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YKL-40 as a new biomarker of disease activity in Takayasu arteritis

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ABSTRACT

Objective: To evaluate the role of YKL-40 as a biomarker of disease activity in patients with Takayasu arteritis (TA).
Methods: The study included 40 patients diagnosed with TA between January 2017 and January 2018. 40 age and sex matched healthy controls were included. Serum levels of YKL-40, as well as IL-6, IL-8, IL-17, sCD163, VEGF, MMP-2, MMP-9, OPN, PTX-3 and IFN-γ, were detected at the base line and end of the 6-month follow-up. Modified Kerr criteria, in which MRA was performed instead of traditional angiography, was used a standard measure of disease activity. The association of the measured biomarkers with disease activity was analysed.
Results: The serum levels of YKL-40, IL-6, IL-8, IFN-γ, MMP-2, MMP-9, PTX-3 and OPN were significantly higher in active disease than in inactive disease. Significant differences in the serum levels of YKL-40, IL-6 and PTX-3 were also observed according to the disease activity degree. Logistic analysis demonstrated that high YKL-40 levels and high IL-6 levels were independent risk factors for active disease. When YKL-40 was combined with IL-6, the specificity and sensitivity for detecting active disease were increased (87.6% and 70.4% respectively); similar findings were obtained when YKL-40 was combined with CRP (72.3% and 84.6% respectively). A predictive model of active disease using ESR, CRP, IL-6, PTX-3 and MMP-9 showed significantly improved diagnostic efficiency when YKL-40 was added to the model (sensitivity: 85.1%; specificity: 94.3%; NRI value: 12.4%; IDI value: 4.6%, $p < 0.05$).
Conclusions: Serum YKL-40 concentrations may be a useful biomarker of disease activity in TA.

1. Introduction

The YKL-40 protein, also called CHI3L1, is a mammalian member of the family of 18-glycosyl hydrolases [1,2]. YKL-40 is a heparin- and chitin-binding lectin that does not exhibit chitinase activity. YKL-40 is usually secreted from activated neutrophils and macrophages in different tissues in response to inflammation [1,3]. Recent research has demonstrated that YKL-40 is also secreted by vascular smooth muscle cells, cancer cells, as well as cancer-associated fibroblasts [2,4–6]. The precise biological function of YKL-40 is not clear; however, there is some indication that it is involved in the inflammation and remodelling of the extracellular matrix after tissue injury [1,2]. Recently, YKL-40 has received attention as a potential biomarker in some pathological conditions characterized by tissue injury and inflammation, such as rheumatoid arthritis and liver fibrosis [7,8]. Furthermore, elevated serum levels of YKL-40 have been positively associated with disease activity and disease severity in patients with giant cell arteritis, atherosclerosis, coronary heart disease as well as Behcet disease [9–12]. Based on these findings, YKL-40 might be a valuable target of investigation in the context of cardiovascular disease.

Takayasu arteritis (TA) is a rare systemic vasculitis that causes stenosis, occlusion, and dilatation of large vessels such as the aorta and its major branches [13]. TA is typically characterized by a prolonged, indolent course with nonspecific manifestations such as fever, malaise, weight loss and headache/dizziness [14]. Further, important organs may gradually become dysfunctional due to stenosis or occlusion of a culprit vessel. TA tends to progress despite treatment with glucocorticoid and/or immunosuppressive agents [15,16]. Therefore, it is crucial to identify more biomarkers to monitor disease activity and possibly predict exacerbations, in order to aid early treatment.

The causes of TA are still unknown, but its prominent pathological feature is an inflammatory infiltrate composed of T lymphocytes, macrophages, as well as fibroblasts, and vascular remodelling and fibrosis in response to early vascular inflammation [17,18]. Several inflammatory cytokines including interleukin (IL)-6, IL-17 and interferon (IFN)-γ have been reported to play an important role in TA [19,20]. Our previous study confirmed the crucial role of IL-6 in the vascular fibrosis of TA, and its relationship with disease activity [19,21]. The expression of YKL-40 has been reported to be regulated by inflammatory cytokines, including IL-6 and IL-17 [1,3]. Based on these findings, it would be interesting to evaluate the serum YKL-40 level in TA and determine its association

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with the levels of associated cytokines such as IL-6 and IL-17. That is, the serum levels of YKL-40 may have potential as a biomarker for the assessment of disease activity in TA.

The present study evaluates the potential of YKL-40 for monitoring disease activity in TA and analyses its association with previously reported inflammatory cytokines associated with TA [22–24], including IL-6, IL-8, IL-17, sCD163, vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-2, MMP-9, osteopontin (OPN), pentraxin (PTX)-3 and IFN-γ.

