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Tumor necrosis factor-alpha stimulated gene-6: A biomarker reflecting disease activity in rheumatoid arthritis

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Abstract

Background: To explore the serum tumor necrosis factor-alpha stimulated gene-6 (TSG-6) level and its association with disease activity in rheumatoid arthritis (RA) patients.

Methods: We recruited 176 RA patients, 178 non-RA patients (lupus erythematosus, osteoarthritis, ulcerative colitis, ankylosing spondylitis and psoriasis) and 71 healthy subjects. Serum TSG-6 levels were detected by enzyme-linked immunosorbent assay (ELISA). RA patients were divided into inactive RA and active RA groups by disease activity score of 28 joints based on C-reactive protein (DAS28-CRP). The receiver operating characteristic (ROC) curve and Spearman's rank correlation test analyzed the correlation between TSG-6 concentration and RA disease activity.

Results: Tumor necrosis factor-alpha stimulated gene-6 levels in the RA group were increased ($p < 0.01$). TSG-6 concentrations indicated an upward tendency with increased disease activity; The area under the curve (AUC) of TSG-6 for diagnosing RA and assessing the severity of RA were 0.78 and 0.80, respectively; The combination of TSG-6 and anti-mutated citrullinated vimentin antibodies (anti-MCV) (sensitivity:98.4%)improved the diagnostic accuracy of RA. Binary logistic regression analysis showed that TSG-6 was an independent risk factor related to the severity of RA, and OR (95% CI) was 1.2 (1.003–1.453).

Conclusion: The TSG-6 levels in RA patients were elevated and related to disease activity. Therefore, TSG-6 may serve as a new potential biomarker for evaluating RA disease activity.

KEYWORDS
anti-mutated citrullinated vimentin antibodies, C-reactive protein, disease activity score of 28 joints, rheumatoid arthritis, tumor necrosis factor-alpha stimulated gene-6

Tingting Hu and Yuhan Liu contributed equally to this work and should be considered co-first authors.

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a common clinical chronic autoimmune disease with joint inflammation.[1] The disease can cause synovitis, articular pannus, cartilage and bone destruction, and ultimately lead to joint deformity and disability.[2,3] At present, the pathogenesis of RA is yet to be illuminated. Therefore, we still need to study its pathological mechanism and seek suitable biomarkers or combinations to diagnose RA.

Tumor necrosis factor-alpha stimulated gene-6 (TSG-6) is a new gene discovered by Lee et al.[4] when screening cDNA expression libraries of human fibroblasts interfered by tumor necrosis factor-alpha. The protein it encodes contains 277 amino acids. TSG-6 is hyaluronan (HA) binding protein associated with inflammation.[5,6] Piling evidence showed that under the induction of growth factors, hormones and pro-inflammatory cytokines, the expression of TSG-6 is significantly increased in the synovial fluid, synovial membrane and chondrocytes of RA patients.[7–9] Interestingly, in a collagen antibody-induced arthritis model,[10] the progression of arthritis and the degree of joint destruction in mice that knocked out the *Tsg-6* gene were more serious. Therefore, TSG-6 may be a potential marker for assessing the severity of RA disease. Although studies have discussed the important role of serum TSG-6 in the pathogenesis of RA, it remains uncertain whether serum TSG-6 can effectively diagnose RA. Thus, we investigated whether TSG-6 was a potential biomarker for the diagnosis of RA.

In this research, we detect the serum TSG-6 levels in the RA group, the non-RA group and healthy control patients (HCs). The correlation between TSG-6 concentration and clinical indicators and laboratory indicators is analyzed to explore its clinical value in the diagnosis of RA.

2 | MATERIALS AND METHODS

2.1 | Study population

A total of 425 subjects who were diagnosed in the outpatient or inpatient department of the Second Affiliated Hospital of Nanchang University from December 2019 to April 2021 were selected. The first group was the RA group (176 cases), all RA patients according to the diagnostic criteria of 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR).[11] The second group was the non-RA group (178 patients with non-RA autoimmune diseases), including 41 cases of lupus erythematosus, 34 cases of osteoarthritis, 36 cases of ulcerative colitis, 36 cases of ankylosing spondylitis and 31 cases of psoriasis. At last, 71 healthy participants in our hospital during the same period were selected as HCs. All participants were from the Han populations of Jiangxi, China. All participants had a clear diagnosis with intact clinical, imaging and laboratory examination data, and the clinical examination of healthy participants was normal. The following subjects were excluded: those with cerebrovascular diseases, liver disease, thyroid disease, infection, pregnancy and malignancy. All subjects were informed and agreed to participate in this study. All work has been approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University (No. Review-2019-075) and complied with the ethical guidelines of the Helsinki Declaration.

