A Metaanalysis of the Increased Risk of Rheumatoid Arthritis-related Pulmonary Disease as a Result of Serum Anticitrullinated Protein Antibody Positivity

Junqing Zhu, Yi Zhou, Xiaoguang Chen, and Juan Li

ABSTRACT. ***Objective.*** An inconsistent association has been reported between the serum anticitrullinated protein antibodies (ACPA) level and rheumatoid arthritis (RA)-related pulmonary disease risk. We conducted a metaanalysis to reveal the association between them.

Methods. An electronic search was performed in PubMed, ScienceDirect, and SpringerLink databases for studies published up to August 2013. The distributions of the serum ACPA level in cases and controls were obtained from eligible studies. The risk of RA-related pulmonary disease associated with serum ACPA positivity was estimated by OR and 95% CI. According to the heterogeneity results, a fixed-effects model or a random-effects model was used to calculate the pooled OR. Publication bias and sensitivity analyses were conducted.

Results. Overall, 243 patients with RA-related pulmonary disease and 1442 RA controls were included in the metaanalysis. The results showed that the pooled OR was 2.621 (95% CI, 1.561–4.403, $p < 0.001$) for the increased risk of RA-related pulmonary disease due to the serum ACPA positivity. In the white population subgroup, an increased OR was 3.453 (95% CI 1.798–6.630, $p < 0.001$), whereas no association was found in the Asian population subgroup. Additionally, we further revealed that serum ACPA positivity indicated a higher risk for interstitial lung disease (ILD) and interstitial pulmonary fibrosis (IPF) among patients with RA (OR 4.679, 95% CI 2.071–10.572, $p < 0.001$). The heterogeneity, publication bias, and sensitivity analyses had no statistical significance in any group.

Conclusion. To our knowledge, this is the first metaanalysis to reveal that serum ACPA positivity is highly associated with the risk of RA-related pulmonary disease, particularly in RA-related ILD and IPF. (First Release June 1 2014; J Rheumatol 2014;41:1282–9; doi:10.3899/jrheum.131341)

Key Indexing Terms:
RHEUMATOID ARTHRITIS RHEUMATOID ARTHRITIS-RELATED PULMONARY DISEASE
INTERSTITIAL LUNG DISEASE INTERSTITIAL PULMONARY FIBROSIS
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Rheumatoid arthritis (RA) is a systemic, inflammatory, autoimmune disease characterized primarily by a persistent synovitis of multiple joints as well as cartilage and bone destruction. It affects about 0.5%–1% of the world population[1]. Notably, extraarticular manifestations (EAM) are common in patients with RA and include vascular, pulmonary, cardiovascular, renal, ophthalmic, skeletal, and nervous disorders[2]. The 10-year and 15-year cumulative incidence of EAM can reach 40.6% and 53%, respectively, in patients with established RA[3,4]. Among these, pulmonary involvement affects 5%–10% of patients with RA and is associated with increased morbidity and early mortality[5]. As a heterogeneous clinical presentation, the common pulmonary involvement in patients with RA includes interstitial lung disease (ILD), interstitial pulmonary fibrosis (IPF), airway disease (AD), pleural disease, nodular lung disease, bronchiolitis obliterans, and arteritis[6]. Although the exact cause and pathogenesis of RA have yet to be identified, the relationship between the onset of RA and its extraarticular involvement is close, and is driven by a combination of several genes and environmental triggers. Smoking, infec-

tious agents, and other environmental stressors can result in the loss of tolerance to self-proteins in patients through posttranslational modification, which is affected by the role of susceptibility genes (e.g., HLA-DR4 alleles)[7]. In this process, antigen-driven T cell and B cell responses combined with the consequential production of rheumatoid factor (RF), anticitrullinated protein antibody (ACPA), and inflammatory cytokines play an important role in the development of RA[8].

