

## Hemostasis in Erysipelas-The Reason for Considering Antithrombotic Therapy

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### Abstract

The indices of external (prothrombin time, INR) and internal (activated partial thromboplastin time (aPTT) coagulation pathways, the degree of disfibrinogenemia (thrombin time, functional fibrinogen activity and D-dimer level), the amount and functional activity of the platelets (aggregation with ADP) and the erythrocytes (aggregation with lanthanoid and protamine sulfate) were studied in 60 patients with erysipelas. Also, we have studied endothelial dysfunction manifested in the decrease of athrombogenicity of vascular wall endothelium (antithrombin III and protein C) and in the increase of adhesive properties of the endothelium (von Willebrand factor).

The comparison groups comprised normal volunteers (n=32) and patients with focus of 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), undergoing in hospital treatment in Moscow 2nd Clinical Hospital for the Infectious Diseases.

The thesis, according to which the rate of hemorrhagic complications in leg erysipelas is by 3,9 times higher than in facial erysipelas, was confirmed by laboratory findings. In particular, a significant decrease of protein C was noted in patients with leg erysipelas and concomitant chronic venous insufficiency. We have found increased D-dimer and decreased  $\alpha$ 2-macroglobulin levels, suggesting potent activation of proteolytic enzymes (plasmin, matrix metalloproteinases, 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 (latent) hemolysis - the decrease of haptoglobin concentration and the increase of indirect bilirubin and LDH blood level; the changes of rheological properties of erythrocytes - the increase of deformability (aggregation with lanthanum chloride) and the decrease of elasticity (aggregation with protamine sulfate) - have been identified as one of the main factors for DIC-like syndrome in erysipelas.

**Keywords:** Facial erysipelas; Lower-limb erysipelas; Latent hemolysis; DIC-like syndrome; Erythrocyte aggregation; Platelet aggregation; Fibrinogen; D-dimer; Protein C; Antithrombin - III; Von Willebrand factor; Haptoglobin;  $\beta$ -hemolytic streptococcus.

### Introduction

To date, clinical research focuses on the study of the relation between the inflammation and the coagulation, as the dysfunction of vascular endothelium, common for these two pathological processes, represents 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 [1-3].

Erysipelas, being an acute infectious disease, is widely spread and does not depend from the level of industrial development and social security in different countries. Common and local predisposing factors form an important pathogenetic aspect of this condition's development. Leg erysipelas is often associated with obesity, type 2 diabetes mellitus, chronic venous insufficiency (CVI) and feet and nail mycoses [4-7]. Facial erysipelas often develops in association with otomycosis and chronic diseases of the ORL- organs [8].

Despite modern methods of treatment, up to 10% cases of erysipelas are complicated by the development of skin necrosis at the sites of hemorrhages and vesicles formation, of venous incompetence (periphlebitis, phlebitis, thrombophlebitis). An increasing rate of hemorrhagic forms of erysipelas is being registered [9-15]. In this view, 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.

### Purpose of the work

The work was aimed at the study of the changes of the system of hemostasis and of blood rheology during the progression of infection in patients with lower-limb and facial erysipelas and to validate the advisability of replacement and/or antithrombotic therapy.

### Material and Methods

#### Characteristics of the patients

We have studied 60 patients aged 25 to 71 years with the diagnosed 2nd degree lower-limb erysipelas (36) and 2nd degree facial erysipelas (24); 67% of patients had primary form of the disease. Erythematous

form of erysipelas was diagnosed in 33% of cases (in 52% of facial erysipelas), erythematous-bullous in 15%, erythematous-hemorrhagic in 22%, and bullous-hemorrhagic in 30% of cases. Erythematous-hemorrhagic (11 cases) and bullous-hemorrhagic (15 cases) erysipelas affected the lower limb more frequently than the face (2 and 3 cases, respectively). The risk of hemorrhagic disturbances was significantly higher in cases with local inflammation process (78%) affecting the legs, than in cases of the face affection (20%); the odds ratio (OR) was 9.9 [2,7,8,34].

Primary facial erysipelas was diagnosed in 92% of cases and was more frequently seen in women (16 women, 8 men). Primary lower-limb erysipelas was registered in 50% of cases, repeated erysipelas in 31% and recurrent erysipelas in 19% of cases, unlike the facial erysipelas: primary (92%), repeated (4%) and recurrent (4%). The risk of erysipelas recurrence was statistically significantly higher when the inflammation focus was located on the leg, in comparison with the face (OR=5.55) (1,2,51),  $p=0.009$ .

Gender ratio in leg erysipelas was comparable (males-17, females - 19). Feet mycosis and onychomycosis were found to be the most frequent (88%) associated diseases. Obesity grade 2 to 4 was present in 11 patients, 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, while 29% of these patients had chronic ORL pathology (otitis, tonsillitis, 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 (Head Physician of the Hospital – Miasnikov V.A., M.D., Ph.D Head of Erysipelas department – Potekaeva S.A., M.D.). Thirty-two patients received antibacterial monotherapy: IM benzylpenicillin novocaine salt twice daily, 1,2 mln ME per day for 7-10 days, another two patients received IM Cephalosporin (Cephazolin) 1 g  $\times$  3 times daily for 5 days. Combined two-antibiotics therapy (IM benzylpenicillin novocaine salt twice daily, 1.2 ml ME per day for 7-10 days and Ciprolet twice daily per os, 1 g per day for 10 days) was used in 14 patients. Twelve patients got a three-antibiotics combination (benzylpenicillin novocaine salt 1.2 ml ME per day for 7 days+IV Ciprofloxacin 800 mg per day for 3 days with subsequent passage to 1 g per day per os for 10 days+IM Cefazolin 1 g three times per day for 5 days). Additionally, the patients received: antihistamine agents (Zodac, Diazolin); topical physiotherapy (UV irradiation therapy and low frequency current (LFC) for facial erysipelas; UV irradiation therapy, LFC and magnetic therapy for lower-limb erysipelas); the focus of erysipelas (on the legs) was regularly treated with tanning solution of potassium permanganate. The patients who participated in the study did not receive any additional drugs capable to influence their hemostasis' state.

