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Value of serum collagen triple helix repeat containing-1(CTHRC1) and 14-3-3η protein compared to anti-CCP antibodies and anti-MCV antibodies in the diagnosis of rheumatoid arthritis

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ABSTRACT

Introduction: Serological markers are important in the diagnosis of rheumatoid arthritis (RA) and other connective tissues diseases This study explored the clinical value of collagen triple helix repeat containing-1 (CTHRC1) and 14-3-3η protein, compared to routine markers, in the diagnosis of RA.

Methods: We recruited 103 RA patients, 105 non-RA patients (osteoarthritis, ankylosing spondylitis, systemic lupus erythematosus) and 59 healthy controls. CTHRC1, 14-3-3η, anti-cyclic citrullinated peptide antibody (anti-CCP), anti-mutated citrullinated vimentin antibody (anti-MCV), rheumatoid factor and erythrocyte sedimentation rate (ESR) levels were measured, and their diagnostic value for RA evaluated and compared.

Results: All laboratory indices were elevated in RA ($P < 0.05$). Of these, anti-MCV had the highest sensitivity (86.4%) and anti-CCP the highest specificity (94.5%). The areas under the curve (AUC) of CTHRC1, 14-3-3η, anti-CCP, anti-MCV, rheumatoid factor and ESR were 0.84, 0.81, 0.89, 0.91, 0.85 and 0.77 respectively (all $P < 0.01$). Anti-CCP and anti-MCV were the most valuable in the diagnosis of RA. The combination of anti-CCP and anti-MCV had the maximum Youden index, followed by the combination of anti-CCP and 14-3-3η. Binary logistic regression analysis showed that 14-3-3η had the largest odds ratio value (95% CI) at 5.1 (2.1–12.5) for RA.

Conclusion: CTHRC1 and 14-3-3η are promising serological indicators of RA, and when combined with anti-CCP, anti-MCV and ESR, can improve the diagnosis of this disease.

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Introduction

Rheumatoid arthritis (RA) is a common clinical autoimmune disease. Its main feature is symmetrical chronic inflammation of extremities and small joints. The clinical manifestations are pain, swelling and decreased function of the affected joints. The diagnosis of RA is mainly based on the patient's symptoms and the results of imaging and laboratory testing. However, due to the diverse manifestations of the disease, some present with atypical early symptoms and negative serology, which leads to misdiagnosis or missed diagnosis and delays the best time for treatment [1]. Therefore, improving the diagnosis of this disease is essential.

Collagen triple helix repeat containing-1 (CTHRC1) promotes bone and cartilage erosion and pannus formation by activating fibroblast-like synoviocytes (FLS) and so may be a marker for the diagnosis of RA [2]. 14-3-3η protein is significantly increased in serum and joint synovial fluid of RA patients and can up-regulate the expression of a variety of RA-related inflammatory factors [3,4], suggesting that it may be involved in pathogenesis. Anti-citrullinated protein antibody (ACPA) testing is a significant breakthrough in RA laboratory diagnosis. Anti-cyclic citrullinated peptide antibodies (anti-CCP) and anti-mutated citrullinated vimentin antibodies (anti-MCV) have high specificity for the diagnosis of RA [5,6]. Rheumatoid factor is the first serological indicator for the diagnosis of RA. Erythrocyte sedimentation rate (ESR) is a simple and reproducible acute phase response indicator. Despite this, these indices are rarely compared in a disease-controlled study. We therefore set out to determine which indices can be used alone or in combination to provide the highest diagnostic value for RA. We selected 103 RA patients, 105 non-RA patients with inflammatory joint disease and 59 healthy controls in the Second Affiliated Hospital of Nanchang University from December 2018 to December 2019, and serum CTHRC1, 14-3-3η protein, ACPA, RF and ESR levels were detected. The analysis results were reported as follows.

