Serum levels of 14-3-3η are associated with increased disease risk, activity and duration of rheumatoid arthritis in Chinese patients

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Abstract. The aim of the present study was to determine the association between serum 14-3-3η expression levels and disease risk, inflammation level and disease duration in Chinese patients with rheumatoid arthritis (RA). A total of 45 Chinese patients with RA, 45 patients with osteoarthritis (OA) and 44 age- and sex-matched (with the RA group) healthy control (HC) subjects were consecutively recruited for the present case-controlled study. In addition, the demographic and clinicopathological characteristics of the patients with RA were collected. Serum samples were obtained from patients with RA, patients with OA and the HCs, and the serum levels of 14-3-3η were determined by ELISA. Compared with that in the OA patients (P=0.006) and HCs (P<0.001), 14-3-3η expression was significantly increased in RA patients, and receiver operating characteristics (ROC) analysis indicated that it served as a potential predictive marker for the risk of RA. In patients with RA, serum levels of 14-3-3η were positively correlated with disease duration (P=0.003), erythrocyte sedimentation rate (P=0.006) and disease activity score in 28 joints (P=0.025). The proportion of rheumatoid factor (RF)-positive patients (P=0.023) and anti-citrullinated protein antibody (ACPA)-positive patients (P=0.002) with RA was increased (when 14-3-3η expression was increased) compared with RF-negative patients or ACPA-negative patients, respectively. Of note, 14-3-3η serum levels were able to distinguish patients with established RA (disease duration, >2 years) from patients with early RA (disease duration, ≤2 years) with an AUC of 0.759 (95% CI, 0.612-0.905), and the sensitivity and the specificity at the best cut-off point (14-3-3η=0.613 ng/ml) were 79.3 and 75.0%, respectively. Furthermore, 14-3-3η was able to differentiate between RF-positive RA patients and RF-negative patients or HCs. In conclusion, circulating 14-3-3η expression may serve as a novel biomarker for disease risk and activity of RA in Chinese patients.

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Introduction

Rheumatoid arthritis (RA) is a debilitating autoimmune disease that affects ~1% of the population worldwide (1-3). Uncontrolled active RA causes severe joint damage, disability and various comorbidities, including cardiovascular diseases and pulmonary complications, and is thus a significant burden on the patient and society (4,5). The prognosis of patients with RA has markedly improved in recent years, possibly due to the treat-to-target strategy and novel therapeutics, including interleukin (IL)-6 inhibitors and Janus kinase inhibitors; however, RA remains a burdensome condition, as it is usually diagnosed at a late stage and patients typically exhibit symptoms for the remainder of their life (6,7). Therefore, it is necessary to explore novel biomarkers that may contribute to early diagnosis, and may assist in disease management of patients with RA.

The 14-3-3 proteins are a family of highly conserved acidic molecules that are widely expressed in eukaryotic cells, and they usually homo- or hetero-dimerize, forming a cup-like structure termed as 'amphipathic groove' (8,9). Through the groove-like structure, 14-3-3 proteins have the ability to interact with >200 signaling proteins, including transmembrane receptors, kinases and phosphatases (10,11). To date, a total of seven isoforms of 14-3-3 proteins have been discovered (α/β, γ, δ/ξ, ε, η, θ/τ and σ), which participate in various biological functions, including regulation of cell proliferation and signal transduction (9,12-14).

14-3-3η levels have been reported to be increased in the serum of patients with RA compared with those in healthy individuals (15), and may therefore be used as an additional marker in serological measurements for the diagnosis of RA alongside rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) (16). However, there are inconsistent results on the association of 14-3-3η levels with systemic inflammation and disease activity (17,18), suggesting that the role of

14-3-3η in disease activity and progression of RA remains to be further clarified. The association between 14-3-3η and RA in Chinese patients remains largely elusive, and to the best of our knowledge, only a small amount of research has been performed to determine its role in the disease (19). Therefore, the aim of the present study was to examine whether there is a correlation between the serum levels of 14-3-3η and the risk of disease, inflammation levels and disease activity in Chinese patients with RA.

