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INVERSE CORELLATION OF CRP AND SERUM IRON IN PATIENTS WITH SIMPLE COMMUNITY-ACQUIRED PNEUMONIA (CAP)

SUMMARY

The inflammatory response associated with infection, synthesis acute phase proteins (APPs), shifts iron from the circulation into storage in order to protect the organism.

The prospective study included 47 patients (15 female, 32 male) with simple Community-aequired pneumonia (CAP) (mean age 62.4 ± 15.4 years). The patients did not use the supplementary iron therapy. We measured the white blood cell count (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total iron binding capacity (TIBC), serum iron and calculated transferrin. Blood samples were analyzed on the first day (group I) and after 6-8 days of hospitalization (group II). The aim of the investigation was to describe segmental changes and correlation of APPs and serum iron during the diagnostic work-up and antibiotic treatment.

CRP (166.95 ± 79.04 mg/L) showed decrease in group II (28.68 ± 26.59 mg/L) ($p<0.001$). TIBC was higher in group II (32.49 ± 6,47 vs. 39.34 ± 6.85 µmol/L) ($p<0.001$), with increase in serum iron (4.89 ± 3.23 vs. 10.04 ± 4.6 µmol/L) ($p<0.001$). ESR showed slower rate of decline ($p>0.001$). WBC showed significant decrease (10.67 ± 3.6 vs. 7.9 ± 2.23x10^3/ml). Serum iron showed negative linear correlation with CRP ($r = -0,625$; $p<0.01$) and positive correlation with TIBC ($r = +0.633$; $p<0.01$).

Serum CRP falls rapidly along with infection subsiding, while transferrin increases. Iron deficiency is common in patients with CAP. It positively correlates with transferrin, and negatively with CRP. The organism temporary responds to infection with hypoferremia, so that iron supplementation during CAP is unnecessary.

Key words: **inflammation, hypoferremia, pneumonia**

INTRODUCTION

During the infection, a complex reaction which is referred to as inflammation is activated with the aim to restore homeostasis and improve survival (1). Different types of macrophages from the site of the local reaction are stimulated to deliver cytokines into the peripherial circulation. These induce the synthesis of acute phase proteins (APPs) (2). These changes are observed within hours after infection or inflammation and are characterized by fever, leukocytosis, raised concentrations of circulating ACTH and glucocorticoids, the activation of the clotting casscade, an increase in the erythrocyte sedimentation rate, negative nitrogen balance, decrease in serum iron and zinc levels, and an increase in serum copper concentration (2). Measuring the alterations in APPs can be a useful clinical marker when an infection or inflammatory response is suspected (1).

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C-reactive protein (CRP) is a positive acute phase protein synthesized by hepatocytes. In response to infection or tissue inflammation, CRP production is rapidly stimulated by interleukin IL-6, IL-1 and TNF-α (3). Serum concentrations increase markedly with acute invasive infections, parallel with the severity of inflammation or tissue injury. This advantage makes CRP a useful marker for the presence of disease, response to therapy, and ultimate recovery (1). Adnet et al. showed that high CRP levels were helpful in the diagnosis of bacterial pneumonia (4). Besides, serial CRP measurements may be useful to evaluate the response to antibiotic treatment and to detect complications in patients with infections as a prognostic and follow-up test (5, 6). CRP is recommended as the first-line method of screening the infection (5).

Transferrin (Tf) is a negative APP synthesized predominantly by hepatocytes. A number of different functions may take place in the lower respiratory tract. Quantitatively, Tf is the main antioxidant in the lung and prevents the occurrence of "free" iron in the respiratory tract in non-smokers, but not in smokers (7). Tf levels as a percentage of total protein in BAL fluid are very high (4-5.6%) compared with values of plasma (8). Decreased transferrin is seen in malnutrition, but serum levels can also be decreased in patients with chronic inflammatory disease or chronic infection and malignancy (9,10). Low transferrin can impair hemoglobin production and therefore lead to anemia. Transferrin is abnormally high in iron deficiency anemia. Since only about one-third of the iron-binding sites of transferrin is occupied by Fe (III) in normal conditions, serum transferrin has considerable reserve iron-binding capacity (UIBC). Transferrin and ceruloplasmin present in plasma and help to keep in order transition metals and to reduce their involvement in free radical reactions (11).

