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Hematološki biljezi anemije i koncentracija C-reaktivnog proteina kod stabilne kronične opstrukcijske plućne bolesti

Hematological markers of anemia and C-reactive protein in patients with stable chronic obstructive pulmonary disease

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Sažetak

Uvod: Cilj istraživanja bio je odrediti vrijednosti hematoloških biljega anemije i koncentracije C-reaktivnog proteina (CRP) kod bolesnika oboljelih od stabilne kronične opstrukcijske plućne bolesti (KOPB), kako bi se utvrdila prisutnost anemije, stupanj sistemske upale i korelacija između analita.

Materijali i metode: U ispitivanje je bilo uključeno 150 bolesnika s KOPB (forsirani ekspiracijski volumen u prvoj sekundi (engl. *forced expiratory volume in 1 second* (FEV_1) = 48 ± 21%) i 51 kontrolni ispitanik (FEV_1 = 106 ± 15%). Prema smjernicama Globalne inicijative za kroničnu opstrukcijsku plućnu bolest (engl. *Global Initiative for Chronic Obstructive Lung Disease,* GOLD) bolesnici su podijeljeni u četiri podskupine, a prema kriterijima SZO za anemiju u dvije skupine: ispitanici s anemijom i bez nje. Koncentracija CRP u serumu određena je imunoturbidimetrijskom metodom, a koncentracija hematoloških biljega protočnom citometrijom.

Rezultati: Nije nađena statistički značajna razlika u broju eritrocita, koncentraciji hemoglobina i vrijednostima hematokrita između skupine svih bolesnika s KOPB i kontrolne skupine, kao ni između podskupina GOLD i kontrolne skupine. Anemija je bila prisutna kod 24% bolesnika. Koncentracija CRP bila je statistički značajno viša kod skupine svih bolesnika s KOPB ($P < 0,001$), svih podskupina GOLD (I. $P < 0,001$; IIA., IIB., III. $P < 0,001$) i podskupina s anemijom i bez nje ($P < 0,001$) u usporedbi s kontrolnom skupinom. Specifičnost i osjetljivost CRP određena je ROC analizom i pokazala je dobru dijagnostičku točnost koncentracije CRP kod bolesnika s KOPB. Koeficijent korelacije između koncentracije CRP i koncentracije hemoglobina odnosno vrijednosti hematokrita otkrio je dobru negativnu korelaciju (CRP i hemoglobin: $r = -0,60$, $P < 0,001$; CRP i hematokrit: $r = -0,59$, $P < 0,001$) samo u podskupini GOLD III.

Zaključak: Koncentracija CRP, biljega sistemske upale, bila je statistički značajno viša kod bolesnika s KOPB i kod svih podskupina GOLD u usporedbi s kontrolnom skupinom. Zabilježena je dobra negativna korelacija između vrijednosti hematokrita i koncentracije hemoglobina te uznapredovale upale (koncentracija CRP) kod podskupine GOLD III. Postotak bolesnika s anemijom bio je u skladu s podacima iz literature.

Abstract

Background: The aim of the study was to determine the values of hematological markers of anemia and of C-reactive protein (CRP) in patients with stable chronic obstructive pulmonary disease (COPD) in order to define the presence of anemia, degree of systemic inflammation and correlations between the analytes.

Material and methods: The study included 150 COPD patients (FEV_1 = 48 ± 21%) and 51 control subjects (FEV_1 = 106 ± 15%). Pursuant to GOLD guidelines, the patients were divided into four subgroups and according to WHO criteria for anemia into two subgroups: subjects with and those without anemia. Serum CRP concentration was determined by immunoturbidimetric method and hematological markers were determined by flow cytometry.

Results: No significant differences were found in the values of erythrocytes, hemoglobin and hematocrit either between the group of all COPD patients and control subjects or between GOLD subgroups and control subjects. Anemia was present in 24% of COPD patients. CRP concentration was significantly higher in the group of all COPD patients, in all GOLD subgroups (I, IIA, IIB and III) and in subgroups with and without anemia as compared to control subjects ($P < 0.001$ all). CRP specificity and sensitivity were determined by ROC analysis and showed good diagnostic accuracy for CRP in COPD patients. Correlation coefficient between CRP and hemoglobin and hematocrit values revealed a good negative correlation ($r = -0.60$ and $r = -0.59$, respectively; $P < 0.001$ both) only in GOLD subgroup III.

Conclusion: CRP concentration, a marker of systemic inflammation, was significantly higher in COPD patients and in all GOLD subgroups as compared to the control group. Good negative correlation was recorded between hematocrit and hemoglobin values and advanced inflammation (CRP concentration) in GOLD subgroup III. The rate of anemia was in agreement with literature data.

Ključne riječi: kronična opstrukcijska plućna bolest; anemija; C-reaktivni protein; smjernice GOLD

Key words: chronic obstructive pulmonary disease; anemia; C-reactive protein; GOLD guidelines

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Uvod

Kronična opstrukcijska plućna bolest (KOPB), jedan od vodećih zdravstvenih problema u razvijenim zemljama, multisistemska je bolest koja započinje oštećenjem morfologije i funkcije pluća te napreduje do višestrukih sistemskih manifestacija kao što su sistemski upalni procesi, imunodeficijencija, česte infekcije dišnog sustava, pothranjenost, osteoporoza, srčanožilne komplikacije (1) i, kao što navode nedavna izvješća, anemija (2). Stoga je opravdano trajno znanstveno i stručno zanimanje za KOPB s ciljem sprječavanja i ranog otkrivanja bolesti, kao i potraga za najučinkovitijim liječenjem, poboljšanjem kvalitete života i produženom stopom preživljavanja. Upala koju obilježava povećani broj neutrofila jedan je od ključnih čimbenika u patogenezi ove bolesti. Upalni odgovor započinje aktivacijom alveolarnih makrofaga koji sintetiziraju neutrofilne kemoatraktante, leukotrien (LTB_4) i interleukin-8 (IL-8). Oni nadalje aktiviraju neutrofile, najvažnije efektorske stanice upalnih događaja kod KOPB. Naime, aktivirani neutrofili sintetiziraju i izlučuju proteolitičke enzime (npr. neutrofilnu elastazu, katepsine i matriks-metaloproteinaze) i reaktivne kisikove spojeve koji izravno oštećuju tkivo pluća (3). Koncentracija proteina akutne faze također je povišena u krvi bolesnika s KOPB. Podaci iz literature ukazuju na povišenu koncentraciju C-reaktivnog proteina (CRP) kao biljega sistemske upale kod ovih bolesnika (4).

