



**Autoantibody Prevalence in Active Tuberculosis Patients:
Reactive or Pathognomonic? A case-control study**

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**Autoantibody Prevalence in Active Tuberculosis Patients: Reactive or
Pathognomonic?**

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Short title: Evident Interaction between chronic active infection and
autoimmunity

Key words: anti-cardiolipin antibody, anti-topoisomerase I, autoantibody,
autoimmune disease, tuberculosis

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ABSTRACT

Background: Autoantibodies can be found in patients with active tuberculosis (TB) but lack of clinical significance. This study aimed to evaluate if autoantibodies in TB are pathognomonic, reactive, or incidental.

Methods: One hundred active TB patients and 100 healthy medical staff were evaluated for the presence of serum autoantibodies, including anti-dsDNA, anti-Ro, anti-La, anti-Sm, anti-RNP, anti-Jo1, anti-Scl-70, anti-centromere, anti-histone, anti-cardiolipin IgG and anti-cardiolipin IgM. For patients with active TB, serum titers were re-evaluated after three months of anti-TB treatment.

Result: Of the 100 TB patients (median age, 63 years; 67 males), 32 had elevated serum autoantibody titers. The most common elevated serum autoantibodies were anti-cardiolipin IgG (n=11), anti-histone (n=11), and anti-Scl-70 (n=6). Compared to the healthy medical staff and general population, TB patients had significantly elevated anti-cardiolipin IgG and Anti-Scl-70 levels. Autoantibody-positive and autoantibody-negative TB patients had similar clinical manifestations of TB and adverse events during anti-TB treatment. Serum anti-cardiolipin IgG and anti-Scl-70 levels returned to normal in seven and five patients, respectively, after anti-TB treatment.

Conclusion: Elevation of autoantibodies is not pathognomonic in active TB patients.

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Caution must be taken in diagnosing autoimmune disease in TB patients.

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Article Summary

Article focus :

We all know that chronic active tuberculosis has immunogenicity that autoantibodies were often found in these patients. Are there disease specific autoantibodies in these patients? Were these disease specific autoantibodies also pathognomic even without corresponding symptoms and request treatment like immune suppressant? Can these autoantibodies, even disease specific, without corresponding symptoms be reactive to stimulation, like tuberculosis?

Key messages :

We revealed that in tuberculosis patients, disease specific autoantibody did exist in some patients, besides non-specific rheumatoid factor or antinuclear antibody. Furthermore, the autoantibody titer may decrease, even return to normal, according to infection activity. This may suggest that these autoantibody reactive to tuberculosis activity, instead of being pathognomic and necessarily of immune suppressant.

Strengths and limitations of this study :

We first elucidate the autoantibody titer change according to infection activity instead of screening the rheumatology symptoms only. However, patient number and difficulty with control group chosen may be limitations of our study.

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INTRODUCTION

Tuberculosis (TB) has become one of the most important diseases in the past two decades. Direct infection not only leads to organ dysfunction but also to a great variety of clinical manifestations that contribute to difficult diagnosis and management. The infection process is also known to stimulate immune response[1].

Previous studies have shown that sera from patients with active TB may contain autoantibodies that are unique in autoimmune diseases. The autoantibodies reported include rheumatoid factor (RF), anti-nuclear antibody (ANA), anti-cardiolipin antibody (ACA) (IgM isotype predominant), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-cyclic citrullinated peptide[2-5]. Some of these are hallmarks of certain autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, and ANCA-associated vasculitis. Some are even disease-specific, such as anti-cyclic citrullinated peptide in rheumatoid arthritis[6-7]. However, no definite correlations between serology and clinical manifestation have been found in these studies, most of which are cross-sectional studies with limited case numbers. Little is known regarding the clinical significance (i.e., pathognomonic, reactive, or incidental) of these autoantibodies in TB patients.

This study aimed to compare sera from patients with active TB to those of healthy controls to evaluate the prevalence of autoantibodies. Dynamic changes of

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7 months of anti-TB treatment determine the clinical significance of elevated
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10 autoantibodies in this clinical entity.
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PATIENTS AND METHODS

Subjects and study protocol

The study was approved by the Institutional Review Board of the National Taiwan University Hospital (NTUH REC: 9561707008). Between January 2007 and December 2009, 933 new cases of culture-confirmed TB were diagnosed at the National Taiwan University Hospital. Among them, 100 patients were enrolled in this study after informed consent had been obtained, including 96 with pure pulmonary TB, two with concomitant pulmonary and extra-pulmonary TB (peritonitis in one and meningitis in another), and two with pure extra-pulmonary TB (neck lymphadenopathy in one and cutaneous TB in another). The first serum samples were collected before the start of anti-TB treatment. The autoantibodies examined were autoantibodies to the Ro antigen, La antigen, centromere protein, double-stranded DNA, topoisomerase I (Scl-70), Smith protein, ribonucleoprotein particle (RNP), histone protein, and histidyl-tRNA synthetase (Jo1). Anti-cardiolipin IgG and anti-cardiolipin IgM were also examined. For those with elevated serum autoantibody levels, follow-up serum samples were collected three months after anti-TB treatment to evaluate the effect of anti-TB treatment on the autoantibody titers.

All of the participants received a standard anti-TB treatment consisting of daily isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) in the

first two months, followed by daily INH and RIF for the next four months[8]. The regimen was modified if necessary by the primary care physician. One hundred healthy medical staff members were enrolled as the control group.

The clinical parameters collected were age, sex, underlying disease, clinical manifestations, and radiographic findings of TB, as well as adverse events during anti-TB treatment. Respiratory symptoms included cough, sputum, hemoptysis, dyspnea, and chest pain. Constitutional symptoms included fever, weight loss, general malaise, and night sweating. The adverse events were classified into 7 categories: 1) rheumatologic events, including cutaneous reaction and arthralgia; 2) gastrointestinal events, including abnormal liver function, gastric discomfort, abdominal pain, and bowel habit change; 3) constitutional events, including fever, malaise, poor appetite, and malaise; 4) renal events, including hyper-uricemia and renal function impairment; 5) neurologic events, including blurred vision, insomnia, delirium, headache, and numbness; 6) respiratory event, including cough, dyspnea, and chest pain; and 7) hematologic events, including leukopenia, thrombocytopenia, and anemia.

Detection of autoantibodies

A commercial test system AtheNA Multi-Lyte® ANA-II Plus Test System was used to test IgG class anti-extractable nuclear antigens, including autoantibody to Ro antigen, La antigen, centromere protein, double-stranded DNA, Scl- 70, Smith protein,

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RNP, histone protein, and Jo1. Serum samples were prepared as 1:21 dilution and enzyme-linked immuno-sorbent assay (ELISA) was performed according to the manufacturer’s instructions.

A commercial available kit QUANTA Lite™ ACA IgM III was used to test anti-cardiolipin IgM. Sera were prepared as 1:101 dilution. The commercial kit Phadia® Varelista Cardiolipin IgG Antibodies EIA kit was used to test anti-cardiolipin IgG at serum dilution of 1:101. All ELISA assays were performed according to the manufacturer’s instructions.

Statistical analysis

Inter-group difference was calculated using independent-samples *t* test for continuous variables and *chi*-square test or Fisher exact test for categorical variables, as appropriate. A two-sided *p* <0.05 was considered significant. All analyses were performed using the SPSS software version 12.0 for Windows.

RESULTS

The median age of TB patients was 63 years (range 19-92 years), with a male-to-female ratio of 2.03. Forty-one TB patients had underlying diseases, including malignancy in 17, diabetes mellitus in 14, end-stage renal disease in nine, and one each with systemic lupus erythematosus (SLE), ankylosing spondylitis, and rheumatoid arthritis. The SLE patient was a middle-aged woman who initially presented as polyarthritides, malar rash, and positive anti-dsDNA and anti-nuclear antibodies. She received disease modifying anti-rheumatic drugs (DMARDs) and corticosteroids.

The patient with ankylosing spondylitis was a young woman diagnosed by clinical symptoms and positive HLA-B27. She received non-steroidal anti-inflammatory drugs (NSAIDs). The patient with rheumatoid arthritis was a young woman who presented as symmetric polyarthritides with positive rheumatoid factor. She received DMARDs and NSAIDs. Before the diagnosis of pulmonary TB, results of autoantibody tests were all negative in three patients. Among the 17 TB patients with malignancies, six were lung cancer, three hematologic malignancies, four airway and lung malignancies, two prostate cancers, one breast cancer, and one pancreatic cancer.

The median age of the control group was 30 years, with a male-to-female ratio of 0.33. None had underlying diseases.

