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THROMBOSE-FIBROUS COMPLICATIONS OF SYSTEMIC ENDOTHELIUM DAMAGE AT COVID-19

RESEARCH GROUP:

Dieieva Yuliia Valerievna
Doctor of Medical Sciences, Professor,
Head of the Department of Otorhinology
Bogomolets National Medical University, Ukraine

Makarova Natalia Nikolaevna
Candidate of Biological Sciences, Senior Lecturer,
Department of Biochemistry
Bogomolets National Medical University, Ukraine

Voroshylova Natalia Mikhailovna
Candidate of Biological Sciences, Major Researcher,
Department of Biochemistry
SI "O.S.Kolomiychenko Institute of Otolarynglology, NAMSU", Ukraine

Verevka Serhij Viktorovych
Doctor of Biological Sciences, Professor,
Head of the Department of Biochemistry
SI "O.S.Kolomiychenko Institute of Otolarynglology, NAMSU", Ukraine

Summary. *The analysis of literature data and our own research of lungs tissues of the persons who died owing to COVID-19 caused fibrosis testify to participation in this pathology of a cascade of disturbances of molecular and cellular levels. Viral damage to endothelial cells causes systemic damage to the vascular glycocalyx, which loses its clotting properties and releases significant amounts of blood clotting factors. The fibrin clot formed under such conditions is characterized by resistance to fibrinolysis and locally blocks blood vessels with the systemic development of endogenous intoxication. Destabilized proteins of the latter form micro- and nano-sized aggregates with a significant content of β-folded structures. This contributes to the increase of fibrin resistance to the proteolytic action of plasmin, causes the development of fibrosis of the tissues affected in this way, and leads to the failure of the functions of the relevant organs.*

Keywords*: hemostatic system, thrombosis, fibrosis, protein aggregation, COVID-19*

The formation of fibrous tissues is a common complication of diseases, mainly of inflammatory origin, which causes more than 800 thousands deaths annually

[1, 2]. Characteristic features of this complication are a wide variety of processes involved at the cellular and molecular levels and the complexity of prevention and treatment [3, 4]. Leading triggers of fibrosis include chronic inflammation, various infections and prolonged coagulation cascade disorders [4].All these factors are inherent in the pathogenesis of COVID-19, which over the past two years has become a pandemic [5]. Therefore, the study of ways and mechanisms of this process is of undoubted interest both from a scientific and cognitive point of view, and from a practical one. Recent experimental data allow us to approach the solution of these issues and substantiate the position of the formation of fibrous tissue as a consequence of complex dysfunction of the hemostatic system due to viral damage to the endothelium of blood vessels. The prevention of hemorrhage in vascular damage and maintenance of blood in a liquid state is ensured by a dynamic balance between the coagulation and fibrinolytic cascades of the hemostatic system. Its vascular, platelet and humoral components are in a complex relationship to each other [6]. Like many multi-link regulatory systems, an integral condition for the proper functioning of the hemostatic system is the regular and strictly limited course of each of its stages (Fig.1).