ACPA have been heavily studied in patients with RA because they are a highly specific biomarker that is comparably sensitive to RF for RA diagnosis[9]. Accordingly, the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 RA classification criteria put forward the serum ACPA titer as one of the diagnostic items for RA[10]. Additionally, ACPA is a reliable marker for predicting severe joint damage and deformity in patients with early or established RA[11] and may be associated with increased risk for further EAM[2]. Data also suggest that tobacco exposure increases the risk of RA-related pulmonary disease through the interplay of smoking, the HLA-DRB1 "shared epitope," and ACPA[12,13]. However, the exact mechanism of ACPA in the development of RA-related pulmonary disease remains unclear. Although many studies have investigated the relationship between ACPA production and the risk for concurrent pulmonary disease in RA, an inconsistent association has been reported between the serum ACPA level and RA-related pulmonary disease risk[14,15]. Some studies have found that serum ACPA positivity is associated with pulmonary disease in patients with RA[12,15], whereas others have failed to confirm those results[14,16]. Therefore, it is essential to collect these inconsistent studies and systematically analyze whether serum ACPA positivity increases the risk of pulmonary disease in patients with RA.

MATERIALS AND METHODS

Search strategy. We performed an electronic search of PubMed, ScienceDirect, and SpringerLink databases for studies published up to August 2013 without any limits. The search terms were the following: "citrullinated peptide," "antibodies to citrullinated protein antigens," "ACPA," "anticitrullinated peptide antibodies," "anti-cyclic citrullinated peptide antibodies," "anti-CCP," "anti-cyclic citrullinated peptide-2 autoantibodies," "CCP2," "rheumatoid arthritis," "RA," "lung disease," "pulmonary disease," and "extra-articular." References were checked to identify repeated studies.

Inclusion and exclusion criteria. To be included in the metaanalysis, eligible studies had to meet all the following inclusion criteria: (1) diagnosis of RA based on the ACR 1987 RA classification criteria or the ACR/EULAR 2010 RA classification criteria; (2) RA-related pulmonary disease, which included ILD, IPF, AD, pleural disease, nodular lung disease, bronchiolitis obliterans, and arteritis, diagnosed by clinical specialists according to the related diagnostic criteria or judged using high-resolution computed tomography (HRCT); (3) case-control or cohort studies; (4) studies with distributions of serum ACPA (positive and negative) in patients with RA-related pulmonary disease and RA controls; and (5) studies reporting basic participant characteristics, e.g., ethnicity.

The exclusion criteria were (1) mechanistic studies, review articles, guidelines, case reports, and metaanalyses; (2) patients with only pulmonary function abnormalities or other pulmonary diseases, including lung tuberculosis, lung infection, and lung cancer; (3) a lack of RA participants without EAM as controls; (4) duplicate data presented in multiple studies; and (5) where the necessary data could not be obtained.

Quality assessment. The methodological quality of the selected studies was independently evaluated by 2 authors (JQZ and YZ) using the Newcastle-Ottawa Scale (NOS) for case-control studies[17]. This scale assesses each study in 3 domains including selection of study groups, comparability of groups, and exposure ascertainment in groups. The maximum total score for a study is 9. It was decided that a score of 7–9 would indicate a "high-quality study", a score of 4–6 a "moderate-quality study", and a score of 1–3 a "low-quality study"[18]. The authors resolved any disagreements through discussion.

Data extraction. The following data were extracted from each article: author, publication year, country and primary race of the patients, type of RA-related pulmonary disease, sample size, other baseline characteristics in cases and controls [including sex, age, RA duration, Disease Activity Score in 28 joints (DAS28), C-reactive protein (CRP), RF positivity, smoking history, using methotrexate (MTX), using biologic agents, and using steroid], and the numbers of patients positive and negative for serum ACPA among cases and controls. Two authors (JQZ and YZ) selected eligible studies according to the inclusion and exclusion criteria, extracted the information given above independently, and resolved any discrepancies by discussion.

Statistical analysis. The risk of RA-related pulmonary disease as a result of serum ACPA positivity was estimated by the OR and 95% CI. According to variables thought to potentially influence the study outcomes (such as quality of studies, ethnicity, RA duration, or pulmonary disease type), subgroups based on 3 or more studies each were analyzed separately. When $p < 0.05$, the risk was considered statistically significant. The OR represented the magnitude of risk in the forest plots. An OR > 1 represented a risk factor and < 1 a protective factor. The between-study heterogeneity was evaluated by a chi-squared Q test and the I^2 statistic[19]. A p value < 0.10 or an I^2 value > 50% was considered statistically significant in the heterogeneity analysis. When the heterogeneity was not significant, a fixed-effects model was performed to calculate the pooled OR. Otherwise, a random-effects model was selected when between-study heterogeneity was identified. Based on "1-study removed" analyses, sensitivity analyses were performed to assess the stability of the final study outcome in each subgroup separately. Begg's test and Egger's test for funnel plot asymmetry were performed to check the publication bias[20]. When $p < 0.10$, the publication bias was considered statistically significant. All p values were 2-sided. Data analyses were conducted using Stata software version 11.0 (Stata Corporation).