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Results of autoantibody tests were summarized in Table 1. Thirty-two TB patients had elevated serum autoantibody levels, including nine with more than one autoantibody. The most prevalent autoantibodies were anti-Scl-70, anti-histone, and anti-cardiolipin IgG. A significantly higher proportion of TB patients had elevated serum anti-cardiolipin IgG titers than the healthy controls ($p<0.001$). Compared to a previous report evaluating the prevalence of autoantibodies in the general population[9], TB patients were more likely to have elevated serum titers of anti-Scl-70 ($p<0.05$) and anti-cardiolipin IgG ($p<0.001$).

Clinical manifestations were similar in the autoantibody-positive and -negative groups (Table 2). Cough and sputum production were the most common respiratory symptoms, whereas fever and weight loss were the most common constitutional symptoms. Sputum samples were smear-positive for acid-fast bacilli in 31 patients. Radiographic findings were also similar in the autoantibody-positive and -negative groups. Among anti-Scl-70-positive and anti-Scl-70-negative groups, pulmonary cavitation was noted in 16% and 9%, respectively ($p=0.441$), whereas pleural effusion was present in 33% and 16%, respectively ($p=0.245$).

Within 3 months of anti-TB treatment, 148 adverse events were recorded in 61 patients (Table 3). The most common were rheumatologic events, followed by gastrointestinal events. There was no significant difference in adverse events between

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4 autoantibody-positive and -negative TB patients.
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7 For the 11 patients with elevated anti-cardiolipin IgG and six patients with
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9 elevated anti-Scl-70 at baseline, serum titers of the autoantibodies were followed-up
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11 after 3 months of anti-TB treatment (Table 4). None of the patients received
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13 immuno-suppressive drugs and DMARDs. Follow-up serum titers of anti-cardiolipin
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15 IgG and anti-Scl-70 returned to normal limits in seven and four patients, respectively.
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18 Among the five patients with persistent elevated anti-cardiolipin IgG or anti-Scl-70
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21 autoantibodies, none had rheumatologic symptoms at the end of the six-month
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24 anti-TB treatment.
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DISCUSSION

The present study has three important findings. First, elevated serum autoantibodies can be found in one-third of patients with active TB. The prevalence of autoantibodies, especially anti-cardiolipin IgG and anti-Scl-70, is significantly higher than that in the general population[9]. Second, consistent with previous studies[2-5], the presence of autoantibodies neither altered the clinical manifestations and radiographic findings of active TB nor changed the risk of developing adverse events during anti-TB treatment. Third, elevated autoantibody levels returned to normal limits simply by anti-TB treatment and not by immunosuppressive therapy. These findings suggest the possibility that increased serum autoantibodies during active TB may not be diagnostic for autoimmune diseases.

Autoantibodies come from a break in self-tolerance whereby fragments of mixed self and pathogen antigens may induce the immune response, as in a mode of epitope spread and autoantibody production[10-11]. Epitope spread occurs when there is chronic inflammation, causing the immune system to produce a variety of antibodies against pathogens of chronic infections. Active TB is one of the most common infectious diseases causing long-term inflammation and tissue destruction. Thus, it is reasonable that autoantibodies are more common in TB patients.

Clinically detectable autoantibodies are often characteristic of certain

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4 autoimmune diseases[12]. However, all autoantibodies, like other clinical
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7 examinations, should be tested under corresponding clinical symptoms and signs.
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10 Most autoantibodies are pathognomonic of certain conditions, such as anti-dsDNA in
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12 SLE [13], anti-cyclic citrullinated peptide antibody in rheumatoid arthritis[7, 14],
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14 anti-Scl-70 in systemic sclerosis[15], and anti-Jo-1 in polymyositis[16]. However,
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16 autoantibodies may also be present in conditions other than autoimmune diseases,
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18 especially when there are no corresponding clinical symptoms, such as rheumatoid
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20 factor in infective endocarditis [17] and anti-phospholipid syndrome secondary to
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22 infection or malignancy[18].
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30 The high prevalence of serum autoantibodies in active TB patients has rarely
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32 been investigated and its explanation remains unclear. If the autoantibodies are
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34 pathognomonic, there should be corresponding rheumatologic symptoms and signs,
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36 especially in those with specific anti-extractable nuclear antigen antibodies. The
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38 presence of autoantibodies does not alter the clinical manifestations and radiographic
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40 presentations of active TB, implying that it is less likely for autoantibodies to have a
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42 pathognomonic role[2-5]. Usually, in autoimmune diseases, serology titers of
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44 autoantibodies change along with disease activity, such as anti-dsDNA in lupus
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46 nephritis[13]. However, the finding that autoantibody titers return to normal limits
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48 after anti-TB treatment in more than two-thirds of TB patients suggests an opposite
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explanation: that the increase in autoantibodies is a reactive change during the disease course of active TB. This is a reminder of a very important point. If a patient is screened and found to have elevated autoantibodies but without corresponding rheumatology symptoms, other possible etiologies should be considered. In TB endemic areas, pulmonary TB should be a very important differential diagnosis. If TB is missed TB and patients are treated as autoimmune diseases using systemic corticosteroids, disseminated TB and TB-related mortality may occur.

The association of active TB and anti-cardiolipin IgG and anti-topoisomerase I has never been reported in literature. Anti-cardiolipin antibody is one of the hallmarks of anti-phospholipid syndrome, which is well known to be secondary to infectious disease[18]. Such antibody may be triggered by an infectious process which is non- β_2 -glycoprotein 1-dependent. In active TB, mycobacterial phenolic glycolipids may activate neutrophils and release containers of ectosomes[19-20]. Vesicles released from activated neutrophils express phosphatidylserine and annexin V may be the epitopes of anti-phospholipid antibodies[21-22]. This may explain the high prevalence of anti-cardiolipin IgG in TB patients. In general, the transient positivity of anti-phospholipid antibody is not considered pathognomonic and does not fulfill the diagnostic criteria of anti-phospholipid syndrome[18].

However, some studies have different observations and show that TB patients

with diffuse alveolar hemorrhage have transient positivity of anti-cardiolipin IgG[23-24]. Moreover, in the present study, anti-cardiolipin IgG remained elevated in four of 11 patients after three months of anti-TB treatment. Although anti-cardiolipin IgG is not diagnostic, a high index of suspicion should be maintained on the clinical manifestations of anti-phospholipid antibody, including thrombosis, hemorrhage, hemolytic anemia, and thrombocytopenia [18, 25] in TB patients with elevated anti-cardiolipin IgG.

Anti-Scl-70 is the first known prevalent autoantibody in patients with progressive systemic sclerosis. The autoantibody targets an antigen that is 70 kDa protein. Later studies reveal that Scl-70 should be the nuclear DNA topoisomerase I[26-27]. Topoisomerase I is an enzyme that relaxes the strain on DNA by nicking and ligating it. One of the notorious manifestations of systemic sclerosis is interstitial lung disease, characterized by lung fibrosis[28]. Although there is no significant difference, the TB patients in this study have elevated anti-Scl 70 and a higher risk of pulmonary cavitation and pleural effusion than the others. The underlying mechanism for the elevated anti-Scl-70 in TB patients is unclear since antigenic topoisomerase I is not a content of leukocyte ectosome[20]. Moreover, it is not released from the TB bacilli because the structure of mycobacterial topoisomerase I is different from those of humans[29-30].

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In the present study, anti-Scl-70 titers returned to normal range after anti-TB treatment in all except one patient. The anti-Scl-70 antibody in active TB is probably secondary to pulmonary inflammation and destruction, which is uncovered by the nuclear topoisomerase I, and trigger the production of autoimmunity. The titer decreases once pulmonary injury is alleviated.

The study has some limitations. First, because using medical staff as the control group, the baseline characteristics were very different from those in the TB group. This may lead to underestimate in the prevalence of autoantibody in non-TB group. However, the autoantibody prevalence was still higher in TB patients than that in general population reported in previous publications[9]. Second, we only obtained follow-up samples in those with elevated serum level of autoantibody before anti-TB treatment. Therefore, the dynamic change of autoantibody may be missed.

Tuberculosis does have a kaleidoscopic of presentations that constantly challenge physicians. In a TB endemic area, there is a significant proportion (32%) of TB patients with elevated autoantibody titers, especially anti-cardiolipin IgG and anti-Scl-70. The phenomenon is likely to be reactive due to lack of clinical correlations and spontaneous regression after control of TB activity. In a TB endemic area, sputum samples for acid-fast smear and mycobacterial culture should be taken in patients with elevated serum autoantibody titers but without the corresponding

rheumatology symptoms.