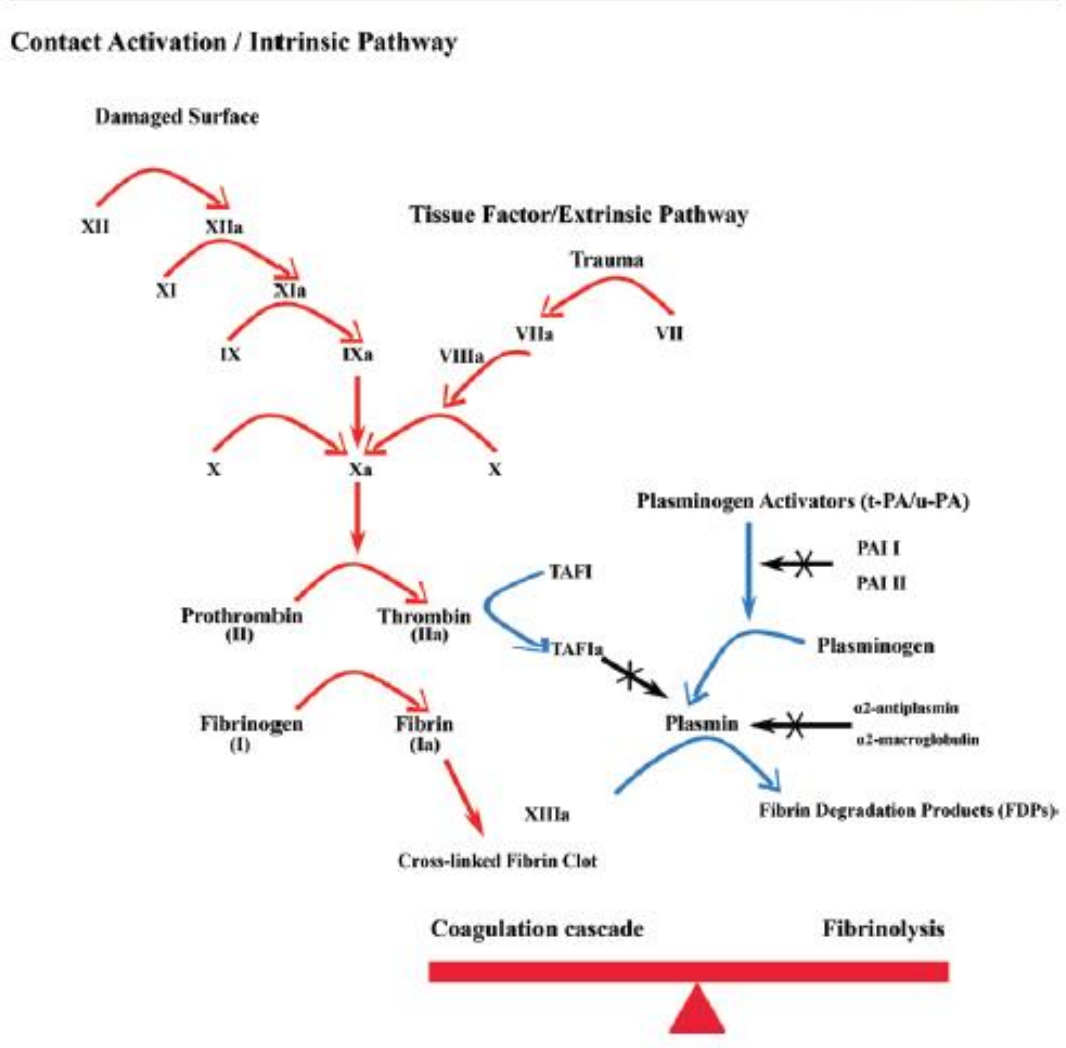

Fig. 1. Simplified scheme of dynamic equilibrium between coagulation and fibrinolytic cascades of hemostatic system *[7]*