2. Materials and methods

2.1. Patients

A total of 40 patients, who first visited our department between January 2017 and January 2018, were enrolled in this study. The inclusion criteria were (i) classified as TA according to the 1990 ACR classification criteria [25]; (ii) absence of other concomitant autoimmune diseases, malignancy, or infection; and (iii) 6-month follow-up. Age and sex matched healthy controls ($n = 40$) were also enrolled.

The study was approved by the Institutional Review Board of Zhongshan Hospital, Fudan University, China, and was conducted with the informed consent of all the participants.

2.2. Follow-up

The frequency of visits was once every month. Detailed information regarding the patient's signs and symptoms were recorded at each visit. Complete blood count, erythrocyte sedimentation rate (ESR), and the levels of C-reactive protein (CRP) and pro-inflammatory cytokines were determined according to standard protocols, and disease activity was analysed using the Kerr score. Drug-related side events were recorded at each follow-up.

All the patients enrolled in this study underwent whole-body contrast-enhanced magnetic resonance angiography (MRA) at the base line. At the end of the 6-month follow-up, a second round of MRA scans were obtained. Patients were grouped according to the revised angiographic classification of the international TA conference in Tokyo (1996) based on lesion distribution [26]: type I, branches of the aortic arch; IIa, ascending aorta, aortic arch, and its branches; IIb, ascending aorta, aortic arch, its branches, and thoracic descending aorta; III, thoracic descending aorta, abdominal aorta, and/or renal arteries; IV, abdominal aorta and/or renal arteries; V, combined features of IIb and IV.

2.3. Disease activity assessment

Modified Kerr's criteria were used as the gold standard for disease activity assessment in this study [27]. Modified Kerr's criteria are a general evaluation based on new/worse symptoms, new/worse physical signs, inflammatory parameters (ESR > 40 mm/h), and positive imaging findings (MRA) instead of traditional angiography. Patients who satisfied ≥2 criteria and had no other infective or inflammatory conditions were considered to have active disease and were assigned to the active group. Positive MRA findings at baseline include vascular wall enhancement, wall edema etc. as defined in one previous study [21].

The presence of new vascular lesions and progression of the stenosis of primary lesions by ≥25% were considered as progressive imaging findings at the end of the 6-month follow-up. Two rheumatologists undertook the evaluation in a systematic and independent manner, and if their initial opinion differed, consensus was achieved by discussion.

2.4. Biochemical analysis

Serum samples of each patient were collected at the base line and at the end of the 6-month follow-up, and stored at −80 °C. Serum concentrations of YKL-40 (R&D Systems Europe Ltd., Abingdon, UK), IL-6 (eBioscience, San Diego, CA, USA), IL-8 (eBioscience, San Diego, CA, USA), IL-17 (R&D Systems Europe Ltd., Abingdon, UK), VEGF (eBioscience, San Diego, CA, USA), OPN (eBioscience, San Diego, CA, USA), IFN-γ (R&D Systems Europe Ltd., Abingdon, UK), sCD163 (eBioscience, San Diego, CA, USA), MMP-2 (eBioscience, San Diego, CA, USA), MMP-9 (eBioscience, San Diego, CA, USA) and PTX-3 (R&D Systems Europe Ltd., Abingdon, UK) were determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions, and the assays were performed in duplicate.

2.5. Statistical analysis

All the statistical analyses were performed using the SPSS statistical software (version 20.0; SPSS Institute Inc., Cary, NC, USA). Discrete variables were described using frequency (percentages), and continuous variables were reported using median (range) or mean (SD). The Mann–Whitney *U* test was used for comparison of serum YKL-40 concentrations between two independent groups. Furthermore, the differences in serum YKL-40 levels in patient subgroups categorized according to disease activity (in terms of the Kerr score) were assessed using one-way ANOVA. Pearson's correlation analysis was used to evaluate the associations between the different parameters. Univariate logistic regression analysis was performed to identify potential biomarkers for predicting active disease. The receiver operating characteristic (ROC) curve was used to determine the cut-off level for YKL-40 and other biomarkers. Based on the determined cut-off value for YKL-40 and other biomarkers, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Predictive models for identifying active disease were created using different biomarkers with or without YKL-40. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were performed to evaluate whether YKL-40 could improve the efficiency of predicting active disease. All probabilities were two-tailed, and statistical significance was set at $p < 0.05$.

3. Results

3.1. Patient characteristics

The study population comprised 40 patients (mean age, 31 ± 4 years; female:male ratio, 9:1; mean duration of disease, 23 ± 17 months). The active group comprised 28 patients, whereas the inactive group comprised 12 patients. The detailed demographic and clinical characteristics of the patients are summarized in Supplementary Table.

