Institut za plućne bolesti, Sremska Kamenica[1]
Institut za onkologiju, Sremska Kamenica[2]
Klinički centar Vojvodine Novi Sad
Klinika za imunologiju i nefrologiju[3]

Originalni naučni rad
Original study
UDK 616.24-002.5-097
DOI:10.2298/MPNS0808333K

AUTOANTITELA KOD BOLESNIKA LEČENIH OD AKTIVNE PLUĆNE TUBERKULOZE

AUTOANTIBODIES IN PATIENTS TREATED FOR ACTIVE PULMONARY TUBERCULOSIS

Svetlana KAŠIKOVIĆ-LEČIĆ[1], Aleksandar KERENJI[2], Slobodan PAVLOVIĆ[1], Vesna KURUC[1], Igor MITIĆ[3] i Tanja ILIĆ[3]

Sažetak - Imunološki odgovor domaćina prema mikobakterijama može biti odgovoran za nastanak autoantitela usmerenih prema sopstvenim tkivnim antigenima. Cilj našeg istraživanja bio je da ispitamo prisustvo autoantitela u serumima obolelih od aktivne plućne tuberkuloze, njihovu učestalost i međusobnu povezanost sa polom, eventualnom hipergamaglobulinemijom i primenjenom terapijom. Tehnikom indirektne imunofluorescencije, uz korišćenje kriostatskih isečaka pacovskih organa i humanih epitelnih ćelija karcinoma larinksa, ispitivano je prisustvo autoantitela (antinuklearnih, antisrčanih, antimitohondrijalnih, antiparijetalnih, antiglatkomišićnih, antitiroidnih i reumatoidnog faktora) u serumima ispitanika tokom lečenja bolesti i posle pet godina od završenog lečenja. Utvrđeno je da se u serumima dela bolesnika mogu detektovati pojedina ispitivana autoantitela u niskom titru i da ne postoji statistički značajna razlika u njihovom prisustvu među polovima. Prisustvo antinuklearnih antitela u serumima bolesnika je pored mogućih mehanizama molekulske mimikrije i poliklonske aktivacije limfocita, bar delimično posledica primene izonijazida. Nalaz drugih autoantitela je nespecifičan i nije u vezi sa infekcijom mikobakterijama, niti sa upotrebom antituberkulotika. Tokom lečenja bolesti dolazi do reverzibilne hiper-gamaglobulinemije, koja je bar delimično posledica prisustva autoantitela u serumima bolesnika.
Ključne reči: Autoantitela; Plućna tuberkuloza; Autoimunitet; Izoniazid

Summary - *The genesis of auto-immune antibodies directed against the own tissue antigens of a host may be due to the host's immune response to mycobacteria. The prospective study included 110 patients treated for active pulmonary tuberculosis and the control group of 60 healthy subjects, volontary blood donors. Applying the method of indirect immunofluorescence and cryostat sections of rat organs and human larynx cancer epithelial cell line (HEp-2 cells), the prescence of the following autoantibodies in the serum of the examined patients was examined: antinuclear (ANA), anticardiac (ACA), antimitochondrial (AMA), antiparietal (APA), anti smooth muscular (ASMA), antithyroidal (ATA), rheumatoid factor (RF). These autoantibodies were determined in the course of treatment and five years later. Low levels of some examined autoantibodies were detected in the serum of a number of the examined patients. No significant difference in the presence of the analysed antibodies was registered between the sexes. In the course of the treatment a reversible hyper-gammaglobulinemia developed, which was at least partially due to the presence of autoantibodies in the patients blood serum. Besides possibly involved mechanisms of molecular mimicry and polyclonal lymphocyte activation, the presence of antinuclear antibodies in the patient's serum is at least partially due to isoniazide treatment. The finding of other autoantibodies is nonspecific and not related to the mycobacterial infection or antituberculosis drug administration.*
Key words: *Autoantibodies; Tuberculosis, Pulmonary; Autoimmunity; Isoniazid*

Uvod

Hronične bakterijske infekcije mogu da dovedu do poremećaja imunološke tolerancije na više načina: promenom sopstvenih antigena (antigenskih determinanti) domaćina i formiranjem novih (kako zbog destrukcije sopstvenih tkiva, tako i zbog uticaja lekova koji se koriste u terapiji), molekulskom mimikrijom između domaćinovih i mikrobnih antigena, poliklonalnom aktivacijom limfocita, pojavom autoreaktivnih klonova na odeljene antigene, poremećajima u sekreciji citokina ili disbalansom u antiidiotipskoj mreži. Najčešće je za nastanak autoimunosti odgovorno istovremeno dejstvo više mehanizama [1-4]. Svi ovi fenomeni mogu da postoje kod tuberkuloznih bolesnika, ali su relativno neispitani parametri.

Cilj našeg istraživanja bio je da utvrdimo da li se u serumima obolelih i lečenih od aktivne, novootkrivene plućne tuberkuloze mogu detektovati:

Introduction

Chronic bacterial infections may induce the immunological tolerance impairment via several mechanisms: alteration of the host's antigens (antigenic determinants) and formation of the new ones (due both to the tissue destruction and effects of the applied drugs), molecular mimicry between the host and microbial antigens, polyclonal lymphocyte activation, emergence of autoreactive clones to separated antigens, impaired cytokine selection or antidiotype network imbalance. Autoimmunity is usually due to the simultaneous activity of several mechanisms [1-4]. All these phenomena may be involved in tuberculosis, but the parameters have still been poorly investigated.

The study was aimed at establishing the presence of antinuclear (ANA), antiparietal (APA), antismoothmuscle (ASMA), antimitochondrial (AMA),

Adresa autora: Asist. mr sc. med. Svetlana Kašiković-Lečić, Institut za plućne bolesti, 21204 Sremska Kamenica, Institutski put 4, E-mail:sklecic@sbb.co.yu

Skraćenice

aAT	- autoantitela
ANA	- antinuklearna antitela
APA	- antiparijetalna antitela
AGMA	- antiglatkomišićna antitela
AMA	- antimitohondrijalna antitela
ASA	- antisrčana antitela
ATA	- antitiroidna antitela
RF	- reumatoidni faktor
ATL	- antituberkulozni lekovi
TIIF	- tehnika indirektne imunofluorescencije
HEp-2 ćelije	- humane epitelne ćelije karcinoma larinksa
UP	- ukupni proteini
A	- albumini
gG	- gama globulini
A/G	- albuminsko-globulinski indeks
BK	- *Bacilus Koch*
H	- izonijazid
R	- rifampicin
Z	- pirazinamid

Abbreviations

aAT	- autoantibodies
ANA	- antinuclear antibodies
APA	- antiparietal antibodies
AGMA	- antismoothmuscle antibodies
AMA	- antimitochondrial antibodies
ASA	- anticardiac antibodies
ATA	- antithyroid antibodies
RF	- rheumatoid factor
ATL	- anti-tuberculous drugs
TIIF	- technique of indirect immunofluorescence
HEp-2 ćelije	- human larynx cancer epithelial cells
UP	- total proteins
A	- albumins
gG	- gama globulins
A/G	- albumin-globulin index
BK	- *Bacilus Koch*
H	- isoniazid
R	- rifampicin
Z	- pyrazinamide

antinuklearna (ANA), antiparijetalna (APA), antiglatkomišićna (AGMA), antimitohondrijalna (AMA), antisrčana (ASA), antitiroidna (ATA) autoantitela i reumatoidni faktor (RF), da utvrdimo njihovu učestalost i međusobnu povezanost sa polom, primenjenom terapijom i eventualnom hipergamaglobulinemijom.

