters.

Methods

Human subjects

This study protocol was approved by the Ethics Committee of Nanjing Drum Tower Hospital Institutional Review Board. We conducted a retrospective analysis of consecutive TED patients in a tertiary referral center (the Affiliated Drum Tower Hospital, Nanjing University School of Medicine) from January 2018 to October 2021. GD was diagnosed based on hyperthyroidism associated with anti-thyrotrophic hormone (TSH) receptor autoantibodies (TRAb). TED was diagnosed by the ophthalmology department based on clinical ophthalmic examinations. TED activity was assessed using the 7-point CAS system, as described previously (3). Patients were eligible for inclusion if they were 18–80 years of age, diagnosed with GD, with active or inactive TED for less than 18 months. We excluded patients who had previous orbital irradiation or surgery for TED, received i.v. glucocorticoids within 3 months before enrollment, or received treatments for GD other than antithyroid drugs. Patients with autoimmune diseases, immunosuppression, or active infection were also excluded to prevent changes in the NLR values due to the patients' background characteristics.

Demographic data, laboratory assays for thyroid function, and autoantibodies

Baseline demographic characteristics including age, gender, history of smoking, duration of GD, and duration of TED were collected. Blood cell counts were obtained and NLR was calculated by dividing the absolute neutrophil count by the lymphocyte count. Serum TSH, free triiodothyronine, free thyroxine, and thyroid autoantibodies (thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb)) concentrations were detected by electrochemical luminescence assays with Cobas Eless 601 (Roche). The reference ranges of TSH, TPOAb, and TgAb were 0.27–4.2 mIU/L, 0–34 IU/mL, and 0–115 IU/mL, respectively. TRAb was measured using a third-generation thyrotrophin binding inhibiting immunoglobulin (TBII) assay with the automated Cobas electrochemiluminescence immunoassay (Roche). Serum thyroid-stimulating antibodies (TSAbs) were measured as TSH receptor-stimulating immunoglobulin with the Siemens IMMULITE 2000 TSI assay. The reference ranges of TRAb and TSAb were 0–1.75 and 0–0.55 IU/L, respectively.

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MR imaging

Orbital MR imaging was performed with a 3.0T scanner (Ingenia, Philips). A retrospective analysis of two-dimensional quantification MR images was performed by one dedicated radiologist for all patients. The radiologist was unaware of the clinical status, as well as of the laboratory results of all patients. Proptosis was defined as the distance between the corneal eminence and the inter-zygomatic line in a horizontal T1-weighted image. The diameters of the EOMs (including inferior rectus, superior rectus, medial rectus, and lateral rectus) were estimated at the sites of their enlargement in the coronal or horizontal section of the T1-weighted images. To quantitively measure orbital muscle inflammation, the signal intensity of muscle tissues was acquired on two sequential coronal images from T2 fluid-sensitive sequences, and the average signal intensity for each muscle was calculated. A signal intensity ratio (SIR) was calculated by setting the signal intensity of each EOM in proportion to that of the ipsilateral temporalis muscle (13). Within each patient, the clinically more severe eye was designated as the study eye. The average EOM diameter and SIR of the study eye were calculated and used in the final analysis.

Statistical analyses

Continuous variables are presented as means ± S.E. and categorical variables as numbers with percentages. The mean baseline NLR value in our cohort was 2.0, thus a cutoff value of 2.0 was set for the analysis. Differences between NLR subgroups were analyzed by using the independent sample *t*-test or Mann–Whitney U test for continuous variables and using χ^2 test for categorical variables. Spearman's correlation was performed to assess the association between NLR, CAS, average SIR of the study eye, and other democratic, serum, and MR imaging parameters. Simple and multiple regression analysis was used to evaluate the relationships between CAS, average SIR of the study eye, and other parameters, with parameters that were statistically significant by simple regression analysis included in multiple regression analysis. As corticosteroid treatment could impact NLR values and MR findings, we performed a separate analysis excluding those who were receiving or have received corticosteroid treatments over 3 months before enrollment. A Cox proportional hazards model was used to identify the independent predictive factors of TED severity, as presented by EUGOGO criteria. A moderate-to-severe or very severe (sight-threatening) form at follow-up was considered as an event. Factors with $P < 0.10$ in the univariate analysis were selected for multivariate analysis. Analyses were implemented using SPSS (version 21.0). Two-tailed P values < 0.05 were considered as significant.

