High Levels of Anti-Cyclic Citrullinated Peptide Autoantibodies Are Associated with Co-occurrence of Pulmonary Diseases with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To investigate whether levels of anti-cyclic citrullinated peptide antibodies (anti-CCP2) in patients with rheumatoid arthritis (RA) are associated with the co-occurrence of lung diseases.

Methods. A total of 252 RA patients were included in a cross-sectional study. Pulmonary disease was confirmed by high-resolution chest computed tomography scan. Circulating anti-CCP2 were quantified using ELISA. Multivariate logistic regression was conducted to identify independent risk factors for lung disease.

Results. Male sex (OR 3.29, 95% CI 1.59–6.80) and high anti-CCP2 levels (OR 1.49, 95% CI 1.25–1.78) were identified as independent risk factors for lung disease in the RA population.

Conclusion. High anti-CCP2 levels are associated with lung disease in the RA population. (First Release March 1 2011; J Rheumatol 2011;38:979–82; doi:10.3899/jrheum.101261)

Key Indexing Terms:

ANTI-CYCLIC CITRULLINATED PEPTIDE AUTOANTIBODIES

PULMONARY DISEASE RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a complex disease driven by a combination of genetic risk factors and environmental events. It is well established that the risk conferred by HLA-DRB1 shared-epitope alleles (HLA-DRB1*SE) is restricted to individuals with RA who are positive for antibodies to citrullinated peptides (ACPA)[1]. The association between tobacco smoking and ACPA-positive RA is well recognized (reviewed by Klareskog, *et al*[1]). The fact that IgA ACPA appears early during the immune response suggests that immunity triggered from mucosal surfaces may be involved in the inception of anticitrullinated protein immunity[2]. Following the observation of citrullinated proteins in the lungs of smokers[3], it was proposed that smoking, in the presence of HLA-DRB1*SE, may trigger immunity to citrullinated proteins and lead to the development of RA[1]. This challenging concept should not be limited to smoking. It could be hypothesized that lung inflammation may lead to the development of anticitrullinated peptide immunity. We hypothesized that the presence of a lung disease with RA could influence the ACPA production; our aim in this study was to examine the link between anti-CCP2 levels and presence of lung disease in a population with RA.

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MATERIALS AND METHODS

Study population and design. We conducted a cross-sectional study among 252 patients with RA fulfilling the American Rheumatism Association 1987 criteria for RA[4]. Clinical and radiological data were obtained from medical records. Patients' characteristics including disease duration, cigarette smoking habit, co-occurrence of Sjögren's syndrome according to the revised European criteria[5], history of lung disease, respiratory symptoms, and chest radiographs were assessed.

When suspected, lung disease was confirmed by a chest high-resolution computed tomography (HRCT) scan. A chest physician blinded to clinical and immunological data reviewed chest HRCT results and classified the lung disease according to the predominant abnormality as follows: interstitial lung disease, bronchiectasis, or rheumatoid nodules.

Autoantibody analysis. Anti-cyclic citrullinated peptide antibodies (anti-CCP2) and rheumatoid factor (RF) were identified and quantified with the Immunoscan-CCPlus ELISA test (Eurodiagnostica, Malmö,

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Sweden) and nephelometry (BNII, Siemens, Marburg, Germany), respectively. Levels above 25 U/ml and 30 UI/ml, respectively, were regarded as positive.

Statistical analysis. Categorical variables were described with numbers and percentages, and continuous variables with median and interquartile range. Comparisons between patients with and those without lung disease were performed using the chi-square or Fisher exact test when appropriate for categorical variables, and with the Wilcoxon rank-sum test for continuous variables. We constructed a multivariate logistic regression model with stepwise selection variables to identify risk factors for lung disease. The variables having a p value < 0.20 in univariate analysis and those that were clinically relevant [i.e., presence of Sjögren's syndrome, tobacco smoking, use of methotrexate and anti-tumor necrosis factor-α (TNF-α), and disease duration] were included in the multivariate models. SAS 9.1 software (SAS Inc., Cary, NC, USA) was used for all statistical analyses.