2.2 | Clinical investigation and data collection

Basic and medical history information was collected by reviewing electronic medical records. Including swollen joint count (SJC), tender joint count (TJC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), disease activity score based on 28 joint counts (DAS28), clinical disease activity index (CDAI) and simplified disease activity index (SDAI). According to DAS28-CRP,[11,12] RA groups were classified as active RA group (DAS28-CRP ≥ 3.2) and inactive RA group (DAS28-CRP < 3.2). There was no statistically significant difference in age and gender of each group ($p > 0.05$).

2.3 | Method

The serum TSG-6 concentration was detected by ELISA reagent (Shanghai Jianglai Biotechnology Co. Ltd.). The detection range was 0.625 ~ 20 ng/ml. First, added 50 μl of the subject's serum to the sample well. Then added 100 μl of horseradish peroxidase-conjugated reagent. The reaction plate was incubated at 37°C for 60 min. The 96-well reaction plate was washed five times. Added substrate solution to each well, incubated at 37°C in the dark for 15 min and added stop solution. The absorbance was measured at 450 nm with a microplate reader, and the concentration of serum TSG-6 was calculated according to the standard curve. All assays were performed strictly according to the instructions of the reagents used and the standard operating procedure (SOP) of the Second Affiliated Hospital of Nanchang University.

2.4 | Statistical analysis

The normality and homogeneity of variance of the measurement data were analyzed by the Kolmogorov–Smirnov test and Levene's test, respectively. Normally distributed data were expressed as mean ± standard deviation; a one-way analysis of variance (ANOVA) test was used to make multiple groups comparisons and followed by the Games–Howell test for pairwise comparisons. Nonnormally distributed data were represented by the medians with interquartile ranges (IQR); the Kruskal–Wallis test was used to make multiple groups comparisons and followed by the Bonferroni–Dunn test for pairwise comparisons. Categorical variables were presented as a percentage. Compare the differences between count data groups by chi-square test. Spearman correlation analysis was used to compare the correlation between serum level of TSG-6 and disease activity. Draw the receiver operating characteristic (ROC)

curve to analyze the clinical value of TSG-6 in the diagnosis of RA. Binary logistic regression was used to analyze the correlation between TSG-6 and the severity of RA. Statistical significance was defined as p-value <0.05. All analyzes were handled with SPSS and GraphPad Prism.

3 | RESULTS

The demographics and laboratory results of all participants are shown in Table 1. There were no significant differences in gender and age among RA patients, non-RA group and HCs. Mann–Whitney U test indicated the levels of plasma TSG-6, anti-mutated citrullinated vimentin antibodies (anti-MCV), anti-cyclic citrullinated peptide antibodies (anti-CCP), Rheumatoid factor (RF), CRP, ESR and neutrophil to lymphocyte ratio (NLR) in the RA group increased dramatically than non-RA group and HCs ($p < 0.01$).

The area under the ROC curve (AUC) of serum TSG-6 for RA is shown in Table 2 and Figure 1A. Among all the indicators, the highest sensitivity and specificity were anti-MCV and anti-CCP, respectively. The lowest sensitivity and specificity were ESR and NLR, respectively. Notably, the combination of TSG-6 and anti-MCV

