C-reaktivni protein i komplement C3 i C4 u djece s latentnom tuberkuloznom infekcijom

C-reactive protein and complement components C3 and C4 in children with latent tuberculosis infection

Slavica Dodig[1], Daniela Galez[2], Ivka Zoričić-Letoja[1], Branka Kristić-Kirin[1], Kornelija Kovač[1], Boro Nogalo[1], Jadranka Živčić[1], Renata Zrinski-Topić[1]

[1]Dječja bolnica Srebrnjak; Referentni centar Ministarstva zdravstva za kliničku alergologiju djece, Zagreb

[1]Srebrnjak Children's Hospital, Reference Center for Clinical Pediatric Allergology of the Ministry of Health and Welfare, Zagreb, Croatia

[2]Opća bolnica Karlovac, Karlovac

[2]Karlovac General Hospital, Karlovac, Croatia

Sažetak

Cilj: U posljednje se vrijeme hsCRP (engl. *high sensitive* CRP) primjenjuje kao prognostički biljeg kronične upale. Cilj ovoga rada bio je ispitati dolazi li do promjene serumske koncentracije hsCRP, C3 i C4 u djece s latentnom tuberkuloznom infekcijom nakon dva mjeseca profilakse izonijazidom.

Ispitanici i metode: Ukupno je ispitano 79-ero djece podijeljene u tri skupine: 1) ispitanici s latentnom tuberkuloznom infekcijom (LTBI); 2) ispitanici s tuberkulozom pluća; 3) klinički zdravi ispitanici upućeni na sistematski pregled, s biokemijsko-hematološkim pokazateljima unutar referentnih vrijednosti za dob – kontrolna skupina. Krv je uzorkovana dvaput: prije započinjanja terapije i poslije dvomjesečne terapije tijekom koje su lijekovi primjenjivani svakodnevno. Imunokemijskim metodama određena je koncentracija hsCRP, C3 i C4.

Rezultati: Koncentracija hsCRP, C3 i C4 u ispitanika s LTBI prije profilakse izonijazidom bila je statistički značajno veća nego u kontrolnoj skupini. Nakon profilakse izonijazidom u osoba s LTBI koncentracija hsCRP bila je statistički značajno manja nego prije primjene izonijazida. Specifičnost je za sve odabrane analite bila veća nego osjetljivost. Granične vrijednosti za hsCRP imale su bolju dijagnostičku učinkovitost nego granične vrijednosti za C3 odnosno C4.

Zaključak: Koncentracija hsCRP može se primijeniti za praćenje bolesnika s LTBI u svrhu procjene odgovora na profilaksu izonijazidom i stupnja aktivnosti bolesti.

Ključne riječi: dijete, komplement C3, komplement C4, C-reaktivni protein, latentna tuberkulozna infekcija, tuberkuloza

Abstract

Aim: Recently, hsCRP (high sensitive CRP) concentration has been used as a prognostic marker of chronic inflammation. The aim of the study was to determine whether two-month isoniazid prophylaxis induced changes in serum concentrations of hsCRP, C3 and C4 in children with latent tuberculosis infection (LTBI).

Subjects and methods: The study included 79 children divided into three groups: 1) subjects with LTBI; 2) subjects with lung tuberculosis; and 3) control group of clinically healthy subjects, referred for systematic examination, with biochemistry-hematology parameters within reference range for age. Blood sampling was performed twice: before drug administration and after a two-month period during which drugs were administered on a daily basis; hsCRP, C3 and C4 were determined by use of immunoassays.

Results: Before prophylactic isoniazid therapy induction, the concentrations of hsCRP, C3 and C4 were significantly higher in LTBI group as compared with control group. After prophylactic therapy, the concentration of hsCRP in LTBI was lower than before isoniazid administration. The specificity was greater than sensitivity for all study analytes. The cut-off value of hsCRP showed higher optimum diagnostic efficiency than the cut-off values of C3 and C4.

Conclusion: The concentration of hsCRP can be used in the follow up of LTBI patients to evaluate response to isoniazid prophylaxis and the level of disease activity.