Results

Overall baseline characteristics

The demographical and clinical characteristics of the patients are presented in Table 1. Of 87 TED patients, 39 were male; the mean age was 48.6 years, mean duration of GD was 32.4 months, and the mean duration of TED was 7.0 months. The mean CAS was 3.3 and the mean NLR was 2.0. Current medications when they were enrolled were methimazole (in 67 patients, median dose 10 mg), propylthiouracil (in 1 patient, dose 150 mg), levothyroxine (in 10 patients, median dose 75 μg), and others were not on antithyroid medications or hormones. No patient was on glucocorticoid treatment at the time of enrollment. Twenty-nine patients (33.3%) had been treated with i.v. glucocorticoids more than 3 months before enrollment.

Patient characteristics stratified by baseline NLR

A baseline NLR ≥ 2.0 was recorded in 37 (42.5%) patients, and NLR < 2.0 in 50 (57.5%) patients. Patients with NLR ≥ 2.0 and NLR < 2.0 were similar in terms of sex, smoking, durations of GD and TED, serum thyroid hormone levels, and serum concentrations of TSAb, TgAb, and TPOAb. Patients with NLR ≥ 2.0 tended to be older (52.9 ± 2.12 vs 45.4 ± 1.96 years, $P=0.011$), showed a higher CAS (3.9 ± 0.19 vs 2.9 ± 0.17, $P<0.001$), average SIR (3.58 ± 0.39 vs 2.7 ± 0.21, $P=0.047$), average EOM diameter (6.0 ± 0.22 vs 5.3 ± 0.17 mm, $P=0.017$), and proptosis (22.5 ± 0.5 vs 20.6 ± 0.36 mm, $P=0.003$), but a lower serum TRAb level (median 5.74 vs 14.73 mIU/L, $P=0.049$), as compared with those with NLR < 2.0 (Table 1).

Variables correlated with CAS and MR parameters

NLR correlated significantly with CAS in the overall cohort ($P=0.001$), as were serum TSH ($P=0.004$) and free thyroxine levels ($P=0.004$) (Table 2). Duration of GD and TED did not correlate with CAS ($P=0.988$ and 0.532, respectively). TRAb and TSAb also did not show a significant correlation with CAS ($P=0.932$ and 0.641, respectively). All MR parameters showed a

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Table 1 Characteristics of the 87 enrolled patients with thyroid eye disease based on NLR.