TABLE 1 Demographics and serological indicators of subjects in each group

Marker	RA (n = 176)	Non-RA (n = 178)	HCs (n = 71)	p
Gender (male/female)	53/123	51/127	21/50	0.991
Age (years) (min, max)	52.0 ± 13.9 (18 ~ 82)	50.0 ± 12.0 (28 ~ 78)	50.0 ± 12.0 (28 ~ 78)	0.300
BMI (kg/m^2)	20.1 ± 4.6	20.3 ± 3.6	21.3 ± 6.2	0.570
TSG-6 (ng/ml)	9.5 (7.3–13.5)*,#	6.7 (6.5–11.2)	6.6 (6.4–11.4)	$p < 0.01$
Anti-CCP (U/ml)	294.5 (148.4–993.5)*,#	22.9 (14.1–34.6)	21.4 (14.4–29.9)	$p < 0.01$
Anti-MCV (U/ml)	142.6 (29.1–552.1)*,#	13.4 (9.0–18.2)	11.2 (6.9–15.4)	$p < 0.01$
Disease activity	—	—	—	—
RF (U/ml)	91.6 (20.6–402.3)*,#	26.7 (11.7–48.6)*	8.0 (7.0–18.3)	$p < 0.01$
ESR (mm/h)	17.0 (9.3–41.0)*,#	14.0 (6.0–27.0)*	7.0 (5.0–9.0)	$p < 0.01$
CRP (mg/L)	5.3 (3.8–14.3)*,#	5.0 (2.9–8.8)*	2.3 (1.9–4.6)	$p < 0.01$
NLR	3.4 (2.3–5.6)*,#	2.5 (1.8–3.8)*	1.4 (1.0–1.8)	$p < 0.01$
Tender joint count (0–28)	2.0 (1.0–5.0)	—	—	—
Swollen joint count (0–28)	1.0 (0.0–2.0)	—	—	—
DAS28-CRP	2.9 (2.4–4.3)	—	—	—
DAS28-ESR	3.2 (2.5–4.9)	—	—	—
SDAI	8.2 (2.6–20.9)	—	—	—
CDAI	7.8 (2.4–18.2)	—	—	—

Note: $p < 0.01$: *Compared with the HCs group; #compared with Non-RA group. Categorical variables were expressed as numbers, continuous variables as mean ± standard deviation. Data for non-normally distributed measurements were expressed as median (interquartile range).

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; anti-MCV, anti-mutated citrullinated vimentin; BMI, body mass index; CDAI, clinical disease activity index; CRP, C-reactive protein; DAS28-CRP, Disease Activity Score of 28 joints based on C-reactive protein; DAS28-ESR, Disease Activity Score of 28 joints based on Erythrocyte sedimentation rate; ESR, erythrocyte sedimentation rate; HCs, healthy control patients; NLR, neutrophil to lymphocyte ratio; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, simplified disease activity index; TSG-6, Tumor necrosis factor-alpha stimulated gene-6.

TABLE 2 Evaluation of diagnostic performance of each marker

Marker	AUC (95%CI)	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
TSG-6 (ng/ml)	0.78 (0.74–0.82)	6.99	86.9	74.7	0.62
Anti-CCP(U/ml)	0.90 (0.86–0.92)	100.0	79.0	96.0	0.75
Anti-MCV(U/ml)	0.89 (0.86–0.92)	21.1	88.1	88.8	0.77
RF(U/ml)	0.76 (0.72–0.80)	43.5	79.9	72.4	0.49
CRP (ng/ml)	0.69 (0.64–0.73)	11.2	64.8	76.2	0.33
ESR (mm/h)	0.72 (0.67–0.76)	16.0	58.0	76.7	0.35
NLR	0.73 (0.68–0.77)	2.8	60.2	68.7	0.37

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; anti-MCV, anti-mutated citrullinated vimentin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil to lymphocyte ratio; RF, rheumatoid factor; TSG-6, Tumor necrosis factor-alpha Stimulated gene-6.

(A) RA vs (Non-RA+Healthy subjects)

