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Monocyte and neutrophil direct counts correlate with C-reactive protein plasma concentration in patients with acute pancreatitis

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Abstract: Acute pancreatitis (AP) is associated with the intensive inflammatory response in white blood cells (WBC) and C-reactive protein (CRP). This paper presents the relationship between the CRP plasma concentration and the direct counts of peripheral WBC in AP during the initial five days. The study consisted of 56 patients with AP, 36 patients with mild form of AP and 20 patients with severe form of AP. ABX VegaRetic hematological analyzer was used to perform the count of blood cells, and the immunonephelometric method was performed to measure the CRP concentration levels. AP patients presented with WBC count values in the range of 3.2 - 22.4 $\times$ $10^3/\mu$l and CRP concentration levels in the range 3.3 - 599.8 mg/l. The WBC count correlates with CRP levels during the entire observation period. The relationship of CRP and WBC is expressed in the following regression equation: WBC ($10^3/\mu$l) = 3.66 + 1.40 $\times$ $\log_e$CRP (mg/l). The highest median neutrophil count (8.15 $\times$ $10^3/\mu$l) was observed on the first day. The count decreased to 5.27 $\times$ $10^3/\mu$l on the fifth day. The most substantial finding in this study involved the values found for the monocytes and CRP (r= 0.53; p<0.001). Day two and day three were the highest (r=0.59, p<0.001). On day two, the regression equation for this relationship is: Monocytes ($10^3/\mu$l) = -0.34 + 0.21 $\times$ $\log_e$CRP(mg/l). The correlation between direct monocyte count and plasma CRP concentration in AP reflect a CRP-dependent stimulation of IL-6 release from activated blood monocytes.

Keywords: Acute pancreatitis, white blood cells, inflammation

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1 Introduction

Mobilization of blood inflammatory cells and migration to the site of tissue damage is a crucial part of the inflammatory process. Proinflammatory cytokines such as interleukin 1 (IL-1β), interleukin 2 (IL-2) and interleukin 8 (IL-8), as well as inflammatory interleukin 6 (IL-6) are responsible for mobilization of bone marrow reserves of blood inflammatory cells and for liver synthesis of acute phase proteins [1, 2]. Acute pancreatitis (AP) is associated with intensive inflammatory response that is associated with a rapid increase in white blood cell (WBC) count. The increase in WBC count is considered to be an early sign of disease development. Thus, the WBC count is also considered a measure of severity in AP [3, 4].

Another indicator used to determine the diagnosis of inflammatory disease is C-reactive protein (CRP). Elevated CRP concentration in plasma is an important sign of inflammation development and yields a measure of inflammatory process intensity [5]. In AP, elevated CRP concentration levels at 48 hours after onset of the symptoms yields a measure of disease severity and risk of developing systemic inflammatory response. [6]. Since CRP synthesis by the liver depends mostly on IL-6 stimulation, CRP concentration is considered as the surrogate indicator. Furthermore, compared to the direct plasma IL-6 measurement, the CRP is easier and less expensive to perform [7]. Through the effects of the same stimuli, both the WBC counts and the CRP plasma concentration levels increase [8]. Phagocytes, which infiltrate the pancreas, are responsible for local tissue damage and further extension of the inflammatory state [9]. On the other hand, CRP protects the tissues surrounding the inflammatory site and participates in harmonization of cellular mechanisms of the inflammatory reaction [10]. Therefore, the increase in the WBC count and CRP concentration are considered the complementary and sensitive markers of developing AP [11]. However, while CRP is a well defined biochemical entity, the WBC count value consists of the combined counts of five cellular populations differing in reactions to inflammatory stimuli. In our report we present studies on the relationship among CRP plasma concentration and the direct counts of individual populations of peripheral WBC in the initial phase of AP.

2 Statistical methods and Experimental Procedures

The experimental group consists of 56 patients admitted to the Second Department of General Surgery, Collegium Medicum Jagiellonian University of Krakow and diagnosed with AP. Of the 56 patients, 21 were females and 35 were males, and fell into the age range of 22 to 92 years (mean: 52.8 ± 16.3). All patients in the experimental group entered the hospital within 48 hours following the onset of acute disease symptoms. The diagnosis of AP was based on clinical symptoms, such as abdominal pain, nausea and vomiting, and with elevated serum amylase activity, which were at least three times above the upper reference limit. The clinical status of each patient was evaluated using Ranson and Glasgow prognostic scores. In addition, ultrasound examination of the abdomen was

conducted, which revealed an enlargement of the pancreas and decreased echogeneity in each patient. Patients with suspected pancreatic necrosis were diagnosed using contrast enhanced abdominal tomography. The severity of AP and associated complications were evaluated based on the Atlanta Classification. The final experimental group consisted of 36 (64,3%) patients diagnosed with mild AP and 20 (35,7%) patients with severe AP. Among the 20 patients with severe AP, 7 (12,5%) developed organ failure and 13 (23,2%) experienced local complications, such as necrosis (n=9), pseudocyst(n=3) and abscess (n=1). Ranson score was above 3 in 10 (17%) patients and Glasgow score was above 3 in 9 (16%) patients.