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Table 1. Prevalence of autoantibody in tuberculosis (TB) patients, healthy medical staff and the general population [9]

Autoantibody	Normal population		TB patients (n=100)	Healthy control (n=100)	<i>p</i> value		
	(n=2181)				3 group	TB vs. normal	TB vs. control
	ELISA-pos	% Pos					
Anti-Ro	58	2.7	2	2	0.855	1	1
Anti-La	5	0.2	0	0	0.795	1	1
Anti-RNP	11	0.5	2	1	0.138	0.108	1
Anti-Sm	0	0	0	0		1	1
Anti-Scl-70	0	0	6	3	< 0.001	< 0.001	0.498
Anti-Jo1	0	0	1	1	< 0.001	0.044	1
Anti-dsDNA	10	0.5	1	0	0.579	0.39	1
Anti-centromere	30	1.4	5	2	0.101	0.059	0.683
Anti-histone	NA		11	7			0.323
Anti-cardiolipin IgM	NA		6	10			0.297
Anti-cardiolipin IgG	NA		11	0			0.001

Table 2. Clinical manifestations in autoantibody-positive and -negative tuberculosis

(TB) patients			
	Autoantibody-positive (n=32)	Autoantibody-negative (n=68)	<i>p</i> value
Respiratory symptoms	22 (69%)	46 (68%)	0.912
Cough	9 (28%)	16 (23%)	0.621
Sputum	4 (13%)	13 (19%)	0.411
Hemoptysis	2 (6%)	3 (4%)	0.654
Dyspnea	5 (16%)	10 (15%)	>0.999
Chest pain	2 (6%)	4 (6%)	>0.999
Constitutional symptoms	14 (44%)	21 (31%)	0.208
Fever	5 (16%)	9 (13%)	0.763
Weight loss	4 (13%)	6 (9%)	0.722
General malaise	3 (9%)	3 (4%)	0.381
Night sweating	2 (6%)	3 (4%)	0.654
Sputum smear grading			
3+ ~ 4+	2 (6%)	8 (12%)	0.495
1+ ~ 2+	6 (19%)	15 (22%)	0.705
Negative	24 (69%)	45 (66%)	0.373
Serum albumin < 3.5 g/dL	6 (19%)	7 (10%)	0.339
Radiographical findings			
Bilateral infiltration	10 (31%)	34 (50%)	0.078
Cavitations	1 (3%)	8 (12%)	0.265
Pleural effusion	5 (16%)	11 (16%)	0.944
Miliary lesion	0	3 (4%)	0.549

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Data are number (%).

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Table 3. Adverse events during anti-TB treatment in autoantibody-positive and -negative TB patients

	Autoantibody-positive (n=32)	Autoantibody-negative (n=68)	<i>p</i> value
Rheumatologic events	10 (31%)	28 (41%)	0.340
Gastrointestinal events	9 (28%)	22 (32%)	0.670
Neurologic events	8 (25%)	16 (24%)	0.872
Renal events	7 (22%)	19 (28%)	0.519
Constitutional events	4 (13%)	19 (28%)	0.087
Respiratory events	2 (6%)	3 (4%)	0.654
Hematologic events	1 (3%)	0	0.320
Total number of events	41	107	

Data are number (%).

Table 4. Serum autoantibody titers in tuberculosis (TB) patients before and after anti-TB treatment

Patient No.	Anti-Scl-70		Patient No.	ACA-IgG	
	Initial	Follow-up		Initial	Follow-up
No. 8	137	155	No. 18	18.1	9.3
			No. 19	33.2	2.6
No. 18	156	35	No. 36	12.3	4.1
			No. 48	14.1	6.7
No. 51	125	28	No. 55	10.8	13.4
			No. 60	23.8	2.6
No. 64	152	55	No. 62	31.1	4.8
			No. 72	18.9	30.9
No. 65	154	79	No. 81	14.6	23.5
			No. 92	21.2	21.1
No. 91	153	68	No. 94	12.8	5.1

Abbreviation: ACA, anti-cardiolipin antibody
Normal reference values for anti-Scl-70 and ACA-IgG are <100 U/mL and <12.5 GPL, respectively

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Authors' Contributions:

Dr. Jann-Yuan Wang and Prof. Song-Chou Hsieh designed the study. Dr. Chieh-Yu Shen, Prof. Chia-Li Yu, Prof. Song-Chou Hsieh, Dr. Jann-Yuan Wang, and Prof. Li-Na Lee all involve manuscript writing and data interpretation. Dr. Chieh-Yu Shen and Dr. Jann-Yuan Wang involve statistical analysis. Prof. Chong-Jen Yu is the director responsible for general organization and instruction.

Conflict of interest statements

All authors declare no financial, professional or other personal interest of any nature or kind in related product, service and/or company.

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Data Sharing

There was no additional unpublished data from this study.

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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Page No in Paper	Recommendation
Title and abstract	1	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		2	(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	4	Explain the scientific background and rationale for the investigation being reported
Objectives	3	4,5	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	6	Present key elements of study design early in the paper
Setting	5	6	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
			(b) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	6,7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/measurement	8*	6,7,8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	6,7,8	Describe any efforts to address potential sources of bias
Study size	10	6	Explain how the study size was arrived at
Quantitative variables	11	Not applicable	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	8	(a) Describe all statistical methods, including those used to control for confounding
		8	(b) Describe any methods used to examine subgroups and interactions
		Not applicable	(c) Explain how missing data were addressed
		Not applicable	(d) If applicable, explain how loss to follow-up was addressed
		8	(e) Describe any sensitivity analyses
Results			
Participants	13*	9, Table 2, Table 3	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		Not applicable	(b) Give reasons for non-participation at each stage
		Not applicable	(c) Consider use of a flow diagram
Descriptive data	14*	9, Table 2,	(a) Give characteristics of study participants (eg demographic,

		Table 3	clinical, social) and information on exposures and potential confounders
		Not applicable	(b) Indicate number of participants with missing data for each variable of interest
		9	(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	9,10, 11, Table 1,Table 4	Report numbers of outcome events or summary measures over time
Main results	16	9, 10, 11, Table 1, Table 4	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		Not applicable	(b) Report category boundaries when continuous variables were categorized
		Not applicable	(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Not applicable	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	11, 12, 13, 14, 15, 16	Summarise key results with reference to study objectives
Limitations	19	15	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	11, 12, 13, 14, 15, 16	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	11, 12, 13, 14, 15, 16	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	17	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.



Autoantibody Prevalence in Active Tuberculosis : Reactive or Pathognomonic?

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Autoantibody Prevalence in Active Tuberculosis: Reactive or Pathognomonic?

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Short title: Evident interaction between chronic active infection and autoimmunity

Key words: *anti-cardiolipin antibody, anti-topoisomerase I, autoantibody, autoimmune disease, tuberculosis*

Word count: Abstract-178; Text-2444

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ABSTRACT

Background: Autoantibodies are found in patients with active tuberculosis (TB) but lack clinical significance. This study aimed to evaluate if autoantibodies in TB are pathognomonic, reactive, or incidental.

Methods: One hundred active TB patients and 100 healthy controls were evaluated for serum autoantibodies, including anti-dsDNA, anti-Ro, anti-La, anti-Sm, anti-RNP, anti-Jo1, anti-Scl-70, anti-centromere, anti-histone, anti-cardiolipin IgG and anti-cardiolipin IgM. In patients with active TB, serum titres were re-evaluated after three months of anti-TB treatment.

Results: Of 100 patients with TB (median age, 63 years; 67 males), 32 had elevated serum autoantibody titres. The most commonly elevated were anti-cardiolipin IgG (n=11), anti-histone (n=11), and anti-Scl-70 (n=6). Compared to healthy controls and the general population, TB patients had significantly elevated anti-cardiolipin IgG and anti-Scl-70 levels. Autoantibody-positive and -negative TB patients had similar clinical manifestations of TB and adverse events during anti-TB treatment. Serum anti-cardiolipin IgG and anti-Scl-70 levels returned to normal in seven and five patients, respectively, after anti-TB treatment.

Conclusions: Autoantibody elevation is not pathognomonic of active TB. Caution must be taken in diagnosing autoimmune disease in TB patients.

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ARTICLE SUMMARY

Article focus

Because chronic active tuberculosis has immunogenicity, autoantibodies are often found in TB patients. Are there disease-specific autoantibodies in these patients? Are these autoantibodies pathognomonic even without the attendant symptomatology? Do they require immuno-suppressant therapy? Can these disease-specific autoantibodies be reactive to stimulation like tuberculosis even without corresponding symptoms?

Key messages

Disease-specific autoantibodies other than rheumatoid factor or anti-nuclear antibody exist in patients with tuberculosis. Autoantibody titres may decrease, even return to normal, as the infection is controlled. These findings suggest that autoantibodies are reactive to tuberculosis instead of being pathognomonic, and do not require immuno-suppressant therapy.

Strengths and limitations of this study

This is the first study to evaluate the clinical significance of autoantibodies by sequential data in tuberculosis. Despite the high probability of TB exposure and infection, medical staff serving as control subjects may have higher prevalences of autoantibodies.

