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Original Article

The anti-mutated citrullinated vimentin antibody as a potential predictor for rheumatoid arthritis associated interstitial lung diseases

Feng Tian[1], Jingyang Li[1], Hangxing Tuo[1], Qing Ling[1], Saiqun Zeng[2], Zhenhua Wen[1], Xiaowen Luo[1]

[1]Department of Rheumatism, Zhuzhou Central Hospital, Zhuzhou 412000, Hunan, China; [2]Department of Ultrasonography, Zhuzhou Central Hospital, Zhuzhou 412000, Hunan, China

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Abstract: Rheumatoid arthritis (RA) is a long lasting autoimmune disorder which is characterized by symmetric arthritis and synovial inflammation. RA associated Interstitial lung diseases (RA-ILD) are common extra-articular manifestations of RA which occurs in between 4 to 50% of the RA patients. However, exact biomarker for predicting the development of RA-ILD is still unclear. In this study, the anti-MCV antibody, anti-CCP antibody, anti-AKA antibody and anti-AFP antibody as well as different Ig forms of rheumatoid factor were systematically evaluated between RA-ILD and RA patients for the correlation of between the RA associated autoantibodies and ILD. Our data demonstrated that the anti-MCP antibodies titer in RA-ILD patients were significantly higher that RA patients without ILD. However, no significant difference was observed for anti-CCP antibodies between two groups. In conclusion, our study suggested that existence of anti-MCV antibodies as well as its level could be a potential predictor for RA-ILD.

Keywords: Rheumatoid arthritis, RA associated interstitial lung diseases, anti-MCV antibody, anti-CCP antibody, autoimmune disease

Introduction

Rheumatoid arthritis (RA) is a long lasting autoimmune disorder which is characterized by symmetric arthritis and synovial inflammation leading to progressive joint erosion and even deformity [1]. Generally, Rheumatoid arthritis results in warm, swollen, and painful joints [1]. The globe prevalence of RA ranges from 0.5% to 1%, and varies based on gender, population and ethnicity [2, 3]. Since William Heberden in described "some degree of heredity" for rheumatoid arthritis (RA) in 1806, Subsequent twin- and family-based studies demonstrated that genetic factors accounts to one third of the susceptibility of RA [4, 5]. Moreover, other environmental factor such as smoking and air pollution contributed to occurrence of RA as well [5, 6]. Furthermore, studies also confirmed a potential association between RA with infections caused by Herpesvirus [7-9]. However, as a chronic autoimmune disorder, the causes of RA are still not completely understood [4].

RA associated Interstitial lung diseases (ILD) are common extra-articular manifestations of RA which occurs in between 4 to 50% of the RA patients according to different reports [10-12]. ILD defines as a group of diseases which is characterized by chronic fibrosis and inflammation of the pulmonary interstitium [13]. Moreover, RA associated interstitial lung disease can be caused by anti-inflammatory and biologic drugs which are used to treat RA [14].

Patients developing RA-ILD demonstrate an increased mortality compared to RA patients without ILD [15]. RA-ILD patients may experience bouts of acute exacerbations (AE) similar to other patients with ILD [16]. The severity of RA as well as increased risk of death was reported to be associated with RA-ILD [17]. Although exact biomarker for predicting the development of RA-ILD is still unclear, data suggested that the severity of RA may be related to the development of ILD, such as presence of erosive joint disease, high levels of erythrocyte sedimentation rate (ESR) and presence of

Table 1. Serum positive ratio for anti-MCV and anti-CCP between RA-ILD and RA group

	RA-ILD (%)	RA (%)	*P*-value
Case (n)	37	38	NA
Anti-MCV positive (n)	100.00 (37/37)	71.05 (27/38)	0.00
Anti-CCP positive (n)	81.08 (30/37)	73.68 (28/38)	0.304

rheumatoid nodules are considered to be contributing factors for the development of ILD [18, 19]. Moreover, there was also report demonstrated that a variety of specific anti-citrullinated peptide (CCP) antibodies and an expanded repertoire of these antibodies were present in patients with RA-ILD with lung function abnormalities, which suggesting a link between autoimmunity with developing of RA-ILD [20].

