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Auto-antibody profiles in patients with active pulmonary tuberculosis

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SUMMARY

OBJECTIVES: To determine the prevalence of a wide array of auto-antibodies in patients with tuberculosis (TB) compared with healthy controls.

MATERIALS AND METHODS: Forty-seven consecutive patients (age 47 ± 21 years, 29 males) with recently diagnosed active pulmonary tuberculosis (PTB) and 39 healthy controls were enrolled. Data collected on a questionnaire included clinical features of the disease, duration of symptoms, presence of fever, cough, arthralgia, myalgia, sicca symptoms and others. Serum samples were collected from the patients' before initiating TB treatment, frozen at −20°C and tested for antinuclear antibodies (ANA), anti-ds DNA, anti-Sm, anti-RNP, anti-Ro, anti-La, and anti-cardiolipin (ACA) (IgG and IgM).

RESULTS: Rheumatic symptoms were relatively rare: arthralgia ($n = 2$), myalgias ($n = 2$), and eye ($n = 1$) and mouth dryness ($n = 4$). The TB patients' mean serum levels of anti-ds DNA, anti-Sm, anti-RNP, anti-SSA (anti-Ro), and anti-ACA-IgM were significantly increased compared with controls ($P < 0.05$ for all). A significantly higher proportion of TB patients had increased pathological levels of anti-ds DNA (32% vs. 2.5%), anti-Sm (38% vs. 0%), anti-RNP (15% vs. 0%), anti-Ro (64% vs. 10%), anti-ACA-IgG (59% vs. 0%) and anti-ACA-IgM (47% vs. 7.7%) ($P < 0.05$ for all).

CONCLUSIONS: Patients with active TB have significantly increased titres of various auto-antibodies, including highly specific serological markers, such as anti-Sm.

RELEVANCE: Differential interpretation of serological studies of patients with systemic manifestations should consider the possibility of PTB.

KEY WORDS: ANA; auto-antibodies; anti-Sm; cardiolipin; tuberculosis

THE PAST TWO DECADES have been marked by a worldwide resurgence of tuberculosis (TB). Approximately 2 billion people have latent TB infection and about 8 million will develop active TB.[1] Extrapulmonary tuberculosis (EPTB) is also on the rise, with approximately 10% involving the bones and joints.[2] Patients with TB may present with a variety of rheumatic symptoms and signs even without evidence of direct musculoskeletal/local involvement. TB and systemic lupus erythematosus (SLE) share many symptoms, such as fever, myalgias, arthralgia/arthritis,[3] rash[4] and multi-organ involvement. *Lupus vulgaris*, which is one of the cutaneous manifestations of TB, may be confounded with an exacerbation of SLE.[5] Poncet's disease is the classical example of TB-induced polyarthritis with prominent systemic involvement, suggestive of auto-immune diseases.[6] The serum of patients with TB may contain rheumatoid factors (RFs) in up to 40% of cases.[7] In addition to RFs, TB patients may present with a variety of auto-antibodies,[8] such as anti-nuclear, found in 15%[9] to 40%[7] of TB patients, anti-cardiolipin, found in almost 50%,[9] and anti-neutrophil cytoplasmatic antibodies, found in about 44%.[10]

The aim of this study was to determine the prevalence and associations of a wide array of auto-antibodies in patients with confirmed pulmonary TB (PTB).

PATIENTS AND METHODS

Forty-seven consecutive patients with recently diagnosed active PTB were included in the study. They were all hospitalised in the Department of Tuberculosis of the Shmuel-Harofeh Geriatric Medical Centre, and had clinical symptoms and radiological signs of TB as well as positive cultures for *Mycobacterium tuberculosis*. All the patients were human immunodeficiency virus (HIV) negative. A special questionnaire was used to record data on the clinical features of the disease, such as duration of symptoms, presence of fever and cough, as well as rheumatological manifestations,

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such as arthralgia/arthritis, myalgia, rash, mucocutaneous symptoms, sicca symptoms (Sjogren's syndrome), spontaneous abortion, history of thrombosis and family history of auto-immune disease. Serum samples were collected before starting treatment for TB and frozen at −20°C. The controls were 39 aged-matched apparently healthy hospital personnel.

