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Disfibrinogenemiya and Hidden Hemolysis - Indicators of Hemorrhagic Syndrome in Erysipelas

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Material and methods: the author studied the indices of external (time of prothrombin (PT), international normalized ratio(INR)) and internal (activated partial thromboplastin time (aPTT)) coagulation pathways, the degree of disfibrinogenemiya (thrombin time (TT), functional fibrinogen activity and D-dimer level), the amount and functional activity of the platelets (aggregation with adenosine diphosphate (ADP) and the erythrocytes (aggregation with lanthanoid (LaCl₃) and protamine sulfate(PS)) in 60 cases of erysipelas. The studied indices also included endothelial dysfunction – the decrease of athrombogenicity of vascular wall endothelium (antithrombin III (AT III) and protein C) and the increase of endothelial adhesive properties (von Willebrand factor(vWf)).

Keywords: facial erysipelas, lower-limb erysipelas, hidden hemolysis, DIC-like syndrome, erythrocyte aggregation, platelet aggregation, fibrinogen, D-dimer, protein C, antithrombin - III, von Willebrand factor, haptoglobin, β – hemolytic streptococcus (β -HS).

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DISFIBRINOGENEMIYAANDHIDDENHEMOLYSISINDICATORSOFHEMORRHAGICSYNDROMEINERYSIPELAS

Strictly as per the compliance and regulations of:



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The comparison groups included normal volunteers (n=32) and patients with inflammation localized on the face (n=24), and the legs (n=36) at various stages of the disease (day 1–3; 4-6; 7-10; and 11-15 from the onset of the disease). These patients underwent in-hospital treatment in 2nd Moscow Clinical Hospital for the Infectious Diseases.

Results and discussion: The thesis, according to which the rate of hemorrhagic complications in lower limb erysipelas (LLE) is by 3,9 times higher than in facial erysipelas (FE), was confirmed by laboratory findings. In particular, a significant decrease of protein C was noted in patients with LLE and concomitant chronic venous insufficiency (CVI). We have found increased D-dimer and decreased α_2 -macroglobulin (α_2 MG) levels, suggesting potent activation of proteolytic enzymes (plasmin, matrix metalloproteinases and neutrophil elastase), which can be one of the causes of bullae, erosion and ulceration formation in the erysipelas focus on the lower limb.

The signs of intravascular (hidden) hemolysis - the decrease of haptoglobin concentration and the increase of indirect bilirubin and lactic dehydrogenase (LDH) blood levels; the changes of rheological properties of erythrocytes – the increase of deformability (aggregation with lanthanum chloride (LaCl₃)) and the decrease of elasticity (aggregation with protamine sulfate (PS)) – have been identified as one of the

main factors for disseminated intravascular coagulation (DIC)-like syndrome in erysipelas.

Keywords: facial erysipelas, lower-limb erysipelas, hidden hemolysis, DIC-like syndrome, erythrocyte aggregation, platelet aggregation, fibrinogen, D-dimer, protein C, antithrombin - III, von Willebrand factor, haptoglobin, β – hemolytic streptococcus (β -HS).

I. INTRODUCTION

To date, clinical research focuses on the study of the relation between the inflammation and the coagulation. The dysfunction of vascular endothelium, common for these two pathological processes, is an early pathophysiological sign and an independent predictor of unfavorable prognosis of most diseases. The prevention of endothelial dysfunction of the microcirculatory bed helps to prevent and to treat many diseases.

Erysipelas is a widely spread acute infectious disease. Its development does not depend on industrial development and social security in different countries. General and local predisposing factors form an important pathogenic aspect of this condition's development. Lower limb erysipelas (LLE) is often associated with obesity, type 2 diabetes mellitus, chronic venous insufficiency (CVI) and feet and nail mycoses [1, 2]. Facial erysipelas (FE) often develops in association with otomycosis and chronic ear, nose and throat (ENT) diseases [3].

Despite modern methods of treatment, up to 10% cases of erysipelas are complicated by skin necrosis at the sites of hemorrhages and vesicles formation, by venous incompetence (periphlebitis, phlebitis and thrombophlebitis). The frequency of hemorrhagic form of erysipelas increased [4, 5]. Hence, the study of the system of hemostasis and blood rheology during the developing infectious process in patients with lower limb and facial erysipelas is of thrilling importance.

a) Purpose of the work

The purpose of this work was to study the changes of hemostasis and of blood rheology during infective process in patients with lower limb and facial erysipelas and to validate the advisability of replacement and/or antithrombotic therapy.

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II. MATERIAL AND METHODS

a) Patient profiles

We have studied 60 patients aged 25 to 71 years. Thirty-six of them had 2nd degree lower limb erysipelas and 24 had 2nd degree facial erysipelas (24). 67% of patients had primary form of the disease. Erythematous form of erysipelas was found in 33% of cases (52% in facial erysipelas), erythematous-bullous in 15%, erythematous-hemorrhagic in 22%, and bullous-hemorrhagic in 30% of cases. Erythematous-hemorrhagic (n=11) and bullous-hemorrhagic (n=15) erysipelas affected the lower limb more frequently than the face (2 and 3 cases, respectively). The risk of hemorrhagic lesions was significantly higher in cases with local inflammatory process (78%) affecting the legs, than in face affection (20%); the odds ratio (OR) = 9,9 (2,8; 34,7).

Primary facial erysipelas diagnosed in 92% of cases was more common in women (16 women, 8 men). LLE was primary in 50% of cases, repeated in 31% and recurrent in 19% of cases. Facial erysipelas was primary in 92%, repeated in 4% and recurrent in 4% of cases. The risk of LLE recurrence was significantly higher than of the facial erysipelas (OR =5,55 (1; 51,2), p=0.009).

Gender ratio in LLE was almost equal (males-17, females - 19). Feet and nail mycosis onychomycosis were the most frequent (88%) concomitant diseases. Eleven patients had grade 2 to 4 obesity, 5 patients had subcompensated type 2 diabetes mellitus.

Skin diseases (retroaural dermatitis, streptoderma, psoriasis) were the underlying pathology in 37,5% of patients with facial erysipelas. 29% of these patients had chronic ENT diseases (otitis, tonsillitis and rhinitis). Four patients had type 2 diabetes mellitus.