Characteristics	Total	High NLR (≥2)	Low NLR (<2)	*P* value
Number of patients	87	37 (42.5)	50 (57.5)	
Age (years)	48.6 ± 1.49	52.9 ± 2.12	45.39 ± 1.96	**0.011**
Male (%)	39 (44.8)	21 (56.8)	18 (36)	0.08
Smoking (%)	27 (31.0)	12 (32.4)	15 (30)	0.34
Duration of TED (months)	7.03 ± 0.53	7.07 ± 0.79	6.97 ± 0.69	0.935
Duration of GD (months)	32.4 ± 4.07	31.3 ± 4.91	33.3 ± 6.19	0.816
Clinical activity score	3.3 ± 0.14	3.86 ± 0.19	2.88 ± 0.17	**<0.001**
TSH level (mIU/L)	0.03 (0.005, 1.11)	0.08 (0.01, 2.06)	0.015 (0.005, 0.485)	0.067
Free triiodothyronine (pmol/L)	6.26 (4.81, 14.86)	5.77 (4.64, 8.98)	7.30 (5.14, 17.12)	0.105
Free thyroxine (pmol/L)	24.5 ± 2.38	20.4 ± 2.86	27.7 ± 3.56	0.131
TRAb (IU/L)	9.34 (3.29, 21.26)	5.74 (2.64, 14.2)	14.73 (4.92, 24.89)	**0.049**
TSAb (IU/L)	6.43 (2.83, 14.2)	4.81 (2.01, 23.6)	6.94 (3.75, 12.3)	0.526
TgAb (IU/mL)	13.69 (10.6, 203.8)	12.5 (10, 44.2)	14.7 (11.2, 496)	0.165
TPOAb (IU/mL)	28.28 (9.78, 271.7)	22.4 (9.2, 226.5)	40.4 (11.9, 285.7)	0.423
CRP (mg/L)	4.08 ± 0.40	4.86 ± 0.35	3.38 ± 0.73	0.071
Average SIR	3.06 ± 0.21	3.58 ± 0.39	2.70 ± 0.21	**0.047**
Average EOM diameter (mm)	5.6 ± 0.14	5.99 ± 0.22	5.32 ± 0.17	**0.017**
Proptosis (mm)	21.4 ± 0.31	22.5 ± 0.5	20.6 ± 0.36	**0.003**
NLR	2.0 ± 0.1	2.88 ± 0.14	1.34 ± 0.06	**<0.001**
TH control status				0.169
Hyperthyroidism	58 (66.7)	21 (56.8)	37 (74.0)	
Hypohyroidism	9 (10.3)	6 (16.2)	3 (6.0)	
Euthyroidism	20 (23.0)	10 (27.0)	10 (20.0)	

Continuous variables are presented as mean ± S.E.M. or median (interquartile range). Categorical variables are presented as number (percentage). CAS, clinical activity score; CRP, C-reactive protein; EOM, extraocular muscle; GD, Graves' disease; NLR, neutrophil-to-lymphocyte ratio; SIR, signal intensity ratio; TED, thyroid eye disease; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TRAb, thyrotrophin receptor antibody; TSAb, thyroid-stimulating antibody; TSH, thyroid-stimulating hormone.

significant correlation with CAS (all $P<0.05$). Stratified analysis excluding patients receiving corticosteroids over 3 months before enrollment revealed a similar and significant correlation between CAS with NLR ($P=0.005$) and MR parameters (Supplementary Table 1, see section on supplementary materials given at the end of this article). However, patients with active TED (CAS ≥ 3) did not differ from those with inactive TED (CAS < 3) with regard to all MR measures (Supplementary Table 2).

NLR correlated significantly with average SIR ($P=0.004$), average EOM diameter ($P=0.007$), and proptosis ($P=0.007$). All MR parameters correlated with each other (all $P<0.05$), suggesting a consistent indication of TED activity or severity (Table 2). Analyses excluding patients receiving corticosteroids over 3 months before enrollment showed largely similar findings (Supplementary Table 1). A positive correlation was found between average EOM diameter (but not other MR parameters) and age ($P<0.001$). Duration of GD, TED, TRAb, and TSAb did not show correlation with MR parameters in our study. There was an inverse correlation between CAS (as well as MR parameters) with serum-free triiodothyronine, but a positive correlation with TSH (Table 2).

Simple and multiple regression analysis for CAS and MR parameters

To further assess the activity and severity of TED, we performed a simple and multiple regression analysis, using CAS and MR parameters as the dependent variable. CAS correlated significantly with free thyroxine ($P=0.034$) and NLR ($P=0.001$) by simple regression analysis, but by multiple regression analysis, the correlation remained only for NLR ($P=0.001$; Table 3). Similarly, simple and multiple regression analysis including NLR and other potentially affecting variables confirmed a significant correlation only between NLR and average SIR ($P=0.029$). NLR was also correlated with proptosis after simple and multiple regression analysis ($P=0.037$). Age remained the only variable significantly correlated with average EOM diameter after multiple regression. These findings suggested that NLR was an independent variable affecting CAS and MR parameters.

