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# Link between Biochemical and Hematological Parameters and their Role as Pre-diagnostic Indicators of Acute Inflammation in Preschool Children

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## Abstract

**Background:** Correlations between biochemical and hematological parameters and acute immune response in children are insufficiently described. The aim of this study was to determine the potential links between selected biochemical and hematological parameters and the development of any type of inflammation in children of preschool age, as well as to determine the concentration ratio between C-reactive protein and serum iron.

**Patients and methods:** The subjects of this research were preschool children suspected of inflammation in the municipality of Novo Sarajevo. All biochemical parameters (C-reactive protein, total iron binding capacity, unsaturated iron binding capacity and serum iron) were determined using a Cobas® 8000 automatic analyzer. Hematological parameters (white blood cell count, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were determined using an automated hematology analyzer Celltac F®.

**Results:** Iron levels were reduced in children who had elevated levels of C-reactive protein. White blood cell counts did not significantly differ from reference limits ($p>0.05$). Hematological parameters (HCT, hemoglobin concentration, MCV, MCH and MCHC) did not deviate from reference values in cases of acute inflammation and therefore cannot be used as valid indicators of inflammation in preschool children. Gender-specific differences have not been established for any parameter.

**Conclusion:** Elevated levels of C-reactive protein in combination with serum iron levels are the best indicator of acute inflammation in preschool children. TIBC and UIBC together with serum iron levels might be a prognostic marker of compensatory anemia development.

**Keywords:** C-reactive protein, serum iron, acute inflammation, preschool children.

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## INTRODUCTION

The sensitivity of child's organism causes slightly different responses to the acute phase of inflammation. The correlation between biochemical and hematological parameters during acute inflammation is still subject of discussion.

C-reactive protein (CRP) is synthesized in the liver in response to a variety of inflammatory processes in the organism. It is an acute phase reactant and its principal role is the activation of complement and the initiating of an immune response. CRP synthesis is induced by cytokines, interleukin-6 (IL-6), interleukin-1 (IL-1) and indirectly by tumor necrosis factor alpha (TNF-α) (1,2). The synthesis of CRP starts very fast and reaches a maximum within 48 hours. During the acute inflammatory reaction, CRP values can be increased up to hundred times. Reference values for CRP are under 5 mg/L (3). Plasma contains about 2.5 mg of iron which is transported bound to a plasma protein transferrin (4). UIBC is a parameter of latent or unsaturated iron-binding capacity while TIBC shows the maximum concentration of iron that transferrin can bind. Reference values for serum iron in preschool children range from 10 to 30 μmol/L and for TIBC varies between 43 and 80 μmol/L (5,6). The number of leukocytes is a dynamic equilibrium between the bone marrow formation and their deterioration and depends on the physiological state of the organism. The number of leukocytes in preschool children ranges from 5.3 to 11.5 x $10^9$ per liter of blood (7). Reference values for hematological parameters in preschool children are: hemoglobin 11.5 to 13.5 g/dL , HCT 31.7 to 39.6%, MCV 72.7 to 86.5 fL, MCH 24.1 to 29.4 pg and MCHC 32 to 35.3 g/dL (7,8,9).

The aim of this study was the assessment of correlations between biochemical and hematological parameters and acute inflammation reactants in preschool children, with special emphasis on the correlation between CRP and other biochemical or hematological parameters.

## PATIENTS AND METHODS

### Subjects

This research was conducted on preschool children from the municipality of Novo Sarajevo (Bosnia and Herzegovina). They were born between 2009 and 2012. Blood samples were drawn between 7 and 10 o'clock in the morning, after 15 minutes of inactivity (Department for Laboratory Diagnostics, Health Center “Omer Maslić”, Sarajevo, Bosnia and Herzegovina).

Our research was conducted in compliance with all applicable guidelines with ensured proper implementation of the safety of persons participating in the scientific research, including Fundamentals of Good Clinical Practice, Declaration of Helsinki 1975, as revised in 2008, and the Law on rights, obligations and responsibilities of patients in Federation of Bosnia and Herzegovina. Analysis was carried out with the consent of all parents.

This study covered pre-school children with symptoms of acute infections e.g. fever, sore throat, malaise, poor appetite–symptoms caused by bacterial or viral infections. The cause of infection varied as shown by analyzed parameters, especially with the values of CRP.

**Sampling**

The blood for analysis was obtained from median cubital vein. In samples for hematological analysis was added anticoagulans (EDTA) while blood samples for biochemical analysis (including CRP) didn't contain anticoagulans.