The patients underwent in-hospital treatment in Erysipelas department of the 2nd Moscow Clinical Hospital for Infectious Diseases. Thirty-two patients received antibacterial monotherapy: intramuscularly (IM) Benzylpenicillin novocaine salt twice daily (1,2 mln ME per day) for 7-10 days. Another two patients received IM Cephazolin three times daily (3 g per day) for 5 days. Combined two-antibiotics therapy (IM Benzylpenicillin novocaine salt twice daily (1,2 mln ME per day) for 7-10 days and Ciprofloxacin twice daily per os (1 g per day) for 10 days) was used in 14 patients. Twelve patients got a three-antibiotics combination (IM Benzylpenicillin novocaine salt (1,2 mln ME per day) for 7 days + intravenously (IV) Ciprofloxacin (800 mg per day) for 3 days with subsequent passage to per os (1 g per day) for 10 days + IM Cephazolin three times daily (3 g per day) for 5 days). Additionally the patients received: antihistamine agents (Cetirizine dihydrochloride); topical physiotherapy (ultraviolet irradiation (UVI) therapy and low frequency current (LFC) for facial erysipelas; UVI therapy, LFC and magnetic therapy for lower limb

erysipelas). The focus of LLE erysipelas was regularly treated with tanning solution of potassium permanganate. The studied patients did not receive medications capable to influence their hemostasis' state.

Mean duration of in-hospital stay was 11,9 + 4,1 days for the patients with LLE and 8,4 + 1,6 days for the patients with FE.

The hemostasis indices were studied in the beginning of the disease (days 1-3) - study point 1, during the course of the disease (days 4-6; 7-10) - points 2 and 3, and during the recovery period (days 11-15) - study point 4. Every third patient with LLE underwent the follow-up examination (5 months after the discharge). It helped to differentiate between erysipelas-induced changes and the underlying concomitant diseases.

The control group comprised 32 normal subjects aged 24 to 50 years, with equal gender distribution.

b) Statistical analysis

The results were processed on Statistica 10.0 for Windows 7.0 software. The statistically significant (reliable) differences between the groups was studied using: Mann-Whitney test for quantitative values, two independent groups; Kruskal-Wallis test for more than two independent groups; Wilcoxon test for quantitative values, related groups (pre-and post-therapy, in dynamics); chi-square test, and, if necessary, two-tailed Fisher's exact test for qualitative values. The differences evaluated with the use of parametric and non-parametric methods were considered statistically significant with confidence level >95% (p<0.05). In case of statistically significant inter-group differences, the odds ratio (OR) and 95 confidence interval (CI) were calculated OR (-95% CI; + 95 CI). The values in the Table are presented as Me (median) and SD - standard deviation.

III. METHODOLOGY

Laboratory investigations of biochemical indices, the parameters of hemostasis, as well as the determination of blood serum protein fractions using electrophoresis were conducted together with the specialists from the express-laboratories of the 2nd Clinical Hospital for Infectious Diseases; the determination of protein C was conducted together with the specialists from the express-laboratories of Filatov' Pediatric City Clinical Hospital. The aggregation properties of the platelets, the elasticity and the deformability of the erythrocytes were studied together with senior researchers of the laboratories of hemostasis of the clinical department of Central Research Institute of Epidemiology of Federal Service on Customers' Rights Protection and Human Well-being Surveillance (Moscow). Aggregation capacity of the platelets (using Born' method) and the erythrocytes (using Sheremetiev'

original technique [6]) was determined by photometric aggregometers SOLAR AP-2110 (Belarus) and BIOLA LA - 230 (RF). Platelet aggregation was induced using 2×10^{-5} M ADP (Reanal); erythrocyte aggregation – using 1% protamine sulfate (RF) solution and LaCl_3 (RF) solution (at a dilution of 150 mg of reagent in 100 ml water).

The indices of the external (prothrombin time (PT), International Normalized Ratio (INR), Quick prothrombin index and the internal (activated partial thromboplastin time (aPTT)) coagulation pathways were turbidimetric determined on an automatic coagulometer ACL ELITE PRO (USA). The degree of disfibrinogenemiya was evaluated by the thrombin time (TT), functional fibrinogen activity (using Clauss method) and D-dimer dimension.

The endothelial dysfunction was determined by the degree of athrombogenicity of the vascular wall endothelium (antithrombin III (AT-III) and protein C levels) as well as by the adhesive properties of the endothelium (using von Willebrand factor). Blood plasma levels of AT-III was determined on ACL ELITE PRO coagulometer; protein C– on SYSMEX CA-500 coagulometer (Siemens Healthcare, USA). Von Willebrand factor (vWf) was determined by manual method (Renam reagents, RF).

Blood serum protein fractions were studied on agarose gel in HYDRASYS analyzer (Sebia, France).

The inflammatory level was estimated on the base of C-reactive protein (CRP) on HITACHI - 902 analyzer (Roche, Japan). The same analyzer was used to determine the content of haptoglobin and lactic dehydrogenase (LDH) in blood serum. According to the design of the study, we determined the substrates and the enzymes of human biochemical passport [1, 7, 8], performed clinical blood and urine analyses.

IV. RESULTS AND DISCUSSION

During acute infection, the disturbances of hemostasis can evolve to hidden or evident signs of disseminated intravascular coagulation with threatening venous thromboses. The mechanisms of venous thromboses can be triggered by tissue lesion (in this case – infectious inflammation caused by β -hemolytic streptococcus (β -HS), the edema of the affected limb, the presence of lymphostasis and chronic venous insufficiency, the excess and/or the hyperactivity of the plasma factors. All the above factors accentuate the already existing dysfunction of the vascular wall endothelium. For this reason, a special attention is given to the study of athrombogenic and adhesive properties of the vascular wall and to the control of native anticoagulants' level (protein C and antithrombin-III) [1, 9, 10, 11].

a) Athrombogenic and adhesive properties of the vascular wall

Baseline level of protein C during the first 3 days of the disease (at admission) in patients with lower limb erysipelas ($81,9 \pm 4,9\%$) was significantly lower than in patients with facial erysipelas ($94,1 \pm 6,0\%$), and reliably below the control values ($100 \pm 5\%$, $p < 0,05$) as shown in Table 1.