Materials and methods

Participants. A total of 45 patients with RA (age range, 28-78 years; males/females, 8/37) that were admitted to the Department of Rheumatology and Immunology at the First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China) between June 2016 and September 2017 were consecutively enrolled in the present case-controlled study. The inclusion criteria were as follows: i) Diagnosis of RA according to the American College of Rheumatology classification of RA from 1987 (20); and ii) patients aged ≥18 years. The exclusion criteria were as follows: i) Severe deformation of a joint; ii) history of hematological malignancy or solid tumors; iii) history of severe infection, renal dysfunction or hepatic dysfunction; or iv) pregnant or lactating females. In addition, 45 patients with osteoarthritis (OA; age range: 43-82 years; males/females, 20/25) admitted between February 2019 and April 2019 and 44 healthy controls (HCs; age range, 34-76 years; males/females, 22/22) who were also presented at the physical examination center at the Department of Rheumatology and Immunology at the First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China) between August 2017 and September 2017 and were age- and sex-matched to the RA patients, were recruited.

Data collection. Following enrollment, comprehensive datasets on the patients with RA were collected which included the following: Age, sex, body mass index (BMI), disease duration, tender joint count (TJC), swollen joint count (SJC); disease activity score in 28 joints (DAS28), RF status, ACPA status and the treatments received within 3 months, including biologics, conventional disease-modifying anti-rheumatic drugs, glucocorticoids and non-steroidal anti-inflammatory drugs. The erythrocyte sedimentation rate (ESR) was determined with an ELITech Excyte™ 20 Automated ESR Analyzer (ELITech).

Sample collection and ELISA. A peripheral blood sample was obtained from each patient with RA and each HC, and the serum was subsequently isolated by centrifugation at 4˚C and 625 x g for 10 min and stored at -80˚C. 14-3-3η expression (cat. no. ml057387) and C-reactive protein (CRP) expression (cat. no. ml057570) in serum were determined by ELISA using commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd) according to the manufacturer's protocol.

Statistical analysis. Statistical analysis was performed using SPSS version 21.0 (IBM Corp.) and GraphPad Prism 6.0 (GraphPad Software Inc.). Values are expressed as the mean ± standard deviation, median (range or interquartile range) or n (%). Comparisons between two groups were performed using Student's t-test, the Wilcoxon rank-sum test or the Chi-squared test. Comparison among three groups was performed using a Kruskal-Wallis H-test followed by Bonferroni correction. Receiver operating characteristic (ROC) curves were drawn to assess the value for predicting the risk of RA, or the ability to distinguish patients with established RA from those with RA at the early stage. A Spearman's rank test was used to evaluate the correlation between 14-3-3η expression and continuous variables in RA patients. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinicopathological characteristics. The demographics, clinical characteristics and treatment history of the patients are presented in Table I. The median disease duration was 5.0 (0-40.0) years, and the median values for TJC, SJC and ESR were 5 (range, 2-12) joints, 5 (range, 1-15) joints and 49.0 (range, 6.0-109.0) mm/h, respectively. The median value for CRP was 18.5 (0.2-136.0) mg/l and the mean DAS28 was 5.05±0.65.

Comparison of 14-3-3η expression between RA patients, OA patients and HCs. Comparison of 14-3-3η expression among RA patients, OA patients and HCs was performed with a Kruskal-Wallis H-test followed by Bonferroni correction, which revealed that the serum level of 14-3-3η in RA patients [1.184 (0.076-6.770) ng/ml] was increased compared with that in OA patients [0.131 (0.056-0.508) ng/ml; $P=0.006$] and HCs [0.041 (0.019-0.056) ng/ml; $P<0.001$; Fig. 1A]. ROC curve analysis revealed that 14-3-3η expression was able to differentiate RA patients from OA patients (Fig. 1B) and HCs (Fig. 1C), with an area under the ROC curve (AUC) of 0.718 (95%CI: 0.609-0.827) and 0.883 (95%CI: 0.813-0.952), respectively.