Zbog prateće hipoksije s pojavom KOPB gotovo se tradicionalno javlja i policitemija (5). Uz policitemiju kod bolesnika s KOPB također se javlja i anemija; prema podacima iz literature javlja se u 5-33% slučajeva (6). Pretpostavlja se da je mehanizam nastanka anemije u KOPB isti kao u anemiji upale (7). Naime, povišene koncentracije upalnih citokina (IL-1, TNF-alfa, IFN-gama itd.) vode ka skraćivanju života eritrocita, poremećenom korištenju željeza iz retikuloendotelnih zaliha i do slabljenja kompenzacijske aktivnosti koštane srži. Stoga koštana srž ne odgovara na pojačano stvaranje eritropoietina (EPO), javlja se otpornost na EPO i razvija se anemija, najčešće normocitna i normokromna, a u rjeđim slučajevima mikrocitna i hipokromna anemija (8). Pretpostavlja se da bi anemija mogla biti značajan prognostički čimbenik kod bolesnika s KOPB, budući da su smanjene koncentracije hemoglobina i niže vrijednosti hematokrita kod ovih bolesnika po-

Introduction

Chronic obstructive pulmonary disease (COPD), one of the leading health problems in developed countries, is a multisystemic disease that starts with lung morphology and function impairment and progresses to multiple systemic manifestations such as systemic inflammation processes, immunodeficiency, frequent respiratory infections, malnutrition, osteoporosis, cardiovascular complications (1) and, as recently reported, to anemia (2).

Continuous scientific and professional interest in COPD is therefore justified and aimed at prevention and early detection of the disease, as well as at search for the most efficient treatment, improvement of the quality of life, and prolonged survival. Inflammation, characterized by an increased neutrophil count, is one of the key factors in the pathogenesis of this disease. Inflammatory response starts with activation of alveolar macrophages, which synthesize neutrophil chemoattractants, leukotriene (LTB_4) and interleukin-8 (IL-8). They further activate neutrophils, the most important effector cells in inflammatory events in COPD. Namely, activated neutrophils synthesize and excrete proteolytic enzymes (e.g., neutrophilic elastase, cathepsins and matrix metalloproteinases) and reactive oxygen molecules that directly damage lung tissue (3). The concentration of acute phase proteins is also elevated in the blood of COPD patients. Literature data point to raised C-reactive protein (CRP) concentration as a marker of systemic inflammation in these patients (4).

Owing to accompanying hypoxia, COPD is almost traditionally associated with polycythemia (5). Besides polycythemia, anemia also occurs in COPD patients; according to literature, its incidence is 5%-33% (6). It is assumed that the mechanism of anemia development in COPD is the same as that of anemia of inflammation (7). Namely, elevated concentrations of inflammatory cytokines (IL-1, TNF-alpha, IFN-gamma, etc.) lead to a shortening of erythrocyte life, disturbed utilization of iron from reticuloendothelial stores, and to impairment of the compensational activity of bone marrow. Hence, bone marrow does not respond to enhanced erythropoietin (EPO) formation, resistance to EPO emerges, and anemia develops, most commonly normocytic and normochromic, seldom microcytic and hypochromic anemia (8). It is assumed that anemia could be a significant prognostic factor

vezane s čestom i dugotrajnom hospitalizacijom i povećanom stopom smrtnosti (9).

Ciljevi ovoga istraživanja bili su: a) odrediti relativni udio anemije kod svih bolesnika s KOPB i razlike u hematološkim biljezima anemije između bolesnika s KOPB i bolesnika iz podskupina raspoređenih prema smjernicama GOLD u usporedbi s kontrolnom skupinom; b) odrediti razlike u koncentraciji CRP kao biljega sistemske upale između svih bolesnika s KOPB i bolesnika iz podskupina raspoređenih prema smjernicama GOLD u usporedbi s kontrolnom skupinom; c) odrediti razlike u koncentraciji CRP kod podskupina bolesnika s anemijom i bez nje; d) procijeniti moguću korelaciju između koncentracije CRP i odabranih hematoloških biljega anemije; i e) odrediti dijagnostičku točnost CRP u razlučivanju bolesnika s KOPB i zdravih ispitanika.