Both the lack and excessive activity of any of its components causes a cascade of disorders with severe functional complications. Thus, excessive blood clotting plays a significant role in the development of atherosclerosis and its complications, myocardial infarction, cerebrovascular disorders, diabetes, malignant neoplasms, pregnancy complications, septicemia and septic shock, hereditary thrombophilic disorders and other postoperative disorders. On the contrary, insufficient blood clotting or excessive fibrinolysis cause a variety of bleeding, which are a serious complication of trauma and surgery. Genetically determined functional insufficiency of blood clotting factors causes hemophilia. The requirements for the regularity of the fibrinolytic process are no less strict. The efficiency of the fibrinolysis process is due to the ability of plasminogen and tissue plasminogen activator (t-PA, E.C.3.4.21.68) to be sorbed on the fibrin network with following activation to plasmin (E.C. 3.4.21.7) and to cleave the fibrin clot into large soluble blocks. That is, both blood clotting and fibrinolysis are due to a sequence of activation and proteolytic processes under the control of highly specific inhibitors. Both the lack and excess of functional activity of any of the components of this system disturbs the hemostatic balance and causes severe complications [6].In the current COVID-19 pandemic, the thrombotic complications inherent in this disease deserve special attention [5]. A characteristic feature of this disease is damage to both macro- and micro-vessels, and, unlike other infectious vasculitis, not venous vessels only but arterial ones also are damaged independently of their size [8].The intracellular SARS-CoV-2 virusesare found in both the middle vessels and the alveolar capillaries of the lungs affected by thrombosis. Viral damage to endothelial cells leads to procoagulation changes in the lumen of blood vessels, the development of immunotrombosis and decreased blood circulation in the body. Endothelial glycocalyx is an antithrombotic surface that effectively blocks the action of thrombin due to the binding of antithrombin III and the heparin sulfate component [9].Viral damage to the glycocalyx causes a shift in the balance between fibrin coagulation and fibrinolysis. Endothelial damage leads to the release into the bloodstream of Weibel-Palade bodies, which are the kind of depot of von Willebrand factor, factor VIII and P-selectin [8, 9]. The first two of them are important components of the platelet link of the blood clotting system, the third one belongs to the proteins of cell adhesion. The lack of these factors is the cause of various complications that cause bleeding, and their excessive action contributes to dysfunctional thrombosis [6, 7]. However, the content in the bloodstream of the main thrombin inhibitor - antithrombin III - does not change significantly [8]. Severe thrombotic complications of COVID-19 indicate a functional insufficiency of the fibrinolytic system to compensate for excessive blood clotting.This may be due to local deficiency of certain components of the hemostatic system (Fig. 1) at violation of the regularity of its activation cascade. However, no less important may be a violation of the regular structure of fibrin, that may be caused by a local excess of coagulation factors and isolation of damaged SARS-CoV-2 vessels. The violation of the regularity of fibrin structure cause for increase of its resistance to fibrinolysis, since the plasmin cleavage of fibrin network into large soluble fragments becomes complicated significantly [10]. Plasmin itself is quite weak trypsin-like serine proteinase. The efficacy of its lysis of fibrin is mediated by the ability of this enzyme to migrate through the fibrin network from one site of cleavage to another one. The plasminolysis-formed C-terminal group becomes a new ligand for plasmin binding sites by directing the catalytic center to the next splittingsite[11]. Under conditions of normal fibrin structure, this provides a limited number of cleavages with the formation of large soluble blocks (Fig. 2).

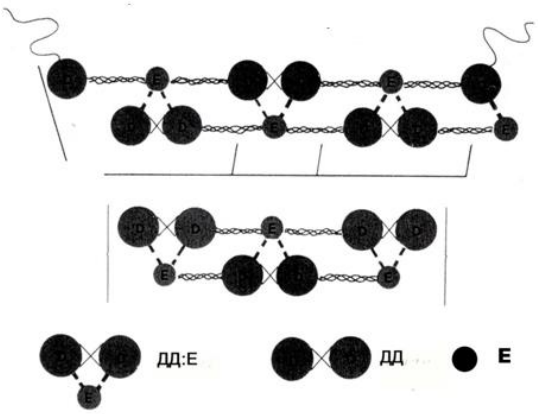

Fig. 2. The scheme of enzymatic cleavage of fibrin by plasmin *[6]*

However, at disturbancesof fibrin structure such migration becomes complicated, which also inhibits the process of fibrinolysis. Thus, it is shown that the fibrin clot formed by contact with foreign surfaces differs significantly in both structure and resistance to plasminolysis [10]. In other word, the violation of the regularity of the process of fibrin clot formation leads to increase of the resistance of fibrin to plasmin lysis. In addition, plasmin in sorbed on undegraded fibrin state is protected from the action of its natural inhibitor - α2-antiplasmin, but retains the proteolytic and activator effect on soluble proteins [11]. Even more, the disruption of protein metabolism in isolated vessels leads to local accumulation of protein and peptide components of endogenous intoxication. The imbalance of their structure creates preconditions for the development of aggregation processes, in particular for the formation of β-structured protein aggregates [12].According to recent data, the formation of such structures is not limited to amyloid complications, but is observed in a number of pathological processes, which to some extent are associated with impaired protein metabolism [13, 14]. Such deposits dramatically increase the resistance of the respective tissues to proteolysis and may contribute to the formation of irregular fibrin with increased resistance to fibrinolysis.

