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The clinical value of serum sirtuin-1 in the diagnosis of rheumatoid arthritis: a pilot study

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ABSTRACT

Introduction: Cell biology studies, animal models and other data suggest a role for sirtuin-1 in the pathogenesis of rheumatoid arthritis (RA). We hypothesized the clinical significance of serum sirtuin-1 in this disease.

Methods: Serum was obtained from 141 RA patients, 144 non-RA patients and 88 healthy controls. Sirtuin-1, anti-mutant citrulline vimentin antibody (anti-MCV), anti-cyclic citrulline polypeptide antibody (anti-CCP), rheumatoid factor and C-reactive protein were measured by immunological methods, and erythrocyte sedimentation rate was determined by the Westergren method.

Results: All markers were higher in the RA group than in the non-RA group and the healthy control group ($P < 0.01$). The specificity of sirtuin-1 for the diagnosis of RA was 97% (the highest among all markers), sensitivity was 71%. In ROC curve analysis, the AUCs (95% CI) of sirtuin-1, anti-CCP and anti-MCV were 0.87 (0.82–0.91), 0.91 (0.88–0.94) and 0.92 (0.89–0.95) respectively (all $p < 0.01$). The combination of sirtuin-1and anti-MCV gave the highest Youden index of 0.79, whilst Cox regression showed sirtuin-1 and rheumatoid factor were the strongest independent predictors of RA.

Conclusions: Serum sirtuin-1 is increased in RA, and may have a place is the diagnosis of this disease when combined with other markers.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease where inflammation of the synovial membrane is caused by abnormal activation of the immune system, which is characterized by the production of numerous inflammatory cells and cytokines in the synovial fluid and the production of various antibodies in the serum [1]. Due to the insidious onset of RA and the lack of specific clinical manifestations, patients often fail to be diagnosed and take effective treatment in time. Whilst rheumatoid factor (RF) is the most common serum marker, anti-cyclic citrulline polypeptide antibody (anti-CCP) and anti-mutant citrulline vimentin antibody (anti-MCV) are also valuable, but the sensitivity and specificity of these markers leave room for additional tools [2].

Sirtuin-1, a member of the sirtuins family [3], is class III deacetylase and regulator of several biochemical processes. It is widely expressed and is the link between extracellular signals and target gene transcription regulation [4]. Sirtuin-1 can deacetylate a series of histone and non-histone proteins and regulate cell energy metabolism, inflammatory response, and oxidative stress. There is considerable literature from cell biology and animal models that point to a potential role for sirtuin-1 in immune regulation and RA [5–8], but a role in clinical laboratory diagnosis is unexplored and is therefore justified. We therefore hypothesized that sirtuin-1 has potential as a marker of this disease.

Materials and methods

We tested our hypothesis in a case–control study of 141 patients with RA (36 males and 105 females, mean/SD 52.7 ± 15.8 years old), 144 in a non-RA group (40 with ankylosing spondylitis [11 males and 29 females, age 52.9 ± 13.9]; 48 with osteoarthritis [14 males, 34 females, aged 55.1 ± 12.2]; and 56 with Sjogren's syndrome [15 males, 41 females, 58.7 ± 15.0]), and a healthy control group consisted of 88 [25 males and 63 females, 52.6 ± 15.2] years old, all of whom were health care practitioners in the physical examination centre of the hospital. The three groups were matched for age ($p = 0.15$) and sex ($p = 0.6$). The patients were all inpatient and outpatient confirmed patients from the Second Affiliated Hospital of Nanchang University between January 2019 and September 2020. All patients met the diagnostic

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criteria of the relevant disease guidelines [9–12]. This study obtained specimens with the patient's informed consent and approved by the ethics committee of the Second Affiliated Hospital of Nanchang University. Inclusion criteria were having signed the informed consent, a clear diagnosis, and the relevant imaging examinations and clinical medical records were complete, and for healthy controls, routine examinations of the health check-ups were normal. Exclusion criteria were other abnormal metabolic diseases, other autoimmune diseases, cancer, severe liver, kidney, heart, or lung system diseases, pregnancy and lactation, and age <18 years