The control group was composed of 30 healthy persons (15 females and 15 males, age 36.6 ± 10.3 years).

The study was approved by the Bioethics Committee of Jagiellonian University. All patients in this study signed an informed consent for their participation.

Venous blood samples for laboratory tests were collected in Becton Dickinson Vacutainer tubes (Beckton Dickinson –USA) from each patient upon admittance and during the period of five consecutive days.

CRP concentrations were measured in serum by the immunonephelometric method with a Behring 100 Nephelometer (Dade-Behring Diagnostics Inc. Westwood, MA, USA). The detection limit was 0.16 mg/l with the reference values 0-3.3 mg/l.

Blood for hematological examination was collected in Vacutainer tubes with $EDTAK_2$ solution. Total erythrocyte count and erythrocyte indices, platelet count, and direct and differential counts of neutrophils, lymphocytes, eosinophils, basophils and monocytes were measured with ABX VegaRetic hematological analyzer using the 5-diff leukocyte differentiation system. The results of the automated blood counting were validated by systematic daily quality control using EightCheck-3WP, ICN blood control samples covering "normal," "high" and "low" ranges.

All normal distributed variables are denoted with "mean ±SD." Variables with non-normal distribution are represented with median and range values. The Mann-Whitney U-test was used to assess differences between groups. The Spearman coefficient was used to assess correlations between variables. *P level* <0.05 was considered statistically significant. Statistical analysis was performed with Statistica 6.0 (StatSoft Inc., Tulsa, USA).

3 Results

The total WBC count values for patients with AP were in the wide range of $3.20 \times 10^3/\mu$l to $22.40 \times 10^3/\mu$l during the entire observation period (Table 1). The total WBC count significantly correlated with CRP plasma levels on all five days of observation (Table 2) the regression equation to express this relationship is: WBC $(10^3/\mu\text{l}) = 3.66 + 1.40 \times \log_e$CRP (mg/l) (Figure 1).

Neutrophils, which typically comprise the largest fraction of WBC, tend to follow the total WBC count changes. The highest median neutrophil direct count value was

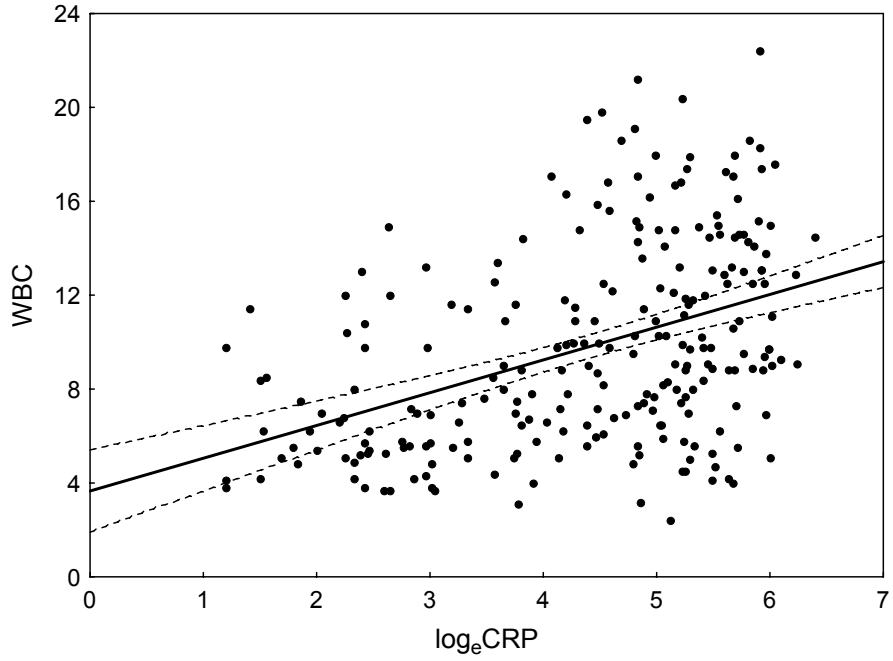

Fig. 1 Regression line for the relationship between WBC count and CRP plasma concentration ($\log_e$-transformed) in AP patients (data from the 5-day observation period). The regression equation: WBC ($\times 10^3/\mu$l) = 3.66 + 1.40 $\times$ $\log_e$CRP (mg/l).

