ORIGINAL ARTICLE

VASCULAR-PLATELET HEMOSTASIS OF INJURED PATIENTS: PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

The aim: We study vascular-platelet hemostasis peculiarities in patients with severe trauma.

Materials and methods: We included 50 patients, who were divided into control (n=15) and study (n=35) groups. The control group included patients without traumatic injuries, study group – patients with severe trauma. The study group was divided into the I subgroup (patients received 1 g tranexamic acid IV at the prehospital stage), and the II subgroup (1 g tranexamic acid IV after hospital admission).

Results: The main changes in the I subgroup started on the 3rd day, while in the II subgroup – on the 1st day. Patients of both subgroups on the 1st and 3rd days had a normal number of platelets in venous blood, however, on the 3rd day, there was a decreasing level of discocytes whereas the level of discoechinocytes, spherocytes, spheroechinocytes, and the sum of active forms of platelets were increased in comparison with the control group ($p<0.05$).

Conclusions: The changes in vascular-platelet hemostasis in patients appeared in the I subgroup on the 3rd day, while in the II subgroup – on the 1st day. For the I subgroup was the decreasing level of discocytes, whereas the level of discoechinocytes, spherocytes, spheroechinocytes, and the sum of active forms of platelets were increased. For the II subgroup on the 1st day, there was an increasing sum of active forms of platelets, on the 3rd day – the level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes, and the sum of active forms of platelets were increased.

KEY WORDS: coagulopathy, severe trauma, platelets activation, vascular-platelet hemostasis

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INTRODUCTION

Traumatic injuries are the third-largest cause of mortality in the general population in Europe and the leading cause of mortality in young patients [1]. Coagulopathy is a common complication after injury and develops independently from iatrogenic, hypothermic, and dilutional causes [2]. Combined tissue injury and shock result in hemostatic failure, which has been identified as a multidimensional molecular, physiologic and clinical disorder termed trauma-induced coagulopathy (TIC) [3]. Blood loss associated with acute traumatic coagulopathy is a leading cause of death following injury [4]. TIC is associated with increased early transfusion requirements, the development of organ failure, and mortality [5]. Owing to the innate crosstalk between coagulation and inflammation, there are widespread adverse downstream inflammatory and immune consequences associated with early trauma coagulopathies, including organ dysfunction and thromboembolic complications [4].

Although, all modern research is devoted to coagulation factors, platelets play an important role in thrombus formation. Careful assessment of platelet dysfunction is hampered by the technical complexity of existing techniques for studying platelet function under conditions that would meet the conditions of the human body. It is also unclear how to use and interpret vascular hemostasis in patients with trauma coagulopathy. Understanding the biology of TIC is of utmost importance, as it is often responsible for uncontrolled bleeding, organ failure, thromboembolic complications, and death.

THE AIM

The aim was to study vascular-platelet hemostasis peculiarities in patients with severe trauma, to single out these changes and find out primary hemostasis pathophysiology specifics in case of trauma-induced coagulopathy. The hypothesis was that morphological changes would not be detected in the first day after receiving the injury in patients with severe trauma.

MATERIALS AND METHODS

In January 2021 – October 2021 it was conducted the prospective observation study at the Department of

Anesthesiology and Intensive Care, Danylo Halytsky Lviv National Medical University; Department of Anesthesiology and Intensive Care at the Municipal Non-Profit Enterprise "8th City Clinical Hospital of Lviv". The reserch was conducted in accordance with the requirements of the Helsinki Declaration of the World Medical Association, the Council of Europe Convention on Human Rights. The reserch was approved by the Bioethics Commission of Danylo Halytsky Lviv National Medical University, protocol No.7, September 20, 2021. Patients were included to the reserch after signing an informed consent.

Criteria for inclusion in the study were: consent of the patient or his legal representatives to participate in the study, polytrauma, administration of tranexamic acid on the first day after injury. Criteria for exclusion from the study were: the patient's refusal to participate in the study, medical history of congenital pathology of the hemostasis system, the agonal state of the patient.

Study included 50 patients aged 19 to 55 years. All patients were divided into 2 groups. First, the control group, included 15 patients of the therapeutic department with trauma, without preconditions for changes and in the absence of laboratory-confirmed disorders in the hemostasis system. Second, the study group included 35 patients with a diagnosis of "polytrauma" (severe trauma), who were admitted to the Hospital Anesthesiology and Intensive Care department. Next, patients with polytrauma were divided into two subgroups, depending on they received tranexamic acid in the prehospital stage or after hospitalization.