With the decrement of the erysipelas focus, the level of protein C recovered gradually in both groups.

Protein C in patients with LLE without CVI ($n=28$) was $99,8 \pm 4,7\%$ during the acute stage of the disease, and increased to $140 \pm 4,5\%$ during the recovery stage $p < 0,001$, (Figure 1). The low baseline level of protein C in lower-limb erysipelas with CVI ($69,8 \pm 8,1\%$) did not significantly change during the recovery ($79,15 \pm 4,0\%$, $p = 0,21$) and remained refractory low during the treatment (Figure 1).

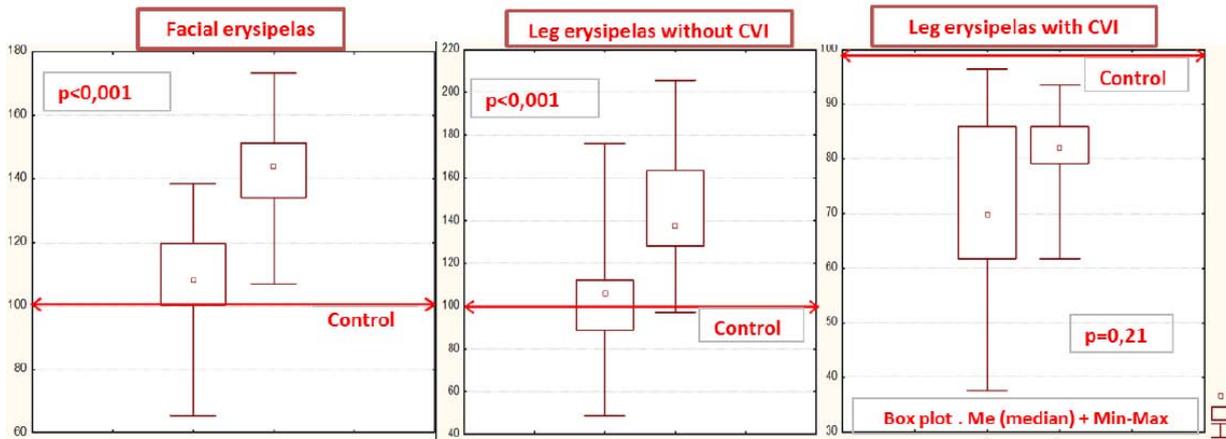


Figure 1 : Protein C in patients with facial erysipelas (1), lower-limb erysipelas without (2) and with CVI (3)

Note: Left columns - at admission (1st week of the disease), right columns – 2nd-3rd weeks of the disease, recovery period (Wilcoxon test).

In facial erysipelas, the level of protein C returned to the norm during the third week of the disease ($108,2 \pm 5,1\%$ at admission and $144 \pm 4,6\%$ at

discharge; $p_{1-4} < 0,001$). In LLE without CVI it recovered during the fourth week ($99,8 \pm 4,7\%$ at admission and $140 \pm 4,4\%$ at discharge; $p_{1-4} < 0,001$). In LLE with CVI,

the level of protein C did not change: $69,8 \pm 8,1\%$ at admission, $79,15 \pm 4,07\%$ at discharge; $p_{1-4}=0,21$), (Figure 1).

In the presence of endothelial dysfunction, the level of protein C in certain patients significantly

increased during recovery - from study point 1 to study point 4 (from $49,7$ to 112% , increase by 125% ; from $48,9$ to $110,7\%$, increase by 126% ; from $65,5$ to $119,7\%$ increase by 83%). This is a good example of the adaptation mechanism (Figure 2).