**Biochemical analysis**

Biochemical parameters (CRP, total serum iron, UIBC and TIBC) were determined with the automatic autoanalyzer Cobas® 8000 (model Cobas c502, Roche Diagnostics, USA). After the preparation procedures, samples were put into the autoanalyzer and the concentrations were measured by turbidimetric or spectrophotometric methods. C-reactive protein concentration was determined by the autoanalyzer Cobas c 502 (imunoturbidimetric principle) (10,11). UIBC and serum iron levels were obtained by FerroZine method (12,13).

**Hematological analysis**

Hematological parameters included total number of leukocytes (WBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean concentration of hemoglobin in erythrocytes (MCH) and mean corpuscular hemoglobin concentration(MCHC). All parameters were analysed by autoanalyzer Celltac F® (Nihon Kohden, Japan). The analysis were based on flow citometry principles (14).

**Statistical analysis**

Statistical methods included analysis of variance (ANOVA), Spearman bivariate correlation and Kolmogorov-Smirnov test. Statistical analysis were performed by SPSS(Version 20.0, SPSS, Inc., Chicago, IL, USA) and MS Excel 2013 statistical program. P values lower than 0.05 ($P<0.05$) were considered as significant and P values lower than 0.01 ($P<0.01$) as highly significant.

## RESULTS

This study included 51 children (29 boys and 22 girls). C reactive protein (CRP) concentration, hematocrit (HCT), hemoglobin concentration (Hb), leukocytes (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), serum iron concentration (Fe), total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were determined in all individuals.The results of biochemical and hematological analysis (including mean and standard deviation) are shown in **Table 1** and compared with reference ranges.

| Parameter (n = 51) | Reference range | Mean ± SD (n = 51) | ♂♂ (n = 29) | ♀♀(n = 22) | P-value ♂♀ |
|---|---|---|---|---|---|
| **CRP (mg/L)** | 0-5 | 29.63 ± 52.35 | 21.03 ± 35.50 | 40.95 ± 67.89 | <0.05* |
| **Fe (µmol/L)** | 10-30 | 7.18 ± 5.64 | 6.98 ± 5.06 | 7.44 ± 6.44 | >0.05 |
| **UIBC (µmol/L)** | 33-50 | 49.87 ± 9.94 | 51.07 ± 9.33 | 48.30 ± 10.71 | >0.05 |
| **TIBC (µmol/L)** | 43-80 | 57.02 ± 8.35 | 58.14 ± 7.67 | 55.55 ± 9.15 | >0.05 |
| **WBC (x $10^9$/L)** | 5-12 | 10.47 ± 4.13 | 10.85 ± 3.77 | 9.96 ± 4.59 | >0.05 |
| **Hb (g/dL)** | 12-14 | 12.2 ± 1.27 | 12.31 ± 1.33 | 12.04 ± 1.21 | >0.05 |
| **Hct (%)** | 32-40 | 35.15 ± 5.57 | 36.17 ± 3.44 | 35.30 ± 3.16 | >0.05 |
| **MCV (fL)** | 73-87 | 75.81 ± 4.45 | 75.35 ± 4.54 | 76.40 ± 4.34 | >0.05 |
| **MCH (pg)** | 24-30 | 25.74 ± 1.87 | 25.64 ± 1.92 | 25.16 ± 1.85 | >0.05 |
| **MCHC (g/dL)** | 32-35 | 33.99 ± 1.00 | 34.01 ± 0.82 | 33.96 ± 1.22 | >0.05 |

*P<0.05 is statistically significant

**Table 1.** Biochemical and hematological parameters of preschool girls and boys accompanied by gender specific analysis and P-values.

| Parameter | Kolmogorov-Smirnov (df = 51) | | Spearman correlation coefficient | |
|---|---|---|---|---|
| | Statistics | Sig. | CRP | |
| **CRP** | 0.292 | 0.00.** | F | Sig. |
| **Fe** | 0.236 | 0.00.** | -0.523 | 0.00.** |
| **UIBC** | 0.076 | 0.200 | 0.175 | 0.22 |
| **TIBC** | 0.143 | 0.011* | -0.116 | 0.418 |
| **WBC** | 0.135 | 0.021* | 0.354 | 0.011* |
| **Hb** | 0.064 | 0.200 | -0.497 | 0.00.** |
| **Hct** | 0.086 | 0.200 | -0.432 | 0.002** |
| **MCV** | 0.103 | 0.200 | -0.315 | 0.024* |
| **MCH** | 0.124 | 0.047* | -0.492 | 0.002** |
| **MCHC** | 0.102 | 0.200 | -0.492 | 0.002** |