RESULTS

Characteristic analysis and quality assessment of the included studies. According to the search strategy as well as the inclusion and exclusion criteria, 7 reports examining the association between serum ACPA positivity and the risk of RA-related pulmonary disease were included in our analysis (Figure 1)[12,14,15,16,21,22,23]. Table 1A-B presents the details for each study, including the author, publication year, country and primary race of the patients, types of RA-related pulmonary disease, sample size, other baseline characteristics in cases and controls, the numbers of patients positive and negative for serum ACPA among cases and controls, and the NOS score.

All studies were case-control designed and used the ACR

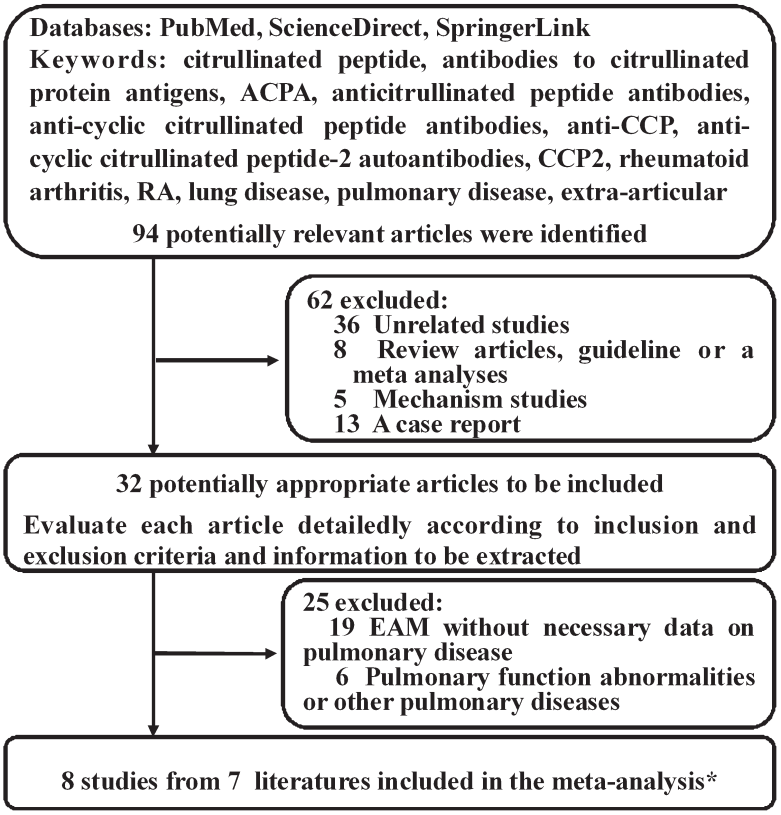

Figure 1. Flow diagram of the selection and nature of the studies. *One study included detailed data on the relationships between serum anticitrullinated protein antibody (ACPA) positivity and the risks of interstitial lung disease and airway disease separately. Anti-CCP: anticyclic citrullinated peptide antibody; RA: rheumatoid arthritis; EAM: extraarticular manifestations.

1987 RA classification criteria as the diagnostic criteria for RA. The studies were published from 2008 to 2013. Overall, 243 patients with RA-related pulmonary disease and 1442 patients with RA as controls were entered to analyze whether the risk of RA-related pulmonary disease is increased by serum ACPA positivity. The most common RA-related pulmonary diseases were ILD and IPF[12,21,23]. Only 1 study reported detailed data on the relationships between serum ACPA positivity and the risks of ILD and AD separately[21]; the other studies reported a single type or mixed types of RA-related pulmonary diseases. Thus, considering the 1 study of ILD and AD as 2 separate studies, we included a total of 8 studies in the metaanalysis. Of the included studies, 4 were conducted in Asian populations[14,16,21] and the other 4 were conducted in European populations[12,15,22,23]. All the included studies were high-quality or moderate-quality studies, based on the NOS assessment (Table 1A).