Key words: child, complement C3, complement C4, C-reactive protein, latent tuberculosis infection, tuberculosis

Pristiglo: 26. lipnja 2007. *Received: June 16, 2007*

Prihvaćeno: 15. siječnja 2008. *Accepted: January 15, 2008*

Uvod

Tuberkuloza (TBC) je bolest označena upalom plućnog parenhima, a uzrokovana je unutarstaničnom bakterijom, mikobakterijem tuberkuloze (*M. tuberculosis*). Oko 2 milijarde ljudi ima latentnu infekciju mikobakterijem tuberkuloze (LTBI) (1). Budući da od infekcije do očitovanja bolesti može proći i dulje vrijeme, važno je inficirane prepoznati prije početka razvoja bolesti kako bi se pravodobnom profilaksom spriječilo razvijanje bolesti. Tuberkulinski kožni test (engl. *tuberculin skin test*, TST) kao „zlatni standard" te *in vitro* određivanje gama-interferona kao jedna od novijih dijagnostičkih metoda (2,3) (uključujući anamnezu, podatak o kontaktu s TBC bolesnikom, kliničku sliku, rendgenogram prsišta) ključne su pretrage za otkrivanje infekcije *M. tuberculosis*. Nuspojave primjene antituberkuloznih lijekova, kako u bolesnika s aktivnom tuberkulozom tako i u osoba s LTBI prate se određivanjem kompletne krvne slike, broja trombocita, te testovima za ispitivanje jetrene funkcije (4), a longitudinalno određivanje koncentracije C-reaktivnog proteina (CRP) može se primijeniti kao pokazatelj aktivnosti bolesti (5).

CRP je dobro poznati biljeg akutne upale, koncentracija kojega se u serumu često određuje radi procjene stupnja sistemske upale (6), primjerice kod reumatskih (7) ili crijevnih bolesti (8) ili kako bi se utvrdila bakterijska etiologija neke upale, primjerice pneumonije, kako u odraslih (9) tako i u djece (10). Posljednjih se godina za određivanje vrlo malih koncentracija CRP primjenjuje imunoturbidimetrijska metoda na lateks česticama. Tom se metodom povećava analitička osjetljivost određivanja koncentracije tzv. visoko osjetljivog, hsCRP (engl. *high sensitive*) u serumu do 0,1 mg/L, što je omogućilo da se određivanje koncentracije hsCRP primjenjuje i kao prognostički upalni biljeg kronične upale u bolesnika s kardiovaskularnim bolestima (11), šećernom bolešću (12,13) i astmom (14,15).

CRP kao protein akutnog odgovora sudjeluje u regulaciji sustava komplementa (16). Ispitivanje uloge sustava komplementa posljednjih godina doživljava preporod zbog njegove uloge ne samo u infekciji, upali ili alergijskoj reakciji, nego i u uklanjanju apoptotičnih stanica (17), u razvoju autoimunih bolesti (18), ali i u mogućoj primjeni u terapijske svrhe (19). Mikobakterij tuberkuloze također može preko humanog monocitnog receptora C1 aktivirati sustav komplementa (20).

Ovim radom željeli smo ispitati dolazi li do promjene u koncentraciji hsCRP, C3 i C4 u djece s latentnom tuberkuloznom infekcijom. Koncentracije hsCRP te komponenata komplementa C3 i C4 određivale su se prije profilakse izonijazidom i poslije dvomjesečne profilakse tijekom koje je izonijazid primjenjivan svakodnevno.

Introduction

Pulmonary tuberculosis is a disease characterized by inflammation of lung parenchyma and is caused by the intracellular bacterium *Mycobacterium (M.) tuberculosis*. There are some two billion people with latent *M. tuberculosis* infection (LTBI) worldwide (1). As a long time may elapse from infection to disease manifestation, identification of those infected before the initial disease development is of utmost importance to prevent it to flare up by timely prophylaxis. Tuberculin skin test (TST) as the gold standard, and *in vitro* determination of interferon gamma (IFN-γ) as one of recent diagnostic methods (2,3) (including history, data on contact with a tuberculosis patient, clinical picture, and chest x-ray) are crucial tests used to detect *M. tuberculosis* infection. Side effects of antituberculotics in patients with active tuberculosis and individuals with LTBI are monitored by determination of complete blood count, platelet count and liver function tests (4), whereas longitudinal determination of C-reactive protein (CRP) concentration can be used as an indicator of disease activity (5).

CRP is an established marker of acute inflammation, and its serum concentration is frequently determined to assess the grade of systemic inflammation (6), e.g., in rheumatic (7) or intestinal (8) diseases, or to verify bacterial etiology of inflammation such as pneumonia in adults (9) and children (10) alike. Recently, the immunoturbidimetric method on latex particles has been used to determine very low CRP concentrations. The method has improved analytical sensitivity in determining serum concentration of high-sensitive CRP (hsCRP) to 0.1 mg/L, thus enabling the use of hsCRP concentration as a prognostic marker of chronic inflammation in patients with cardiovascular disease (11), diabetes mellitus (12, 13) and asthma (14, 15).