The severity of the patients' condition was assessed by the Injury Severity Score (ISS). We studied: platelet count and hematocrit; indicators of vascular-platelet hemostasis (intravascular platelet activation, platelet aggregation induced by adrenaline and adenosine diphosphate) were determined by turbinometric method, using a phase contrast microscope. The main stages of the study were: prehospital stage (1st hour after injury), 1st, 3rd, 5th day after admission. Also we analyzed the volume of crystalloids and colloids at all stages of the study, the use of tranexamic acid in the prehospital stage and on the 1st day after admission to treatment, the volume of fresh frozen plasma and erythrocyte mass on the 1st, 3rd, 5th that day from the moment of admission to treatment. The presence of disseminated intravascular coagulation syndrome was performed by the ISTH Scale (International Society on Thrombosis and Haemostasis Scale) using a medical calculator [7]. We used Kruskal-Wallis test. The obtained data were checked for the normality of the distribution, they did not correspond to it, therefore the description was carried out using statistical indicators for data that do not correspond to the normal distribution.

RESULTS

The severity of patient`s state was assessed by the ISS scale and we found that, less than 9 points were not received by any of the patientsof the main group, 9–15 points were evaluated by 17 % of patients with polytrauma, 16–25 points were 71 % patients, more than 25 points –12 % of patients. The severity of patients` state is given in Table I-III.

We found no significant differences in age, body weight, body mass index (BMI) between patients in control and study groups.

Changes of vascular-platelet hemostasis in patients with severe trauma (study group) (tabl 3) at all stages of the study had significant differences from those in the control group. Main changes in I subgroup started on 3rd day, while in II subgroup – on 1st day. Patients of both subgroups on 1st and 3rd days had normal number of platelets in venous blood, however, on 3rd day there were decreasing level of discocytes, whereas level of discoechinocytes, spherocytes, spheroechinocytes and sum of active forms of platelets were increased. On the other hand, in II subgroup against the background of normal count of platelets on 1st day there was increasing sum of active forms of platelets, on 3rd day – level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes and the sum of active forms of platelets were increased.

After analyzing the frequency of transfusion of erythrocyte mass and fresh-frozen plasma, it was found that transfusion throughout treatment was performed in 15 cases out of 25, which was 60 % of all cases of polytrauma. In 9 cases out of 25 –transfusion was performed on the first day, which is 36 %. The ratio of erythrocyte mass to fresh-frozen plasma volume on the first day of treatment was 1:1, erythrocyte mass was 475±35 ml, and fresh-frozen plasma volume was 458±15 ml. Analyzing the literature, it should be noted that coagulopathy is one of the most common complications of polytrauma. The reason is a combination of shock caused by bleeding, tissue damage–associated with the regulation of thrombomodulin, the generation of throm-bin-thrombomodulin complex, activation of anticoagu-lant and fibrinolytic systems [5]. Disorders of hemostasis after injury are associated not only with iatrogenic causes –impaired thrombin production and platelet function due to hypothermia, acidosis and dilution coagulopathy, and with modern hemostatic treatments dilution coagulopathy is rare. To induce clinical manifestations of trauma-induced coagulopathy, the trigger is tissuedamage (as a consequence, activation of the coagulation cascade, thrombin production and stimulation of anticoagulant pathways), which must be combined with tissue hypoperfusion (it is believed that this leads to contact, induced expression of thrombo-

Table I. Assessment of the severity of patients by Injury Severity Score (ISS)

ISS	Study group (n, %)
<9 points	-
9-15 points	6 (17%)
16-25 points	25 (71%)
> 25 points	4 (12%)

Table II. Anthropometric characteristics of patients (Me [Q1; Q3])

Parameters	Control group (n=15)	Study group (n=35)
Age, years	39.3 [29.1; 43.5]	36.6 [31.3; 44.8]
Body weight, kg	74.6 [63.4; 81.9]	74.9 [68.2; 84.5]
BMI, kg / m^2	24.9 [19.8; 27.4]	26.1 [21.3; 30.1]

Table III. Indicators of intravascular platelet activation (Me [Q1; Q3])

