Study of the relationship between anti-citrullinated protein antibodies and occurrence of interstitial lung disease in patients with rheumatoid arthritis

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Introduction
Rheumatoid arthritis (RA) is a systemic inflammatory disorder that most commonly affects the joints. Interstitial lung disease (ILD) is the most common pulmonary manifestation of RA-associated ILD. Patients with RA typically have circulating auto-antibodies, the most common being rheumatoid factor and anti-cyclic citrullinated protein antibodies (ACPA).
Aim
To determine the occurrence of ILD in patients with RA and its relation to anti-citrullinated protein antibodies.
Patients and methods
The study was conducted on 40 patients diagnosed according to ACR/EULAR 2010 criteria for diagnosis of RA. They were divided into two groups according to ACPA positivity: group I included 20 patients with RA who were ACPA positive, and group II included 20 patients with RA who were ACPA negative.
Exclusion criteria
Patients with interstitial pneumonia, asbestos exposure, other connective tissue diseases such as systemic lupus erythematosus and systemic sclerosis, autoimmune hepatitis, and hepatitis C virus infection were excluded.
Patients and methods
The participates underwent thorough medical history taking, full clinical examination, disease activity score-28 based on C-reactive protein and functional assessment questionnaire (Health Assessment Questionnaire) score, complete blood count, SGPT, SPOT, urea, creatinine, erythrocyte sedimentation rate first hour and rheumatoid factor, ACPA titer, high-resolution computed tomography of the chest, radiological examination for both hands and feet, BMI, ECHO, pulmonary function tests, and assessment of pulmonary artery pressure. An informed consent was taken from all patients in the study.
Results
ACPA-positive patients with RA are accompanied with a statistically significant restrictive pattern of pulmonary function tests. Positive high-resolution computed tomography findings indicate RA-ILD.
Conclusions
In RA, high titer of ACPA may be related to the development of ILD.

Keywords:
anti-cyclic citrullinated protein antibodies, interstitial lung fibrosis, rheumatoid arthritis, rheumatoid factor

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disorder that most commonly affects the joints; causes progressive, symmetric, and erosive destruction of cartilage and bone; and is usually associated with auto-antibody production [1,2]. Although RA is more common among females, rheumatoid arthritis-associated interstitial lung disease (RA-ILD) occurs more frequently in males, with a male to female ratio as high as 2 : 1 in some studies [3]. Onset of lung disease typically occurs in the fifth to sixth decade of life. The incidence of RA-ILD may increases as newer agents allow better disease control and consequently, increased life expectancy [4]. Age has consistently been shown to be a risk factor for the development of ILD, and another major risk factor is the history of smoking [5]. However, high levels of rheumatoid factor (RF) are a known risk factor for extra-articular manifestations of RA-ILD [5]. The exact mechanism for this has not been