* P<0.05 is statistically significant

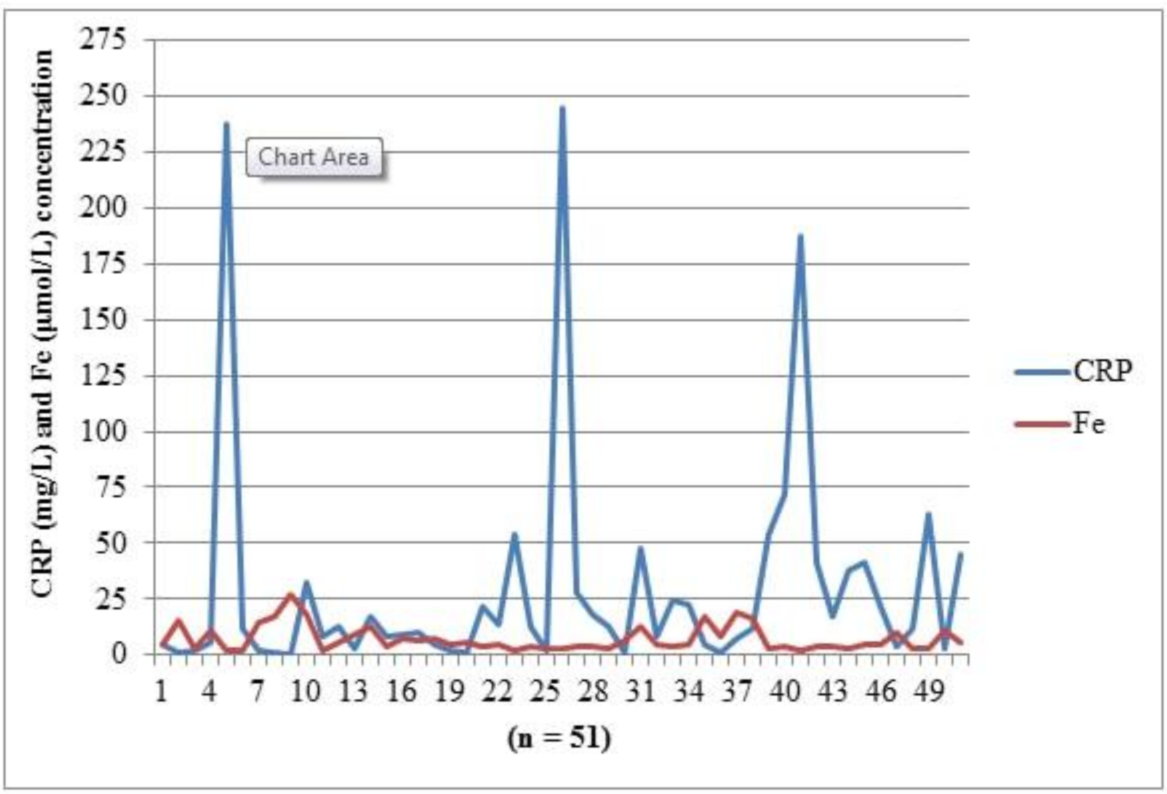
** P<0.01 is statistically significant

**Table 2.** Results of Spearman bivariate correlation test between CRP and other parameters and Kolmogorov-Smirnov normality test.

The highest deviation in comparision with reference values was obtained for CRP (29.63 ± 52.35 mg/L) and for the concentration of serum iron (7.18 ± 5.64μmol/L). Other parameters were within reference ranges. Boys had higher values of obtained parameters but without significant differences in comparison to girls, except for CRP and serum iron values. Normal frequency distribution test (Kolmogorov-Smirnov test) and CRP correlations with other parameters (Spearman correlation coefficient) are shown in Table 2.

Statistically significant differences ($P<0.05$) for CRP, Fe, TIBC, WBC and MCH values indicate deviation from the normal data distribution of the analyzed parameters (KS test). Between CRP and almost all parameters (except WBC and UIBC) a negative correlations were found while significant differences were determined for the WBC and MCV ($P<0.05$), Fe, Hb, HCT, MCH and MCHC ($P<0.01$).The correlation of serum iron and CRP values are presented in Figure 1.