Indicators	Control group (n=15)	Study group (n=35)					
		1st day (d1)		3rd day (d3)		5th day (d5)	
		Subgroup					
		I (n=6)	II (n=29)	I (n=6)	II (n=29)	I (n=29)	II (n=29)
The number of platelets in the venous blood, 10^9/ l	200.7 [183; 213]	169 [141; 198]*	168 [136; 184]	161 [136; 184]*	140 [122; 151.4]	173.3 [151; 191]*	150 [133; 156]
Discocytes, %	81.4 [73; 93]*	81.6 [69.1; 94.5]	77.1 [63; 85]*	76.5 [65.3; 85.2]	74.5 [64; 83]*	82.2 [67.1; 93.4]	86.2 [67; 93]*
Discoechinocytes, %	8.4 [4.8; 8.8]	17.0 [12.1; 28.5]	18.2 [13.7; 24.1]	18.4 [11.3; 28.5]	18.8 [13.2; 23.9]*	12.1 [6.3; 24.4]	12.7 [8.8; 16.4]
Spherocytes, %	5.6 [3.8; 8.5]	1.6 [1.1; 6.5]	1.7 [1.1; 3.6]*	3,2 [2.3; 6.2]	3,6 [2.2; 6.1]*	3.0 [2.3; 7.7]*	3.2 [2.3; 8.6]*
Spheroechinocytes, %	3.0 [2.3; 5.6]	4.5 [2.1; 6.7]	4.6 [2.3; 8.6]	2.8 [1.3; 6.6]	3 .1 [1.3; 5.2]	1.1 [0.4; 5.8]*	1.5 [1.1; 3.6]*
Bipolar forms, %	0	0	0	0	0	0	0
The sum of the active forms of platelets, %	12.7 [10.1; 16.5]	17.3 [12.1; 25.3]	23 [21.1; 28.7]*	22.2 [15.1; 29.5]	24.2 [21.2; 30.4]*	19.3 [13.2; 27.3]	19.3 [11.2; 26.4]*
The number of platelets involved in the aggregates, %	14 [10; 18]*	3.2 [1.2; 6.4]*	3.4 [1.2; 6.5]	6.5 [4.3; 9.4]*	6.6 [3.2; 9.4]**	7.6 [4.2; 11.4]*	7.5 [4.4; 9.5]**

Note: * –$p<0.05$ compared with the control group of patients
** –$p<0.01$ compared with the control group of patients

modulin and endothelial protein C on the surface of the endothelium to activate protein C) [3]. Identifying distinct pathways implicated in TIC is critical to tailoring targeted resuscitation practices for improved outcomes after injury, and the practice of transfusing platelets in equal ratios to blood and plasma has become standard of care regardless of platelet count because platelets are known to play a pivotal role in normal coagulation and maintenance of endothelial integrity. The effect of trauma and shock on vascular platelet hemostasis remains unexplored [6], platelets in hemostasis in polytrauma play a critical role, and their low level predicts mortality [7]. Kornblith L. Z. et. al. demonstrated that the contribution of platelets to the strength of clots in trauma is higher than the contribution of fibrinogen.

In patients with trauma, decreased platelet responsiveness to ADP secondary to downregulation of platelet $P2Y_{12}$ receptor (it is a G-protein coupled receptor that binds adenosine diphosphate (ADP). Consequently down regulation of this receptor or antagonist blockade inhibits ADP-mediated platelet aggregation. Thrombin when bound to TM increases anticoagulant activity through activation of protein C. But thrombin-TM interactions also promote antifibrinolytic activity by thrombin-mediated activation of TAFI (thrombin-activatable fibrinolysis inhibitor). Activated TAFI interferes with plasminogen binding to fibrin clots, which is required for plasminogen conversion to plasmin by plasminogen activators [8]. Anemia caused by bleeding or dilution due to fluid resuscitation may also affect platelet adhesion.

DISCUSSION

Thus, our research confirmed the significant the role of platelets in the pathogenesis of post-traumatic coagulopathy. It was established that the level of discocytes decreases, the level of discoechinocytes, spherocytes, spheroechinocytes and the amount of active forms of platelets increase in I subgroup on 3rd day. For II subgroup on 1st day there was increasing sum of active forms of platelets, on 3rd day – level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes and the sum of active forms of platelets were increased. There is a little knowlagge about ascular-platelet hemostasis in trauma patients, but a similar study was conducted by George MJ et al. (2020), measuring the force of platelet contraction (as a platelet contraction test). Their results show that platelet hyperfunction is observed in trauma patients who survive, and platelet dysfunction in patients who died. [9]. Hofer V et al. (2019) was analyzed platelet function on Platelet Function Analyzer (PFA 100) with adenosine diphosphate (ADP) and epinephrine as activation factors. They research discovered, that approximately one quarter to one third of primarily admitted trauma patients without long-term anticoagulation medication showed a delayed platelet activation in the PFA-100 test. By considering all trauma patients an even higher rate can be expected [10]. The contribution of platelet disorders to trauma-induced coagulopathy is not sufficiently understood and these aspects should be the subject of further research and our research try to reveal the dark side of the issue of platelet disorders in severe trauma patients.