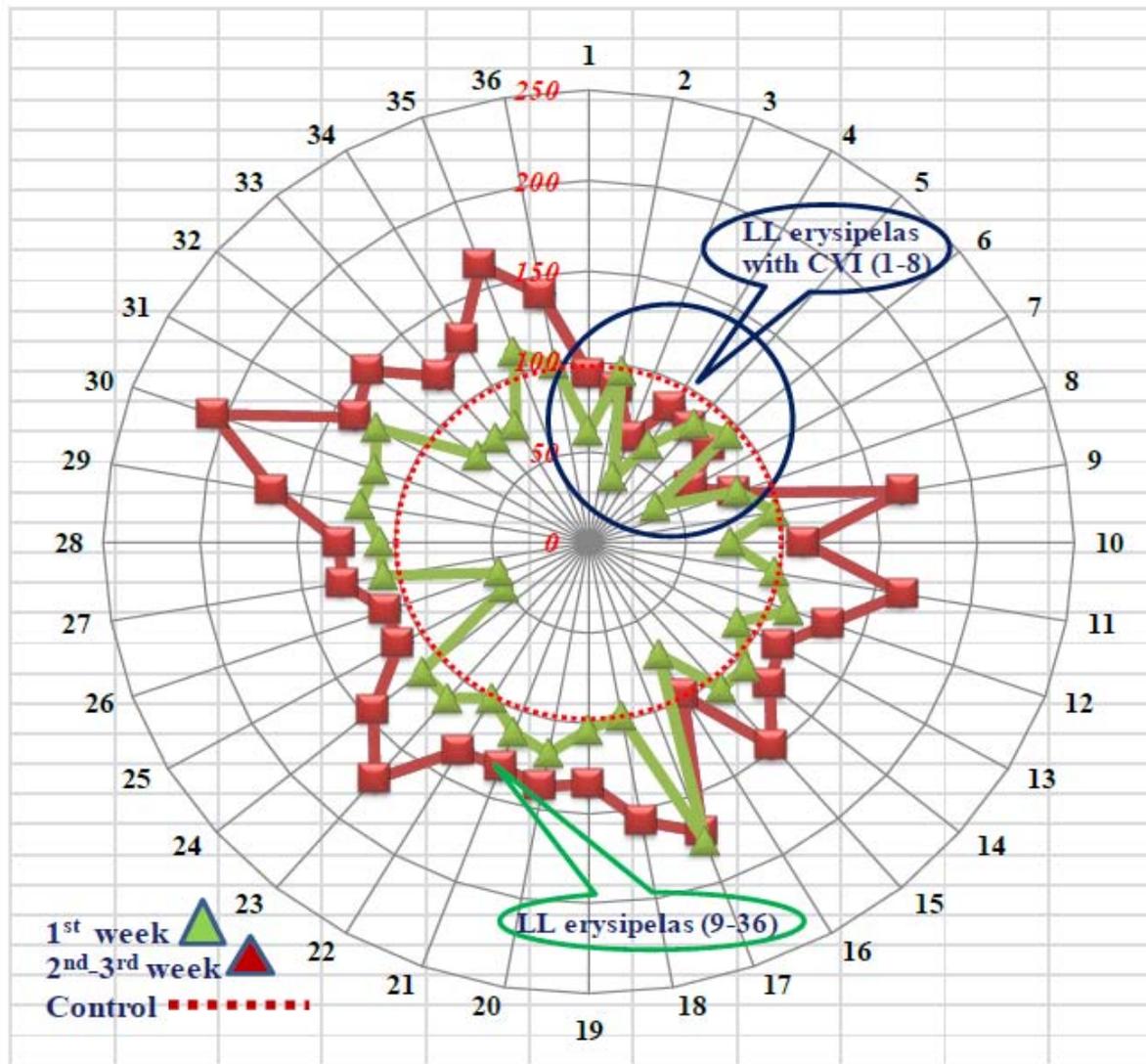


Figure 2 : Changes of protein C levels in patients with lower limb erysipelas

Note: 1-8 with CVI, 9-36 without CVI, LL - lower limb

The recovery in LLE without CVI (and with positive dynamics of protein C level) was more favorable than in LLE with concomitant CVI. According to some authors [1, 4, 9, 10, 12], CVI leads to the prolongation of the healing time of erysipelas focus and of the recovery. We have shown that in the presence of normal protein C ($100 \pm 5\%$), the chances for the favorable course of LLE are significantly higher ($OR=2,89$ ($0,15; 55$)), than in erysipelas with low protein C and concomitant CVI.

We determined also the level of another, not less important natural anticoagulant – the antithrombin - III. According to the literature [13, 14], the thrombosis (strokes, infarctions) develop when AT-III level is 80-

90%. AT-III deficit occurs in the presence of clinical signs of disseminated intravascular coagulation and the multiorgan failure syndrome.

AT-III deficit was more pronounced in LLE, than in facial erysipelas (Table №1). The baseline values of AT-III in patients with LLE ($87,6 \pm 2,5\%$) were significantly lower than in facial erysipelas ($91,8 \pm 2,5\%$), and lower than the control values ($p < 0,05$). AT-III level did not recover up to the discharge (Table №1). Bigger AT-III deficit seen in patients with LLE can explain higher incidence of hemorrhagic forms of erysipelas in this group of patients in comparison with facial erysipelas (78% in LLE and 22% in FE). Besides, AT-III (as well as

fibrinogen, CRP, α 1-acid glycoprotein) is an acute-phase protein. Our previous studies had shown that the level of acute-phase proteins in patients with lower limb erysipelas was higher than in patients with facial erysipelas [8].

The concentration of α 2-macroglobulin decreased by 25%. The minimal level of α 2-MG (an inhibitor of various proteases, including plasmin) was seen at the end of the 1st week of the disease: $3,78 \pm 0,16\%$ in LLE and $3,96 \pm 0,16\%$ in FE.

β -hemolytic streptococcus produces pathogenicity factors as streptokinase, hyaluronidase, streptodornase, etc. These factors destroy the protective level of heparansulfate, lining the vascular endothelium. This is accompanied by an increase of prothrombogenic properties of the vascular wall, the release of von Willebrand factor (vWf) and the decrease of AT-III activity. We noted an increase of vWf (187% to 220%) during 1st week of the disease in all patients with erysipelas. With the extinction of the inflammatory focus, high values of vWf tended to decrease, however they never reached normal values (Table 1).

Thus, the results of the study of endothelial markers in erysipelas patients are suggestive of a compromised endothelium-related hemostasis regulation. It concerned not only antithrombotic (decreased levels of protein C and AT- III), but also adhesive characteristics (high level of vWf). The deficit of natural anticoagulants was more pronounced in LLE cases. The refractivity of protein C in patients with LLE and CVI is a diagnostic marker of CVI and indication for vascular replacement therapy [13, 14].

It is known, that the body uses natural anticoagulants for the isolation (delimitation) of infectious inflammation area. The decrease of anticoagulants' concentration «opens the gate» for the generalization of infectious inflammation [11, 15, 16, 17].

b) Changes in plasma hemostasis and disfibrinogenemiya

We have found the following shifts in the indices of the external (prothrombin time, prothrombin index, INR) and the internal (aPTT) coagulation pathways, in the degree of disfibrinogenemiya (thrombin time, functional platelets' activity and D-dimer level) in patients with erysipelas (Table 2):

- The activation of coagulation cascade during the acute stage of the disease (decreased TT in facial and lower-limb erysipelas during the days 1-3 of the disease in comparison with the control ($p < 0,05$). It means the active processes of thrombin and fibrin formation in the blood flow;
- The activation of the external coagulation pathway (increased PT time in lower- limb erysipelas in comparison with the control ($p = 0,033$), decreased prothrombin index and increased INR);

- The activation of the internal coagulation pathway (the increase of aPTT at admission and during recovery in LLE. In facial erysipelas, the baseline aPTT was below the control ($p < 0,05$) and increased by the end of the 1st week of the disease ($p < 0,025$);
- The disfibrinogenemiya (decreased TT with the activation of fibrin polymerization process and the appearance of a great amount of D-dimers in the patient's blood (Table № 3). The fibrinogen level in LLE was higher than in FE: $8,0 \pm 2,3$ g/l vs $5,9 \pm 1,8$ g/l ($p = 0,008$) in 1- 6th days and $6,6 \pm 2,4$ g/l vs. $4,3 \pm 1,0$ g/l ($p < 0,0001$) in 7-15th days. Fibrinogen is acute-phase protein, as well as: AT-III, CRP, presepsin, procalcitonin, α - tumor necrosis factor, interleukin - 6 [16, 14].