Materijal i metode

U prospektivnoj kliničkoj studiji ispitano je 110 bolesnika lečenih od aktivne plućne tuberkuloze. Ovi bolesnici su podeljeni u dve grupe (grupa A i B). U grupi A je odabrano 70 bolesnika koji su prvi put hospitalizovani u Institutu za plućne bolesti u Sremskoj Kamenici, zbog aktivne, novootkivene, plućne tuberkuloze pozitivne na *Bacilus Koch* (BK). U grupi B je oda- brano 40 ispitanika koji su pre 5 godina prvi put lečeni od aktivne, BK pozitivne plućne tuberkuloze u Institutu za plućne bolesti u Sremskoj Kamenici. Posle postizanja negativizacije sputuma, dalje le- čenje se sprovodilo u regionalnim dispanzerima za plućne bolesti, gde su vršene redovne kontrole obolelih do završetka lečenja i povremene kontrole posle tog perioda. Posle prekida uzimanja antituberkuloznih lekova (ATL), ispitanici ove grupe nisu imali recidive bolesti, a pri našem pregledu bili su dobrog opšteg stanja, bez kliničkih, radioloških i bakterioloških znakova aktivnog oboljenja. Ova grupa je formirana sa ciljem da se ispita prisustvo autoantitela u serumima bolesnika, posle višegodišnjeg prekida primene ATL.

Zasejavanjem sputuma na Lowenstain-Jensenove podloge, pri prijemu na bolničko lečenje, kod svih ispitanika je kao uzročnik bolesti izolovan *Mycobacterium tuberculosis.*

Svi ispitanici su lečeni kombinovanom trojnom antituberkulotskom terapijom (H - izonijazid 5 mg/kg/dan, R-rifampicin 10 mg/kg/dan i Z-pirazinamid 15-30 mg/kg/dan), po šestomesečnom terapijskom

anticardiac (ACA), antithyroid (ATA) antibodies and the rheumatoid factor (RF) in the sera from patients treated for active, new-detected pulmonary tuberculosis, and defining their frequency and correlation to the sex, applied therapy, and possible hyper-gammaglobulinemia.

Material and methods

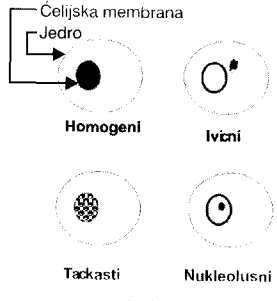
The prospective study included 110 patients treated for active pulmonary tuberculosis, subdivided into two groups (Group A and Group B). Group A included 70 patients hospitalized in the Institute for Pulmonary Diseases, Sremska Kamenica due to active, new-detected, mycobacterium positive pulmonary tuberculosis. Group B included 40 patients who were treated first time 5 years ago for active pulmonary tuberculosis. Once the negative sputum finding was achieved, their treatment was continued in the regional pulmonary dispensaries where they were submitted to regular controls until the treatment was completed, as well as to occasional controls afterwards. Upon discontinuation of the treatment with antituberculous drugs (ATD), these patients developed no relapses and were in a good general condition on controls, free of any clinical, radiologic, or bacteriological signs of the active disease. This group was formed in order to examine the presence of autoantibodies in the patients' sera several yeas after the AT therapy discontinuation.

On admission, these patients had their sputum samples cultivated in Lowenstain-Jensen's medium, which showed the presence of Mycobacterium tuberculosis as the inducing agent of their disease.

All the patients of this group received the six month (2 HRZ + 4 HR) combined three-drug antituberculous therapy (H - isoniazid 5 mg/kg/day, R -rifampicin 10 mg/kg/day and Z -pyrazinamide 15-30 mg/kg/day). In this period, the patients received the

režimu (2 HRZ + 4 HR). Tokom lečenja bolesti bolesnici nisu uzimali druge lekove (samo ATL). Kod svih je lečenje bolesti bilo uspešno (poboljšanje opšteg stanja obolelog, postizanje negativizacije sputuma, tj. odsustva *M. tuberculosis* iz sputuma, regresija promena uočenih na radiološkom snimku grudnog koša) i završeno posle 6 meseci. Na primenjenu terapiju nije došlo do pojave neželjenih efekata (poremećaji funkcije jetre, krvne slike, alergija).

Od svakog ispitanika u grupi A, uzimano je tri puta (kod ispitanika grupe B i kontrolne grupe, samo jedan put) po 5 ml krvi, za imunološke analize i to: u nultom momentu, tj. pre započinjanja lečenja dokazane aktivne plućne tuberkuloze, posle mesec dana od započinjanja terapije, kao i na kraju sprovedenog lečenja (posle 6 meseci). Primenom tehnike indirektne imunofluorescencije (TIIF) ispitivano je prisustvo odgovarajućih autoantitela u serumima bolesnika [5-7]. Kao antigena podloga za dokazivanje autoantitela korišćeni su 4u debeli kriostatski isečci pacovske jetre, srca, želuca, bubrega i štitnjače (tzv. zbirni preparat). Ispitivani serumi su najpre testirani u početnom razblaženju 1:32. Serumi bolesnika koji su bili ANA pozitivni u titru 1:32, ispitivani su dalje do razblaženja 1:128. Za ispitivanje prisustva ANA u serumima bolesnika pored tkivnog korišćen je i ćelijski supstrat, tj. kulture HEp-2 ćelija (humane epitelne ćelije karcinoma larinksa) na komercijalnom kitu *Quantafluor*, firme *Kallested* iz Pariza. Korišćenjem HEp-2 ćelija mogu se detektovati i antitela protiv onih antigena kojih inače nema na tkivnim supstratima, a koji mogu da se zapaze samo u toku deobe ćelije. Kod bolesnika koji su imali pozitivan titar ANA vršeno je određivanje i tipa jedarne fluorescencije (Slika 1). Dokazivanje RF vršeno je lateks aglutinacionim testom [8]. Kod većine bolesnika grupe A urađena je elektroforeza serumskih proteina. Određivane su relativne vrednosti pojedinih frakcija proteinograma kod dela bolesnika oba pola, radi poređenja pojave autoantitela sa eventualnom hiper-gamaglobulinemijom.



