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Prevalence of Autoantibodies (ACPA) and Rheumatoid Factor among Rheumatoid Arthritis Patients in Sulaymaniyah

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Abstract

Background and objectives: Rheumatoid factor and anticitrullinated protein antibodies are the most characteristic autoantibodies for rheumatoid arthritis. Our aims were to evaluate the prevalence of Rheumatoid factor and anticitrullinated protein antibodies and their association with demographic and clinical characteristics of patients with RA.

Methods: This is a cross-sectional study which was conducted at Rheumatology and Rehabilitation Center/Sulaymaniyah from February 2022 to November 2022. The study included patients with rheumatoid arthritis from both sexes. Socio-demographic and clinical data were collected. Enzyme linked immunosorbent assay was used to estimate serum levels of rheumatoid factor and anticitrullinated protein antibodies antibody titer.

Results: The prevalence of rheumatoid factor was 72.61%. Each of high disease activity, mean DAS28 scores, rheumatoid nodules and deformities were significantly associated with rheumatoid factor positivity (P= 0.015, P= 0.025, P= 0.007 and P<0.001, respectively). The prevalence of anticitrullinated protein antibodies was 68.87%. High disease activity, rheumatoid nodules and deformities were significantly associated with anticitrullinated protein antibodies (P= 0.014, P<0.001 and P<0.001, respectively).

Conclusions: The prevalence of rheumatoid factor and anticitrullinated protein antibodies among patients with rheumatoid arthritis was within the global context. High disease activity and the presence of rheumatoid nodules are positively associated with rheumatoid factor-positivity. Number of tender joints and the presence of rheumatoid nodules were positively associated with anticitrullinated protein antibodies-positivity.

Keywords: Anticitrullinated protein antibodies, Rheumatoid arthritis, Rheumatoid factor, Risk factors

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Introduction

Rheumatoid arthritis (RA) is the most common autoimmune joint disease, with the primary sites of pathology being joint tissues impacted by chronic synovial inflammation, synovial hyperplasia, and bone degradation.

A variety of extra-articular disease symptoms occur regularly, with cardiovascular problems being related with an elevated mortality risk in RA patients.[1]The etiology of RA involves a complicated interplay between B cells, T cells, and dendritic cells. A variety of environmental and genetic variables cause loss of tolerance to proteins with citrulline residues, resulting in the generation of autoantibodies such as anti-cyclic citrullinated protein/peptide antibody (ACPA) and rheumatoid factor (RF).[2]

The presence or absence of RFs, as well as their titers and isotypes, have crucial consequences for the diagnosis and prognosis of RA. Seropositive (RF-positive) RA patients may have more severe and erosive joint disease, as well as extra-articular symptoms such as rheumatoid nodules and vasculitis, than seronegative patients (RF-negative).[3]

In RA patients, RF testing has been shown to have a sensitivity of 60% to 90% and a specificity of 85%. According to each study population, the sensitivity could, however, fluctuate anywhere from 26% to 90%.[4]

Anticitrullinated protein antibodies testing have been introduced to improve the specificity of the RA categorization criteria. The 2010 ACR/EULAR criteria for RA diagnosis includes both RF and ACPA. ACPA and RF sensitivities have been found to be similar in studies, while ACPA offers higher specificity than RF for early RA. Combining the positive results of both ACPA and RF provides more sensitivity and is more helpful in leading to a diagnosis.[5] There is a great conflict in literatures regarding the impact of ACPA and RF on disease activity. Positive ACPA [6] and positive RF [7] have been linked to more active illness in certain studies, while positive ACPA has been linked to low clinical disease activity in others.[8] Rheumatoid factor of Immunoglobulin (Ig) A rather than IgM predicts a worse outcome.[9]

The aim of this study was to determine the prevalence of RF and ACPA antibodies, as well as their relationship to demographic and clinical parameters of RA patients.