Mean duration of in-hospital stay was 11.9 + 4.1 days for the patients with leg erysipelas and 8.4 + 1.6 days for the patients with facial erysipelas.

The hemostasis indices were studied in the initial stage of the disease (1<sup>st</sup> to 3<sup>rd</sup> days) - point 1, during the progression of the disease (days 4-6, 7-10) – points 2 and 3 and during the recovery period (days 11-15) – point 4 of the study. Every third patient with leg erysipelas underwent the follow-up study (in 5 months after the discharge), which allowed to separate the changes induced by erysipelas from the underlying concomitant diseases.

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

The obtained results were proceeded with the use of Statistica 10.0 for Windows 7.0 software. The evaluation of statistically significant (reliable) differences between the groups was performed 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 [17].

Laboratory investigations of biochemical indices, the parameters of coagulogram, as well as the determination of blood serum protein fractions using electrophoresis were conducted together with the specialists from the express-laboratories of the 2<sup>nd</sup> Clinical Hospital for Infectious Diseases (Head of Laboratories T.N. Molotilova); the determination of protein C was performed together with the specialist in laboratory diagnosis Dr. T.N. Lagutina in the express-laboratories of Filatov Pediatric City Clinical Hospital 13 (Head of Laboratories T.N. Lagutina, M.D.). The study of aggregation properties of the platelets, the elasticity and the deformability of the erythrocytes was conducted together with senior researcher of the laboratories of hemostasis of the clinical department of Central Research Institute of Epidemiology, O.S. Astrina, the determination of von Willebrand factor-together with the research fellow N.I. Alioshina, (Head of clinical department of Central Research Institute of Epidemiology of Federal Service on Customers' Rights Protection and Human Well-being Surveillance – Professor A.V. Gorelov, M.D.).

Aggregation capacity of the platelets (using Born method) and the erythrocytes (using original technique suggested by Yu.A. Sheremetiev [18]) was determined using photometric technique with the help of SOLAR AP 2110 (Belarus) and LA 230 (scientific-production association BIOLA, RF) aggregometers.

Platelet aggregation was induced using  $2 \times 10^{-5}$  M ADP (Reanal, Hungary); erythrocyte aggregation using 1% protamine sulfate (PS) solution (Ellara, RF) and water solution of lanthanum chloride (LaCl<sub>3</sub> Khimik, RF, at a dilution of 150 mg of the chemical agent in 100 ml H<sub>2</sub>O).

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

The endothelial dysfunction was determined by the degree of athrombogenicity of the vascular wall endothelium (antithrombin III and protein C levels) as well as by the adhesive properties of the endothelium (using von Willebrand factor). Blood plasma levels of antithrombin-III (AT-III, AT) was determined using automatic coagulometer ACL ELITE PRO; of protein C (PC) using automatic coagulometer SYSMEX CA-500 (Siemens Healthcare, USA, chemical

agents Siemens AG, Germany). Von Willebrand factor (fvW) was determined using the manual method (1982) and chemical agent Renam (RF).

Blood serum protein fractions were studied using electrophoretic method applied to agarous gel in HYDRASYS analyzer (Sebia, France).

The level of the inflammatory reaction was estimated on the base of C-reactive protein (CRP) content using HITACHI 902 analyzer (Roche, Japan). The same analyzer was used for the determination of the content of transferrin, ceruloplasmin and haptoglobin, LDH and LDH1-2 fractions in blood serum. According to the design of the study, we have determined the substrates and the enzymes of biochemical passport [19,20], evaluated clinical blood and urine analyses.

## Results and Discussion

The disturbances of hemostasis system in acute infectious process can evolve to a latent or evident picture of disseminated intravascular coagulation with threatening venous thromboses. The mechanisms of venous thromboses development can be triggered by: tissue damage (in this case – erysipelas), the edema of the affected limb, the presence of lymphostasis and chronic venous insufficiency (CVI), the excess and/or the hyperactivity of the plasma factors. All the above factors enhance the 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 the level of native anticoagulants (protein C and antithrombin-III) [21-26]. Curiously, the picture was quite different for the red blood cells. Initially normal amount of erythrocytes at admission (point 1) was decreased by the days 7-10 of the disease (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, the index of ESR rose to maximal value:  $53.7 \pm 8.5$  mm/hour in lower-limb erysipelas and  $26.3 \pm 5.8$  mm/hour in facial erysipelas,  $p=0.006$ .