In this context, it is of peculiar interest to study the protein structure of the lungs of people who died from COVID-19. It is known that in this pathology the functional failure of the lungs is due to fibrous lesions, that in varying degrees ischaracteristic for this disease (Fig. 3).

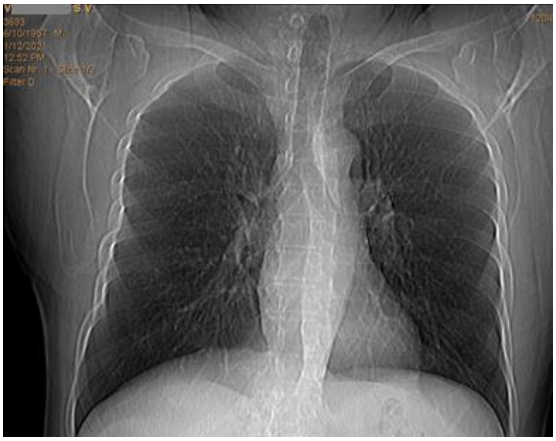

Fig. 3. Computed tomography of the lungs of a person who underwent COVID-19 in mild form

The aim of the work was to study the structure of the lung tissues of persons, who died as a result of COVID-19, in order to identify the possible inclusions in their composition of β-structured protein aggregates.

The methodical part included the selection of surgical material with fixation in 10% formaldehyde solution and staining of tissue microsections with specific for amyloid-like structures Congo Red dye. The obtained preparations were examined in light, polarization and fluorescence microscopes.Congo Red-stained β-structured protein aggregates become brick-red in light microscopy, apple-green in polarization microscopy, and emit red light in ultraviolet excitation under fluorescence microscopy. The latter circumvents the Abbe's diffraction limit, according to which objects smaller than 0.61 of wave-length become invisible.

Results. As a result of the study, it was found that all the studied preparations contain β-structured protein aggregates. In light and polarization microscopes, these deposits showed varying degrees of severity. When using the same luminescent microscopy (Fig. 4) in all studied preparations a dense red glow throughout the affected tissue were observed.

