Original article

IMPORTANCE OF ACUTE PHASE INFLAMMATION SERUM LEVEL MARKERS FOR EARLY DETECTION, FOLOW-UP AND INITIAL PROGNOSIS OF BACTERIAL LOW RESPIRATORY TRACT INFECTIONS IN PATIENTS WITH ALCOHOL LIVER CIRRHOSIS

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Combined metabolic and haemodynamic changes in patients (pts) with alcohol liver cirrhosis induce a significant loss of immune response, which represents the main cause of bacterial infections, primarily of low respiratory tract, with high risk of mortality.

Considering the influence of bacterial low respiratory tract infections on the course and prognosis of liver cirrhosis, comparing the level of general inflammation response, the clinical data of 67 alcohol liver cirrhosis pts in Child B stage of disease were retrospectively analyzed, diagnosed and treated from September 2001 till February 2006. Regarding the presence of infection, pts were divided in two groups: I-experimental including 37 pts and II-control group with 30 pts.

In I group of pts, a significant initial increase of C-reactive protein and fibrinogen serum level ($p<0.001$) were reported as well as increased erythrocyte sedimentation rate ($p<0.05$) compared to the control group. The same values were significantly decreased after antibiotic treatment. Gram-negative bacteria were dominant in the culture isolates, the total proteins and albumins serum levels were initially significantly lower ($p<0.05$), while alanin-aminotranspherasis and lactate dehydrogenase were increased ($p<0.05$) compared to the control group, with further normalizing tendency at the end of antibiotic treatment.

Early detection of bacterial low respiratory tract infections in patients with alcohol liver cirrhosis, by determination of acute phase inflammation serum level markers, is important in achieving the effective and prolonged remission of disease, but it still remains the missing link in complex chains of the unfavorable disease course activation.
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***Key words**: liver, cirrhosis, alcohol, infections*

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Introduction

Liver cirrhosis is a chronical disease, characterised by hepatocyte necrosis, nodular regeneration of liver parenchyma with pseudolobuli, increase colagenous adhesive tissue, and lobuli architectonics disorders (1). An important characteristic of liver cirrhosis is the inflammatory process, which, in the hepatocytes necrosis area, releases various cytokines and inflammation factors. By further activation of lymphocytes and macrophags, it stimulates the generation of fibroblasts and adhesive colagenous tissue, which characterises alcohol cirrhosis, where myofibroblasts make up to 60% of all mezenchim liver cells. Thus, because of hyaline sclerosing of central veins and generating centrolobular fibrosis in alcohol liver disease, the pressure in the portal vein system increases and manifested cirrhosis evolves (1,2).

Hepatocyte necrosis and combined metabolism-chemodynamic changes in the organisms of the patients with cirrhosis induce a high level of weakening or absolute absence of defensive mechanisms, in the area of secondary imunodefficiency, primarily generated as reticuloendothelial system damage (lymphoreticural tissue). This state is the main cause of bacteriological infections of dominant respiratory tract, as one of the complications, and increases the mortality rate of these patients (3,4). Reticuloendothelial system functions as defensive mechanism through phagocyte activity towards bacteria and endotoxines which enter the liver through intestinal mucosa and portal vein, thus preventing further invasion of infective agents. Decreased number and function of Kupffer cells in cirrhosis, as well as weaker chemotaxis and phagocytic activity of lymphocytes,

increase the possibility for successful appearance and development of bacterial infection in these patients. On the other hand, malnutrition and decreased synthetic function of the liver in cirrhosis, contribute to further cell and humoral immunity disorders, through complement components insufficiency or their exessive consumption in the states with expressed functional glomerulopathy (hepatorenal syndrome) (5). Patients with cirrhosis have, as a complication, Gram-negative bacterial infection of lower respiratory tract, which is present in 30-50% of the hospitalized (6). Graudal et al. showed in their study that the infection development in these patients does not correlate with their survival duration directly, but that the infection is more frequent in the patients with progressed disease and fatal outcome in 15.4% of cases. In other patients, the infection induces further disease aggravation in terms of starting of liver, respiratory and renal insufficiency, which influences bad disease prognosis (7).