RESULTS

RA population. Among the 252 RA patients studied, 23.4% had a lung disease established clinically and confirmed by HRCT. The lung disease pattern was heterogeneous, including interstitial lung disease (ILD; 38.9%), bronchiectasis (33.8%), and nodules (32.2%). All the characteristics of patients with RA are summarized in Table 1.

Univariate analysis. An increased frequency of anti-CCP2 positivity was observed in the subset of patients with RA with lung disease compared to the patients without lung disease: 96.6% versus 86.0%, respectively (p = 0.0256). In agreement with our hypothesis, the median level of anti-CCP2 was found to be strongly increased in the individuals with lung disease compared to those without lung disease: 2157 U (range 1207–3320 U) versus 176 U (range 57–1103 U), respectively (p < 0.0001; Table 1). This association was not restricted to a specific lung disease pattern and remained after exclusion of individuals having lung nodules (p = 0.0008; data not shown).

Similarly, both the frequency of RF positivity and levels of RF were increased in the RA group with lung disease (Table 1). Male gender was found to be associated with the co-occurrence of lung disease in our RA population: 44.1% of patients with lung disease were male compared to 24.4% of individuals with RA without lung disease (p = 0.0035; Table 1). No association was detected between levels of anti-CCP2 and tobacco smoking, presence of Sjögren's syndrome, methotrexate and anti-TNF-α therapy, and RA disease duration, regardless of the pattern of lung disease that was investigated.

Multivariate analysis. Multivariate analysis with stepwise logistic regression identified both male gender (OR 3.29, 95% CI 1.59–6.80, p = 0.0013) and increased level of anti-CCP2 (OR 1.49, 95% CI 1.25–1.78, for a 1000 U/ml increase of anti-CCP2; p < 0.0001) as independent risk factors for lung disease in our RA population (Table 2, Figure 1).

DISCUSSION

This cross-sectional study demonstrated that anti-CCP level and male gender are independent predictive factors of active lung disease in patients with RA. An advantage of our methodological approach is the avoidance of confounding factors such as the co-occurrence of Sjögren's syndrome, tobacco smoking, and methotrexate or anti-TNF-α therapy, which are well known risk factors for ILD[6]. We found that 23.4% of the RA patients had a symptomatic lung disease with an abnormal HRCT scan. This prevalence is in good agreement with the estimated frequency of RA-related lung disease[7,8]. Nonetheless, the "true" prevalence of lung disease during RA would not be identified, as most of the lung disease detected by a systematic HRCT scan is asymptomatic[9].

We identified male gender as an independent risk factor for RA-related lung disease, consistent with previous findings[9,10]. Regarding anti-CCP2 positivity and RA-related lung disease, the conflicting results could be explained by a

Table 1. Characteristics and comparison of RA subgroups with and without lung disease detected by high resolution computed tomography.

Variable	RA, n = 252	RA without Lung Disease, n = 193	RA with Lung Disease, n = 59	p*
Male, %	29.0	24.4	44.1	0.0035
Median disease duration, yrs (IQR)	8 (3–16)	8 (3–18.5)	9 (4–16)	0.93
Bone erosion, %	68.1	66.3	72.8	0.87
RF-positive, %	72.3	69.6	82.0	0.08
Median RF levels, UI/ml (IQR)	68 (0–238)	64 (0–221)	123 (32–402)	0.0476
Anti-CCP2-positive. %	88.5	86.0	96.6	0.0256
Median anti-CCP2 levels, U/ml (IQR)	231 (84–1978)	176 (57–1103)	2157 (1206–3320)	< 0.0001
Cigarette smoking, ever, %	33.5	33.9	33.2†	0.81
Median tobacco smoking, pack-yrs (IQR)	0 (0–2)	0 (0–2)	0 (0–15)	0.87
Sjögren's syndrome, %	25.0	23.8	28.8	0.44
Methotrexate use, %	60.0	62.2	54.2	0.27
TNF-α inhibitor use, %	31.0	30.1	33.9	0.58

*Chi-square test or Fisher exact test when appropriate for categorical variables; Wilcoxon rank-sum test for continuous variables. † Regarding the following lung disease patterns, nodules, bronchiectasis, and interstitial lung disease: 26.3%, 66.7%, and 43.7%, respectively, were smokers. Anti-CCP2: anti-cyclic citrullinated protein/peptide 2 antibodies: TNF-α: tumor necrosis factor-α; IQR: interquartile range.