As for other baseline characteristics in cases and controls, statistically significant differences were found between cases and controls in some studies, particularly regarding sex[15,21], age[21], RA duration[21,22], RF positivity[12,21], smoking history[12,14,21], use of biologic agents[12], and use of steroids[12]. But the other studies did not report such differences for those factors. Those factors may potentially affect the risk of RA-related pulmonary disease, but not all subgroup analyses were performed according to these variables because of the limitations of the original studies. No statistically significant differences in DAS28, CRP, and using MTX were found between cases and controls in the studies described. All those confounding factors are described in Table 1B.

Quantitative synthesis and heterogeneity analysis. We pooled all 8 studies to evaluate whether serum ACPA positivity increased the risk of RA-related pulmonary disease. The pooled OR was 2.621 (95% CI 1.561–4.403, $p < 0.001$) with the fixed-effects model ($Q = 9.62$, $p = 0.211$, $I^2 = 27.2\%$), indicating that the heterogeneity was not significant (Figure 2A). Further, we performed subgroup analyses according to quality of studies, ethnicity, RA duration, and type of RA-related pulmonary disease, because those factors could have affected the metaanalysis results.

We performed subgroup analysis according to quality of

Table 1A. Demographic characteristics of the individual studies for RA related pulmonary disease included in the metaanalysis.

First Author	Year	Country/ Primary Race	RA-PD	Cases/ Controls, n	Male/ Female, n[&]	Age, Yrs, Mean ± SD or (range)[&]	RA Duration, Yrs, Mean ± SD or (range)[&]	NOS Score	ACPA RA-PD P	ACPA RA-PD N	ACPA RA P	ACPA RA N
Giles	2013	USA/W	ILD	57/120	28/29–43/77	61 ± 9/58 ± 8	9 (5–19)/8 (4–16)	7	51	6	82	38
Mori-ILD*	2012	Japan/A	ILD	24/302	12/12–70/232[#]	73 (64–76)/59 (52–68)[#]	1.5 (0–6.3)/0 (0–6)	7	24	0	267	35
Mori-AD*	2012	Japan/A	AD	30/302	3/27–70/232	65 (56–71)/59 (52–68)	7.5 (4–12)/0 (0–6)[#]	7	27	3	267	35
Jearn	2012	Korea/A	IPF, BC, and RN	31/345	NA	NA	NA	6	30	1	333	12
Aubart	2011	France/W	ILD, BC, and RN	59/193	26/33–47/146[#]	NA	9 (4–16)/8 (3–18.5)	6	57	2	166	27
Skare	2011	Brazil/W	RPPL	27/44	23/4–40/4	49 ± 9.6/45 ± 11.6	13.9 ± 6.7/10.6 ± 6.9[#]	6	10	3	17	2
Inui	2008	Japan/A	PD	18/36	10/8–11/25	58 (33–78)/57 (25–77)	NA	6	16	2	32	4
Alexiou	2008	Greece/W	IPF	11/125	NA	NA	NA	6	10	1	73	52

Table 1B. Clinical characteristics from the individual studies for RA related pulmonary disease included in the metaanalysis.