Hence, the disfibrinogenemiya and the activation of coagulation cascade were higher in lower limb erysipelas (see above). The peak changes occurred at 4-6th days (study point 2), (Table 3).

The initial fibrinogen level in LLE ($7,7 \pm 0,38$ g/l) was by 18% higher than in FE ($6,53 \pm 0,49$ g/l). D-dimer level in lower-limb erysipelas (399 ± 46 ng/ml) was more than twofold higher in comparison with FE patients ($160,2 \pm 41$ mg/ml) and by 27 times (!) higher than in control group ($14,5 \pm 3,18$ ng/ml, $p < 0,001$).

The documented differences in D-dimer level allowed us to conclude that the processes of intravascular coagulation in lower limb erysipelas are more intense than in facial erysipelas. The presence of the clots of polymerized fibrin is a necessary condition for the increase of D-dimer level, as the process of plasminogen transformation to plasmin takes place inside these clots [5, 11, 13].

The increase of D-dimers and the decrease (expenditure) of α 2-macroglobulin also are suggestive of a potent local activation of proteolysis enzymes (plasmin, matrix metalloproteinases and neutrophil elastase). These enzymes destroy the extracellular matrix and induce the process of erosions, ulcers and necrosis formation in the area of infectious inflammation.

Starting from the 2nd week of disease, with the improvement of patients' condition, the levels of acute-phase proteins (fibrinogen, CRP, α 1-antitripsin (α 1-AT) etc.) decreased (Table 3).

The presence of disfibrinogenemiya, the activation of external and internal coagulation pathways, as well as the increase of acute-phase proteins suggest a close relation between the systemic inflammatory response and the compromised hemostasis there. The risk of severe (erythematous-bullous, erythematous-hemorrhagic, bullous-hemorrhagic) forms of LLE was significantly higher compared with FE (OR= 4,9 (1,5; 16)).

c) *Platelets' aggregation activity and erythrocytes' rheological properties*

In normal settings, the circulating platelets do not interfere with the internal surface of the vessel, covered by a thin layer of heparansulfate, that confers athrombogenic and anti-adhesive properties to vascular endothelium. The vascular wall injury results in the exposition of subendothelium components, mainly collagen, into the blood flow. In case of the participation of vWf (interaction with the platelet GP1b receptors) and of fibrinogen (interaction with the platelet GPIIb/IIIa receptors), the processes of platelet adhesion and aggregation are significantly enhanced [Ошибка! Источники ссылки не найден., 15].

In our study, the ADP-induced platelet aggregation was minimal at 1-3th days: $41,7 \pm 4\%$ in facial erysipelas and $64,8 \pm 3,9 \%$ in LLE ($p < 0,05$), which is statistically lower in comparison with platelets' functional activity in normal subjects ($76 \pm 3,1\%$, $p < 0,05$). At 11-15th days of the disease, the platelets' functional activity tended to recover ($71,7 \pm 3,4\%$ in LLE and $59 \pm 3,2\%$ in FE). The number of platelets increased in both groups: from $224 \pm 10 \times 10^9/l$ to $408 \pm 21 \times 10^9/l$ ($p = 0,002$) in lower limb erysipelas and from $234 \pm 30 \times 10^9/l$ to $317 \pm 29 \times 10^9/l$ ($p = 0,04$) in facial erysipelas. Hence, the platelets' amount and their functional activity in erysipelas occurred on the 2nd week of disease.

Herewith, the initially normal amount of erythrocytes at admission (study point 1) decreased by the days 7-10 of the disease (study points 2 and 3). The number of cells decreased by 7% of the initial value (from 4,7 to $4,39 \times 10^{12}/l$) in facial erysipelas and by 5% (from 4,6 to $4,37 \times 10^{12}/l$) in lower limb erysipelas. Simultaneously, erythrocyte sedimentation rate (ESR) rose to the maximum: $53,7 \pm 8,5$ mm/hour in lower limb erysipelas and $26,3 \pm 5,8$ mm/hour in facial erysipelas ($p = 0,006$).

Earlier, we have found an increase of indirect bilirubin and LDH blood levels in patients with erysipelas. Together with the decreased of haptoglobin found in this study ($3,86 \pm 0,26$ g/l in FE and $3,79 \pm 0,3$ g/l in LLE ($p < 0,05$ with the control), it suggests the presence of hidden hemolysis [7]. Further changes of haptoglobin level confirm this conclusion. The level of this protein increased by 153 % of the initial values in facial erysipelas and by 61% — in LLE (Figure 3).

Hence, intravascular (hidden) hemolysis is one of the leading pathogenetic mechanism of DIC-like syndrome. This syndrome is often described as a clinically unapparent (local) disseminated intravascular coagulation. In our study, we have seen the transformation of DIC-like syndrome to the classic DIC in three cases - 5% of 60 studied patients [8].