Slika 1. Tipovi fluorescencije jedra
Fig. 1. *Types of nuclear fluorescence*

Kontrolnu grupu sačinjavalo je 60 zdravih osoba (30 muškaraca i 30 žena), dobrovoljnih davalaca

AT therapy only, achieving a good treatment response (improved general health condition, negative sputum finding free of M. tuberculosis, radiological regression), so the treatment was discontinued after six month. No undesirable side effects of the applied treatment (impaired liver function or blood count, or allergy) were registered.

Three 5 ml blood samples were taken from each Group A patient (in Group B and Control Group subjects only one blood sample was taken) for immunological assays - prior to initiating the AT therapy, after one-and six-month treatment. Applying the method of indirect immunofluorescence (IIF), the presence of respective autoantibodies in the serum of the patients was examined [5-7]. Cryostat sections (4u thick) of rat organs (including the liver, heart, stomach, kidneys and thyroid), the so called cumulative preparation, was used as the antigenic medium. The initial testing dilution rate of the examined sera was 1:32. The sera titrated at 1:32 positive to ANA were submitted to further tests, reaching the dilution rate of 1:128. (The presence of ANA in the patients' sera was examined using both the tissue and the cellular substrate, i.e. the HEp-2 cell cultures (human larynx cancer epithelial cells), using a commercial kit Quantafluor, produced by Kallested, Paris. The HEp-2 cell cultures also enabled detection of antibodies against those antigens otherwise absent from the tissue substrates and which may be noticed exclusively in the course of the cellular division. In the patients with a positive ANA titre, the type of nuclear fluorescence was determined. The RF was confirmed by the latex aglutination test [8]. In most Group A patients elecrophoresis of the serum proteins was performed. The relative values of certain proteingram fractions were determined in a number of patients of both sexes in order to correlate the emergence of autoantibodies and possible hyper-gammaglobulinemia.

The Control Group included 60 healthy subjects (30 males and females), voluntary blood donors at the Blood Transfusion Institute of Novi Sad. The groups were formed on the sex criteria in order to evaluate th impact of sex on the test results.

All the subjects included in the study denied any personal or family history of autoimmune diseases or atopy, and had not received immunosuppressive or any other therapy, vaccines or sera in the recent one-year period.

The obtained data were analysed by the methods of parametric statistics (t test, regressive analysis and correlation tests). The obtained results are presented in tables. We used a commercial software package *Excel* 5.0 of the Programme *Windows* 95 Programe. The obtained results are given in tables and graphs.

krvi u Zavodu za transfuziju krvi u Novom Sadu. Ispitanici su podeljeni na podgrupe sačinjene od muškaraca ili žena da bi se ispitao uticaj pola na rezultate ispitivanih parametara.

Svi ispitanici su negirali postojanje neke od autoimunih ili atopijskih bolesti (kako kod njih, tako i kod članova svoje familije), nisu koristili imunosupresivne i dr. lekove, niti primali vakcine i serume u poslednjih godinu dana.

Za obradu podataka koristili smo metode parametrijske (t-test, regresiona analiza i korelaciona ispitivanja) statistike. Koristili smo se komercijalnim softverskim paketom *Excel* 5.0 u okviru programa *Windows* 95. Dobijeni rezultati su prikazani tabelarno i grafički.

Rezultati

U prospektivnom ispitivanju obuhvaćeno je 110 bolesnika lečenih od aktivne plućne tuberkuloze (koji su podeljeni u dve grupe: A i B) i 60 zdravih osoba kontrolne grupe.

Na Tabeli 1 prikazane su opšte karakteristike ispitanika po polu i njihova prosečna starost po ispitivanim grupama.

Rezultati prisustva autoantitela u serumima ispitanika

Na Tabeli 2 prikazani su rezultati prisustva autoantitela u serumima ispitanika

Primenom TIIF na zbirnom preparatu, utvrđeno je da tokom lečenja bolesti dolazi do porasta broja bolesnika sa prisutnim ANA u serumu, a da opada broj bolesnika sa prisutnim AGMA i APA autoantitelima u serumu. Nije utvrđena statistički značajna razlika između procenata pozitivnosti ovih autoantitela određivanih tokom lečenja bolesti. AMA su nađena samo inicijalno kod jednog (1,4%) bolesnika. Nije utvrđeno prisustvo ATA i ASA u ispitivanim serumima pre i tokom lečenja plućne tuberkuloze.

Ispitivanjem prisustva autoantitela u serumima ispitanika grupe B utvrđeno je prisustvo AGMA kod jednog bolesnika (2,5%), dok druga ispitivana autoantitela nisu detektovana.

Ispitivanjem prisustva autoantitela u serumima zdravih osoba kontrolne grupe utvrđeno je prisustvo APA u serumu dva (3,33%) ispitanika. Ostala ispitivana autoantitela nisu detektovana u serumima kontrolne grupe.

Našim ispitivanjem nije utvrđena statistički značajna razlika u prisustvu ispitivanih autoantitela među polovima.

Svi serumi su primenom TIIF za određivanje ANA, testirani na tkivnim krioisečcima, u razblaženjima do 1:128. Od ukupno 15 pozitivnih seruma 2 su bila pozitivna do titra od 1:128, a 10 ih je bilo pozitivno do titra 1:64. Tokom lečenja titar autoantitela se nije menjao, sem kod dva bolesnika na kraju lečenja. Kulturom ćelija HEp-2 potvrđeno je prisustvo ANA u serumima bolesnika kod kojih smo i prethodno na tkivnim krioisečcima uočili njihovo

Results

The prospective study included 110 patients treated for active pulmonary tuberculosis (subdivided into two groups - A and B), and the Control Group of 60 healthy subjects.

The general data of the subjects included in the study (including their sex distribution and the mean age of the respective groups) are presented in Table 1.

Serum Autoantibody Test Findings

The frequency of autoantibodies' findings in the patients' serum are presented in Table 2.

Applying the TIF on a cumulative preparation, an increase in the number of patients with the ANA serum finding in the course of the treatment was registered, while the number of patients with AGMA and APA serum findings decreased. No statistically significant difference was established regarding the percentage of positive findings of these autoantibodies determined in the course of the treatment. The AMA were found only initially in one patient (1.4%). The presence of ATA or ASA was not registered in the examined sera prior to, or in the course of the treatment of pulmonary tuberculosis.