First Author	Year	DAS28, Mean (range)[&]	CRP, mg/l, Mean (range)[&]	RF Positivity, n (%)[&]	Smoking History, n (%)[&]	Using MTX, n (%)[&]	Using Biologic Agents, n (%)[&]	Using Steroids, n (%)[&]
Giles	2013	3.8 (3.2–4.4)/ 3.5 (2.8–4.3)	3.5 (1.2–8.4)/ 2.3 (1.0–7.1)	43 (75.0)/ 71 (59.0)[#]	43 (75.0)/ 63 (53.0)[#]	33 (58.0)/ 81 (68.0)	33 (58.0)/ 49 (41.0)[#]	29 (51.0)/ 38 (32.0)[#]
Mori-ILD*	2012	NA	NA	23 (95.8)/225 (74.5)[#]	11 (45.8)/62 (20.5)[#]	3 (12.5)/37 (12.3)	0 (0)/1 (0.3)	NA
Mori-AD*	2012	NA	NA	26 (86.7)/225 (74.5)	3 (10.0)/62 (20.5)	4 (13.3)/37 (12.3)	0 (0)/1 (0.3)	NA
Jearn	2012	NA	NA	NA	NA	NA	NA	NA
Aubart	2011	NA	NA	48 (82.0)/134 (69.6)	20 (33.2)/65 (33.9)	32 (54.2)/120 (62.2)	20 (33.9)/58 (30.1)	NA
Skare	2011	NA	NA	19 (76.0)/31 (70.4)	9 (37.5)/16 (36.3)	14 (58.3)/31 (72)	4 (14.8)/3 (6.8)	NA
Inui	2008	NA	22 (13–32)/ 22 (7–43)	NA	12 (66.7)/11 (30.6)[#]	NA	NA	NA
Alexiou	2008	NA	NA	NA	NA	NA	NA	NA

* One study contained detailed data regarding the relationships between serum ACPA positivity and the risks of ILD and AD separately. [&]Comparison of cases and controls. [#] $p < 0.05$. ILD: interstitial lung disease; AD: airway disease; IPF: interstitial pulmonary fibrosis; BC: bronchiectasis; RN: rheumatoid nodules; RPPL: reticular pattern pulmonary lesions; PD: pulmonary disease; RA: rheumatoid arthritis; W: white; A: Asian; ACPA: anticitrullinated protein antibody; P: positive; N: negative; NOS: Newcastle-Ottawa Scale; DAS28: 28-joint Disease Activity Score; CRP: C-reactive protein; RF: rheumatoid factor; MTX: methotrexate; NA: not available.

studies. The pooled OR was 2.937 (95% CI, 1.429–6.035, p = 0.003) using the fixed-effects model (Q = 2.75, p = 0.252, $I^2 = 27.4\%$) in subgroups with 3 high-quality studies. The pooled OR was 2.301 (95% CI 1.088–4.865, p = 0.029) by using the fixed-effects model (Q = 6.49, p = 0.165, $I^2 = 38.4\%$) in subgroups with 5 moderate-quality studies.

In the white population subgroup, which was examined by 4 studies, an OR of 3.453 was revealed (95% CI 1.798–6.630, $p < 0.001$) by the fixed-effects model (Q = 5.46, p = 0.141, $I^2 = 45.1\%$), which was increased compared with the 8 pooled studies (Figure 2B). However, our metaanalysis did not identify a significant association between serum ACPA positivity and the risk of RA-related pulmonary disease in the Asian population subgroup, which was examined by 4 studies. The pooled OR was 1.562 (95% CI 0.666–3.662, p = 0.305) in the fixed-effects model (Q = 1.53, p = 0.674, $I^2 = 0.0\%$; Figure 2C).

Additionally, we performed subgroup analysis according to RA duration. A total of 5 studies have reported the data of RA duration in such metaanalysis. We pooled all those studies to evaluate whether serum ACPA positivity increased the risk of RA-related pulmonary disease. The pooled OR was 2.723 (95% CI, 1.510–4.910, p = 0.001) with the fixed-effects model (Q = 7.00, p = 0.136, $I^2 = 42.9\%$). After removing 2 studies that had significant statistical difference between RA-related pulmonary disease and RA controls in RA duration, the pooled OR of the other 3 studies increased to 4.375 (95% CI 2.044–9.365, $p < 0.001$) with the fixed-effects model (Q = 0.13, p = 0.937, $I^2 = 0.0\%$). The results of the heterogeneity analysis showed no statistical significance in either subgroup above.