In the active group, prednisone treatment at a dosage of 0.8–1.0 $mg \cdot kg^{-1} \cdot day^{-1}$ (p.o.) was started. After 4 weeks, the prednisone dose was tapered gradually to a maintenance dose of 0.1–0.2 $mg \cdot kg^{-1} \cdot day^{-1}$ within the next 5 months. Additionally, one type of immunosuppressant was used as decided by the attending physician: cyclophosphamide (0.5–0.75 g/m^2, i.v., usually 0.8 g) was used in 13 cases, methotrexate (MTX, 10–15 mg/week, p.o.) in 4 cases, azathioprine (AZA, 50 mg/day, p.o.) in 2 cases and leflunomide (LEF, 20 mg/day, p.o.) in 9 cases. In the inactive group, the prednisone dose was 0.1–0.2 $mg \cdot kg^{-1} \cdot day^{-1}$, and MTX (10–15 mg/week, p.o.), AZA (25–50 mg/day, p.o.) or LEF (10–20 mg/day, p.o.) were used as maintenance drugs.

3.2. Comparison of baseline serum YKL-40 and other inflammation biomarker levels between the active and inactive groups

YKL-40 levels were significantly higher in patients with TA than that in healthy controls (94.1 ng/ml vs. 56.7 ng/ml, $p = 0.04$). At the baseline, the serum levels of YKL-40 were significantly higher in the active group than in the inactive group (109.7 ± 33.7 vs. 63.3 ± 46.6 ng/ml, $p = 0.02$). The active group also had significantly higher serum levels of IL-6 (11.7 ± 10.4 vs. 5.7 ± 6.1 pg/ml, $p < 0.01$), IL-8 (10.3 ± 6.5 vs. 8.1 ± 2.5 pg/ml, $p = 0.04$), IFN-γ (3.1 ± 1.1 vs. 1.3 ± 0.6 pg/ml, $p < 0.01$), MMP-2 (95.3 ± 40.9 vs. 70.1 ± 33.7 ng/ml, $p = 0.04$), MMP-9 (399.5 ± 159.5 vs. 225.4 ± 97.6 ng/ml, $p = 0.03$), PTX-3 (13.2 ± 5.6 vs. 7.3 ± 4.3 ng/ml, $p = 0.02$) and OPN (38.9 ± 41.1 vs. 18.1 ± 12.3 ng/ml, $p < 0.01$) (Fig. 1).

To further analyse the differences in the above biomarkers between patients with different levels of disease activity, the patients were divided into four groups according to their modified Kerr scores: Kerr score ≤ 1 ($n = 12$), Kerr score = 2 ($n = 14$), Kerr score = 3 ($n = 9$) and Kerr score = 4 ($n = 5$). The results demonstrated significant differences in the serum levels of YKL-40, IL-6 and PTX-3 among the four groups (Fig. 1 and Table 1).

3.3. Correlations between serum YKL-40 levels, other serum biomarkers and disease activity

Pearson's correlation analysis showed that the YKL-40 level was positively correlated with the modified Kerr criteria (Rho = 0.67, $p < 0.01$), ESR levels (Rho = 0.55, $p = 0.04$) and CRP levels (Rho = 0.48, $p = 0.03$). Additionally, the serum level of YKL-40 was also positively correlated with IL-6, MMP-9, PTX-3 and OPN (Rho = 0.58, 0.59, 0.62, and 0.71 respectively; $p < 0.05$ for all the correlations). The other correlations between the different factors were not significant.