Aim of the study

The aim of this study was the estimation of influence and the establishment of the level of correlation of bacterial respiratory infection of lower respiratory tract in hospitalized patients with moderate alcoholic liver cirrhosis, to the further development of the disease, reviewing various causes of respiratory infection, characters and functional liver damage, in relation to the level of general inflammatory response of organic structure, by quantitative determination of certain non-specific serum markers of inflammation.

Material and methods

The research was done as a retrospective clinical study, through analysis of clinical data about 67 patients with verified moderate alcohol liver cirrhosis, diagnosed and treated at the Clinic of gastroenterology and hepatology and the Clinic for pulmonary diseases and tuberculosis, Clinical Center of Nis, in the period from September, 2001 to February, 2006.

Patients – research subjects

The patients were divided into two groups, I - experimental group consisted of 37 patients with clinical, radiological, laboratory and microbiologically verified bacterial respiratory infection of lower respiratory tract and moderate alcohol liver cirrhosis. The II - control group consisted of 30 patients with moderate alcohol liver cirrhosis without infective complications. I - group patients were treated with non-specific antimicrobial chemotherapy along with hepatoprotective and supportive-substitutional therapy, while the II - group patients were treated only with hepatoprotective and supportive-substitutional therapy.

Diagnosis of alcohol liver cirrhosis was based on clinical (positive personal anamnesis on ten-year long consumation of alcohol in the amount of 80 g of ethanol per day, physical examination), echosonographic (Toshiba Ecossee 96, 3.75 MHz convex catheter, 1996, Japan) and laboratory parameters of liver damage (serume markers of hepatocelular insufficiency).

Bacterial infections of lower respiratory tract are here defined as a set of: clinical symptoms, physical signs, laboratory parameteres (positive biogram – morning sputum culture and/or bronchoalveolar lavage and / or chemoculture), with pneumonic parenchimic pulmonal lesions on the standard postero-anterior chest radiograph. Endoscopic exploration (fiberoptic bronchoscopy) of bronchial tree with taking of bronchoalveolar lavat was done in some patients because of atypical radiological finding and inconvincing clinical picture. The examination did not include all the febrile patients and those with increased values of C-reactive protein, whose cause was non-bacterial infection (viral pneumonia, oropharyngeal, gastrointestinal and systemic candidiasis, autoimmune diseases, rheumatism and malignancies). All the examinees were HIV-seronegative.

Determination of the level of liver cirrhosis severeness.

The level of liver cirrhosis severeness was evaluated by scoring system of functional liver damage by using Child-Pough classification, which includes determination of three biochemical parameters – serum level of total albumines, bilirubines, as well as determining prothrombin time, and two clinical parameters – presence/absence of ascites and clinically manifested hepatic encephalopathy. Range between minimal (5 points) and maximal (15 points) values of the obtained score categorizes each patient into one of the three groups: 5-7 points – Child A-mild cirrhosis, 9-11 points – Child B-moderate liver cirrhosis and >11 points – Child C-severe liver cirrhosis.

Laboratory examinations

Chematological analysis, in all patients, followed the values of:

Eritrocyte sedimentation rate (ES) – by standard chematological procedure – with ESD timers, Bekton-Dickenson, UK,

Complete peripherial blood examination with leukocyte formula, on chematologic Analyzer AVL 816 analyzer, USA, with impedance and spectropfotometric methods.

Coagulation screening – determination of prothrombin time or INR (ACL-7000, Family, 2004, USA).

Biochemical analysis followed the serum level of:

- C-reactive protein (CRP) – by quantitative turbidimetric method on the Olympus AV 400 analyzer, Japan;
- Fibrinogen – with quantitative turbidimetric method according to Parfontie, spectrophotometric analysis on the Beckman DU 650 analyzer, Germany;
- Total bilirubin – by photometric colour test, Olympus, AU 400, 2003, Japan;
- Total proteins – by photometric colour test, Olympus, AU 400, 2003, Japan;
- Albumin – by photometric colour test Olympus, AU 400, 2003, Japan;
- Aspartate - aminotrasferase and alanin-aminotranspherasis – by kinetic UV test, Olympus, AU 400, 2003, Japan;

- Lactate dehydrogenase (LDH) – by kinetic UV test, Olympus, AU 400, 2003, Japan;
- Total cholesterol – by enzyme colour test, Olympus, AU 400, 2003, Japan.

