RATIONALITY IN DETERMINATION OF INFLAMMATORY PARAMETERES IN SUSPECTED ACUTE INFLAMMATION

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Introduction: Inflammation represents tissue homeostasis, body's most powerful defense mechanism, almost completely non-specific with respect to the quality of noxae, and is present in any tissue. Inflammation can be acute or chronic. Acute inflammation has rapid action and short duration, from a few minutes to several days, and is characterized by exudation of fluid and plasma proteins, as well as by a dominant accumulation of neutrophils. Acute phase inflammatory markers commonly used for diagnostic purposes are C-reactive protein (CRP), erythrocyte sedimentation rate, and white blood cell count also known as leukocytes.

Materials and Methods: Materials used in this study were patients' laboratory results collected from laboratories in the medical institution " Health Center Donji Vakuf" in DonjiVakuf in the period from 1st February to 1st May 2015. For the purposes of this paper we analysed the values of leukocytes, neutrophils, erythrocyte sedimentation rate and the CRP. The study was carried out on a sample of 200 participants - 100 adults and 100 children. The process of determining the values of leukocytes and neutrophils was done by an automated hematology analyzer Sysmex electronic XP300 produced by the Sysmex company (Japan). To determine CRP values in adults participants, we used their blood serum and ran it through the biochemical analyzer BT 1500. Determination of CRP values in children participants was carried out from capillary blood samples using the NycoCard READER II. The erythrocyte sedimentation rate was measured using a modified Westergren method.

Results: The outcome of the statistical analysis has shown that the mean value of leukocytes in children is higher compared to the same value in adults. This parameter is more varied in children (46.91%) than in adults (45.68%). The mean value of neutrophils in children is also higher compared to the same value in adults. This parameter had a higher variation coefficient in adults (58.87%) than in children (51.40%). However, the mean value of the erythrocyte sedimentation rate, after the first and second hours in adult patients is higher than in children. Variation coefficients of this parameter are higher in adult patients after the first hour, whereas they appear to be higher in children after the second hour. The Pearson bivariate correlation has shown the existence of a statistically significant relationship between the values of leukocytes and neutrophils in both studied groups. A positive correlation in the group of adult patients is present in the values of leukocytes and erythrocyte sedimentation rate after the first and second hours, but this difference was not statistically significant.

Conclusion: The results of this study have shown a high percentage of positive results with erythrocyte sedimentation rate after the 1st and 2nd hours and CRP (95%, 81% and 61%), and quite a low percentage of the reference values of leukocytes and neutrophils (33% and 26%) for the total number of participants. Partial correlation has shown that elevated values of white blood cells, as a control parameter, are associated with the combination of the values of leukocytes and neutrophils in adults (p = 0.030), as well as in children (p = 0.000). This data confirms the results of the Pearson bivariate correlation, implying that with an elevated leukocyte count, comes an increase of the value of neutrophils and CRP.

Keywords: inflammation, inflammatory parameters, leukocytes, neutrophils, CRP, erythrocyte sedimentation rate

INTRODUCTION

Inflammation can be either acute or chronic. Acute inflammation occurs rapidly and has a short duration, lasting from several minutes to as much as several days, and is characterized by exudation of fluid and plasma proteins and accumulation of predominantly neutrophilic leukocytes (1). Indication of the presence, severity and chronicity of inflammation can be detected

through white blood cell count, sedimentation rate and C-reactive protein. Although these differentiation markers lack specificity among the many possible causes of inflammation, they are a simple, economical and valuable diagnostic tool for distinguishing inflammatory from non-inflammatory conditions, and providing a follow-up of the response to treatment of a number of disorders.

C-reactive protein (CRP) is a plasma protein, an acute phase reactant of inflammation produced by the liver and adipocytes. It is a highly sensitive (86%), and low-specific (46-61%, depending on the study) biomarker significant for distinguishing bacterial from viral infections. The synthesis of CRP begins in the hepatocytes. After the latency of approximately 6 hours, the serum level is doubled every 8 hours, and the highest concentration (1000 to 10,000 times the baseline) reaches 36 to 48 h duration of the inflammatory process. In case of a successful antibiotic therapy, the CRP concentration is reduced to 50% in the first two days, which allows the follow-up of the disease and the treatment success. There are data on the importance of measuring the serum concentration of CRP in case of suspicion of sepsis, wherein the measurement should be repeated with a distance of 12-24 h. The normal concentration of CRP levels in humans has a median of 0.8 mg / l, and is below 10 mg / l in 99% of normal samples. Levels above this value are abnormal and indicate the presence of disease (2,3).