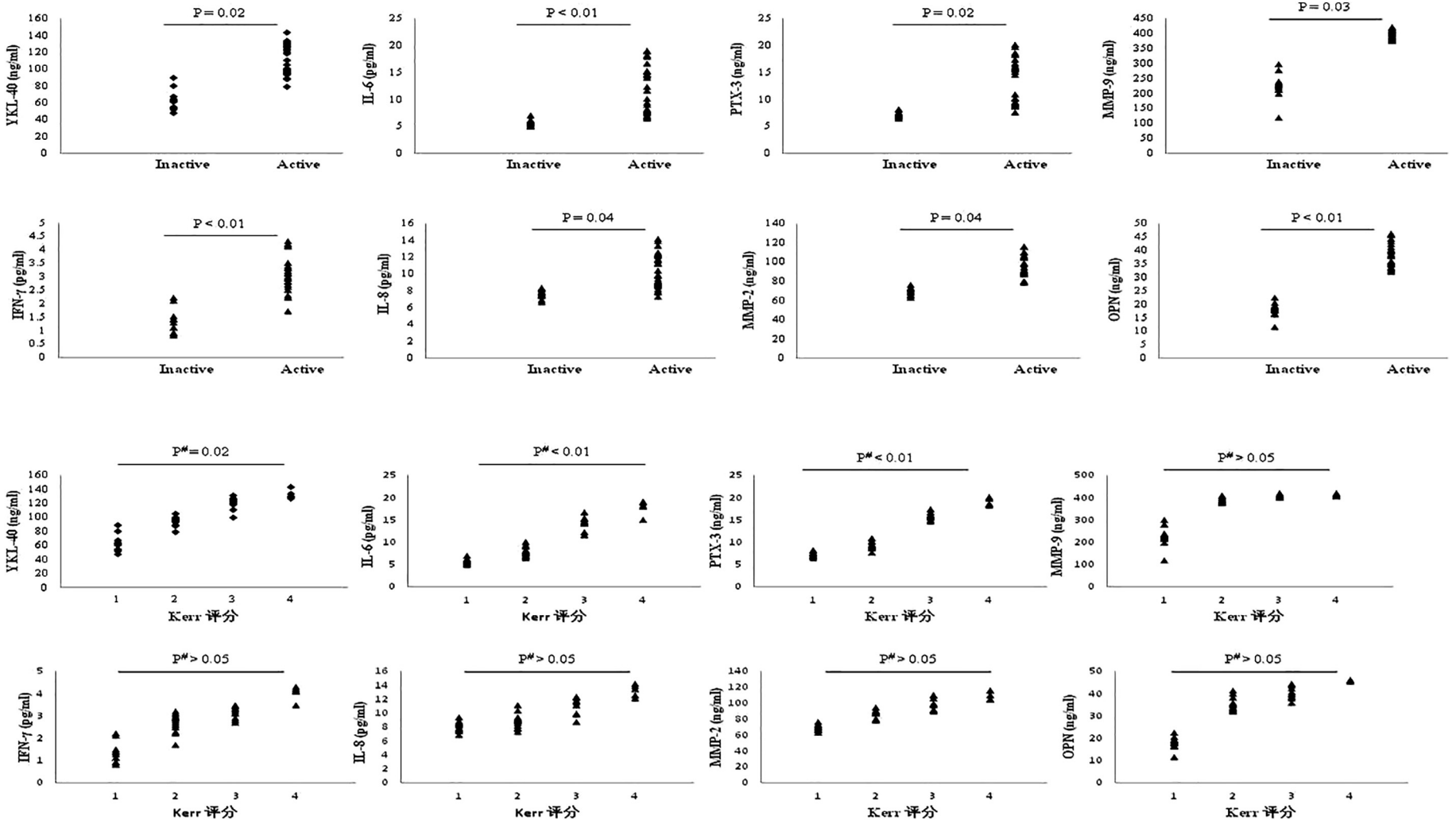

Fig. 1. Comparison of serum YKL-40 levels and other biomarkers between patients with different disease activity. *P*-value: comparison between active and inactive group, $P < 0.05$ was considered to indicate statistical significance; $P^{\#}$-value: comparison between patients according to Kerr score, $P^{\#}$-value <0.05 was considered to indicate statistical significance.

3.4. Potential risk factors for active disease

The parameters that were different between the active and inactive groups at a significance level of $p < 0.10$ at the baseline were chosen as candidate indicators: systemic symptoms, nervous system symptoms, cardiovascular symptoms, asymmetric blood pressure, and the ESR, CRP, YKL-40, IL-6, IL-8, IFN-γ, MMP-2, MMP-9, PTX-3 and OPN levels.

The results of logistic analysis demonstrated that high YKL-40 levels (>80.1 ng/ml, OR = 2.31 [95% CI = 1.08–20.45], $p = 0.01$) and high IL-6 levels (>7.4 pg/ml, OR = 2.03 [95% CI = 1.01–11.47], $p = 0.02$) were independent risk factors for active disease.

3.5. YKL-40 as an indicator of disease activity

ROC analysis showed that at a cut-off value of 80.1 ng/ml, YKL-40 had a sensitivity of 73.2%, a specificity of 82.4%, a PPV of 90.7%, an NPV of 61.6%, and an AUC of 0.81 for identifying active TA (Supplementary Fig. 1). Furthermore, serial and parallel tests were conducted using YKL-40 combined with other potential biomarkers that were increased in patients with active disease, as shown in Table 2. The results indicated that when YKL-40 was combined with IL-6 (cut-off value: 7.4 pg/ml), the specificity improved to 87.6% (sensitivity: 70.4%, PPV: 94.1%, NPV: 65.1%, AUC: 0.86), and when YKL-40 was combined with CRP (cut-off value: 9.5 mg/L), the sensitivity was improved to 84.6% (specificity: 72.3%, PPV: 62.4%, NPV: 92.4%, AUC: 0.82) (Table 2).