Correlation of 14-3-3η expression with clinicopathological characteristics of patients with RA. There was no correlation between 14-3-3η expression and age, sex, BMI, TJC, SJC, CRP levels or therapeutic treatments (all $P>0.05$; Fig. 2A-C, E, F, H and L-O). The expression of 14-3-3η was positively correlated with disease duration ($P=0.003$; Fig. 2D), ESR level ($P=0.006$; Fig. 2G) and DAS28 ($P=0.025$; Fig. 2I). In addition, 14-3-3η levels were elevated in RF-positive patients compared with those in RF negative patients ($P=0.023$; Fig. 2J) and increased in ACPA-positive patients compared with those in ACPA-negative patients ($P=0.002$; Fig. 2K). These results suggest that 14-3-3η may serve as a novel biomarker for disease activity and disease course in patients with RA.

Value of 14-3-3η levels to distinguish patients with established RA from patients with early RA. As the serum levels of 14-3-3η were positively correlated with disease duration, it was next determined whether the may be used to distinguish between patients with established RA (disease duration, >2 years) and patients with early RA (disease duration, ≤2 years). ROC curve analysis determined that serum 14-3-3η levels were able to differentiate between patients with established RA and patients with early RA (AUC, 0.759; 95%CI, 0.612-0.905),

Table I. Characteristics of RA patients (n=45).

Parameter	Value
Age (years)	57.51±12.42
Female sex	37 (82.2)
BMI (kg/m^2)	21.97±3.19
Disease duration (years)	5.0 (0-40.0)
TJC (joints)	5 (2-12)
SJC (joints)	5 (1-15)
ESR (mm/h)	49.0 (6.0-109.0)
CRP (mg/l)	18.5 (0.2-136.0)
DAS28 score	5.05±0.65
RF positive	32 (71.1)
ACPA positive	33 (73.3)
Biologics	8 (17.8)
cDMARDs	25 (55.6)
Glucocorticoids	11 (24.4)
NSAIDs	30 (66.7)
No treatment	12 (26.7)

Values are expressed as the mean ± standard deviation, median (range) or n (%). RA, rheumatoid arthritis; BMI, body mass index; TJC, tender joint count; SJC, swollen joint count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; cDMARDs, conventional disease modifying anti-rheumatic drugs; NSAIDs, non-steroidal anti-inflammatory drugs.

and the sensitivity and the specificity at the best cut-off point (14-3-3η=0.613 ng/ml) was 79.3 and 75.0%, respectively (Fig. 3).

Comparison of 14-3-3η expression between RF-positive and RF-negative RA patients, as well as HCs. Of the 45 RA patients, 32 were RF-positive, 10 were RF-negative and the remaining 3 patients lacked data. 14-3-3η expression was increased in RA patients with RF-positive status compared with that in RA patients with RF-negative status (P=0.046) and HCs (P<0.001; Fig. 4A). In addition, 14-3-3η was able to distinguish RA patients with RF-positive status from RA patients with RF-negative status (AUC, 0.738; 95%CI, 0.550-0.925), and the sensitivity and the specificity at the best cut-off point (14-3-3η=1.537 ng/ml) was 62.5 and 90.0%, respectively (Fig. 4B). Furthermore, 14-3-3η was able to differentiate between RA patients with RF-positive status and HCs (AUC, 0.760; 95%CI, 0.631-0.890), with a sensitivity and specificity at the best cut-off point (14-3-3η=0.062 ng/ml) of 65.2 and 86.4%, respectively (Fig. 4C).

Comparison of the predictive value for RA risk among 14-3-3η, CRP and ESR. The predictive value of 14-3-3η for the risk of RA was then compared with that of CRP and ESR by using ROC curves. In this ROC analysis, the AUCs for 14-3-3η, CRP and ESR were 0.883 (95% CI: 0.813-0.952), 0.888 (95% CI: 0.817-0.956) and 0.961 (95% CI: 0.919-1.000), respectively (Fig. 5). The AUC for 14-3-3η was lower than those for CRP and ESR, but there was no statistical significance between 14-3-3η and CRP, or between 14-3-3η and ESR. Therefore, the predictive value of 14-3-3η regarding the risk of RA was close to that of CRP and ESR.