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Materials and methods

We recruited 208 inpatients or outpatients in the Second Affiliated Hospital of Nanchang University from December 2018 to December 2019, including 103 with RA and 105 non-RA patients (36 with osteoarthritis (OA), 29 with ankylosing spondylitis (AS) and 40 with systemic lupus erythematosus (SLE)). All RA patients fulfilled the classification criteria of the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [7]. Fifty-nine healthy subjects were recruited as a control group. All subjects had complete clinical and imaging data for definite diagnoses, and all were of the Han nationality from Jiangxi, China. Inclusion criteria were signing an informed consent agreement and voluntary participation, complete clinical and imaging data for definite diagnoses, no other autoimmune diseases, Exclusion criteria were severe lung, liver, heart or other systemic diseases, systemic infectious diseases, malignant tumours, severe metabolic disorders, pregnant or lactating women. The study was approved by the ethics committee of the Second Affiliated Hospital of Nanchang University.

Fasting venous blood (3 ml) was centrifuged at 3000 × g for 10 minutes after standing for 30 minutes. Serum was assayed immediately or aliquoted and stored samples at −20°C. Rheumatoid factor was detected by rate nephelometry by a Siemens Healthcare Diagnostics autoanalyser. The Westergren method was used to measure the ESR. CTHRC1 (Huzhen Industrial Co. Ltd, Shanghai, China), 14-3-3η protein (American Flarebio Biotechnology Co. Ltd, CA, USA) anti-CCP (Kexin Biotechnology Co. Ltd, Shanghai, China), anti-MCV (from Xiupeng Biotechnology Development Co. Ltd, Tianjin, China) were measured by ELISAs.

SPSS 25.0 statistical software and MedCalc drawing software were used for data analysis. Distribution and homoscedasticity were verified by the Kolmogorov-Smirnov test and Levene's test, respectively. Normal distribution data is expressed as mean with standard deviation (SD), analysis of variance (ANOVA) and Games-Howell for multiple and pairwise comparisons; Non-normal data is expressed as median with interquartile ranges (IQR), and the level difference between groups was nonparametric Kruskal-Wallis test, and then used Bonferroni to make a pairwise comparison. Categorical data is expressed as a percentage, and the data comparison was performed by the χ^2 test. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+ LR), negative likelihood ratio (-LR) and the Youden index (sensitivity + specificity −1, and index of diagnostic accuracy; 0 = no value, 1 = excellent) were calculated to evaluate the diagnostic efficiency of each marker for RA. Receiver operating characteristic (ROC) curves were assessed the diagnostic value of various indicators for RA. Binary logistic regression analysis was used to evaluate the risk factors related to RA. A *P*-value<0.05 was taken to imply statistical significance.

Results

Demographic and laboratory data are shown in Table 1. The groups were matched for age and sex. Serum CTHRC1, 14-3-3η protein, anti-CCP, anti-MCV, rheumatoid factor and ESR levels in RA patients were significantly higher than those in non-RA patients and healthy controls. Rheumatoid factor and ESR levels of patients in the non-RA group and 14-3-3η protein levels of OA and AS patients in the non-RA group were significantly higher than those in healthy controls.

The area under the ROC curve (AUC) data are shown in Table 2 and Figure 1. Anti-MCV had the highest AUC, followed by anti-CCP, although (with the exception of ESR), 95% CIs of all indices overlapped. Anti-MCV had the highest sensitivity and anti-CCP the highest specificity. PPV, +LR and the Youden Index also indicated that anti-CCP was the best determinant of RA, followed by anti-CCP.

Table 3 shown analysis of combinations of markers. In almost all cases, this improved the Youden index, and in almost all cases, the index was higher in parallel measurement than in series measurement. When analysed in parallel, the combination of anti-CCP and anti-MCV had the highest Youden

Table 1. Demographic and laboratory data.