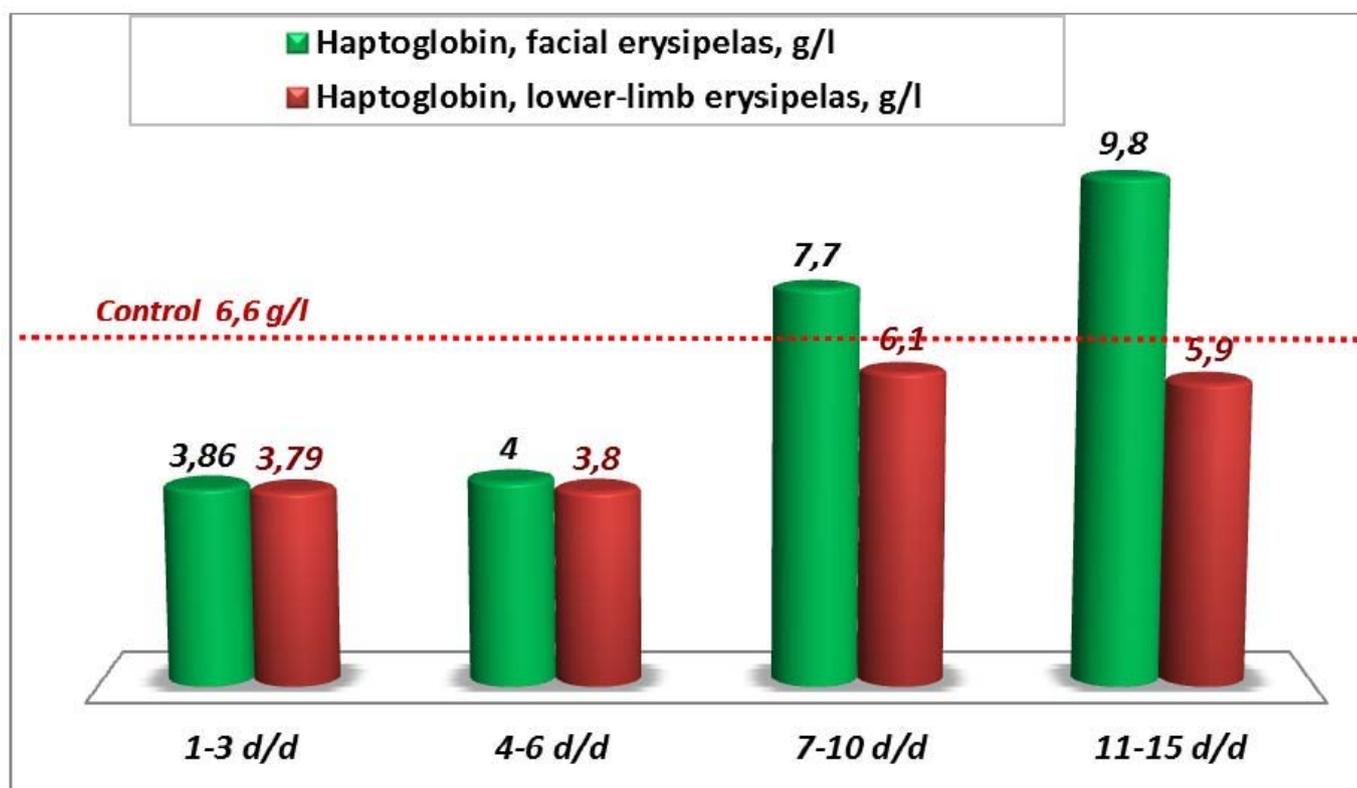


Figure 3 : Changes of haptoglobin level (g/l) in facial and lower limb erysipelas at 1st-2nd week of the disease

Note: d/d - days of the disease

We studied also the changes of rheological properties of erythrocytes – their elasticity (the

aggregation with protamine sulfate) and deformability (the aggregation with lanthanum chloride) in erysipelas.

The degree of blood cells' elasticity and deformability in normal subjects is almost equal: $62 \pm 4,9$ % for PS and $66,4 \pm 4,2$ % for LaCl₃.

In erysipelas, the cells' elasticity decreased twofold (aggregation with PS), while the deformability (aggregation with LaCl₃) increased by 37% (Table 4). The aggregation with two types of inductors (LaCl₃ and PS) was recorded simultaneously on aggregometer «BIOLA». The aggregates were 3,6 times bigger in size and 7,8 times higher in aggregate's degree on LaCl₃ compared with PS.

After addition of LaCl₃, the erythrocytes of erysipelas patients interacted faster (3-5 minutes) and quickly precipitated as large conglomerates [1, 8].

Some authors also described the changed conformation properties of the erythrocytes' membrane, the cells' form transformation from concave-discoid to spherical. Our experiments showed that with the addition of LaCl₃ to the erythrocytes of erysipelas

patients, cytoarchitectonics of cellular membranes is disturbed. It leads to fast adhesion between the cells and to the formation of cell conglomerates. The aggregation with LaCl₃ helps to reproduce the picture of erysipelas-associated hidden hemolysis and increased erythrocyte "frailness" in vitro.

The mechanism of protamine sulfate action is different. It does not induce conformational rebuilding of the erythrocytes' membrane. With the loss of negative charge of the membrane (preventing cells adhesion to each other), the erythrocytes sediment and form the coin columns. The aggregates on the PS look smaller and softer, and the aggregation time is slower (≥ 10 minutes) [1, 8].

The found differences in erythrocytes' elasticity and deformability persisted during the recovery period. The high "frailness" of erythrocyte membrane persisted up to the end of the 2nd week, and its low "plasticity"- up to the discharge (Figure 4).