Serum ACPA positivity increased the risk of ILD and IPF in patients with RA. Additionally, according to the clinical similarity of pathophysiological processes, we pooled studies examining the relationships between serum ACPA positivity and the risks of ILD, IPF, and reticular pattern pulmonary lesions on HRCT. The pooled OR was 3.394 (95% CI 1.675–6.879, p = 0.001) for this subgroup, which was examined by 4 studies, in the fixed-effects model (Q = 5.48, p = 0.140, $I^2 = 45.3\%$; Figure 2D), but it was 2.621 for all 8 studies. Although the heterogeneity analysis demonstrated no statistical significance, the I^2 value reached 45.3%. Therefore, we recalculated the OR and 95% CI after excluding 1 study that examined the association between

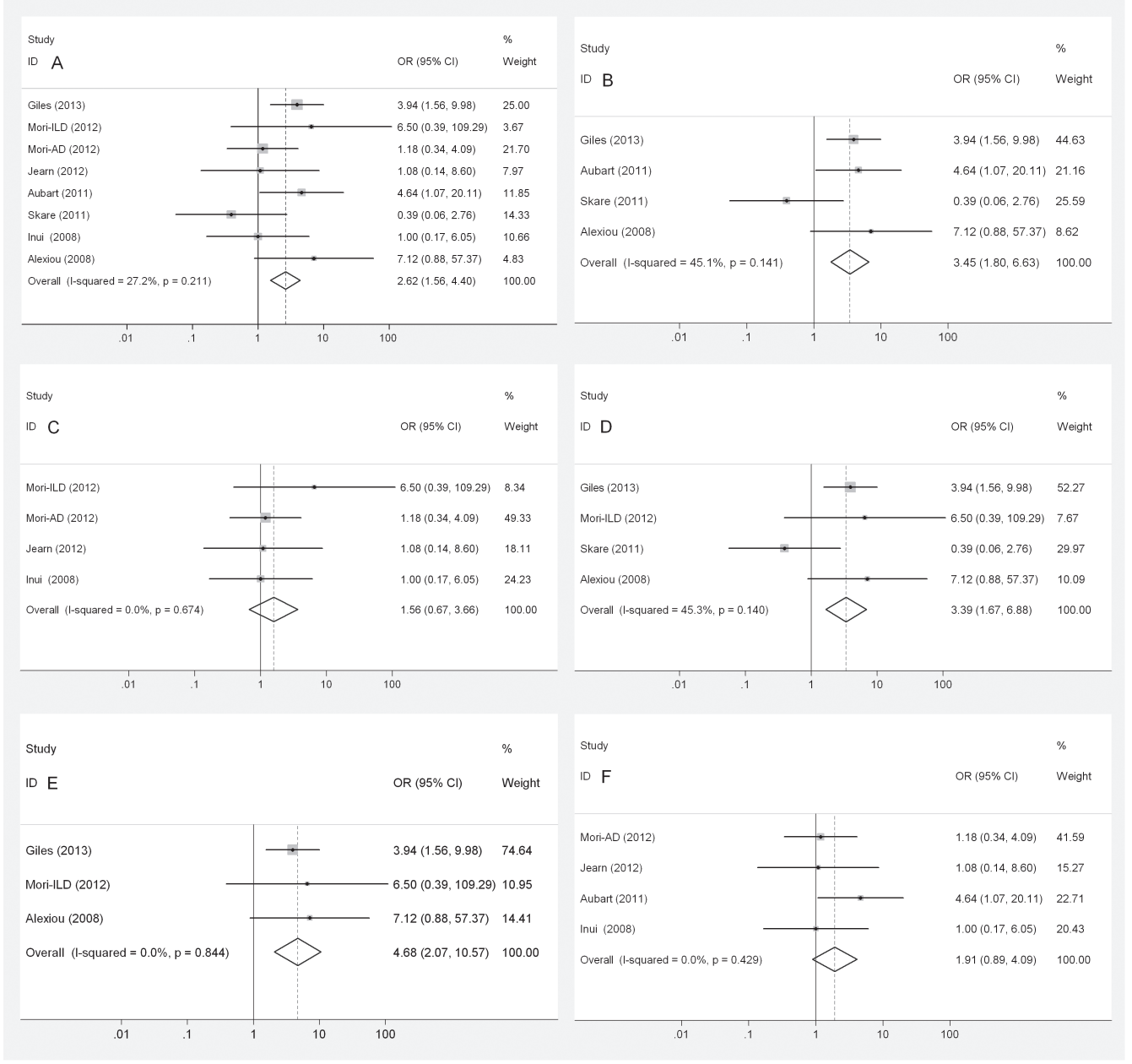

Figure 2. Forest plot of the association between anticitrullinated protein antibody (ACPA) positivity and the risk of rheumatoid arthritis (RA)-related pulmonary disease. A. Pooled risk of RA-related pulmonary disease with serum ACPA positivity. B. Pooled risk of RA-related pulmonary disease with serum ACPA positivity in the white population subgroup. C. Pooled risk of RA-related pulmonary disease with serum ACPA positivity in the Asian population subgroup. D. Pooled risk of RA-related interstitial lung disease (ILD) and interstitial pulmonary fibrosis (IPF) with serum ACPA positivity. E. Pooled risk of RA-related ILD and IPF with serum ACPA positivity when one study that examined the association between serum ACPA positivity and the risk of reticular pattern pulmonary lesions on high-resolution computed tomography was excluded. F. Pooled risk of RA-related pulmonary disease with serum ACPA positivity, excluding ILD and IPF. All these pooled risks were estimated by fixed-effects models. AD: airway disease.

serum ACPA positivity and the risk of reticular pattern pulmonary lesions on HRCT. As a result, the pooled OR increased to 4.679 (95% CI, 2.071–10.572, $p < 0.001$) in the fixed-effects model, and the I^2 decreased to 0.0% ($Q = 0.34$, $p = 0.844$; Figure 2E). Additionally, the fixed-effects model indicated a pooled OR of 1.913 (95% CI 0.894–4.094, $p = 0.095$) in the subgroup of 4 studies that examined the relationship between serum ACPA positivity and the risk of other RA-related pulmonary diseases ($Q = 2.77$, $p = 0.429$, $I^2 = 0.0\%$; Figure 2F). Because of the limited number of related studies, other subgroup analyses according to RA-related pulmonary diseases type were not performed in our metaanalysis.

Publication bias and sensitivity analysis. Both Begg's test

and Egger's test for funnel plot asymmetry showed that no publication bias existed in any of the pooled groups (data not shown). Similarly, sensitivity analyses showed no changes in the direction of the effect when any 1 study was excluded.