Three ml fasting venous blood from all subjects was collected with a separating gel vacuum accelerating blood collection tube, standing for 30 min, centrifuged at 1,026 × g for 15 min, and the upper serum was collected for use. Sirtuin-1 was determined by ELISA (Hefei Laier Biotechnology, Hefei, Anhui Province, China); Anti-CCP on a Shenzhen YHLO Biotech Co., Ltd. (Shenzhen City, Guangdong Province, China) iFlash 3000-C chemiluminescence immunoassay analyser and supporting reagents; Anti-MCV by ELISA (Tianjin Super Biotechnology, Tianjin, China); CRP and RF were determined by the rate-scattering turbidimetric method using the Beckman Coulter IMMAGE 800 automatic specific protein analysis system and supporting reagents, and ESR by the Widmanstat method using Roller 20, an automatic rapid erythrocyte sedimentation rate analyser.

Data analysis used SPSS 23.0 statistical software, normal distribution data are represented by mean/SD, non-normal distribution data are represented by the median and interquartile range (IQR), and dichotomous variables are compared by χ^2 test. The non-parametric Kruskal–Wallis test was used to compare continuous variables. Receiver operating characteristic (ROC) with area under the curve (AUC) data, sensitivity, specificity, Youden index, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were determined by standard methods. Further calculations determined the sensitivity, specificity, Youden index in series and parallel to evaluate the combined diagnostic value of each group of indicators. Cox regression analysis was used to determine independent predictors of RA. $P < 0.05$ indicated that the difference was statistically significant.

Results

Table 1 shows that serum levels of sirtuin-1, anti-MCV, anti-CCP, RF, ESR and CRP in RA patients are higher than those in non-RA patients and healthy controls. Table 2 shows data on the diagnostic value of the markers. The significance of the AUC of the markers was, in order, anti-MCV, anti-CCP, sirtuin-1, RF, CRP and ESR, with no difference between sirtuin-1, anti-CCP and anti-MCV as 95% confidence intervals overlapped. Sirtuin-1 had the highest specificity and positive predictive value, whereas anti-MCV had the highest sensitivity and negative predictive value. These data were improved by combinations of markers (Table 3). The parallel combination of sirtuin-1 and anti-CCP had the highest Youden index and specificity. Cox regression analysis (Table 4) showed that sirtuin-1 and RF levels were both strong independent predictors of RA.

Discussion

RA is a common autoimmune disease, the global prevalence rate is between 0.4% and 1.3%, most patients having multiple autoantibodies [13,14]. RF is widely used in clinical practice because of simple, convenient, and high positive rate. However, its specificity for RA diagnosis is low, which makes the diagnosis of some early RA patients who lack typical manifestations difficult. Anti-CCP and anti-MCV are better markers but also suffer from imperfect diagnostic ability [15–17].

Overexpression of sirtuin-1 in RA synovial cells promotes chronic inflammation by stimulating the production of inflammatory cytokines and inhibiting cell apoptosis [17]. Previous studies have provided numerous links between RA and sirtuin-1. For example, osteoblast-specific sirtuin-1 gene knockout mice

Table 1. Levels of markers in each study group.

Marker	Healthy Controls (n = 88)	Non-RA (n = 144)	RA (n = 141)
SIRT1 (ng/ml)	32.1 ± 10.6	40.0 ± 8.4	55.3 ± 13.7
RF (U/ml)	13.4 (10 ~ 39.5)	44.8 (10 ~ 57.6)	143.9 (71 ~ 541)
anti-CCP (U/ml)	15.9 (0.5 ~ 14.2)	21.7 (0.5 ~ 23.1)	327.1 (64.2 ~ 633)
anti-MCV (U/ml)	19.0 (3.7 ~ 22.7)	26.3 (0.5 ~ 30.4)	441.4 (59.9 ~ 1000)
ESR (mm/h)	9.9 ± 4.6	34.1 ± 19.3	48.5 ± 26.7
CRP (mg/L)	6.3 ± 1.3	20.1 ± 8.2	31.3 (19.6 ~ 42.7)

Data presented as mean ± SD or median (IQR). Each marker overall difference $p < 0.01$ (ANOVA). All healthy controls/RA differences significant at $p < 0.01$. All healthy controls/non-RA, and RA/non-RA differences significant at $p < 0.05$ (posthoc test).

Table 2. Diagnostic values of research indices.