As with many other factors involved in inflammation, it is very important that an appropriate iron balance is maintained (3). Free iron in the lung exerts toxic effects through its ability to catalyze highly reactive hydroxyl radicals from less reactive super oxide and hydrogen peroxide via the Fenton and Haber-Weiss reaction (12). Iron overload leads to inhibition of IFN-γ, TNF-α, IL-12, and nitric oxide formation as well as impairment of macrophage, neutrophil, and T-cell function (13).

The ability of bacteria, especially P. aeruginosa to obtain extracellular iron from host tissues for growth and enhancement of virulence by the secretion of iron-binding granules, called siderophores, suggests that this organism could play a direct role in depleting the body of iron stores (14). Iron deficiency (ID) in patients with cystic fibrosis has been well described, although its clinical significance is uncertain. The prevalence of ID increases from approximately 33% of pediatric cases to > 60% in the adult population, and it is normally attributed to a combination of chronic inflammation, gastrointestinal factors, and poor dietary intake (10, 15). Read at al. in their study find that iron deficiency is very common in the adult cystic fibrosis population and is directly related to the severity of suppurative lung disease, FEV1, ferritin/CRP ratio, body mass index, daily sputum volume, transferrin saturation, inverse re-lated with CRP (14).

In this study, we tested the hypothesis that ID in CAP may be directly related to severity of infection. We measured serum iron status and determined relationship of positive and negative acute phase proteins and biochemical markers of inflammation, such as CRP and Tf, in 47 patients with simple CAP. We tested the hypothesis that ID is common in acute infection, and decreases with its decline.

MATERIAL AND METHODS

The prospective study comprised 47 adult subjects (age range 16-83 years; 15 female, 32 male) with simple CAP, mean age 62.4±15.4 years. Patients were admitted to the Clinic for Lung Disease, Knez Selo, Clinical Centre Nis, during the ten-month period. Clinical history, symptoms and physical signs, pulmonary infiltrations on chest radiographs, blood laboratory parameters, sputum microbiology analysis were used as diagnostic standard. The history of iron deficiency anemia and supplementary iron therapy before hospitalization excluded patients from the investigation.

Blood samples were received for complete blood count, erythrocyte sedimentation rate (ESR), CRP, total iron binding capacity (TIBC), serum iron. All venous blood samples were collected in the morning. Samples collected on the first day of hospitalization presented group I, and samples collected after 6-8 days presented group II. White blood cell counts (WBC), ESR were studied by routine methods (Analyzer AVL 816), CRP by turbidometry method (Olympus AU 400). The serum iron and UIBC was determined by photometric color test (Olympus AU 400). UIBC measurements were used in conjunction with serum iron concentration to ob-tain the TIBC. TIBC correlates well with Tf concen-tration, and the theoretical ratio used. TIBC (mmol/L) = 25.1 x Tf (g/L) (16).

Statistical Analysis: Student t-test was used to compare the mean values of laboratory parameters in groups, while Pearson coefficient of linear corelation was used to describe the level of correlation between APP-s and serum iron.

AIMS

The aim of study was to describe segmental changes and use of WBC, ESR, CRP, Tf and serum iron, during the diagnostic work-up and antibiotic treatment in patients with CAP; to determine the level of correlation between CRP, serum iron and Tf and to show the role of temporary ID during the infection.

RESULTS

WBC were increased in the acute phase in patients with community-acquired pneumonia on the first day of hospitalization to the level 10.67 ± 3,6 x 10^3/ml. After 6-8 days there was a decline to 7.9 ± 2.23 x 10^3/ml (Figure 1) with significant difference ($p<0.001$).

