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Clinical diagnostic significance of 14-3-3η protein, high-mobility group box-1, anti-cyclic citrullinated peptide antibodies, anti-mutated citrullinated vimentin antibodies and rheumatoid factor in rheumatoid arthritis

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ABSTRACT

Introduction: Circulating markers of rheumatoid arthritis (RA) include the 14–3-3η protein, high-mobility group box-1 (HMGB1), anti-cyclic citrullinated peptide (anti-CCP) antibodies, anti-mutated citrullinated vimentin (anti-MCV) antibodies and rheumatoid factor (RF). We set out to determine which two markers in combination provided best discriminatory power for this disease.

Methods: We recruited 108 RA patients, 102 non-RA patients (SLE, AS, Sjogren's syndrome, MCTD) and 90 healthy controls. Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and the Youden index of each analyte were calculated and binary logistic regression analysis and receiver operating characteristic (ROC) curve were performed to evaluate their diagnostic value for RA alone and in paired combination.

Results: As expected, all markers were elevated in RA patients ($P < 0.05$). Binary logistic regression analysis showed that 14–3-3η had the highest odds ratio (95% CI) at 2.4 (1.9–2.8). Anti-CCP and anti-MCV had the highest areas under the curves [AUC (95% CI)] at 0.85 (0.78–0.90) and 0.85 (0.78–0.91) respectively (both $P < 0.001$). In serial detection (one marker followed by another), no combination had a Youden index >0.6. In parallel analysis (both considered together) several combinations had a Youden index >0.7, of which the highest (0.78) was anti-CCP with anti-MCV, with a sensitivity of 93.3% and specificity of 84.7%.

Conclusions: Despite individual increases in serum 14–3-3η, HMGB1, anti-CCP, anti-MCV and RF, the combination of anti-CCP and anti-MCV might be of great help for diagnostic in RA, and so should be considered as routine tests for this disease.

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by inflammatory lesions in synovial tissue, subsequent joint destruction leading to loss of joint function, and disability, with a global prevalence of 0.51% [1]. Joint erosions develop quickly in 25% of RA patients during the first 3 months of the disease and in about 75% of patients during the first 2 years [2]. Early diagnosis combined with an accurate prognostic assessment is a core principle in the effective management of RA patients. At present, the specific pathogenesis of RA has not been clarified, but cytokines and inflammatory mediators are involved [3].

The 14–3-3 family of highly conserved regulatory proteins consists of seven isoforms: α/β, γ, δ/ζ, ε, η, θ/τ and σ, with functions of cell cycle regulation, apoptosis, transcription regulation, immune inflammation and stress response [4,5]. 14–3-3η is widely expressed in eukaryotes and involved in the occurrence and development of RA [6]. Serum 14–3-3η up-regulates cytokines and enzymes and allows local and systemic inflammation to persist, leading to the joint injury [7]. Thus, serum 14–3-3η is a new clinical RA marker with high specificity related to the severity of the disease and is an addition to the existing markers in the diagnosis of RA [5].

High-mobility group box-1 (HMGB1) is a ubiquitous highly conserved single polypeptide in all mammal eukaryotic cells which exists mainly within the nucleus and acts as a DNA chaperone [8]. HMGB1 binds to related cell signal transduction receptors, such as RAGE, TLR2, and TLR4, and becomes a proinflammatory cytokine that participates in the development and progression of many diseases including RA. Levels of HMGB1 are elevated in RA [9].

Anticitrullinated peptide antibodies (ACPAs) are the most specific autoantibodies of RA and are associated with increased clinical disease activity and progression of structural damage. RF is one of the most common laboratory indices in RA which is widely

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used in clinical practice. ACPAs and RF have high weight in the final scoring system of the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) RA classification criteria [10]. ACPAs have been shown to predict joint damage and are associated with more severe disease and extra-articular manifestations [11]. Anti-cyclic citrullinated peptide (anti-CCP) antibodies, and anti-mutated citrullinated vimentin (anti-MCV) antibodies are the most common clinical tests for ACPAs, which reflect the level of ACPAs in patients' serum.

Despite the above, these markers have rarely been compared in a well-powered case and disease-controlled study. Accordingly, we set out to establish which markers, alone or in combination, would provide the best data for the diagnosis of RA.

Materials and methods

We recruited 210 outpatient or inpatient cases from the Second Affiliated Hospital of Nanchang University from January 2018 to May 2019, including 108 RA patients and 102 non-RA patients. The 102 non-RA patients were diagnosed with systemic lupus erythematosus (n = 26), ankylosing spondylitis (20), Sjogren's syndrome (27) and mixed connective tissue disease (29). Ninety healthy subjects were recruited as a control group. All RA patients met the diagnostic criteria of ARA/EULAR [10]. All participants had a clear diagnosis with intact clinical and imaging data, and the clinical examination of healthy participants was normal. Exclusion criteria were other autoimmune diseases, serious liver, kidney, lung, heart, thyroid or other systemic diseases, and malignant tumours. All specimens were obtained with patients' informed consent and the study was approved by the hospital ethics committee of the Second Affiliated Hospital of Nanchang University.