Discussion

In the present study, it was demonstrated that the serum level of 14-3-3η was increased in RA patients compared with that in OA patients and HCs. Serum 14-3-3η levels may additionally be a good predictor for the risk of RA. The 14-3-3η expression in serum was positively correlated with disease duration, ESR and DAS28 score, and elevated in RF-positive patients and ACPA-positive patients compared with that in RF-negative patients or ACPA-negative patients. Furthermore, it was possible to distinguish patients with established RA from those with early RA, as well as RF-positive RA patients from HCs based on the serum levels of 14-3-3η. The results suggest that 14-3-3η may be used as a novel biomarker to monitor disease activity and the course of disease for Chinese patients with RA.

14-3-3η serves varying physiological roles, including protection against mitochondria-mediated apoptosis (21), prevention of cardiac dysfunction (22) and reduction of α-synuclein-mediated cellular toxicity (23). Increased expression of 14-3-3η was first observed in Canadian patients with joint inflammation (15). Subsequent studies from Canada and Japan demonstrated that 14-3-3η expression was increased in patients with early and established RA compared with that in healthy subjects, and it was a good predictor for the risk of RA (17,18,24,25). However, the predictive value of 14-3-3η expression regarding the risk of RA has not been previously studied in a Chinese population, to the best of our knowledge. The present study demonstrated that, compared with those in OA patients and HCs, 14-3-3η levels in the serum were increased in Chinese RA patients, and they were capable of distinguishing between RA patients and OA patients or HCs. The ability of 14-3-3η to distinguish patients with RA from OA patients and HCs may be associated with the ability of 14-3-3η to upregulate the levels of pro-inflammatory cytokines, which are closely associated with inflammatory progression in RA (15,24,26-32). A clinical study from Canada indicates that serum 14-3-3η levels in patients with RA are positively associated with matrix metalloprotease (MMP)-1 and MMP-3 (15). Another study by the same group further demonstrated that stimulation of THP-1 cells by 14-3-3η induced the release of inflammatory transcripts, including IL-1β, IL-6, MMP-1, MMP-3 and receptor activator of NF-κB ligand (RANKL) (24). Numerous studies have indicated that IL-1β and IL-6 participate in the inflammatory processes of RA (26-28), while MMP-1, MMP-3 and RANKL have been demonstrated to exert important roles in joint damage (29-32). Therefore, 14-3-3η may increase inflammatory activity and joint damage in patients with RA through regulating IL-1β, IL-6, MMP-1, MMP-3 and RANKL, which may explain its predictive effect for the risk of RA revealed in the present study.

14-3-3η expression levels are positively correlated with DAS28, clinical disease activity index, simple disease activity index (SDAI), joint space narrowing, TJC, SJC, CRP and ESR

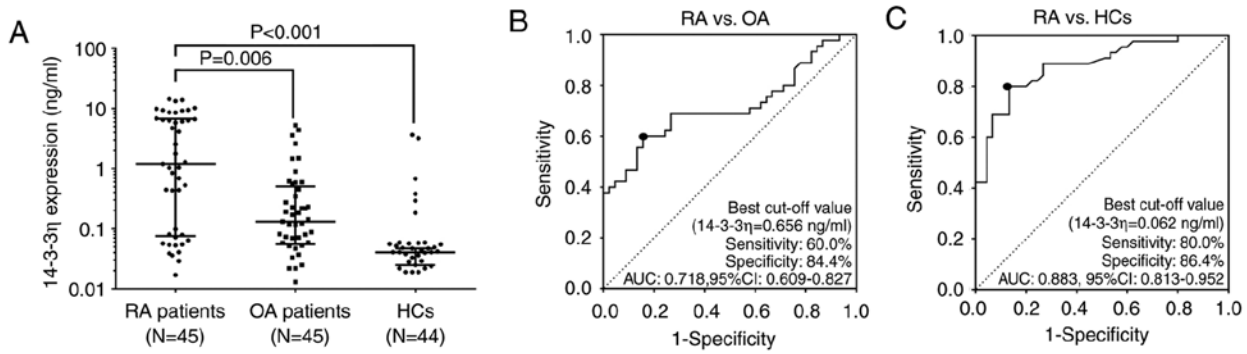

Figure 1. 14-3-3η expression in RA patients, OA patients and HCs. (A) Serum level of 14-3-3η expression; (B) Predictive value of 14-3-3η in RA patients and OA patients; (C) Predictive value of 14-3-3η in RA and HCs. Receiver operating characteristic curves were drawn to assess the ability of 14-3-3η expression to distinguish RA patients from OA patients and HCs. RA, rheumatoid arthritis; OA, osteoarthritis; HCs, healthy controls; AUC, area under curve.