With the account of the increased blood levels of indirect bilirubin and LDH in patients with erysipelas, found in our earlier studies, as well as of the decreased level of haptoglobin found in this study ( $3.86 \pm 26$  g/l in facial erysipelas and  $3.79 \pm 0.3$  g/l in lower-limb erysipelas,  $p<0.05$  with the control), the picture of latent hemolysis in erysipelas becomes evident [16,38]. This conclusion is confirmed by further changes of haptoglobin level. The level of this protein increased twofold by ricochet: 253% of the initial values in facial erysipelas and 161% - in lower-limb erysipelas (Figure 3). Baseline values of protein C during the first 3 days of the disease (at admission) in the group of patients with lower-limb erysipelas ( $81.9 \pm 4.9\%$ ) were significantly lower, than in patients with facial erysipelas ( $94.1 \pm 6.0\%$ ), and reliably below the control values ( $100 \pm 5\%$ ,  $p<0.05$ ) (Table 1).

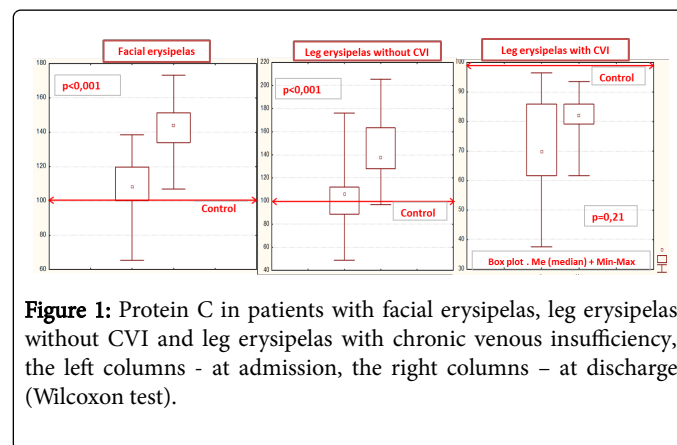
Hence, intravascular (latent) hemolysis is one of the leading pathogenetic mechanisms of DIC-like syndrome. Some authors describe this syndrome in erysipelas as a clinically inapparent (local) DIC [4,11,12,14,40,43,47]. In our study, we have seen the transformation of DIC-like syndrome to the classical DIC in three (5%) of 60 studied patients [16,38]. We studied also rheological properties of erythrocytes, their elasticity (as judged by the aggregation with protamine sulfate) and deformability (by the aggregation with lanthanum chloride) in facial and lower-limb 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  $LaCl_3$ . With the decrement

of the erysipelas focus, the level of protein C recovered gradually in both groups:  $119.6 \pm 3.1$  at day 4-6 of the disease,  $129 \pm 6.4\%$  at day 7-10,  $153 \pm 4.4\%$  at day 11-15 in patients with facial erysipelas ( $p<0.05$  between the study points 1/4, 2/4 and 3/4); and  $103 \pm 3.2\%$ ,  $134.5 \pm 4.7\%$ ,  $139 \pm 6.7\%$  in patients with leg erysipelas ( $p<0.05$  between the study points 1/4, 2/4).

The level of protein C in patients with lower-limb erysipelas 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). In lower-limb erysipelas with CVI, the low baseline level of protein C ( $69.8 \pm 8.1\%$ ) did not significantly change during the therapy –  $79.15 \pm 4.0\%$ ,  $p=0.21$  and remained below the level seen during the recovery in patients with erysipelas without CVI (Figure 1).

The level of protein C recovered to the normal value during the third week of the disease in patients with facial erysipelas ( $108.2 \pm 5.1\%$  at admission and  $144 \pm 4.6\%$  at discharge;  $p$  for points 1/4 $<0.001$ ); and during the fourth week in patients with leg erysipelas without CVI ( $99.8 \pm 4.7\%$  at admission and  $140 \pm 4.4\%$  at discharge;  $p$  for points 1/4 $<0.001$ ). In patients with leg erysipelas with CVI, the level of protein C remained refractory low during the follow-up:  $69.8 \pm 8.1\%$  at admission,  $79.15 \pm 4.07\%$  at discharge;  $p$  for points 1/4=0.21) (Table 1).

The changes in the level of protein C in the settings of endothelial dysfunction in certain patients (from 49.7% (point 1) to 112% (point 4) increase 125%; from 48.9% (point 1) to 110.7% (point 4) increase 126%; from 65.5% (point 1) to 119.7% (point 4) increase 83% deserves attention as an effective mechanism of native sanogenesis factors' work during the recovery (Figure 2).