elucidated, but the formation of circulating immune complexes may be involved [6]. Although joint disease is the main presentation, there are a number of extra-articular manifestations, including subcutaneous nodule formation, vasculitis, and inflammatory eye disease [7]. Of these manifestations, lung involvement is a major contributor to morbidity and mortality. Respiratory symptoms may precede articular symptoms in 10–20% of cases; however, they may be masked by poor functional status from joint disease or owing to the presence of chronic inflammation [8]. Most respiratory manifestations occur within first 5 years from disease onset [8]. There are a variety of pulmonary manifestations of RA, including pulmonary parenchymal disease (ILD), and inflammation of the pleura (pleural thickening and effusions), airways, and pulmonary vasculature (vasculitis and pulmonary hypertension) [9]. Patients with RA may present with manifestation of airway obstruction (bronchiolitis obliterans). Anti-cyclic citrullinated peptide (anti-CCP) antibodies have also been associated with the development of airway disease. In patients with RA and in the absence of clinical suspicion for infection or any respiratory complications, high-resolution computed tomography (HRCT) may be used to make a diagnosis of interstitial pneumonia. Airway disease in RA may reflect chronic immune activation, increased susceptibility to infection often is related to immune modulatory medications or direct toxicity from disease modifying anti - rheumatic drugs or biological therapy [10,11]. Patients with RA typically have circulating auto-antibodies, the most common being RF and anti-CCP [12]. These antibodies may be present in the serum for several years before clinical disease onset [13,14]. The presence of both RF and anti-CCP has been linked to the development of ILD, particularly when present in high titers [2,15–19]. There is growing evidence that RA begins in the lungs, a theory supported by a subgroup of patients who are anti-CCP positive with lung disease but with no articular manifestations [20,21]. Most patients with RA-ILD will have a restrictive pattern on pulmonary function tests, with or without decreased diffusing capacity of the lung for carbon monoxide and hypoxemia [5]. The impairment of both forced vital capacity and diffusing capacity of the lung for carbon monoxide is associated with poorer prognosis [7]. A variety of patterns are seen on HRCT in RA, with the most common being usual interstitial pneumonia [22,23]. Lung involvement in RA differs from other connective tissue disorders, in which a nonspecific interstitial pneumonia pattern is most frequently seen [24,25]. In the usual interstitial pneumonia, HRCT scan shows subpleural, basal-predominant, reticular abnormalities with honey-combing and traction bronchiectasis but with a relative absence of ground-glass opacities [26].

The nonspecific interstitial pneumonia is characterized by basal predominant ground-glass opacification and absence of honey-combing. Additional patterns less commonly seen in RA include organizing pneumonia, diffuse alveolar damage, lymphocytic interstitial pneumonia, and desquamative interstitial pneumonia [27,28]. Combined pulmonary fibrosis and emphysema has also been demonstrated on HRCT scan in patients with RA [29]. Patients typically have centrilobular or paraseptal emphysema in conjunction with lower lobe fibrosis, which is associated with an increased risk of pulmonary hypertension [30]. Pattern of reactive lymphoid tissue known as inducible bronchus-associated lymphoid tissue has been found in patients with RA-ILD and is associated with local production of inflammatory cytokines and elevated titer of anti-CCP antibodies [22]. Patients with high BMI have been shown in large studies to have higher disease activity, to be less likely to respond to treatment, and to have more extensive disability [23].

Methotrexate is the most common first-line agent used to treat RA and prevents joint destruction [31]. Chronic progressive pulmonary fibrosis has been described in the setting of methotrexate treatment. In addition, it is known to provoke rheumatoid nodule formation, and this may reflects an increase in the diagnosis of methotrexate-induced lung injury. The presence of poorly formed non-necrotizing granulomas and scattered eosinophils may suggest atypical finding of methotrexate-induced hypersensitivity pneumonitis [32,33].

Aim

The aim of the work was to determine the occurrence of ILD in patients with RA and its relation to anti-citrullinated protein antibodies.

Patients and methods

The study was conducted on 40 patients diagnosed according to ACR/EULAR 2010 criteria for diagnosis of RA [34]. An informed consent was taken from all participants. Patients were recruited from Alexandria Main University Hospital and were divided into two groups according to anti-cyclic citrullinated protein antibodies (ACPA) positively. Group I included 20 patients with RA who were ACPA positive. Group II included 20 patients with RA who were ACPA negative.

Inclusion criteria

Patients presenting with RA having at least 5-year disease duration were included.

Exclusion criteria

Newly diagnosed patients with RA, other causes of ILD such as interstitial pneumonia, asbestos exposure, other autoimmune connective tissue diseases known to affect the level of ACPA like systemic lupus erythematosus, systemic sclerosis, autoimmune hepatitis, and hepatitis C virus infection were excluded.