Patients and methods

This is a cross-sectional study conducted at Rheumatology and Rehabilitation Center/Sulaymaniyah during the period from February 2022 to November 2022. The study was approved by Kurdistan Council of Medical Specialties (KHCMS). A total of 230 consecutive patients diagnosed to have RA according to 2010 ACR /EULAR classification criteria.[3,4]

Patients were included in this study. Patients >30 yrs old and have adequate cognitive status as determined by communicating with the patients. Patients with cognitive impairment, pregnancy or breast feeding, and those who refused to give a consent were excluded from the study.

Each participant in this study provided informed consent in accordance with the Helsinki Declaration. The Ethics Committee of the Kurdistan Council of Medical Specialties granted ethical approval.

Data collection of patients were done through interview and questionnaires. The following data were collected: Socio-Demographic data: including age, gender, smoking status, residence, educational status, comorbidities, height in centimeters and weight in kilograms, from which body mass index (BMI) was calculated according to the

equation BMI=weight in Kg/ height in meter squared

Clinical data including disease duration, DAS28 score, disease activity, family history of RA, the presence of rheumatoid nodules and deformity were also collected.

Five mL of venous blood were collected from all patients in plain tubes. Sera were separated by centrifugation. Ready kits were used to measure serum level of ACPA and RF (Aeskulisa/Germany) using enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions. ACPA-CCP2 levels >20 IU/mL were considered positive, while AF> 15 IU/ml was considered positive.

The SPSS statistical software, version 25 (IBM Corporation, USA) was used for data entry and all statistical analyses. The mean and standard deviation (SD) of quantitative values were reported. Independent t-test was used to differentiate between two means. Counts and percentages were used to express categorical variables, and then chi-square association test was used for categorical variables. The significant level of statistics was considered when $p<0.05$.

Results

Out of 230 patients, 167 patients (72.61%) were positive for RF, while 63 patients (27.39%) were negative.

No significant difference was noticed between the two groups regarding age, gender, disease duration, BMI, smoking, educational level, and comorbid diseases. However, rural residents were more common among RF-positive than RF-negative patients (26.35% vs. 14.29%) with a significant difference (P= 0.05), Table (1).

Table (1): Demographic characteristics according to the positivity of rheumatoid factor

Variables	RF-positive N=167	RF-negative N=63	p value
Age, years	55.07±10.65	56.86±10.06	0.351
Gender			
Male	38(22.75%)	22(34.92%)	0.061
Female	129(77.25%)	41(65.08%)	
Disease duration, years	9.14±6.29	10.78±7.06	0.083
BMI, Kg/m^2	28.20±4.90	28.9±5.08	0.856
Smoking			
Yes	30(17.96%)	18(28.57%)	0.077
No	137(82.04%)	45(71.43%)	
Residency			
Urban	123(73.65%)	54(85.71%)	0.050
Rural	44(26.35%)	9(14.29%)	
Education level			
Illiterate	64(38.32%)	26(41.27%)	0.683
Literate	103(61.68%)	37(58.73%)	
Comorbid diseases*			
Hypertension	59(35.33%)	22(34.92%)	0.954
Diabetes mellitus	27(16.17%)	13(20.63%)	0.425
Ischemic heart disease	20(11.98%)	3(4.76%)	0.104

Thyroid disease	18(10.78%)	5(7.94%)	0.522
Dyslipidemia	20(11.98%)	5(7.94%)	0.380

* patient may have more than one comorbid disease, RF = rheumatoid factor

The median number of tender and swelling joints was higher among RF-positive (7.0 and 6.0, respectively) than RF-negative patients (4.0 and 5.0, respectively) with significant differences (P= 0.009 and P= 0.008, respectively). Similarly, 54.49% of RF-positive patients had high disease activity compared with 42.86% of RF-negative patients with a significant difference. Furthermore, the mean DAS28 scores in patients positive and negative for RF was 5.18±1.3 and 4.58±1.6 respectively with a significant difference (P= 0.025). Finally, among RF-positive patients 10.78% had rheumatoid nodules and 51.5% of them had joint deformities, while patients with RF-negative with 0.0% rheumatoid nodules and only 19.05% joint deformities. The differences between two groups was significant (P= 0.007), Table (2).