CRP as an acute phase protein is involved in the regulation of the complement system (16). In the last few years, research into the role of complement system has been revived because of its role not only in infection, inflammation or allergic reaction but also in the apoptotic cell clearance (17) and development of autoimmune disorders (18), and for its potential therapeutic use (19). *M. tuberculosis* can also activate complement system *via* C1 human monocyte receptor (20).

The objective of the present study was to assess changes in the concentration of hsCRP, C3 and C4 in children with latent *M. tuberculosis* infection. Concentrations of hsCRP as well as complement components C3 and C4 were determined before drug administration and after a two-month period during which drugs were administered on a daily basis.

Ispitanici i metode

Ispitanici

Ukupno je analizirano 79 ispitanika u dobi od 2,5 mjeseca do 18 godina, koji su u razdoblju od listopada 2005. do siječnja 2007. godine bili upućeni u Dječju bolnicu Srebrnjak, Zagreb radi sistematskog pregleda ili u svrhu otkrivanja moguće infekcije bakterijom *M. tuberculosis*. Ispitanici su prema dijagnozi svrstani u tri skupine:

1. ispitanici s LTBI (N = 26, dob 1–18 godina) koji su bili u bliskom kontaktu s tuberkuloznim bolesnikom i imaju pozitivan nalaz tuberkulinskog kožnog testa s pročišćenim proteinskim derivatom (engl. *purified protein derivative,* PPD); ispitanici su kao profilaksu su dobivali izonijazid;
2. djeca s tuberkulozom pluća (N = 18, dob 2,5 mjeseca do 18 godina); dijagnoza je postavljena na temelju kliničke slike, pozitivnog nalaza tuberkulinskoga kožnog testa s PPD, nalaza specifičnih tuberkulotskih promjena na radiogramu pluća, te mikrobiološkog nalaza *M. tuberculosis* u sputumu, bronhalnom aspiratu ili ispirku želuca. Bolesnici su kao terapiju dobivali 4 antituberkulotika (izonijazid, rifampicin, etambutol, pirazinamid).
3. klinički zdravi ispitanici (N = 35, dob 4–18 godina) upućeni na sistematski pregled, s biokemijsko-hematološkim pokazateljima unutar referentnih vrijednosti za dob.

Krv za analizu uzimana je između 8 i 12 sati. Dva mjeseca nakon uzimanja propisanih lijekova krv je ponovno uzimana u ispitanika s LTBI i bolesnika s TBC. Istraživanje je odobrilo etičko povjerenstvo bolnice, a roditelji su potpisali informirani pristanak.

Metode

Određivanje koncentracije hsCRP, C3 i C4

Koncentracija hsCRP (imuno-turbidimetrijska metoda na lateks česticama) te koncentracija C3 i C4 komponente komplementa (imuno-turbidimetrijska metoda) određivane su na biokemijskom analizatoru Olympus AU 400 (Olympus, Tokyo, Japan) uz primjenu reagensa istog proizvođača (Olympus System Reagents, Olympus Diagnostica, Hamburg, Germany).

Statističke metode

Pohrana podataka i priprema za statističku analizu učinjena je u programu Excel 2000 programskog paketa Microsoft Office (Microsoft, SAD). Obrada podataka učinjena je u programu za statističku obradu MedCalc 9.4.1.0 (Medisoftware, Mariakerke, Belgium) (21). Varijable koje su slijedile normalnu raspodjelu opisane su aritmetičkom sredinom ($\bar{x}$) i standardnom devijacijom (SD), dok su varijable koje nisu slijedile normalnu raspodjelu prikazane

Subjects and methods

Subjects

The study included 79 subjects aged 2.5 months to 18 years, referred between October 2005 and January 2007 to Srebrnjak Children's Hospital in Zagreb for systematic examination or detection of possible *M. tuberculosis* infection. The subjects were divided into three groups according to diagnosis:

1. subjects with LTBI, with a positive history of close contact with a tuberculosis patient and purified protein derivative (PPD) hyperresponsiveness (N = 26, aged 1–18 years) and were administered isoniazid for prophylaxis;
2. subjects with lung tuberculosis (N = 18, aged 2.5 months to 18 years), diagnosed on the basis of clinical picture, positive PPD test finding, specific tuberculotic lesions on chest x-ray, and microbiological finding of *M. tuberculosis* in sputum, bronchial aspirate or gastric lavage. Patients with tuberculosis received therapy consisting of four antituberculotics (isoniazid, rifampicin, ethambutol and pyrazinamide);
3. control group, clinically healthy subjects (N = 35, aged 4–18 years), referred for systematic examination, with biochemistry and hematology parameters within the reference range for age.