Methods

All patients were subjected to the following:

(1) Thorough medical history taking with specific stress on age, sex, disease duration, duration of mourning stiffness, number of swollen and tender joints, history of smoking, symptoms related to pulmonary system involvement, manifestations of extra-articular involvement such as rheumatoid nodules, iritis, mouth ulcers, and drug history (methotrexate) [35].
(2) Full clinical examination with specific stress on examination of musculoskeletal system.
(3) Determination of disease activity using disease activity score-28 (DAS-28) based on C-reactive protein (CRP) [35] and functional assessment using Health Assessment Questionnaire score [36].
(4) General examination for detection of extra-articular manifestations associated with RA, such as skin (rheumatoid nodules), nervous system (to exclude carpal tunnel syndrome), and cardiac examination for detection of any cardiac abnormalities.
(5) Routine laboratory investigations: complete blood count with differential white blood cell count [37], liver function tests including SGPT (AST) and SPOT (ALT) [38], kidney function tests such as blood urea and serum creatinine [39], erythrocyte sedimentation rate (mm/1st h) [40], and CRP (mg/dl) [41].
(6) Immunological profile: RF titer [42] and ACPA titer [43].
(7) HRCT scan of the chest without contrast [44], radiological examination for both hands and feet [45], and measurement of the BMI.
(8) ECHO cardiography and Doppler assessment of pulmonary artery pressure [46].
(9) Pulmonary function tests (Spirometry) [47].

Statistical analysis

Data are shown as the mean±SEM. All statistical analyses were performed using SPSS statistical software version 18 (SPSS; SPSS Inc., Chicago, Illinois, USA). Data were analyzed between two groups using Student *t* test, whereas among more than two groups, data were analyzed by the one-way analysis of variance method. Differences of *P* value less than 0.05 were considered as statistically significant.

Results

Table 1 comparison between the two studied groups according to Demographic data, Smoking index (Pack/year), Disease duration (years), CBC, Renal Function Tests, and Body Mass Index (BMI). Table 2 comparison between the two studied groups according to Drug History. Table 3 comparison between the two studied groups according to Extra-articular manifestations, DAS-28 Score and HAQ Score. Table 4 comparison between the two studied groups according to the level of ESR (mm/hr), CRP (mg/dl) and Rheumatoid Factor titre. Table 5 comparison between the two studied groups according to pulmonary involvement manifestations, ECHO findings and HRCT of the chest; ACPA positive rheumatoid arthritis patients is accompanied with a statistically significant restrictive pattern of pulmonary function tests, positive HRCT findings indicate rheumatoid arthritis associated lung disease (RA-ILD).

Discussion

Higher levels of anti-CCP antibodies have been found in patients with RA-ILD compared with patients with RA only, but the role of such antibodies in the pathogenesis of RA-ILD is not clear. As in the other ILDs, the inflammatory response activates cytokines, chemokines, and growth factors, such as tumor necrosis factor-alpha, vascular endothelial growth factor, platelet-derived growth factor, and interleukins. These contribute to a differentiation and proliferation of fibroblasts, increased synthesis and deposition of extracellular matrix, and increased activity of matrix metalloproteinases resulting in ILD.

Our study demonstrated that ACPA-positive patients with RA are accompanied with radiograph findings in hands and feet, which are statistically significant from ACPA-negative patients (P=0.026). A statistically significant restrictive pattern of pulmonary function tests and HRCT changes in ACPA-positive patients indicate RA-ILD.

The age ranges are in agreement with Gamal *et al*. [48] who reported that the age at disease onset presents a peak around the fourth decade of life.

Table 1 Comparison between the two studied groups according to demographic data, smoking index (pack/year), disease duration (years), complete blood count, renal function tests, and BMI