Materijali i metode

Ispitanici

U istraživanje je bilo uključeno 150 bolesnika oboljelih od KOPB i 51 ispitanik kao kontrolna skupina. U skupini bolesnika s KOPB bilo je 105 (70%) muškaraca i 45 (30%) žena, medijan dobi 71 (63–76) godina. Svi su bolesnici bili liječeni na Odjelu za plućne bolesti Opće bolnice „Dr. Ivo Pedišić" u Sisku u razdoblju od svibnja 2007. do lipnja 2008. KOPB je dijagnosticirana na temelju podataka iz anamneze, kliničkih nalaza i nalaza funkcijskog spirometrijskog testa. Iz istraživanja su isključeni bolesnici s drugim plućnim bolestima, akutnim upalnim bolestima, bolestima jetre, šećernom bolešću i poviješću karcinoma. Temeljem stadija bolesti, odnosno spirometrijskog nalaza, koji se smatra zlatnim standardom kod dijagnosticiranja ove bolesti, bolesnici su podijeljeni u 4 podskupine prema smjernicama GOLD (engl. *Global Initiative for Chronic Obstructive Lung Disease*, GOLD): podskupina GOLD I, IIA, IIB i III (GOLD I blagi oblik, forsirani ekspiracijski volumen u prvoj sekundi (engl. *forced expiratory volume in 1 sec*, FEV_1) ≥ 80% od očekivane vrijednosti; GOLD IIA umjereni oblik, 50% ≤ FEV_1 < 80% od očekivane vrijednosti; GOLD IIB umjereni oblik, 30% ≤ FEV_1 < 50% od očekivane vrijednosti; GOLD III težak oblik, FEV_1 < 30% od očekivane vrijednosti ili FEV_1 < 50% od očekivane vrijednosti sa zastojem u disanju ili kliničkim znacima zatajivanja desne strane srca (10). U podskupini GOLD I bilo je 17 (11%) ispitanika, u GOLD IIA 47 (31%), u GOLD IIB 50 (33%) i u GOLD III 36 (24%) ispitanika. Bolesnici su nadalje podijeljeni u dvije podskupine prema kriterijima Svjetske zdravstvene organizacije (SZO) za anemiju: vrijednost hematokrita < 0,390 L/L za muškarce i < 0,360 L/L za žene (11). U podskupini A bilo je 36 bolesnika s anemijom (36/150 = 24%), a u podskupini B 114 bolesnika koji nisu imali anemiju (114/150 = 76%).

in COPD patients, because decreased hemoglobin and hematocrit concentrations in these patients are associated with frequent and long-term hospitalization and increased mortality (9).

The goals of this study were: (a) to establish the relative proportion of anemia in all COPD patients and differences in hematological markers of anemia between COPD patients and patient subgroups according to The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines in comparison with control group; (b) to establish differences in CRP concentration as a marker of systemic inflammation between all COPD patients and patient subgroups according to the GOLD guidelines in comparison with control group; (c) to establish differences in CRP concentration between the subgroups of patients with and without anemia; (d) to assess the possible correlation between CRP concentration and selected hematological markers of anemia; and (e) to determine diagnostic accuracy of CRP in discriminating between patients with COPD and healthy subjects.

Materials and methods

Subjects

The study included 150 COPD patients and 51 control subjects. In the group of COPD patients there were 105 (70%) male and 45 (30%) female patients, median age 71 (63 and 76, respectively). All patients were treated at Department of Pulmonary Diseases, Dr. Ivo Pedišić General Hospital, Sisak, during the period from May 2007 to June 2008. COPD was diagnosed on the basis of history data, clinical findings, and results of functional spirometric tests. Patients with other pulmonary diseases, acute inflammatory diseases, liver diseases, diabetes mellitus and cancer history were excluded from the study. Based on the stage of the disease, i.e. spirometry result, considered as the "gold standard" in diagnosing this disease, patients were allocated to four subgroups according to the GOLD guidelines: I, IIA, IIB, and III (GOLD I mild form, FEV_1 (forced expiratory volume in 1 sec) ≥ 80% of anticipated value; GOLD II A moderate form, 50% ≤ FEV_1 < 80% of anticipated value; GOLD II B moderate form, 30% ≤ FEV_1 < 50% of anticipated value; and GOLD III severe form, FEV_1 < 30% of anticipated value or FEV_1 < 50% of anticipated value with respiratory failure or with clinical signs of right heart failure (10).

GOLD I subgroup comprised 17 (11%), subgroup IIA 47 (31%), subgroup IIB 50 (33%) and subgroup III 36 (24%) patients. Study patients were further divided into two subgroups according to the World Health Organization (WHO) criteria for anemia: hematocrit value < 0.390 L/L for males, and < 0.360 L/L for females (11). Subgroup A (with anemia) included 36 (36/150 = 24%) patients, and subgroup B (without anemia) 114 (114/150 = 76%) patien-

U kontrolnu skupinu bio je uključen 51 ispitanik, 21 (41%) muškarac i 30 (59%) žena, medijan dobi 52 godine (46–56). Odabrani su među ispitanicima koji su prošli redovni sistematski pregled u zdravstvenim centrima u Sisku i Petrinji te nisu pokazivali znakove akutnih ni kroničnih stanja. Svi su dobrovoljci prošli standardne funkcijske testove respiracijskog sustava kao glavni kriterij uključivanja u istraživanje. Temeljem normalnih spirometrijskih nalaza dobrovoljci su bili uključeni u istraživanje kao ispitanici kontrolne skupine.
Svi su ispitanici dali svoj obaviješteni pismeni pristanak za sudjelovanje u istraživanju koje je odobrio i Etički odbor Opće bolnice „Dr. Ivo Pedišić" u Sisku.

Uzorci

Za hematološke testove rabila se puna krv uzeta u epruvetu s K_2EDTA kao antikoagulansom. Odredio se broj eritrocita, koncentracija hemoglobina, vrijednost hematokrita, parametri eritrocita: MCV (engl. *mean cell volume of red cells*; srednji stanični volumen), MCH (engl. *mean cell hemoglobin content of red cells*; srednja vrijednost staničnog hemoglobina), MCHC (engl. *mean cell hemoglobin concentration*; srednja koncentracija staničnog hemoglobina) i broj retikulocita.
Za određivanje koncentracije CRP rabio se serum u epruvetama bez antikoagulansa centrifugiran 10 minuta na 1300 x g.

Metode

Hematološki testovi napravljeni su standardnim mjernim postupkom po načelu protočne citometrije na automatskom brojaču stanica Cell Dyn 3200 CS (Abbott Diagnostics). Broj retikulocita određen je na istom uređaju nakon bojanja bojama New Methylene Blue N. Koncentracija CRP određena je imunoturbidimetrijskom metodom na automatskom analizatoru Dimension Xpand Plus (Siemens Healthcare Diagnostics) s reagensima istoga proizvođača.

