

# THE SIGNIFICANCE OF PLATELET-DERIVED GROWTH FACTOR CONCENTRATIONS IN ENDOTHELIOTROPIC NATURAL FOCI INFECTIONS

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## ABSTRACT

In recent years, the clinical symptomatology of Astrakhan spotted fever and coxiellosis has worsened. The pathogenesis of these two natural focal, endothelium-dependent infections is rather complicated, multifactorial and not properly studied. The current clinical symptomatology is characterized by severe intoxication syndrome, frequently signs of multiple organ failure, development of hemocoagulation and vascular complications [3, 4, 5]. To date, no early markers of endothelial vascular damage have been proposed for use in practical health care, which enable accurately assessing the severity degree and predicting the further course of the rickettsiae and coxiellosis.

Platelet-derived growth factor (PDGF), which is widely used in the diagnosis and prognosis of cardiovascular diseases, various injuries and diabetes mellitus, may become such a marker.

**Keywords:** Astrakhan fever rickettsiae, Astrakhan spotted fever, Q fever, coxiellosis, platelet-derived growth factor (PDGF), endothelial damage.

## INTRODUCTION

In spite of numerous investigations of epidemiology, clinical, diagnostic and treatment issues of Astrakhan spotted fever (ASF) and coxiellosis, the morbidity of these infections is still high and stable. In recent years there have appeared severe forms of SARS, including lethal, which generally correlates with global trends in the aggravation of the clinical course of tick-borne spotted fevers [1, 2, 9]. This can be explained by the sensitivity of rickettsiae to global changes in the ecological environment, mediated by anthropurge human activity and changing the living conditions of pathogens in natural foci. Increased adaptive properties of rickettsiae and coxsies probably lead to an increase in their infectiousness, which is reflected in the clinical picture of patients.

The modern clinical symptomatology of coxiellosis is widely discussed by the medical community, which actualizes the search for early predictors of severe course and complications of these infections [9, 12].

Since in LPS and coxiellosis the main pathogenetic mechanism determining the severity of infections is endothelial damage of the microcirculatory bed, with the development of nonspecific vasculitis, platelet-derived growth factor (PDGF) may become such a predictor.

The source of PDGF in serum is platelet alpha granules, although macrophages and endothelial cells can also produce it. Receptors to PDGF are located in the vascular wall on fibroblasts and smooth muscle cells [14, 17]. In macrophage monocytes and fibroblasts PDGF stimulates chemotaxis, proliferation and expression of many genes [7]. PDGF significantly increases the inflow of immune cells and fibroblasts into the damaged

tissues, stimulates collagen production and thereby reduces healing time. Under the influence of PDGF on endothelial cells there is a regulation of their division and proliferation, formation of new cells, which generally affects the healing process in all endotheliotropic infections [17].

Thus, PDGF is involved in the regulation of alteration, exudation and proliferation of acute inflammation. Increased PDGF values in serum can be associated with hemorrhage, anemia, thrombocytopenia, and inflammatory paresis of the vascular wall [13, 15, 16].

PDGF is the best studied representative of the group of protein growth factors. PDGF synthesis and processing is carried out in megakaryocyte cells of bone marrow, platelet precursors and is stored in  $\alpha$ -granules of platelets. While PDGF is inside platelets, it is inaccessible to other cells; however, upon interaction with thrombin, platelet activation occurs followed by release of its contents into the serum [7]. Platelets are the main source of PDGF in the organism [10, 18].

It is known that rickettsiae, unlike other intracellular pathogens, effectively infect bone marrow dendritic cells, with the possibility of localization in them even after phagocytosis [19].

The general damaging effect on vascular endothelium, synthesis of endothelium-dependent clotting factors, intensifies platelet synthesis and aggregation leading to increased concentration of PDGF. This may reflect the severity of vascular damage in endotheliotropic tick-borne fevers and serve as a predictor of the severe course of these infections. And if in the phase of endothelial alteration by rickettsiae and CYC with coxies, platelet hypersecretion and hyperaggregation are protective mechanisms providing hemostasis. Whereas in the phase of exudation and proliferation, platelet and PDGF functions universally solve antibacterial, regenerative and proliferative processes [6, 11]. Accordingly, PDGF deficiency can affect insufficient endothelial regeneration, involvement of new cells and spreading of infectious agents [20, 21].

All the above defined is the purpose of the present study.

**Objective of the investigation:** to study clinical diagnostic and prognostic significance of platelet-derived growth factor concentrations in patients with Astrakhan spotted fever and coxiellosis depending on infection degree and severity.