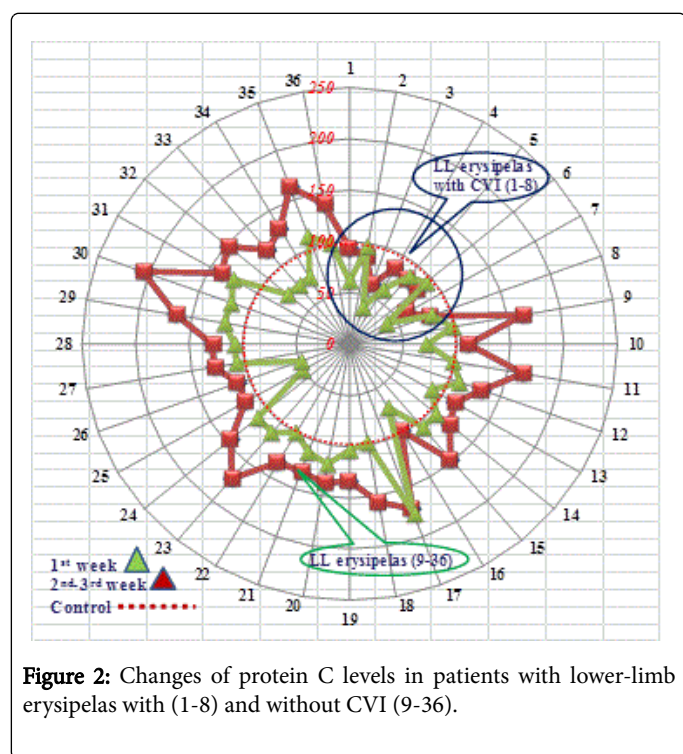
**Figure 1:** Protein C in patients with facial erysipelas, leg erysipelas without CVI and leg erysipelas with chronic venous insufficiency, the left columns - at admission, the right columns - at discharge (Wilcoxon test).

According to some authors, CVI leads to the prolongation of the healing time of erysipelas focus and of the recovery period [4,13-15,21]. We were able to prove, that in the presence of normal protein C levels ( $100 \pm 5\%$ ), the chances for the favorable course of leg erysipelas are significantly higher (OR=2.89) (0,15,55) than in erysipelas with low protein C level and associated chronic venous insufficiency. In the absence of CVI, the recovery in our patients with lower-limb erysipelas was shorter.

Besides protein C, we have determined the level of another, not less important natural anticoagulant – the antithrombin III (AT-III). According to the literature, the thromboses (strokes, infarctions) develop when AT-III level is 80-90%. Antithrombin deficiency occurs in the presence of clinical manifestations of disseminated intravascular

coagulation (DIC) and of the developing of multiorgan failure syndrome [13,25,28,40,43].

Antithrombin deficiency was more manifested in patients with lower-limb erysipelas, than with facial erysipelas (Table 1). The baseline values of AT-III in patients with leg erysipelas ( $81.6 \pm 2.5\%$ ) were statistically lower than in facial erysipelas ( $91.8 \pm 2.5\%$ ) ( $p < 0.05$  between the groups, the value in the control group being  $97.3 \pm 0.38\%$ ), and did not recover before the 15th day of the disease (Table 1). Antithrombin-III deficiency seen in patients with leg erysipelas can explain higher incidence of hemorrhagic forms of erysipelas (78%) in this group of patients in comparison with the patients with facial erysipelas (22%) (Table 1).



**Figure 2:** Changes of protein C levels in patients with lower-limb erysipelas with (1-8) and without CVI (9-36).

Besides, AT-III, (as well as fibrinogen, CRP, orosomucoid) is an acute-phase protein. Our previous studies have shown that the level of acute-phase proteins in patients with lower-limb erysipelas was higher than in patients with facial erysipelas [6,16]. The concentration of  $\alpha 2$ -macroglobulin ( $\alpha 2$ -MG) decreased by 25%. The minimal level of  $\alpha 2$ -MG – an universal inhibitor of various proteases, including plasmin – was seen at the end of the 1st week of the disease:  $3.78 \pm 0.16\%$  in facial erysipelas and  $3.96 \pm 0.16\%$  in leg erysipelas.

It is known that  $\beta$ -hemolytic streptococcus ( $\beta$ -HS) produces several pathogenicity factors (streptokinase, hyaluronidase, streptodornase), that destroy the protective level of heparansulfate, lining the vascular wall endothelium. This is accompanied by an increase of prothrombogenic properties of the vascular wall endothelium, the release of von Willebrand factor and the decrease of antithrombin III activity [9,10,12,16,27]. We have noted an increase of fvW within the interval from 220% to 187% during the first week of the disease in all patients with erysipelas, irrespective of the localization of the inflammation focus (face  $187 \pm 4.2\%$ , lower limb  $190 \pm 2\%$ ,  $p < 0.05$  for the control value:  $150.4 \pm 3.9\%$ ). With the extinction of the erysipelas focus, there was a tendency for the decrease of high values of von Willebrand factor, however the studied index was not fully normalized (Table 1).

Thus, the obtained results of the study of endothelial markers in patients with erysipelas suggest that their endothelium-related hemostasis regulation was compromised. It concerned not only antithrombotic (decreased levels of protein C and antithrombin III), but also adhesive characteristics (high level of von Willebrand factor). The deficiency of natural anticoagulants was statistically more significant in leg erysipelas in comparison with facial erysipelas. The detected protein C deficiency without a tendency for recovery in the settings of conducted therapy, on the one hand, serves as a laboratory indicator of existing CVI in the presence of the lower-limb erysipelas, and, on the other hand, can be an indication for the prescription of vascular replacement therapy in patients with CVI [30-35]. It is known that the body uses natural anticoagulants for the isolation (delimitation) of infective inflammation area, and the decrease of anticoagulants' concentration lowers the resisting barrier and opens the gate for the generalization of infective inflammation [29,30].