The serum samples were tested for a wide array of auto-antibodies, including antinuclear antibodies (ANAs), anti-cardiolipin antibodies (ACA), anti-double-stranded DNA (anti-ds DNA), anti-β2 glycoprotein 1, anti-Smith (anti-Sm), anti-ribonucleoprotein (anti-RNP), anti-Ro (anti-SSA [anti-Sjogren's syndrome A]) and anti-La (anti-SSB [anti-Sjogren's syndrome B]) (IgG) antibodies. These antibodies were chosen because they represent the most frequently used auto-antibodies in the diagnosis of SLE.

Detection of antinuclear antibody

Serum diluted 1:80 in phosphate buffered saline (PBS) was overlaid onto fixed HEp-2 cells. The slides were washed twice for 5 min each with PBS, overlaid with fluorescent-labelled conjugate (anti-human IgG heavy and light chain specific) and incubated for an additional 30 min. A coverslip was placed over the twice-washed slide and it was read by a fluorescent microscope at 40× power. The fluorescent intensity was scored semiquantitatively from 1+ to 5+ relative to the intensity of a negative and a positive (4+) control. The fluorescence pattern was also recorded.

Detection of anti-double-stranded DNA antibodies

A commercially available kit (Zeus Scientific, Raritan, NJ, USA) was used for the detection of IgG anti-ds DNA antibodies. The enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacture's instructions. Briefly, 100 μl of calibrators, controls and patient samples (1:100) were dispensed into the appropriate wells. After 20 min of incubation, the fluid was aspirated from the wells and the wells were washed three times with buffer. One hundred μl of conjugate (anti-human IgG gamma chain-specific, coupled with horseradish peroxidase) was then added to the wells. After 20 min incubation, the wells were washed three times with buffer, and 100 μl of enzyme substrate (3,3′,5,5′-tetramethylbenzidine) was dispensed into the wells. After 10 min of incubation in the dark, 50 μl of stop solution (0.5 M H_2SO_4) was dispensed into all wells. The absorbance (optical density) was read at 450 nm. Positive and negative controls were used as indicated by the manufacturer. According to the manufacturer's instructions, a result of >25 IU/ml was considered positive.

Detection of anti-cardiolipin antibodies (IgG and IgM) and anti-β2 glycoprotein 1

A commercially available kit (Zeus Scientific, Raritan, NJ) was used for the detection of ACA (IgG and IgG) and anti-β2 glycoprotein 1. The ELISA was performed according to the manufacturer's instructions. The procedure followed that used for the detection of anti-ds DNA antibodies. According to the manufacturer's instructions, a result of >48 IU/ml was considered positive for IgG-cardiolipin, >44 IU/ml was positive for IgM-cardiolipin and >25 IU/ml for anti-β2 glycoprotein 1.

Detection of anti-Sm, anti-RNP, anti-Ro and anti-La (IgG) antibodies

A commercially available kit (Zeus Scientific) was used for the detection of anti-Sm, anti-RNP, anti-Ro and anti-La (IgG) antibodies. The ELISA was performed according to the manufacturer's instructions. The procedure followed that used for the detection of anti-ds DNA antibodies. According to the manufacturer's instructions, the normal values for anti-Ro, La, RNP and Sm are <25 IU/ml.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc, Chicago, IL, USA). As the auto-antibody levels were not in a Gaussian distribution, we used the Median test to compare antibody titres between groups. The χ^2 test was used to compare positivity rates between TB patients and controls. The Pearson correlation coefficient was used for correlations between clinical and laboratory data. $P < 0.05$ (two tail) was considered significant.