observed on the first day of disease (median 8.15 $\times$ $10^3/\mu$l; range 1.50 - 16.20 $\times$ $10^3/\mu$l). Following day one, the neutrophil direct count steadily decreased to the median value 4.46 $\times$ $10^3/\mu$l on day four of the disease (Table 1). The scatter range remained constant during the entire studied period (Figure 2). The direct neutrophil counts correlate significantly with CRP levels on days two through four (Table 2), with the strongest correlation on day four (Figure 3).

The strongest correlations with CRP levels were observed for monocytes. Direct monocyte counts in the initial days of AP are significantly higher than those in healthy individuals. The scatter range of the monocyte counts increased on the days following admittance (Table 1). Significant correlations between CRP level and the direct monocyte count were observed during the entire observation period and were strongest on days two and three (r= 0.68, p<0.0001) (Table 2). This relationship became apparent on the second day with the following regression equation: Monocytes ($10^3/\mu$l) = -0.34 + 0.21 $\times$ $\log_e$CRP (mg/l) (Figure 4). A correlation between monocyte and neutrophil counts was also observed (Table 3).

The lymphocyte counts in AP are illustrated in Table 1. Although patients with AP had generally lower median direct lymphocyte count values than those in healthy individuals, no significant trends of changes were observed in the studied days.

The neutrophil – lymphocyte (N/L) and neutrophil – monocyte (N/M) ratios in patients of experimental group are also illustrated in Table 1. On the first day after admittance, both N/L and N/M ratio were high and decreased steadily on the days following.

Table 1 WBC counts in control group and experimental group. Control group consists of healthy persons. Experimental group consists of patients diagnosed with AP. The WBC count for the study group was taken over the period of five consecutive days after admittance to the Second Department of General Surgery, Collegium Medicum Jagiellonian University of Krakow. Data are expressed as median and range. Day one is the day of patient admittance.

Parameter studied	Patients group (n=56) Day 1	Day 2	Day 3	Day 4	Day 5	Control group (n=30)
Total WBC count ($10^3/\mu$l)	11.40 3.20-21.20	9.70 2.40-22.40	8.70 3.70-17.60	7.40 3.10-19.50	7.65 3.80-18.60	5.6 3.5-11.7
Neutrophils ($10^3/\mu$l)	8.15 1.50-16.20	6.10 1.12-16.30	5.86 2.13-15.69	4.46 1.85-13.10	5.27 2.21-14.00	3.26 2.95-6.51
Lymphocytes ($10^3/\mu$l)	1.10 0.39-3.27	1.15 0.22-2.61	1.25 0.32-3.24	1.11 0.49-2.63	1.12 0.64-2.28	2.20 2.00-2.92
Monocytes ($10^3/\mu$l)	0.75 0.25-1.56	0.54 0.20-1.40	0.57 0.18-2.12	0.52 0.17-2.54	0.58 0.13-2.05	0.40 0.35-0.57
Neutrophil – lymphocyte ratio	7.47 2.99-40.50	5.16 1.73-26.53	4.64 1.09-18.31	4.09 1.23-18.33	4.43 1.74-12.44	1.50 1.03-2.95
Neutrophil – monocyte ratio	12.03 3.18-31.15	11.00 1.11-32.87	11.03 2.59-33.70	10.00 2.52-41.15	8.05 3.33-23.94	8.77 6.40-13.85
CRP (mg/l)	94.45 <3.3-328.9	182.10 11.3-440.0	148.45 10.3-599.8	90.4 5.4-398.4	61.4 <3.3-506.5	<3.3

Table 2 Correlations of total WBC counts with CRP plasma levels in AP patients.