DISCUSSIONS

It is well known that ACPA aimed at citrulline-containing epitopes are an efficient and useful marker for early RA. The sensitivity and specificity for RA diagnosis of the first- and second-generation anticyclic citrullinated peptide antibody tests (anti-CCP1, anti-CCP2) can reach 68%–80% and 97%–98%, respectively[24,25]. Many studies have demonstrated that the serum ACPA level can predict the severity of joint damage and the possibility of extraarticular involvement[26,27]. However, the exact risk of RA-related pulmonary disease owing to serum ACPA positivity was not known. Thus, we conducted this metaanalysis and showed that the overall risk of RA-related pulmonary disease caused by serum ACPA positivity was 2.621. We further revealed that serum ACPA positivity indicated a greater risk for patients with RA to develop ILD and IPF (OR 4.679). Because the sample sizes in the pooled studies were relatively modest, our result demonstrating an increased risk for RA-related pulmonary disease due to serum ACPA positivity should be considered preliminary. Even so, to the best of our knowledge, this is the first metaanalysis to evaluate the effect of serum ACPA positivity on the risk of RA-related pulmonary disease.

The exact mechanism of the increased risk of RA-related pulmonary disease due to serum ACPA positivity remains unclear. A possible explanation is that a pathogenic interaction results in pulmonary tissue damage mediated by smoking, the HLA-DRB1 shared epitope, and ACPA[12,13]. Although smoking would accelerate the citrullination of autoantigens in the lungs of both normal subjects and patients with RA[28], tobacco exposure increases the risk for developing ACPA only in shared epitope-positive patients with RA[29]. Thus, we hypothesize that the circulating pathogenic autoantibodies may target citrullinated proteins in the lung because they share common antigenic epitopes, such as those present on synovial tissue. However, serum ACPA-positive patients with RA are at greater risk of developing ILD and IPF than other RA-related pulmonary diseases (OR 4.679 vs 1.913). Therefore, more complex mechanisms must exist. Another possible explanation is that ACPA are also associated with higher levels of multiple systemic inflammatory cytokines in RA[30], which play an important role in the pathologic processes of ILD and IPF[31]. Those cytokines, including tumor necrosis factor-α, interleukin 1β, and vascular endothelial growth factor, are embedded in the extracellular matrix, where they influence and regulate fibrosis, angiogenesis, and tissue repair in IPF, which is a progressive ILD characterized by an aberrant wound-healing process[32]. Because of the complexity of biological systems, additional mechanistic studies will be required in patients with RA-related pulmonary disease.