Table (2): Association of clinical characteristics with RF-positivity

Variables	RF-positive N=167	RF-negative N=63	p value
DAS28 score components			
ESR, mm/hr	32 (2-105)	35.0 (2-98)	0.588*
Tender joints	7.0(0-28)	4.0(0-22)	0.009
Swelling joints	6.0 (0-18)	5.0(0-20)	0.008
VAS score	6.23±2.19	5.7±2.19	0.897
Total DAS28 score	5.18±1.3	4.58±1.6	0.025
Disease activity			
Remission	1(0.06%)	1(1.59)	
Low	11(6.59%)	13(19.4%)	0.015
Moderate	64(38.32%)	22(34.92%)	
High	91(54.49%)	27(42.86%)	
Family history of RA			
Yes	84(50.3%)	28(44.44%)	0.428
No	83(49.7%)	35(55.565)	
Rheumatoid nodules			
Yes	18(10.78%)	0(0%)	0.007
No	149(89.22%)	63(100%)	
Joint deformity			
Yes	85(51.5%)	12(19.05%)	<0.001
No	81(48.5%)	51(80.95%)	

*Data were expressed as median and range, RF = rheumatoid factor; ESR = Erythrocyte sedimentation rate; DAS28 = disease activity score (number of joints examined); VAS = Visual Analogue Scale

Out of 230 patients, 140 patients (68.87%) were positive for ACPA antibodies, while 90 patients (39.13%) were negative.

Patients were categorized into anti- ACPA positive (140, 68.87%) and ACPA negative (90, 39.13%). No significant difference was notice between these subgroups regarding their age, gender, disease duration, BMI, smoking, residency, educational level, and comorbid diseases. However, IHD was more frequent among ACPA positive than and ACPA negative patients (13.57% vs. 4.44%) with a significant difference (P= 0.018), Table (3).

Table (3): Association of demographic characteristics with ACPA positivity

Variables	ACPA-positive N=140	ACPA-negative N=90	p value
Age, years	55.24±0.66	56.06±10.29	0.489
Gender			
Male	34(24.29%)	26(28.89%)	0.438
Female	106(75.71%)	64(71.11%)	
Disease duration, years	9.6±6.75	9.58±6.42	0.194
BMI, Kg/m^2	28.16±4.51	28.75±5.57	0.092
Smoking			
Yes	28(20%)	20(22.22%)	0.686
No	112(80%)	70(77.78%)	
Residency			
Urban	105(75%)	72(80%)	0.380
Rural	35(25%)	18(20%)	
Education level			
Illiterate	53(37.86%)	37(41.11%)	0.622
Literate	87(62.14%)	53(58.89%)	
Comorbid diseases*			
Hypertension	48(34.29%)	33(36.67%)	0.712
Diabetes mellitus	23(16.43%)	17(18.89%)	0.631
Ischemic heart disease	19(13.57%)	4(4.44%)	0.018
Thyroid disease	15(10.71%)	8(8.89%)	0.652
Dyslipidemia	18(12.86%)	7(7.78%)	0.227

* patient may have more than one comorbid disease, ACPA = anti-citrullinated protein antibodies

The median number of tender swollen joints was significantly higher in those with positive as compared to those with negative ACPA (p =0.013). Likewise, the disease activity was significantly different between the two subgroups (p =0.014). Additionally, rheumatoid nodules and joint deformities were reported in 12.86% and 55%, respectively in ACPA negative patients compared with 0% and 22.22% of ACPA negative patients with significant differences (P<0.001), Table (4).

Table (4): Association of clinical characteristics with ACPA positivity

Variables	ACPA-positive N=140	ACPA-negative N=90	p value
DAS28-ESR components			
ESR, mm/hr	32(2-105)	32.5(2-98)	0.343*
Tender joints	8.0(0-28)	4.0(0-22)	0.013
Swelling joints	6.0(0-18)	6.0(0-20)	0.162
Disease activity			
Remission	0(0%)	2(2.22%)	0.014
Low	9(6.43%)	15(16.67%)	
Moderate	54(38.57%)	32(35.56%)	
High	77(55%)	41(45.56%)	
VAS score	6.33±2.13	5.7±2.27	0.444
Total DAS28 score	5.26±1.29	4.64±1.52	0.096
Family history of RA			
Yes	69(49.29%)	43(47.78%)	0.823
No	71(50.71%)	47(52.22%)	
Rheumatoid nodules			
Yes	18(12.86%)	0(0%)	<0.001
No	122(87.14%)	90(100%)	
Joint deformity			
Yes	77(55%)	20(22.22%)	<0.001
No	63(45%)	70(77.78%)	