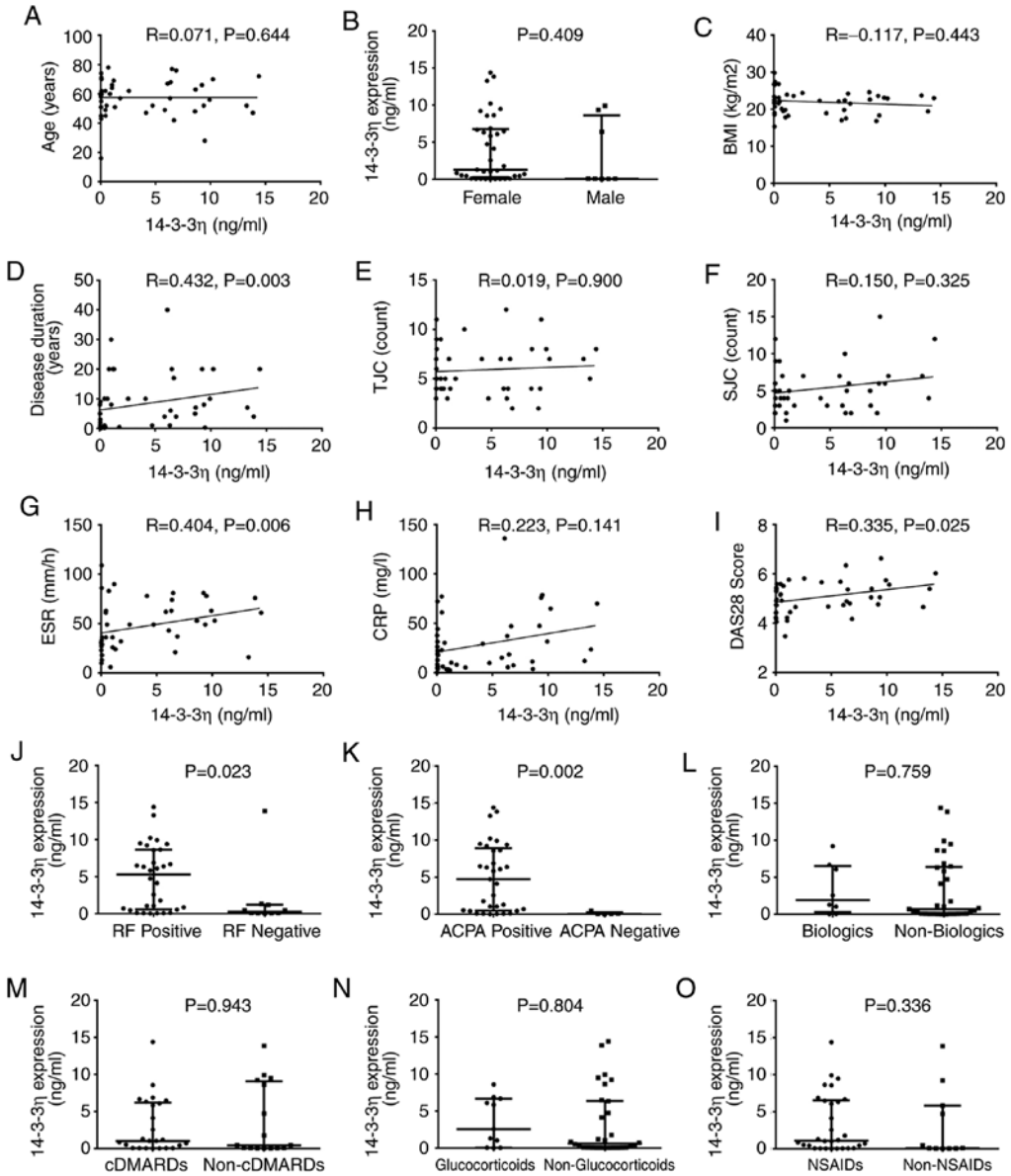

Figure 2. Correlation of 14-3-3η expression in serum with clinicopathological characteristics of patients with RA. The correlation of 14-3-3η expression with (A) age, (B) sex, (C) BMI, (D) disease duration, (E) TJC, (F) SJC, (G) ESR, (H) CRP (I) DAS28, (J) RF positive (K) ACPA positive, (L) Biologicals, (M) cDMARDs, (N) Glucocorticoids and (O) NSAIDs for RA are presented. RA, rheumatoid arthritis; HCs, healthy controls; BMI, body mass index; TJC, tender joint count; SJC, swollen joint count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; cDMARDs, conventional disease-modifying anti-rheumatic drugs; NSAIDs, non-steroidal anti-inflammatory drugs.

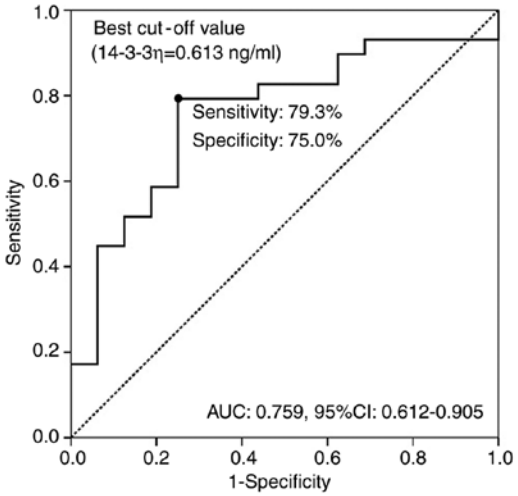

Figure 3. ROC curve of 14-3-3η expression in serum for distinguishing between patients with established RA (>2 years) from patients with early RA (≤2 years). Expression of 14-3-3η between patients with established RA from patients with early RA with an area under the curve of 0.759; 95% CI, 0.612-0.905 at the appropriate cut-off point (the expression of 14-3-3η was 0.613 ng/ml). ROC curves were constructed to calculate the predictive value of 14-3-3η expression for RA risk. RA, rheumatoid arthritis; ROC, receiver operating characteristic curve; AUC, area under curve.

in Japanese RA patients (17). In addition, a study from Canada revealed that increased 14-3-3η levels in the serum were associated with worse radiographic progression of RA, even in patients with SDAI remission (25). However, another study performed in Canada demonstrated that 14-3-3η levels are not correlated with DAS28 or CRP (24), contradicting the results obtained in the present study. These studies indicate that the correlation of 14-3-3η expression levels with disease activity in patients with RA is variable.