	Total (*N*=40)	ACPA titer		Test of significant	*P*
		Negative (*N*=20)	Positive (*N*=20)		
Sex [*n* (%)]					
Male	13 (32.5)	8 (40.0)	5 (25.0)		
Female	27 (67.5)	12 (60.0)	15 (75.0)	χ^2=1.026	0.311
Age (years)					
Minimum–maximum	37.0–65.0	41.0–65.0	37.0–64.0		
Mean±SD	53.28±7.41	54.65±6.55	51.90±8.11		
Median	53.0	55.0	50.0	*t*=1.180	0.245
Smoking index					
Minimum–maximum	0.0–33.0	0.0–30.0	0.0–33.0		
Mean±SD	5.12±956	6.21±10.37	4.03±8.80		
Median	0.0	0.0	0.0	MW=0.717	0.473
Disease duration					
Minimum–maximum	6.0–25.0	6.0–21.0	6.0–25.0		
Mean±SD	12.40±4.49	12.65±4.27	12.15±4.80		
Median	12.0	12.5	11.0	MW=0.583	0.560
Hb (g/dl)					
Minimum–maximum	9.50–14.0	9.90–14.9	9.50–13.80		
Mean±SD	11.33±1.07	11.61±0.93	11.05±1.14		
Median	11.20	11.45	11.0	*t*=1.700	0.097
WBC total ($\times10^6$)					
Minimum–maximum	4.70–17.0	4.70–12.0	5.0–17.0		
Mean±SD	9.08±2.83	8.71±2.57	9.46±3.09		
Median	9.0	8.95	9.75	*t*=0.840	0.406
Platelets ($\times10^3$)					
Minimum–maximum	150.0–430.0	150.0–430.0	154.0–400.0		
Mean±SD	250.18±70.97	251.45±72.15	248.90±71.61		
Median	239.50	255.0	233.50	*t*=0.112	0.911
Blood urea					
Minimum–maximum	15.0–69.0	21.0–66.0	15.0–69.0		
Mean±SD	45.15±11.90	39.65±11.08	40.65±12.93		
Median	41.0	39.0	42.0	*t*=0.263	0.794
Creatinine					
Minimum–maximum	0.56–1.52	0.65–1.50	0.56–1.52		
Mean±SD	1.02±0.24	1.04±0.25	1.0±0.24		
Median	1.0	1.0	1.0	*t*=0.514	0.610
BMI (kg/m^2) [*n* (%)]					
<18.5	5 (12.5)	3 (15)	2 (10)	χ^2=1.236	^{FE}P=0.648
18.5 to <25	16 (40)	7 (35)	9 (45)		
25 to <30	11 (27.5)	5 (25)	6 (30)		
≥30	8 (20)	5 (25)	3 (15)		

χ^2, *P*, χ^2 and *P* values for c^2 test for comparing between the groups. ACPA, anti-cyclic citrullinated protein antibodies; FE, Fisher exact for c^2 test; Hb, hemoglobin; MW, Mann–Whitney test; WBC, white blood cell.

Table 2 Comparison between the two studied groups according to drug history

	Total (*N*=40)	ACPA titer		χ^2	^{Mc}P
		Negative (*N*=20)	Positive (*N*=20)		
Drug history [*n* (%)]					
MTX	13 (32.5)	8 (40.0)	5 (25.0)		
MTX then Avara	11 (27.5)	7 (35.0)	4 (20.0)		
MTX then biologics	4 (10.0)	1 (5.0)	3 (15.0)		
Avara	6 (15.0)	2 (10.0)	4 (20.0)		
Avara then biologics	3 (7.5)	0	3 (15.0)		
MTX then Avara then biologics	3 (7.5)	2 (10.0)	1 (5.0)	6.087	0.307

χ^2, *P*, χ^2 and *P* values for c^2 test for comparing between the groups. ACPA, anti-cyclic citrullinated protein antibodies; MC, Monte–Carlo for c^2 test; MTX, methotrexate.

Table 3 Comparison between the two studied groups according to extra-articular manifestations, disease activity score-28 score, and Health Assessment Questionnaire score