*Data were expressed as median and range, ACPA = anti-citrullinated protein antibodies; ESR = Erythrocyte sedimentation rate; DAS28 = disease activity score (number of joints examined); VAS = Visual Analogue Scale

Discussion

In the current study, 72.61% of RA patients were found to be seropositive for RF. This is almost identical to a recent local study including 1493 Iraqi patients with RA, of whom 72.2% were positive for RF. [10] The observed present rate is within the range of most recent cohort studies indicating that about 60-80% of RA patients were seropositive.[11] However, this percent is higher than that reported by others who documented a 47% and 52% of patients to show positive RF.[12,13] This may be attributed to differences in demographic and ethnic characteristics of the patients.

According to the result of the current study, RF-positivity was significantly associated with the number of tender joints, disease activity and DAS28. These finding harmonizes the results of Seri and coauthors [10]. In contrast, Zemri et al.[14]demonstrated no statistically meaningful link. This could be explained by the time elapsed between the commencement of the ailment and the presentation to the clinic. Another study disclosed that in early arthritis, the seronegative group had a higher DAS28 score.[15] This could be linked to a delay in diagnosis because seronegative patients require more clinical symptoms to be diagnosed with RA using the 2010 ACR/EULAR criteria. Further, patients seropositive for RF had more joint deformities relative to those with seronegative. This finding also reported by other recent studies.[13]

Rheumatoid nodules were exclusively found in patients positive for RF denoting that RF-seropositive RA patients tend to have more extra-articular manifestations. This was in accordance with the finding of other study.[16]

In the present study, the prevalence of ACPA antibodies was 68.87% which is in line with the result of Rhida et al.[11] who reported a prevalence of 70.% of ACPA among Iraqi patients with RA. Additionally, the present prevalence is within the context of the global prevalence, as many literatures have indicated that (70-80%) of patients with RA were positive for these antibodies.[17]

The present finding revealed that ACPA antibodies was significantly associated with IHD. This result is in line with the study of Geraldino-Pardilla et al.[18] who measure ACPAs in 270 patients with RA. There was a statistically significant association between ACPA positivity and IHD (P=0.001). This association was mainly attributed to the immune complexes that containing citrullinated proteins which have the capacity to initiate inflammation, including neutrophil activation, and to the potential role of anti-Cit-histone antibodies in the evolution of atherosclerosis in RA.[19].

In the present study, ACPA was significantly associated with disease activity, number of tender joints and rheumatoid nodules. Different studies worldwide revealed different results. Several studies have indicated a direct link between ACPA positivity with poor patient's outcomes, such as increased disease activity (DAS28 score), radiographic progression and disability.[20]

Ursum et al.[21] on the other hand, evaluated 545 Dutch patients with early arthritis. They displayed no link between antibody levels and early illness outcomes such as DAS28 disease activity, Health Assessment Questionnaire (HAQ) functional status, or radiographic progression. Murata et al.[22] also assessed ACPA titer in a total of 3286 patients, of whom 1806 patients were ACPA-positive. The ACPA titer level and disease activity had a very poor connection.

This discrepancy between different studies could be explained by several factors, the most important of which are ethnic variation and differences in clinical characteristic of the patients.

The current study showed that rheumatoid nodules were reported in 12.86% of ACP-positive versus ACPA negative patients. The association between high levels of anti-CAPA antibodies and rheumatoid nodules alludes to the characteristics of rheumatoid nodules. They contain fibrin and IgG, as previously stated, but nothing is known regarding the specificity of those IgG.