The speed with which the erythrocyte sedimentation occurs in clinical practice is a measure to assess the health status of individuals. Tests have shown that in many acute and chronic diseases, there occurs an accelerated erythrocyte sedimentation. The sedimentation rate depends on several factors: the number of erythrocytes, haemoglobin content, the tendency of erythrocyte to accumulate (adhesion and agglutination), the composition of blood plasma, and especially on the quantitative relationship of certain proteins in the blood plasma. External factors that influence the sedimentation rate are temperature and seasons. At higher temperatures, sedimentation is accelerated, and at lower temperatures, it is slowed down (4). In the study of patients with unclear inflammatory sources, mild to moderately elevated erythrocyte sedimentation rate alone has no specificity and has no significant diagnostic importance. Significantly elevated erythrocyte sedimentation (i.e. greater than 100 mm / h), on the other hand, is almost always a significant marker (5).

Leukocytes are the body's moving protective units. They are partially formed in the bone marrow (granulocytes and monocytes, and fewer lymphocytes), and partly in lymphocytic tissue (lymphocytes and plasma cells). Leukocytes participate in cellular and humoral defence of organisms from foreign agents. The main reason for the presence of leukocytes in the blood is, simply, their transmission from the bone marrow or lymphatic tissue to the areas of the body where they invade. When released from the bone marrow, granulocytes live normally in the blood for 4-8 hours, and another 4 to 5 days in the tissues (65). Neutrophil granulocytes or neutrophils are granulocyte leukocytes (granulocytes) whose essential role is defending organisms from bacterial and fungal infections. Basic properties of neutrophils are the ability of spontaneous motion (migration), the ability to pass through the blood vessels, to respond to hematocytic stimuli, phagocytosis and degranulation. Degranulation is the release of granule contents (generally enzymes, mediators and reactive oxygen intermediate) in the extracellular space to destroy the intruders (7,8).

MATERIALS AND METHODS

A retrospective-descriptive analysis was used as a scientific method for this study. The materials used in this study represent patients' biochemical analyses collected from the laboratory at "Dom zdravlja Donji Vakuf" in Donji Vakuf in the period from 1st February to 1st May 2015. For the purposes of this study we have been tracking the values of leukocytes and, neutrophils, the erythrocyte sedimentation rate and the values of the CRP. The study had 200 respondents, of which 100 adults and 100 children. In the classification and statistical processing of data, the age of the patients was taken into account and accordingly classified into groups. Data on leukocyte counts, neutrophils, erythrocyte sedimentation and CRP sedimentation were compared in order to determine their value for suspected acute inflammation.

The analysis of leukocyte and neutrophils counts, erythrocyte sedimentation and CRP in adults was performed on venous blood samples taken in the morning from the cubic vein (in the latent ditch) using a flash system, supplemented with EDTA anticoagulants to the tube, to prevent blood coagulation. As in all laboratory analyses, blood extraction was performed on subjects who had not taken food for 12 hours, or "on an empty stomach". The analysis of leukocyte and neutrophil counts and the erythrocyte sedimentation rate in children was also performed on venous blood samples, while the determination of CRP in children was done from capillary blood samples.

The process of determination of leukocyte and neutrophil values was performed by an automated electronic haematological analyzer Sysmex XP300 (made by the Japanese company Sysmex). The determination of CRP in adults was performed from the blood serum on the BT 1500 biochemical analyzer. Determination of CRP in children was performed from capillary blood samples on the NycoCard READER II apparatus. Determination of erythrocyte sedimentation rate was performed by a modified Westergreen method.

Statistical methods Based on the obtained values of inflammatory parameters, their statistical processing was performed and the results were presented in tabular and graphical form. All the results were processed using descriptive and analytical statistics using Microsoft Office Excel 2010 and Statistics 7 software. Individual relationships between individual groups were tested

by a student's test (for normal distribution). Values p <0.05 were considered statistically significant.

RESULTS

The study encompassed 200 respondents, of whom 100 were children and 100 adults. In the total sample, female gender (124; 62%) was dominant over male gender (76; 38%) (p <0.0007). The dominance of the female gender was also manifested in adults, where women comprised 56, 56% compared to men (44; 44%) (p> 0,230) and also in children (68; 68%) compared to male (32, 32%) (p <0.0003). The average age of adult subjects was 44.4 years, and of children 7.3 years.

In all subjects, the average leukocyte count was 9.506 (x 109 / L). The maximum value for leukocytes was 30.6 (x 109 / L). For neutrophils, the average value was 4,818 (x 109 / L). As for erythrocyte sedimentation, the mean value was 30/51. The maximum CRP value measured was 212 (mg / L). The lowest standard deviation value was observed in neutrophil values (2,692). This means that the least variations were observed in these parameters, while in all other parameters a fairly high standard deviation or concentration variability was observed. Table 1 shows the results of statistical processing of total data.