AUC=0.784
Sensitivity:86.9%
Specificity:74.7%

(B) Inactive RA vs Active RA
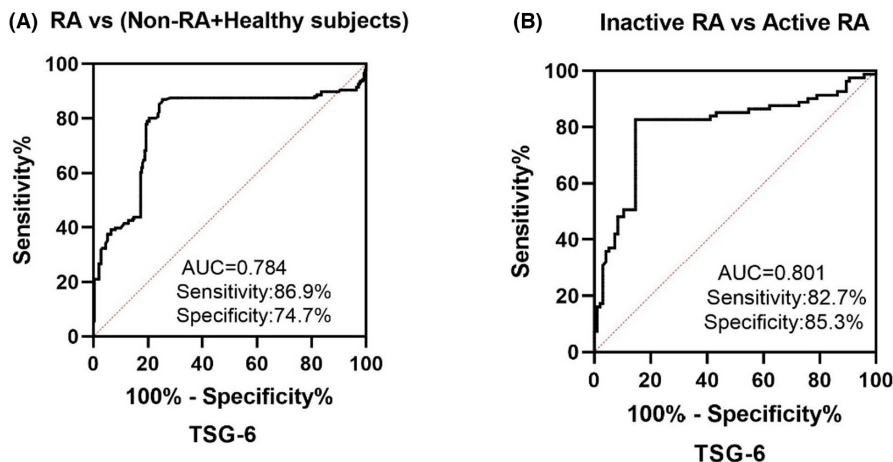
AUC=0.801
Sensitivity:82.7%
Specificity:85.3%

FIGURE 1   The clinical value of serum TSG-6 in diagnosing RA and evaluating the condition of RA. (A) ROC curve of serum TSG-6 for the diagnosis of RA; (B) ROC curve of serum TSG-6 for the diagnosis of RA disease activity. RA, rheumatoid arthritis; TSG-6, Tumor necrosis factor-alpha Stimulated gene-6
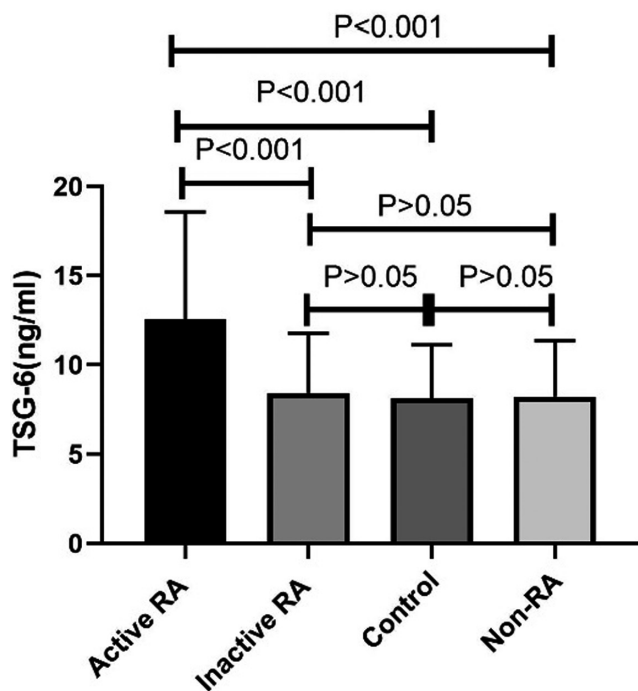

FIGURE 2   Serum TSG-6 levels in the active RA group, inactive RA group, healthy control group and non-RA group.RA, rheumatoid arthritis; TSG-6, Tumor necrosis factor-alpha Stimulated gene-6

(98.4%) had the highest sensitivity when connected in parallel, and the combination of anti-MCV and anti-CCP (99.60%) had the highest specificity when connected in series.