Association between high level anti-CAPA antibodies and rheumatoid nodules refers to rheumatoid nodules nature. As previously described, they contain fibrin and IgG but nothing is known about the specificity of those IgG. Citrullinated proteins and RF were found in the necrotic center.[23] As such, these antibodies could form immune complexes with citrullinated fibrin that are then converted into macroimmune complexes by RF and constitute the source of rheumatoid nodules. ACPAs have been shown to react with type II collagen, causing proteoglycan depletion and severe arthritis [24]. Given the evidence of complement activation in antibody-cartilage surface interactions in RA patients, it is possible that ACPA binding to type II collagen causes joint inflammation and structural damage by activating the complement cascade.[25] In contrast, Murata et al.[22] found no change in tender or swollen joint counts. Those with extraarticular symptoms, such as rheumatic nodules, are more likely to be RF positive, according to

universal consensus. Likewise, RF positivity, but not ACPA positivity, appeared to be more common in patients with extra-articular symptoms than in patients without.[26] The treatment regimen could explain the differences across trials. It was previously observed that DMARDs, particularly methotrexate and TNF-alpha inhibitors, could hasten rheumatoid nodulosis.

Conclusions: Rheumatoid factor and ACPA may contribute to disease perpetuation by potentiating immune complex formation and complement fixation. Clinically, RA patients with RF-positivity and ACPA-positivity should be considered to have more aggressive disease and treated accordingly.

Conflict of interest: The authors declare that they have no conflict of interest

References:

1. Kurowska W, Kuca-Warnawin EH, Radzikowska A, Maśliński W. The role of anti-citrullinated protein antibodies (ACPA) in the pathogenesis of rheumatoid arthritis. Cent Eur J Immunol. 2017;42(4):390-8. DOI: 10.4103/tcmj.tcmj_116_18
2. De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, Lebeer K, et al. Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Ann Rheum Dis. 2004;63(12):1587-93. DOI: 10.1136/ard.2003.017574
3. .Scott DL, Smith C, Kingsley G. Joint damage and disability in rheumatoid arthritis: an updated systematic review. Clin Exp Rheumatol. 2003;21(5 Suppl 31):S20-7.
4. Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, Saigo K. et al. diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med. 2007;146(11):797-808. DOI: 10.7326/0003-4819-146-11-200706050-00008
5. Ingegnoli F, Castelli R, Gualtierotti R. Rheumatoid factors: clinical applications. Dis Markers. 2013;35(6):727-34. DOI: 10.1155/2013/726598
6. Aletaha D, Alasti F, Smolen JS. Rheumatoid factor, not antibodies against citrullinated proteins, is associated with baseline disease activity in rheumatoid arthritis clinical trials. Arthritis Res Ther. 2015;17:229. DOI: 10.1186/s13075-015-0736-9
7. Barra L, Pope JE, Orav JE, Boire G, Haraoui B, Hitchon C, et al. Prognosis of seronegative patients in a large prospective cohort of patients with early inflammatory arthritis. J Rheumatol. 2014;41:2361–9. DOI: 10.3899/jrheum.140082
8. Seri A, Mohamed H, Adam ME, Elagib EM, Eltahirm NIA, Mansour SMA, et al. Analysis of serum immune markers in seropositive and seronegative rheumatoid arthritis among Sudanese patients and the relation between the serotype and joint involvement: a cohort study. Open Access Rheumatol. 2021; 13:325-332. DOI: 10.2147/OARRR.S339134
9. Sung W, Tsai W. Rethink about the role of rheumatoid factor and anti-