**Figure 1.** Concentration and correlation of CRP and serum iron (Fe) in preschool children.



The highest value of CRP fits the lowest concentration of serum iron and vice versa. Figure 2 and Figure 3 show the normal probability plot for CRP and serum iron (Fe). Based on Q-Q plot it can be observed that most of the results for both parameters were not normally distributed and also deviation of CRP is much higher in comparison to serum iron.

**Figure 2.** Probability or Q-Q plot of Serum Iron (Fe)

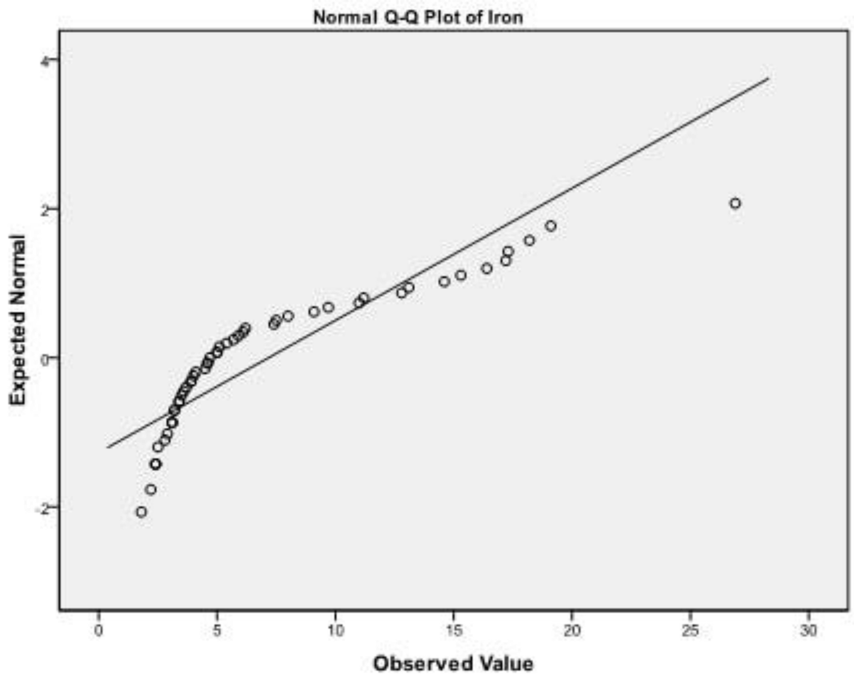
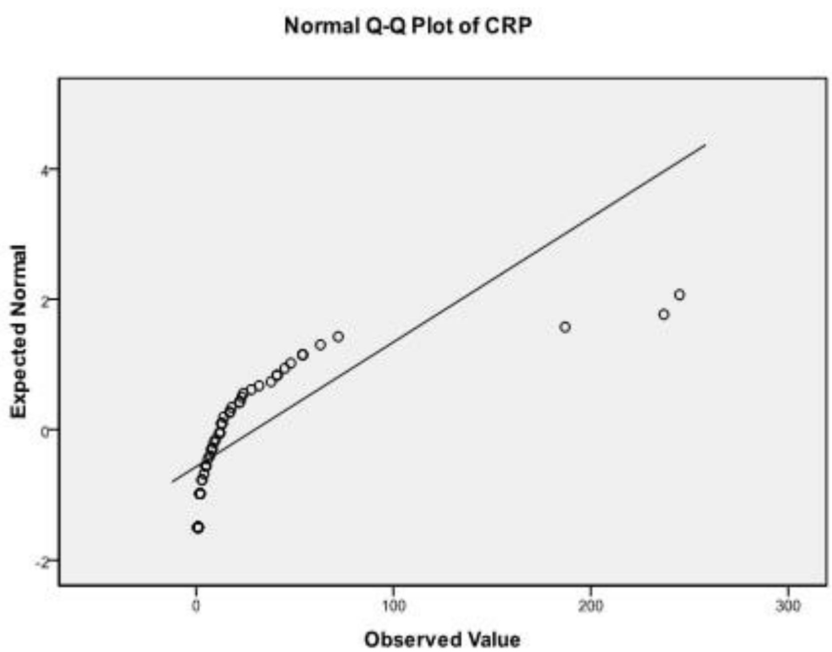