The expression of serum TSG-6 was increased in the active RA group (IQR 12.7 [9.6–14.5]) compared to the inactive RA group (IQR
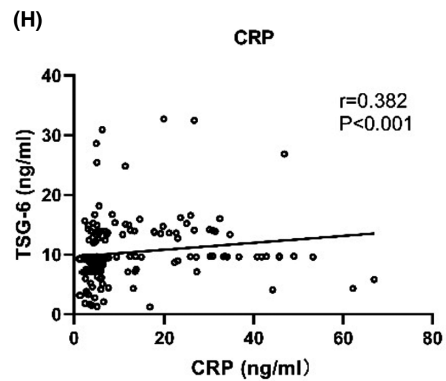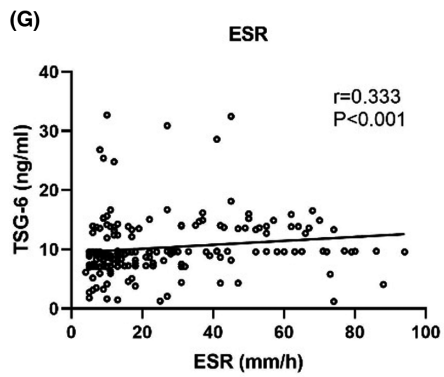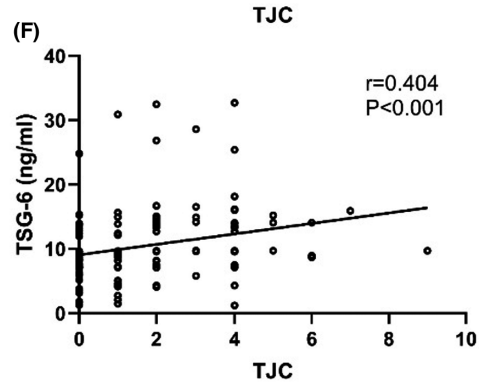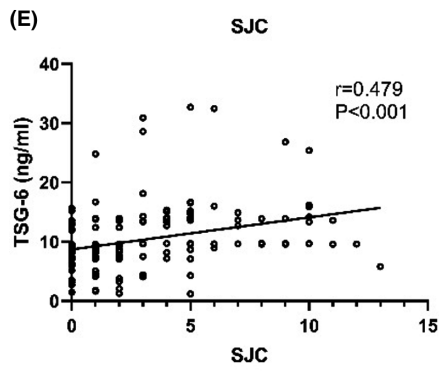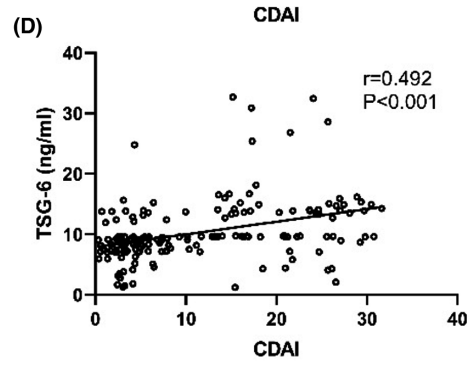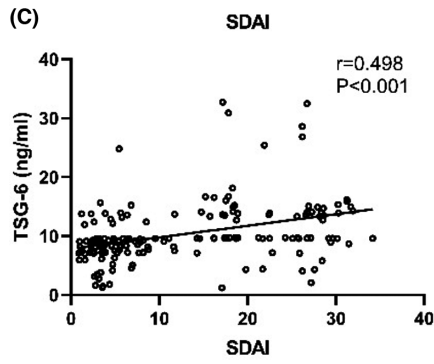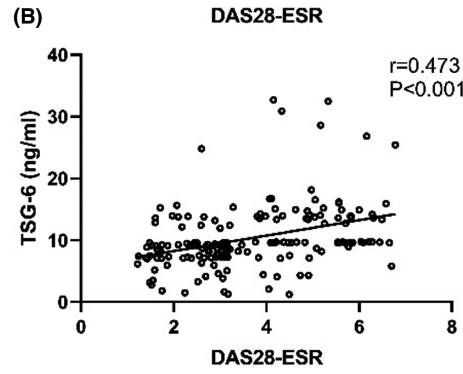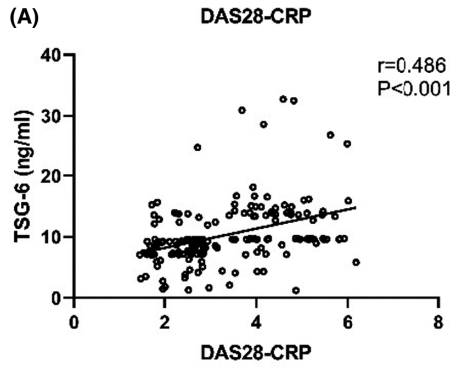
8.3 [7.1–9.4], $p < 0.01$) (Figure 2). The AUC for the differentiation of patients with activity RA from those in inactivity RA was 0.80, the sensitivity was 82.7%, the specificity was 85.3%, and the Youden index was 0.68 (Figure 1B). Moreover, the correlation analysis showed that TSG-6 levels were positively related to comprehensive disease activity scores and most serological indicators, including DAS28-CRP, DAS28-ESR, SDAI, CDAI, TJC, SJC, CRP and ESR (Figure 3). Univariate and multivariate logistic regression analyzes showed that TSG-6 was an independent risk factor related to RA disease activity (odds ratio = 1.2, 95% CI: 1.003–1.453, $p = 0.028$) (Table 3).