INTRODUCTION

Tuberculosis (TB) has become one of the most important diseases in the past two decades. It leads to organ dysfunction, mortality, and various clinical manifestations. Previous studies have shown that sera from patients with active TB may contain autoantibodies that are unique in autoimmune diseases. The reported autoantibodies include rheumatoid factor (RF), anti-nuclear antibody (ANA), anti-cardiolipin antibody (ACA) (IgM isotype predominant), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-cyclic citrullinated peptide [1-4]. Some of these are hallmarks of certain autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus (SLE), and ANCA-associated vasculitis. Some are even disease-specific, such as anti-cyclic citrullinated peptide in rheumatoid arthritis [5, 6]. However, most studies are cross-sectional with limited case numbers and without definite correlations between serology and clinical manifestations. Little is known on the clinical significance (i.e., pathognomonic, reactive, or incidental) of autoantibodies in TB and the necessity of corticosteroid therapy.

Since TB patients present with non-specific symptoms like fever, malaise, and weight loss, clinicians may simultaneously order mycobacterial studies (acid-fast smear and mycobacterial culture) and autoimmune serology. Results of the latter usually become available earlier than results of the former. Thus, some TB patients

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are put on systemic corticosteroids rather than anti-TB treatment, rendering the further dissemination of *Mycobacterium tuberculosis* bacilli. In this prospective cohort study, the prevalence of autoantibodies in patients with active TB was evaluated and compared to those of healthy controls. Dynamic changes in the autoantibodies were also monitored to investigate their clinical significance in TB patients.

PATIENTS AND METHODS

Subjects and the study protocol

The Institutional Review Board of the National Taiwan University Hospital (NTUH) approved this study (NTUH REC: 9561707008). To have a power of 0.8 and an alpha error of 0.05 in a two-sided test where the prevalence of anti-nuclear antibody in TB patients and the general population was 33% and 20%, respectively [1], the calculated sample size was 83 for each. Therefore, from the 933 new cases of culture-confirmed TB diagnosed at the NTUH between January 2007 and December 2009, 100 were enrolled. All of the study participants provided written informed consent.

Among the 100 TB patients, 96 had pure pulmonary TB, two had concomitant pulmonary and extra-pulmonary TB (peritonitis in one and meningitis in another), and two had extra-pulmonary TB only (neck lymphadenopathy in one and cutaneous TB in another). The first serum samples were collected before the start of anti-TB treatment. Blood was examined for autoantibodies to the Ro antigen, La antigen, centromere protein, double-stranded DNA, topoisomerase I (Scl-70), Smith protein, ribonucleoprotein particle (RNP), histone protein, and histidyl-tRNA synthetase (Jo1). Anti-cardiolipin IgG and anti-cardiolipin IgM were also examined. For those with elevated serum autoantibody levels, follow-up serum samples were collected three

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months after anti-TB treatment to evaluate its effect on the autoantibody titres.

All of the TB patients received standard anti-TB treatment consisting of daily isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) in the first two months, followed by daily INH and RIF for the next four months [7]. The regimen was modified by the primary care physician if necessary. One hundred healthy medical staff members were enrolled as the control group.

The clinical parameters collected were age, sex, underlying disease, clinical manifestations, and radiographic findings of TB, as well as adverse events during anti-TB treatment. Respiratory symptoms included cough, sputum, hemoptysis, dyspnea, and chest pain, while constitutional symptoms were fever, weight loss, general malaise, and night sweats. The adverse events were classified into 7 categories: 1) rheumatologic, including cutaneous reaction and arthralgia; 2) gastrointestinal, including abnormal liver function, gastric discomfort, abdominal pain, and change in bowel movement; 3) constitutional, including fever, poor appetite, and malaise; 4) renal, including hyper-uricemia and impaired renal function; 5) neurologic, including blurred vision, insomnia, delirium, headache, and numbness; 6) respiratory, including cough, dyspnea, and chest pain; and 7) hematologic, including leukopenia, thrombocytopenia, and anaemia.

Because latent TB infection was more common in the medical staff than in the

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4 general population [8], 100 health care workers were recruited as the control group
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13 confounded by similar environment and genetic components as the TB cases.

14 15 **Detection of autoantibodies**

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18 A commercial test system AtheNA Multi-Lyte® ANA-II Plus Test System was used
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21 to test IgG class anti-extractable nuclear antigens, including autoantibodies to the Ro
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24 antigen, La antigen, centromere protein, double-stranded DNA, Scl- 70, Smith protein,
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27 RNP, histone protein, and Jo1. Serum samples were prepared at 1:21 dilution and
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33 manufacturer's instructions.

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36 A commercially available kit QUANTA Lite™ ACA IgM III was used to test
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39 anti-cardiolipin IgM. Sera were prepared at 1:101 dilution. The commercial kit
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42 Phadia® Varelisa Cardiolipin IgG Antibodies EIA kit was used to test anti-cardiolipin
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45 IgG at serum dilution of 1:101. All ELISA assays were performed according to the
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49 50 **Statistical analysis**

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as appropriate. Statistical significance was set at $p<0.05$. All analyses were performed using the SPSS software version 12.0 for Windows.

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RESULTS

The clinical characteristics of the 100 TB patients were summarized in Table 1.

The median age was 63 years (range, 19-92 years), with a male-to-female ratio of 2.03. Forty-one TB patients had underlying diseases such as malignancy (n=17), diabetes mellitus (n=14), end-stage renal disease (n=9), and one each with systemic lupus erythematosus (SLE), ankylosing spondylitis, and rheumatoid arthritis. Among the 17 TB patients with malignancies, six had lung cancer, three had hematologic malignancies, four had airway and lung malignancies, two had prostate cancer, one had breast cancer, and one had pancreatic cancer.

The SLE patient was a middle-aged woman who presented as polyarthritis, malar rash, and positive anti-dsDNA and anti-nuclear antibodies. She received disease modifying anti-rheumatic drugs (DMARDs) and corticosteroids. The patient with ankylosing spondylitis was a young woman diagnosed by clinical symptoms and positive HLA-B27. She received non-steroidal anti-inflammatory drugs (NSAIDs). The patient with rheumatoid arthritis was a young woman with symmetric polyarthritis with positive rheumatoid factor. She received DMARDs and NSAIDs. Before the diagnosis of pulmonary TB, results of autoantibody tests were all negative in these three patients.

Thirty-two TB patients had elevated serum autoantibody levels, including nine

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with more than one autoantibody (Table 1). The clinical manifestations and radiographic findings of the autoantibody-positive and -negative groups were similar. Cough and sputum production were the most common respiratory symptoms, while fever and weight loss were the most common constitutional symptoms. Thirty-one patients were sputum smear positive for acid-fast bacilli.

Detailed results of the autoantibody tests were shown in Table 2. The most prevalent autoantibodies in TB patients were anti-Scl-70, anti-histone, and anti-cardiolipin IgG. A significantly higher proportion of TB patients had elevated serum anti-cardiolipin IgG titres than the healthy controls ($p<0.001$). Compared to a previous report on the prevalence of autoantibodies in the general population [10], TB patients were more likely to have elevated serum titres of anti-Scl-70 ($p<0.05$) and anti-cardiolipin IgG ($p<0.001$). Among the anti-Scl-70-positive and -negative groups, 16% and 9%, respectively, had pulmonary cavitation ($p=0.441$) and 33% and 16%, respectively, had pleural effusion ($p=0.245$).

The median age of the healthy controls was 30 years, with a male-to-female ratio of 0.33. None had underlying diseases. Though not statistically significant, the control group had a higher prevalence of anti-cardiolipin IgM than the TB group (Table 2). Of the 10 healthy controls positive for anti-cardiolipin IgM, eight had borderline titres that just passed the cut-off value. Among them, one was positive for

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4 anti-RNP and another for anti-histone antibodies. Of the six TB patients positive for
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7 anti-cardiolipin IgM, four had borderline titres and one was positive for anti-histone
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12 Within three months of anti-TB treatment, 61 patients had 148 adverse events
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14 (Table 3). The most common were rheumatologic events, followed by gastrointestinal
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17 events. There was no significant difference in adverse events between
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21 autoantibody-positive and -negative TB patients.
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24 For the 11 patients with elevated anti-cardiolipin IgG and six patients with
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27 elevated anti-Scl-70 at baseline, serum titres were followed-up after three months of
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30 anti-TB treatment (Table 4). None of the patients received immunosuppressants and
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33 DMARDs. Follow-up serum titres of anti-cardiolipin IgG and anti-Scl-70 returned to
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36 normal limits in seven and four patients, respectively. Among the five patients with
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39 persistently elevated anti-cardiolipin IgG or anti-Scl-70 autoantibodies, none had
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42 rheumatologic symptoms at the end of the six-month anti-TB treatment.
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DISCUSSION

The present study has three important findings. First, one-third of patients with active TB had elevated serum autoantibodies. The prevalences of their autoantibodies, especially anti-cardiolipin IgG and anti-Scl-70, were significantly higher than those of the general population [10]. Second, consistent with previous studies [1-4], the presence of autoantibodies neither altered the clinical manifestations and radiographic findings of active TB nor changed the risk of developing adverse events during anti-TB treatment. Third, the elevated autoantibody levels returned to normal limits simply by anti-TB treatment and not by immunosuppressive therapy. These findings suggest that increased serum autoantibodies during active TB may not be diagnostic of autoimmune diseases. Clinical correlation and follow-up are still necessary.