Day of the observation	Spearman correlation coefficient r WBC counts	neutrophil direct counts	monocyte direct counts	N/L ratio	N/M ratio
1	0.35 (p<0.05)	NS	0.43 (p<0.05)	NS	NS
2	0.43 (p<0.01)	0.47 (p<0.01)	0.68 (p<0.0001)	NS	NS
3	0.48 (p<0.001)	0.56 (p<0.001)	0.68 (p<0.0001)	0,49 (p<0.01)	NS
4	0.54 (p<0.0001)	0.67 (p<0.0001)	0.59 (p<0.001)	0.58 (p<0.001)	NS
5	0.59 (p<0.0001)	0.58 (p<0.001)	0.66 (p<0.001)	0.46 (p<0.05)	-0.40 (p<0.05)

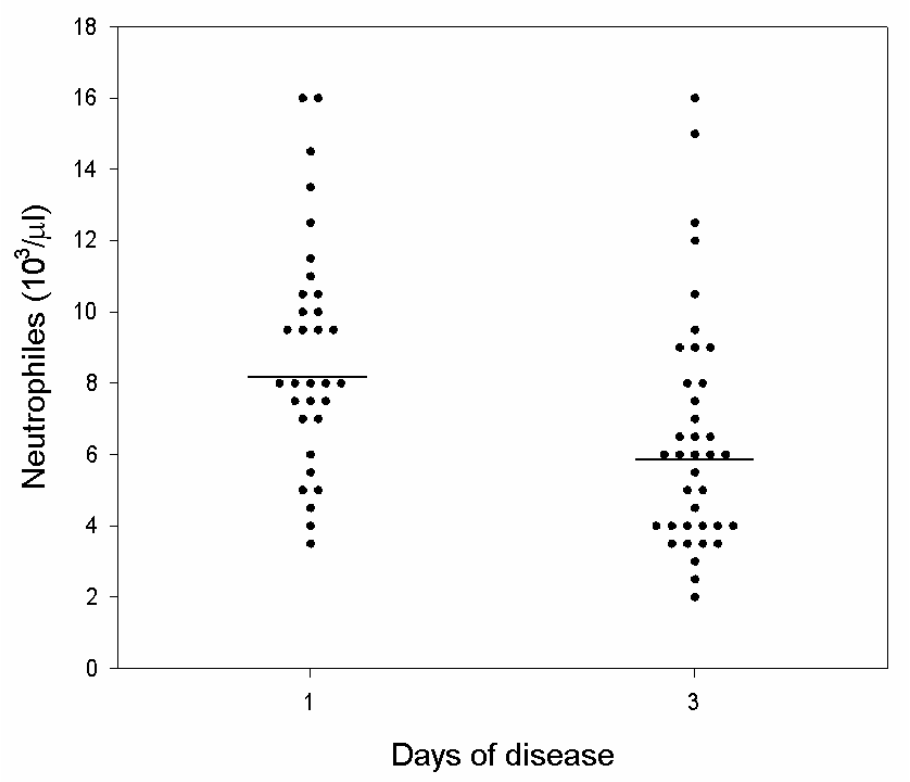

Fig. 2 The distribution of neutrophil direct count in AP patients on the 1^{st} and 3^{rd} day after hospital admission.

Table 3 Monocyte-neutrophil correlations in AP patients. Spearman correlation coefficient (r) and *p level*.

	Day 1	Day 2	Day 3	Day 4	Day 5
r	0.46 (p<0.01)	0.60 (p<0.001)	0.38 (p<0.05)	0.54 (p<0.01)	0.71 (p<0.001)

The N/L ratio differed significantly between AP patients and control subjects, but N/M ratio differed only in days one through three (Table 1). N/L ratio correlates with CRP concentration on days three through five (Table 2). There is a reverse correlation between N/M and CRP concentration on day five (Table 2).

No significant correlations between the lymphocyte, eosinophil and basophil direct counts and CRP were found.

4 Discussion

There is a well-known interrelation between the CRP plasma concentration and the WBC count [3, 12]. Our study confirms a significant, strong correlation between the total WBC count and CRP plasma concentrations in AP patients during the entire five days of observation (Table 2). The most pronounced correlation was found for CRP and