Univariate and multivariate analysis for predictors of final TED activity

During a median follow-up of 25 months, 70% of patients (55 of 79) had mild activity, while the others had moderate-

Table 2 Correlation analyses of NLR and CAS with demographic, serum, and MR imaging parameters.

Variables	NLR		CAS		Average SIR		Average EOM diameter		Proptosis	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
NLR	1	NA	0.341	**0.001**	0.326	**0.004**	0.306	**0.007**	0.297	**0.007**
CAS	0.341	**0.001**	1	NA	0.262	**0.02**	0.248	**0.029**	0.337	**0.002**
Age	0.251	**0.02**	0.034	0.755	0.122	0.292	0.394	**<0.001**	0.087	0.440
Duration of GD	−0.026	0.809	−0.002	0.988	−0.164	0.151	−0.161	0.160	−0.002	0.988
Duration of TED	−0.085	0.435	0.068	0.532	−0.075	0.514	0.008	0.945	0.110	0.324
Free thyroxine	−0.044	0.69	−0.303	**0.004**	−0.035	0.758	−0.170	0.137	−0.211	0.057
Free triiodothyronine	−0.124	0.257	−0.198	0.068	−0.250	**0.028**	−0.199	0.082	−0.272	**0.014**
TSH	0.185	0.089	0.302	**0.004**	0.220	0.053	0.252	**0.026**	0.306	**0.005**
TRAb	−0.235	**0.031**	0.009	0.932	0.113	0.333	0.037	0.754	0.060	0.599
TSAb	−0.056	0.744	0.079	0.641	0.294	0.087	0.015	0.931	0.260	0.126
CRP	0.081	0.520	−0.045	0.720	0.009	0.946	−0.156	0.252	−0.025	0.851
Proptosis	0.297	**0.007**	0.337	**0.002**	0.307	**0.006**	0.372	**0.001**	1	NA
Average EOM diameter	0.306	**0.007**	0.248	**0.029**	0.257	**0.023**	1	NA	0.372	**0.001**
Average SIR	0.326	**0.004**	0.262	**0.02**	1	NA	0.257	0.023	0.307	**0.006**

CAS, clinical activity score; CRP, C-reactive protein; EOM, extraocular muscle; GD, Graves' disease; NLR, neutrophil-to-lymphocyte ratio; SIR, signal intensity ratio; TED, thyroid eye disease; TRAb, thyrotrophin receptor antibody; TSAb, thyroid-stimulating antibody; TSH, thyroid-stimulating hormone.

to-severe or very severe activity. Univariate analysis revealed a significant difference in age, gender, smoking status, and NLR between patients with mild activity and those with moderate-to-severe or very severe activity. However, only NLR remained a significant predictor of final TED activity (hazard ratio (HR) 3.7, 95% CI 1.22–11.2, $P=0.02$) (Table 4).

Discussion

To our knowledge, our study remains the first study to demonstrate the relationship between a readily available inflammatory marker in clinical practice – NLR – with objective and quantitative parameters of TED activity and severity from MR imaging. We confirmed a significant correlation between MR measures and CAS and showed a significant correlation between NLR and CAS, as well as all MR measures including proptosis, the average SIR, and EOM diameter of the study eye. This positive correlation was validated by both simple and multiple regression analysis. In addition, a high NLR at baseline was associated with a worse clinical outcome of TED at follow-up.