Tabela 1. Opšte karakteristike ispitanika i njihova prosečna starost u ispitivanim grupama

Table 1. *Patients' general features and mean age*

Grupa *Group*	Muški *Males*	Prosečna starost (god)/*Mean age (years)*	Ženski *Females*	Prosečna starost(god)/*Mean age (years)*	Ukupno *Total*
A	49	46,3	21	34,6	70
B	28	44,1	12	36,8	40
Kontrolna/*Control*	30	44,7	30	35,1	60

Tabela 2. Učestalost prisustva autoantitela u serumima ispitanika

Table 2. *The frequency of autoantibody finding in the patients' serum*

	Ispitanici grupe A/*Group A*						Grupa B *Group B*	
	pri prijemu *on admission*		posle 1 mesec *after 1 month*		posle 6 meseci *after 6 months*		posle 5 godina *after 5 years*	
Autoantitela *Autoantibodies*	broj	%	broj	%	broj	%	broj	%
Antinuklearna *Antinuclear*	3	4,3	5	7,3	7	14,3	0	0
Antiglatkomišićna/*Anti smooth muscular*	4	5,7	2	2,9	1	2,0	1	2,5
Antiparijetalna *Antiparietal*	3	4,3	1	1,5	1	2,0	0	0
Antimitohondrijalna/*Antimitochondrial*	1	1,4	0	0	0	0	0	0
Broj ispitanika *Number of tested*	70		68		49		40	

prisustvo. Koristeći kulture ćelija HEp-2 prisustvo autoantitela je određivano samo u razblaženjima seruma od 1:32, odnosno nisu rađena testiranja seruma u većim razblaženjima (iz finansijskih razloga). Određivanjem tipova jedarne fluorescencije utvrđeno je da je kod 9 bolesnika postojao tačkasti tip, a kod 6 bolesnika homogeni tip. Ovakav nalaz jedarne fluorescencije potvrđen je i korišćenjem kulture ćelija HEp-2.

Sva ostala autoantitela: AGMA, APA i AMA su detektovana u titru 1:32.

Na Tabeli 3 prikazani su rezultati prisustva RF u serumima ispitanika. Pokazano je da su pre započinjanja lečenja plućne tuberkuloze utvrđene povišene serumske vrednosti RF kod 4 bolesnika (5,7%) grupe A. Kod tri bolesnika, izmerena serumska vrednost RF je bila 40 IU/ml, a kod jednog 80 IU/ml. Posle mesec dana lečenja, RF su detektovani u serumima kod 5 bolesnika (7,1%). Kod dva bolesnika izmerena vrednost RF u serumu bila je 40 IU/m, kod dva 160 IU/ml, a kod jednog bolesnika 80 IU/ml (došlo je do porasta serumskih vrednosti RF). Po završenom lečenju, nisu utvrđene povišene vrednosti RF u serumima ispitivanih bolesnika. Dva bolesnika kod kojih su pri ranijim ispitivanjima seruma utvrđene povišene serumske vrednosti RF se nisu odazvala na kontroni pregled posle završenog lečenja, pa ni vrednosti RF u serumu nisu mogli biti određene. Jedan od ovih bolesnika nije imao povišene serumske vrednosti RF pri prijemu, a posle mesec dana izmerena vrednost je iznosila 160 IU/ml. Kod drugog bolesnika je pri prijemu na bolničko lečenje izmerena vrednost RF iznosila 40 IU/ml, a posle mesec dana vrednost RF bila je u granicama referentnih vrednosti.

Određivanjem vrednosti RF u serumima ispitanika grupe B utvrđene su povišene vrednosti kod jednog ispitanika (3,6%), a izmerena vrednost RF u serumu je iznosila 40 IU/ml. Nije utvrđeno prisustvo RF u serumima zdravih ispitanika kontrolne grupe.

Tabela 3. Rezultati prisustva reumatoidnog faktora u serumima ispitanika

Table 3. *Rheumatod factor test findings in the patients' serum*

	Reumatoidni faktor/*Rheumatoid factor*			
		Grupa A *Group A*		Grupa B *Group B*
Broj ispitanika *Number of tested*	pri prijemu *on admission*	posle 1 meseca *after 1 month*	posle 6 meseci *after 6 month*	posle 5 godina *after 5 year*
Broj ispitanika *Number of tested*	70	70	60	40
Ispitanici sa RF* *Pos. RF finding**	4 (5,7%)	5 /7,1%)	0 (0%)	1 (2,5%)
Koncentracija RF (IU/ml)/*Concentration RF (IU/ml)*	40-80	40-160	0	0

Ispitanici sa prisutnim RF - prisustvo RF u serumu u koncentraciji >20 IU/ml

Positive RF finding - Serum RF concentrationin > 20 IU/ml

By examining the presence of autoantibodies in the sera of the Group B patients, the presence of AGMAs was been registered in one patient (2.5%), while other examined autoantibodies were not detected.

By examining the presence of autoantibodies in the sera of the Control Group subjects, the presence of APAs was registered in the sera of two subjects (3.33%), while other examined autoantibodies were not detected.

Our investigation established no statistically significant sex-related differences in the presence of the examined autoantibodies.

By applying the TIF for ANA determination, all serum samples were tested on the tissue cryosections, at 1:28 dilution rate. Of the total of positive sera, two and 10 were positive at the titres of up to 1:128 and 1:64 respectively. During the treatment, the titre of autoantibodies remained unchanged. By HEp-2 cellular cultivation, the presence of ANAs was established in the sera from those patients in whom their presence had been formerly registered in the tissue cryosections. The presence of antibodies by HEp-2 cultivation was examined only at the serum dilution rate of 1:32, that is more diluted serum samples were not tested due to economic reasons. Determination of the nuclear fluorescence type established the dot-like and homogenous pattern in nine and six patients respectively. These findings were confirmed by HEp-2 cell cultivation.

All other autoantibodies (AGMA, APA and AMA) were detected at the titre of 1:32.

The RF test findings are presented in Table 3. Table 3 reveals that four A Group patients (5.7%) had increased serum RF levels prior to initiation of the treatment for pulmonary TB. The measured serum RF level was found to be 40 IU/ml in three patients, and 80 IU/ml in one patient. After one-month treatment, the RF was detected in the serum from 5 patients (7.1%), in the levels of 40IU/ml and 160 IU/ml in two patients respectively, and in the level of 80 IU/ml in one patient (the serum RF levels increased). Once the applied therapy had been completed, no increased RF levels were detected in the patients' sera. Two patients with formerly increased serum RF levels did not come for the scheduled control examination after completing the treatment, so their RF levels could not be measured. One of these patients wasn't presented with an elevated serum RF level on admission, while one month later he had the measured RF level of 160 IU/ml. Another patient had the RF level of 40 IU/ml on admission, and one month later it fell within the baseline.

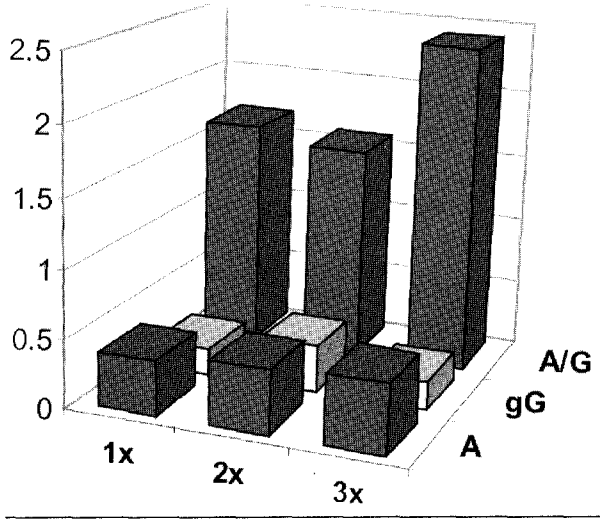
Among the Group B patients, the elevated serum RF level was measured in one patient (3.6%), amounting to 40 IU/ml. No elevated serum RF levels were registered in the Control Group healthy patients.