In the present study, serum 14-3-3η levels exhibited moderate to strong associations with prolonged disease duration, increased ESR level and higher DAS28 score with high R values (R values for the correlation were all >0.3). In addition, 14-3-3η levels were able to differentiate between patients with early RA and those with established RA. Therefore, 14-3-3η may serve as a novel marker for monitoring disease activity and disease duration in Chinese patients with RA. As discussed above, elevated 14-3-3η expression induces overexpression of IL-1β, IL-6, MMP-1, MMP-3 and RANKL, which in turn may aggravate the inflammatory response in addition to cartilage and bone destruction, thus resulting in elevated disease activity and prolonged disease duration (26-32).

A clinical study performed in Canada demonstrated that the utilization of RF along with 14-3-3η increased the detection rate from 57 to 75% compared with the use of RF alone for the diagnosis of RA at the early stage and utilization of ACPA along with 14-3-3η increased the detection rate from 59 to 72% compared with the use of ACPA alone (18). Serum 14-3-3η levels were also observed to be positively associated with RF and ACPA levels (18). Similar results have been obtained in other studies, which suggest that 14-3-3η expression may be correlated with RF and ACPA levels in RA patients (24,33). In the present study, the serum levels of 14-3-3η were positively correlated with RF and ACPA levels in Chinese patients with RA, which was partially consistent with the above-mentioned studies. This consistency may be due to the fact that 14-3-3η, RF and ACPA are all involved in the release of pro-inflammatory factors, including IL-1β and IL-6, which are correlated with inflammation in RA (34-36). Therefore, it was expected for 14-3-3η serum levels to exhibit a positive correlation with RF and ACPA levels in patients with RA in the present study. Furthermore, as indicated by ROC curve analysis, 14-3-3η was also able to distinguish RF-positive RA patients from RF-negative RA patients and HCs, suggesting that it may be utilized as a biomarker for predicting active disease of RF-positive RA patients. This may be explained as follows: As mentioned above, 14-3-3η is able to induce the release of proinflammatory cytokines and cause joint damage, which has a considerable probability to increase RF expression and then elevate the risk of RA.