RESULTS

Patients

Table 1 summarises the demographic and clinical characteristics of the TB patients and healthy controls. The patients had a mean duration of symptoms of 4.4 ± 1.7 months, 34 (73%) had fever and 44 (94%) presented with cough. Few patients had other symptoms, such as arthralgia (4%), myalgias (4%), or eye and

Table 1 Demographic and clinical characteristics of the study cohort

	Patients with TB (n = 47) n (%)	Healthy controls (n = 39)
Age, years ± SD	52.3 ± 17	47 ± 21
Sex, male/female	29/21	13/26
Duration of symptoms (months) ± SD	4.4 ± 1.7	—
Symptoms		
Fever	34 (73)	—
Cough	44 (94)	—
Arthralgia	2 (4)	—
Myalgia	2 (4)	—
Eye dryness	1 (2)	—
Mouth dryness	4 (8)	—

TB = tuberculosis; SD = standard deviation.

Table 2 Serum levels of auto-antibodies in patients with TB

Serum levels	TB	Controls	*P* value
ds-DNA	23 (13–39)	12 (11–17)	<0.001
Sm	22 (11–36)	12 (10–26)	<0.05
RNP	10 (8–12)	7 (5–9)	<0.001
Ro	50 (26–99)	23 (13–27)	<0.001
La	12 (10–24)	12 (10–24)	NS
IgG-ACA	63 (29–131)	57 (28–143)	NS
IgM-ACA	39 (32–63)	23 (18–32)	<0.001
β2 glycoprotein	2.3 (1.8–3.4)	3.7 (2.8–5.9)	<0.001

Values are all in units/ml. Median (interquartile) *P* by median test (two-tail) comparing TB patients with controls.
TB = tuberculosis; ds = double-stranded; Sm = Smith; RNP = ribonucleoprotein; Ro = anti-SSA (anti-Sjogren's syndrome A); La = anti-SSB (anti-Sjogren's syndrome B); IgG = immunoglobulin G; ACA = anti-cardiopolin antibodies; IgM = immunoglobulin M; NS = non-significant.

mouth dryness (2% and 8%, respectively). None had mucocutaneous aphthae or skin manifestations or a history of spontaneous abortion, thrombosis or known first-degree familial auto-immune disease.

Serum levels of ANA, anti-ds DNA, anti-Sm, anti-RNP, anti-Ro and La, anti-ACA-IgG and IgM and anti-β2 glycoprotein 1

The median serum levels of anti-ds DNA, anti-Sm, anti-RNP, anti-Ro, anti-ACA-IgM were significantly increased in the TB patients compared to the controls (Table 2). There was no difference in anti-La and anti-ACA-IgG antibodies. The levels of β2 glycoprotein 1 were higher in the control group.

ANA at a titre >1/80 was found in 33% of patients compared to 20% for controls (P = non-significant). A significantly higher proportion of TB patients presented levels above the normal range of anti-ds DNA (32% vs. 2.5%), anti-Sm (38% vs. 0%), anti-RNP (15% vs. 0%), anti-Ro (64% vs. 10%), anti-ACA-IgG (59% vs. 0%) and anti-ACA-IgM (47% vs. 7.7%) (Table 3).

Correlations between serological parameters and clinical manifestations

There was a statistical correlation between the presence of anti-RNP and anti-Ro ($P = 0.01$), anti-La ($P = 0.003$) and anti-ds DNA (0.01). Anti-Ro antibodies were associated with anti-ACA-IgG ($P < 0.001$), anti-ds DNA ($P < 0.001$) and anti-RNP ($P < 0.01$). No significant correlation was found between clinical symptoms and serological findings.