	Total (*N*=40) [*n* (%)]	ACPA titer [*n* (%)]		χ^2	^{Mc}P
		Negative (*N*=20)	Positive (*N*=20)		
Extra-articular manifestations					
No	24 (60.0)	16 (80.0)	8 (40.0)		
Rheumatoid nodules	3 (7.5)	2 (10.0)	1 (5.0)		
Interstitial lung disease	11 (27.5)	2 (10.0)	9 (45.0)		
Carpal tunnel syndrome	2 (5.0)	0	2 (10.0)		
Eye manifestations	0	0	0		
Vasculitis	0	0	0		
Atlanto-axial subluxation	0	0	0	9.037*	0.010*
DAS-28 score					
Minimum–maximum	2.10–5.50	2.10–5.50	2.50–5.50		
Mean±SD	3.51±0.85	3.44±0.87	3.59±0.84	*t*=0.563	0.576
Median	3.30	3.30	3.50		
HAQ score					
Minimum–maximum	0.30–2.40	0.55–2.20	0.30–2.40		
Mean±SD	1.37±0.54	1.34±0.50	1.39±0.60	*t*=0.302	0.765
Median	1.33	1.20	1.43		

χ^2, *P*, χ^2 and *P* values for c^2 test for comparing between the groups. ACPA, anti-cyclic citrullinated protein antibodies; DAS-28, disease activity score-28; HAQ, Health Assessment Questionnaire; MC, Monte–Carlo for c^2 test. *Statistically significant at *P* value less than or equal to 0.05.

Table 4 Comparison between the two studied groups according to the level of erythrocyte sedimentation rate (mm/h), C-reactive protein (mg/dl), and rheumatoid factor titer

	Total (*N*=40)	ACPA titer		*t*	*P*
		Negative (*N*=20)	Positive (*N*=20)		
ESR 1st h (mm/h)					
Minimum–maximum	3.0–45.0	5.0–41.0	3.0–45.0		
Mean±SD	18.13±10.58	16.65±9.75	19.60±11.42	0/879	0/385
Median	17.0	14.0	19.0		
ESR 2nd h (mm/h)					
Minimum–maximum	9.0–90.0	9.0–55.0	12.0–90.0		
Mean±SD	32.90±16.90	30.10±12.98	35.70±20.03	1.049	0.301
Median	29.50	25,0	34.0		
CRP (mg/dl)					
Minimum–maximum	2.0–26.0	2.0–22.0	2.0–26.0		
Mean±SD	9.80±6.41	8.80±5.84	10.80±6.95	MW=0.800	0.423
Median	9.0	6.0	9.50		
Rheumatoid factor titer					
Minimum–maximum	5.0–20.0	7.0–18.0	6.0–20.0		
Mean±SD	10.43±3.65	10.35±3.05	10.50±4.25	0.128	0.899
Median	9.0	9.50	9.0		

t, *P*, *t* and *P* values for Student *t* test for comparison between the two groups. ACPA, anti-cyclic citrullinated protein antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MW, Mann–Whitney test.

Eltokhy *et al*. [49] and Salinnas *et al*. [50] showed an increased incidence of extra-articular manifestations among ACPA-positive patients with rheumatoid arthritis.

In disagreement with our study, Lopez *et al*. [51] and Porto *et al*. [52] found no increased incidence of occurrence of extra-articular disease in relation to ACPA-positive patients with RA.

Colglazier and Sutej [53] found that there were a significant difference in erythrocyte sedimentation rate level and Health Assessment Questionnaire score in patients with RA positive for RF and ACPA, than those with positive RF only. The previous studies showed conflicting results regarding DAS-28-CRP in ACPA positive and negative patients. Although these results are not in agreement with the studies carried out by Shakiba *et al*. [54], these results are in agreement with the

Table 5 Comparison between the two studied groups according to pulmonary involvement manifestations, ECHO findings, and high-resolution computed tomography of the chest findings

	Total (*N*=40) [*n* (%)]	ACPA titer [*n* (%)]		χ^2	^{Mc}P
		Negative (*N*=20)	Positive (*N*=20)		
Pulmonary manifestations					
No	22 (55.0)	9 (45.0)	13 (65.0)		
Dyspnea	7 (17.5)	4 (20.0)	3 (15.0)		
Cough	5 (12.5)	3 (15.0)	2 (10.0)		
Cough and dyspnea	6 (15.0)	4 (20.0)	2 (10.0)		
Cyanosis	0	0	0		
Crepitation	0	0	0		
Finger clubbing	0	0	0	1.828	0.669
ECHO findings					
Normal	37 (92.5)	19 (95.0)	18 (90.0)		
Mild MR	3 (7.5)	1 (5.0)	2 (10.0)		
PHT	0	0	0	0.360	^{FE}P=1.000
HRCT findings					
HRCT +ve ILD	15 (37.5)	6 (30.0)	9 (45.5)		
HRCT –ve ILD	25 (62.5)	14 (70.0)	11 (55.0)	1.784	^{FE}P=0.013