Rezultati proteinograma

Na Tabeli 4 prikazan je zbirni proteinogram (za bolesnike oba pola), određivan kod bolesnika tri puta, tokom lečenja plućne tuberkuloze. Uočava se da ukupni proteini tokom lečenja neznatno opadaju, ali su u granicama referentnih vrednosti (60-80 g/l). Albumini konstantno rastu tokom terapije i njihove srednje vrednosti se statistički značajno razlikuju na početku i na kraju terapije (p=0,031). Relativna vrednost gama globulinske frakcije proteinograma bila je povišena (>0,18%) prilikom sva tri određivanja. Vrednosti gama-globulina rastu tokom terapije, tako da se srednje vrednosti globulina prilikom kontrole posle mesec dana statistički značajno razlikuju (p=0,042), a na kraju terapije se vraćaju na početne vrednosti. Pad nivoa globulina (odnosno njegove srednje vrednosti) na kraju terapije u odnosu na vrednosti posle mesec dana lečenja takođe je statistički značajan (p=0,00005). Srednja vrednost albuminsko-globulinskog indeksa je niža prilikom ispitivanja posle mesec dana u odnosu na početne vrednosti, da bi na kraju terapije bila viša, prvenstveno zahvaljujući porastu albumina tokom terapije. Ta razlika je statistički signifikantna (p=0,0099).

Tabela 4. Prikaz srednjih vrednosti pojedinih frakcija proteinograma kod bolesnika tokom lečenja plućne tuberkuloze

Table 4. *Mean levels of certain proteingram fractions during TB treatment*



Zbirni (muški i ženski) proteinogram/*Cummulative (male and female) proteingram*												
	pri prijemu/*on admission*				posle 1 mesec/*after 1 month*				posle 6 meseci/*after 6 months*			
	UP	A	gG	A/G	UP	A	gG	A/G	UP	A	gG	A/G
n		30				8				24		
x	70,94	0,40	0,20	1,67	70,91	0,47	0,34	1,57	68,82	0,51	0,21	2,41
sd	8,7	0,1	0,1	1,0	8,1	0,1	0,3	0,9	9,6	0,1	0,1	1,0

Legenda/*legend*: UP-ukupni proteini/*total proteins (g/l)*; A-albumini/*albumins* (u relativnim vrednostima/*in relative values*); gG-gama globulini/*gama globulins* (u relativnim vrednostima/*in relative values*); A/G-albumunsko-globulinski index/*albumin-globulin index;*

Da bismo uporedili pojavu autoantitela (aAT) sa hiper-gamaglobulinemijom, sve bolesnike smo

Proteinogram findings

Table 4 shows the cumulative proteinogram (from patients of both sexes), examined three times during the treatment of pulmonary tuberculosis.

Table 4 reveals a mild decrease of total proteins during the treatment, but they remained at the baseline (60-80 g/l). Albumins were increasing constantly in the course of the treatment, exhibiting a statistically significant difference of their mean level at the beginning and the end of the treatment (p=0.031). The relative value of gamma-globulins increased (>0.18%) at all three measurements. The values of gamma-globulins were rising during the treatment, so the average value of globulins after 1 month was significantly different (p=0.042), but at the end of the treatment these values decreased to the initial level. There was also a statistically significant difference between the values of globulins measured at the and of the treatment and after one month of the treatment (p=0.00005). The mean albumin-globulin index was lower after one month of the treatment than on admission but at the end of the treatment it increased, primarily due to increasing albumin levels during the treatment. That difference was statistically significant (p=0.0099).

To correlate the emergence of auto-antibodies (aAT) to hyper-gammaglobulinemia, we classified the examined patients into two groups, depending on their serum aAT findings. Group I included the patients with a confirmed serum aAT finding, and Group II consisted of those with no evidence of serum aAT. Table 5 reviews some mean values of certain proteinogram fractions in patients with and without serum aAT, determined in the course of the treatment.

Tabela 5. Prikaz srednjih vrednosti pojedinih frakcija proteinograma kod bolesnika sa i bez prisutnih autoantitela u serumima tokom lečenja plućne tuberkuloze

Table 5. *Mean levels of certain proteingram fractions in patients with and without serum autoantibodies during TB treatment*

Autoantitela - gama globulini/*Autoantibodies - gama globulins*												
	pri prijemu *on admission*				posle 1 mesec *after 1 month*				posle 6 meseci *after 6 months*			
Bolesnici/*Patients*	UP	A	gG	A/G	UP	A	gG	A/G	UP	A	gG	A/G
sa aAT/*with aAB*	73	0,43	0,25	1,55	74	0,4	0,5	0,9	69	0,44	0,26	1,66
bez aAT *without aAB*	63	0,47	0,19	1,74	66	0,5	0,2	1	69	0,54	0,2	2,78

Legenda/*legend*: UP-ukupni proteini/*total proteins* (g/l); A-albumini/*albumins* (u relativnim vrednostima/*in relative values*); gG-gama globulini/*gama globulins* (u relativnim vrednostima/*in relative values*); A/G-albumunsko-globulinski index/*albumin-globulin index;* aAT/aAB - autoantitela/*autoantibodies*

It may be noticed from the table that the mean gamma-globulin values were elevated at each proteinogram taking (>0.18%), and they were higher in the patients with serum aAT finding.

podelili u dve grupe prema nalazu aAT u serumima. Prvu grupu su činili bolesnici sa dokazanim prisustvom aAT u serumu, a drugu bolesnici bez prisustva aAT u serumu. Na Tabeli 5 prikazane su srednje vrednosti pojedinih frakcija proteinograma kod bolesnika sa i bez prisutnih autoantitela u serumima, određivane tokom lečenja bolesti.

Iz Tabele 5 se uočava da su pri svakom određivanju proteinograma, srednje vrednosti gama-globulina bile povišene (>0,18%) i da su bile više u serumima bolesnika kod kojih je utvrđeno prisustvo aAT u serumu.

Diskusija

Infektivni agensi su neki od najvažnijih faktora sredine koji mogu da bude povezani sa autoimunošću. Mikroorganizmi kao što su virusi, bakterije i paraziti mogu da budu pokretači poremećaja tolerancije i razvoja autoimunološke bolesti [1,9]. Hronične infekcije mogu biti povezane sa pojavom različitih autoantitela [2]. Kod većine osoba, tokom infekcije, prisustvo autoantitela je asimptomatsko. Po izlečenju infektivne bolesti, autoantitela se brzo gube. U nekim slučajevima su zabeležene autoimunološke reakcije od kliničkog značaja [9,10].