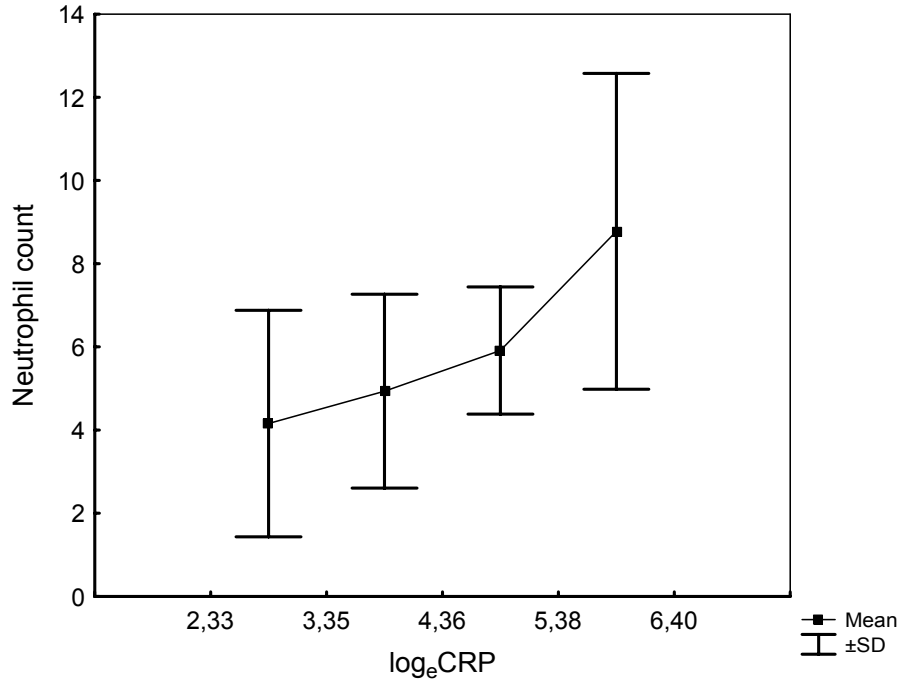

Fig. 3 Relationship between neutrophil count and CRP plasma concentration ($\log_e$-transformed) in AP patients on the 4^{th} day after admission.

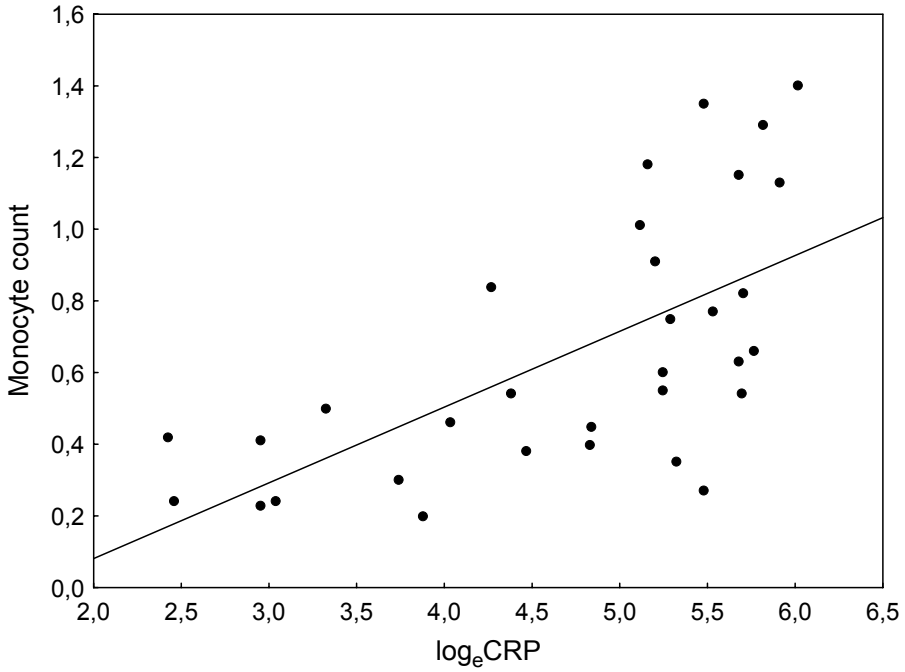

Fig. 4 Regression line for the relationship between monocyte count and CRP plasma concentration ($\log_e$-transformed) in AP patients on the 2^{nd} day after admission. The regression equation: Monocytes ($\times$ $10^3/\mu$l) = -0.34 + 0.21 $\times$ $\log_e$CRP (mg/l).

monocyte direct counts (reaching r=0.68 on days two and three) (Table 2), while CRP – neutrophil correlations were less pronounced.