Blood sampling was performed between 8.00 and 12.00 a.m. At two months of therapy, repeat blood samples were collected in LTBI subjects and tuberculosis patients. The investigation was approved by the hospital ethics committee and a written informed consent was obtained from parents.

Methods

Determination of hsCRP, C3 and C4 concentrations

The concentrations of hsCRP (immunoturbidimetric method on latex particles), and C3 and C4 complement components (immunoturbidimetric method) were determined on an Olympus AU 400 biochemistry analyzer (Olympus, Tokyo, Japan), using Olympus System Reagents (Olympus Diagnostica, Hamburg, Germany).

Statistical analysis

Data were stored and prepared for statistical analysis by use of the Microsoft Office Excel 2000 software (Microsoft, USA). On data processing, MedCalc (Medisoftware, Mariakerke, Belgium) (21) was used. The variables with normal distribution were described by arithmetic mean ($\bar{x}$) and standard deviation (SD), and those not showing normal distribution were presented by median (M) and interquartile range (IQR). Paired Student's t-test and Wilcoxon test were used for comparison of dependent variables (for normal distribution and asymmetric distribu-

medijanom (M) i interkvartilnim rasponom (engl. *interquartile range*, IQR). Za usporedbu zavisnih varijabla primijenjeni su su parni Studentov t-test (za normalnu razdiobu) i Wilcoxonov test (za asimetričnu razdiobu). Za usporedbu više nezavisnih skupina primijenjen je ANOVA test, odnosno neparametrijski Kruskal-Wallis test za raspodjele koje nisu bile normalne. Vrijednosti P < 0,05 smatrane su statistički značajnima. ROC (engl. *Receiver Operating Characteristic*) analiza primijenjena je za određivanje optimalne granične vrijednosti, površine ispod ROC krivulje (engl. area under the curve, AUC), specifičnosti, osjetljivosti i prediktivne vrijednosti.

Rezultati

Rezultati u ispitanika prikazani su u tablici 1. Statistička analiza pokazala je normalnu razdiobu za sve analite osim za hsCRP u skupini LTBI, uzorak 1. ANOVA test za C3 odnosno C4 pokazao je statistički značajnu razliku između ispitivanih skupina (P = 0,007 odnosno P = 0,001). Koncentracija hsCRP, C3 i C4 u ispitanika s LTBI prije profilakse izonijazidom (uzorak 1) bila je statistički značajno veća nego u djece kontrolne skupine (tablica 2.). Koncentracija hsCRP, C3 i C4 je u bolesnika s TBC prije terapije (uzorak 1) bila statistički značajno veća nego u djece kontrolne skupine.

Nakon profilakse izonijazidom (uzorak 2) u djece s LTBI koncentracija hsCRP bila je statistički značajno manja nego prije primjene izonijazida. Jednako tako je koncentracija hsCRP u bolesnika s TBC nakon dvomjesečne terapije (uzorak 2) bila statistički značajno manja (ali je ostala značajno veća u usporedbi s vrijednostima u kontrolnoj skupini). *Post hoc* test ukazao je na razlike u vrijednostima između uzoraka 1 u skupini LTBI i skupini TB. Kruskal-Wallis test za hsCRP ukazao je na značajne razlike između ispitivanih skupina (P < 0,001). Nije postojala značajna

tion, respectively). ANOVA test was used for comparison of multiple independent groups, and non-parametric Kruskal-Wallis test for distributions that were not normal. The values of P < 0.05 were considered statistically significant. ROC (Receiver Operating Characteristic) analysis was used to determine optimal cut-off values, area under the ROC curve (AUC), specificity, sensitivity and predictive values.