All the listed methods of chematological and biochemical blood and serum examinations are accepted by International Federation for Clinical Chemistry – IFCC.

Bacteriological analysis done in I-group (experimental) patients:

- Biogram of morning sputum (KP, XE, 24-hour incubation) and
- Chemoculture (prepared finished substrates, 10-day incubation with expectance of the second day).

HIV screening was done in all the patients by enzyme-linked immunosorbent assay – ELISA test, and in cases of positive results by Western blot test with previously obtained written concordance of the examinees.

Follow–up of the examinees

Clinical data (the results from standard, clinical, echosonographic and laboratory processing) for every patient were used for verification of etiology and the level of liver cirrhosis severeness, and, in accordance with inclusion and exclusion research criteria, they were categorized into one of the above listed groups, experimental and/or control group, and follow - up during the hospitalization in relation to the defined parameters.

Determination of serum values of non-specific inflammation markers (acute phase inflammation markers) was done at the begining of patient's hospitalization and after antimicrobial treatment, i.e. after 2 weeks in I-group patients. For the II-group patients, their values were used.

Statistical processing of examination results

Statistical processing of obtained results was done by calculating average values and standard deviation for the above listed parameters of epidemiological, clinical and laboratory examination, as well as by methods of descriptive statistics. Statistic significance of the obtained individual and group results was determined by Student t-test of the average difference using little independant samples. P values smaller than 0,05 were considered statistically significant (8,9,10).

Results

Analysis of clinical data were done for 67 patients with verified moderate alcohol liver cirrhosis, diagnosed and treated at the Clinic of Gastroenterology and Hepatology and the Clinic for Pulmonary Diseases and TBC, Clinical Center Nis, in the period from September, 2001 to February, 2006. The patients were divided into two groups, I – experimental group which consisted of 37 patients with clinically, radiologically, laboratory and microbiologically verified bacterial respiratory infection of lower respiratory tract and Child B-moderate alcohol liver cirrhosis, and II – control group which consisted of 30 patients with Child B-moderate alcohol liver cirrhosis without manifested infective syndrome.

It is statistically significant that there were more male patients (95,52%, p< 0,05), of average age 49,21 ± 6,71 years. In I-group patients, respiratory symptoms included cough (75,67%), coughing out mucoid (71,42%) or purulent (28,58%) sputum and febrility (48,64%). Average duration of respiratory symptoms was 2,43 ± 1,28 weeks. At the moment of hospitalization, all the examinees from the experimental group had increased values of white bloodline cells - average values 12,09 ± 3,45 G/l, while neutrophil leukocytes (70-86%) dominated in differential formula.

In patients of I-experimental group serum levels of C-reactive protein, fibrinogen (p< 0,001) and erythrocyte sedimentation rate (p<0,05) were significantly higher than the control one.

Table 1. Initial serum levels of acute phase inflammation markers in observed patients