Autoantibodies come from a break in self-tolerance whereby fragments of mixed self and pathogen antigens may induce immune response, as in a mode of epitope spread and autoantibody production [11, 12]. Epitope spread occurs when there is chronic inflammation, causing the immune system to produce a variety of antibodies against pathogens of chronic infections. Active TB is one of the most common infectious diseases causing long-term inflammation and tissue destruction. Thus, it is reasonable that autoantibodies are more common in TB patients.

Clinically detectable autoantibodies are often characteristic of certain autoimmune diseases [13]. However, like in other clinical examinations, all autoantibodies should be tested based on the corresponding clinical symptoms and signs. Most autoantibodies are pathognomonic of certain conditions, such as anti-dsDNA in SLE [14], anti-cyclic citrullinated peptide antibody in rheumatoid arthritis [6, 15], anti-Scl-70 in systemic sclerosis [16], and anti-Jo-1 in polymyositis [17]. However, autoantibodies may also be present in conditions other than autoimmune diseases, especially when there are no corresponding clinical symptoms, such as rheumatoid factor in infective endocarditis [18] and anti-phospholipid syndrome secondary to infection or malignancy[19].

The high prevalence of serum autoantibodies in active TB patients has rarely been investigated and thus, remains unclear. If the autoantibodies are pathognomonic, there should be corresponding rheumatologic symptoms and signs, especially in those with specific anti-extractable nuclear antigen antibodies. Since the presence of autoantibodies does not alter the clinical manifestations and radiographic presentations of active TB, it is unlikely that autoantibodies have a pathognomonic role [1-4]. In autoimmune diseases, serology titres of autoantibodies often change along with disease activity, such as anti-dsDNA in lupus nephritis [14]. However, the finding that autoantibody titres return to normal limits after anti-TB

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treatment in more than two-thirds of TB patients suggests the opposite - that the increase in autoantibodies is a reactive change during the disease course of active TB.

This is a very important reminder.

If a patient has elevated autoantibody levels but no typical or multiple rheumatologic symptoms, other possible aetiologies should be considered. For a patient in TB endemic areas, sputum samples for acid-fast smear and mycobacterial culture should be performed. If TB is missed and patients are diagnosed as having autoimmune diseases and treated with systemic corticosteroids, disseminated TB and TB-related mortality may occur.

The association of active TB and anti-cardiolipin IgG and anti-topoisomerase I has not been previously reported. Anti-cardiolipin antibody is one of the hallmarks of anti-phospholipid syndrome, which is known to be secondary to infectious diseases [19]. Such antibody may be triggered by an infectious process that is non- β_2 -glycoprotein 1-dependent. In active TB, mycobacterial phenolic glycolipids may activate neutrophils and release containers of ectosomes [20, 21]. Vesicles released from activated neutrophils that express phosphatidylserine and annexin V may be the epitopes of anti-phospholipid antibodies [22, 23]. This may explain the high prevalence of anti-cardiolipin IgG in TB patients. In general, the transient positivity of anti-phospholipid antibody is not considered pathognomonic and does not meet the

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4 diagnostic criteria of anti-phospholipid syndrome [19].
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7 However, some studies have different findings and show that TB patients with
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10 diffuse alveolar haemorrhage have transient anti-cardiolipin IgG positivity [24, 25].
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12 Moreover, in the present study, anti-cardiolipin IgG remained elevated in four of 11
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14 patients even after three months of anti-TB treatment. Although anti-cardiolipin IgG
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16 is not diagnostic, a high index of suspicion should be maintained regarding their
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18 clinical manifestations, including thrombosis, haemorrhage, haemolytic anaemia,
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20 and thrombocytopenia [19, 26] in TB patients with elevated anti-cardiolipin IgG.
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27 Anti-Scl-70 is the first known prevalent autoantibody in patients with
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29 progressive systemic sclerosis. The autoantibody targets an antigen that is a 70 kDa
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31 protein. Later studies reveal that Scl-70 should be the nuclear DNA topoisomerase I
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33 [27, 28]. Topoisomerase I is an enzyme that relaxes the strain on DNA by nicking and
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35 ligating it. One of the notorious manifestations of systemic sclerosis is interstitial lung
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37 disease, characterized by lung fibrosis [29]. Although there is no significant difference,
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39 the TB patients in this study have elevated anti-Scl 70 and higher risk of pulmonary
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41 cavitation and pleural effusion. The underlying mechanisms involved are unclear
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43 since antigenic topoisomerase I is not a component of leukocyte ectosome [21].
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47 Moreover, it is not released from the TB bacilli because the structure of
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49 mycobacterial topoisomerase I is different from those of humans [30, 31].
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In the present study, anti-Scl-70 titres returned to normal range after anti-TB treatment in all except one patient. The anti-Scl-70 antibody in active TB is probably secondary to pulmonary inflammation and destruction, which is uncovered by the nuclear topoisomerase I, thereby triggering the production of autoimmunity. The titre decreases once pulmonary injury is alleviated.

This study has some limitations. First, because the control group was composed of medical staff members, their baseline characteristics were very different from those of the TB group. This might lead to uncertainty in the prevalence of autoantibody in non-TB groups. Nonetheless, the autoantibody prevalence was still higher in TB patients than in the general population reported in a previous publication [10]. Second, follow-up samples were only obtained in those with elevated serum levels of autoantibodies before anti-TB treatment. Dynamic changes in autoantibodies might have been missed.

Tuberculosis has a kaleidoscopic of presentations that constantly challenge physicians. In TB endemic areas, a significant proportion (32%) of TB patients has elevated autoantibody titres, especially anti-cardiolipin IgG and anti-Scl-70. This phenomenon is likely to be reactive due to the lack of clinical correlations, as well as the spontaneous regression after TB treatment. Mycobacterial studies should be performed in patients with elevated serum autoantibody titres but without the

typical or multiple manifestations of autoimmune diseases.

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Table 1. Clinical characteristics of autoantibody-positive and -negative tuberculosis

(TB) patients			
	Autoantibody-positive (n=32)	Autoantibody-negative (n=68)	<i>p</i> value
Respiratory symptoms	22 (69%)	46 (68%)	0.912
Cough	9 (28%)	16 (23%)	0.621
Sputum	4 (13%)	13 (19%)	0.411
Hemoptysis	2 (6%)	3 (4%)	0.654
Dyspnea	5 (16%)	10 (15%)	>0.999
Chest pain	2 (6%)	4 (6%)	>0.999
Constitutional symptoms	14 (44%)	21 (31%)	0.208
Fever	5 (16%)	9 (13%)	0.763
Weight loss	4 (13%)	6 (9%)	0.722
General malaise	3 (9%)	3 (4%)	0.381
Night sweating	2 (6%)	3 (4%)	0.654
Sputum smear grading			
3+ ~ 4+	2 (6%)	8 (12%)	0.495
1+ ~ 2+	6 (19%)	15 (22%)	0.705
Negative	24 (69%)	45 (66%)	0.373
Serum albumin < 3.5 g/dL	6 (19%)	7 (10%)	0.339
Radiographic findings			
Bilateral infiltration	10 (31%)	34 (50%)	0.078
Cavitations	1 (3%)	8 (12%)	0.265
Pleural effusion	5 (16%)	11 (16%)	0.944
Miliary lesion	0	3 (4%)	0.549

Data are number (%).

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Table 2. Prevalences of autoantibodies in tuberculosis (TB) patients, healthy controls and the general population [10]

Autoantibody	Normal population		TB patients (n=100)	Healthy control (n=100)	<i>p</i> value		
	(n=2181)				3 group	TB vs. normal	TB vs. control
	ELISA-pos	% Pos					
Anti-Ro	58	2.7	2	2	0.855	1	1
Anti-La	5	0.2	0	0	0.795	1	1
Anti-RNP	11	0.5	2	1	0.138	0.108	1
Anti-Sm	0	0	0	0		1	1
Anti-Scl-70	0	0	6	3	<0.001	<0.001	0.498
Anti-Jo1	0	0	1	1	<0.001	0.044	1
Anti-dsDNA	10	0.5	1	0	0.579	0.39	1
Anti-centromere	30	1.4	5	2	0.101	0.059	0.683
Anti-histone	NA		11	7			0.323
Anti-cardiolipin IgM	NA		6	10			0.297
Anti-cardiolipin IgG	NA		11	0			0.001

Table 3. Adverse events during anti-TB treatment in autoantibody-positive and -negative TB patients

	Autoantibody-positive (n=32)	Autoantibody-negative (n=68)	<i>p</i> value
Rheumatologic events	10 (31%)	28 (41%)	0.340
Gastrointestinal events	9 (28%)	22 (32%)	0.670
Neurologic events	8 (25%)	16 (24%)	0.872
Renal events	7 (22%)	19 (28%)	0.519
Constitutional events	4 (13%)	19 (28%)	0.087
Respiratory events	2 (6%)	3 (4%)	0.654
Hematologic events	1 (3%)	0	0.320
Total number of events	41	107	

Data are number (%).