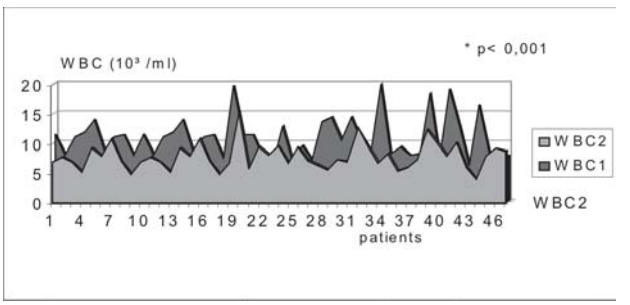


Figure 1. WBC on the first day and after 6-8 days of hospitalization

ESR showed slower rate of decline. In group I, ESR was 79.4 ± 24.04 mm/h; at control measurement ESR showed the value of 63.55 ± 28.59 mm/h, without significant difference ($p>0.001$) (Figure 2).

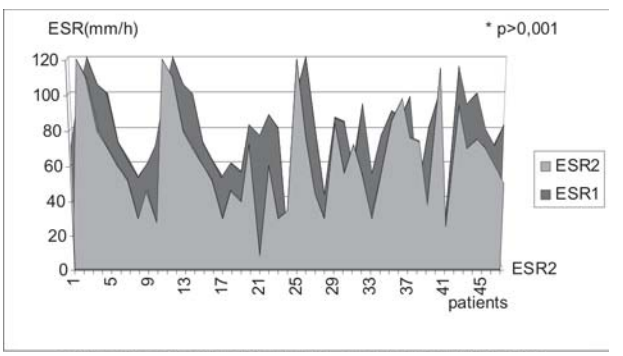


Figure 2. ESR on the first day and after 6-8 days of hospitalization

The changes in the concentrations of acute-phase proteins are largely due to changes in their production by hepatocytes. The acute-phase response, an important pathophysiological phenomenon, replaces the normal homeostatic mechanisms with new set points that presumably contribute to defensive or adaptive capabilities. CRP as positive protein of acute phase, increased greatly at first measurement, showed the value of 166.95 ± 79.04 mg/L (Figure 3). As we expected in control group, CRP fell rapidly with decline in infection to the level of 28.68 ± 26.59 mg/L ($p<0,001$).

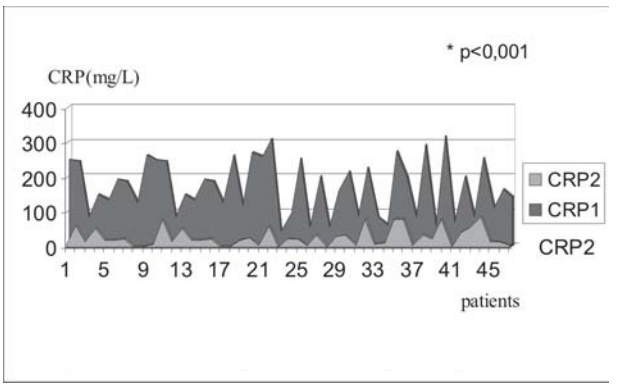


Figure 3. CRP on the first day and after 6-8 days of hospitalization

CRP decreased rapidly, until transferrin showed increase, with decline in infection and resolution of condition. TIBC, as indirect parameter of transferrin, was higher in group II (Figure 4), with value of 39.34 ± 6.85 μmol/L, than in the group I (32.49 ± 6.47 μmol/L), with statistically significant difference ($p<0.001$).