Glavni problem kod obolelih od plućne tuberkuloze je u nemogućnosti imunološkog odgovora da ispolji baktericidnu funkciju [11]. Zbog toga je sterilizacija lezija zavisna od hemioterapije koja mora da se sprovodi najmanje 6 meseci. Oboleli od plućne tuberkuloze se leče kombinovanom hemioterapijom, koja u većini slučajeva podrazumeva obaveznu primenu izonijazida kao moćnog baktericidnog sredstva [11]. Poznato je da izonijazid može da indukuje stvaranje ANA kod dela bolesnika koji su ga uzimali tokom lečenja bolesti, mada u različitim izveštajima sa različitom učestalošću: 4% [12], 11% [13], 15% [14], 17% [15], 24% [16] 47% [2] i 59% [17]. Moguće je da su ove razlike uslovljene razlikama u kriterijumima korišćenim za determinisanje pozitivnosti [13]. Istraživanja su pokazala da je incidencija autoantitela viša među sporim akceleratorima izonijazida, nego među brzim akceleratorima [7,15]. Svi bolesnici uključeni u naše istraživanje su lečeni izonijazidom tako da se ne može isključiti mogućnost da je prisustvo ANA, bar delimično, pored mogućih mehanizama molekulske mimikrije i poliklonske aktivacije limfocita, posledica medikacije.

Istraživanjima Cannata i Seligmanna [19] je zaključeno da se ANA pojavljuju kod nekih bolesnika posle 5 meseci od započinjanja lečenja i iščezavaju 6 meseci po prekidu uzimanja lekova. Ovi rezultati se ne slažu sa našim rezultatitma, jer smo utvrdili raniju pojavu ANA u serumima bolesnika. Mirčetić [7] smatra da ANA izazvana lekovima nestaju iz seruma vrlo brzo posle prekida uzimanja leka koji je indukovao njihovu pojavu. S obzirom da u grupi bolesnika koji su pre 5 godina lečeni od plućne tuberkuloze (grupa B) nismo utvrdili

Discussion

Infective agents are some of the most important environmental factors which may affect autoimmunity. Microorganisms such as viruses, bacteria and parasites, may induce impaired tolerance and generate autoimmunity [1,9]. Chronic infections may be associated with the emergence of diverse autoantibodies [2]. During infection, the presence of autoantibodies remains asymptomatic in most subjects. As soon as the infection is overcome, autoantibodies quickly disappear. However, clinically relevant autoimmune reactions have been registered in some cases [9,10].

The major problem of patients with tuberculosis is the failure of their immune response to manifest its bactericidal function [11]. Sterilization of the lesions therefore depends on chemotherapy, which has to be administered for at least six months. Pulmonary tuberculosis is treated by the combined 6-month chemotherapy, which in most cases obligatorily includes isoniazid as a potent bactericidal agent [11]. It is known that isoniazid may induce ANA formation in a number of patients receiving it during the treatment, but there is a great variety of its reported frequency: 4% [12], 11% [13], 15% [14], 17% [15], 24% [16], 47% [2] and 59% [17]. These differences may be due to the criteria applied to determine positiveness [13]. The autoantibody incidence has been reported to be greater with slow isoniazid accelerators than with the fast ones [7,15]. All the patients included in our investigation were treated with isoniazid, so the presence of ANA, may, at least partially, be attributed to this medication, besides to the possible mechanisms of molecular mimicry and polyclonal lymphocyte activation.

Cannat and Seligmann [19] have reported that ANA emerge five months after initiation of the treatment in some patients and disappear six months after its discontinuation. These findings do not correlate with those obtained in our study, as we have found an early appearance of ANA in the patients' sera. Mircetic [7] believes that the drug-induced ANA disappear from the serum shortly after discontinuing the relevant drug. As in our study ANA were not registered in the group of patients treated for pulmonary tuberculosis five years ago (Group B), it is supposed that, if they had existed, they disappeared, correlating quite well to these authors' observations.

Our study results established no statistically significant sex-related differences regarding the presence of ANA. This is in accordance with to the findings of some authors [13], and deviates from some other [17] authors' findings, who found the prevalence of ANA in females.

The serum samples examined for ANA presence were diluted at the ratio up to 1:128. Of the total of 15 positive sera, two were positive at the titre of up to 1:128, while 10 were positive at the titre of up to 1:64, meaning the titre of antibodies did not increase during the treatment, except in two patients at the

postojanje ANA u serumima ispitanika, možemo pretpostaviti da su ANA (ukoliko su i postojala kod njih) iščezla, što može biti u saglasnosti sa nalazima spomenutih autora.

Našim istraživanjem nije utvrđena statistički značajna razlika u prisustvu ANA među polovima. Ovo je u saglasnosti sa rezultatima pojedinih autora [13], a u suprotnosti sa rezultatima drugih [17] koji nalaze veću prevalenciju ANA u žena.

Svi serumi bolesnika su ispitivani na prisustvo ANA u razblaženjima do 1:128. Od ukupno 15 pozitivnih seruma, 2 su bila pozitivna do titra od 1:128, a 10 ih je bilo pozitivno do titra 1:64. Znači, nije dolazilo do porasta titra antitela tokom lečenja bolesti, sem kod dva bolesnika na kraju lečenja. Naši rezultati su u saglasnosti sa rezultatima Vasquez-del Mercado [13] koja detektuje ANA u titrovima 1:50 i 1:100 i istraživanjima Cannat i Seligmann [19] koji detektuju ANA u serumima bolesnika u titrovima 1:10, 1:50 i 1:100. I u ovim istraživanjima su kod nekih bolesnika titrovi ostali isti, dok je kod drugih došlo do porasta titra. Dlugovitzky i saradnici [12] u svojim istraživanjima detektuju ANA u znatno većim titrovima (1:20 do 1:320), što je u suprotnosti sa našim rezultatima.

U našem radu je utvrđeno prisustvo tačkastog i homogenog tipa jedarne fluorescencije, s tim da je dominirao tačkasti tip (u odnosu 9:6). Ovakav nalaz jedarne fluorescence je potvrđen i korišćenjem kulture ćelija HEp-2, što je u saglasnosti sa nalazom Rothfielda i ostalih [20], a u suprotnosti sa nalazima koje su dobili Dlugovitzky i saradnici [12] i Vasquez-del Mercado [13] kod kojih je dominirao homogeni tip jedarne fluorescence, nad tačkastim tipom.

Ispitivanjem prisustva drugih autoantitela (APA, AGMA, AMA, ATA i ASA) u serumima bolesnika dokazano je prisustvo APA, AGMA i AMA autoantitela, dok ASA i ATA nisu utvrđena. U nama dostupnoj literaturi nije bilo podataka o prisustvu ovih autoantitela u serumima obolelih i lečenih od aktivne plućne tuberkuloze.