Three ml blood from each participant was collected in the morning after an overnight fast for 8 h. Serum was obtained after centrifugation at 1026 g for 15 min. 14–3-3η, anti-CCP, anti-MCV, HMGB1 were measured using commercial ELISA kits (14–3-3η American Flarebio Biotechnology Co. Ltd, CA, USA, anti-CCP and anti-MCV Kexin Biotechnology Co. Ltd, Shanghai, China, and HMGB1 Hengyuan Biotechnology Co. Ltd, Shanghai, China). RF was measured by rate nephelometry (Siemens Healthcare Diagnostics).

Statistical analyses were performed using SPSS 24.0 (SPSS Inc., Chicago, USA) for Windows. Categorical data were described as number and percentage. Comparisons of categorical data were done using the χ2 test. Distribution and homoscedasticity were verified by the Kolmogorov–Smirnov test and Levene's test, respectively. Normally distributed data were presented as mean with standard deviation (SD) and analysed using independent-samples T-tests for two groups and analysis of variance (ANOVA) and Student-Newman-Keuls tests for multiple and pairwise comparisons. Non-normal data were presented as medians with inter-quartile ranges (IQR) and compared using Kruskal–Wallis test with Bonferroni for multiple and pairwise comparisons. Receiver operating characteristic (ROC) curves analysis was conducted to explore the diagnostic performance of variables for RA. A P-value<0.05 indicates statistical significance.

Results

The three groups were matched for age and sex (Table 1). In comparison to the patients with non-RA and healthy controls, patients with RA had higher levels of 14–3-3η, anti-CCP, anti-MCV, RF and HMGB1. Furthermore, patients with non-RA had higher levels of 14–3-3η, RF and HMGB1 than healthy controls. 14–3-3η had by far the greatest differentiation power, as defined by the highest F value, with RF being the second-best discriminator.

Binary logistic regression analysis was performed to explore risk factors associated with the presence of RA. The results showed that 14–3-3η, HMGB1, anti-CCP, anti-MCV and RF were identified independently associated with RA. Odd's ratios (95% CI) were 2.3 (1.93–2.83), 1.005 (1.002–1.008), 1.006 (1.003–1.009), 1.32 (1.14–1.54) and 1.03 (1.01–1.05) respectively (all $P < 0.01$). The ROC curves of the five analytes for RA diagnosis were determined (Table 2). All analytes were highly significant, with considerable overlap, but the areas under the curves (AUC) of anti-CCP and anti-MCV were the largest.

Table 1. Demographic and laboratory data.

Variables	RA (n = 108)	Non-RA (n = 102)	Controls (n = 90)	P
Age (years)	51.7 ± 12.1	49.4 ± 13.4	50.6 ± 12.8	0.311
Sex (F/M)	32/76	34/68	30/60	0.199
14–3–3η (ng/ml)	3.83$^{\#}$ (2.66–5.35)	1.68$^{\#}$ (0.93–2.55)	0.75 (0.50–1.46)	<0.001
anti-CCP (U/ml)	370$^{\#}$ (72–1411)	24 (13–83)	20 (19–25)	<0.001
anti-MCV (U/ml)	400$^{\#*}$ (83–946)	18 (14–37)	18 (16–26)	<0.001
HMGB1 (pg/ml)	12.8$^{\#*}$ (9.8–15.1)	8.5$^{\#}$ (5.3–12.6)	2.8 (2.3-3.4)	<0.001
RF (U/ml)	277$^{\#*}$ (74–636)	26$^{\#}$ (15–44)	12 (6–16)	<0.001

Results are represented as n, mean with SD or median (IQR). By ANOVA, χ^2 test and Kruskal–Wallis test. *Compared with non-RA $P < 0.05$, #Compared with non-RA, control $P < 0.01$. AUC, area under the curve; anti-CCP, anti-cyclic citrullinated peptide; anti-MCV, anti-mutated citrullinated vimentin; HMGB1, high-mobility group box-1; RF, rheumatoid factor.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), negative likelihood ratio (-LR) and Youden index (YDI) of each index were calculated according to the cut-off value of ROC curve. Anti-MCV and RF had the highest sensitivity and anti-CCP had the highest specificity, PPV, +LR and YDI indicating that anti-CCP is of great diagnostic value for RA (Table 3). Combinations of markers were compared (Table 4). The parallel combination of anti-CCP and anti-MCV provided the highest Youden index.