		Non-RA (n = 105)				
Marker	RA (n = 103)	OA (n = 36)	AS (n = 29)	SLE (n = 40)	Control (n = 59)	*P*
Gender (male/female)	31/72	11/25	9/20	11/29	18/41	0.991
Age (year)	52.0 ± 13.9	52.6 ± 12.0	47.1 ± 10.6	48.0 ± 10.3	50.0 ± 12.0	0.300
CTHRC1 (ng/ml)	397 (269–607) *#	160 (111–289)	168 (94–225)	168 (117–243)	158 (94–221)	*P* < 0.001
14-3-3η (ng/ml)	3.9 (2.7–5.0) *#	3.1 (1.8–3.5) *	2.8 (2.1–3.1) *	1.4 (0.7–1.8)	1.2 (0.4–1.4)	*P* < 0.001
Anti-CCP (U/ml)	320 (141–990) *#	24 (15–43)	27 (16–64)	24 (10–84)	21 (14–28)	*P* < 0.001
Anti-MCV (U/ml)	395 (87–1000) *#	14 (9–18)	15 (10–19)	11 (7–19)	11 (8–17)	*P* < 0.001
RF(U/ml)	144 (79–378) *#	27 (15–96)*	39 (16–108) *	16 (8–91) *	8 (7–16)	*P* < 0.001
ESR (mm/h)	50 ± 29*#	28 ± 16*	34 ± 26*	33 ± 19*	10 ± 5	*P* < 0.001

Results are represented as n, mean with SD or interquartile ranges (IQR). By ANOVA, χ^2 test and Kruskal–Wallis test. #Compared with the non-RA groups (OA, AS, SLE) $P < 0.05$, *Compared with control group $P < 0.01$. CTHRC1, collagen triple helix repeat containing-1; anti-CCP, anti-cyclic citrullinated peptide; anti-MCV, anti-mutated citrullinated vimentin; RF, rheumatoid factor; ESR, erythrocyte sedimentation rate.

Table 2. ROC curve parameters and diagnostic values of research indices.

Marker	AUC (95%CI)	Cut-off value	Sensitivity (%)	Specificity (%)	PPV	NPV	+LR	-LR	Youden Index
CTHRC1 (ng/ml)	0.84 (0.80–0.88)	234.6	84.5	75.6	68.5	88.6	3.5	0.2	0.6
14-3-3η (ng/ml)	0.81 (0.75–0.85)	3.7	61.2	92.7	84	79.2	8.4	0.4	0.54
Anti-CCP (U/ml)	0.89 (0.80–0.92)	102.4	78.6	94.5	90	87.6	14.3	0.2	0.73
Anti-MCV (U/ml)	0.91 (0.87–0.94)	31.8	86.4	87.2	80.9	91.1	6.8	0.2	0.74
RF (U/ml)	0.85 (0.80–0.89)	58.6	80.6	73.2	65.4	85.7	3	0.3	0.54
ESR (mm/h)	0.77 (0.72–0.82)	40	59.2	82.9	68.5	76.4	3.5	0.5	0.42

Note: Series sensitivity = A sensitivity × B sensitivity; Series specificity = A specificity+ [(1-A specificity) ×B specificity]; Parallel sensitivity = A sensitivity+ [(1-A sensitivity) ×B sensitivity]; Parallel specificity = A specificity × B specificity, where A = first marker, B = second marker. See table 1 for abbreviations