Changes by days/Index	Facial erysipelas (n=24)			Lower-limb erysipelas (n=36)		
	Protein C, %	AT-III,%	fvW,%	Protein C, %	AT-III,%	fvW,%
Days 1-3 of the disease (point 1)	$94.1 \pm 6.0^{***}$	$91.8 \pm 2.5^*$	$187.0 \pm 4.2^{**}$	$81.9 \pm 4.9^{***}$	$87.6 \pm 2.5^*$	$190 \pm 2.0^{**}$
Days 4-6 of the disease (point 2)	$119.6 \pm 3.1^{***}$	$96.4 \pm 0.8^{**}$	$183.0 \pm 2.7^{**}$	$103.0 \pm 3.2^{***}$	$91.3 \pm 1.7^{***}$	$187.6 \pm 2.9^*$
Days 7-10 of the disease (point 3)	$129.0 \pm 6.4^*$	$91.4 \pm 2.2^*$	$175.6 \pm 6.2^*$	$134.5 \pm 4.7^*$	$89.6 \pm 1.5^*$	$180.0 \pm 2.8^*$
Days 11-15 of the disease (point 4)	$153.0 \pm 4.4^*$	$93.0 \pm 1.1^*$	$174.8 \pm 4.4^*$	$139.0 \pm 6.7^*$	$89.4 \pm 3.2^*$	$176.0 \pm 3.1^*$
Follow-up (in 5 months)	-	-	-	$106.4 \pm 10.2$	$93.0 \pm 1.2^*$	$167.0 \pm 3.7^*$
Normal subjects (n=32)	$100 \pm 5.0$	$97.3 \pm 0.38$	$150.4 \pm 3.9$	$100.0 \pm 5.0$	$97.3 \pm 0.38$	$150.4 \pm 3.9$

Note: \*-significant difference with the control value; \*\*- reliable differences between the groups ( $p < 0.05$ ).

**Table 1:** Changes in protein C, AT-III and fvW levels in lower-limb and facial erysipelas.

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 disfibrinogenemia (thrombin

time, functional platelets' activity and D-dimer concentration) in our patients with the lower-limb and facial erysipelas:

- The activation of coagulation cascade during the acute stage of the disease - the shortening of thrombin time to  $12.2 \pm 0.5$  sec in facial erysipelas and to  $10.9 \pm 0.36$  sec in leg erysipelas during the days 1-3 of the disease ( $p < 0.05$  with control index of  $14.6 \pm 0.26$  sec), suggesting the presence of active processes of thrombin and fibrin formation in the blood flow;
- The activation of the external coagulation pathway - the lengthening of prothrombin time to  $15.4 \pm 0.5$  sec in lower-limb erysipelas and to  $12.2 \pm 0.7$  sec in facial erysipelas (normal subjects  $10.9 \pm 0.14$  sec,  $p = 0.033$  with the control value), the decrease of prothrombin index and the increase of international normalized ratio (INR), (Table 2);
- The activation of the internal coagulation pathway - the lengthening of aPTT:  $39.5 \pm 1.4$  sec (point 1) at admission and  $37.4 \pm 1.3$  sec during the recovery period in leg erysipelas (Table 2). In facial erysipelas the baseline aPTT was shortened:  $28.5 \pm 1.3$  sec ( $p < 0.05$  with the control value:  $33.7 \pm 0.66$  sec) and lengthened to  $45.1 \pm 2.7$  sec ( $p = 0.025$ ) by the end of the 1st week of the disease;
- The disfibrinogenemia - the shortening of thrombin time with the activation of the process of fibrin polymerization and the appearance of a great amount of D-dimers in the patient's blood.

During the 1st week of the disease, the fibrinogen level was higher (than during the 2nd week) in patients with erysipelas localization on the lower limb in comparison with the patients with facial erysipelas (Day 1-6 of the disease  $-8 \pm 2.3$  g/l leg vs  $5.9 \pm 1.8$  g/l face,  $p = 0.008$ , Day 7-15 of the disease  $6.6 \pm 2.4$  g/l leg vs  $4.3 \pm 1.0$  g/l face,  $p < 0.0001$ ), and by 2.7 times higher than the control values ( $2.96 \pm 0.09$  g/l,  $p < 0.001$ ).

Higher level of fibrinogen, an acute-phase protein (together with AT-III, C-reactive protein, presepsin, procalcitonin, TNF-alpha and interleukin-6), can be considered as a more pronounced manifestation of inflammatory events [28,29,33,37]. Hence, the degree of disfibrinogenemia and the activation of the external and internal coagulation pathways were higher, when the erysipelatosus focus was located on the lower limb. The peak of hemocoagulation changes was registered at days 4-6 of the disease (point 2 of the study), (Tables 2 and 3). While fibrinogen level at days 1-3 of lower-limb erysipelas ( $7.7 \pm 0.38$  g/l) was higher than in facial erysipelas ( $6.53 \pm 0.49$  g/l) by 18%, the concentration of plasma D-dimers in patients with lower-limb erysipelas ( $399 \pm 46$  ng/ml) was more than twofold in comparison with the patients with facial erysipelas ( $160.2 \pm 41$  mg/ml), and by 27 times higher than in normal subjects ( $14.5 \pm 3.18$  ng/ml,  $p < 0.001$ ).