Results

Results of patients and controls are shown in Table 1. Statistical analysis indicated that there was normal distribution for all analytes, except for hsCRP in LTBI group, sample 1. ANOVA test for C3 and C4 showed a statistically significant difference between study groups (P = 0.007 and P < 0.001, respectively). Before prophylactic isoniazid therapy induction (sample 1), the concentration of hsCRP, C3 and C4 was significantly higher in LTBI subjects in comparison with control group (Table 2). Also, in the group of tuberculosis patients, pretherapeutic concentration of hsCRP, C3 and C4 (sample 1) was statistically significantly higher than the concentration recorded in control group. After prophylactic therapy the concentration of hsCRP in LTBI (sample 2) was lower than before isoniazid administration. Also, after two-month therapy (TB group, sample 2), the concentration of hsCRP decreased significantly (but remained statistically significantly higher in comparison with control group). *Post hoc* test indicated differences between LTBI sample 1 *vs*. control group and tuberculosis sample 1 *vs*. control group. Kruskal-Wallis test for hsCRP indicated significant differences between study groups (P < 0.001). There was no significant difference between pretherapeutic and post-therapeutic values of C3 and C4 either in LTBI or in tuberculosis group. However, a significant difference between pretherapeutic and post-thera-

Tablica 1. Koncentracije hsCRP, C3 i C4 u kontrolnoj skupini, skupini djece s LTBI i djece s TBC prije (uzorak 1) i poslije (uzorak 2) terapije.

Table 1. Concentrations of hsCRP, C3 and C4 in control group, LTBI group and tuberculosis group of children before (sample 1) and after (sample 2) therapy.

	Control	LTBI		TB	
		Sample 1	Sample 2	Sample 1	Sample 2
	N = 35	N = 26		N = 18	
hsCRP (mg/L)	0.35 ± 0.22	0.49 (0.29-1.06)*	0.42 ± 0.21	20.97 ± 29.39	3.7 ± 5.66
C3 (g/L)	1.10 ± 0.12	1.19 ± 0.21	1.21 ± 0.14	1.26 ± 0.22	1.19 ± 0.22
C4 (g/L)	0.23 ± 0.05	0.25 ± 0.06	0.25 ± 0.07	0.34 ± 0.10	0.28 ± 0.10

LTBI = latent tuberculosis infection; TB = active tuberculosis
Values are presented with $\bar{x}$ ± SD; *median (interquartile range)

Tablica 2. Statistička značajnost razlike u koncentraciji hsCRP, C3 i C4 između kontrolne skupine i ispitanika s LTBI i TBC (ANOVA test i Kruskal-Wallis test)

Table 2. Statistical significance of differences in hsCRP, C3 and C4 values between control subjects and subjects with LTBI and tuberculosis (ANOVA test and Kruskal-Wallis test)

		P		
		hsCRP	**C3**	**C4**
LTBI	Control: sample 1	< 0.001	0.007	< 0.001
	Control: sample 2	< 0.05	< 0.05	< 0.05
	Sample 1: sample 2	0.014	0.743	0.717
TB	Control: sample 1	< 0.001	0. 007	< 0.001
	Control: sample 2	< 0.001	< 0.05	< 0.05
	Sample 1: sample 2	0.026	0.312	0.122

LTBI = latent tuberculosis infection; TB = active tuberculosis; sample 1 = before therapy; sample 2 = after therapy
Post hoc tests was used to compare control *vs*. sample 1 and control *vs*. sample 2.
Paired t test was used to compare sample 1 *vs*. sample 2.

razlika u koncentraciji C3 i C4 prije i poslije terapije niti u skupini LTBI niti u skupini TB. Međutim, postojala je značajna razlika u vrijednostima hsCRP prije i poslije terapije u objema skupinama, LTBI (P = 0,014) i TB (P = 0,026).
Obrađene su ROC krivulje za vrijednosti prije terapije u skupinama LTBI i TB (tablica 3.). U skupini bolesnika s TBC površina ispod krivulje (AUC) bila je statistički značajno veća (P = 0,037) nego u kontrolnoj skupini samo za hsCRP, a u skupini djece s LTBI nije bilo statistički značajne razlike ni za jedan ispitivani parametar (P > 0,05).

peutic values was found for hsCRP values in both LTBI (P = 0.014) and tuberculosis (P = 0.026) groups.
ROC curves were elaborated in LTBI and tuberculosis groups at pretherapeutic values (Table 3). In tuberculosis group, only hsCRP showed a statistically significantly greater area under curve (AUC) (P = 0.037) as compared with control group. In LTBI group, no statistically significant difference was recorded for any of the study parameters (P > 0.05).