Table 4. Serum autoantibody titres in tuberculosis (TB) patients before and after

anti-TB treatment

Patient No.	Anti-Scl-70		Patient No.	ACA-IgG	
	Initial	Follow-up		Initial	Follow-up
No. 8	137	155	No. 18	18.1	9.3
			No. 19	33.2	2.6
No. 18	156	35	No. 36	12.3	4.1
			No. 48	14.1	6.7
No. 51	125	28	No. 55	10.8	13.4
			No. 60	23.8	2.6
No. 64	152	55	No. 62	31.1	4.8
			No. 72	18.9	30.9
No. 65	154	79	No. 81	14.6	23.5
			No. 92	21.2	21.1
No. 91	153	68	No. 94	12.8	5.1

Abbreviation: ACA, anti-cardiolipin antibody

Normal reference values for anti-Scl-70 and ACA-IgG are <100 U/mL and <10 GPL, respectively.

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Author Contributions

Dr. Jann-Yuan Wang and Prof. Song-Chou Hsieh designed the study. Dr. Chieh-Yu Shen, Prof. Chia-Li Yu, Prof. Song-Chou Hsieh, Dr. Jann-Yuan Wang, and Prof. Li-Na Lee all contributed to the manuscript writing and data interpretation. Dr. Chieh-Yu Shen and Dr. Jann-Yuan Wang were involved in the statistical analysis. Prof. Chong-Jen Yu was directly responsible for the general organization and instruction.

Conflict of interest disclosure

All authors declare no financial, professional or other personal interest of any nature or kind in related products, services and/or companies.

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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Page No in Paper	Recommendation
Title and abstract	1	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		2	(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	4, 5	Explain the scientific background and rationale for the investigation being reported
Objectives	3	4,5	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	6	Present key elements of study design early in the paper
Setting	5	6	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	6, 7	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		Not applicable	(b) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	6,7,8	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8	7,8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	8	Describe any efforts to address potential sources of bias
Study size	10	5	Explain how the study size was arrived at
Quantitative variables	11	8,9	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	9	(a) Describe all statistical methods, including those used to control for confounding
		not applicable	(b) Describe any methods used to examine subgroups and interactions
		not applicable	(c) Explain how missing data were addressed
		not applicable	(d) If applicable, explain how loss to follow-up was addressed
		not applicable	(e) Describe any sensitivity analyses
Results			
Participants	13	10	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		Not applicable	(b) Give reasons for non-participation at each stage
		Not applicable	(c) Consider use of a flow diagram
Descriptive data	14	10, table 1, table 3	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		not applicable	(b) Indicate number of participants with missing data for each variable of interest
		Not applica	(c) Summarise follow-up time (eg, average and total amount)

Outcome data	15	10,11,12	Report numbers of outcome events or summary measures over time
Main results	16	10, 11, 12, Table 2, Table 4	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		Not applicable	(b) Report category boundaries when continuous variables were categorized
		Not relevant	(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Table 1, table 3	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	13	Summarise key results with reference to study objectives
Limitations	19	17	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	13, 14,15,16,17	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	17,18	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	24	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.

Autoantibody Prevalence in Active Tuberculosis: Reactive or Pathognomonic?

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ABSTRACT

Background: Autoantibodies are found in patients with active tuberculosis (TB) but lack clinical significance. This study aimed to evaluate if autoantibodies in TB are pathognomonic, reactive, or incidental.

Methods: One hundred active TB patients and 100 healthy controls were evaluated for serum autoantibodies, including anti-dsDNA, anti-Ro, anti-La, anti-Sm, anti-RNP, anti-Jo1, anti-Scl-70, anti-centromere, anti-histone, anti-cardiolipin IgG and anti-cardiolipin IgM. In patients with active TB, serum titres were re-evaluated after three months of anti-TB treatment.

Results: Of 100 patients with TB (median age, 63 years; 67 males), 32 had elevated serum autoantibody titres. The most commonly elevated were anti-cardiolipin IgG (n=11), anti-histone (n=11), and anti-Scl-70 (n=6). Compared to healthy controls and the general population, TB patients had significantly elevated anti-cardiolipin IgG and anti-Scl-70 levels. Autoantibody-positive and -negative TB patients had similar clinical manifestations of TB and adverse events during anti-TB treatment. Serum anti-cardiolipin IgG and anti-Scl-70 levels returned to normal in seven and five patients, respectively, after anti-TB treatment.

Conclusions: Autoantibody elevation is not pathognomonic of active TB. Caution must be taken in diagnosing autoimmune disease in TB patients.

ARTICLE SUMMARY

Article focus

Because chronic active tuberculosis has immunogenicity, autoantibodies are often found in TB patients. Are there disease-specific autoantibodies in these patients? Are these autoantibodies pathognomonic even without the attendant symptomatology? Do they require immuno-suppressant therapy? Can these disease-specific autoantibodies be reactive to stimulation like tuberculosis even without corresponding symptoms?

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Key messages

Disease-specific autoantibodies other than rheumatoid factor or anti-nuclear antibody exist in patients with tuberculosis. Autoantibody titres may decrease, even return to normal, as the infection is controlled. These findings suggest that autoantibodies are reactive to tuberculosis instead of being pathognomonic, and do not require immuno-suppressant therapy.

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Comment [u3]: We add this phrase for better explanation our purpose.

Strengths and limitations of this study

This is the first study to evaluate the clinical significance of autoantibodies by sequential data in tuberculosis. Despite the high probability of TB exposure and infection, medical staff serving as control subjects may have higher prevalences of autoantibodies.

Comment [u4]: We rewrite this part to clarify our strength and limitation.

INTRODUCTION

Tuberculosis (TB) has become one of the most important diseases in the past two decades. It leads to organ dysfunction, mortality, and various clinical manifestations. Previous studies have shown that sera from patients with active TB may contain autoantibodies that are unique in autoimmune diseases. The reported autoantibodies include rheumatoid factor (RF), anti-nuclear antibody (ANA), anti-cardiolipin antibody (ACA) (IgM isotype predominant), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-cyclic citrullinated peptide [1-4]. Some of these are hallmarks of certain autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus (SLE), and ANCA-associated vasculitis. Some are even disease-specific, such as anti-cyclic citrullinated peptide in rheumatoid arthritis [5, 6]. However, most studies are cross-sectional with limited case numbers and without definite correlations between serology and clinical manifestations. Little is known on the clinical significance (i.e., pathognomonic, reactive, or incidental) of autoantibodies in TB and the necessity of corticosteroid therapy.

Since TB patients present with non-specific symptoms like fever, malaise, and weight loss, clinicians may simultaneously order mycobacterial studies (acid-fast smear and mycobacterial culture) and autoimmune serology. Results of the latter usually become available earlier than results of the former. Thus, some TB patients

are put on systemic corticosteroids rather than anti-TB treatment, rendering the further dissemination of *Mycobacterium tuberculosis* bacilli. In this prospective cohort study, the prevalence of autoantibodies in patients with active TB was evaluated and compared to those of healthy controls. Dynamic changes in the autoantibodies were also monitored to investigate their clinical significance in TB patients.

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PATIENTS AND METHODS

Subjects and the study protocol

The Institutional Review Board of the National Taiwan University Hospital (NTUH) approved this study (NTUH REC: 9561707008). To have a power of 0.8 and an alpha error of 0.05 in a two-sided test where the prevalence of anti-nuclear antibody in TB patients and the general population was 33% and 20%, respectively [1], the calculated sample size was 83 for each. Therefore, from the 933 new cases of culture-confirmed TB diagnosed at the NTUH between January 2007 and December 2009, 100 were enrolled. All of the study participants provided written informed consent.

Comment [u6]: We add this paragraph to explain the sample size chosen and institution review board.

Among the 100 TB patients, 96 had pure pulmonary TB, two had concomitant pulmonary and extra-pulmonary TB (peritonitis in one and meningitis in another), and two had extra-pulmonary TB only (neck lymphadenopathy in one and cutaneous TB in another). The first serum samples were collected before the start of anti-TB treatment. Blood was examined for autoantibodies to the Ro antigen, La antigen, centromere protein, double-stranded DNA, topoisomerase I (Scl-70), Smith protein, ribonucleoprotein particle (RNP), histone protein, and histidyl-tRNA synthetase (Jo1). Anti-cardiolipin IgG and anti-cardiolipin IgM were also examined. For those with elevated serum autoantibody levels, follow-up serum samples were collected three

months after anti-TB treatment to evaluate its effect on the autoantibody titres.