Statistička analiza

Normalnost raspodjele ispitana je Kolmogorov-Smirnovljevim statističkim testom. Podaci s normalnom raspodjelom izraženi su kao aritmetička sredina ± standardna devijacija ($\bar{x} \pm SD$), dok su podaci koji nisu slijedili normalnu raspodjelu izraženi kao medijan i interkvartilni raspon. Statistička značajnost razlike između skupina ispitana je parametrijskim nezavisnim t-testom i neparametrijskim Mann-Whitneyevim testom.
Za usporedbu svih ispitivanih analita između četiri podskupine GOLD primijenjena je jednosmjerna analiza varijance ANOVA (engl. *one-way analysis of variance*) i neparametrijski Kruskal-Wallisov test. Svaka vrijednost $P < 0,05$ smatrala se statistički značajnom.

ts. Control group included 51 subjects, 21 (41%) males and 30 (59%) females, median age 52 (46 and 56, respectively). They were selected among the individuals presenting for regular medical check up at Sisak and Petrinja Health Centers, and were without any signs of acute or chronic condition. All volunteers were subjected to the standard pulmonary function test as the main criterion for inclusion in the study. Namely, volunteers were included in the study as controls on the basis of normal spirometric finding.
All patients and control subjects gave a written consent to be included in the study, which was approved by the Ethics Committee of Dr. Ivo Pedišić General Hospital.

Samples

Whole blood samples collected with K_2EDTA as anticoagulant were used for hematologic tests, i.e. erythrocytes, hemoglobin, hematocrit, red blood cell constants: mean cell volume of red cells (MCV), mean cell hemoglobin content of red cells (MCH), mean cell hemoglobin concentration (MCHC), and reticulocytes.
CRP concentration was determined in sera obtained by centrifugation at 1300 x g for 10 minutes in test tubes without anticoagulant.

Methods

Hematological tests were done according to standard procedures based on flow cytometry on an automatic Cell Dyn 3200 CS cell counter (Abbott Diagnostics). Reticulocyte count was determined with the same device after staining with New Methylene Blue N. CRP concentration was determined by the immunoturbidimetric method on an automated Dimension Xpand Plus analyzer (Siemens Healthcare Diagnostics) using test reagents of the same manufacturer.

Statistical analysis

Each set of data were analyzed for normality by the Kolmogorov-Smirnov statistical test. Normally distributed data were expressed as arithmetic mean ± standard deviation ($\bar{x} \pm SD$), while the non-normally distributed data were expressed as median and interquartile range. Significance of between-group differences was tested by the parametric independent t-test and nonparametric Mann-Whitney test.
To compare all study analytes among the four GOLD subgroups, the one-way analysis of variance (ANOVA) and nonparametric Kruskal-Wallis test were used. Statistical significance was set at $P < 0.05$.
To determine the correlation between CRP concentration and hematologic indicators of anemia (hemoglobin, hematocrit, reticulocytes) in the group of COPD patients and in the subgroups, Pearson's and Spearman's coefficients of correlation were calculated. The Receiver

Kako bi se odredila korelacija između koncentracije CRP i hematoloških pokazatelja anemije (hemoglobina, hematokrita i retikulocita) u skupini bolesnika s KOPB te u podskupinama, izračunati su Pearsonov i Spearmanov koeficijent korelacije. Napravljena je ROC analiza (engl. *receiver operating characteristic*, ROC) kako bi se odredila dijagnostička točnost CRP u razlučivanju bolesnika s KOPB i ispitanika kontrolne skupine. Statističke analize provedene su programskim paketom Med Calc 9.6.0.0. za Windows 98.

Rezultati

Osnovne demografske osobine skupine bolesnika s KOPB, podskupina bolesnika raspodijeljenih prema smjernicama GOLD i kontrolne skupine ispitanika prikazane su u tablici 1.

Operating Characteristic (ROC) analysis was performed to estimate diagnostic accuracy of CRP in discriminating between COPD patients and control group. Statistical analyses were performed using the Med Calc 9.6.0.0 for Windows 98 software.

Results

Baseline demographic characteristics of the group of COPD patients, patient subgroups according to GOLD and control group are presented in Table 1.
In the group of COPD patients, the values of body mass index (BMI) differed significantly ($P < 0.001$) from the values recorded in control subjects. The difference was also statistically significant when GOLD subgroups I, IIB and III were compared with control group ($P = 0.027$, $P = 0.001$ and $P < 0.001$, respectively).

Tablica 1. Opće demografske značajke svih bolesnika s KOPB, podskupina bolesnika raspoređenih prema smjernicama GOLD i kontrolnih ispitanika

Table 1. Baseline demographic characteristics of all patients with chronic obstructive pulmonary disease (COPD), subgroups of patients according to GOLD classification and control subjects

	GOLD I (N = 17)	GOLD IIA (N = 47)	GOLD IIB (N = 50)	GOLD III (N = 36)	COPD All (N = 150)	Controls (N = 51)
Age (years)	74 (53–86)*	71 (46–94)*	72 (49–83)*	70 (26–83)*	71 (63–76)*	52 (46–56)
Males, N (%)	10 (58.8)	29 (61.7)	37 (74.0)	26 (72.2)	105 (70)	21 (41)
BMI (kg/m^2)	26.0 (23.5–27.9)*	26.6 (23.5–30.3)	24.5 (21.5–27.5)*	22.3 (19.9–24.7)*	25.1 ± 5.2*	28.7 ± 4.6
FEV_1(%)	87 (83–91)*	59 (52–65)*	41 (36–45)*	24 (20–25)*	48 ± 21*	106 ± 15

GOLD – Global Initiative for Chronic Obstructive Lung Disease; BMI – body mass index; FEV_1 – forced expiratory volume in 1 sec;
*marked difference between the group of patients and group of controls; $P < 0.05$
Data are presented as arithmetic mean ± standard deviation or as median and interquartile range.