Table 3 Proportion of study patients with levels above the normal range of auto-antibodies

Proportion of patients with pathological levels of	Study patients %	Controls %	*P* value
ds-DNA	32	2.5	<0.001
Sm	38	0	<0.001
RNP	15	0	0.015
SSA	64	10	<0.001
SSB	15	14	0.95
IgG-ACA	59	0	<0.001
IgM-ACA	47	7	<0.001
β2 glycoprotein 1	0	2.6	0.453

ds = double-stranded; Sm = Smith; RNP = ribonucleoprotein; SSA = anti-Sjogren's syndrome A; anti-SSB = anti-Sjogren's syndrome B; IgG = immunoglobulin G; ACA = anti-cardiopolin antibodies; IgM = immunoglobulin M.

DISCUSSION

In the present study, we observed that a significantly higher proportion of TB patients had pathologically increased levels of a variety of auto-antibodies, including anti-ds DNA, anti-Sm, anti-RNP, anti-Ro, anti-ACA-IgG and anti-ACA-IgM. The presence of several auto-antibodies has been previously reported in TB. Isenberg et al. studied the presence of RF and ANA in patients with TB, klebsiella and other gram-negative sepsis, and reported a rate of 15–40%.[7] Others have demonstrated anti-cardiolipin positivity in up to 30% of TB patients, although, in contrast to our results, they did not find an increased proportion of anti-ds DNA.[9]

The role of infection in the pathogenesis of auto-immune diseases is a controversial issue. Although there are considerable data supporting the role of infections in a variety of auto-immune diseases, this role has been conclusively established in only a few auto-immune diseases.[11,12] Among the auto-antibodies, the ACAs have mostly been reported in the context of infectious diseases. There is considerable clinical literature to demonstrate the association between a wide variety of infectious agents and ACAs, including mycoplasma pneumonia, malaria, Lyme disease, HIV, rubella, varicella and parvovirus.[13–16] Infection-associated ACA tends to be transient, of lower titre and more often of the IgM type.[17] There is, however, evidence that links ACA-associated thrombo-embolic complication in active infection with parvovirus,[18] hepatitis C[19] and others.[20]

Anti-Sm antibodies are present in 5–30% of patients with SLE. Although the sensitivity of this test is low, it is considered to have a specificity of more than 95%.[21] Interestingly, we could show that a substantial proportion of patients (more than one third) were anti-Sm-positive. This antibody has not previously been reported in active TB or in other infectious diseases, although Sabbatini et al. have shown that a population of anti-Sm from SLE patients is able to bind an epitope shared by the auto-antigen and the viral antigen Epstein-Barr virus-encoded nuclear antigen (EBNA I), suggesting a mechanism of antigen mimicry.[22]

We are aware of the limitations of our study, which included a relatively small number of patients. Likewise, the fact that we tested in-patients may indicate widespread disease, suggesting a bias in our results. We are also well aware that the ELISA kits used may have influenced the results, as there are wide variations in the levels of auto-antibodies between different kits.[23] The finding of a high proportion of patients with high levels of anti-Sm requires confirmation in further studies.

Tuberculosis is a multifaceted disease, which may present with a variety of symptoms, such as fever, myalgia, musculoskeletal symptoms,[6] rash[5] and lung involvement, sometimes imitating auto-immune diseases. A significant proportion of our present series of patients with active TB had high titres of a variety of auto-antibodies, including highly specific ones, such as anti-Sm. The auto-antibody profile of TB patients does not help in differentiating this infectious syndrome from auto-immune disorders, but these findings should be borne in mind when interpreting serological studies in the differential diagnosis of patients with systemic manifestations.

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RÉSUMÉ

OBJECTIFS : Déterminer la prévalence d'une grande variété d'auto-anticorps chez les patients atteints de tuberculose (TB) par comparaison avec les sujets-contrôle sains.