CAS is the most widely used scoring system for TED activity evaluation but is limited by the lack of ability to reflect the inflammatory status of deep orbital structures. Orbital MR imaging provides additional information regarding the activity of TED, including morphological and quantitative evaluation of deep orbital structures (5). Specific MR sequences have been proposed in the latest European guidelines for TED management, which might help quantify disease activity and predict response to anti-inflammatory treatment and outcome of TED (2). In agreement with previous reports, our study confirmed a significant correlation between MR measures and CAS. According to current guidelines, TED is defined as active if CAS ≥ 3/7 and inactive if CAS < 3/7 (2). However, based on this dichotomic category, we found that patients with active TED (CAS ≥ 3) did not differ from patients with inactive TED, with regard to all MR measures. This discrepancy between continuous evaluation (showing significant correlation with MR measures) and dichotomic assessment of CAS (showing no difference in MR measures) highlights the limitation of CAS-based TED activity categorical classification: while a loose correlation might exist, the overlap is too extensive to allow for separation among groups. A modification of the CAS classification or other categorical strategy, for instance, incorporating MR parameters and other variables, is warranted.

Although 1 preliminary study with 58 TED patients compared NLRs in patients with higher and lower CAS

Table 3 Simple and multiple regression analyses of the associations between clinical variables with CAS and MR parameters.*

Variables	CAS		Average SIR		Average EOM diameter		Proptosis	
	Simple regression	Multiple regression	Simple regression	Multiple regression	Simple regression	Multiple regression	Simple regression	Multiple regression
Duration of GD	0.285	—	0.628	—	0.104	—	0.694	—
Duration of TED	0.899	—	0.412	—	0.931	—	0.441	—
TH control status	**0.046**	0.178	**0.050**	0.053	0.140	—	**0.068**	0.068
Age	0.942	—	0.445	—	**<0.001**	**0.001**	0.269	—
Free thyroxine	**0.034**	0.224	0.404	—	0.09	—	**0.033**	**0.037**
NLR	**0.001**	**0.001**	**0.023**	**0.029**	**0.031**	0.10	**0.028**	**0.037**

CAS, clinical activity score; EOM, extraocular muscle; GD, Graves' disease; NLR, neutrophil-to-lymphocyte ratio; SIR, signal intensity ratio; TED, thyroid eye disease; TH, thyroid hormone.
**P* values from simple and multiple regression analyses were provided.

(12), the relationship between an elevated NLR and clinical and MR imaging parameters in patients with TED has not been reported yet. On MR images of TED patients, increased volumes of EOMs are characteristic, as well as MR signal intensities which indicate inflammation of the orbital soft tissues (14). Our study confirmed these anatomical changes in TED patients. These average SIR changes occurred in conjunction with changes in proptosis and average diameters of EOMs in our analysis, suggesting that these MR parameters of orbital soft tissues might provide cumulative values for prediction of TED activity. The consistent correlation of NLR with CAS and all MR parameters evidenced that NLR may be a predictive biomarker for TED activity and severity. It is interesting that C-reactive protein, another indicator of inflammation, did not correlate with TED activity in our study. Therefore, the mechanisms underlying the association between NLR and TED activity might not only be considered as general inflammatory-related but might also be affected by inflammation types. We also found that patients with a higher NLR at baseline tended to have a more severe form of TED, at a median follow-up of 25 months. Our results might provide additional useful information regarding the pre-existing indicators to determine the activity and severity of TED patients and predict their response to treatments. However, it remains unclear to which extent an incremental predictive power of NLR would improve the predictive value of CAS, e.g. in predicting response to therapy. Further prospective studies are needed.

Interestingly, we observed an inverse correlation between CAS (as well as MR parameters) with serum-free triiodothyronine, but a positive correlation with TSH. These findings were in agreement with that of Dr Profilo and colleagues, which showed an inverse correlation between NOSPECS score and serum triiodothyronine (15). Although NOSPECS in that study differed with CAS in our study, with the former reflecting TED severity and the latter indicating TED activity, the MR parameters in our study might be an indicator of both activity and

Table 4 Univariate and multivariate analyses for final disease severity of TED.