In this study, we evaluated the correlations between the RA associated autoantibodies and ILD. Our data demonstrated that the anti-MCP antibodies titer in RA-ILD patients were significantly higher that RA patients without ILD. However, no significant difference was observed for anti-CCP antibodies between two groups. In conclusion, our study suggested that existence of anti-MCV antibodies as well as its level could be a potential predictor for RA-ILD.

Materials and methods

Ethics statement and general information

Institutional Ethics Board approval was obtained from the Medical Ethics Committee of Zhuzhou Central Hospital. All participating patients were formally informed for the purpose of this study and the written informed consents were obtained from all participants. There were totally 75 cases of RA (29 males and 46 females) enrolled in this study. All patients were subjected to the criteria of 2010 ACR/EULAR Classification Criteria for RA. Among 75 patients, there were 37 cases of RA-ILD (subjecting to Interstitial lung diseases diagnosis criteria of American Thoracic Society/European Respiratory Society). The other 38 cases of RA only patients were served as control group. Exclusion criteria included: 1) RA patients with lung infection, pulmonary tuberculosis, chronic obstructive pulmonary disease, bronchiectasis or lung tumor; 2) RA patients with pneumoconiosis or interstitial lung disease due to Inhalation of organic dusts; 3) RA patients with cardiopulmonary insufficiency.

Serum sample collection

The fasting blood samples (3 mL for each patient) from all patients were collected without anticoagulants in the morning before any drinking or eating. All blood samples were kept at room temperature for 2 hours and then centrifuged at 3000 rpm for 10 min for serum collection.

ELISA assays

ELISA assays for indicated antibody were conduct on VICTOR3™ Multilabel Counter (Perkin-Elmer, Waltham, MA, USA) according manufacturer's instructions. The Anti-mutated Citrullinated vimentin antibody (anti-MCV) ELISA kit (ORGENTEC, Diagnostika GmbH, Mainz, Germany), Anti-cyclic citrullinated peptide antibody (anti-CCP) ELISA kit (EUROIMMUNE, Medizinische Labordiagnostika, AG, Lubeck, Germany), Anti-keratin antibody (anti-AKA) ELISA kit (Jiechuangxin Biotech, Beijing, China), Anti-Alpha Fetoprotein antibody (anti-AFP) ELISA kit (Jiechuangxin Biotech), IgA, IgG and IgM rheumatoid factor ELISA kits (EUROIMMUNE) were used for serum screening of indicated antibodies. All ELISA assay were conducted according to instruction of kit manufacturer. CRP test were also conducted by using CRP assay kit (Shanghai Upper Bio-Tech Pharma, Shanghai, China) and Qpad digital quantification system (Shanghai Upper Bio-Tech Pharma).

Statistical analysis

Statistical analysis was conducted in SPSS program (Version 17.0). All data was subjected to Kolmogorov-Smirnov test for analysis of normality and variance. If the data complied with normality, the data were presented as Average ± Standard deviation, the difference in indicators between two groups were subjected to the Student's t test. The Single factor analysis of variance analysis was conducted for multiple groups' comparisons. A two tailed *P*-value of less than 0.05 was considered significant. If the data complied with partial distribution, the data will be analyzed for quartiles and means, as well as subjected to Wilcoxon rank sum test. In this case, the Kruskal-Wallis test will be applied to comparison between more than two groups. For the different indicators, if the data

Table 2. The positive ratio and level of anti-MCV and anti-CCP antibodies in RA-ILD and RA groups

Antibody	Group	Case (n)	Average	SD	Median	QUARTILE
MCV	RA-ILD	37	1482.65 U/mL	427.32 U/mL	1630 U/mL	781.5
	RA	38	319.05 U/mL	516.63 U/mL	80 U/mL	469.25
CCP	RA-ILD	37	475.22 RU/mL	551.81 RU/mL	265 RU/mL	791
	RA	38	332.03 RU/mL	418.63 RU/mL	197.5 RU/mL	440.25

Table 3. Statistic analysis of anti-MCV and anti-CCP antibodies level in RA-ILD and RA patients