4 | DISCUSSION

Rheumatoid arthritis is a chronic autoimmune disease of unknown etiology. Due to the diverse clinical manifestations of RA, patients with early serologic negative or lack of typical symptoms were often misdiagnosed and delayed the best treatment time. Therefore, it is vital to supplement and find suitable biomarkers to improve the diagnosis level of RA.

Tumor necrosis factor-alpha stimulated gene-6 is a 35KD HA binding protein, consisting of a short N-terminal fragment, adjacent link and CUB modules, and a C-terminal region.[13] A large amount of TSG-6 protein has been detected in the synovial fluid and synovial tissue of the inflamed joints of RA patients.[7-9,14] The binding of TSG-6 protein with ligand HA causes conformational changes, thereby participating in the pathological mechanism of RA.[15-19] The data analysis of this study showed that the serum TSG-6 levels in the

FIGURE 3   The correlation between clinical indicators of RA and serum TSG-6 levels. The y-axis represents the concentration of serum TSG-6 in ng/ml. The x-axis reflects the DAS28-CRP score, the DAS28-ESR score, the SDAI score, the CDAI score, the swollen joint count (0–28) score, the tender joint count (0–28) score, ESR and CRP. (A) TSG-6 and DAS28-CRP; (B) TSG-6 and DAS28-ESR; (C) TSG-6 and SDAI; (D) TSG-6 and CDAI; (E) TSG-6 and Swollen joint count (0–28); (F) TSG-6 and Tender joint count (0–28); (G) TSG-6 and ESR; (H) TSG-6 and CRP. The Spearman test was used to assess the relationship between variables. The trend line indicates linear correlation. Each graph shows Spearman's correlation coefficient (r) and the corresponding p-value. CDAI, clinical disease activity index; CRP, C-reactive protein; DAS28, disease activity score based on 28 joint counts; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; SDAI, simplified disease activity index; TSG-6, Tumor necrosis factor-alpha Stimulated gene-6

(A) DAS28-CRP

r=0.486
P<0.001

(B) DAS28-ESR

r=0.473
P<0.001

(C) SDAI

r=0.498
P<0.001

(D) CDAI

r=0.492
P<0.001

(E) SJC

r=0.479
P<0.001

(F) TJC

r=0.404
P<0.001

(G) ESR

r=0.333
P<0.001

(H) CRP

r=0.382
P<0.001

TABLE 3 Analysis of independent risk factors associated with RA disease activity

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*	OR (95% CI)	*p*
TSG-6 (ng/ml)	1.269 (1.149–1.401)	<0.001	1.199 (1.003–1.453)	**0.028**
Anti-CCP (U/ml)	1.000 (1.00–1.001)	0.072	—	—
CRP (ng/ml)	1.814 (1.424–2.311)	<0.001	1.702 (1.313–2.206)	<0.001
ESR (mm/h)	1.215 (1.143–1.291)	<0.001	1.216 (1.090–1.357)	<0.001
Anti-MCV (U/ml)	1.002 (1.001–1.003)	<0.001	1.001 (1.000–1.003)	0.134
NLR	1.211 (1.069–1.372)	**0.003**	0.983 (0.756–1.278)	0.9
RF (U/ml)	1.001 (1.000–1.002)	**0.009**	1.000 (0.998–1.002)	0.716

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; anti-MCV, anti-mutated citrullinated vimentin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil to lymphocyte ratio; RF, rheumatoid factor; TSG-6, Tumor necrosis factor-alpha Stimulated gene-6. The bold values indicate *p* < 0.05, which is statistically significant.

RA group were significantly higher than that in the non-RA group and the HCs. ROC analysis showed that serum TSG-6 concentration had strong overall performance characteristics in distinguishing RA patients from other subjects (non-RA and HCs), with an AUC of 0.78. When the cut-off value was 6.99, the specificity and sensitivity of TSG-6 for RA diagnosis were 86.9% and 74.7%, respectively. When connected in parallel, the combination of TSG-6 and anti-MCV had the highest sensitivity (98.4%), which was significantly higher than the single index. It is suggested that TSG-6 can improve the sensitivity of RA diagnosis, which is of great significance in improving the level of RA diagnosis.