Tablica 3. ROC analiza prijeterapijskih vrijednosti hsCRP, C3 i C4 u ispitanika s LTBI i bolesnika s tuberkulozom

Table 3. ROC analysis of pretherapeutic values of hsCRP, C3 and C4 in LTBI subjects and tuberculosis patients

	LTBI/Control			**TB/Control**		
	hsCRP (mg/L)	**C3 (g/L)**	**C4 (g/L)**	**hsCRP (mg/L)**	**C3 (g/L)**	**C4 (g/L)**
Cut - off value	0.46	1.29	0.23	0.92	1.26	0.28
AUC	0.731	0.607	0.526	0.900	0.734	0.834
Standard error	0.057	0.074	0.076	0.052	0.077	0.066
95% CI	0.602-0.836	0.473-0.729	0.393-0.657	0.786-0.965	0.595-0.846	0.705-0.923
Sensitivity (%)	53.8	38.5	56.0	83.3	55.6	82.4
Specificity (%)	85.7	100.0	57.1	100.0	91.4	85.7
PPV (%)	73.7	100.0	48.3	100.0	76.9	73.7
NPV (%)	71.4	58.6	64.5	92.1	80.0	90.9

LTBI = latent tuberculosis infection; TB = active tuberculosis; AUC = area under the ROC curve; Sensitivity = probability that test result will be positive when the disease is present (true positive rate); Specificity = probability that test result will be negative when disease is not present (true negative rate); PPV = positive predictive value, the proportion of patients with positive test result; NPV = negative predictive value, the proportion of subjects with negative test result

Rasprava

Rezultati istraživanja pokazali su da su, u odnosu na kontrolnu skupinu, koncentracije hsCRP, C3 i C4 statistički značajno veće u djece s LTBI (prije profilakse), te u djece s TBC (prije i poslije dvomjesečne antituberkulotske terapije). Istodobno su vrijednosti hsCRP bile značajno manje nakon dvomjesečne terapije (usporedba s vrijednostima prije terapije) u objema skupinama (LTBI odnosno TB).

Bajaj i sur. su još 1989. godine ukazali na mogućnost primjene CRP u procjeni aktivnosti tuberkuloze (5). Međutim, podatci o određivanju koncentracije CRP u serumu bolesnika s tuberkulozom su proturječni. Neki autori opisuju bolesnike s aktivnom tuberkulozom bez povećane koncentracije CRP (22), dok drugi opisuju bolesnike s povećanom koncentracijom CRP (23,24). Naši su rezultati također pokazali da se praćenje koncentracije hsCRP može primijeniti za praćenje upale u bolesnika s TBC odnosno LTBI. U djece s LTBI veću vrijednost ima određivanje koncentracije CRP metodom visoke osjetljivosti (hsCRP), jer može ukazati na umjerene upalne promjene, što metode manje analitičke osjetljivosti ne omogućuju. U tom slučaju granične vrijednosti koje bi mogle razlikovati zdrave od bolesnih značajno su manje (15). U dostupnoj literaturi nismo našli podataka o koncentraciji hsCRP u djece inficirane *M. tuberculosis*.

Komponenta komplementa C3 može aktivirati alternativni put (20,25), pričemu, čini se, receptor komplementa CR3 na površini makrofaga može posredovati povezivanje *M. tuberculosis* s makrofagom, ali ne i unutarstanično umnožavanje mikobakterija (26,27). Prema Stokesu i sur. jačina vezanja mikobakterija i makrofaga ovisi o fenotipu makrofaga (28). Dubaniewicz i sur. su opisali povećanje koncentracije C3, ali ne i C4, u bolesnika s aktivnom tuberkulozom (27). U istom istraživanju bolesnici s inaktivnom tuberkulozom nisu imali promijenjene vrijednosti C3 i C4. U našem istraživanju je koncentracija i C3 i C4 bila povećana u osoba s LTBI te u bolesnika s TB.

Činjenica da su vrijednosti za specifičnost bile manje nego vrijednosti za osjetljivost ukazuje na to da se temeljem graničnih vrijednosti mogu bolje izdvojiti zdrave osobe (uistinu negativni rezultati).

Većina djece s početnom tuberkuloznom infekcijom nema simptoma ni komplikacija infekcije (29). Lezije plućnog parenhima nisu vidljive na radiogramu pluća, limfni čvorovi nisu uvećani. Dijete ima samo pozitivan tuberkulinski test. Ovi preliminarni rezultati ukazali su na to da latentna kronična upala nije vjerojatna u djece s pozitivnim PPD i koncentracijama hsCRP, C3 i C4 manjima od graničnih vrijednosti. Konačnu procjenu o profilaksi donijet će liječnik.

