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CYTOKINES AND C-REACTIVE PROTEIN-TRIGGER OF IMBALANCE OF THE HEMOSTASIS SYSTEM DURING INFLAMMATION

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Recent studies have confirmed hypothesis about crucial role of cytokines and C-reactive protein (CRP) in progression of inflammation (C.M. Ballantyne et al., 2004; B. Paimany, 2002; J.W. Steinke, 2006; J. Volanakis, 2001). Cytokines are pluripotent short-distant molecules that are synthesized by activated cells of immune system. CRP has been evaluated as «gold marker» of inflammation and predictor of various pathological states. It has been proved experimentally that pro-inflammatory cytokines such as IL-1, IL-6, IL-12, IL-3, TNF-α realized their effects through direct stimulation of CRP expression (B. Paimany, 2002). On the other hand, reaction of inflammation often results in disorders of hemostasis system. Nowadays, cytokines and CRP are considered as highly relevant factors which trigger both inflammation and hypercoagulation (T. van der Poll, et al., 2011). Nevertheless, mechanisms of the interplay between cytokines and CRP, triggering inflammation reaction and factors of hemostasis, are still unclear and require following investigations.

The aim of the research was to study pro-inflammatory or anti-inflammatory links of cytokines' network and CRP concentration as well as parameters of hemostasis system in conditions during inflammatory process.

Materials and methods. For the research, blood samples of patients with acute inflammation (paratonsillar abscess, n=25) were used; control cohort was represented by healthy people (without acute or chronic inflammation, n=20). Parameters of hemostasis system such as fibrinogen concentration, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR) and activity of antithrombin III (AT III) have been defined in blood plasma with the help of standard kits by routine methods. Concentrations of cytokines as IL-1β, IL-4, IL-6, IL-1RA and TNF-α have been determined in blood sera by the immunoenzyme method. CRP concentration has been defined by the turbid metric method. Statistical processing of data has been used for figures assessment (STATISTICA 10.0, Excel for Windows 10).

Results and discussion. It has been found out that in blood sera of patients with paratonsillar abscess concentrations of pro-inflammatory or anti-inflammatory cytokines as well as CRP were elevated in comparison with control cohort and also results from acute inflammation process. Investigated parameters of hemostasis system have demonstrated signs of hypercoagulation in patients with paratonsillar abscess. Concentration of fibrinogen has been elevated, APTT and PT has been shortened down, and INR has been reduced ($p<0.05$). Elevations in fibrinogen levels are associated with an increased risk of thrombotic disease. Activation of PT in inflammation process can be regarded as evidence that cytokines are involved into activation of extrinsic mechanism of thrombin generation. Shortening of APTT has revealed that cytokines may contribute into hypercoagulation by activation of intrinsic pathway. Suppressed activity of AT III can be explained by the ability of inflammatory cytokines to decrease concentration of heparin-like molecules (M. Levi et al., 2003) which are natural cofactor of AT III and, thus, results in delayed inhibition of coagulation enzymes that is favorable for intravascular coagulation.

Conclusions and prospects for further investigation. The results of our research have confirmed that elevated levels of cytokines such as IL-1β, IL-4, IL-1RA, IL-6 and TNF-α as well as CRP in inflammation are associated with imbalance of hemostasis system: Increased concentration of fibrinogen, shortening of APTT and PT, reduced INR are markers of amplification of coagulation cascade. Decreased activity of AT III sustains the suppression of anticoagulant system, and probably results from low regulation or degradation of heparin-like cofactor molecules of AT III by cytokines. These disorders of hemostasis system can be complicated by risk of thrombosis and disseminated intravascular coagulation in patients with paratonsillar abscess. Elevated concentrations of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α as well as CRP associated with hypercoagulation may be used as predictors of DIC syndrome risk in patients with systemic inflammation.

Keywords: inflammation; cytokines' network; interleukins IL-1β, IL-4, IL-6, IL-1RA, TNF-α; C-reactive protein; hemostasis.