Vrijednosti indeksa tjelesne mase u skupini bolesnika s KOPB bile su statistički značajno ($P < 0,001$) različite od vrijednosti izračunatih za kontrolne ispitanike. Također se dobila statistički značajna razlika nakon usporedbe vrijednosti podskupina GOLD I., IIB. i III. s kontrolnom skupinom ($P = 0,027$, $P = 0,001$ i $P < 0,001$).
Skupina bolesnika s KOPB i skupina kontrolnih ispitanika nisu bile jednake dobi. Kontrolni ispitanici bili su mlađi, a razlika je bila statistički značajna ($P < 0,001$) i kod skupine bolesnika s KOPB i kod skupina bolesnika podijeljenih prema smjernicama GOLD. Razlika u vrijednostima FEV_1

The groups of COPD patients and control subjects were not age matched the latter being significantly younger from both all COPD patients and GOLD subgroups ($P < 0.001$). FEV_1 values yielded significant difference between the group of all COPD patients, GOLD subgroups of patients and control group ($P < 0.001$).
The values of hematological indicators and CRP in the group of all COPD patients, GOLD subgroups and control group are presented in Table 2.
The values of erythrocyte constants MCV and MCHC differed significantly between the group of COPD patients

Tablica 2. Vrijednosti hematoloških biljega i koncentracije CRP kod svih bolesnika s KOPB, podskupina bolesnika raspoređenih prema smjernicama GOLD i kontrolnih ispitanika

Table 2. Values of hematologic markers and CRP concentration in patients with chronic obstructive pulmonary disease (COPD), COPD patients distributed in GOLD subgroups, and control subjects

	GOLD I (N = 17)	GOLD IIA (N = 47)	GOLD IIB (N = 50)	GOLD III (N = 36)	COPD (N = 150)	controls (N = 51)
RBC (x 10^{12}/L)	4.77 (4.15–5.18)	4.58 ± 0.56	4.59 ± 0.59	4.77 (4.13–5.44)	4.63 ± 0.63	4.61 ± 0.49
HGB (g/L)	146 (127–154)	141 ± 19	139 ± 20	144 (130–160)	140 ± 20	142 ± 18
HTC (L/L)	0.43 (0.39–0.45)	0.414 ± 0.052	0.413 ± 0.052	0.43 (0.39–0.48)	0.418 ± 0.060	0.409 ± 0.045
MCV (fL)	88.8 (85–92)	91.3 ± 5.3*	90.2 ± 6.9	93.1 (88.5–96.2)*	90.6 ± 6.4 *	88.7 ± 3.6
MCH (pg)	30.2 (28.2–30.7)*	31.0 ± 2.5	30.3 ± 2.9	30.6 (29.6–32.2)	30.5 ± 2.7	30.7 ± 1.8
MCHC (g/L)	335 (324–342)*	339 ± 13*	336 ± 13*	337 (329–340)*	336 ± 12 *	346 ± 12
RTC (%)	1.65 (1.54–1.88)*	1.61 ± 0.56	1.64 ± 0.82	1.39 (1.11–1.8)	1.49 (1.21–1.86)	1.41 (1.30–1.72)
CRP (mg/L)	10.12 (6.99–28.68)*	12.20 (7.82–26.34)*	12.73 (7.42-60.07)*	21.77 (10.46–43.69)*	12.92 (8.01–41.32)*	7.70 (3.58–9.43)

GOLD – Global Initiative for Chronic Obstructive Lung Disease; RBC – red blood cells; Hgb – hemoglobin; Htc – hematocrit; MCV – mean cell volume of red cells; MCH – mean cell hemoglobin content of red cells, MCHC – mean cell hemoglobin concentration; Rtc – reticulocytes, CRP – C-reactive protein; *marked difference between the group of patients and group of controls; P < 0.05
Data are presented as arithmetic mean ± standard deviation or as median and interquartile range.

između skupine svih bolesnika s KOPB, podskupina bolesnika podijeljenih prema klasifikaciji GOLD i kontrolnih ispitanika bila je statistički značajna (P < 0,001).

Vrijednosti hematoloških pokazatelja i koncentracije CRP u skupini bolesnika s KOPB, podskupinama bolesnika podijeljenih prema smjernicama GOLD i skupini kontrolnih ispitanika prikazane su u tablici 2.

Vrijednosti eritrocitnih parametara MCV i MHCH u skupini bolesnika s KOPB statistički su se značajno (P = 0,012; P < 0,001) razlikovale od vrijednosti kod kontrolnih ispitanika. Medijan koncentracije CRP bio je za oko 50% viši u skupini bolesnika s KOPB, te je razlika u odnosu na kontrolnu skupinu bila statistički značajna (P < 0,001).

Razlike u broju eritrocita, koncentraciji hemoglobina i vrijednosti hematokrita nisu bile statistički značajne u usporedbi bilo koje od podskupina GOLD s kontrolnom skupinom.

Vrijednost MCV bila je statistički značajno viša samo u podskupini GOLD IIA. (P < 0,008), MCH samo u podskupini GOLD I. (P < 0,039), dok je vrijednost MCHC bila statistički značajno niža kod svih podskupina GOLD u usporedbi s kontrolnom skupinom (P < 0,001; P = 0,018; P < 0,001 i P < 0,001).

Medijan koncentracije CRP rastao počevši od podskupine GOLD I. prema GOLD III., gdje je iznosio 21,77 mg/L, što je

and control subjects (P = 0.012 and P < 0.001, respectively). The median of CRP concentration was by about 50% higher in the group of COPD patients, yielding a significant difference from the control group (P < 0.001).

The values of erythrocytes, hemoglobin and hematocrit did not differ significantly between any of the GOLD patient subgroups and control subjects.

MCV value was significantly higher in the GOLD IIA subgroup (P < 0.008) and MCH in the GOLD I subgroup (P < 0.039) only, whereas MCHC was significantly lower in all GOLD subgroups as compared to control group (P < 0.001, P = 0.018, P < 0.001 and P < 0.001, respectively). Thee median CRP concentration increased from GOLD I subgroup towards GOLD III subgroup, where it was 21.77 mg/L or threefold median CRP concentration recorded in control group (subgroup I, P < 0.001; IIA, P < 0.001; and IIB and III, P < 0.001).