Predictive models for identifying active disease were created using different biomarkers with or without YKL-40. Following this, NRI and IDI were performed to evaluate whether YKL-40 could improve the efficiency of the models. The predictive model using ESR, CRP, IL-6, PTX-3 and MMP-9 demonstrated a sensitivity of 82.3%, a specificity of 92.7% and an AUC of 0.89. When YKL-40 was added to this model, the sensitivity was 85.1%, the specificity was 94.3%, and the AUC was 0.92. The NRI value was 12.4%, with a z-value of 1.52 and a *p*-value of 0.04. The IDI value was 4.6%, with a z-value of 1.53 and a p-value of 0.04. For the predictive model using all the biomarkers (biomarkers shown in Table 2), the sensitivity was 81.3%, the specificity was 89.4% and the AUC was 0.88. When YKL-40 was added to this model, the sensitivity was 82.9% and the specificity was 91.3%, with an AUC of 0.90. The NRI value was 10.4%, with a z-value of 1.32 and a p-value of 0.06. The IDI value was 3.6%, with a z-value of 1.42 and a p-value of 0.05.

3.6. Changes in YKL-40 and other biomarkers during the 6-month follow-up

After 6-month treatment with prednisone and immunosuppressants, in all the patients, a significant decrease was observed in YKL-40 (54.3 ± 40.4 vs. 95.8 ± 92.3 ng/ml [baseline], $p = 0.02$), IL-8 (6.4 ± 4.1 vs. 9.6 ± 5.7 pg/ml, p = 0.02), IL-6 (5.3 ± 2.0 vs. 9.9 ± 7.1 pg/ml, $p < 0.01$), PTX-3 (6.1 ± 4.3 vs. 11.4 ± 9.3 ng/ml, p = 0.02), MMP-9 (189.7 ± 90.7 vs. 347.3 ± 213.2 ng/ml, $p = 0.03$), and IFN-γ (0.9 ± 1.1 vs. 2.6 ± 1.1 pg/ml, p < 0.01) (Supplementary Fig. 2).

Whole-body contrast-enhanced MRA was performed at the end of the 6-month treatment in all patients. One patient had a new vascular lesion of left subclavian artery (case 1). The other case (case 2) had progression of the renal artery lesion (luminal stenosis: 30% at base line vs. 65% at the end of 6-month).

Case 1. In this patient, active disease was diagnosed at the baseline with a modified Kerr score of 3. During the 6-month treatment, disease remission was achieved with a modified Kerr score of 1, and the ESR and CRP decreased to normal levels (ESR [6-month vs. baseline]: 17 vs. 53 mm/H; CRP: 3.0 vs. 7.6 mg/L). Throughout the treatment, the YKL-40 levels were higher than the cut-off value (6-month vs. baseline: 92.7 vs. 134.2 ng/ml), while the levels of the other biomarkers decreased to below the cut-off value, including IL-6 (6.8 vs. 10.2 pg/ml), MMP-9 (273.9 vs. 391.2 ng/ml), PTX-3 (8.7 vs. 17.4 ng/ml), IFN-γ (1.2 vs. 3.1 pg/ml), and IL-8 (7.1 vs. 9.4 pg/ml).

Case 2. Active disease was diagnosed at the base line with a modified Kerr score of 2. After the 6-month treatment, the modified Kerr score was 1 and the serum levels of ESR and CRP decreased to normal levels (ESR: 15 vs. 49 mm/H; CRP: 2.1 vs. 9.8 mg/L). Furthermore, a decrease in the YKL-40 levels (88.9 vs. 109.3 ng/ml) and IL-6 levels (5.5 vs. 8.2 pg/ml) was indicated, while similar levels of PTX-3 (6.1 vs. 7.4 ng/ml), IL-8 (6.7 vs. 6.9 pg/ml), MMP-9 (297.4 vs. 288.3 ng/ml) and IFN-γ (1.6 vs. 1.7 pg/ml) were observed. Similar to Case 1, the YKL-40 levels were higher than the cut-off value throughout the 6-month treatment.

4. Discussion

To our knowledge, this is the first study to demonstrate the value of YKL-40 as a new biomarker of disease activity in patients with TA.

In a study on giant cell arteritis, the serum levels of YKL-40 were found to be positively correlated to disease activity and treatment response [9]. In our investigation, the YKL-40 level was not only increased in active disease, but also related to the level of disease activity. Moreover, the serum levels of YKL-40 were still higher than the cut-off value (80.1 ng/ml) in the two patients with imaging progression, while the ESR and CRP levels had both decreased to normal after the treatment. These results further demonstrate that YKL-40 could be a better biomarker than CRP and ESR for monitoring disease activity and

Table 1
Comparison of serum YKL-40 levels and other inflammation biomarkers between patients according to Kerr score.