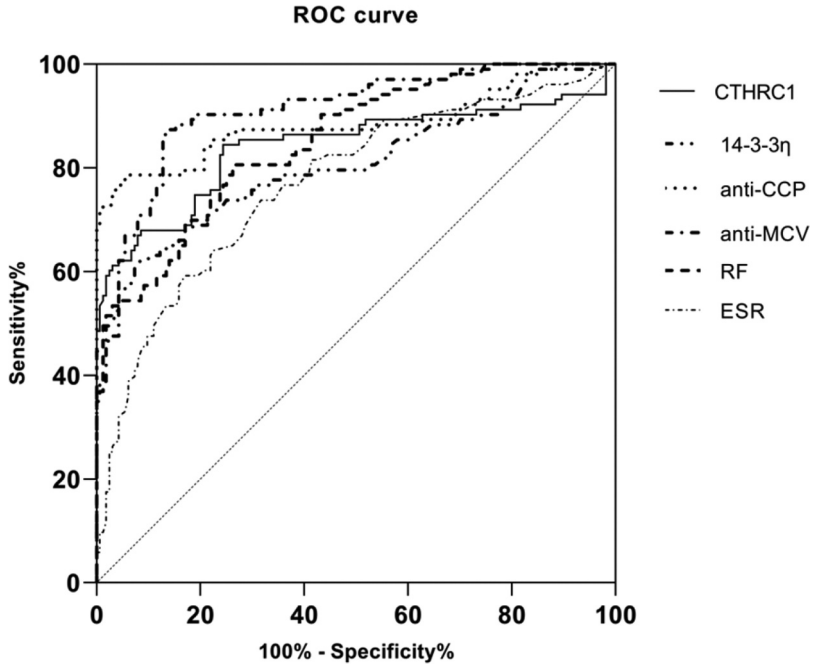

Figure 1. ROC curve of each marker.

Table 3. Clinical evaluation of combinations of indicators.

Marker	In series Sensitivity (%)	In series Specificity (%)	In series Youden Index	In parallel Sensitivity (%)	In parallel Specificity (%)	In parallel Youden Index
CTHRC1/14-3-3η	51.7	98.2	0.5	94	70.1	0.64
CTHRC1/anti-CCP	66.4	98.7	0.65	96.7	71.5	0.68
CTHRC1/anti-MCV	73	96.9	0.7	97.9	65.9	0.64
CTHRC1/RF	68.1	93.5	0.62	97	55.3	0.52
CTHRC1/ESR	50	95.8	0.46	93.7	62.7	0.56
14-3-3η/anti-CCP	48.1	99.6	0.48	91.7	87.6	0.79
14-3-3η/anti-MCV	52.9	99.1	0.52	94.7	80.8	0.76
14-3-3η/RF	49.3	98	0.47	92.5	67.8	0.6
14-3-3η/ESR	36.2	98.8	0.35	84.2	76.9	0.61
anti-CCP/anti-MCV	68	99.3	0.67	97.1	82.4	0.8
anti-CCP/RF	63.4	98.5	0.62	95.9	69.2	0.65
anti-CCP/ESR	46.6	99.1	0.46	91.3	78.4	0.7
anti-MCV/RF	69.6	96.6	0.66	97.4	63.8	0.61
anti-MCV/ESR	51.2	97.8	0.49	94.5	72.3	0.67
RF/ESR	47.7	95.4	0.43	92.1	60.7	0.53

AUC, the area under the curve. PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio. See Table 1 for abbreviations.

Index, followed by the combination of anti-CCP and 14-3-3η protein. The parallel combination of CTHRC1 and anti-MCV had the highest sensitivity, and the series combination of anti-CCP and 14-3-3η had the highest specificity.

Binary logistic regression analysis showed that serum 14-3-3η protein (odds ratio [95% CI] 5.1 [2.1–-12.5]), anti-CCP (1.011 [1.003–1.019]), anti-MCV (1.006 [1.002–1.01]) and rheumatoid factor (1.015 [1.005–-1.026]) were all independently associated with RA.

Discussion

RA is a chronic and progressive autoimmune disease, which mainly involves the synovial tissue of the joint, and is accompanied by hyperplasia of the pannus and progressive bone destruction, which eventually leads to loss of joint function. Its primary aetiology and pathogenesis are still unclear. Rheumatoid factor, ESR and anti-CCP are routine serological test indicators for the diagnosis of RA. The combination of clinical manifestations and imaging examinations can help to diagnose RA patients. However, increased rheumatoid factor is often detected in sera of inflammatory diseases, other autoimmune diseases and even healthy older adults [8], resulting in its low specificity for RA. Therefore, more sensitive and specific serological indicators would be of value in the diagnosis of RA.