All of the TB patients received standard anti-TB treatment consisting of daily isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) in the first two months, followed by daily INH and RIF for the next four months [7]. The regimen was modified by the primary care physician if necessary. One hundred healthy medical staff members were enrolled as the control group.

The clinical parameters collected were age, sex, underlying disease, clinical manifestations, and radiographic findings of TB, as well as adverse events during anti-TB treatment. Respiratory symptoms included cough, sputum, hemoptysis, dyspnea, and chest pain, while constitutional symptoms were fever, weight loss, general malaise, and night sweats. The adverse events were classified into 7 categories: 1) rheumatologic, including cutaneous reaction and arthralgia; 2) gastrointestinal, including abnormal liver function, gastric discomfort, abdominal pain, and change in bowel movement; 3) constitutional, including fever, poor appetite, and malaise; 4) renal, including hyper-uricemia and impaired renal function; 5) neurologic, including blurred vision, insomnia, delirium, headache, and numbness; 6) respiratory, including cough, dyspnea, and chest pain; and 7) hematologic, including leukopenia, thrombocytopenia, and anaemia.

Because latent TB infection was more common in the medical staff than in the

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general population [8], 100 health care workers were recruited as the control group for comparison. Household contacts of TB patients might also have a high probability of latent TB infection but if relatives were used as control[9], the results might be confounded by similar environment and genetic components as the TB cases.

Detection of autoantibodies

A commercial test system AtheNA Multi-Lyte® ANA-II Plus Test System was used to test IgG class anti-extractable nuclear antigens, including autoantibodies to the Ro antigen, La antigen, centromere protein, double-stranded DNA, Scl- 70, Smith protein, RNP, histone protein, and Jo1. Serum samples were prepared at 1:21 dilution and enzyme-linked immuno-sorbent assay (ELISA) was performed according to the manufacturer’s instructions.

A commercially available kit QUANTA Lite™ ACA IgM III was used to test anti-cardiolipin IgM. Sera were prepared at 1:101 dilution. The commercial kit Phadia® Varelisa Cardiolipin IgG Antibodies EIA kit was used to test anti-cardiolipin IgG at serum dilution of 1:101. All ELISA assays were performed according to the manufacturers’ instructions.

Statistical analysis

Inter-group difference was calculated using independent-samples *t* test for continuous variables and *chi*-square test or Fisher exact test for categorical variables,

Comment [u7]: We add this paragraph to explain reason for control group.

as appropriate. Statistical significance was set at $p < 0.05$. All analyses were performed using the SPSS software version 12.0 for Windows.

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RESULTS

The clinical characteristics of the 100 TB patients were summarized in Table 1.

Comment [u8]: The clinical characteristic was added at table 1.

The median age was 63 years (range, 19-92 years), with a male-to-female ratio of 2.03. Forty-one TB patients had underlying diseases such as malignancy (n=17), diabetes mellitus (n=14), end-stage renal disease (n=9), and one each with systemic lupus erythematosus (SLE), ankylosing spondylitis, and rheumatoid arthritis. Among the 17 TB patients with malignancies, six had lung cancer, three had hematologic malignancies, four had airway and lung malignancies, two had prostate cancer, one had breast cancer, and one had pancreatic cancer.

The SLE patient was a middle-aged woman who presented as polyarthritis, malar rash, and positive anti-dsDNA and anti-nuclear antibodies. She received disease modifying anti-rheumatic drugs (DMARDs) and corticosteroids. The patient with ankylosing spondylitis was a young woman diagnosed by clinical symptoms and positive HLA-B27. She received non-steroidal anti-inflammatory drugs (NSAIDs). The patient with rheumatoid arthritis was a young woman with symmetric polyarthritis with positive rheumatoid factor. She received DMARDs and NSAIDs. Before the diagnosis of pulmonary TB, results of autoantibody tests were all negative in these three patients.

Thirty-two TB patients had elevated serum autoantibody levels, including nine

with more than one autoantibody (Table 1). The clinical manifestations and radiographic findings of the autoantibody-positive and -negative groups were similar. Cough and sputum production were the most common respiratory symptoms, while fever and weight loss were the most common constitutional symptoms. Thirty-one patients were sputum smear positive for acid-fast bacilli.

Comment [u9]: We summarized the examination result.

Detailed results of the autoantibody tests were shown in Table 2. The most prevalent autoantibodies in TB patients were anti-Scl-70, anti-histone, and anti-cardiolipin IgG. A significantly higher proportion of TB patients had elevated serum anti-cardiolipin IgG titres than the healthy controls ($p<0.001$). Compared to a previous report on the prevalence of autoantibodies in the general population [10], TB patients were more likely to have elevated serum titres of anti-Scl-70 ($p<0.05$) and anti-cardiolipin IgG ($p<0.001$). Among the anti-Scl-70-positive and -negative groups, 16% and 9%, respectively, had pulmonary cavitation ($p=0.441$) and 33% and 16%, respectively, had pleural effusion ($p=0.245$).

Comment [u10]: We adjusted the detailed result at table 2.

The median age of the healthy controls was 30 years, with a male-to-female ratio of 0.33. None had underlying diseases. Though not statistically significant, the control group had a higher prevalence of anti-cardiolipin IgM than the TB group (Table 2). Of the 10 healthy controls positive for anti-cardiolipin IgM, eight had borderline titres that just passed the cut-off value. Among them, one was positive for

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anti-RNP and another for anti-histone antibodies. Of the six TB patients positive for anti-cardiolipin IgM, four had borderline titres and one was positive for anti-histone antibody.

Comment [u11]: We add this paragraph for detail of control group and result of anticardiolipin IgM.

Within three months of anti-TB treatment, 61 patients had 148 adverse events (Table 3). The most common were rheumatologic events, followed by gastrointestinal events. There was no significant difference in adverse events between autoantibody-positive and -negative TB patients.

For the 11 patients with elevated anti-cardiolipin IgG and six patients with elevated anti-Scl-70 at baseline, serum titres were followed-up after three months of anti-TB treatment (Table 4). None of the patients received immunosuppressants and DMARDs. Follow-up serum titres of anti-cardiolipin IgG and anti-Scl-70 returned to normal limits in seven and four patients, respectively. Among the five patients with persistently elevated anti-cardiolipin IgG or anti-Scl-70 autoantibodies, none had rheumatologic symptoms at the end of the six-month anti-TB treatment.

DISCUSSION

The present study has three important findings. First, one-third of patients with active TB had elevated serum autoantibodies. The prevalences of their autoantibodies, especially anti-cardiolipin IgG and anti-Scl-70, were significantly higher than those of the general population [10]. Second, consistent with previous studies [1-4], the presence of autoantibodies neither altered the clinical manifestations and radiographic findings of active TB nor changed the risk of developing adverse events during anti-TB treatment. Third, the elevated autoantibody levels returned to normal limits simply by anti-TB treatment and not by immunosuppressive therapy. These findings suggest that increased serum autoantibodies during active TB may not be diagnostic of autoimmune diseases. Clinical correlation and follow-up are still necessary.

Comment [u12]: We add this paragraph to emphasize our point.

Autoantibodies come from a break in self-tolerance whereby fragments of mixed self and pathogen antigens may induce immune response, as in a mode of epitope spread and autoantibody production [11, 12]. Epitope spread occurs when there is chronic inflammation, causing the immune system to produce a variety of antibodies against pathogens of chronic infections. Active TB is one of the most common infectious diseases causing long-term inflammation and tissue destruction. Thus, it is reasonable that autoantibodies are more common in TB patients.

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Clinically detectable autoantibodies are often characteristic of certain autoimmune diseases [13]. However, like in other clinical examinations, all autoantibodies should be tested based on the corresponding clinical symptoms and signs. Most autoantibodies are pathognomonic of certain conditions, such as anti-dsDNA in SLE [14], anti-cyclic citrullinated peptide antibody in rheumatoid arthritis [6, 15], anti-Scl-70 in systemic sclerosis [16], and anti-Jo-1 in polymyositis [17]. However, autoantibodies may also be present in conditions other than autoimmune diseases, especially when there are no corresponding clinical symptoms, such as rheumatoid factor in infective endocarditis [18] and anti-phospholipid syndrome secondary to infection or malignancy[19].

The high prevalence of serum autoantibodies in active TB patients has rarely been investigated and thus, remains unclear. If the autoantibodies are pathognomonic, there should be corresponding rheumatologic symptoms and signs, especially in those with specific anti-extractable nuclear antigen antibodies. Since the presence of autoantibodies does not alter the clinical manifestations and radiographic presentations of active TB, it is unlikely that autoantibodies have a pathognomonic role [1-4]. In autoimmune diseases, serology titres of autoantibodies often change along with disease activity, such as anti-dsDNA in lupus nephritis [14]. However, the finding that autoantibody titres return to normal limits after anti-TB

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7 treatment in more than two-thirds of TB patients suggests the opposite - that the
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9 increase in autoantibodies is a reactive change during the disease course of active TB.