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Actuality: Recent studies have confirmed hypothesis about crucial role of cytokines and C-reactive protein (CRP) in progression of inflammation [2, 12, 16, 20]. Cytokines are pluripotent short-distant molecules that are synthesized by activated cells of immune system. They mediate intercellular communications as well as stimulation or inhibition of cell growth, their differentiation, functional activity and apoptosis, etc. [1, 16]. In cell culture inflammatory cytokines, especially tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), are major mediators that can elicit changes in cell phenotype [4]. CRP has been evaluated as «gold marker» of inflammation [13] and predictor of various pathological states such as myocardial infarction, acute renal and cardiac insufficiency, acute coronary syndrome, sepsis, neoplasia of different localizations [16]. It has been proved experimentally that pro-inflammatory cytokines such IL-1β, IL-6, IL-12, IL-3, TNF-α realized their effects through direct stimulation of CRP expression [12, 14]. On the other hand, reaction of inflammation, accompanying appearance of foreign antigens in the organism (independently from their origin: whether these are bacteria and viruses, or self-cells malignization), often results in disorders of hemostasis system. Severe infections and inflammation almost invariably lead to hemostatic abnormalities, ranging from insignificant laboratory changes to severe disseminated intravascular coagulation (DIC) [8, 15, 17, 19]. Imbalance develops due to the extra activation of coagulation mechanisms with simultaneous regulation of anticoagulant pathways and suppression of fibrinolysis [7, 9, 18]. Nowadays, cytokines and CRP are considered as highly relevant factors which trigger both inflammation and hypercoagulation [19]. Nevertheless, mechanisms of the interplay between cytokines and CRP, triggering inflammation reaction and factors of haemostasis, are still unclear and require following investigations.

The aim of our research was to study of pro-inflammatory and anti-inflammatory links of cytokines' network and CRP concentration as well as parameters of hemostasis system in conditions of inflammation process.

Materials and methods: For the research, blood samples of patients with acute inflammation (paratonsillar abscess, n=25) were used; control cohort was represented by healthy people (without acute or chronic inflammation, n=20). Parameters of hemostasis system such as fibrinogen concentration, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR) and activity of antithrombin III (AT III) have been defined in blood plasma with the help of standard kits («Renam», Russia) by routine methods.

Concentrations of cytokines such as IL-1β, IL-4, IL-6, IL-1RA and TNF-α have been determined in blood serum by the immunoenzyme method («Vector-best», Russia). CRP concentration has been defined by the turbidimetric method (kits «Vital Diagnostics», Russia). Statistical processing of data has been used for figures assessment (STATISTICA 10.0, Excel for Windows 10).

Results and the discussion: It has been found out that in blood sera of patients with paratonsillar abscess concentrations of both pools of cytokines as well as CRP were elevated in comparison with control cohort which therefore results from acute inflammation process (**table 1**).

Table 1 – Concentrations of Interleukins and CRP

Parameter	Control cohort, n=20	Acute inflammation, n=25
IL-1β, pg/L	4.37±1.84	12.41±2.88*
IL-6, pg/L	3.89±1.81	9.99±1.76*
TNF-α, pg/L	3.11±1.23	11.35±1.76*
IL-4, pg/L	7.04±2.66	17.45±2.45*
IL-1RA, pg/L	521.83±180.6	2330.8±605.0*
CRP, mg/L	2.78±0.38	5.55±0.63*

Note: * – $p<0.05$.

Thus, concentration of IL-1β has been increased in 2.8 folds, concentration of IL-6 has exceeded control values in 2.6 times; and concentration of TNF-α has been elevated in 3.6 times ($p<0.01$). Concentration of CRP has been increased in 2.0 folds ($p<0.01$). Concentrations of anti-inflammatory cytokines' pool have been increased as well. Yet, IL-4 concentration has rose in 2.5 folds, and IL-1RA content in blood sera has been elevated approximately in 4.5 times.

It has been noted that neutrophils and monocytes that migrate into the focus of inflammation as well as tissue macrophages are able to generate endogenous pyrogens. It is proved that IL-1, IL-6 and TNF-α are potential pyrogens [1, 16]. Progression of macrophagous reaction in the inflammatory focus leads to generating of highly immunogenic antigen determinants on the surface of macrophages' membranes, stimulation of T- and B-lymphocytes, and, eventually, synthesis of specific antibodies, elevation of their level in blood, activation of killer-effect and enhanced production of cytokines. Intercellular communications between mononuclear phagocytes and immunocompetent cells are mediated by cytokines release. Cytokines participate in the integration of immune system components as well as in the systemic reaction of

acute inflammation [2, 16]. One of the manifestations of acute phase of inflammation is the synthesis of specific proteins in liver such as CRP, α_1-antitrypsin, transferrin, C3 component of complement system, etc. Various cytokines (IL-1β, IL-6, IL-8, IL-11, TNF-α) are mediators which initiate synthesis of acute phase proteins [3, 16, 20]. Therefore, such changes of cytokines' profile in our research reflect compensatory-adaptive response of an organism by way of immune system activation in acute inflammation process. This is in accordance to other researchers' figures [1, 2, 8, 10, 14].