Changes by days/ Index	Facial erysipelas (n=24)					Lower-limb erysipelas (n=36)				
	PT, sec	INR	Prothrombin, %	TT, sec	aPTT, sec	PT, secv	INR	Prothrombin, %	TT, sec	aPTT, sec
Days 1-3 of the diseases (point 1)	$12.2 \pm 0.7^{**}$	$1.27 \pm 0.04^*$	$79.2 \pm 4.5^{**}$	$12.2 \pm 0.5^{**}$	$28.5 \pm 1.3^{**}$	$15.4 \pm 0.5^{**}$	$1.47 \pm 0.04^{**}$	$59.3 \pm 2.8^{**}$	$10.9 \pm 0.36^{**}$	$39.5 \pm 1.4^{**}$
Days 4-6 of the diseases (point 2)	$14.9 \pm 0.6^*$	$1.42 \pm 0.05^*$	$68.7 \pm 4.3^*$	$12.7 \pm 0.7^{**}$	$45.1 \pm 2.7^{**}$	$15.1 \pm 0.4^*$	$1.47 \pm 0.04^*$	$61.3 \pm 3.8^*$	$11.9 \pm 0.5^*$	$38.5 \pm 1.7^{**}$
Days 7-10 of the diseases (point 3)	$12.2 \pm 0.7^{**}$	$1.27 \pm 0.04^*$	$79.2 \pm 4.5^{**}$	$12.2 \pm 0.5^{**}$	$28.5 \pm 1.3^{**}$	$15.4 \pm 0.5^{**}$	$1.47 \pm 0.04^{**}$	$59.3 \pm 2.8^{**}$	$10.9 \pm 0.36^{**}$	$39.5 \pm 1.4^{**}$
Days 11-15 of the diseases (point 4)	$12.2 \pm 0.7$	$1.16 \pm 0.03$	$79.4 \pm 4.8^*$	$12.2 \pm 0.3^{**}$	$37.3 \pm 3.0^{**}$	$13.3 \pm 0.25^{**}$	$1.31 \pm 0.03^{**}$	$73.3 \pm 2.8^{**}$	$12.1 \pm 0.26^*$	$37.4 \pm 1.3^{**}$
Follow-up (in 5 months)	-	-	-	-	-	$11.2 \pm 0.3$	$1.1 \pm 0.02$	$93.2 \pm 2.9$	$13.4 \pm 0.15$	$30.4 \pm 1.0$
Normal subjects (n=32)	$10.9 \pm 0.14$	$1.11 \pm 0.02$	$98.2 \pm 1.8$	$14.6 \pm 0.26$	$33.7 \pm 0.66$	$10.9 \pm 0.14$	$1.11 \pm 0.02$	$98.2 \pm 1.8$	$14.6 \pm 0.26$	$33.7 \pm 0.66$

Note: x-significant difference with the control value; \*- Reliable differences between the groups facial erysipelas and lower-limb erysipelas,  $p < 0.05$ .

**Table 2:** Changes of the indices of hemostasis' plasmatic element in facial and lower-limb erysipelas.

These differences in D-dimers blood concentration allowed us to conclude that the processes of intravascular coagulation in lower-limb erysipelas are more intense, as a necessary condition for the increase of D-dimer level consists in the presence of the clots of polymerized fibrin in the blood. The process of plasminogen transformation to plasmin, occurring on the principle "right here, right now", takes place inside these clots [28,32,35,41].

The increase of D-dimer level and the decrease (expenditure) of alpha-2-macroglobulin are also suggestive of a potent local activation of the system of proteolytic enzymes (plasmin, matrix metalloproteinases, neutrophil elastase), inducing the process of the erosion, ulcer and necrosis formation in the infectious inflammation focus [11,28-31].

Changes by days/Index	Facial erysipelas (n=24)				Lower-limb erysipelas (n=36)			
	Fibrinogen, g/l	D-dimer, ng/ml	CRP, mg/l	$\alpha$ 1-AT orosomucoid, %	Fibrinogen, g/l	D-dimer, ng/ml	CRP, mg/l	$\alpha$ 1-AT orosomucoid, %
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: 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 disfibrinogenemia (fibrinogen and D-dimer concentration) and the level of acute-phase proteins in facial and lower-limb erysipelas.

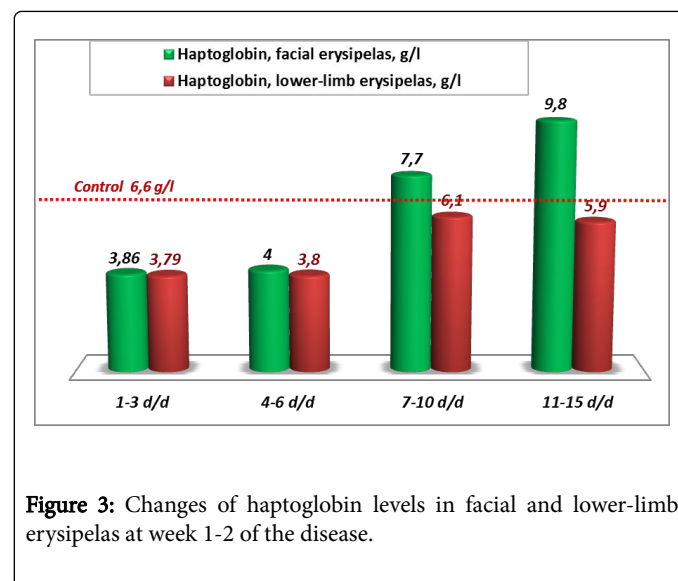
Starting from the second week of the disease, the levels of acute-phase proteins (fibrinogen, CRP,  $\alpha$ 1-antitrypsin ( $\alpha$ 1-AT) and orosomucoid) (=  $\alpha$ 1- acid glycoprotein) decreased with the improvement of patients' condition (Table 3).