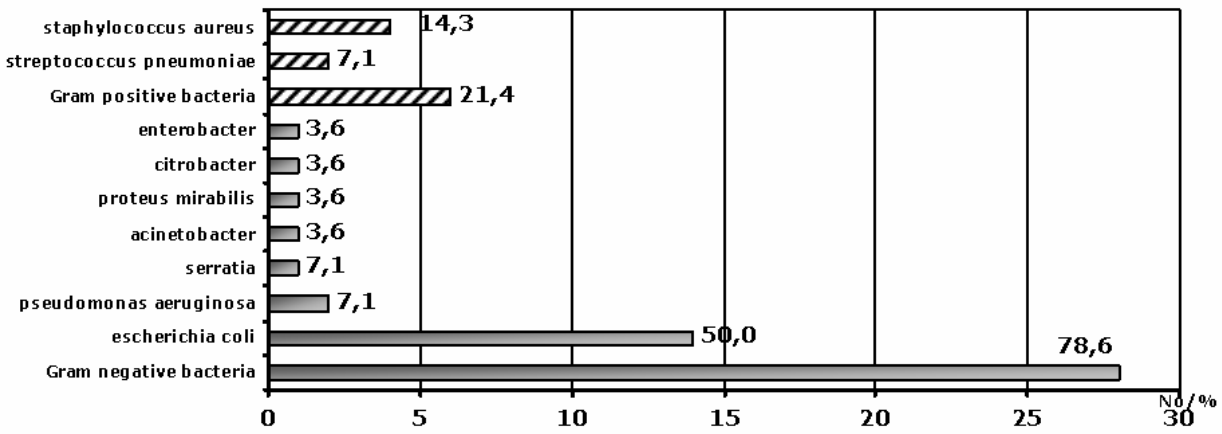
	Erythrocyte sedimentation rate (1/2h)	C-reactive protein (mg/l)	Fibrinogen (g/l)	Leukocyte count (G/l)
I (exper.) group	68,64±22,45 46,23±14,23	54,46±13,75	18,45±7,86	12,09±3,4
II (control) group	12,32±9,61/ 16.41±7.54	6,32±3,58	3,41±1,26	7,09±1,56
Significance	p<0,05	p<0,001	p<0,001	-

Serum values of non-specific inflammation markers (C-reactive protein and fibrinogen) at the begining of hospitalization and after antimicrobial treatment (2 weeks) registred significant decrease (p<0.05), in I-experimental group of patients as presented in Table 2.

Table 2. Serum values of non-specific inflammation markers at the begining of hospitalization and after antimicrobial treatment

I (experim.) group	Eriythrocyte sedimentation rate (1/2h)	C-reactive protein (mg/l)	Fibrinogen (g/l)	Leukocyte count (G/l)
Begining of antimicrobial treatment	68.64±22.45 46.23±14.23	54.46±13.7	18.45±7.86	12.09±3.45
End of antimicrobial treatment	48.44±11.42 26.21±8.36	11.16±3.85	7.65±4.95	8.09±1.63
Significance	-	p<0.05	p<0.05	-

Gram-negative bacterial flora (78,6%) was dominant in the biogram of morning sputum in 28 I-group patients, while *Escherichia coli* (50,0%), *Pseudomonas aeruginosa* (7,1%) and *serratia* (7,1%), were more isolated than other causes , while most often Gram positive bacteria were Staphylococcus aureus (14,3%).



Graph 1. Culture isolats of morning sptum specimens in patients of I (experimental) group

In seven patients of I - group, because of atypical radiological presentation and inconvincing clinical signs, fiberoptic bronchoscopy was done with taking of bronchoalveolar lavage, and its cultivation isolated Gram-negative bacteria (*Escherichia coli* 28.6%, *Pseudomonas aureuginosa* 57.1% and *acinetobacter* 14.3%). In other two patients, verification of causes was determined by chemoculture (*Staphylococcus aureus*). Relation between the serum hepatocelular insufficiency markers in the examined groups of patients is presented in Table 3. Significantly decreased initial values of total serum proteins and albumins and increased ones of alanine-aminotransferase and lactate - dehydrogenase were registered in I-group patients, improving after the antimicrobial treatment ($p<0.05$).

Table 3. Serum levels of hepatocellular failure markers before and after the antimicrobial treatment.

groups / serum markers	i/k*	I (exper.) group	II (control) group	p
total proteins level (g/l))	i	51,32±4,3	60,4±3,1	p<0,05
	k	59,3±2,4		
albumin level (g/l)	i	21,3±2,1	29,1±2,7	p<0,05
	k	29,2±3,1		
aspartate-aminotrasferase level (U/l)	i	54,6±15,7	48,4±23,2	-
	k	64,3±17,8		
alanin-aminotranspherasis level (U/l)	i	186,4±32,1	49,5±18,7	p<0,05
	k	48,5±15,6		
lactate dehydrogenase level (U/l)	i	567,8±45,4	267,86±65,4	p<0,05
	k	387,7±45,7		

i/k* Initially (i) / End of AB treatment (e)