χ^2, *P*, χ^2 and *P* values for c^2 test for comparing between the groups. ACPA, anti-cyclic citrullinated protein antibodies; FE, Fisher exact for c^2 test; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; MC, Monte–Carlo for c^2 test; MR, mitral regurgitation; PHT, pulmonary hypertension.

studies carried out by Porto *et al*. [52] and Sendaroflu *et al*. [55].

In disagreement with our study, Arnab *et al*. [56] showed that CRP titer is significantly higher among ACPA-positive patients with RA than ACPA negative patients with RA.

Inui *et al*. [57] found that there was no significant difference in ACPA positivity or negativity among the patients with RA regarding the occurrence of ILD, reported from HRCT scan of the chest.

The study by Mori *et al*. [58] was done for comparison of pulmonary abnormalities on HRCT in long-standing patients with RA and found that the associated interstitial lung abnormalities were frequently observed in RA even in the absence of respiratory symptoms. They explain that the HRCT findings do not necessarily represent clinically manifest lung disease but could be an indirect sign of a subclinical inflammatory process. Imaging findings are relatively non- specific, with diffuse pulmonary opacities or patchy consolidation seen on chest radiographs and HRCT. Bronchoalveolar lavage and lung biopsy are more helpful in ruling out alternative causes of lung involvement such as infection than establishing the diagnosis of methotrexate-induced lung injury. Although the presence of poorly formed non-necrotizing granulomas and scattered eosinophils may suggest methotrexate-induced hypersensitivity pneumonitis, these are not typical findings in RA-ILD. Bergstrom *et al*. [59] did not observe any differences in lung function test results between the patients with RA who are ACPA positive or those who are ACPA negative and stated that pulmonary dysfunction was demonstrated to have no value in the prediction of ILD in patients with RA. Wilsher *et al*. [60] demonstrated a modest correlation between pulmonary function test results and HRCT findings in patients with RA-ILD. The present study demonstrated the presence of parenchymal changes representing ILD in 45% of ACPA-positive patients with RA and in 30.0% of ACPA-negative patients with rheumatoid arthritis (which was statistically significant), with no significant pulmonary complaints and with normal pulse oximetry. Patients with a high BMI may have a reduced risk of severe joint damage. The underlying pathways could be related to differences in adipokine production, or other metabolic or hormonal factors, and should be furthered studied. The worse clinical symptoms observed in obese patients with RA may be owing to other mechanisms, including nonspecific pain, comorbidities, and immobility [61].

Methotrexate is the most common first-line agent used to treat RA that prevents joint destruction. In 1983, many cases of acute and subacute hypersensitivity pneumonitis have been reported. Higher dose of methotrexate is more likely to be associated with pulmonary toxicity [62]. This typically occurs within the first year of treatment and is felt to represent a hypersensitivity reaction. Symptoms include dyspnea and nonproductive cough with or without systemic symptoms.

There was no significant difference in the ACPA positivity or negativity between the patients receiving methotrexate treatment and those without methotrexate treatment. Thus, methotrexate treatment may not affect the levels of serum ACPA in patients with RA.

Conclusions

Pulmonary involvement is a common and important complication in patients with rheumatoid arthritis; the most common pulmonary complication is ILD. Presence of anti-CCP antibodies positivity is a good biomarker for the development of ILD, and all patients with RA especially those with high titer of anti-CCP antibodies should be routinely evaluated with parenchymal HRCT to assess their pulmonary status.

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Conflicts of interest

There are no conflicts of interest.

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