Relationship between the direct monocyte count and CRP plasma concentration levels in AP has not yet been studied. There is an interrelation between CRP stimulation and IL-6 release from activated blood monocytes [13, 14]. In this study, approximately a two-fold increase in direct monocyte count in the initial phase of acute pancreatitis correlated with increased CRP plasma concentrations. This relationship reflects mutual functional interdependence between monocytes and CRP in inflammation development. CRP directly interacts with blood mononuclears and plays a regulatory role in the inflammatory process. CRP specific receptors are most probably located on monocytes and neutrophils [15]. There are data indicating that CRP directly induces the expression of adhesion molecules and release proinflammatory cytokines by endothelial cells [10] as well as by cultured human monocytes [14, 15]. As shown in the recent studies, monocytes from healthy subjects stimulated by CRP significantly increase IL-6 output in a dose-dependent manner [16]. This effect was associated with elevated baselines of proinflammatory cytokines, which may cooperate with CRP influence on peripheral blood monocytes [17]. Thus, CRP can enhance the release of IL-6 which further stimulates the mobilization of bone marrow neutrophil progenitors. This manifests as an increase in the direct neutrophil count in acute inflammation or post surgery trauma [9].

The observed correlation between the WBC count and CRP concentration level may be explained by the fact that neutrophils comprise an overwhelming part of total WBC count, accounting for 60-90% of all WBC in AP. Both WBC and CRP are considered as indirect markers of generalized body response to inflammatory cytokine stimulation [9]. The majority of AP clinical scoring systems employ WBC count value (upon admittance and after 48 hours) as a predictor of severity [18]. As a matter of fact, both IL-6 and CRP concentration in plasma significantly correlate with pancreatic ribonuclease activity, which is a direct marker of pancreatic tissue damage [19]. Recently, Hamalainen et al [3]. have shown that the WBC count increases earlier than CRP in AP. However, if both the WBC count and the CRP plasma concentration are within the normal range, the development of life threatening clinical course of AP can be reduced.

The activation of neutrophils is secondary to stimulation of blood monocytes, which release the proinflammatory cytokines such as IL-6, IL-1β, TNFα and IL-8 [2, 13, 14] (Figure 5). Thus, the relationship between CRP and the neutrophil direct counts observed in our studies may reflect the mutual functional interdependence between neutrophils, tissue macrophages and blood monocytes [9, 20] (Figure 5). IL-6 enhances CRP synthesis in hepatocytes while the CRP stimulates mononocytes for the synthesis and release of IL-6 and IL-8, which in turn stimulate neutrophils for migration and phagocytosis in the inflammatory site [21–23] (Figure 5). Therefore, CRP plays a role that is auxiliary to cytokines in function coordination of various inflammatory cells during inflammation development.

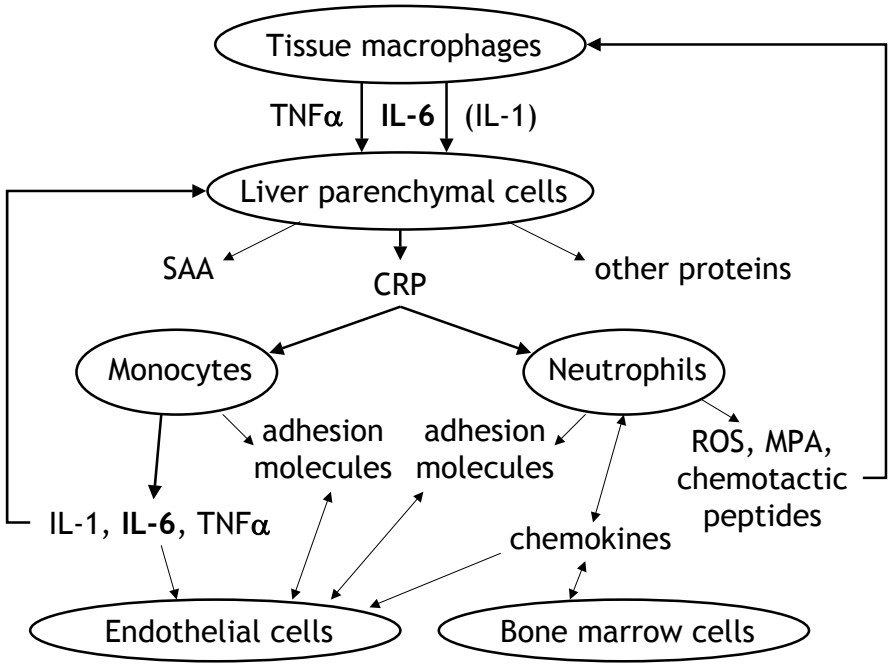

Fig. 5 Hypothetic scheme of CRP-dependent stimulation of monocytes in AP. Abbreviations used: CRP – C-reactive protein, IL – interleukin, TNFα – tissue necrosis facor alpha, SAA – serum amyloid A, ROS – reactive oxygen species, MPA – myeloperoxidase.

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