Values of total serum cholesterol in I-group patients (2.80±0.8 / 3.25±0.6 μmol/l) comparing to the II-group patients (2.98±0.4 μmol/l), were not significantly different, as well as the values of total serum bilirubin level (I group: 16.7±5.6 / 18.2±4.6 μmol/l vs. II group: 17.4±6.8 μmol/l). Prothrombin time in the I-group patients (4.3±0.9 sec/ 5.2±0.4 sec), was not significantly different from the control-group patients (4.9±0.6 sec). Initially, all the patients from the I-experimental group were treated with parenteral wide-range penicilin antibiotics (Ampicilin), and after the verification of causes, the treatment was continued with antibiotic according to antibiogram.

Discussion

The main characteristic of alcohol liver cirrhosis is the inflammatory process, in which in the area of hepatocyte necrosis variuos mediators and cytokynes are being released. Further activation of immunocompetent cells, especially lymphocytes and macrophags, stimulates the appearance of fibroblasts adhesive-colagenous tissue, where myofibroblasts represent up to 60% of all mesenchyma liver cells. Hyalin sclerosing of central veins and the development of centrolobular fibrosis lead to the increase of pressure in portal vein system and the development of clinically manifested cirrhosis in alcohol liver disease (1,2).

Combined metabolical-chemodynamic changes in organisms of the patients with alcohol liver cirrhosis, induce high level of weakening or absolute absence of defensive mechanisms. So, in the field of secondary immunodeficiency, primarily generated as a damage of reticuloendotelial liver system (lymphoreticular tissue), phagocyte activity toward bacteria and endotoxines which enter the liver through intestinal mucosis and portal vein, is stopped, which is the main cause of bacterial infections, especially of respiratory tract, as one of the complications, and increases the mortality rate in these patients (3,4). On the other hand, malnutrition and decreased sintetic function of liver in cirrhosis contribute to further cell and humoral immunity disorders through component complements insufficiency or their excessive consumation in states with expressed functional glomerulopathy (hepatorenal syndrome) (5). These patients often have Gram-negative bacterial infection of lower respiratory tract as a complication, which is present in 30-50% of hospitalized patients. Kuo et al. confirm that in 75.6% of patients with cirrhosis, Gram-negative bacilli are the most frequent cause of infection (6). In our research, in 78.6% of the examinees and seven patients with atypical

radiological presentation of pulmonary infection in bronchoalveolar lavage, Gram-negative cause of infection was isolated in the biogram of morning sputum. Graudal et al. emphasize that the frequency and level of severness of respiratory infection are more frequent in patients with advanced disease stage and represents high risk of fatal outcome in even 15.4% of cases (7). In the rest of the patients, these infections induce further aggravation of this disease in terms of liver motion, respiratory and/or nodule insufficiency, so they are themselves bad prognostic signs (8).

In our research, the level of severness of alcohol liver cirrhosis, at the begining of examination of experimental-group patients, was correlated with the level of severness in control-group patients. It was significantly lower after antibiotic and hepatoprotective treatment, in terms of cirrhosis score, but within the limits of Child B – moderate alcohol liver cirrhosis, which indirectly emphasizes the significance of forehand recognition of infection and adequate therapy. Study of Navasa and Caly confirms this statement (7,8). The presence of bacterial respiratory infection is a bad prognostic factor of survival of these patients (8,9). Mortality in these patients goes from 6% to 30%, according to different studies, but it points out that the presence of bacterial respiratory infection is a bad prognostic factor of survival for these patients. Higher risk of development of this infection was registered in patients with low level of serum albumin and cholesterol, with increased values of serum lactat-dehydrogenasis and total bilirubin, as well as decreased prothrombin time in correlation with disease stage, according to Child-Pough classification (11,12). Lahnborg and Yoneyama say that higher risk of these infections is present in patients with low levels of serum albumin (< 2.65 g/dl) and cholesterol, increased values of serum lactat-dehydrogenises and total bilirubin, as well as lowered prothrombin time, in correlation with the severness of the disease, according to Child-Pough classification (3,5). Our research confirmed that all the patients from experimental group initially showed statistically decreased values of total serum proteins and albumins, while alanin-aminotransferasis and lactat dehydrogenises ones were increased in I-group patients, which improved after antimicrobial treatment ($p<0.05$), which again corresponds with the results of Rosa and al. study (4). On the other hand, initial parameter values of non-specific serum inflammation markers (reactors of acute inflammation phase) were statistically significantly increased in experimental-group patients than in control-group patients ($p<0.001$ and $p<0.05$). The same values were, after the antimicrobial treatment (after 3 weeks), significantly decreased in experimental group patients, which indirectly emphasizes good prognosis of main disease, similar to Graudal and al. study and Yoneyama and Caly's researches (5,7,9).