It is well known that vascular-platelet hemostasis begins with reflex spasm of arterioles, due to the release of platelets of catecholamines and seretonin, followed by adhesion and aggregation, synthesis, accumulation and secretion during activation of substances and the formation of the final platelet thrombus. Even before contact with unmasked collagen, platelets begin to change their form from discoid (D) to activated cells of discoechinocytes (DE), spherocytes (S) and/or spheroehfnocytes (SE) [11]. DE differs from D by the presence of single and short processes, which appear after activation within the first second, and are the result of internal pressure on the plasma membrane of actin filaments. S is a more spherical cell, and SE is a spherical cell with a larger number of long processes. During the contact phase, the processes of activated platelets interact with the elements of the basement membrane of the vascular wall. The direct contact of platelet processes with collagen and the contact of platelets with collagen through Willebrand factor are important. On the surface of D, under the influence of collagen, factor XI is activated without changing its form. Thus D, which is incapable of direct development of aggregation, secretion, refraction, can be subjected to receptor membrane activation and, accordingly, the altered forms are caused by substances for which there are specific receptors on the platelet membrane, thrombin, collagen, adenosine phosphate (ADP), serotonin and other agonists. The hemostatic activity of platelets appears with the conversion of D to DE, and DE is capable of both pronounced adhesion and aggregation, due to exposure in this phase on the plasma membrane of fibrinogen receptors. Platelet aggregation is caused by the appearance of substances of aggregates of platelet or non-platelet origin. The most important are ADP, which is released from damaged cells of the vascular wall, hemolyzed erythrocytes, platelets; thromboxane A, adrenaline, serotonin, platelet aggregation factor, thrombin. The appearance of appendages also promotes aggregation, increasing the likelihood of platelet collisions, which is necessary for this process. In the future, with the formation of a significant amount of SE, the aggregation activity decreases slightly and begins to develop refractoriness of the cell, which is most pronounced in SE. In traumatized patients, platelets lose aggregative function as part of acute coagulopathy, which develops within minutes of injury, increases bleeding, and has a major impact on the risk of multiple organ failure and mortality. The decrease in the ability of platelets to aggregation occurs in parallel with the increase in their procoagulant function.

The main number of studies of primary hemostasis were performed using thromboelastography. Although the question of early platelet dysfunction in coagulopathy due to polytrauma remains unclear, a number of studies suggest that attenuation of platelet stimulation to adenosine diphosphate agonism may be secondary. Fecher A, et al. were assessed platelet function in thrombus composition and stability [12] and found that platelet dysfunction is detected after serious injury and before significant fluid or blood injections. In our research we studied the functional ability of platelets induced by epinephrine and ADP, which can be extrapolated to conditions similar to platelet activation in a trauma patient.

Stalker et al. (2013), in a mouse model [13], described a hemostatic plug consisting of a "core" region of tightly packed platelets surrounded by an outer "shell" region of more loosely packed platelets. Whereas platelets in the core region are isolated from the plasma and exposed to high levels of thrombin and collagen, the shell region is exposed to circulating plasma and grows by platelet-ADP interactions. Some degree of inhibition along the ADP pathway may therefore be normal after

trauma to counterbalance widespread activation of procoagulant mechanisms; another finding supporting this hypothesis is that platelet inhibition along the ADP pathway increases clot sensitivity to tPA-mediated fibrinolysis. Alternatively, platelet assays may inherently select for more dysfunctional platelets because functional platelets were removed from the circulation and incorporated into clots [14].

In most patients with trauma-induced coagulopathy, the number of platelets in the blood remains activated, but paradoxically impaired aggregation reactions exvivo. This phenomenon is described as "platelet depletion" caused by trauma and shock [15]. This phenomenon driven by endothelial release of TF, platelet activating factor and vWF [16]. In our research we learned pathophysiological changes of vascular-platelet hemostasis in patients with severe trauma, our future research will be able to show how these changes affect the treatment of this patients.

STUDY LIMITATIONS

The presented fragment of the study aimed to examine whether the indicators of vascular-platelet and coagulation hemostasi in patients with trauma, compared with almost healthy individuals of the same age. In addition, the study was conducted on a very small sample size, which is insufficient to substantiate the role of vascular platelet hemostasis as a marker for post-traumatic coagulopathy. Prospects for further research include continu-ing to study the indicators of vascular platelet hemostasis, which, accordingly, may lead to the development of new early diagnostic and therapeutic measures for the prevention and treatment of post-traumatic coagulopathy and its consequences.

CONCLUSIONS

The main pathophysiological changes of vascular-platelet hemostasis in patients with severe trauma appeared in I subgroup on 3rd day, while in II subgroup – on 1st day. For I subgroup the specific changes were decreasing level of discocytes, whereas level of discoechinocytes, spherocytes, spheroechinocytes and sum of active forms of platelets were increased. For II subgroup on 1st day there was increasing sum of active forms of platelets, on 3rd day – level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes and the sum of active forms of platelets were increased. For both subgroups on 1st and 3rd days bipolar forms of platelets were not observed.

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The Authors declare no conflict of interest.

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