U serumima zdravih osoba naše kontrolne grupe APA su nađena kod 3,33% ispitanika. Mrđa [21] nalazi u zdravoj populaciji APA sa učestalošću od 4%. Kod zdravih osoba mlađih od 60 godina APA se javljaju sa učestalošću do 16% [22]. S obzirom na učestalost APA u zdravoj populaciji i na činjenicu da naši bolesnici pre hospitalizacije i tokom lečenja plućne tuberkuloze nisu bolovali od perniciozne anemije, niti od neke druge bolesti (za koju su APA karakteristična), smatramo da je nalaz APA u serumima obolelih i lečenih od plućne tuberkuloze nespecifičan i da nije povezan sa infekcijom *M. tuberculosis,* niti sa upotrebom ATL.

Poznato je da AMA mogu da budu prisutna u serumima do 5% zdravih osoba [22,23]. S obzirom da naši bolesnici nisu bolovali od primarne bilijarne ciroze (za koju je karakterističan nalaz AMA u serumima obolelih), niti od hroničnog aktivnog hepatitisa (za koji je karakterističan nalaz AGMA u

end of the treatment. Our results accord with those of Vasquez-del Mercado [13], who detected ANA at the titres of 1:50 and 1:100, as well as to those reported by Cannat and Seligmann [19], who detected ANA at the titres of 1:10, 1:50 i 1:100 in the patients' sera. Our results are in accordance with those obtained by some authors [13] who detected ANA in the patients' sera titrated at 1:50 and 1:100. These studies also reported the titres elevated during the treatment in some patients, while in the others they remained unchanged. Our results do not accord with those obtained by some authors [12], who in their investigations detected ANA at significantly higher titres (1:20 - 1:320).

The dot-like and homogenous types of nuclear immunofluorescence were detected in our study, with the prevalence of the former type (9:6), This pattern of nuclear immunofluorescence was established by HEp-2 cell cultivation, that being in accordance with the results reported by Rothfield et al. [20], but opposing those reported by Dlugovitzky et al. [12], and Vasquez-del Mercado [13], who found the homogenous type of nuclear immunofluorescence dominate the dot-like pattern. Similar findings regarding the nuclear immunoflurescence were obtained by HEp-2 cell cultivation, with agreeing with the findings of Rothfield at al. [20], and deviating from those obtained by Dlugovitzky at al. [12] and Vasquez-del Mercado [13], who established the homogenous pattern for the dominant nuclear immunofluorescence type.

By examining the presence of other autoantibodies (APA, AGMA, AMA, ATA and ASA) in the patients' sera, the presence of APA, AGMA and AMA was established, but no evidence of ASA and ATA was obtained. In the available literature we found no evidence of the presence of these autoantibodies in the sera of the patients treated for active pulmonary tuberculosis.

In the Control Group, APA were detected in 3.33% of the healthy subjects' sera. Mrđa [21] established the 4% frequency rate of APA in healthy population. Among the healthy subjects younger than 60, APA were detected at the frequency rate of up to 16% [22]. Regarding the frequency of APA in healthy population and the fact that our patients did not suffer from pernicious anemia (or any other disease characterized by the presence of APA) prior to hospitalization or in the course of the treatment, we consider the APA finding in the sera of patients treated for active pulmonary tuberculosis nonspecific and not related to either the infection with *M. tuberculosis,* or antituberculosis drug administration.

It is well known that AMA may be present in the sera of up to 5% of healthy subjects [22,23]. As our patients suffered from neither the primary biliary cirrhosis (characterized with the serum AMA finding), nor the chronic active hepatitis (characterized by the serum AGMA finding), we also consider the

serumima obolelih), takođe smatramo da je nalaz AGMA i AMA u serumima ispitanika nespecifičan i da nije u vezi sa infekcijom *M. tuberculosis*, niti sa upotrebom antituberkulotika.

Nizak nivo autoantitela nastaje i kod zdravih osoba tokom imunološkog odgovora na strane antigene [10,24]. Detekcijom takvih "prirodnih" antitela potvrđuje se ideja da i normalno postoji potencijal za autoreaktivnost. Drugo moguće objašnjenje prisustva ovih autoantitela u serumima bolesnika se povezuje sa poliklonalnom aktivacijom B-limfocita u sklopu opšte imunološke reakcije.

U pokušaju da objasnimo prisustvo RF u serumima obolelih od plućne tuberkuloze došli smo do podataka u literaturi koji ukazuju na prisustvo abnormalnog IgG (nazvanog "agalaktozil IgG" - Gal (0) IgG) kod tuberkuloznih bolesnika [22,25]. To je IgG kome nedostaje terminalna galaktoza na CH2 domenu teškog lanca IgG. Smatra se da ovaj glikozilacioni defekt može da dovede do konformacionih promena u Fc regionu IgG, što čini molekule mnogo imunogenijim i uslovljava da se oni vezuju sa većom avidnošću za RF-e, te ima ulogu u produkciji autoantitela. U našem radu nismo ispitivali prisustvo abnormalnog "agalaktozil IgG", ali možemo da pretpostavimo da je on prisutan bar kod dela naših bolesnika i odgovoran za indukciju RF kod njih. Po našem saznanju ne postoje podaci u literaturi koji se odnose na određivanje Gal (0) IgG u serumima bolesnika od plućne tuberkuloze tokom primene terapije.

U radu su analizirani proteinogrami bolesnika određivani u tri navrata. Utvrđeno je da dolazi do promena u gama-globulinskoj frakciji, odnosno do reverzibilne hiper-gamaglobulinemije. Brojne studije su izveštavale o povišenim koncentracijama imunoglobulina kod 1/3 do 1/2 tuberkuloznih bolesnika [13,17,26]. Hiper-gamaglobulinemija kod obolelih sa aktivnom tuberkulozom može da se objasni poznatim adjuvantnim efektom mikobakterija na imunološki sistem što može da vodi poliklonskoj aktivaciji B-limfocita, koja može da bude jedan od mehanizama u nastanku autoimunosti. Ispitujući međusobnu zavisnost pojave autoantitela sa hiper-gamaglobulinemijom može da se zaključi da je prisutna hiper-gamaglobulinemija kod obolelih od plućne tuberkuloze bar delimično posledica prisustva autoantitela u serumima obolelih.

Zaključak

U serumima dela bolesnika lečenih od aktivne plućne tuberkuloze mogu da se detektuju pojedina autoantitela u niskom titru. Nije utvrđena statistički značajna razlika u njihovom prisustvu među polovima. Prisustvo antinuklearnih antitela u serumima bolesnika je pored mogućih mehanizama molekulske mimikrije i poliklonske aktivacije limfocita, bar

serum AGMA and AMA findings nonspecific and not related to *M. tuberculosis* infection or administration of antituberculosis drugs.

The low autoantibody level may be registered in healthy subjects as well in the course of an immune response to alien antigens [10,24]. The detection of these "natural" antibodies confirms the hypothesis of autoreactivity potential generally present in normal conditions. The presence of these autoantibodies in patients' sera may be explained by the B lymphocyte activation included in the general immunological reaction.