Conclusion

Forehand prevention of potential respiratory infection of lower respiratory tract in patients with alcohol liver cirrhosis is highly significant, in terms of improving their life quality and survival extension, as well as of achieving the remission of main disease.

Quantitative determination of non-specific serum inflammation markers (acute inflammation phase reactors) as specific risk factors, development and estimation of severness of bacterial infection in these patients, represent the significant but still insufficiently defined link in the chain of complex pathophysical mechanisms of reactivation of inappropriate development of this disease.

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ZNAČAJ SERUMSKIH VREDNOSTI REAKTANATA AKUTNE FAZE ZAPALJENJA U RANOM OTKRIVANJU, PRAĆENJU I INICIJALNOJ PROGNOZI TOKA BAKTERIJSKIH INFEKCIJA DONJEG RESPIRATORNOG TRAKTA KOD BOLESNIKA SA ALKOHOLNOM CIROZOM JETRE

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Kombinovane metaboličko-hemodinamske promene u organizmu kod obolelih od alkoholne ciroze jetre indukuju znatan stepen slabljenja odbrambenih mehanizama, što predstavlja osnovni uzrok za nastanak i razvoj bakterijskih infekcija, dominantno respiratornog trakta i povećavaju stopu mortaliteta.

U cilju procene uticaja bakterijske respiratorne infekcije donjih disajnih puteva bolesnika sa alkoholnom cirozom jetre, na tok i prognozu osnovne bolesti, a u odnosu na stepen opšte inflamatorne aktivnosti organizma, retrospektivno smo analizirali kliničke podatke 67 bolesnika sa verifikovanom Child B - umereno teškom alkoholnom cirozom jetre, dijagnostikovanom i lečenom u periodu od septembra 2001. do februara 2006. godine. Zavisno od postojanja infekcije, bolesnici su podeljeni u dve grupe: I - eksperimentalnu od 37 ispitanika i II - kontrolnu od 30 ispitanika.

Utvrdjeno je da kod bolesnika I grupe postoje signifikantno povišene inicijalne vrednosti C-reaktivnog proteina i fibrinogena ($p< 0,001$) i brzine sedimentacije eritrocita ($p<0,05$) u odnosu na ispitanike kontrolne grupe, dok su ove vrednosti, po završenom antimikrobnom tretmanu, bile u značajanom padu. Dominirala je infekcija Gram negativnim bakterijama, dok su inicijalno registrovane signifikantno niže vrednosti ukupnih proteina i albumina ($p<0,05$), odnosno povišene vrednosti alanin aminotransferaze i laktat dehidrogenaze ($p<0,05$) u odnosu na bolesnike kontrolne grupe, s tendencijom normalizacije po završetku kauzalnog antimikrobnog tretmana.

Pravovremena detekcija, praćenje i prognoza toka respiratornih infekcija donjih disajnih puteva kod obolelih od alkoholne ciroze jetre, određivanjem nespecifičnih serumskih markera inflamacije, značajni su u cilju postizanja što efikasnije remisije osnovne bolesti, ali i dalje predstavljaju nedovoljno definisanu kariku u lancu kompleksnih patofizioloških mehanizama aktivacije nepovoljnog toka bolesti. *Acta Medica Medianae 2008;47(2):38-43.*

Ključne reči: *jetra, ciroza, alkohol, infekcija*