	RA-ILD		RA			
Antibody	Median	QUARTILE	Median	QUARTILE	Z-value	*P*-value
MCV	1630	781.5	80	469.25	3.523	0.000
CCP	265	791	197.5	440.25	0.714	0.687

Table 4. Positive ratio of autoantibodies between RA-ILD and RA groups

Antibody	RA-ILD (n=37)	RA (n=38)	*P*-value
RF-IgA	78.38 (29/37)	71.05 (27/38)	0.002
RF-IgG	21.62 (8/37)	10.53 (4/38)	0.190
RF-IgM	89.19 (33/37)	86.84 (33/38)	1*
AKA	54.05 (20/37)	34.21 (13/38)	0.083
APF	72.97 (27/37)	63.16 (24/38)	0.362

*Continuity Correction.

was complied with normality, Pearson test will be used for analysis, otherwise, Kendall's Tau-b will be used for data analysis. The identification of risk factors for RA-ILD was subjected to logistic regression analysis.

Results

Comparison of anti-MCV antibody and anti-CCP antibody between RA-ILD and RA patients

We first evaluated the serum positive ratio for anti-MVC and anti-CCP between RA-ILD and RA patients. The positive cut off values for anti-MCV antibody and anti-CCP antibody were ≥20 U/ml and ≥25 RU/ml, respectively. Based on our data, all RA-ILD patients (n=37, 100%) were positive for anti-MCV antibody while 27 out of 38 patients (71.05%) were positive for anti-MCV antibody (**Table 1**). For RA-ILD group, 30 out of 37 patients were positive for anti-CCP antibody while 28 out of 38 patients from RA group were positive for anti-CCP antibody (**Table 1**). Based on X^2 test, compared with RA group, the 100% positive ratio (P=0) of anti-MCV antibody in RA-ILD group is significantly higher than it in RA group, which suggested a strong correlation between the anti-MVC antibody and RA-ILD (**Table 1**). However, no statistical significance was observed for positive ratio of anti-CCP antibody between these groups (P=0.304) (**Table 2**).

To further investigate in relationship of anti-MCV and anti-CCP antibodies between two groups of patients, the serum antibody level were compared as well. As it shown in **Tables 2**, **3**, statistical analysis of anti-MCV antibody level is significant higher (1630 U/mL) for RA-ILD patients than RA patients (319.05 U/mL). On the other hand, average level of anti-CCP antibody in RA-ILD group is a little bit higher (475.22 RU/mL) than RA group (332.03 RU/mL) but demonstrated no statistically difference (P=0.687).

Comparison of other autoantibodies between RA-ILD and RA patients

Rheumatoid factor (RF) is the autoantibody found during the rheumatoid arthritis and is defined as an antibody against the Fc portion of IgG [21]. Also predominant form of RF is IgM, RF can be of any isotype of immunoglobulins such as. IgA, IgG, IgE and IgD [21-23]. Therefore, we also evaluated different RA forms as well as anti-AKA and APF antibodies from RA-ILD and RA patients (**Table 4**). Based on X^2 test, for all these antibodies, only RF-IgA positive ratio demonstrated statistically significance for RA-ILD group than RA group (P=0.002), while no significant difference was observed for other RFs, as well as anti-AKA and anti-APF antibodies.

Evaluations of risk factor for RA-ILD

To systematically evaluate the risk factor for developing RA-ILD, we summarized out data and potential factor for comparison, these potential factors and well as their assignment were listed as **Table 5**. Logistic regression analysis was conducted for these potential factors to identify the most related risk factor of RA-ILD. Based on our result, RF-IgA, anti-MCV antibody,