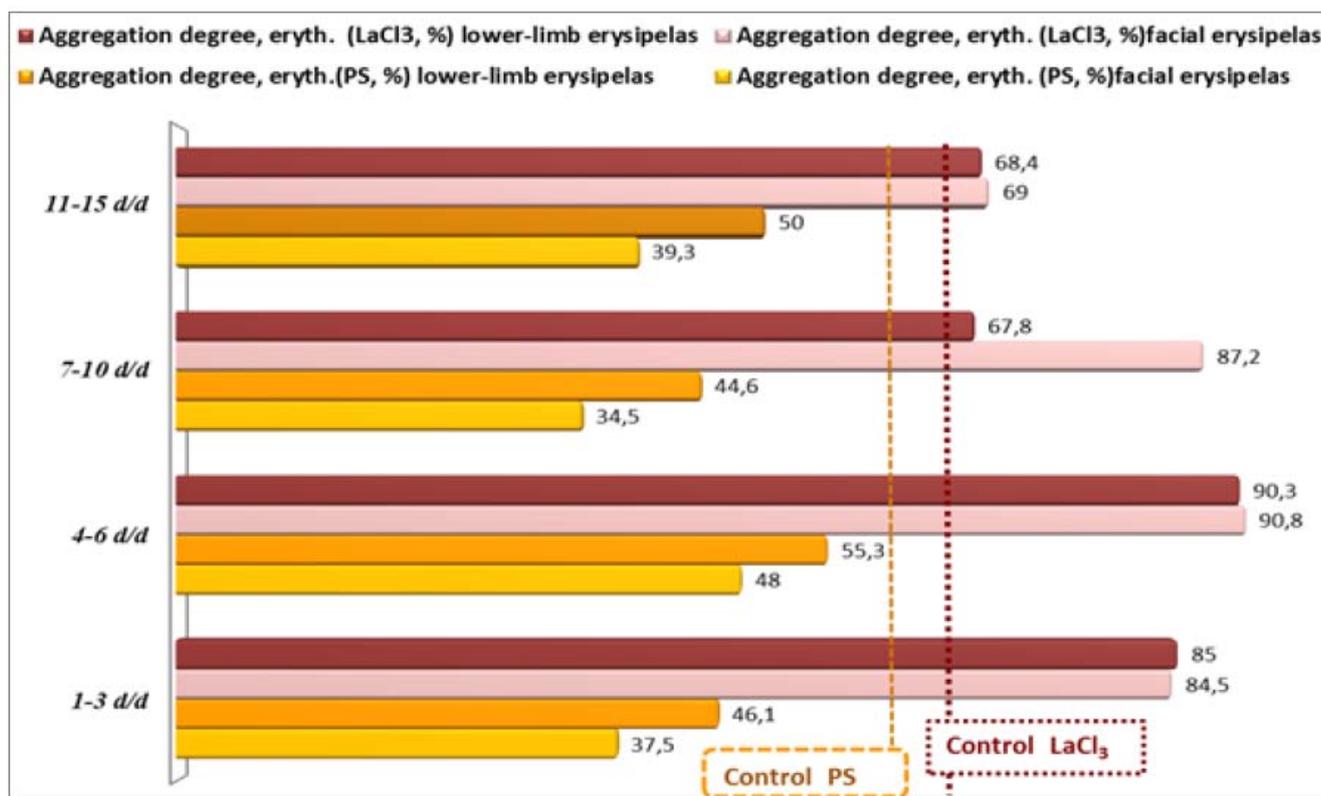


Figure 4 : Erythrocytes' deformability (LaCl₃) and elasticity (PS) in aggregation tests in facial and lower limb erysipelas

Note: d/d — days of the disease

One has to note, that the erythrocytes play not only the role of oxygen transporter in the blood flow. They also serve as a potent buffer of the system of hemostasis and as a second, in order of importance (after albumin), detoxification barrier. The body uses the erythrocyte pool for supplementary refilling of protein deficit in the blood flow [7]. The discoid form allows the erythrocytes to perform their functions with maximal

effectiveness. For this reason, the recovery of erythrocytes' elasticity is of clinical interest for certain therapeutic practices.

V. CONCLUSION AND RECOMMENDATIONS

1. Laboratory studies confirmed that clinical hemorrhagic complications are by 3,9 times more

common in lower limb erysipelas than in facial erysipelas.

- The results of determination of the markers of hemostatic endothelial function in erysipelas patients suggest the disturbances of endothelium-related regulation of hemostasis, as judged by antithrombotic (decreased levels of protein C and of AT-III), as well as by adhesive indices (high level of von Willebrand factor).
- Increased D-dimer and decreased alpha-2-macroglobulin levels suggest potent activation of the system of proteolysis enzymes (plasmin, matrix metalloproteinases and neutrophil elastase). This is pathogenetically associated with the formation of bullae, erosions and ulcers in the erysipelas focus.
- Low level of protein C, persisting during standard therapeutic procedures, is not only a laboratory indicator of chronic venous insufficiency in lower limb erysipelas, but also serves as a marker of the development of DIC-like syndrome.
- Documented evidence of protein C deficit can be an indication for the consideration of antithrombotic therapy. Maximally early start of the replacement therapy (with protein C products) can contribute to the delimitation of the inflammation area and to the decrease of the severity of local proteolysis reactions.
- We found the signs of intravascular (hidden) hemolysis and of disturbed rheological properties of erythrocytes – their increased deformability (aggregation with lanthanum chloride) and decreased elasticity (aggregation with protamine sulfate). The disturbed erythrocytes elasticity is an indication for the supplementation of standard erysipelas therapy with the agents contributing to the increase of erythrocytes' plasticity and to the improvement of blood rheology (Pentoxifylline).
- Clinically important risk of hemorrhagic complications in lower limb erysipelas is higher, than in facial erysipelas, OR = 9,88 (2,81; 34,7).

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Table 1 : Changes in protein C, AT-III and vFw levels in facial and lower-limb erysipelas (Me ±SD)

Changes by days/Index	Facial erysipelas (n=24)			Lower-limb erysipelas (n=36)		
	Protein C, %	AT-III,%	vFw,%	Protein C, %	AT-III,%	vFw,%
Days 1-3 of the disease (point 1)	94,1±6,0*,**	91,8±2,5*	187,0±4,2**	81,9±4,9*,**	87,6±2,5*	190±2,0**
Days 4-6 of the disease (point 2)	119,6±3,1*,**	96,4±0,8**	183,0±2,7**	103,0±3,2*,**	91,3±1,7*,**	187,6±2,9*
Days 7-10 of the disease (point 3)	129,0±6,4*	91,4±2,2*	175,6±6,2*	134,5±4,7*	89,6±1,5*	180,0±2,8*
Days 11-15 of the disease (point 4)	153,0±4,4*	93,0±1,1*	174,8±4,4*	139,0±6,7*	89,4±3,2*	176,0±3,1*
Follow-up (in 5 months)	_____	_____	_____	106,4±10,2	93,0±1,2*	167,0±3,7*
Normal subjects (n=32)	100±5,0	97,3±0,38	150,4±3,9	100,0±5,0	97,3±0,38	150,4±3,9

Note: *— significant difference with the control value, **— reliable differences between the groups ($p < 0,05$)

Table 2 : Changes in plasma hemostasis in facial and lower-limb erysipelas (Me ±SD)