	Univariate analysis			Multivariate analysis	
	Mild	Moderate-to-severe or very severe	*P* value	Hazard ratio (95% CI)	*P* value
No.	55	24			
Age	46.5 ± 1.83	53.38 ± 2.61	**0.037**	0.996 (0.956, 1.039)	0.862
TSH	4.59 ± 2.31	3.79 ± 1.64	0.830		
Free triiodothyronine	11.72 ± 1.29	9.07 ± 2.08	0.268		
Free thyroxine	24.87 ± 2.93	23.5 ± 5.15	0.808		
NLR	1.73 ± 0.11	2.65 ± 0.24	<0.001	3.697 (1.217, 11.23)	**0.021***
Duration of GD	32.2 ± 5.59	36.08 ± 7.26	0.691		
Duration of TED	6.82 ± 0.65	7.35 ± 1.05	0.657		
CRP	4.3 ± 0.5	3.97 ± 0.88	0.724		
Gender (male)	19	17	**0.004**	0.53 (0.158, 1.778)	0.304
Smoking (yes)	6	11	**0.002**	2.33 (0.722, 7.519)	0.157
TH control status (yes)	16	11	0.198		

CRP, C-reactive protein; GD, Graves' disease; NLR, neutrophil-to-lymphocyte ratio; TED, thyroid eye disease; TH, thyroid hormone; TSH, thyroid-stimulating hormone.
*NLR ≥ 2.0 vs NLR < 2.0.

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severity (4). These findings were also in keeping with the knowledge that hypothyroidism can be associated or it may determine a progression of TED (15, 16) and that iatrogenic hypothyroidism in treating patients with GD should be avoided (2).

We also found that older TED patients had a higher NLR and a larger EOM diameter, but the average SIR or proptosis was not different. Multiple regression analysis also confirmed the positive correlation between EOM diameter and age in TED patients. This does not suggest a more active state in older TED patients, as EOM dimension measurements do not correlate with the inflammatory activity of the disease, and muscle enlargement can also be found in chronic stages of TED (4, 6). It is notable that in our study, no correlation was found between CAS and MR parameters with either serum TRAb or TSAb. Evidence regarding the correlation of TRAb with TED activity is somewhat equivocal. Several studies with a moderate sample size of TED patients found a correlation between TRAb and CAS (17, 18), but others with a similar number of patients did not find an association (15, 19). In contrast, TSAb levels seem to be consistently reported to be correlated with the severity and activity of TED (17, 19, 20). The discrepancy of TSAb correlation in our study with others remains unclear but might be partially due to the change of TSAb levels under antithyroid drugs. Also, in addition to antibodies targeting the thyrotropin receptor, other autoantigens and antibodies are involved in the development of TED, among which, the insulin-like growth factor I receptor (IGF-I) receptor plays a central role (21, 22). Future studies are needed to test the possible correlation between IGF-I pathway and TED activity.

Several limitations should also be acknowledged in our study. First, the retrospective nature of the study made it impossible to avoid potential confounding, although the MR images were reevaluated by one experienced radiologist who was blind to the clinical status and laboratory findings of all patients. Second, also due to the retrospective nature of this study, the effect of NLR on the response of patients to anti-inflammatory treatment for TED needs to be confirmed in prospective studies. Third, MR data were obtained from two-dimensional images, thus three-dimensional volumetric analyses of the orbital fat and the orbital cavity were unavailable (23), which might produce less variance (7). Fourth, it is unclear to which extent an incremental predictive power of NLR would improve the predictive value of CAS, and further studies are needed. Fifth, thyroid function control in our TED cohort was inadequate, which is largely due to the lack of follow-up examinations of thyroid function and timely adjustment of medication dosages in most patients because they might be free from GD-related severe symptoms before they developed symptoms of TED. However, multiple regression analysis controlled for thyroid hormone control status revealed similar significant associations between NLR and CAS and MR parameters.

In conclusion, in patients with GD and TED, there was a significant correlation between NLR and clinical and MR imaging parameters. A high NLR at baseline was associated with a worse clinical outcome of TED at follow-up. Future prospective studies are needed to validate our findings and determine the incremental predictive power of NLR in addition to CAS in predicting response to therapy.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-22-0260.