CRP and ESR are sensitive indicators for inflammation and their increments contribute to predicting RA risk in clinical practice (3,6). For instance, a clinical study determined that CRP and ESR levels were increased in patients with erosive RA, and they have a good predictive value regarding the risk of erosive RA (37). In order to compare the predictive value of 14-3-3η, CRP and ESR for the risk of RA, ROC curves for differentiating RA patients from HCs were drawn, and it was determined that the AUC of 14-3-3η was numerically lower than that of CRP and ESR, implying that 14-3-3η may be inferior to CRP and ESR in differentiating RA patients from HCs. A possible explanation of this result may be as follows: CRP and ESR levels may rapidly increase if tissue injury is present, even if the tissue injury is mild, while 14-3-3η levels may increase in a slower manner when tissue injury occurs (38-41). Hence, CRP and ESR might be more sensitive than 14-3-3η in predicting the risk for RA. However, the 95%CI of the AUC for 14-3-3η (95% CI: 0.813-0.952) was crossed with that of CRP (95% CI: 0.817-0.956) and ESR (95% CI: 0.919-1.000), suggesting that there was no significant difference regarding the sensitivity in differentiating RA patients from HCs between 14-3-3η and CRP/ESR. Furthermore, due to the fact that CRP and ESR levels are increased in numerous diseases other than RA (including infections, myocardial infarction and systemic lupus erythematosus), the specificity of CRP and ESR regarding the prediction of the risk of RA may be lower than that of 14-3-3η. However, this notion requires further investigation. As for the comparison of the predictive value for the risk of RA between 14-3-3η, RF and ACPA, additional investigation is required due to the lack of data on RF or ACPA for HCs in the present study.

Of note, a previous study performed in China yielded similar results to those of the present study, including the increase of 14-3-3η levels in RA patients compared with HCs, and their correlation with increased disease activity (19). However, the present study was more comprehensive than this previous study. The present study not only compared the levels of 14-3-3η between RA patients and HCs, but also between RA patients and OA patients, and revealed that 14-3-3η was able to distinguish RA patients from OA patients. Furthermore, the present study not only assessed the association of 14-3-3η expression with disease activity, but also the association of 14-3-3η expression with the level of inflammation in RA patients. Therefore, the present study provided more