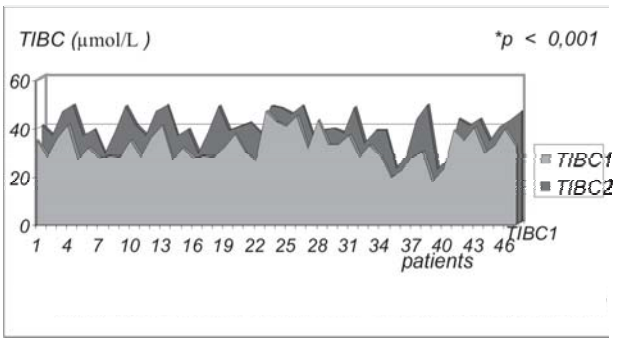


Figure 4. TIBC on the first day and after 6-8 days of hospitalization

In addition, serum transferrin showed low value in first measurement of 1.293 ± 0.257 g/L, with slow increase after 6-8 days to 1.567 ± 0.272 g/L ($p<0.001$) (Figure 5).

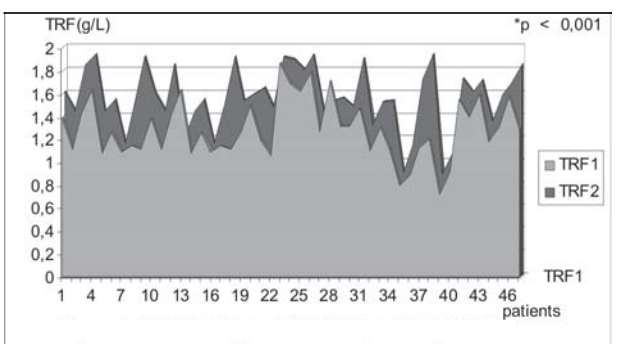


Figure 5. TRF on the first day and after 6-8 days of hospitalization

With resolution of infection, there was an increase of serum iron (4.89 ± 3.23 vs 10.04 ± 4.6 μmol/L) ($p<0.001$) (Figure 6).

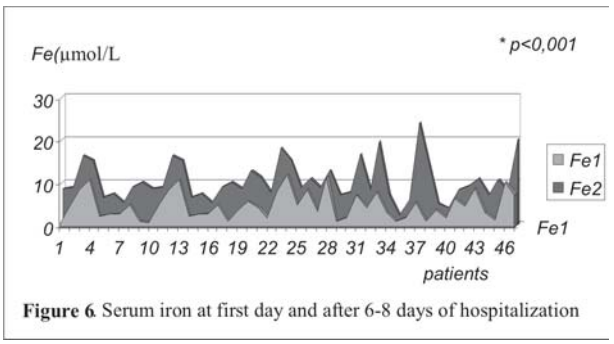
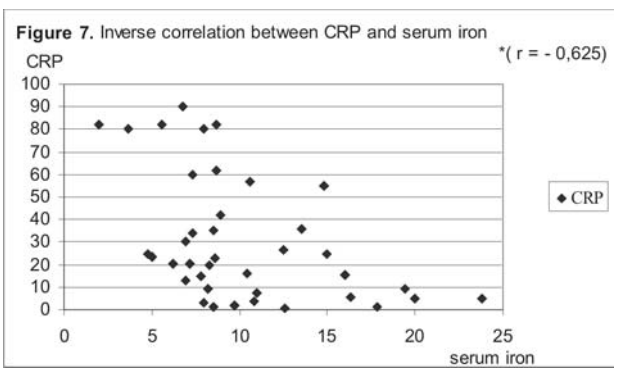


Figure 6. Serum iron at first day and after 6-8 days of hospitalization

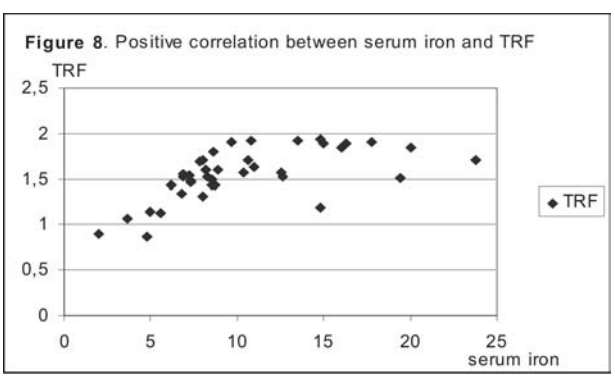
Figure 6. Serum iron on the first day and after 6-8 days of hospitalization

In our study, there was no difference between female and male group in observed laboratory parameters.