Table 5. The possible risk factors for RA-ILD and their assignment

Risk Factor	Variable factor name	Assignment
Sex	X_1	Male=0, Female=1
Age (year)	X_2	<45=1, 45~54=2, 55~64=3, 65~=4
Duration (month)	X_3	≤6=1, 7~12=2, 13~24=3, >24=5
DAS28	X_4	≤2.6=1, 2.61~3.2=2, 3.21~5.1=3, >5.1=4
RF-IgA	X_5	≤20=1, 21~60=2, 61~200=3, >200=4
RF-IgG	X_6	≤20=1, 21~60=2, 61~200=3, >200=4
RF-IgM	X_7	≤20=1, 21~60=2, 61~200=3, >200=4
Anti-CCP positive	X_8	≤25=1, 26~75=2, 76~100=3, 101~300=4, >300=5
Anti-MCV positive	X_9	≤20=1, 21~60=2, 61~100=3, 101~300=4, >300=5
Anti-AKA	X_{10}	Positive=1, negative=0
Anti-APF	X_{11}	Positive=1, negative=0
RA-ILD	Y	Positive=1, negative=0

Table 6. The OR and *P*-value of RA-ILD risk factors

Factor	OR value	*P*-value
RF-IgA	5.358	0.046
Anti-MCV antibody	1039.388	0.001
DAS28	13.409	0.024
Age	1.607	0.002

Disease activity score in 28 joints (DAS28) and age were identified as the potential risk factor for developing RA-ILD (**Table 6**). Moreover, it is notable that the anti-MCV antibody level is a highly risky factor for RA-ILD with OR-value of 1039.388 and *P*-value of 0.001. Taken together, our data suggested that existence of anti-MCV antibodies as well as its level could be a potential predictor for RA-ILD.

Discussion

As a systematic autoimmune disorder, RA could result extra-articular manifestations and lung is the frequent extra-articular target of RA [24, 25]. The extra-articular manifestations of RA in lung could be presented as variety forms, such as pulmonary rheumatoid nodules, rheumatoid pleuritis, Caplan's syndrome, bronchiectasis and ILD [26-29]. The cause and mechanism of RA associated secondary lung manifestations are still elusive, and the contributing factor may include genetic factors as well as environmental factors [30, 31]. Study had suggested that Silica exposure during the working environment is associated with increased risk of developing rheumatoid arthritis and its associated lung manifestations [32-34]. Other reports demonstrated that clinically manifest ILD was correlated with high DAS28 score, high titer of rheumatoid factor and anti-cyclic citrullinated peptide antibodies, age as well as carriage of HLA-DRB1*1502 mutation [35, 36].

Currently, researchers still hold some argument about the correlation of and anti-cyclic citrullinated peptide antibodies and development of RA-ILD. Some reports proposed that genetic factor and smoking could result generate more citrulline related proteins in lung of RA patients which leads to the development of anti-cyclic citrullinated peptide antibodies, and anti-cyclic citrullinated peptide antibodies could be viewed as an independent factor for developing RA-ILD [37, 38]. However, in another report which focus on evaluating the relationships between and anti-cyclic citrullinated peptide antibodies and pulmonary diseases, no significant differences were found for the prevalences and levels of anti-cyclic citrullinated peptide antibodies between RA patients with and without ILD and follicular bronchiolitis [39].

In this study, we evaluated the correlations between the RA associated autoantibodies and ILD. Our data demonstrated that the anti-MCP antibodies titer in RA-ILD patients were significantly higher that RA patients without ILD. However, no significant difference was observed for anti-CCP antibodies between two groups. Moreover, the positive ratio of RF-IgA is also higher in RA-ILD groups than RA patient, while the positive ratio of RF-IgG, RF-IgM anti-AKA antibodies, anti-APF antibodies and anti-CCP antibodies demonstrated little difference between these two groups. Therefore, our data suggested that anti-MCV antibodies may be used as a predictor for RA-ILD rather than anti-CCP antibodies. As a result, if the higher titer of anti-MCV antibodies were observed in RA

patients, this may imply the possible RA-related manifestation of lung and chest CT may be needed for further examination to confirm existence of ILD. However, in our study, the smoking was not considered as another factor for evaluation and large scale study may needed to validation our speculation. In conclusion, our study demonstrated that anti-MCV antibodies could be a potential predictor for RA-ILD and may provide valuable information for clinical diagnosis of RA-ILD.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jingyang Li, Department of Rheumatism, Zhuzhou Central Hospital, Zhuzhou 412000, Hunan, China. E-mail: lijingyang2015@sina.cn

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