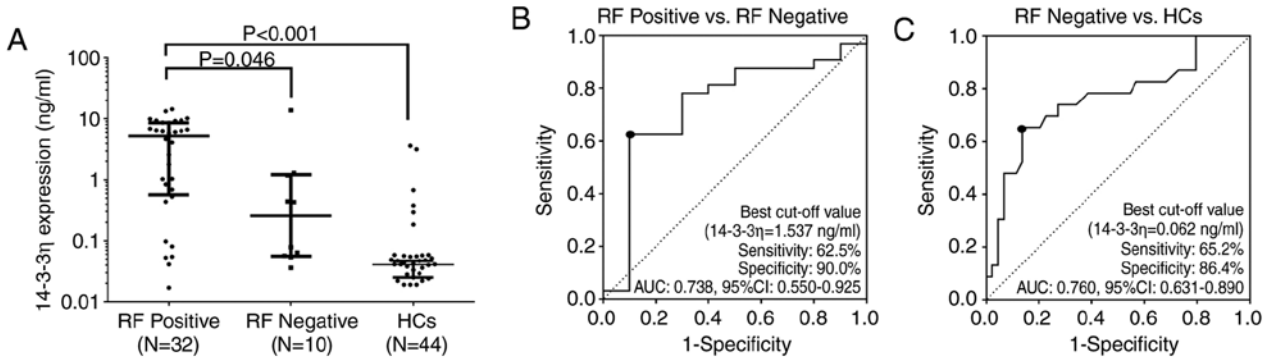

Figure 4. Comparison between RA patients with RF-positive and RF-negative status as well as HCs regarding 14-3-3η expression. (A) 14-3-3η expression in RA patients with RF-positive status was increased compared with that in RA patients with RF-negative status (P=0.046) and HCs (P<0.001). (B) Furthermore, 14-3-3η was able to distinguish RA patients with RF-positive status from RA patients with RF-negative status (AUC, 0.738; 95% CI, 0.550-0.925), and the sensitivity and the specificity at the best cut-off point (14-3-3η=1.537 ng/ml) was 62.5 and 90.0%, respectively. (C) 14-3-3η was also able to differentiate RA patients with RF-positive status from HCs (AUC, 0.760; 95%CI, 0.631-0.890), with a sensitivity and specificity at the best cut-off point (14-3-3η=0.062 ng/ml) of 65.2 and 86.4%, respectively. Comparison among groups was performed by using the Kruskal-Wallis H-test followed by Bonferroni correction. P<0.05 was considered to indicate a statistically significant difference. Receiver operating characteristic curves were drawn to assess the ability of 14-3-3η expression to distinguish RF positive RA patients from the RF negative RA patients and HCs. RA, rheumatoid arthritis; RF, rheumatoid factor; HCs, healthy controls; AUC, area under the curve.

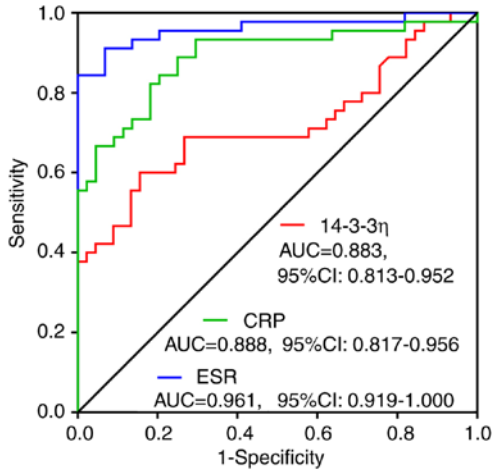

Figure 5. Comparison of the predictive value for the risk of RA among 14-3-3η, CRP and ESR. The AUCs for 14-3-3η, CRP and ESR were 0.883 (95%CI: 0.813-0.952), 0.888 (95%CI: 0.817-0.956) and 0.961 (95%CI: 0.919-1.000), respectively, in predicting the risk of RA. Receiver operating characteristic curves were drawn to assess the ability of 14-3-3η, CRP and ESR expression to distinguish RA patients from HCs. RA, rheumatoid arthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; AUC, area under the curve; HCs, healthy controls.

comprehensive information about the value of 14-3-3η levels in Chinese RA patients.

Of note, the present study had several limitations. The association of 14-3-3η expression with pro-inflammatory factors, including IL-1β and IL-6, was not evaluated, and it remains to be determined whether 14-3-3η expression is associated with the inflammatory response in Chinese patients with RA. The present study was a case-control study without follow-up, and a follow-up study on the same groups of patients with RA/HCs regarding 14-3-3η levels is required in order to evaluate the association of 14-3-3η expression with the therapeutic efficacy. The sample size in the present study was relatively small, and that the results may have been affected by certain abnormal values, leading to decreased statistical power. The patients of the present cohort were enrolled between June 2016 and September 2017, and the results should be clarified with data collected from newly enrolled patients with RA and HCs. The sensitivity and specificity of 14-3-3η for the diagnosis of RA in comparison with other biomarkers, including RF and ACPA, requires further investigation. In addition, the recruitment of patients at a single institute may have introduced selection bias.

In conclusion, circulating 14-3-3η may serve as a novel biomarker for disease risk, activity and duration of RA in Chinese patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JT, XC and LS designed the study, MD, AP and CL performed the experiments, and YZ and XX analyzed the data. All authors wrote and revised the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) and all of the participants provided informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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