CTHRC1 can participate in the remodelling of RA synovial tissue by promoting FLS migration. CTHRC1 expression can be detected in the synovial pannus [2], and can also participate in FLS activation, bone resorption and bone destruction in the course of RA synovitis by activating the classic Wnt/β-catenin signal pathway. Therefore, CTHRC1 can activate FLS in multiple ways to promote bone and cartilage erosion and pannus formation. With a cut-off value was 234.6 ng/ml, the sensitivity and specificity of CTHRC1 for RA diagnosis are 84.5% and 75.6%, respectively, slightly different from other research results [9]. The reason may be related to the geographical difference of the test subjects and the reagents used. The parallel combination of CTHRC1 and anti-MCV had the highest sensitivity for RA whilst the positive rate of CTHRC1 for RA was second only to anti-MCV, suggesting that CTHRC1 combined with other indicators can increase the detection rate of RA. Myngbay et al. [9] have shown that CTHRC1 levels are positively correlated with rheumatoid factor, ACPA, C-reactive protein and disease activity, and are also closely related to interleukin-1β, interleukin-6, interleukin-8 and interferon-γ. In general, serum CTHRC1 is a sensitivity marker and is expected to be an indicator for the diagnosis of RA.

Our data confirms the increase of serum 14-3-3η protein RA patients [10]. It is closely related to matrix metalloproteinase-3 and matrix metalloproteinase-1, indicating that 14-3-3η may be related to RA bone destruction [3,4]. Furthermore, levels of 14-3-3η protein in patients with OA and AS is significantly higher than those in the healthy controls, indicating that 14-3-3η protein is associated with the occurrence of all inflammatory joint disease, especially RA. The high odds ratio of 14-3-3η is notable, and its specificity was second only to anti-CCP, results consistent with those of Xun et al. [11], suggesting that 14-3-3η protein has potential value in the diagnosis of RA. When connected in parallel, the combination of anti-CCP and anti-MCV had the highest Youden index, followed by the combination of anti-CCP and 14-3-3η protein [12,13]. The series combination of anti-CCP and 14-3-3η had the highest specificity, more elevated than each single index, and is consistent with other research results showing that ACPA is a highly specific antibody for RA diagnosis, and also suggests that 14-3-3η protein can improve the sensitivity of RA diagnosis, a conclusion consistent with data from El-Sherif et al. [14].

ACPA is an antibody produced against self-denatured proteins. It can activate other immune cells and stimulate the production of cytokines through Fc receptors. The potential inflammatory cascade leads to clinically evident arthritis [15]. It is usually based on the detection of anti-CCP and anti-MCV, which has been widely used in clinical. In our hands, anti-MCV had the highest sensitivity and anti-CCP had the highest specificity for RA. The combined detection of these two antibodies can override potential deficiencies and increase the sensitivity of RA diagnosis, a view consistent with Sun et al. [16]. According to the results of the ROC curve, CTHRC1, 14-3-3η, anti-CCP, anti-MCV, rheumatoid factor and ESR had a moderate or higher value for the diagnosis of RA, and anti-MCV had the highest AUC, followed by anti-CCP, indicating that ACPA has the highest value on diagnosing RA. However, interpretation must be cautious as there is considerable overlap in the CIs for these curves.

Rheumatoid factor and ESR have for decades been major serological indicators for the diagnosis of RA, but these indices were consistently out-performed by the newer markers. However, the combination of anti-CCP and ESR had the 4th highest Youden index, indicating minor value where 14-3-3η cannot be determined.

This work represents an advance in biomedical science because it shows that certain combinations of CTHRC1, 14-3-3η, anti-CCP, anti-MCV and ESR, with a Youden index ≥0.68, can improve the diagnosis of RA.

Summary table

What is known about this subject:
- Increased individual levels of serum CTHRC1, 14-3-3η, ACPA, rheumatoid factor and ESR are useful in the diagnosis of RA.

What this paper adds:
- 14-3-3η has a high odds ratio (95% CI) of 5.1 (2.1–12.5) for RA
- The combinations of any two of CTHRC1, 14-3-3η, anti-CCP, anti-MCV, rheumatoid factor and ESR can improve the diagnosis of RA
- The combination of anti-CCP and anti-MCV has the highest overall sensitivity, specificity and Youden index for diagnosing RA.

Disclosure statement

All authors report that they have no conflict of interest.

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