Partial oxygen pressure (pO_2) in COPD patients (9.38 ± 1.93 kPa) was significantly lower as compared to the reference values (10.00-13.33 kPa). COPD patients also showed an increase in leukocyte count (10.24 ± 4.21 x 10^9/L) (results not shown).

The values of hematological markers and CRP in COPD patients divided into subgroups according to the WHO criteria for anemia are presented in Table 3. Anemia was present in 36 (24%) patients.

tri puta više nego medijan koncentracije CRP kod kontrolne skupine (podskupina I. P < 0,001; IIA. P < 0,001; IIB. i III. P < 0,001).
Parcijalni tlak kisika (pO_2) kod bolesnika s KOPB (9,38 ± 1,93 kPa) bio je statistički značajno niži u usporedbi s referentnim vrijednostima (10,00-13,33 kPa), a broj leukocita je kod bolesnika bio povećan (10,24 ± 4,21 x 10^9/L) (rezultati nisu prikazani).
Vrijednosti hematoloških biljega i koncentracije CRP kod bolesnika s KOPB podijeljenih u podskupine prema kriterijima SZO za anemiju prikazani su u tablici 3. Anemija je bila prisutna kod 36 bolesnika (24% ukupnog broja bolesnika).

In group A patients (with anemia), reticulocyte count was significantly higher (P = 0.003), while median CRP concentration was threefold that recorded in group B patients (without anemia) (P < 0.001).
Comparison of the study analytes in all four GOLD subgroups showed no statistically significant difference.
Determination of the coefficient of correlation between hemoglobin concentration and CRP and between hematocrit and CRP in the group of COPD patients produced a weak negative correlation (r = -0.28; P < 0.001 and r = -0.27; P = 0.001, respectively) (12), while there was no correlation between reticulocyte count and CRP concentration. The strongest negative correlation was obtained

Tablica 3. Vrijednosti hematoloških biljega i koncentracija CRP kod bolesnika s KOPB raspoređenih u skupine prema kriterijima SZO za anemiju

Table 3. Values of hematologic markers and CRP concentrations in patients with chronic obstructive pulmonary disease (COPD) distributed in groups according to World Health Organization criteria for anemia

	COPD Patients with anemia (N = 36)	COPD Patients without anemia (N = 114)
RBC (x 10^{12}/L)	3.94 (3.75-4.21)	4.83 ± 0.51
Hgb (g/L)	118 (108-127)	149 ± 15
MCV (fL)	88.5 (84.9-93.1)	91.4 ± 5.2
MCH (pg)	29.9 (28.1-31.9)	30.9 ± 2.2
MCHC (g/L)	334 (327-340)	338 ± 11
Rtc (%)	1.81 (1.31-2.04)*	1.52 ± 0.53
CRP (mg/L)	33.94 (18.39-79.98)*	11.32 (7.76-27.67)

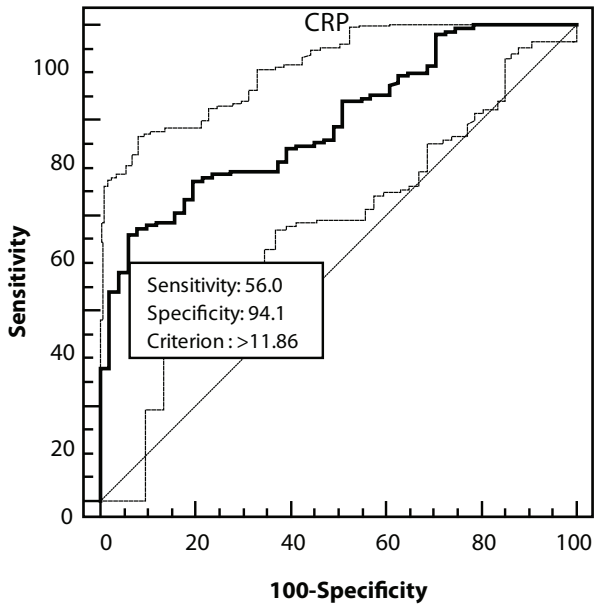
RBC – red blood cells; Hgb – hemoglobin; Htc – hematocrit; MCV – mean cell volume of red cells; MCH – mean cell hemoglobin content of red cells, MCHC – mean cell hemoglobin concentration; Rtc – reticulocytes; CRP – C-reactive protein; *marked difference between groups of patients with and without anemia, P < 0.05
Data are presented as arithmetic mean ± standard deviation or as median and interquartile range.

Broj retikulocita bio je statistički značajno viši kod bolesnika iz skupine A (P = 0,003), dok je medijan koncentracije CRP bio tri puta viši kod bolesnika s anemijom (skupina A) nego kod bolesnika koji nemaju anemiju iz skupine B (P < 0,001).
Nije bilo statistički značajne razlike kod usporedbe ispitivanih analita u svim podskupinama GOLD.
Izračunom koeficijenta korelacije između koncentracije hemoglobina i CRP te vrijednosti hematokrita i CRP u skupini bolesnika s KOPB dobivena je slaba negativna korelacija (hemoglobin i CRP: r = -0,28; P < 0,001 te hematokrit i CRP: r = -0,27; P = 0,001) (12), dok između broja retikulocita i koncentracije CRP nije bilo korelacije. Najbolja negativna korelacija dobivena je određivanjem koeficijenta korelaci-

by determination of the correlation coefficient between hemoglobin concentration and CRP (r = -0.60; P < 0.001), and between hematocrit and CRP (r = -0.59; P < 0.001) (12) in the GOLD III subgroup.
Although CRP concentration was significantly higher in patients with anemia than in those without it, there was no correlation between CRP concentration and hemoglobin or hematocrit, and no correlation between CRP concentration and reticulocyte count either in the group of patients with anemia.
Good diagnostic efficiency of CRP was determined by ROC analysis, comparing the group of all COPD patients with control group (area under the curve, AUC = 0.795; 95%CI = 0.732-0.849; P < 0.001; at a cut-off concentration