Our metaanalysis revealed that serum ACPA positivity was highly associated with RA-related pulmonary disease, particularly ILD and IPF (OR 2.621 vs 4.679). But no association was found in the Asian population subgroup (OR 1.562, p = 0.305). Even not considering ethnicity, the inconsistencies indicate that the effect of serum ACPA positivity on the risk of RA-related pulmonary disease may be complex because of the presence of stratification factors and confounding factors. For example, some studies included in this metaanalysis have described that sex, age, RA duration, RF positivity, smoking history, use of biologic agents, and use of steroids have statistically significant differences between cases and controls. After removing 2 studies that had significant statistical differences between RA-related pulmonary disease and RA controls in RA duration, the increased OR value (from 2.723 to 4.375) and decreased heterogeneity (from 42.9% to 0.0%) indicated that the balance of RA duration between them is very important when evaluating the risk of RA-related pulmonary disease from serum ACPA positivity.

Also, based on the data of the current included studies, we performed preliminary analyses for RF positivity in correlation with RA-related pulmonary disease. The results showed that the pooled OR were 1.667 (95% CI 0.884–3.141, p = 0.114) and 2.335 (95% CI 1.451–3.759, p < 0.001) in the random-effects model (Q = 15.18, p = 0.01, I^2 = 67.1%) and the fixed-effects model (Q = 3.16, p = 0.206, I^2 = 36.7%) for the risk of RA-related pulmonary disease and ILD owing to serum RF positivity, respectively (data not shown). But the previous study revealed that RA-ILD patients have a higher prevalence of RF compared with the natural history of cryptogenic fibrosing alveolitis[33]. Those partial contradictions could be explained by the different control populations and disease types. Additionally, other EAM or lung infections in RA may also correlate with RA-related pulmonary disease[12], and treatments with disease-modifying antirheumatic drugs and biological agents might have reduced the levels of ACPA[34]. Those could obscure the potential association to some extent.

Although all those confounding factors may potentially affect the risk of RA-related pulmonary disease from serum ACPA positivity, not all balanced baseline subgroup analysis was performed because of the limitations from the original research. Even so, the risk of RA-related pulmonary disease also may be associated with the serum ACPA titers in addition to serum ACPA positivity. Giles, *et al* reported that a high serum ACPA titer is also a potent indicator of RA-related ILD, even after adjusting for possible confounding factors (age, sex, current and former smoking, and RF)[12]. Therefore, further studies that have handled the stratification and confounding factors well and provide more

convincing evidence are necessary to determine the association between the serum ACPA level and the risk of RA-related pulmonary disease.

Several limitations should be considered in our metaanalysis. First, most of the studies included in the analysis were of moderate quality, based on the NOS assessment. High-quality studies formed a smaller percentage primarily because hospital controls were applied instead of community controls and because confounding factors were not well controlled. Second, the heterogeneity existed in the pooled group with RA-related pulmonary disease, although it showed no statistical significance. Because the number of included studies was limited, subgroup analyses were performed only for patients with RA and concurrent ILD or IPF. Other RA-related pulmonary disease risk analyses were conducted using pooled data. Not all further subgroup analysis was performed according to the confounding factors because of the limitations from the original research. Therefore, the association of certain subgroups and serum ACPA positivity may have been masked. Third, the assessment of disease risk is not applicable to African populations, because no study has been performed in that group. Therefore, caution should be exercised when interpreting the results of this metaanalysis.

To our knowledge, this is the first metaanalysis to evaluate the effect of serum ACPA positivity on the risk of RA-related pulmonary disease. Our metaanalysis revealed that serum ACPA positivity was highly associated with RA-related pulmonary disease, particularly with ILD and IPF, although no association was found in the Asian population subgroup. Further, more careful prospective studies with larger sample sizes and well-controlled confounding factors are required to confirm our findings.

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