Table 2. Results of statistical processing of total data of children and adults

Parameter	Total (children and adults)			
	Leukocytes	Neutrophils	Erythrocyte sedimentation	
			1h	2h
Mean value	9,506	4,818	30	51
Minimum value	3,2	1,5	1	3
Maximum value	30,6	11,7	130	128
Standard deviation	4,485	2,692	24,315	30,350
Coefficient of variation %	47,19	55,87	80,67	59,62
Percentage of people with elevated values	33%	26%	95%	81%

Based on statistical analysis, the mean value of leukocyte counts in children is higher in relation to the same value in adults. This parameter varied more in children (46.91%) than in adults (45.68%). The mean number of neutrophils in children is also higher in relation to the same value in adults. This parameter varied more in adults (58.87%) than in children (51.40%). However, the mean values of erythrocyte sedimentation, after the first and second hours in adult subjects, are higher than in children. Coefficients of variation of this parameter after the first hour are higher in adult subjects, while after the second hour they appear to be higher in children (Table 2).

Table 2. Comparative results of statistical data processing of children and adults

Parameter	Leukocytes		Neutrophils		Erythrocyte sedimentation				CRP
					1hour		2hour		children -
Age group	Children	Adults	Children	Adults	children	adults	children	adults	
Number of respondents	100	100	100	100	100	100	100	100	100
X min	3,5	3,2	1,2	1,5	3	1	5	3	4,14
X max	29	30,6	11,7	11,2	102	130	127	128	203,2
Mean value	10,249	8,762	5,349	4,287	27	33	47	55	35,477
Standard deviation	4,808	4,002	2,749	2,524	21,334	26,599	29,319	30,859	45,090
Coefficient of variation %	46,91	45,68	51,40	58,87	61,95	79,93	67,15	56,31	127,09
Percentage of people with elevated values	41%	25%	33%	18%	96%	85%	76%	60%	62%
T test	-2,36		-2,83		1,832		1,820		0,394
P value	0,0190		0,005		0,068		0,070		0,693

Of our greatest interest was to conclude that there is a certain correlation between the concentration of leukocytes and neutrophils; leukocytes and CRPs; leukocytes and erythrocyte sedimentation after 1h and 2h, and leukocytes, neutrophils and CRPs in elderly subjects. Table 3 shows the results of the correlation between determined parameters in adult respondents. Statistical analysis has shown a positive correlation between leukocyte / neutrophil counts; leukocytes / CRP, leukocytes / erythrocyte sedimentation after 1h and 2h (0.782, 0.225, 0.091, 0.090 in sequences), which means that in the case of leukocyte growth, these parameters increase. In the case of correlation between leukocyte / neutrophil and leukocyte / CRP values, the correlation is statistically significant (p <0.000, p <0.025). The obtained values of correlations leukocyte / erythrocyte sedimentation after the 1st and 2nd hour do not represent statistically significant results (P> 0.370, p> 0.371).

Table 3. Statistical correlation of leukocyte counts / neutrophils; Leukocyte / CRP, leukocyte / erythrocyte sedimentation after the 1st and 2nd hour in adults

Group of adult respondents				
Parameter	Leukocytes	Neutrophils	Erythrocyte sedimentation (1h)	Erythrocyte sedimentation (2h)
Mean value	8,762	4,287	33	55
	Pearson correlation		P value	
Leukocytes / Neutrophils	0,782		0,000	
Leukocytes / CRP	0,225		0,025	
Leukocytes / Erythrocyte sedimentation (1h)	0,091		0,370	
Leukocytes / Erythrocyte sedimentation (2h)	0,090		0,371	
	Partial correlation		P value	
Leukocytes/ Neutrophils /CRP	0,180		0,030	

Table 4 shows the correlation between the parameters in children. Statistical analysis has shown to have had a positive correlation between leukocyte / neutrophil counts; leukocytes / CRP, leukocyte / erythrocyte sedimentation after 1h and 2h (0.784; 0.377; 0.267; 0.320 in the order), which means that in the case of leukocyte growth, these parameters increase. In the case of correlation analysis between leukocyte / neutrophil count, leukocyte / CRP and leukocyte / erythrocyte sedimentation after the 1st and 2nd hour correlation was statistically significant (p <0,000; p <0,0001; p <0,007; p <0,001).

Table 4. Statistical correlation of leukocyte / neutrophil counts; Leukocyte / CRP, leukocyte / erythrocyte sedimentation after 1h and 2h in children

Group of children respondents				
Parameter	Leukocytes	Neutrophils	Erythrocyte sedimentation (1h)	Erythrocyte sedimentation (2h)
Mean value	10,249	5,349	27	47
	Pearson correlation		P value	
Leukocytes / Neutrophils	0,784		0,000	
Leukocytes / CRP	0,377		0,0001	
Leukocytes / Erythrocyte sedimentation(1h)	0,267		0,007	
Leukocytes / Erythrocyte sedimentation(2h)	0,320		0,001	
	Partial correlation		P value	
Leukocytes / Neutrophils /CRP	0,442		0,000	