je između koncentracija hemoglobina i CRP (r = -0,60; P < 0,001) te vrijednosti hematokrita i koncentracije CRP (r = -0,59; P < 0,001) (12) u podskupini GOLD III.
Iako je koncentracija CRP kod bolesnika s anemijom bila statistički značajno viša nego kod bolesnika bez anemije, nije pronađena korelacija između koncentracije CRP i koncentracije hemoglobina ili vrijednosti hematokrita u skupini bolesnika s anemijom, kao niti korelacija između koncentracije CRP i broja retikulocita.
ROC analizom ustanovljena je dobra dijagnostička učinkovitost CRP u usporedbi svih skupina bolesnika s KOPB s kontrolnom skupinom - AUC (engl. *area under the curve*, površina ispod krivulje) = 0,795; 95%-tni interval pouzdanosti (engl. *confidence interval*, CI) = 0,732-0,849; P < 0,001; kod granične koncentracije od 11,86 mg/L dijagnostička osjetljivost 56% i specifičnost 94,1%) (slika 1.).
ROC analiza je također napravljena za sve hematološke pokazatelje anemije, uspoređujući skupinu svih bolesnika s KOPB s kontrolnom skupinom; međutim, dobivena dijagnostička učinkovitost nije bila zadovoljavajuća za sve ispitane parametre (AUC ≤ 0,5).

of 11.86 mg/L, diagnostic sensitivity of 56% and specificity of 94.1% were determined) (Figure 1).
ROC analysis was also done for all hematological indicators of anemia, comparing the group of all COPD patients with control group; however, the diagnostic efficiency thus obtained was unsatisfactory for all study parameters (AUC ≤ 0.5).



Slika 1. ROC analiza C-reaktivnog proteina (CRP) u skupini bolesnika s kroničnom opstrukcijskom plućnom bolešću (KOPB).

Figure 1. ROC analysis of C-reactive protein (CRP) in the group of all patients with chronic obstructive pulmonary disease.

Rasprava

U našoj skupini bolesnika s KOPB anemija (snižene vrijednosti hematokrita i koncentracije hemoglobina, po-

Discussion

In the group of COPD patients included in the study, anemia (decreased hematocrit and hemoglobin values, increased proportion of reticulocytes, lower MCH and MCHC values along with normal or decreased MCV values) was present in 36 (24%) patients. CRP concentration was significantly higher in the group of all patients, as well as in all GOLD subgroups as compared to control group, confirming the role of CRP as a marker of systemic inflammation in this disease. CRP concentration was significantly higher in the subgroup of COPD patients with anemia as compared to the patient subgroup without anemia, pointing to the possible association of anemia with the presence of inflammation in this disease. Reticulocyte count was also higher in the subgroup of COPD patients with anemia. The results of this study also pointed to negative correlation between hematocrit and hemoglobin values and CRP concentration (a marker of inflammation),

višen broj retikulocita, niže vrijednosti MCH i MCHC uz normalne ili snižene vrijednosti MCV) bila je prisutna kod 36 (24%) bolesnika. Koncentracija CRP bila je statistički značajno viša u skupinama svih bolesnika, kao i u podskupinama GOLD u usporedbi s kontrolnim ispitanicima, što potvrđuje značenje CRP kao biljega sistemske upale kod ove bolesti. Koncentracija CRP je bila statistički značajno viša kod podskupina bolesnika s KOPB koji su imali anemiju u usporedbi s podskupinama bolesnika koji nisu imali anemiju, što ukazuje na moguću povezanost anemije s prisutnošću upale kod ove bolesti. Broj retikulocita također je bio viši kod podskupina bolesnika s KOPB koji su imali i anemiju. Rezultati ovoga istraživanja također su pokazali negativnu korelaciju između vrijednosti hematokrita i koncentracije hemoglobina te koncentracije CRP (biljega upale), naročito u podskupini GOLD III. u kojoj su bili bolesnici s teškim oblikom bolesti i znatno smanjenom plućnom funkcijom ($r = -0,60$, $P < 0,001$; $r = -0,59$, $P < 0,001$), rezultat kakav su također opisali Richmond i Kiss (13).

Dijagnostička specifičnost i osjetljivost koncentracije CRP ispitana je ROC analizom. Kod granične vrijednosti od 11,86 mg/L dijagnostička je točnost, odnosno sposobnost testa da prepozna bolest kod određene osobe, iznosila je 56%, dok je dijagnostička specifičnost, odnosno vjerojatnost da će rezultati testa isključiti bolest kod zdravih osoba iznosila 94%. Prema dobivenoj ROC krivulji i području ispod krivulje (AUC) ustanovljena je dobra diskriminacijska učinkovitost koncentracije CRP, odnosno dobra dijagnostička točnost. Pinto-Plata i sur. (14) su došli do zaključka da bi CRP mogao biti biljeg sistemskog upalnog procesa koji se javlja kod bolesnika s KOPB.

Anemija prisutna kod bolesnika s KOPB uglavnom je bila normocitna i normokromna (83,4%) te u manjem postotku mikrocitna i hipokromna (16,6%), što odgovara slici anemije upale (15). Naime, uz nedostatak željeza, kroničnе upalne bolesti najčešća su stanja povezana s anemijom (anemija upale, anemija kroničnih bolesti). Stoga je KOPB nedavno dodana popisu kroničnih upalnih bolesti povezanih s anemijom, zajedno s infekcijama, tumorima, autoimunim bolestima i bubrežnim bolestima (7). Similowski i sur. (7) su izvijestili da je KOPB bolest koja se prije uglavnom povezivala s policitemijom (incidencija 5-8%) i tek se nedavno, iako se to čini paradoksalnim, počela povezivati s anemijom (16), incidencija koje je 10-15% (6). Omjer bolesnika s anemijom u ovom istraživanju slaže se s literaturnim podacima, budući da su neki autori izvijestili o pojavi anemije kod čak 33% bolesnika (17). Policitemija je bila prisutna kod 8% bolesnika s KOPB, što se također slaže s rezultatima drugih autora (17,18).