## MATERIALS AND METHODS

The study had a prospective, cohort character. Ethical principles of the Helsinki Declaration of the World Medical Association (1964, 2000) were observed in work with the patients. The scientific study was approved by the local ethical committee of Astrakhan State Medical University, (Russia) (protocol No 3, 2018). Voluntary informed consent was obtained from all patients. The platelet-derived growth factor study group included 433 patients, 223 of whom were patients with AFS, 210 of whom were observed with the diagnosis of Q fever (coxiellosis).

Blood sampling to study PDGF concentrations was performed during the period of fever, on day 5-6 of the disease.

The age of the examined patients ranged from 18 to 88 years. The average age was  $48.25 \pm 33.7$ . Among the patients men prevailed - 229 persons (52,86%). There were 73 patients with ASF of severe course and 150 of moderate course. Among the patients with coxiellosis of a severe course there were 46 persons, of a moderate course 164.

For quantitative determination of platelet-derived growth factor-BB (PDGF-BB) we used Human PDGF-BB Elisa immunoassay kit (Bender MedSystems. Supplier in RF, BioChemMac, Moscow).

Antibodies to human PDGF were sorbed at the bottom of microtiter plate wells. PDGF of standards and control samples were bound to antibodies at the bottom of the wells. The injected biotin antibody conjugate to PDGF antibodies bound to PDGF-BB conjugated to the first antibodies. After incubation and washing, the unbound biotin conjugate was removed from the wells, and the conjugate, streptavidin, was added to the wells. After the second incubation, the substrate solution was added to the vacated wells and interacted with the enzyme complex to stain the medium. The staining intensity was measured at 450 nm and was directly proportional to the concentration of PDGF-BB present in the samples tested. The concentration of PDGF-BB in the samples was determined using a standard curve constructed using seven dilutions of the standard prepared.

The minimal detectable concentration of human PDGF-HB was 4,6 pg/ml, which was determined as the concentration of the analyte. The method was used to detect both natural and recombinant human PDGF-HB.

The results were statistically processed using StatTech v. 1.2.0 (developed by Stattech, Russia) and IBM SPSS Statistics 26.0 (USA); Excel 10.

Statistical analysis of the results was performed using variation statistics, calculating mean values (M),

standard deviation, or arithmetic mean error ( $\sigma$ ). Significance of differences between separate characteristics was estimated by Student's t-test with significance of differences at  $p < 0,05$ .

We compared populations by qualitative characteristics by means of confidence interval (CI) and analysis of arbitrary correlation tables using chi-square test. Correlation coefficient between separate dependent variables was calculated by Pearson correlation and regression analysis, and the closeness (strength) of the relationship was determined using the Cheddock scale. Significance of differences in the studied features was determined at  $p < 0,05$ .

## RESULTS AND DISCUSSION:

Based on the data presented in the table, PDGF-BB, as a cytokine released during endothelial damage, was twice as high in patients with a severe course of ASF, compared with a moderate course –  $10503,75 \pm 1623,0$  versus  $5189,5 \pm 1210,37$  ( $p < 0,01$ ,  $t = 2,62$ ). At the same time the level of thrombocytopenia was statistically significantly more expressed in patients with severe form in comparison with the moderate form –  $118,24 \pm 40,79$  VS  $262,25 \pm 50,2$  ( $p < 0,05$ ,  $t = 2,23$ ). Level of leukocytosis, CRP also prevailed with statistical significance in patients with severe course of infection –  $p < 0,05$ ,  $t = 2,25$  and  $p < 0,05$ ,  $t = 2,06$ , respectively. Fibrinogen level at severe form was statistically significantly lower in comparison with the average form of the disease:  $2,47 \pm 0,58$  VS  $4,32 \pm 0,38$  –  $p < 0,01$ ,  $t = 2,67$  (Table 1).

*Table 1. The values of platelet-derived growth factor (PDGF-BV), platelets, leukocytes, CRP, fibrinogen in patients with Astrakhan spotted fever of severe and moderate severity*

Degree of severity of the ASF disease	PDGF-BV pg/ml	Platelets (x109; g/l) M+m	Leukocytes (x109; g/l) M+m	SRB mg/L	Fibrinogen g/l
APL severe course (n=73)	$10503,75 \pm 1623,31^{**}$	$118,24 \pm 40,79^*$	$13,87 \pm 2,51^*$	$48,94 \pm 10,12^*$	$2,47 \pm 0,58^{**}$
Moderate degree ASF (n=150)	$5189,5 \pm 1210,37^{**}$ $p < 0,01$ , $t = 2,62$	$262,25 \pm 50,2^*$	$6,7 \pm 1,97^*$	$19,7 \pm 9,97^*$	$4,32 \pm 0,38^{**}$
Credibility	$p < 0,01$ , $t = 2,62$	$p < 0,05$ , $t = 2,23$	$p < 0,05$ , $t = 2,25$	$p < 0,05$ , $t = 2,06$	$p < 0,01$ , $t = 2,67$