Changes by days/Index	Facial erysipelas (n=24)					Lower-limb erysipelas (n=36)				
	PT, sec	INR	Prothrombin, %	TT, sec	aPTT, sec	PT, sec	INR	Prothrombin, %	TT, sec	aPTT, sec
Days 1-3 of the disease (point 1)	12,2±0,7*,**	1,27±0,04*	79,2±4,5*,**	12,2±0,5*,*	28,5±1,3*,*	15,4±0,5*,*	1,47±0,04*,**	59,3±2,8*,**	10,9±0,36*,**	39,5±1,4*,**
Days 4-6 of the disease (point 2)	14,9±0,6*	1,42±0,05*	68,7±4,3*	12,7±0,7*,*	45,1±2,7*,*	15,1±0,4*	1,47±0,04*	61,3±3,8*	11,9±0,5*	38,5±1,7*,**
Days 7-10 of the disease (point 3)	12,2±0,7*,**	1,27±0,04*	79,2±4,5*,**	12,2±0,5*,*	28,5±1,3*,*	15,4±0,5*,*	1,47±0,04*,**	59,3±2,8*,**	10,9±0,36*,**	39,5±1,4*,**
Days 11-15 of the disease (point 4)	12,2±0,7	1,16±0,03	79,4±4,8*	12,2±0,3*,*	37,3±3,0*,*	13,3±0,25*,**	1,31±0,03*,**	73,3±2,8*,**	12,1±0,26*	37,4±1,3*,**
Follow-up (in 5 months)	—	—	—	—	—	11,2±0,3	1,1±0,02	93,2±2,9	13,4±0,15	30,4±1,0
Normal subjects (n=32)	10,9±0,14	1,11±0,02	98,2±1,8	14,6±0,26	33,7±0,66	10,9±0,14	1,11±0,02	98,2±1,8	14,6±0,26	33,7±0,66

Note: PT — Prothrombin time, INR — International Normalized Ratio, TT — Thrombin time, aPTT — activated Partial Thromboplastin time; *— significant difference with the control value, **— reliable differences between the groups ($p < 0,05$).

Table 3 : Signs of disfibrinogenemiya, (Me ±SD)

Changes by days/Index	Facial erysipelas (n=24)				Lower-limb erysipelas (n=36)			
	Fibrinogen, g/l	D-dimer, ng/ml	CRP, mg/l	α_1 -AT + α_1 -acid GP, %	Fibrinogen, g/l	D-dimer, ng/ml	CRP, mg/l	α_1 -AT + α_1 -acid GP, %
Days 1-3 of the disease (point 1)	6,53±0,49*,**	160,2±4*,**	126±14*	3,63±0,2*	7,7±0,38*	399±46*,**	126±8,5*	3,79±0,29*
Days 4-6 of the disease (point 2)	5,62±0,47*,**	164±2*,*	44,5±7,1*,**	3,34±0,08*	6,75±0,42*	371±88*,**	117±13*,**	4,2±0,3*,**
Days 7-10 of the disease (point 3)	5,0±0,33*	147,7±27*,**	1,2±0,2*	3,22±0,14*	5,3±0,17*	381±43*,**	45,7±15*	3,45±0,2*
Days 11-15 of the disease (point 4)	4,2±0,2*,**	88,6±12,7*,**	2,0±0,3*,**	2,98±0,13*	5,6±0,4*,**	467±46*,**	9,4±3*,**	3,2±0,24*
Follow-up (in 5 months)	—	—	—	—	5,1±0,6*	202,6±5*	1,7±0,4*	2,5±0,2
Normal subjects (n=32)	2,96±0,09	14,5±3,18	0,6±0,2	2,6±0,1	2,96±0,09	14,5±3,18	0,6±0,2	2,6±0,1

Note: CRP — C reactive protein, α_1 -AT — α_1 -antitrypsin, α_1 -acid GP — α_1 -acid GP glycoprotein;

*— significant difference with the control value, **— reliable differences between the groups ($p < 0,05$).

Table 4 : Platelets' number and aggregation activity and erythrocytes' rheological properties in facial and lower-limb erysipelas, (Me ±SD)

Changes by days/Index	Platelets, $\times 10^9/l$	ADP-aggregation degree: platelets, %	Erythrocytes, $\times 10^{12}/l$	ESR, mm/hour	LaCl ₃ -aggregation degree: erythrocytes, %	PS -aggregation degree: erythrocytes, %	Haptoglobin, g/l
Facial erysipelas (n=24)							
Days 1-3 of the disease (point 1)	234±30,0	41,7±4*,**	4,5±0,08*	20,4±1,8*,* *	84,5±6,4*,**	37,5±3,7*,**	3,86±0,26*,* *
Days 4-6 of the disease (point 2)	249±9,4	67,2±5,1	4,7±0,08	18±2,8*,**	90,8±5,3*,**	48±3,1*,**	4,0±0,4*,**
Days 7-10 of the disease (point 3)	296±39	47±2,6*,**	4,39±0,08	26,3±5,8*,* *	87,2±3,6*,**	34,5±1,7*,**	7,7±1,2*,**
Days 11-15 of the disease (point 4)	317±29	59±3,2*,**	4,3±0,2	16,5±2,3*	69±7,3	39,3±3,9*,**	9,8±,4*,**
Lower-limb erysipelas (n=36)							
Days 1-3 of the disease (point 1)	224±10	64,8±3,9*,**	4,7±0,06*	33,5±3,9*,* *	85±4,5*,**	46,1±2,7*,**	3,79±0,3*,**
Days 4-6 of the disease (point 2)	253±14	68,2±4,3	4,6±0,08	35,2±3,7*,* *	90,3±4,8*,**	55,3±5,0	3,8±0,4*,**
Days 7-10 of the disease (point 3)	314±12,8	53,7±3,0*,**	4,37±0,14	53,7±8,5*,* *	67,8±5*,**	44,6±3,4*,**	6,1±0,8
Days 11-15 of the disease (point 4)	408±21	71,7±3,4*	4,5±0,1	33,7±6,4*,* *	68,4±4,9	50,0±6,0*,**	5,9±0,6
Follow-up (in 5 months)	—	52,7±5,5*	—	—	63,0±6,4	36,7±3,4*,**	5,4±0,8
Normal subjects (n=32)	250±5,2	76±3,1	4,5±0,13	≤ 10	66,4±4,2	62±4,9	6,6±0,5

Note: ESR - erythrocyte sedimentation rate, *— significant difference with the control value, **— reliable differences between the groups ($p < 0,05$)

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