Declaration of interest

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

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Data availability statement

The data of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author contribution statement

Xiaowen Zhang and Chen Han were co-first authors and contributed to the study design, data acquisition, data analysis, data interpretation and drafting the manuscript. Hongwei Wang, Xinghong Sun, Xin Dou, Xueying He, and Di Wu contributed to the data acquisition. Shanmei Shen contributed to the data interpretation. Dalong Zhu, Xinlin Zhang and Yan Bi contributed to the study design, and data interpretation. All authors approved the final version submitted for publication.

References

1 Kahaly GJ. Management of Graves thyroidal and extrathyroidal disease: an update. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** 3704–3720. (https://doi.org/10.1210/clinem/dgaa646)

2 Bartalena L, Kahaly GJ, Baldeschi L, Dayan CM, Eckstein A, Marcocci C, Marino M, Vaidya B, Wiersinga WM & EUGOGO. The 2021 European Group on Graves' Orbitopathy (EUGOGO) clinical practice guidelines for the medical management of Graves' orbitopathy. *European Journal of Endocrinology* 2021 **185** G43–G67. (https://doi.org/10.1530/EJE-21-0479)

3 Mourits MP, Koornneef L, Wiersinga WM, Prummel MF, Berghout A & van der Gaag R. Clinical criteria for the assessment of disease activity in Graves' ophthalmopathy: a novel approach. *British Journal of Ophthalmology* 1989 **73** 639–644. (https://doi.org/10.1136/bjo.73.8.639)

4 Politi LS, Godi C, Cammarata G, Ambrosi A, Iadanza A, Lanzi R, Falini A & Bianchi Marzoli S. Magnetic resonance imaging with diffusion-weighted imaging in the evaluation of thyroid-associated orbitopathy: getting below the tip of the iceberg. *European Radiology* 2014 **24** 1118–1126. (https://doi.org/10.1007/s00330-014-3103-3)

5 Siakallis LC, Uddin JM & Miszkiel KA. Imaging investigation of thyroid eye disease. *Ophthalmic Plastic and Reconstructive Surgery* 2018 **34** S41–S51. (https://doi.org/10.1097/IOP.0000000000001139)

6 Muller-Forell W & Kahaly GJ. Neuroimaging of Graves' orbitopathy. *Best Practice and Research: Clinical Endocrinology and Metabolism* 2012 **26** 259–271. (https://doi.org/10.1016/j.beem.2011.11.009)

7 Jain AP, Gellada N, Ugradar S, Kumar A, Kahaly G & Douglas R. Teprotumumab reduces extraocular muscle and orbital fat volume in thyroid eye disease. *British Journal of Ophthalmology* 2022 **106** 165–171. (https://doi.org/10.1136/bjophthalmol-2020-317806)

8 Cupp MA, Cariolou M, Tzoulaki I, Aune D, Evangelou E & Berlanga-Taylor AJ. Neutrophil to lymphocyte ratio and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies. *BMC Medicine* 2020 **18** 360. (https://doi.org/10.1186/s12916-020-01817-1)

9 Marques P, de Vries F, Dekkers OM, Korbonits M, Biermasz NR & Pereira AM. Serum inflammation-based scores in endocrine tumors. *Journal of Clinical Endocrinology and Metabolism* 2021 **106** e3796–e3819. (https://doi.org/10.1210/clinem/dgab238)

10 Kim M, Kim BH, Jang MH, Kim JM, Kim EH, Jeon YK, Kim SS & Kim IJ. High neutrophil-to-lymphocyte ratio is associated with relapse in Graves' disease after antithyroid drug therapy. *Endocrine* 2020 **67** 406–411. (https://doi.org/10.1007/s12020-019-02137-y)

11 Yamazaki H, Sugino K, Matsuzu K, Masaki C, Akaishi J, Hames K, Tomoda C, Suzuki A, Uruno T, Ohkuwa K, *et al.* Inflammatory biomarkers and dynamics of neutrophil-to-lymphocyte ratio in anaplastic thyroid carcinoma. *Endocrine* 2020 **70** 115–122. (https://doi.org/10.1007/s12020-020-02313-5)