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Table 2. Factors independently associated with the presence of pulmonary disease with RA in multivariate analyses. Multivariate logistic regression analysis adjusted for the presence of Sjögren's syndrome, tobacco smoking, use of methotrexate, and the disease duration.

Variable	p	OR (95% CI)
Anti-CCP2 levels (for a 1000 U/ml increase)	< 0.0001	1.49 (1.25–1.78)
Gender		
Female	—	1 (ref)
Male	0.0013	3.29 (1.59–6.80)
Tobacco smoking		
Never	—	1 (ref)
Ever	0.83	0.93 (0.43–1.96)
Sjögren's syndrome		
No	—	1 (ref)
Yes	0.71	1.16 (0.53–2.55)
Methotrexate intake		
No	—	1 (ref)
Yes	0.09	0.55 (0.27–1.11)
Disease duration (for a 1-year duration)	0.68	1.01 (0.97–1.05)

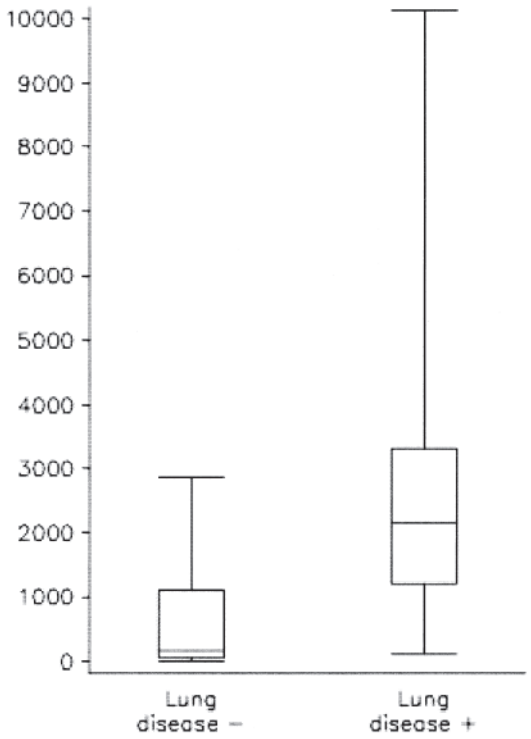

Figure 1. The intensity of anti-CCP2 production is associated with the co-occurrence of lung disease with RA. A comparison of anti-CCP2 levels (U/ml) between RA patients with lung disease (n = 59) and without lung disease (n = 193). Anti-CCP2 production was independently associated with the co-occurrence of lung disease: OR = 1.49 for a 1000 U/ml increase (95% CI 1.25–1.78); p < 0.0001.

restricted qualitative analysis[11,12], whereas our study focused on quantitative analysis, and it corroborates a previously reported correlation between high serum anti-CCP2 and lung disease[13]. These findings suggest that mucosal surfaces such as the lung may be involved in triggering anti-citrulline immunity. Interestingly, local ACPA production has been observed in inductible bronchus-associated lymphoid tissue (iBALT) of RA patients[14]; and exposure to cigarette smoke was previously linked to induction of iBALT[15], supporting the link between ACPA production and RA-related lung disease.

Identification of markers of RA-related lung disease could be relevant. Indeed, the contribution of ILD to mortality in patients with RA was found to be similar to that of cardiovascular disease[9].

Our study identified both an increase of anti-CCP2 production and male gender as independent risk factors for co-occurrence of lung disease in RA. A prospective study focusing on ILD with systematic chest HCRT scans would be required to assess the link between RA-related ILD and the production of ACPA.

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