Marker	AUC 95% CI	Cut-off value	Sensitivity (%)	Specificity (%)	PPV	NPV	PLR	NLR	Youden Index
SIRT1 (ng/ml)	0.87 (0.83 ~ 0.91)	49	70.9	97.0	92.6	83.9	9.1	0.3	0.68
RF (IU/ml)	0.82 (0.77 ~ 0.87)	54	80.1	72.8	63.8	85.7	3	0.3	0.53
Anti-CCP (U/ml)	0.91 (0.88 ~ 0.94)	120	74.5	95.7	90.1	86	17.3	0.3	0.7
Anti-MCV (U/ml)	0.92 (0.89 ~ 0.95)	34	85.1	86.2	78.4	90.4	6.2	0.2	0.71
ESR (mm/h)	0.75 (0.70 ~ 0.81)	43	63.1	82.3	68.5	77.7	3.6	0.4	0.45
CRP (mg/L)	0.78 (0.74 ~ 0.84)	25	59.6	84.9	70.6	76.6	3.9	0.5	0.45

AUC, area under curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

Table 3. Clinical evaluation of mutual index combination test.

	In series			In parallel		
Marker	Sensitivity (%)	Specificity (%)	Youden Index	Sensitivity (%)	Specificity (%)	Youden Index
SIRT1/anti-CCP	52.8	99.9	0.53	92.6	92.8	0.85
SIRT1/anti-MCV	60.3	99.6	0.60	95.7	83.6	0.79
anti-CCP/anti-MCV	63.4	99.4	0.63	96.2	82.5	0.79

Table 4. Cox regression of the markers in the prediction of RA.

	β	S.E.	Wald	Sig.
SIRT1	0.15	0.043	12.399	0.001
RF	−0.015	0.003	23.513	0.001
Anti-CCP	0.014	0.008	3.174	0.021
Anti-MCV	0.026	0.012	4.504	0.034
ESR	−0.22	0.065	11.426	0.075
CRP	0.154	0.077	3.963	0.047

Abbreviations: S.E., Standard Error; Sig, significance

reduce osteoblast differentiation and lead to a decrease in bone mass, while osteoclast-specific SIRT1 gene knockout mice activate the NF-κB signalling pathway and promote osteoclast differentiation, resulting in bone loss [8,18,19].

Our principal result is of increased serum sirtuin-1 in RA compared to non-RA connective tissue diseases and healthy controls. However, this was also the cases for other serum markers, although sirtuin-1, anti-CCP and anti-MCV all had broadly equivalent AUCs and Youden indices. When testing these three markers in series, the specificity for RA was excellent, but sensitivity was poor, and so the Youden index was little improved. However, when tested in parallel, sensitivity, specificity and Youden indices all improved, the combination of sirtuin-1 and anti-CCP giving the superior outcome. Despite this, Cox's regression analysis showed that sirtuin-1 was the best of the three in predicting RA. Further studies in large numbers are required to provide more robust data on the implications of our findings. For example, it is as yet unclear whether sirtuins-1 levels reflect disease activity and are influenced by anti-inflammatory therapy. A secondary result is that sirtuin-1 is also increased in certain other connective tissue diseases, i.e. ankylosing spondylitis, osteoarthritis and Sjogren's syndrome. This finding may also prove to be useful in these diseases.

In summary, our pilot data indicate that, like anti-CCP and anti-MCV, sirtuin-1 may be of value in the diagnosis of RA. The specificity of situin-1 has advantages compared with other indicators, and its combined detection with anti-CCP can significantly improve the diagnostic accuracy of RA. This work represents an advance in biomedical science because it shows that SIRT1 might be useful for diagnostic in RA and certain other connective tissue diseases.

Summary table

What is known about this subject:

- RA is a chronic inflammatory autoimmune disease characterized by joint destruction leading to loss of joint function, and disability.
- RF, anti-CCP and anti-MCV have certain diagnostic value for RA, ESR and CRP have clear diagnostic value for RA activity.

What this paper adds

- Serum sirtuin-1 is elevated in patients with RA, and compared with other indicators, has higher specificity.
- Sirtuin-1 has potential in the diagnosis of other connective tissue diseases
- The combined measurement of sirtuin-1 and anti-CCP have a superior Youden index compared to other combination and each marker alone.

Disclosure statement

Authors declared no interest conflict.

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