Prema našim spoznajama ovo je prvi prikaz hsCRP u djece s LTBI. Nedostatak ovoga istraživanja bio je mali broj ispitanika, čemu možemo pripisati i nedovoljnu statističku

Discussion

Study results indicated the concentrations of hsCRP, C3 and C4 to be statistically significantly higher in children with LTBI (before prophylaxis) and children with tuberculosis (pre-therapeutically and after 2-month antituberculotic therapy) as compared with the control group of healthy children. Also, the values of hsCRP were significantly lower after two months of therapy (as compared with pretherapeutic values) in both LTBI and tuberculosis groups.

As early as 1989, Bajaj *et al.* pointed to the possible use of CRP in the assessment of tuberculosis activity (5). However, literature data on the determination of CRP concentration in serum of tuberculosis patients are contradictory. Some authors describe patients with active tuberculosis without increase in CRP concentration (22), whereas others report on patients with elevated CRP concentration (23,24). Also, our results indicated that monitoring of hsCRP concentration could be used in the follow up of patients with tuberculosis and LTBI. In LTBI children, determination of CRP concentration by a high sensitivity method (hsCRP) is of special value because it may point to moderate inflammatory lesions that cannot be identified by the methods of lower sensitivity. In this case, borderline values discriminating healthy subjects from affected ones are significantly lower (15). We found no reports on hsCRP concentration in children with *M. tuberculosis* infection in the available literature.

Complement component C3 can activate alternative pathway (20,25), whereby the CR3 complement receptor on the macrophage surface appears to be involved in mediating *M. tuberculosis* binding to macrophages but not intracellular replication of *M. tuberculosis* (26,27). According to Stokes *et al.*, the intensity of *M. tuberculosis* binding to macrophages depends on the macrophage phenotype (28). Dubaniewicz *et al*. describe elevated concentration of C3 but not of C4 in patients with active tuberculosis (27). In their study, the levels of C3 and C4 were not changed in patients with inactive tuberculosis. In our study, both C3 and C4 were increased in subjects with LTBI and in patients with tuberculosis.

The fact that specificity was lower than sensitivity indicates that healthy subjects (i.e. true negative rates) could be better distinguished on the basis of optimal cut-off values of selected analytes.

In most cases of initial tuberculosis infection in children there are no symptoms and no complications (29). The lesions of lung parenchyma are not visible on chest x-ray, while lymph nodes remain normal in size. The child only shows positive tuberculin skin test. These preliminary results have suggested that latent chronic inflammation is not probable in children with positive PPD, and with hsCRP, C3 and C4 concentration less than cut-off values. Definitive decision on the prophylaxis will be made by physician.

ku značajnost razlike analize ROC krivulje. Potvrde li se rezultati ovoga rada u nekom neovisnom istraživanju na većem broju ispitanika, hsCRP mogao bi se smatrati korisnim biljegom za praćenje bolesnika s LTBI u svrhu procjene odgovora na profilaksu izonijazidom i stupnja aktivnosti bolesti.

Adresa za dopisivanje:

Slavica Dodig
Dječja bolnica Srebrnjak
Srebrnjak 100
10000 Zagreb
e-pošta: slavica.dodig@zg.t-com.hr
tel: 01 6391 100
faks: 01 2430 784

This is, to our knowledge, the first report on hsCRP in children with LTBI. The limitation of the present study was the small number of study subjects, to which the inadequate statistical significance of difference on ROC curve analysis could have also been ascribed. However, may the results of this study be confirmed in an independent study including a greater number of subjects, hsCRP could be considered a useful marker in the follow up of LTBI patients, to evaluate the response to isoniazid prophylaxis and the level of the disease activity.