Discussion

RA is one of the most common chronic inflammatory joint diseases [12], and critically ill patients may die from multiple organ involvement [13]. Due to the diverse clinical manifestations of RA, the lack of typical symptoms and negative serology in the early stage, patients are often misdiagnosed. Therefore, improvement of the serum diagnostic level of RF and early therapeutic intervention to prevent joint lesions are of great value. Levels of 14–3-3η protein and HMGB1 in serum and joint synovial fluid of RA patients are higher than that in healthy controls [14,15]. In addition, anti-CCP, anti-MCV and other autoantibodies may be detected in the early stage of RA [16], suggesting that 14–3-3η, HMGB1, anti-CCP, anti-MCV and RF may participate in the development of RA. We confirm much of this, reporting that patients with RA have markedly higher levels of 14–3-3η protein, HMGB1, anti-CCP, anti-MCV and RF than those in non-RA patients and healthy controls [16–18]. Furthermore, we show that patients with a mixture of non-RA diseases had higher levels of 14–3-3η, HMGB1 and RF than healthy controls. Anti-CCP appears in the early stage of RA and has favourable predictive effect on the severity and prognosis of RA [19]. Furthermore, Harre et al. [20] found that the presence of anti-MCV may increase osteoclastogenesis and bone resorption. A rat model of RA has increased expression of HMGB1 by synoviocytes, which gives support to our data of increased serum levels in our own species [21].

The Youden indices of all markers were poor (consistently <0.6) when assessed in series, despite excellent specificities (all >0.95). However, in parallel analyses, the Youden index was >0.7 in several combinations, with far improved sensitivities, the best being that of anti-CCP with anti-MCV, with the combination of anti-CCP with 14–3-3η being a close second, results consistent with those of others [22,23], suggesting that anti-CCP and anti-MCV are high-specific antibodies for RA diagnosis. The study by Gong et al. [24] has shown that the AUC of 14–3-3η for diagnosing RA was 0.98, with a sensitivity of 91.7% and a specificity of 99.6%, when the cut-off value was 0.88 ng/ml. The sensitivity, specificity and AUC were much higher, and the cut-off point much lower than the results of this study, possibly because of the reagents or the nature of the patients we recruited. Barouta et al. [16] showed that 52% of RA were positive for anti-MCV, 44% for anti-CCP, consistent with this study. There has been a great deal of research [10,15] regarding

Table 2. ROC curve for research indices.

Marker	AUC (95% CI)	Cut-off value
14–3-3η	0.83 (0.79–0.87)	2.6 ng/ml
anti-CCP	0.85 (0.78–0.90)	95.5 U/ml
anti-MCV	0.85 (0.78–0.91)	79.2 U/ml
HMGB1	0.80 (0.69–0.89)	10.0 pg/ml
RF	0.82 (0.75–0.88)	46.2 U/ml

AUC, area under the curve. See Table 1 for other abbreviations.
All significant at $P < 0.001$.

Table 3. Diagnostic values of research indices.

Marker	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR	Youden index
14–3-3η	63.3	91.1	82.6	77.8	7.10	0.40	0.54
anti-CCP	71.6	94.3	93.6	68.1	12.65	0.30	0.66
anti-MCV	76.5	89.8	89.7	69.4	7.50	0.26	0.66
HMGB1	73.9	78.1	63.0	83.8	3.37	0.33	0.52
RF	76.5	78.7	81.6	71.2	3.59	0.30	0.55

PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio. See Table 1 for other abbreviations.

Table 4. Clinical evaluation of combinations of markers.

	In series			In parallel		
Groups	Sensitivity (%)	Specificity (%)	Youden index	Sensitivity (%)	Specificity (%)	Youden index
14–3-3η/anti-CCP	45.3	99.5	0.45	89.6	85.9	0.76
14–3-3η/anti-MCV	48.5	99.1	0.48	91.4	81.8	0.73
14–3-3η/HMGB1	46.8	98.0	0.45	90.4	71.1	0.62
14–3-3η/RF	48.5	98.1	0.47	91.4	71.7	0.63
anti-CCP/anti-MCV	54.8	99.4	0.54	93.3	84.7	0.78
anti-CCP/HMGB1	52.9	98.8	0.52	92.6	73.6	0.66
anti-CCP/RF	54.8	98.8	0.54	93.3	74.2	0.68
anti-MCV/HMGB1	56.6	97.8	0.54	93.9	70.1	0.64
anti-MCV/RF	58.6	97.8	0.56	94.5	70.7	0.65
HMGB1/RF	56.6	95.3	0.52	96.5	61.4	0.58

See other tables for abbreviations.

the association between HMGB1 and RA from the viewpoint that HMGB1 acts as an important proinflammatory cytokine participant in the development and progression of RA disease, but in our hands, it compares poorly with other markers.

This work represents an advance in biomedical science because it shows that of the combinations of any two from 14–3-3η protein, HMGB1, anti-CCP, anti-MCV and RF, that of anti-CCP and anti-MCV have the highest discriminatory power for the detection of RA, and might be useful for diagnostic and therapeutic strategies in this disease.

Summary Table

What is known about this subject:
- Increased individual levels of serum 14-3-3η, HMGB1, anti-CCP, anti-MCV and RF have certain diagnostic value for RA.

What this paper adds
- 14-3-3η, followed by RF, has the highest discriminatory power for RA versus non-RA connective tissue diseases and healthy controls.
- The combination of anti-CCP and anti-MCV had the highest overall sensitivity, specificity and Youden index for diagnosing RA.

Disclosure statement

No potential conflict of interest was reported by the authors.

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