DISCUSSION

This study has shown that the value of erythrocyte sedimentation statistically significantly increases with age. This fact was confirmed by the studies of Osei-Bimpong and Sar. (2007) who conducted a study on 295 blood samples from men and women who had not been hospitalized six weeks before the study, nor did their family doctor find any pathological symptoms. Respondents were divided into two groups, those below and over 40 years of age. This study confirms that after the age of 40 years there is an age-related increase in erythrocyte sedimentation, which is constantly increasing, especially after the age of 60 years. CRP also affects age, but to a lesser extent (9). CRP variation is quite large, but it is insignificant in children (127.09%), compared to adults (127.07%). The high variation of this parameter is confirmed by the study (Mohammed A. and Sar., 2004) where it is stated that CRP is normally present in healthy serum concentrations less than 5 mg / L, but grows rapidly in response to infections or inflammatory conditions when it can reach a concentration a 1000 times greater than basal (10).

Our research has also confirmed that there is a positive correlation between the value of leukocytes, neutrophils, CRP and acute inflammation. A study conducted in Benzage (Libya) at Al Fateh Teaching Hospital in the period from October 2001 to May 2002 included 216 patients with a suspicion of acute appendicitis. Blood samples for CRP analysis, leukocytes and neutrophil counts were immediately sent to the laboratory. There were 139 male and 77 female patients at the age of 3 to 13 years. Those with

appendicitis had 114 patients with CRP values greater than (8 μg / ml) and 109 patients had a leukocyte count greater than (11,000 / μL) and 111 patients with a neutrophil rate of more than 75 %. 112 of 130 patients with acute appendicitis had all three positive tests and only 8 out of 86 patients who did not have acute appendicitis had all three positive tests. This study is consistent with other authors' research that shows that clinical evaluation of clinical signs and symptoms combined with the analysis of three inflammatory parameters can improve diagnostic precision and significantly reduce the perforation frequency and the rate of negative laparotomy (10).

Correlation of these three parameters was also investigated by other authors (Xharra, S. et al., 2012) on the example of acute appendicitis. The study was conducted in 173 patients who were surgically treated for acute appendicitis. Leukocytes, neutrophils, and CRPs are randomly collected preoperatively from all patients involved. In those with positive appendicitis, CRP and leukocyte values were elevated in 126 patients (72.8%), and neutrophils percentage was greater than 75% in 117 patients (67.6%). Of 106 patients with triple positive inflammatory parameters, 101 (95.2%) had acute appendicitis. The elevated CRP value was directly related to the severity of inflammation (p-value <0.05). CRP monitoring increases diagnostic accuracy of acute appendicitis. Diagnostic accuracy of CRP is not significantly higher than leukocytes and neutrophils. The combination of these three tests significantly increases accuracy (11). The correlation between CRP and erythrocyte sedimentation was investigated by professors at the Faculty of Medicine and Pharmacy at Ibn Zohr University Agadir and Hassan II University

Casablanca in Morocco (El Maataoui, A., 2015). The purpose of this study was to establish the correlation between these two parameters. A retrospective study was conducted. A total of 76 patients were selected from the hospital database. They all had a referral for CRP and erythrocyte sedimentation. Another important criterion for inclusion was taking blood samples for CRP and sedimentation on the same day and the same hour. Values of erythrocyte and CRP sedimentation correlated significantly with each other (Pearson r = 0.516, p <0.001). Multiple regression analyzes were performed to determine the combination of variables that now correlate with CRP and sedimentation values. There was a positive correlation of neutrophils and leukocytes with CRP (12).

What we also need to look at is the difference in positive values in children and adults, so we can notice that all analyzed parameters have a higher percentage of positive results in children compared to adults. It is also important to note that positive leukocyte and neutrophil counts in children and adults (41%, 25%, and 33%, 18%) were present in less than half of the subjects. In contrast, positive values of erythrocyte sedimentation in children and adults are present in a very high percentage (96%, 85% and 76% and 60%). Probes of positive CRP values in children and adults are present in more than half of the respondents (62% and 60%). The study conducted by Leila Unkila-Kallio et al. (1993) shows that leukocytes compared with CRP and erythrocyte sedimentation in children in the diagnosis of acute hematogenic osteomyelitis have the lowest probability value. Forty-four children aged 2 weeks to 14 years with bacteriologically confirmed acute hematogenic osteomyelitis were tested. Erythrocyte sedimentation was elevated (≥20 mm / h) initially in 92% of cases; The mean value is 45 mm / h. CRP was elevated (> 19 mg / L) at the time of receiving in 98% of cases, mean value was 71 mg / L. The number of leukocytes was a poor indicator of acute hematogenic osteomyelitis, as only 35% of children had leukocytosis (leukocytes> 12 x 10^9 / L) at time of inoculation (13).

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