Disfibrinogenemia, activated external and internal coagulation pathways, as well as increased level of acute-phase proteins found in our study confirm a close relation between the systemic inflammatory response and the compromised hemostasis with their mutual potentiation [1,2,3,25,28,30,33,37,40]. The risk of the development of hemorrhagic (erythematous-hemorrhagic, bullous-hemorrhagic) erysipelas was significantly higher, when the focus of inflammation was located on the legs, than on the face (OR=9.88) (2,34,73,81). The risk of severe forms of erysipelas development on the lower limb (erythematous-bullous, erythematous-hemorrhagic, bullous-hemorrhagic) also was higher in comparison with the face (OR=4.9) [1,5,16].

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 fvW (interaction with the platelet GPIb receptors) and of fibrinogen (interaction with the platelet GPIIb/IIIa receptors), the processes of platelet adhesion and aggregation are significantly enhanced [27,28,41,42,46].

In our study, the ADP-induced platelet aggregation was minimal at days 1-3 of the disease: 41.7 ± 4% in facial erysipelas and 64.8 ± 3.9% in leg erysipelas (p<0.05), which is statistically lower in comparison with platelets' functional activity in normal subjects (76 ± 3.1%, p<0.05). At days 11-15 of the disease, there was a tendency for the recovery of platelets' functional activity (71.7 ± 3.4% in lower-limb erysipelas and 59 ± 3.2% in facial erysipelas). The number of platelets increased in both groups: from 224 ± 10 × 10<sup>9</sup>/l (point 1) to 408 ± 21 × 10<sup>9</sup>/l (point 3), (p=0.002) in leg erysipelas and from 234 ± 30 × 10<sup>9</sup>/l to

317 ± 29 × 10<sup>9</sup>/l (p=0.04) in facial erysipelas. Hence, the recovery of the number and the functional activity of the platelets in erysipelas occurred by the end of the second week of the disease.



**Figure 3:** Changes of haptoglobin levels in facial and lower-limb erysipelas at week 1-2 of the disease.

In erysipelas, the cells' elasticity decreased twofold (aggregation with PS), while the deformability (aggregation with LaCl<sub>3</sub>) increased by 37% (Table 4). We used aggregometer Biola to measure the size of erythrocytic aggregates and the height of aggregation curve simultaneously with two types of inductors (LaCl<sub>3</sub> and PS). The aggregates were 3.6 times bigger, and the degree of aggregation by 7.8 times higher with LaCl<sub>3</sub> than with PS. With the addition of LaCl<sub>3</sub>, the erythrocytes of erysipelas patients interacted faster (3-5 minutes), and formed the blocks, that quickly precipitated to the bottom of the test-tube forming large conglomerates apparent to the naked eye [16].

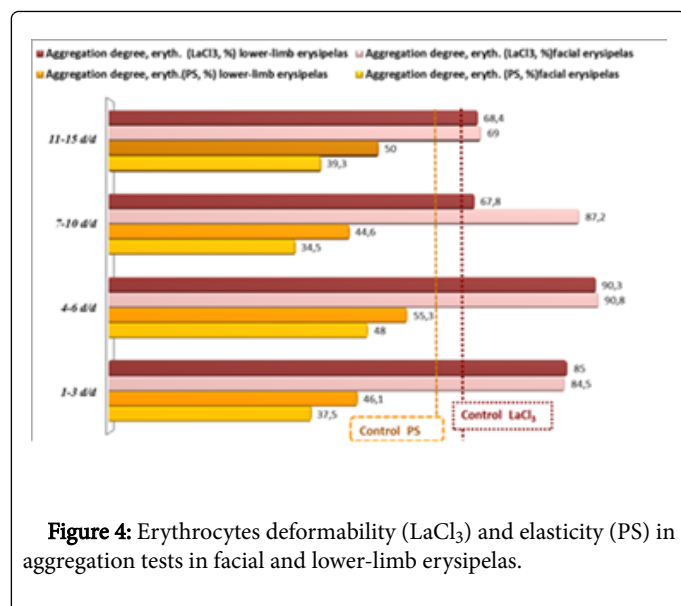
Other authors also have noted erysipelas-related changes of the erythrocyte membrane with cells' form transformation from concave-discoid to spherical [4,14,18,47]. Our experiments confirm that with the addition of lanthanoid, the erythrocytes of erysipelas patients undergo a conformational rebuilding (the destruction of membrane's cytoarchytectonics), which leads to fast adhesion between the cells and to the formation of cell conglomerates. Thus, the aggregation with LaCl<sub>3</sub> helps to reproduce the picture of erysipelas-associated intravascular hemolysis in vitro. Protamine sulfate does not induce

conformational rebuilding of the membrane. It displaces, in a stepwise manner, various previously adsorbed substrates from the erythrocyte membrane, with their subsequent replacement by native proteins. This is accompanied by the repolarization of the erythrocyte membrane. With the loss of negative charge of the membrane that prevents cells adhesion to each other, the erythrocytes form the rouleaux. Visually, the aggregates on the PS are smaller and softer, and the aggregation reaction develops by several times slower ( $\geq 10$  minutes), than on the lanthanoid [18,38].