12 Celik T. Neutrophil-to-lymphocyte ratio in thyroid ophthalmopathy. *Bratislavske Lekarske Listy* 2017 **118** 495–498. (https://doi.org/10.4149/BLL_2017_095)

13 Kirsch EC, Kaim AH, De Oliveira MG & von Arx G. Correlation of signal intensity ratio on orbital MRI-TIRM and clinical activity score as a possible predictor of therapy response in Graves' orbitopathy – a pilot study at 1.5 T. *Neuroradiology* 2010 **52** 91–97. (https://doi.org/10.1007/s00234-009-0590-z)

14 Higashiyama T, Nishida Y & Ohji M. Relationship between magnetic resonance imaging signal intensity and volume of extraocular muscles in thyroid-associated ophthalmopathy with methylprednisolone pulse therapy. *Clinical Ophthalmology* 2016 **10** 721–729. (https://doi.org/10.2147/OPTH.S105096)

15 Profilo MA, Sisti E, Marcocci C, Vitti P, Pinchera A, Nardi M, Rocchi R, Latrofa F, Menconi F, Altea MA, *et al.* Thyroid volume and severity of Graves' orbitopathy. *Thyroid* 2013 **23** 97–102. (https://doi.org/10.1089/thy.2012.0379)

16 Bahn RS. Graves' ophthalmopathy. *New England Journal of Medicine* 2010 **362** 726–738. (https://doi.org/10.1056/NEJMra0905750)

17 Jang SY, Shin DY, Lee EJ, Choi YJ, Lee SY & Yoon JS. Correlation between TSH receptor antibody assays and clinical manifestations of Graves' orbitopathy. *Yonsei Medical Journal* 2013 **54** 1033–1039. (https://doi.org/10.3349/ymj.2013.54.4.1033)

18 Nicoli F, Lanzolla G, Mantuano M, Ionni I, Mazzi B, Leo M, Sframeli A, Posarelli C, Maglionico MN, Figus M, *et al.* Correlation between serum anti-TSH receptor autoantibodies (trabs) and the clinical feature of Graves' orbitopathy. *Journal of Endocrinological Investigation* 2021 **44** 581–585. (https://doi.org/10.1007/s40618-020-01353-y)

19 Subekti I, Boedisantoso A, Moeloek ND, Waspadji S & Mansyur M. Association of TSH receptor antibody, thyroid stimulating antibody, and thyroid blocking antibody with clinical activity score and degree of severity of Graves ophthalmopathy. *Acta Medica Indonesiana* 2012 **44** 114–121.

20 Hiromatsu Y, Eguchi H, Matsuo Y, Kato T, Tani J, Watanabe S, Teshima Y & Araki N. Role of a new bioassay for thyroid-stimulating antibodies (aequorin TSAb) in Graves' ophthalmopathy. *Endocrine Journal* 2020 **67** 347–352. (https://doi.org/10.1507/endocrj.EJ19-0398)

21 Pritchard J, Han R, Horst N, Cruikshank WW & Smith TJ. Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway. *Journal of Immunology* 2003 **170** 6348–6354. (https://doi.org/10.4049/jimmunol.170.12.6348)

22 Smith TJ & Janssen JAMJL. Insulin-like growth factor-I receptor and thyroid-associated ophthalmopathy. *Endocrine Reviews* 2019 **40** 236–267. (https://doi.org/10.1210/er.2018-00066)

23 Douglas RS, Kahaly GJ, Patel A, Sile S, Thompson EHZ, Perdok R, Fleming JC, Fowler BT, Marcocci C, Marino M, *et al.* Teprotumumab for the treatment of active thyroid eye disease. *New England Journal of Medicine* 2020 **382** 341–352. (https://doi.org/10.1056/NEJMoa1910434)

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