	Inactive group		Active group		P-value	P#-value
	Kerr ≤1 (*n* = 12)	Kerr = 2 (n = 14)	Kerr = 3 (n = 9)	Kerr = 4 (*n* = 5)		
YKL-40 (ng/ml)	63.3 ± 46.6	94.7 ± 78.9	120.1 ± 67.9	132.8 ± 114.1	0.02	0.02
IL-6 (pg/ml)	5.7 ± 6.1	7.9 ± 5.6	14.3 ± 7.7	17.8 ± 3.8	<0.01	0.01
IL-8 (pg/ml)	8.1 ± 2.5	8.9 ± 7.7	11.1 ± 4.6	13.2 ± 5.7	0.04	0.15
IL-17A (pg/ml)	1.2 ± 0.8	1.2 ± 0.9	1.7 ± 1.1	2.1 ± 1.4	0.79	0.44
IFN-γ (pg/ml)	1.3 ± 0.6	2.7 ± 1.5	3.1 ± 2.3	4.0 ± 1.9	<0.01	0.09
sCD163 (ng/ml)	479.3 ± 203.4	534.3 ± 211.3	582.6 ± 310.9	589.9 ± 117.9	0.88	0.49
VEGF (ng/ml)	137.2 ± 84.4	120.3 ± 90.7	107.8 ± 100.7	90.8 ± 78.4	0.59	0.15
MMP-2 (ng/ml)	70.1 ± 33.7	87.3 ± 33.6	99.4 ± 41.7	110.4 ± 57.6	0.04	0.23
MMP-9 (ng/ml)	225.4 ± 97.6	389.3 ± 111.1	407.9 ± 231.4	413.1 ± 173.2	0.03	0.87
PTX-3 (ng/ml)	7.3 ± 4.3	9.3 ± 3.7	15.9 ± 2.8	19.1 ± 6.4	0.02	<0.01
OPN (ng/ml)	18.1 ± 12.3	35.1 ± 32.0	40.3 ± 22.7	45.9 ± 34.7	<0.01	0.69

YKL-40 protein: a chitinase-like protein, IL: interleukin-, VEGF: vascular endothelial growth factor, MMP: matrix metalloproteinase, OPN: osteopontin, PTX-3: pentraxin, IFN-γ: interferon-γ; P-value: comparison between the active and inactive group, P-value <0.05 was considered to indicate statistical significance; P#-value: comparison between patients according to Kerr score, P#-value < 0.05 was considered to indicate statistical significance.

Table 2
Value of YKL-40 with/without other biomarkers for indicating disease activity.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)
YKL-40 (80.1 ng/ml)	73.2%	82.4%	90.7%	61.6%	0.81 (0.78–0.92)
IL-6 (7.4 pg/ml)	75.0%	83.3%	91.6%	60.8%	0.83 (0.76–0.95)
PTX-3 (8.8 ng/ml)	77.8%	81.2%	84.4%	55.9%	0.80 (0.66–0.85)
IFN-γ (1.7 pg/ml)	78.5%	70.4%	65.7%	55.7%	0.77 (0.61–0.84)
IL-8 (8.4 pg/ml)	55.9%	57.4%	47.8%	47.2%	0.54 (0.43–0.61)
MMP-2 (78.6 ng/ml)	67.7%	75.2%	73.5%	58.7%	0.68 (0.59–0.74)
MMP-9 (325.4 ng/ml)	69.7%	74.6%	70.1%	60.3%	0.69 (0.53–0.81)
OPN (30.4 ng/ml)	52.3%	55.4%	42.3%	43.9%	0.53 (0.42–0.67)
ESR (40 mm/H)	63.9%	70.2%	63.1%	56.5%	0.61 (0.55–0.66)
CRP (9.5 mg/L)	81.3%	67.7%	54.3%	89.3%	0.71 (0.61–0.77)
Serial test					
YKL-40 and IL-6	70.4%	87.6%	94.1%	65.1%	0.86 (0.81–0.94)
YKL-40 and PTX-3	72.7%	85.3%	92.7%	63.3%	0.82 (0.77–0.89)
YKL-40 and IFN γ	73.1%	75.8%	67.8%	64.8%	0.79 (0.69–0.86)
YKL-40 and IL-8	52.3%	69.3%	64.8%	44.1%	0.61 (0.54–0.71)
YKL-40 and MMP-2	60.8%	78.4%	81.2%	52.3%	0.70 (0.62–0.78)
YKL-40 and MMP-9	63.7%	78.2%	79.3%	56.1%	0.71 (0.65–0.76)
YKL-40 and OPN	48.4%	63.7%	51.9%	36.9%	0.58 (0.47–0.62)
YKL-40 and ESR	62.3%	76.7%	74.6%	55.5%	0.69 (0.61–0.74)
YKL-40 and CRP	72.6%	75.2%	66.6%	63.8%	0.78 (0.68–0.83)
Parallel test					
YKL-40 or IL-6	79.4%	70.6%	55.8%	87.2%	0.74 (0.69–0.77)
YKL-40 or PTX-3	80.7%	73.8%	65.1%	89.2%	0.77 (0.73–0.81)
YKL-40 or IFN γ	82.3%	75.1%	69.5%	90.1%	0.78 (0.69–0.84)
YKL-40 or IL-8	61.2%	56.1%	42.7%	54.2%	0.62 (0.55–0.70)
YKL-40 or MMP-2	70.8%	66.4%	68.2%	61.8%	0.70 (0.66–0.75)
YKL-40 or MMP-9	74.3%	60.3%	61.3%	65.3%	0.72 (0.63–0.77)
YKL-40 or OPN	62.7%	50.7%	40.1%	52.9%	0.58 (0.46–0.63)
YKL-40 or ESR	69.6%	62.9%	47.6%	58.4%	0.64 (0.59–0.71)
YKL-40 or CRP	84.6%	72.3%	62.4%	92.4%	0.82 (0.76–0.88)