Corresponding author:

Slavica Dodig
Srebrnjak Children's Hospital
Srebrnjak 100
10000 Zagreb
Croatia
e-mail: slavica.dodig@zg.t-com.hr
phone: +385 1 6391 100
fax: +385 1 2430 784

Literatura/References

1. *World Health Organization. Global tuberculosis control: surveilleance, planning, financing. WHO Report 2005. Geneva, 2005.*
2. *Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Latif M, Conlon CP, et al. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T-cells. Am J Respir Crit Care Med 2001;163:824-8.*
3. *Mazurek GH, LoBue PA, Daley CL, Bernardo J, Lardizabal AA, Bishai WR et al. Comparison of a whole-blood interferon γ assay with tuberculin skin testing for detecting latent Mycobacterium tuberculosis infection. JAMA 2001;286:1740-7.*
4. *Targeted Tuberculin Testing and Treatment of Latent Tuberculosis Infection. ATS/CDC Statement Committee on Latent Tuberculosis Infection Membership List, June 2000. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr4906a1.htm. Accessed December 27th 2007.*
5. *Bajaj G, Rattan A, Ahmad P. Prognostic value of C-reactive protein i tuberculosis. Indian Pediatr 1989;26:1010-3.*
6. *Pepys MB, Baltz MC. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv Immunol 1983;34:141-212.*
7. *Emery P, Luqmani R. The validity of surrogate markers in rheumatic disease. Br J Rheumatol 1993;32:Suppl 3:3-8.*
8. *Walker-Smith JA. Chronic inflammatory bowel disease in children: a complex problem in management. Postgrad Med J 2000;76:469-72.*
9. *Lagerstrom F, Engfeldt P, Holmberg H. C-reactive protein in diagnosis of community-acquired pneumonia in adult patients in primary care. Scand J Infect Dis 2006;38:964-9.*
10. *Korppi M. Non-specific host response markers in the differentiation between pneumococcal and viral pneumonia: what is the most accurate combination? Pediatr Int 2004;46:545-50.*
11. *Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-9.*
12. *Coulon J, Willems D, Dorchy H. Increase in C-reactive protein plasma levels during diabetes in infants and young adults. Presse Med 2005;34:89-93.*
13. *Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327-34.*
14. *Takemura M, Matsumoto H, Niimi A, Ueda T, Matsuoka H, Yamaguchi M, et al. High sensitivity C-reactive protein in asthma. Eur Respir J 2006;27:908-12.*
16. *Galez D, Dodig S, Raos M, Nogalo B. CRP in children with asthma and allergic rhinitis. Biochemia Medica 2006;16(2):163-9.*
16. *Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003:111:1805-12.*
17. *Botto M. Links between complement deficiency and apoptosis. Arthritis Res 2001;3:207-10.*
18. *Boackle SA, Holers VM. Role of complement in the development of autoimmunity. Curr Dir Autoimmun 2003;6:154-6.*
19. *Boackle SA. Complement and autoimmunity. Biomed Pharmacother 2003;57:269-73.*
20. *Schlesinger LS, Bellinger-Kawahara CG, Payne NR, Horwitz MA. Phagocytosis of Mycobacterium tuberculosis is mediated by human monocyte complement receptors and complement component C3. J Immunol 1990;144:2771-80.*
21. *MedCalc Download, Available at: www.medcalc.be/download.php. Accessed December 27th 2007.*
22. *Ito KM, Yoshiyama T, Wada M, Ogata H. C-reactive protein in patients with bacteriological positive lung tuberculosis. Kekkaku 2004;79:309-11. Available at; http://www.ncbi.nlm.nih.gov/pubmed/ Accessed August 7th 2007.*
23. *Peroš-Golubičić T. C-reactive protein in sarcoidosis. Acta Med Iugosl 1991;45:169-74.*
24. *Koyanagi A, Kuffö, Gresely L, Shenkin A, Cuevas LE. Relationships between serum concentrations of C-reactive protein and micronutrients in patients with tuberculosis. Ann Trop Med Parasitol 2004;98:391-9.*
25. *Mueller-Ortiz SL, Wanger AR, Norris SJ. Mycobacterial protein HbhA binds human complement component C3. Infect Immun 2001; 69: 7501-11.*
26. *Velasco-Velazquez MA, Barrera D, Gonzalez-Arenas A, Rosales C, Agramonte-Hevia J. Macrophage – Mycobacterium tuberculosis interactions: role of complement receptor 3. Microb Pathog 2003;35:125-31.*
27. *Dubaniewicz A, Sztaba-Kania M, Hoppe A. Analysis of some immunological parameters in pulmonary tuberculosis. Pol Merkur Lekarski 2004;16:123-7.*
28. *Stokes RW, Haidl ID, Jefferies WA, Speert DP. Mycobacteria – macrophage interactions. Macrophage phenotype determines the nonopsonic binding of Mycobacterium tuberculosis to murine macrophages. J Immunol 1993;151:7067-76.*
29. *Starke JR. Tuberculosis in children. Semin Respir Crit Care Med 2004; 25:353-64.*