While attempting to explain the RF presence in the sera of pulmonary TB patients, we came across the literature data suggesting the presence of abnormal IgG (labelled "agalactosole IgG" - Gal (0) IgG) levels in TB patients [22,25]. This IgG type lacks the terminal galactose on the CH2 domain of the heavy IgG chain. It is considered that this glucosylation defect may induce conformational changes in the Fc IgG region, making the molecules much more immunogenic and causing them to bind to RF-e with greater avidity, and it also plays a role in autoantibody production. In our study we did not examine the presence of abnormal "agalactosyl IgG", but it is supposed to be present in at least a portion of our patients in whom it is responsible for the RF induction. As far as we know, there are no literature data referring to determination of Gal (0) IgG in the serum of the patients with pulmonary tuberculosis in the course of the treatment.

The study analysed the patients' proteinograms taken on three occasions. Alterations were evidenced in the gamma-globulin fraction, that is, a reversible hyper-gammaglobulinemia was verified. Numerous studies reported increased ummunoglobulin concentrations in one third to one half of TB patients [13,17,26]. Hyper-gammaglobulinemia in patients with active tuberculosis may be explained by the well-known adjuvant effect of mycobacteria onto the immune system, inducing the polyclonal B lymphocyte activation which may be one of the possible mechanisms of the development of autoimmunity. By examining the mutual correlation between the emergence of auto-antibodies and hyper-gammaglobulinemia, it may be concluded that the present hyper-gammaglobulinemia in patients with tuberculosis results, at least partially, from the presence of auto-antibodies in the patients' sera.

Conclusion

Low titres of certain autoantibodies may be registered in the serum of a number of patients with active pulmonary tuberculosis. No significant difference in the presence of the analysed autoantibodies was registered between the sexes. The presence of antinuclear antibodies in the serum may, at least partially (besides molecular mimicry and polyclonal lymphocyte activation) be due to isoniazid admi-

delimično posledica primene izonijazida. Nalaz drugih autoantitela je nespecifičan i nije u vezi sa infekcijom mikobakterijama, niti sa upotrebom antituberkulotika. Tokom lečenja bolesti dolazi do reverzibilne hiper-gamaglobulinemije, koja je bar delimično posledica prisustva autoantitela u serumima bolesnika.

nistration. The finding of other autoantibodies is nonspecific and correlated neither to the mycobacterial infection, nor to the antituberculosis therapy. In the course of the treatment a reversible hypergammaglobulinemia developed, which was at least partially due to the presence of autoantibodies in the patients' blood serum.

Literatura

1. Mc Devit HU, Wakeland EK. Autoimmunity. Curr Opin Immunol 1998;10:647-8.
2. Lindquist KJ, Coleman RE, Osterland CK. Autoantibodies in chronic pulmonary tuberculosis. J Chronic Dis ,1970;22: 717-25.
3. Roitt IM. Autoimmunity and autoimmune disease. In: Roitt IM, Brostoff J, Male DK. Immunology. London: Mosby; 1996.
4. Falcone M, Sarvetnick N. Cytokine that regulate autoimmune responses. Curr Opin Immunol 1999;11:670-6.
5. Cavallaro JJ. Laboratory methods for the detection of antinuclear antibodies: a joint effort of the centers for disease control and the arthritis foundation. Atlanta, Georgia; 1987;732: 143-84.
6. Kavanaugha A, Tomar R, Reveille J, et al. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. Arch Pathol Lab Med 2000; 124:71-81.
7. Mirčetić V, Savić-Đurković R, Petrović M, Vukojević P. Antinukleusna antitela. Beograd: Medicinska knjiga; 1995.
8. Lučić A. Funkcijsko ispitivanje krvi. U: Borota R, Lučić A. Priručnik praktičnih i seminarskih vežbi iz patološke fiziologije. Novi Sad: Medicinski fakultet; 1998:405-6.
9. Schenfeld Y, Isenberg DA. Mycobacteria and autoimmunity. Immunol Today 1988;9:178-82.
10. Grabar P. Autoantibodies and the physiological role of immunoglobulins. Immunol Today 1983;4:337-40.
11. Popovac D. Bolesti pluća. Beograd: Data Status; 1999: 277-350.
12. Dlugovitzky D, Torres A, Hourquescos MC. Low occurrence of arthritic manifestation in patients with pulmonary tuberculosis: T cell subsets and humoral studies. Mem Inst Oswaldo Cruz 1995;90:623-8.
13. Vasquez-del Mercado M, Casiano CA, Rubin RL. IgA antihistone antibodies in Isoniazid-treated tubrculosis patients. Autoimmunity 1995;20:105-11.
14. Rapoport BL, Morrison RC, Sher R, Dos Santos L. A study of antibodies in chronic mycobacterial infections. Int J Lepr Other Mycobact Dis 1990;58:518-25.
15. Price-Evans DA, Bullen MF, Houston J. Antinuclear factors in rapid and slow acetylator patients treated with Isoniazid. J Med Genet 1972;9:53-6.
16. Pradhan VD, Badakere SS, Ghosh K, Pawar AR. Spectrum of anti-neutrophil cytoplasmic antibodies in patients with pulmonary tuberculosis overlaps with that of Wegener 's granulomatosis. Indian J Med Sci 2004;58:283-8.
17. Alargacon-Segovia D, Fishbein E. Serum immunoglobulins in pulmonary tuberculosis. Chest 1971;60:133-6.
18. Demkow U, Zielonka TM, Radomska D. Influence of Isoniazid on selected parameters of immunological response. Pol Merkuriusz Lek 2000;7:27-30.
19. Cannat A, Seligmann M. Possible induction of antinuclear antibodies by Isoniazid. Lancet 1966:1:185-7.
20. Rothfield NF, Bierer WF, Garfield JW. Isoniazid induction of antinuclear antibodies. Ann Intern Med 1978;88:650-2.
21. Mrda Z. Klinički i socioekonomski aspekti infekcije Helicobacter pylori (doktorska disertacija). Novi Sad: Medicinski fakultet; 2000.
22. Brostoff J, Scadding KG, Male D, Roitt IM. Clinical Immunology. London: Mosby; 1994:5-10.
23. Glišić B, Stefanović D, Popović M, Mitrović D. Klinički značaj odredivanja autoantitela u autoimunim bolestima. Vojnosanit Pregl 1999;56:407-17.
24. Stites DR, Terr AI, Parslow TG. B cell development and the humoral immune response. In: Medical Immunology. Stamford: Appletion&Lange; 1993:115-30.
25. Rook GAW, Stanford JL. Slow bacterial infection or autoimmunity? Immunol Today 1992;13:160-4.
26. Radosavljević T. Promene parametara celularnog i humoralnog imuniteta u toku aktivne plućne tuberkuloze (magistarski rad) Beograd: Medicinski fakultet; 1994.

Rad je primljen 28. III 2006.
Prihvaćen za štampu 3. V 2006.
BIBLID.0025-8105:(2008):LXI:7-8:333-342.