Investigated parameters of hemostasis system have demonstrated signs of hypercoagulation in patients with paratonsillar abscess (**table 2**).

Table 2 – Parameters of Coagulation

Parameter	Control cohort, n=20	Acute inflammation, n=25
Fibrinogen, g/L	3.15±0.43	5.11±0.64*
APTT, sec	46.89±4.98	38.70±2.99*
PT, sec	15.3±0.6	11.84±0.58*
INR	1.19±0.06	0.9±0.05*
AT III, per cent	100.8±6.7	77.6±4.5*

Note: * – p<0.05.

Concentration of fibrinogen has been elevated; it reached 5.11±0.64 g/L (p<0.01). Increase of fibrinogen concentration results from inflammation due to the fact that fibrinogen is an acute phase reactant [5]. Elevations in fibrinogen levels are associated with an increased risk of thrombotic disease.

Such parameters as APTT and PT have been shortened down; they have constituted correspondently 38.7±2.99 sec and 11.1±0.47 sec (p<0.05). INT has been reduced till 0.84±0.04 (p<0.05).

Due to the fact that PT and INR reflect events of extrinsic pathway of coagulation cascade, their activation in inflammation process can be regarded as evidence that cytokines are involved into activation of this mechanism. This is in concern with results of multiple researches that have demonstrated that tissue factor (TF), which is the most important initiator of the extrinsic coagulation cascade, belongs to class II cytokine receptor family. It is the cofactor for the activated plasma clotting factor VII (FVIIa) which catalyzes the activation of factor X and IX and leads to the generation of thrombin and thus, finally, of a fibrin clot. Under physiologic conditions TF is abundantly expressed only in the adventitia, nevertheless in many pathologic conditions its activation is induced by several inflammatory mediators such as IL-6, IL-1β and CRP [3, 10]. On the other hand, the expression of TF on monocytes and macrophages is markedly stimulated by the presence of activated platelets and granulocytes. The cellular interactions between these cells result in enhanced production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) [4, 7], so that, creates pathological positive feedback leading to severe hypercoagulation.

Shortening of APTT, which is the parameter for the assessment of intrinsic mechanism of coagulation cascade, has reviled that cytokines may contribute into hypercoagulation by activation of this pathway as well. In accordance to scientific figures, inflammatory mediators presumably increase the number of microparticles in circulation, i.e. phospholipids for prothrombinase complex generation, through leucocyte activation, so that they can lead to factor XII activation and involvement of intrinsic mechanism of coagulation through kallikrein-kinin system. These events can contribute to vessels injury, vasodilation in a variety disease including sepsis [4, 6].

Meanwhile, our research has shown that activity of AT III has been reduced to 77.6±4.5% (p<0.05). Suppressed activity of AT III can be explained by the experimental data *in vitro* that reviled ability of inflammatory cytokines and neutrophil activation products to decrease concentration of heparin-like molecules [6, 8] which are natural cofactor of AT III. In human severe sepsis, these heparin-like molecules have been shown to be down-regulated or degraded, further diminishing the activity of natural anticoagulants [6, 11]. Antithrombin inhibitory activity markedly decreases during severe sepsis, often to less than 50 per cent of normal levels [7, 8]. Decreased AT III concentrations result in approximately proportional decreases in the rates of inhibition of target proteases that mediate activation of endothelial receptors with following adhesion of leukocytes and platelets. Thus, this decrease in antithrombin concentration results in delayed inhibition of coagulation enzymes that favor intravascular coagulation [11].

Conclusions and prospects for further investigation: The results of our research have confirmed that elevated levels of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α as well as CRP in inflammation are associated with imbalance of hemostasis system:

1. Increased concentration of fibrinogen, shortening of APTT and PT, reduced INR are markers of amplification of coagulation cascade.
2. Decreased activity of AT III sustains the suppression of anticoagulant system, and probably results from low regulation or degradation of heparin-like cofactor molecules of AT III by cytokines.
3. These disorders of hemostasis system can be complicated by risk of thrombosis and DIC in patients with paratonsillar abscess.
4. Elevated concentrations of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α as well as CRP associated with hypercoagulation may be used as predictors of DIC syndrome risk in patients with systemic inflammation.