PPV: positive predictive value; NPV: negative predictive value; AUC: area under ROC curve; 95% CI: 95% confidence interval; YKL-40 protein: a chitinase-like protein; IL: interleukin, MMP: matrix metalloproteinase, OPN: osteopontin, PTX-3: pentraxin, IFN-γ: interferon-γ, ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

may reflect other aspects of the inflammatory response that are not indicated by CRP or ESR. Additionally, in our study, we used NRI and IDI to compare predictive models with or without YKL-40, and the results confirmed that YKL-40 could improve the effectiveness of the models for disease activity assessment.

In our former study, infiltration of macrophages as well as fibroblasts/myofibroblasts in the adventitia and media of the involved vessels was observed in TA [19]. This could mean that the interaction between macrophages and fibroblasts/myofibroblasts might promote vascular fibrosis and remodelling in TA. Further, according to research on chronic renal fibrosis, YKL-40 might promote tissue fibrosis by regulating the interaction between macrophages and fibroblast/myofibroblasts [28,29]. Research on breast cancer also indicated that YKL-40 could regular the interaction between macrophages and cancer fibroblast [30]. The present study demonstrated higher YKL-40 levels in patients with imaging progression; thus, YKL-40 might be associated with the vascular fibrosis and remodelling that occur subsequent to inflammation (shown in Fig. 2). However, the mechanism by which YKL-40 may control vascular fibrosis in TA is unclear. The role of YKL-40 in vascular fibrosis via regulation of the interaction between macrophages and fibroblasts/myofibroblasts and the specific mechanisms associated with it would be an interesting and valuable topic of study in the future.

Our previous studies have demonstrated that IL-6 and MMPs are involved in vascular fibrosis and remodelling and are positively associated with disease activity in TA [19,21]. In this study, we have investigated the relationship between YKL-40 and these factors for the assessment of disease activity. We confirmed the association of IL-6 and MMP-9 with disease activity; however, we found that neither was associated with the grade of disease activity. Further, the sensitivity and specificity of MMP-9 for the diagnosis of active TA were lower than the sensitivity and specificity of IL-6 and YKL-40, but the values were similar to those reported for MMP-9 in a Japanese study [31]. Nonetheless, we found a positive correlation between the serum levels of YKL-40 and IL-6 and MMP-9. Given the important roles of IL-6 and MMP-9 in vascular inflammation and the subsequent fibrosis, this finding confirms that YKL-40 not only reflects the inflammatory status, but also reflects the status of chronic vascular fibrosis in TA.

Through immunohistochemical analysis, in our previous study, we found higher expression of IFN-γ in the TA-involved vessels [19]. In the present study, we found that the serum IFN-γ level was significantly increased in active disease. Thus, the T-lymphocyte immune response may play a role in the pathogenesis of TA. YKL-40 has been reported to play important roles in regulating the T-lymphocyte immune response. For example, it was shown that YKL-40 could maintain the balance of the Th-1 and Th-2 lymphocyte immune response in many pathophysiologic conditions [32]. Further, a study on rheumatoid arthritis demonstrated that YKL-40 could influence the expression of IFN-γ and the balance of the Th-1/Th-2 response [33,34]. These findings point to the possible role of YKL-40 and the T-lymphocyte response in TA (shown in Fig. 2). In the future, if YKL-40 does emerge as a regulator of the T-lymphocyte immune response in TA, it might have potential not only as a biomarker for disease activity, but also as a treatment target.