MATERIELS ET MÉTHODES : On a enrôlé dans l'étude 47 patients consécutifs (âge 47 ± 21 ans, 29 hommes) atteints de TB pulmonaire active récemment diagnostiquée ainsi que 39 sujets-contrôle sains. Les données colligées sur un questionnaire ont inclus les signes cliniques de la maladie, la durée des symptômes, la présence de fièvre, de toux, d'arthralgie, de myalgie, de symptômes de dessèchement des muqueuses et d'autres. Les sérums des patients ont été recueillis avant le début du traitement TB, congelés à −20°C et testés à la recherche d'anticorps antinucléaires (ANA), anti-ds DNA, anti-Sm, anti-RNP, anti-Ro, anti-La, anti-cardiolipine (ACA) (IgG et IgM).

RÉSULTATS : Les symptômes rhumatismaux ont été relativement rares : arthralgie ($n = 2$), myalgie ($n = 2$), sécheresse des yeux ($n = 1$) et de la bouche ($n = 4$). Chez les patients tuberculeux, les niveaux sériques moyens en ce qui concerne les anti-ds DNA, les anti-Sm, les anti-RNP, les anti-SSA et les anti-ACA-IgM sont significativement augmentés par rapport aux sujets-contrôle ($P < 0,05$ pour tous). Une proportion significativement plus élevée de patients TB ont des niveaux pathologiques accrus d'anti-ds DNA (32% vs. 2,5%), d'anti-Sm (38% vs. 0%), d'anti-RNP (15% vs. 0%), d'anti-Ro (64% vs. 10%), d'anti-ACA-IgG (59% vs. 0%) et d'anti-ACA-IgM (47% vs. 7,7%) par rapport aux sujets-contrôle ($P < 0,05$ pour l'ensemble).

CONCLUSIONS : Chez les patients atteints de TB active, les taux différents auto-anticorps sont significativement augmentés, et particulièrement ceux des marqueurs sérologiques hautement spécifiques comme l'anti-Sm.

RESUMEN

OBJETIVOS : Determinar la prevalencia de una amplia gama de autoanticuerpos en pacientes con tuberculosis (TB) y compararla con la de un grupo de testigos sanos.
MATERIAL Y MÉTODOS : Se incluyeron en el estudio 47 pacientes consecutivos (edad 47 ± 21 años, 29 hombres) con diagnóstico reciente de TB y 39 testigos sanos. Los datos recogidos en un cuestionario abarcaron las características clínicas de la enfermedad, la duración de los síntomas, la presencia de fiebre, tos, artralgias, mialgias, síntomas de síndrome seco (síndrome de Sjörgren) y otros. Antes de comenzar el tratamiento antituberculoso se recogieron muestras de suero de los pacientes, que se congelaron a −20°C y se analizaron a fin de detectar la presencia de anticuerpos antinucleares, anticuerpos dirigidos contra el ácido desoxirribonucleico nativo o bicatenar (anti-ADN nativo), contra el antígeno Smith (anti-Sm), contra ribonucleoproteínas (anti-RNP), anti-Ro, anti-La y anticuerpos anticardiolipínicos de tipo IgG e IgM.
RESULTADOS : Los síntomas reumáticos fueron relativamente raros : artralgias ($n = 2$), mialgias ($n = 2$) y xeroftalmia ($n = 1$) y xerostomía ($n = 4$). En los pacientes con TB, la concentración sérica media de anticuerpos anti-ADN nativo, anti-Sm, anti-RNP, anti-SSA (anti-Ro), y anticardiolipínicos de tipo IgM fue más alta que en los testigos, en forma estadísticamente significativa ($P < 0,05$ para todos los anticuerpos). Una proporción significativamente mayor de pacientes con TB presentó concentraciones altas patológicas de anticuerpos anti-ADN nativo (32% contra 2,5%), anti-Sm (38% contra 0%), anti-RNP (15% contra 0%), anti-Ro (64% contra 10%), anticardiolipínicos de tipo IgG (59% contra 0%) y anticardiolipínicos de tipo IgM (47% contra 7,7%) ($P < 0,05$ para todos).
CONCLUSIONES : Los pacientes con TB activa presentan títulos altos de diversos auto-anticuerpos, incluidos marcadores serológicos sumamente específicos, como el anti-Sm.