Veliko francusko istraživanje društva ANTADIR (*Association Nationale pour le Traitement a Domicile des Insuffisants Respiratoires*) (9) pokazalo je snažnu korelaciju između koncentracije hemoglobina i smrtnosti kod bolesnika s KOPB.

notably in GOLD subgroup III, which includes patients with the severe form of the disease with greatly reduced pulmonary function ($r = -0.60$, $P < 0.001$; $r = -0.59$, $P < 0.001$), as also reported by Richmond and Kiss (13).

Diagnostic specificity and sensitivity of CRP were determined by ROC analysis. At the cut-off value of 11.86 mg/L, diagnostic sensitivity, i.e. test ability to recognize COPD patient, was 56%, while diagnostic specificity, i.e. the probability that the test result would exclude the disease in a healthy person, was 94%. The ROC curve and area under the curve (AUC) indicated good discriminatory efficiency of CRP, i.e. its good diagnostic accuracy. Pinto-Plata *et al*. (14) report that CRP could be a marker of a systemic inflammatory process present in COPD patients.

The anemia found in the group of COPD patients was mainly normocytic and normochromic (83.4%), and to a lesser extent microcytic and hypochromic (16.6%) anemia, which corresponds to the picture of anemia of inflammation (15). Namely, after iron deficiency, chronic inflammatory diseases are the most common conditions associated with anemia (anemia of inflammation, anemia of chronic disease). Thus, COPD has recently been added to the list of chronic inflammatory diseases associated with anemia, along with infections, tumors, autoimmune diseases and kidney diseases (7). Similowski *et al*. (7) report that COPD is a disease that used to be mainly associated with polycythemia (incidence 5%-8%) and, although it may seem paradoxical, it has only recently started to be associated with anemia (16), the incidence of which is 10%-15% (6). The proportion of patients with anemia in this study was consistent with literature data, since some authors report on the incidence of anemia of up to 33% (17). Polycythemia was present in 8% of our COPD patients, which is also in accordance with the results reported by other authors (17,18).

The large French ANTADIR (Association Nationale pour le Traitement a Domicile des Insuffisants Respiratoires) study (9) showed strong correlation between hemoglobin concentration and mortality in COPD patients. In the same study, hematocrit value was defined as an independent and most important prognostic indicator of COPD patient survival. A higher survival rate was recorded in patients with polycythemia, while higher mortality was determined in the group of patients with low hematocrit values. In recent years, polycythemia is less common in these patients, which may be attributed to better medical care of these patients, as well as to the increased utilization of angiotensin-converting enzyme (ACE) inhibitors that are widely used to treat cardiac complications in COPD patients (19). It is also assumed that hemoglobin concentration in patients with COPD reflects the balance between erythropoiesis stimulation by hypoxia (most notable in GOLD subgroup III, representing the highest degree of disease progression) and its suppression due

U istom su istraživanju vrijednosti hematokrita definirane kao nezavisan i najvažniji prognostički pokazatelj preživljavanja kod bolesnika s KOPB. Viša je stopa preživljavanja zabilježena kod bolesnika s policitemijom, dok je viša stopa smrtnosti ustanovljena u skupini bolesnika s niskim vrijednostima hematokrita. Prošlih godina policitemija je bila rjeđa kod bolesnika s niskim vrijednostima hematokrita, što se može pripisati boljoj medicinskoj skrbi bolesnika, kao i povećanoj primjeni inhibitora angiotenzin konvertirajućeg enzima (engl. *angiotensin-converting enzyme*, ACE) koji su u širokoj uporabi u liječenju srčanih komplikacija kod bolesnika s KOPB (19). Također se pretpostavlja da koncentracija hemoglobina kod bolesnika s KOPB odražava ravnotežu između eritropoeze stimulirane hipoksijom (što se najbolje vidi kod podskupine GOLD III. koja predstavlja najviši stupanj napredovanja bolesti) i njene supresije zbog prisutnosti upale (20). Kako su vrijednosti pO_2 kod bolesnika s KOPB u ovom istraživanju bile niže od referentnih vrijednosti, a koncentracije CRP povišene, kao i broj leukocita, rezultati našega istraživanja slažu se sa spomenutom pretpostavkom.

Ovo preliminarno istraživanje potvrdilo je značenje određivanja koncentracije CRP kao biljega sistemske upale kod bolesnika s KOPB, kao i moguću povezanost povišene koncentracije CRP s napredovanjem bolesti. Granična vrijednost od 11,86 mg/L, dobivena ROC analizom, mogla bi biti dodatni biokemijski parametar koji bi mogao pomoći u razlučivanju bolesnika s KOPB i zdravih osoba. Priličan broj bolesnika ima anemiju sa značajkama koje odgovaraju anemiji upale. Međutim, ovo je istraživanje bilo ograničeno malim brojem ispitanika u svakoj podskupini, pa su stoga potrebna daljnja opsežnija istraživanja s većim brojem ispitanika kako bi se objasnio mehanizam razvoja anemije, odredilo značenje anemije kao biljega i posrednika patofizioloških događaja kod KOPB te kao prognostičkog čimbenika kod bolesnika s KOPB.

to the presence of inflammation (20). Since pO_2 values in COPD patients are lower than the reference interval, along with raised concentrations of CRP and leukocytes, the results of this study comply with this assumption.

This preliminary investigation confirmed the importance of CRP concentration as a marker of systemic inflammation in COPD patients as well as the possible association of an increase in CRP concentration with disease progression. The cut-off value of 11.86 mg/L, obtained by ROC analysis, might be an additional biochemical parameter that could help discriminate between COPD patients and healthy individuals. An appreciable proportion of patients have anemia with characteristics corresponding to those of anemia of inflammation. This study was, however, limited by the small number of subjects in the subgroups and further more extensive studies in a greater number of subjects are necessary to elucidate the mechanism of anemia development, to determine the importance of anemia as a marker or mediator of pathophysiological events in COPD, and as a prognostic factor in COPD patients.

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