This study found that the serum TSG-6 concentrations showed an upward trend with increased disease activity. The TSG-6 concentrations were significantly higher in active RA than in inactive RA, which was a little different from the study of Gyorgy Nagyeri.[20] The cause may be correlated with differences in the kits used and species differences. This tendency was confirmed in RA patients defined by DAS28-ESR, SDAI and CDAI. At the same time, ROC analysis indicated that the AUC of TSG-6 in the active RA group vs. the inactive RA group was 0.80, indicating that TSG-6 can effectively evaluate the severity of RA. Spearman correlation analysis directed that TSG-6 concentrations in RA patients were significantly positively correlated with DAS28-CRP, DAS28-ESR, SDAI, CDAI, SJC, TJC, ESR and CRP. Binary logistic regression analysis also showed that the increase of TSG-6 levels was independently correlated with the severity of RA (*p* < 0.05). These results showed that TSG-6 might serve as a biomarker in reflecting the activity of RA disease.

Possible explanations for this positive correlation will be complex. Serum levels of TSG-6 are elevated in many inflammatory diseases.[5,21–26] Szántó S[10] et al. revealed that in the collagen antibody-induced arthritis model, the joints of mice with TSG-6 gene defects are more severely damaged. Interestingly, in an inflammatory environment, the TSG-6 concentrations in FLS were markedly increased. TSG-6 also has an anti-inflammatory function and chondroprotective effect.[27] Therefore, TSG-6 may become an attractive therapeutic target for RA patients. Nevertheless, further investigations are required to explore the development and therapeutic role of TSG-6 in RA.

5 | CONCLUSIONS

The TSG-6 concentrations in RA patients were markedly higher than that in the non-RA group and HCs, and it was positively related to the activity of RA disease. The combination of TSG-6 and anti-MCV can significantly improve the diagnosis of RA. Therefore, it is possible that TSG-6 could be used in the next future as a biomarker for diagnosing RA and assessing disease activity.