There is an excellent correlation between serum iron and APPs. Serum iron showed inverse correlation with CRP ($r = -0.625$; $p<0.01$) (Figure 7).



Tf are also present in plasma and help to sequester transition metals (X). Concentration of serum iron was in positive correlation with Tf ($r = +0.633$; $p<0.01$) (Figure 8).



DISCUSSION

The results of this study confirm that serum CRP values were significantly higher on the first day of hospitalization than after 6-8 days of antibiotic treatment in patients with CAP. At the beginning of antibiotic therapy, serum Tf and serum iron and diagnostic work-up were significantly decreased. The ID was associated with decline in infection. There is an excellent inverse correlation between serum iron and CRP, and positive correlation between serum iron and Tf.

CAP was defined as the presence of a new infiltrate on the chest radiograph along with appropriate clinical history and physical signs of lower respiratory tract infection in a patient not hospitalized within the previous month (17). Community-acquired pneumonia refers to any pneumonia contracted outside hospital. Early and appropriate treatment of CAP patients is one of the most important factors to reduce morbidity and mortality (18).

Several investigators (4,6) have reported that CRP has often higher sensitivity, specificity, and predictive values than WBC and ESR in the diagnosis of bacterial infections. Korppi at al. recommended C-reactive protein as the first-line method of screening in pneumococcal pneumonia in children (19). Walmary (20) showed that characteristics of CRP suggest its superiority over WBC and ESR as a detector of bacterial infections. Deodhare at al. clear up that ESR can be influenced by concentrations of fibrinogen, monoclonal proteins and red cell morphology, sex, where as CRP has no cross-interfaces (21).

In our study, on the first day of hospitalization, WBC showed increase, and after 6-8 days showed decline to normal range with significant differences. However, 19 patients (40.4%) showed normal range of WBC at the beginning of therapy. WBC can not preset the intensity of inflammation. ESR, as indirect parameter of acute phase protein changes, showed slow decrease during antibiotic therapy without statistical difference. The patients examined were elderly (62.4 ± 15.4 years) and 22 patients (46.7%) had comorbidity. For the same reasons, there was no significant difference between male and female group. ESR was affected by a multitude of factors. Acute-phase changes reflected the presence and intensity of inflammation, so early determination of serum CRP is useful and has advantages over the traditional strategy of measuring the WBC and ESR (22). This indicates that CRP is the best of the parameters studied for use in diagnostic work-up and in follow-up.

Cell and tissue damage resulting from an oxidative stress can ultimately be the consequence of a disruption of normal iron metabolism and an increased availability of catalytically active metal (23). Transferrin and ceruloplasmin are also present in plasma and help to keep in order transition metals, so as to reduce their involvement in free radical reactions (11). Measured serum iron concentration is principally the Fe (III) bound to serum transferrin

and does not include the iron contained in serum as free hemoglobin. Iron metabolism is of crucial importance in the biology and pathophysiology of the lower respiratory tract (24).

Hansson at al. proved that CRP showed the greatest amplitude of changes, and together with iron and saturation percentage of transferring, it also showed the earliest response to recovery in patients with CAP (9). Bacteria use iron for metabolism and grow up. Neiland concluded that the ability of P.aeruginosa to obtain extracellular iron from host tissues for growth and enhancement of virulence by the secretion of iron-binding granules, called siderophores, suggests that this organism could play a direct role in depleting the body of iron stores (25). The results of Page study on other bacteria show that decline of Tf and ID serves as an iron trap, probably to protect the cells from oxidative damage mediated by H_2O_2 and the Fenton reaction (26). Modun at al. explore the contribution of the staphylococcal receptor to the acquisition of iron from human transferrin (27). Read at al showed that ID is very common in the adult cystic fibrosis population and is directly related to the severity of suppurative lung disease (14).