	Changes by days/Index	Days 1-3 of the disease (point 1)	Days 4-6 of the disease (point 2)	Days 7-10 of the disease (point 3)	Days 11-15 of the disease (point 4)	Follow-up (in 5 months)	Normal subjects (n=32)
<b>Facial erysipelas (n=24)</b>	Platelets, $\times 10^9/l$	234 $\pm$ 30.0	249 $\pm$ 9.4	296 $\pm$ 39	317 $\pm$ 29	-	250 $\pm$ 5.2
	ADP -aggregation degree, platelets, %	41.7 $\pm$ 4***	67.2 $\pm$ 5.1	47 $\pm$ 2.6***	59 $\pm$ 3.2***	-	76 $\pm$ 3.1
	Erythrocytes, $\times 10^{12}/l$	4.5 $\pm$ 0.08*	4.7 $\pm$ 0.08	4.39 $\pm$ 0.08	4.3 $\pm$ 0.2	-	4.5 $\pm$ 0.13
	ESR, mm/hour	20.4 $\pm$ 1.8***	18 $\pm$ 2.8***	26.3 $\pm$ 5.8***	16.5 $\pm$ 2.3*	-	< 10
	LaCl <sub>3</sub> -aggregation degree: erythrocytes, %	84.5 $\pm$ 6.4***	90.8 $\pm$ 5.3***	87.2 $\pm$ 3.6***	69 $\pm$ 7.3	-	66.4 $\pm$ 4.2
	PS -aggregation degree: erythrocytes, %	37.5 $\pm$ 3.7***	48 $\pm$ 3.1***	34.5 $\pm$ 1.7***	39.3 $\pm$ 3.9***	-	62 $\pm$ 4.9
	Haptoglobin, g/l	3.86 $\pm$ 0.26***	4.0 $\pm$ 0.4**	7.7 $\pm$ 1.2***	9.8 $\pm$ 4***	-	6.6 $\pm$ 0.5
<b>Lower-limb erysipelas (n=36)</b>	Platelets, $\times 10^9/l$	224 $\pm$ 10	253 $\pm$ 14	314 $\pm$ 12.8	408 $\pm$ 21	-	250 $\pm$ 5.2
	ADP -aggregation degree, platelets, %	64.8 $\pm$ 3.9***	68.2 $\pm$ 4.3	53.7 $\pm$ 3.0***	71.7 $\pm$ 3.4*	52.7 $\pm$ 5.5*	76 $\pm$ 3.1
	Erythrocytes, $\times 10^{12}/l$	4.7 $\pm$ 0.06*	4.6 $\pm$ 0.08	4.37 $\pm$ 0.14	4.5 $\pm$ 0.1	-	4.5 $\pm$ 0.13
	ESR, mm/hour	33.5 $\pm$ 3.9***	35.2 $\pm$ 3.7***	53.7 $\pm$ 8.5***	33.7 $\pm$ 6.4***	-	< 10
	LaCl <sub>3</sub> -aggregation degree: erythrocytes, %	85 $\pm$ 4.5***	90.3 $\pm$ 4.8***	67.8 $\pm$ 5***	68.4 $\pm$ 4.9	63.0 $\pm$ 6.4	66.4 $\pm$ 4.2
	PS -aggregation degree: erythrocytes, %	46.1 $\pm$ 2.7***	55.3 $\pm$ 5.0	44.6 $\pm$ 3.4***	50.0 $\pm$ 6.0***	36.7 $\pm$ 3.4***	62 $\pm$ 4.9
	Haptoglobin, g/l	3.79 $\pm$ 0.3***	3.8 $\pm$ 0.4**	6.1 $\pm$ 0.8	5.9 $\pm$ 0.6	5.4 $\pm$ 0.8	6.6 $\pm$ 0.5

Note: ESR - erythrocyte sedimentation rate; \*— 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.



**Figure 4:** Erythrocytes deformability (LaCl<sub>3</sub>) and elasticity (PS) in aggregation tests in facial and lower-limb erysipelas.

## Conclusion and Recommendations

Laboratory studies confirmed the hypothesis that lower-limb erysipelas is complicated by clinical hemorrhagic manifestations by 3.9 times more commonly, than facial erysipelas.

The results of determination of the markers of hemostatic endothelial function in patients with erysipelas suggest the disturbances of endothelium-related regulation of hemostasis, as judged by antithrombotic (decreased levels of protein C and of antithrombin III), as well as by adhesive indices (high level of von Willebrand factor).

Increased level of D-dimer and decreased level of alpha-2-macroglobulin suggest potent activation of the system of proteolytic enzymes (plasmin, matrix metalloproteinases, neutrophil elastase), with 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 CVI in leg erysipelas, but also serves as a marker of the development of DIC-like syndrome.

Documented protein C deficiency can serve as an indication for the solution of the question of antithrombotic therapy application. Maximally early start of the replacement therapy (with recombinant protein C) can contribute to the delimitation of the area of inflammatory focus and to the decrease of the severity of local proteolysis reactions in erysipelas.

We found the signs of intravascular (latent) hemolysis and of disturbed rheological properties of erythrocytes – 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 red blood cells flexibility and to the improvement of rheological properties of the blood (e.g., Pentoxifylline).

The risk of hemorrhagic complications in lower-limb erysipelas is higher, than in facial erysipelas, OR = 9.88 [2,7,34].

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