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ЦИТОКІНИ ТА С-РЕАКТИВНИЙ БІЛОК-ТРИГГЕРИ ДИСБАЛАНСУ СИСТЕМИ ГЕМОСТАЗУ ПРИ ЗАПАЛЕННІ

Кармазіна І. С., Аль-Ріхані Х.

Резюме. У статті аналізуються результати дослідження впливу цитокінової мережі та С-реактивного білку (СРБ) на дисбаланс системи гемостазу при запальному процесі на моделі паратонзилярного абсцесу. У 25 зразках сироватки крові хворих на паратонзилярний абсцес досліджено вміст цитокінів ІЛ-1β, ІЛ-4, ІЛ-6, ІЛ-1РА, ФНП-α та СРБ; у зразках плазми крові визначали показники коагулограми: концентрацію ЗФ, АЧТЧ, ПЧ, МНВ та активність АТ III. Виявлено достовірне підвищення вмісту як прозапальних, так і протизапальних цитокінів, а також СРБ у сироватках крові хворих на паратонзилярний абсцес, що відображає компенсаторно-адаптаційну реакцію організму у відповідь на запалення. Результати дослідження показали підвищення ЗФ, скорочення АЧТЧ та ПЧ, зменшення МНВ та зниження активності АТ III. Таким чином, одержані дані підтверджують, що цитокіни та СРБ можуть спричиняти дисбаланс системи гемостазу через активацію прокоагулянтної ланки з одночасним пригніченням антикоагулянтних механізмів при запальному процесі, що може ускладнювати перебіг хвороби та супроводжуватися ризиком розвитку

тромбозів та ДВЗ-синдрому. Збільшення концентрацій прозапальних цитокінів ІЛ-1β, ІЛ-6, ФНП-α, а також СРБ, асоційовані з гіперкоагуляцією можуть використовуватися у якості предикторів розвитку ДВС синдрому у пацієнтів з системним запаленням.

Ключові слова: запалення; цитокінова мережа; інтерлейкіни ІЛ-1β, ІЛ-4, ІЛ-6, ІЛ-1РА, ФНП-α; С-реактивний білок; система гемостазу.

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ЦИТОКИНЫ И С-РЕАКТИВНЫЙ БЕЛОК-ТРИГГЕРЫ ДИСБАЛАНСА СИСТЕМЫ ГЕМОСТАЗА ПРИ ВОСПАЛЕНИИ

Кармазина И. С., Аль-Рихани Х.

Резюме. В статье анализируются результаты исследования влияния цитокиновой сети и С-реактивного белка (СРБ) на дисбаланс системы гемостаза при воспалительном процессе на модели паратонзиллярного абсцесса. В 25 образцах сыворотки крови больных с паратонзиллярным абсцессом исследовано содержание цитокинов ИЛ-1β, ИЛ-4, ИЛ-6, ИЛ-1РА, ФНО-α и СРБ; в образцах плазмы крови определяли концентрацию ОФ, АЧТВ, ПВ, МНО и активность АТ III. Обнаружено достоверное повешение содержания как провоспалительных, так и противовоспалительных цитокинов, а также СРБ в сыворотках крови больных с паратонзиллярным абсцессом, что отражает компенсоторно-адаптационную реакцию организма в ответ на воспаление. Результаты исследования показали повышение ОФ, укорочение АЧТВ и ПВ, уменьшение МНО и снижение активности АТ III. Таким образом, полученные данные подтверждают, что цитокины и СРБ могут вызывать дисбаланс системы гемостаза через активацию прокоагулянтного звена с одновременным угнетением атикоагулянтных механизмов при воспалительном процессе, что может сопровождаться риском развития тромбозов и ДВС-синдрома. Увеличение концентраций провоспалительных цитокинов ИЛ-1β, ИЛ-6, ФНО-α, а также СРБ, ассоциированные с гиперкоагуляцией могут быть использованы в качестве предикторов развития ДВС синдрома у пациентов с системным воспалением.

Ключевые слова: воспаление; цитокиновая сеть; интерлейкины ИЛ-1β, ИЛ-4, ИЛ-6, ИЛ-1РА, ФНО-α; С-реактивный белок; система гемостаза.

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