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REFERENCES

1. Cush J-J. Rheumatoid arthritis: early diagnosis and treatment. *Med Clin North Am.* 2021;105(2):355-365.
2. Koopman F-A, Chavan S-S, Miljko S, et al. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc Natl Acad Sci USA.* 2016;113(29):8284-8289.
3. Huang J, Zeng T, Zhang X, et al. Clinical diagnostic significance of 14-3-3eta protein, high-mobility group box-1, anti-cyclic citrullinated peptide antibodies, anti-mutated citrullinated vimentin antibodies and rheumatoid factor in rheumatoid arthritis. *Br J Biomed Sci.* 2020;77(1):19-23.
4. Lee T-H, Lee G-W, Ziff E-B, et al. Isolation and characterization of eight tumor necrosis factor-induced gene sequences from human fibroblasts. *Mol Cell Biol.* 1990;10(5):1982-1988.
5. Wisniewski H-G, Colon E, Liublinska V, et al. TSG-6 activity as a novel biomarker of progression in knee osteoarthritis. *Osteoarthritis Cartilage.* 2014;22(2):235-241. doi:10.1016/j.joca.2013.12.004
6. Milner C-M, Day A-J. TSG-6: a multifunctional protein associated with inflammation. *J Cell Sci.* 2003;116(Pt 10):1863-1873.
7. Robinson L-J, Tourkova I, Wang Y, et al. FSH-receptor isoforms and FSH-dependent gene transcription in human monocytes and osteoclasts. *Biochem Biophys Res Commun.* 2010;394(1):12-17.
8. Bayliss M-T, Howat S-L, Dudhia J, et al. Up-regulation and differential expression of the hyaluronan-binding protein TSG-6 in cartilage and synovium in rheumatoid arthritis and osteoarthritis. *Osteoarthritis Cartilage.* 2001;9(1):42-48.
9. Wisniewski H-G, Vilcek J. TSG-6: an IL-1/TNF-inducible protein with anti-inflammatory activity. *Cytokine Growth Factor Rev.* 1997;8(2):143-156.
10. Szanto S, Bardos T, Gal I, et al. Enhanced neutrophil extravasation and rapid progression of proteoglycan-induced arthritis in TSG-6-knockout mice. *Arthritis Rheum.* 2004;50(9):3012-3022. doi:10.1002/art.20655
11. Aletaha D, Neogi T, Silman A-J, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010;62(9):2569-2581.
12. Wells G, Becker J-C, Teng J, et al. Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann Rheum Dis.* 2009;68(6):954-960.
13. Day A-J, Milner C-M. TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties. *Matrix Biol.* 2019;30(11):60-83.
14. Maier R, Wisniewski H-G, Vilcek J, et al. TSG-6 expression in human articular chondrocytes. Possible implications in joint inflammation and cartilage degradation. *Arthritis Rheum.* 1996;39(4):552-559.
15. Lesley J, English N-M, Gal I, et al. Hyaluronan binding properties of a CD44 chimera containing the link module of TSG-6. *J Biol Chem.* 2002;277(29):26600-26608.
16. Lesley J, Gal I, Mahoney D-J, et al. TSG-6 modulates the interaction between hyaluronan and cell surface CD44. *J Biol Chem.* 2004;279(24):25745-25754. doi:10.1074/jbc.M313319200
17. Hutas G, Bajnok E, Gal I, et al. CD44-specific antibody treatment and CD44 deficiency exert distinct effects on leukocyte recruitment in experimental arthritis. *Blood.* 2008;112(13):4999-5006. doi:10.1182/blood-2008-04-150383
18. Dyer D-P, Thomson J-M, Hermant A, et al. TSG-6 inhibits neutrophil migration via direct interaction with the chemokine CXCL8. *J Immunol.* 2014;192(5):2177-2185.
19. He Z, Hua J, Qian D, et al. Intravenous hMSCs ameliorate acute pancreatitis in mice via secretion of tumor necrosis factor-alpha stimulated gene/protein 6. *Sci Rep.* 2016;6(12):6384-6391.
20. Nagyeri G, Radacs M, Ghassemi-Nejad S, et al. TSG-6 protein, a negative regulator of inflammatory arthritis, forms a ternary complex with murine mast cell tryptases and heparin. *J Biol Chem.* 2011;286(26):23559-23569.
21. Li Q, Song W-J, Ryu M-O, et al. TSG-6 secreted by human adipose tissue-derived mesenchymal stem cells ameliorates severe acute pancreatitis via ER stress downregulation in mice. *Stem Cell Res Ther.* 2018;9(1):255-268.
22. Zhang N, Luo X, Zhang S, et al. Subconjunctival injection of tumor necrosis factor-alpha pre-stimulated bone marrow-derived mesenchymal stem cells enhances anti-inflammation and anti-fibrosis in ocular alkali burns. *Graefes Arch Clin Exp Ophthalmol.* 2021;259(4):929-940.
23. Yang H, Wu L, Deng H, et al. Anti-inflammatory protein TSG-6 secreted by bone marrow mesenchymal stem cells attenuates neuropathic pain by inhibiting the TLR2/MyD88/NF-kappaB signaling pathway in spinal microglia. *J Neuroinflammation.* 2020;17(1):154-175.
24. Koike Y, Li B, Lee C, et al. The intestinal injury caused by ischemia-reperfusion is attenuated by amniotic fluid stem cells via the release of tumor necrosis factor-stimulated gene 6 protein. *FASEB J.* 2020;34(5):6824-6836.
25. Wang M, Zhang M, Fu L, et al. Liver-targeted delivery of TSG-6 by calcium phosphate nanoparticles for the management of liver fibrosis. *Theranostics.* 2020;10(1):36-49.
26. Lee R-H, Pulin A-A, Seo M-J, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell.* 2009;5(1):54-63.
27. Bardos T, Kamath R-V, Mikecz K, et al. Anti-inflammatory and chondroprotective effect of TSG-6 (tumor necrosis factor-alpha-stimulated gene-6) in murine models of experimental arthritis. *Am J Pathol.* 2001;159(5):1711-1721.