citrullinated protein antibody in rheumatoid arthritis. Rheumatol Immunol Res. 2021; 2: 19-25. DOI.org/10.2478/rir-2021-003
10. Ridha A, Hussein S, AlJabban A, Gunay LM, Gorial FI, Al Ani NA. The clinical impact of seropositivity on treatment response in patients with rheumatoid arthritis treated with etanercept: a real-world Iraqi experience. Open Access Rheumatol. 2022;14;14:113-121. doi: 10.2147/OARRR.S368190.
11. Ingegnoli F, Castelli R, Gualtierotti R. Rheumatoid factors: clinical applications. Dis Markers. 2013; 35(6):727-34. DOI: 10.1155/2013/726598.
12. Hannech E, Ben Tekaya A, Saidane O, Bouden S, Leila R, Tekaya R, et al. Comorbidities profiles in seropositive rheumatoid arthritis versus seronegative rheumatoid arthritis. Ann Rheum dis. 2022; 81(suppl. 1). DOI.org/10.1136/1nnrheumdis-2022-eular.336
13. Baka Z, Buzás E, Nagy G. Rheumatoid arthritis and smoking: putting the pieces together. Arthritis Res Ther. 2009;11(4):238. DOI.org/10.1186/ar3751
14. Ouali S, Zemri K, Sellam F, Harir N, Beniassa Z, Herbi S, et al. Is there an association between anti-citrullinated peptide antibodies and the severity of rheumatoid arthritis parameters in Algerian patients? J Drug Deliv Therapeut. 2020;10(4):17–24). DOI: 10.1002/acr.23106
15. Barra L, Pope JE, Orav JE, Boire G, Haraoui B, Hitchon C, et al. Prognosis of seronegative patients in a large prospective cohort of patients with early inflammatory arthritis. J Rheumatol. 2014;41(12):2361–2369). DOI: 10.3899/jrheum.140082
16. Sarikaya Y, Sandal Uzun G, Ata EB, Arslan S, Ekici M, Durhan G, et al. Pulmonary rheumatoid nodules: does serologic status matter? Ann Rheum Dis. 2022; 81 (suppl. 1). doi.org/10.1136/annrheumdis-2022-eular.2526
17. Lee DM, Schur PH. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. Ann. Rheum. Dis. 2003, 62, 870–874. DOI: 10.1136/ard.62.9.870
18. Geraldino-Pardilla L, Giles JT, Sokolove J, Zartoshti A, Robinson WH, Budoff M, et al. Association of anti-citrullinated peptide antibodies with coronary artery calcification in rheumatoid arthritis. Arthritis Care Res (Hoboken). 2017 Aug;69(8):1276-1281. DOI: 10.1002/acr.23106
19. Sohn DH, Rhodes C, Onuma K, Zhao X, Sharpe O, Gazitt T, et al. Local joint inflammation and histone citrullination in a murine model of the transition from preclinical autoimmunity to inflammatory arthritis. Arthritis Rheumatol 2015; 67:2877–87. DOI.org/10.1002/art.39283
20. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. 2011, 365, 2205–2219. DOI: 10.1056/NEJMra1004965.
21. Ursum J, Bos WH, van Dillen N, Dijkmans BA, van Schaardenburg D. Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor

are not associated with outcome in early arthritis patients: a cohort study. Arthritis Res Ther. 2010;12(1):R8. DOI: 10.1186/ar2907

22. Murata K, Ito H, Hashimoto M, Murakami K, Watanabe R, Tanaka M, et al. Fluctuation in anti-cyclic citrullinated protein antibody level predicts relapse from remission in rheumatoid arthritis: KURAMA cohort. Arthritis Res Ther 2020;22, 268. doi.org/10.1186/s13075-020-02366-x
23. Anquetil F, Clavel C, Offer G, Serre G, Sebbag M. IgM and IgA rheumatoid factors purified from rheumatoid arthritis sera boost the fc receptor- and complement-dependent effector functions of the disease-specific anti-citrullinated protein autoantibodies. J Immunol 2015; 194:3664–74DOI: 10.4049/jimmunol.1402334
24. Ge C, Tong D, Liang B, Lönnblom E, Schneider N, Hagert C, et al. Anti-citrullinated protein antibodies cause arthritis by cross-reactivity to joint cartilage. JCI Insight 2017, 2. DOI: 10.1172/jci.insight.93688
25. Wu CY, Yang HY, Lai JH. Anti-citrullinated protein antibodies in patients with rheumatoid arthritis: biological effects and mechanisms of immunopathogenesis. Int J Mol Sci. 2020 Jun 4;21(11):4015. DOI: 10.3390/ijms21114015
26. De Rycke L, Peene I, Hoffman IEA, Kruithof E, Union A, Meheus L, et al. Rheumatoid factor and anti-citrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extraarticular manifestations. Ann Rheum Dis 2004;63:1587 – 93 DOI: 10.1136/ard.2003.017574