Our results show decrease of transferrin at the start of infection. We can explain that with greater need of the organism for antioxidant transferrin function during the infection and inflammation. Transferrin is essential for iron transport. During bacterial infection, pathogen and host compete for iron. The inflammatory response associated with infection shifts iron from the circulation into storage to prevent activation of iron in Fenton reaction. Iron overload leads to inhibition of IFN-γ, TNF-α, IL-12, and nitric oxide formation as well as impairment of macrophage, neutrophil, and T-cell function (13). The function of iron deficiency (ID) is to prevent the collapse of the defense mechanism. Our study shows that there is an excellent inverse correlation between serum CRP level and serum iron level. CRP production is rapidly stimulated, rises early before the onset of clinical symptoms, and declines with the resolution of infection. This acute phase protein could be used as a tool for monitoring the effect of antibiotic therapy and decline in infection. Humans respond to infection with inflammatory cytokine-induced hypoferremia (13). With the resolution of condition, we got increase of serum iron and normalization of CRP level.

CONCLUSION

The inflammatory response associated with infection, synthesis positive APP, shifts iron from the circulation into storage. There is an excellent inverse correlation between serum iron and CRP during decline in infection. Iron deficiency is common in patients with CAP. The values of transferrin and serum iron increases with the resolution of condition. The role of temporary iron deficiency in impaired immunity and decreased microbial virulence calls into question the value of iron supplementation during inflammation and infection.

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NEGATIVNA KORELACIJA C-REAKTIVNOG PROTEINA I SERUMSKOG GVOŽĐA KOD BOLESNIKA SA VANBOLNIČKI STEČENOM PNEUMONIJOM (VSP)

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SAŽETAK

Inflamacija uzrokovana infekcijom dovodi do produkcije proteina akutne faze (PAF), koji u toku odbrambenih mehanizama pomažu u otklanjanju serumskog gvožđa iz cirkulacije. Prospektivna studija uključuje 47 bolesnika (15 žena, 32 muškaraca) sa vanbolnički stečenom pneumonijom (VSP) (starosti 62.4±15.4 god). Bolesnici nisu koristili supstitucionu terapiju gvožđa. Određivana je vrednost broja leukocita (Le), brzina sedimentacije eritrocita (SE), C-reaktivnog proteina (CRP), ukupnog kapaciteta transferina za vezivanje gvožđa (TIBC), serumskog gvožđa i izračunata vrednost transferina. Krv je analizirana na prijemu (Grupa I) i nakon 6-8 dana hospitalizacije (Grupa II). Cilj studije bio je da analizira promene i korelaciju PAF-e sa serumskim gvožđem tokom dijagnostike i antibiotskog tretmana.

CRP (166.95±79.04 mg/L) pokazuje pad vrednosti u Grupi II (28.68±26.59 mg/L) ($p<0.001$). TIBC pokazuje veće vrednosti u Grupi II (32.49±6.47 prema 39.34±6.85 µmol/L) ($p<0.001$), uz uočljiv porast vrednosti gvožđa (4.89±3.23 prema 10.04 ± 4.6µmol/L) ($p<0.001$). SE pokazuje lagani pad vrednosti u Grupi II ($p>0.001$). Leukociti pokazuju značajniji pad (10.67±3.6 prema 7.9±2.23x10^3/ml). Serumsko gvožđe pokazuje negativnu linearnu korelaciju sa CRP-om ($r = -0.625$; $p<0.01$) i pozitivnu korelaciju sa TIBC-om, odnosno transferinom ($r=+0.633$; $p<0.01$).

CRP pada brzo sa smanjenjem infekcije, dok vrednost transferina pokazuje porast. Deficit gvožđa je uobičajan kod bolesnika sa VSP, pozitivno koreliše sa transferinom a negativno sa vrednošću CRP-a. Organizam tokom infekcije privremeno sklanja gvožđe iz cirkulacije kao odbrambeni mehanizam, te je supstituciona terapija tokom VSP suvišna i nepotrebna.