**Figure 3.** Probability or Q-Q plot of C-reactive protein (CRP)



## DISCUSSION

CRP is present in low concentrations (<5 mg/L) in healthy individuals. In the course of anti-inflammatory response the concentration of CRP rises very fast, reaching its maximum in two days. CRP concentrations exceeding 100 mg/L are certainly linked with serious bacterial infections and autoinflammatory diseases (15, 16,17,18). A special aspect of our study was focused on analysis of serum iron whose values were elevated during acute inflammatory processes in children. Values of serum iron in children in our study were lower in comparison to other studies (19,20,21,22). Decreased serum iron levels were monitored and they were followed by decreased concentrations of TIBC and UIBC. The reason lies in the fact that during an inflammatory process hepcidin synthesis increases and it incorporates into the cell membranes involved in iron metabolism. The process of synthesis and membrane incorporation of hepcidin, prompted by IL-6, causes the iron to be trapped mainly in the enterocytes, macrophages and cells of the liver. Low serum iron values are considered to be the body's defending mechanism against infection, because the reduction of iron also reduces the available iron that microorganisms use in many metabolic pathways (23, 24). Soluble transferrin receptor (sTfR) is generally unaffected by inflammatory status, whereas ferritin increases along with acute-phase response in children (25). A mutation of NLRC4 gene can lead to a serious inflammatory flare that is similar to, or a form of, the macrophage activation syndrome (MAS). In such cases the biochemical analysis during MAS-like flares included elevation of C-reactive protein in serum and an extreme hyperferritinemia (26). White blood cell count is an important parameter during infection, although some previous studies have not shown significant changes of this value (27). In our study the number of leukocytes also did not deviate from the reference value, although patients with an inflammatory process often have an increased number of leukocytes (7). No correlation between the level of leukocytes and CRP values was observed in our study. Weak leukopoiesis response and little or no increase in the number of leukocytes in children is most likely a result of the time necessary for their mobilization, activation and adequate immuno-modulating chemotaxis in peripheral circulation, which is very short in the acute process. Hemoglobin concentrations were within reference ranges. According to the localization of hemoglobin in erythrocytes and due to the fact that it is not a participant in the anti-inflammatory response, changes in hemoglobin concentrations were not observed during acute inflammatory processes. Hematocrit values were within the reference range (7). Red blood cells parameters (MCV, MCH and MCHC) were within reference ranges, as confirmed by previous studies carried out during acute inflammatory conditions (7,22).

However, during chronic inflammatory processes the changes of these parameters were evident as a result of compensatory response of hematopoietic centers (28,29,30,31,32). No statistically significant differences were observed between genders. However, higher CRP and iron values and lower concentrations of UIBC and TIBC were observed in females. These results are confirmed by previous studies (19,20). Lower iron concentration and higher TIBC and UIBC concentrations in males compared to females indicate that iron concentration is an interesting diagnostic marker in the inflammatory process. Hemoglobin concentrations were higher in males, which is a result of increase of the total amount of iron (Fe + UIBC). High concentrations of CRP correlated with a decreased serum iron observed in our study (2), which is considered a result of intensive and invasive immune responses. The ratio of CRP to serum iron shows that the concentration of serum iron directly depends on CRP values. It is evident that the patients with maximum CRP values have the lowest concentration of serum iron and vice versa. Amounts of free iron are only available during inflammatory processes. Spearman's coefficient of variation showed a statistically significant correlation between serum iron and CRP ($P<0.01$). CRP and cytokines affect the redistribution of iron in the liver and the mononuclear phagocyte system. As a consequence, a reduction of iron-binding proteins in plasma occurs. Decreased serum iron prevents its use by microorganisms and prevents the emergence of pro-oxidation potential of iron, which often leads to tissue damage due to the creation of ROS (33). Furthermore, inadequate values of hs-CRP were associated with severe obesity and high systolic blood pressure (35). These markers can be used to identify children and adolescents with higher risk of developing atherosclerosis later in life. Another study (35) concluded that obese children and adolescents have significantly increased hs-CRP compared with a normal weight group. However, the limitations of this study were the absence of a control group and an insufficient number of patients, so further research should be conducted on this topic.

## CONCLUSIONS

Significant increase of CRP, slight increase of leukocytes and a decrease of serum iron during an inflammatory process in children were evident in our study. Values of TIBC and UIBC did not show diagnostic importance. A higher number of leukocytes in chronic response is expected. Increased CRP associated with lower serum iron levels might be considered as a biomarker of inflammatory processes in children. Furthermore, TIBC and UIBC together with serum iron levels might be prognostic markers of anemia due to chronic inflammation status. However, these findings need to be confirmed by large prospective cohort studies.

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