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11 This is a very important reminder.

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14 If a patient has elevated autoantibody levels but no typical or multiple
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16 rheumatologic symptoms, other possible aetiologies should be considered. For a
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18 patient in TB endemic areas, sputum samples for acid-fast smear and mycobacterial
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20 culture should be performed. If TB is missed and patients are diagnosed as having
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22 autoimmune diseases and treated with systemic corticosteroids, disseminated TB
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24 and TB-related mortality may occur.

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summary and highlight our point.

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30 The association of active TB and anti-cardiolipin IgG and anti-topoisomerase I
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32 has not been previously reported. Anti-cardiolipin antibody is one of the hallmarks of
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34 anti-phospholipid syndrome, which is known to be secondary to infectious diseases
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36 [19]. Such antibody may be triggered by an infectious process that is non-
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38 β_2 -glycoprotein 1-dependent. In active TB, mycobacterial phenolic glycolipids may
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40 activate neutrophils and release containers of ectosomes [20, 21]. Vesicles released
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42 from activated neutrophils that express phosphatidylserine and annexin V may be
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44 the epitopes of anti-phospholipid antibodies [22, 23]. This may explain the high
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46 prevalence of anti-cardiolipin IgG in TB patients. In general, the transient positivity of
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48 anti-phospholipid antibody is not considered pathognomonic and does not meet the
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diagnostic criteria of anti-phospholipid syndrome [19].

However, some studies have different findings and show that TB patients with diffuse alveolar haemorrhage have transient anti-cardiolipin IgG positivity [24, 25]. Moreover, in the present study, anti-cardiolipin IgG remained elevated in four of 11 patients even after three months of anti-TB treatment. Although anti-cardiolipin IgG is not diagnostic, a high index of suspicion should be maintained regarding their clinical manifestations, including thrombosis, haemorrhage, haemolytic anaemia, and thrombocytopenia [19, 26] in TB patients with elevated anti-cardiolipin IgG.

Anti-Scl-70 is the first known prevalent autoantibody in patients with progressive systemic sclerosis. The autoantibody targets an antigen that is a 70 kDa protein. Later studies reveal that Scl-70 should be the nuclear DNA topoisomerase I [27, 28]. Topoisomerase I is an enzyme that relaxes the strain on DNA by nicking and ligating it. One of the notorious manifestations of systemic sclerosis is interstitial lung disease, characterized by lung fibrosis [29]. Although there is no significant difference, the TB patients in this study have elevated anti-Scl 70 and higher risk of pulmonary cavitation and pleural effusion. The underlying mechanisms involved are unclear since antigenic topoisomerase I is not a component of leukocyte ectosome [21]. Moreover, it is not released from the TB bacilli because the structure of mycobacterial topoisomerase I is different from those of humans [30, 31].

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7 In the present study, anti-Scl-70 titres returned to normal range after anti-TB
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9 treatment in all except one patient. The anti-Scl-70 antibody in active TB is probably
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11 secondary to pulmonary inflammation and destruction, which is uncovered by the
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13 nuclear topoisomerase I, thereby triggering the production of autoimmunity. The
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15 titre decreases once pulmonary injury is alleviated.
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19 This study has some limitations. First, because the control group was composed
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21 of medical staff members, their baseline characteristics were very different from
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23 those of the TB group. This might lead to uncertainty in the prevalence of
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25 autoantibody in non-TB groups. Nonetheless, the autoantibody prevalence was still
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27 higher in TB patients than in the general population reported in a previous
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29 publication [10]. Second, follow-up samples were only obtained in those with
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40 Tuberculosis has a kaleidoscopic of presentations that constantly challenge
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42 physicians. In TB endemic areas, a significant proportion (32%) of TB patients has
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44 elevated autoantibody titres, especially anti-cardiolipin IgG and anti-Scl-70. This
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46 phenomenon is likely to be reactive due to the lack of clinical correlations, as well as
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typical or multiple manifestations of autoimmune diseases.

Comment [u15]: We summarized and highlight our result and point of view for this study.

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Table 1. Clinical characteristics of autoantibody-positive and -negative tuberculosis

(TB) patients			
	Autoantibody-positive (n=32)	Autoantibody-negative (n=68)	<i>p</i> value
Respiratory symptoms	22 (69%)	46 (68%)	0.912
Cough	9 (28%)	16 (23%)	0.621
Sputum	4 (13%)	13 (19%)	0.411
Hemoptysis	2 (6%)	3 (4%)	0.654
Dyspnea	5 (16%)	10 (15%)	>0.999
Chest pain	2 (6%)	4 (6%)	>0.999
Constitutional symptoms	14 (44%)	21 (31%)	0.208
Fever	5 (16%)	9 (13%)	0.763
Weight loss	4 (13%)	6 (9%)	0.722
General malaise	3 (9%)	3 (4%)	0.381
Night sweating	2 (6%)	3 (4%)	0.654
Sputum smear grading			
3+ ~ 4+	2 (6%)	8 (12%)	0.495
1+ ~ 2+	6 (19%)	15 (22%)	0.705
Negative	24 (69%)	45 (66%)	0.373
Serum albumin < 3.5 g/dL	6 (19%)	7 (10%)	0.339
Radiographic findings			
Bilateral infiltration	10 (31%)	34 (50%)	0.078
Cavitations	1 (3%)	8 (12%)	0.265
Pleural effusion	5 (16%)	11 (16%)	0.944
Miliary lesion	0	3 (4%)	0.549

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Data are number (%).

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Table 2. Prevalences of autoantibodies in tuberculosis (TB) patients, healthy controls and the general population [10]

Autoantibody	Normal population		TB patients (n=100)	Healthy control (n=100)	<i>p</i> value		
	(n=2181)				3 group	TB vs. normal	TB vs. control
	ELISA-pos	% Pos					
Anti-Ro	58	2.7	2	2	0.855	1	1
Anti-La	5	0.2	0	0	0.795	1	1
Anti-RNP	11	0.5	2	1	0.138	0.108	1
Anti-Sm	0	0	0	0		1	1
Anti-Scl-70	0	0	6	3	<0.001	<0.001	0.498
Anti-Jo1	0	0	1	1	<0.001	0.044	1
Anti-dsDNA	10	0.5	1	0	0.579	0.39	1
Anti-centromere	30	1.4	5	2	0.101	0.059	0.683
Anti-histone	NA		11	7			0.323
Anti-cardiolipin IgM	NA		6	10			0.297
Anti-cardiolipin IgG	NA		11	0			0.001

Table 3. Adverse events during anti-TB treatment in autoantibody-positive and -negative TB patients

	Autoantibody-positive (n=32)	Autoantibody-negative (n=68)	<i>p</i> value
Rheumatologic events	10 (31%)	28 (41%)	0.340
Gastrointestinal events	9 (28%)	22 (32%)	0.670
Neurologic events	8 (25%)	16 (24%)	0.872
Renal events	7 (22%)	19 (28%)	0.519
Constitutional events	4 (13%)	19 (28%)	0.087
Respiratory events	2 (6%)	3 (4%)	0.654
Hematologic events	1 (3%)	0	0.320
Total number of events	41	107	

Data are number (%).

Table 4. Serum autoantibody titres in tuberculosis (TB) patients before and after

anti-TB treatment

Patient			Patient		
Anti-Scl-70			ACA-IgG		
No.	Initial	Follow-up	No.	Initial	Follow-up
No. 8	137	155	No. 18	18.1	9.3
			No. 19	33.2	2.6
No. 18	156	35	No. 36	12.3	4.1
			No. 48	14.1	6.7
No. 51	125	28	No. 55	10.8	13.4
			No. 60	23.8	2.6
No. 64	152	55	No. 62	31.1	4.8
			No. 72	18.9	30.9
No. 65	154	79	No. 81	14.6	23.5
			No. 92	21.2	21.1
No. 91	153	68	No. 94	12.8	5.1

Abbreviation: ACA, anti-cardiolipin antibody

Normal reference values for anti-Scl-70 and ACA-IgG are <100 U/mL and <10 GPL,

respectively.

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Author Contributions

Dr. Jann-Yuan Wang and Prof. Song-Chou Hsieh designed the study. Dr. Chieh-Yu Shen, Prof. Chia-Li Yu, Prof. Song-Chou Hsieh, Dr. Jann-Yuan Wang, and Prof. Li-Na Lee all contributed to the manuscript writing and data interpretation. Dr. Chieh-Yu Shen and Dr. Jann-Yuan Wang were involved in the statistical analysis. Prof. Chong-Jen Yu was directly responsible for the general organization and instruction.

Conflict of interest disclosure

All authors declare no financial, professional or other personal interest of any nature or kind in related products, services and/or companies.

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