*Note: "\*" sign indicates reliability of differences  $p < 0,05$ ; "\*\*\*" sign  $p < 0,01$ ; "\*\*\*\*" sign –  $p < 0,001$*

There was a correlation between PDGF-BB, platelets, leukocytes, CRP and fibrinogen. PDGF-BB level was in direct correlation, statistically significant ( $p < 0,05$ ) relationship between leukocyte level, CRP level in patients with severe ASF. In patients with moderate infection the correlation between PDGF-BB index and leukocytes, although high, was not statistically significant ( $p > 0,05$ ). It was noteworthy that not all patients with severe and moderate ASF had higher than normal increase in the results of traditional methods of investigation.

In all patients with severe and moderate ASF a statistically significant inverse correlation on Cheddock's scale of strong severity between PDGF-BB index, platelet and fibrinogen levels was revealed, which proves the involvement of PDGF-BB in pathogenesis of endothelial dysfunction in ASF.

It seems that the degree of direct toxic damage of endothelium by rickettsiae leads to a more significant release of PDGF-HB, correlates with the degree of disease severity, intensity of petechial rash caused by statistically significant decrease in platelets and fibrinogen.

We also studied PDGF-BB in patients with Q fever in patients with severe and moderate infection (Table 2).

*Table 2 - Platelet-derived growth factor (PDGF-BB), platelets, leukocytes, CRP, and fibrinogen in patients with Q fever with moderate to severe course*

Severity of coxivirus disease	PDGF-BV pg/ml	Platelets (x10 <sup>9</sup> ; g/l) M+m	Leukocytes (x10 <sup>9</sup> ; g/l) M+m	SRB mg/L	Fibrinogen g/l
Fever-Ku severe course (n=46)	10378,94±4583,881*	101,87±20,4 **	13,32±4,12*	45,2±11,4	3,63±0,81
Coxiellosis of moderate severity (n=164)	4895,78±1754,22* p<0,05, t=1,86	268,5±64,2 ** p<0,01, t=2,33	4,95±1,78 * p<0,05, t=1,96	25,4±10,79 p>0,05 t=1,28	4,06±0,32 p>0,05 t=0,48

Note: "\*" sign indicates reliability of differences  $p<0,05$ ; "\*\*" sign  $p<0,01$ ; "\*\*\*" sign -  $p<0,001$

When comparing parameters of PDGF-BB, platelets, leukocytes, CRP and fibrinogen in patients with Q fever depending on severity degree, statistically significant differences concerned only parameters of PDGF-BB ( $p<0,05$ ), platelets ( $p<0,01$ ) and leukocytes ( $p<0,05$ ). While analysis of CRP and fibrinogen parameters in patients with coxiellosis showed no statistically significant differences -  $p>0,05$ . In our opinion, this indicates a higher diagnostic value of PDGF-BB determination as a marker of inflammatory vascular, endotheliotropic infection.

There was a correlation between PDGF-BB indices and traditional examination results of patients with Q fever - level of leukocytes, platelets, CRP and fibrinogen. Similarly to the established correlation relation of PDGF-BB index with platelets and fibrinogen in ALS, inverse proportional correlation relation of significant degree of severity was established in patients with both severe and moderate course of coxiellosis -  $p<0,05$ .

In our opinion, the similarity of correlation parallels of the studied infections is obvious and explained by common pathogenetic mechanisms of rickettsia and coxies penetration into the endothelium of the vascular bed, with the development of pathognomonic changes in organs and tissues of patients.

Establishment of strong direct and inverse statistically significant correlations between the studied marker and the results of traditional examination of patients (leukocytes, platelets, fibrinogen and CRP) depending on the severity of AFS and coxiellosis gives the grounds to conclude about diagnostic value of PDGF-BB determination in these patients. While traditional methods of investigation not always correlated with the severity of infection, determination of PDGF-BB statistically significantly increased even at intermediate degrees of infection severity in comparison with normal values.

## CONCLUSION

PDGF-BB concentration was studied in patients with AFS and coxiellosis of moderate and severe course in correlation with the results of traditional methods of investigation. PDGF-BB proved to be a sensitive marker for vascular endothelium damage in AFS and coxiellosis. It can be used as an additional diagnostic criterion allowing specifying the infection degree and severity, estimating endothelial dysfunction expression and, thus, predicting the disease progression.

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## CONFLICT OF INTEREST

The authors declare no obvious and potential conflicts of interest related to the publication of this article.

## AUTHORS' CONTRIBUTIONS

Vasil'kova V.V. - Material collection, statistical processing of results, manuscript writing; Kantemirova B.I. - research design, general editing of the article; Zhidovinov A.A. - methodological and consulting assistance.

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