Other potential biomarkers as reported in other studies [22–24], including IL-8, IL-17, PTX-3, OPN, VEGF, MMP-2 and sCD163, were also analysed in our study. Among these biomarkers, PTX-3 was confirmed as a relatively satisfactory biomarker for monitoring disease activity. Several previous studies have also demonstrated the value of PTX-3 for disease assessment in TA with the specificity of 87% to 94% and sensitivity of 82% to 90% [35–38]. In the present study, PTX-3 was found to have a specificity and sensitivity of 81.2% and 77.8% respectively, at a cut-off value of 8.8 ng/ml. The slight difference between the three studies may be due to differences in the study population and the cut-off values.

Our study had several limitations. First, it is still lack of a universally recognised gold standard for disease activity assessment for TA. Kerr criteria had low sensitivity and specificity to identity active vascular

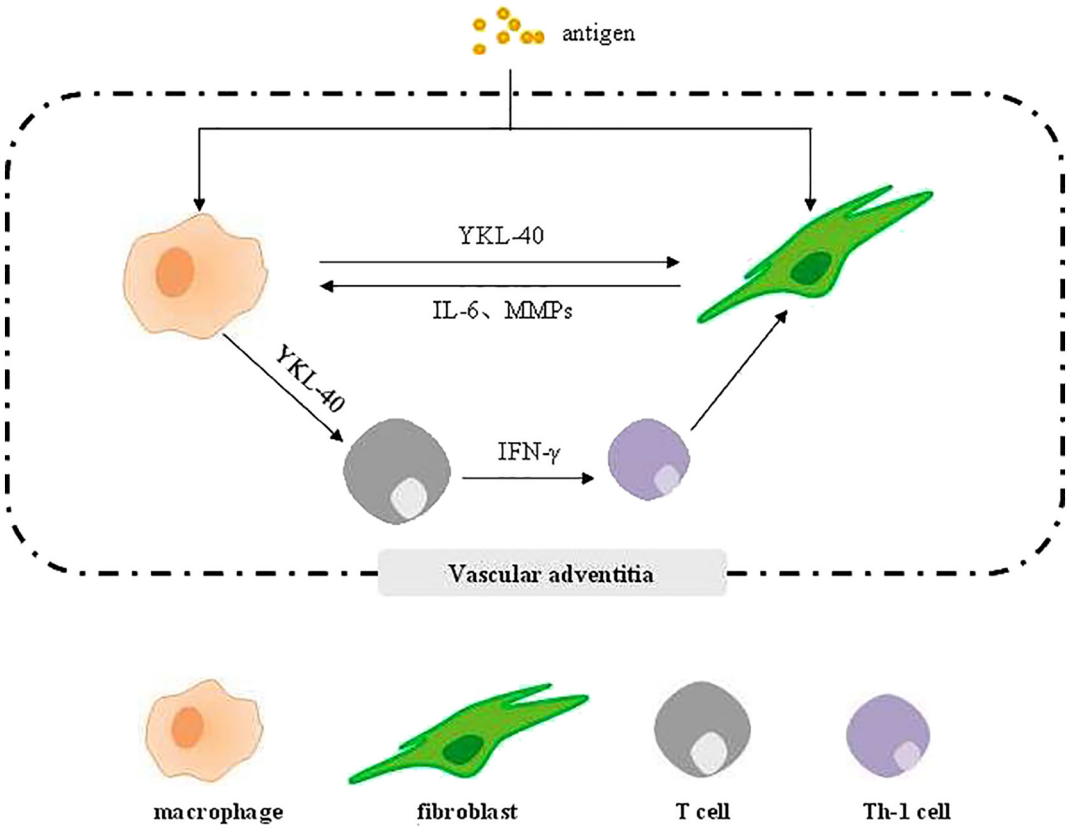

Fig. 2. The possible role of YKL-40 in regulating the interaction among macrophage, T cell and fibroblast in the vascular adventitia in Takayasu arteritis.

inflammation. Thus, other approaches should also be taken into account for identifying active vascular inflammation [39–41]. Second, the sample size was relatively small. Thirdly, immunohistochemical analysis was not performed to determine the origin of YKL-40 and the relationship between local YKL-40 and serum YKL-40 levels. A study on giant cell arteritis has shown that YKL-40 could be derived from giant cells and macrophages in the inflamed arteries, which may contribute to the elevated serum YKL-40 level [9]. This means that apart from being an indicator of systematic inflammation, YKL-40 may also reflect the level of local inflammation in the involved vessels. The last limitation of this study was that the follow-up duration was only 6 months; it would be interesting to monitor the changes in YKL-40 over a longer period. Despite these limitations, the present study has for the first time demonstrated that YKL-40 is a potential biomarker of disease activity in TA, and lays the foundation for confirmatory and more in-depth studies into this protein marker.

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