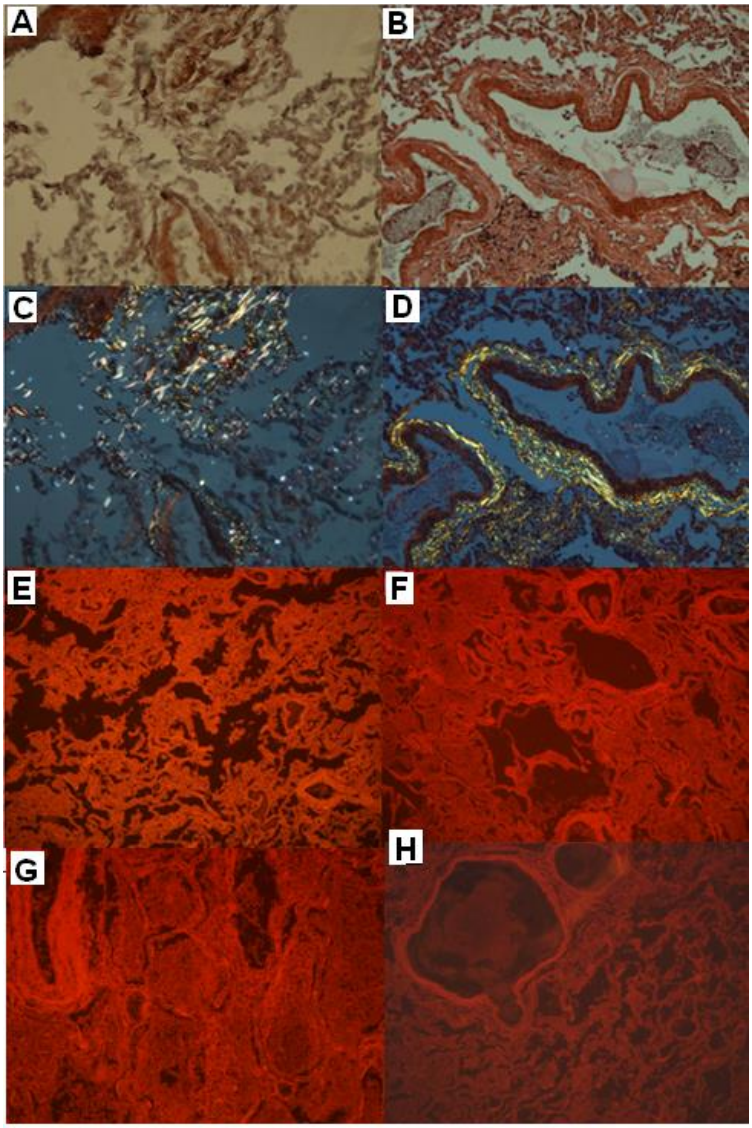

Fig. 4. Lung preparations of persons who died as a result of COVID-19-induced fibrosis: A, B – light microscopy; C, D – polarization microscopy; E, F, G, H – fluorescence microscopy. Congo Red.× 200

This indicates the finely dispersedcharacter of the protein aggregates that were included in fibrosis-affected tissues. They aremainly are represented by particles smaller than 0.61 of the light wavelength, which corresponds to approximately 240 nm. This makes them invisible under light and polarization microscopy due to the Abbe diffraction constraint. On contrary, at fluorescence microscopy this limitation is circumvents. Traditional hematoxylin and eosin staining as well as Movat's pentachromic one do not provide such opportunities[2, 15].

It is clear that the formation of such inclusions affectson the structure and functional ability of the surrounding tissues. Upon contact with fibrin, such inclusions disrupt the structure of the fibrin clot, which increases its resistance to plasmin cleavage. It has been shown recently that β-structured protein components of SARS-CoV-2 virus induce the formation of fibrin with increased resistance to fibrinolysis [16, 17]. This is in good agreement with the data on the increased resistance to proteolysis of the clot formed by contact with surfaces capable of inducing denaturation [10].

Discussion. Submitted data indicate the existence of a cascade of processes caused by viral infection and lead to failure of the affected organs. Prolonged both in time and in space viral infection of endothelial cells causes systemic damage to the glycocalyx, that leads to the loss of the latter of anticoagulant properties and the massive release of coagulation factors.The fibrin clot formed under such conditions is characterized by an irregular structure, which increases its resistance to fibrinolysis. At the same time, local blockage of the vessel ensures the accumulation of products of endogenous intoxication, in particular proteins with impaired non-functional proteolysis structure. Such destabilized proteins are prone to aggregation and are able to form micro- and nano-sized aggregates with a significant content of β-folded structures. The latter circumstance not only contributes to the further increase of fibrin resistance to the proteolytic action of plasmin, but also causes the development of fibrosis of the tissues affected in this way. In other words, the β-structured protein aggregates found in the damaged tissues are both a consequence and a cause of the development of the fibrous process, which causes a functional deficiency of the damaged organ.It is also clear that these mechanisms do not exhaust the multifaceted cascade of COVID-19 disorders of molecular and cellular levels, but they are an important prerequisite for the development of oxidative stress, cytokine storm, non-functional proteolysis, disruption of biosynthesis of important regulatory factors, and many other processes which together cause functional inferiority of the affected tissues.

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