ORIGINAL ARTICLE

FEATURES OF COAGULOPATHY AND SYSTEMIC INFLAMMATION IN PATIENTS AFTER COVID-19 INFECTION

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ABSTRACT

The aim: To determine the peculiarities of laboratory data concerning blood coagulation and systemic inflammation in COVID-19 patients in three months after discharge and recovery. The state of coagulation, anticoagulation, and fibrinolytic systems, as well as their prognostic value having been well studied in hospitalized COVID-19 patients, their state three months after hospitalization, are not yet well understood.

Materials and methods: Methods of randomization, anthropometry, ECG, standard clinical blood testing, immunoenzymometry, immunoanalysis, and primary statistical analysis were used in the study. Anthropometric measurements of patients (n=20), blood samples, blood serum samples, urine samples, and statistical data were the materials of the study.

Results: Indices of coagulation and systemic inflammation in studied patients after COVID-19 were obtained (PTT, s ; PATPT, s; Fibrinogen, g/L; Platelets ×109 /L; PCT, ng/mL; DD, µg/L; CRP, mg/L; IL -6, pg/mL; IL -10, pg/mL; Cortisol (nM/L); CIC (IU/mL); Ig A (g/L).

Conclusions: Summing up the results obtained, it is possible to assert micro- and macro-vascular thromboses to be common in COVID-19 cases; they are associated with poor prognosis for diseased patients and are not completely investigated; the role of thromboses in COVID-19 course and complications are to be studied as well as the strategies of fibrinolytic therapies for such condition are to be justified. The presence of specific rheological and serological changes in patients even three months after surviving COVID-19 needs further study to understand the necessity of anti-thrombolytic drug uptake for a relatively long time.

KEY WORDS: COVID-19, Inflammation, Thrombolytic Therapy, Blood Platelets, Immunoglobulins, Interleukins

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INTRODUCTION

Viral respiratory infections including coronavirus agent of severe acute respiratory syndrome (SARS-CoV), coronavirus agent of Middle East respiratory syndrome (MERS) as well as SARS-CoV-2 pandemic agent initiate coagulopathies, lead to intravascular thrombi and fibrinogen deposits formation [1], this infection consequences being associated with unique pro-thrombotic pathophysiology [2].

In COVID-19 patients, usual blood coagulation anomalies imitate often other systemic coagulopathies associated with severe conditions such as disseminated intravascular coagulation (DIC); however, coagulation anomalies in COVID-19 patients possess some marked differences [3].

Deep vein thromboses (DVT) and lung artery embolism (LAE) are found in 20-30% of COVID-19 patients [2, 3]; in Holland, the cumulative incidence of large vessels thromboses reached 49%, the majority of them being LAE; in Italy the level of such events was 21% (27.6% of them were found in intensive care units and 6.6% in general population); in France the thromboses level was as high as 20.6%. In meta-analysis including 49 investigations having been realized with 18,093 patients, the common LAE incidence reached 17.0% (9.8-33.0%) [4]; in large randomized controlled studies 6-10% of LAE were found in cases of anticoagulants use in prophylactic doses; when anticoagulant compounds have been used in therapeutic doses, the LAE levels were lower (4-8%) [5]; other systemic reviews indicate the LAE incidence to be 28%, its fluctuations reaching 19-24% in cases of clinical diagnostics and 36-46% for routine screening [6]. All the authors agree the blood coagulation system to be activated and disturbed in SARS-CoV-2 infected humans; however, the properties of coagulopathies associated with this condition are different from other well-known blood coagulation disorders [7]. Such disorders include immunomediated thrombotic mechanisms, complement activation, syndrome of macrophages activation, anti-phospholipid antibodies syndrome, hyper-ferritinemia, and regulative disturbance of renin-angiotensin system [1].

J. Connors, J. Levy [8] have summed up the data being associated with the SARS-CoV-2 coagulopathy; they have found such a coagulopathy to possess some specific features including a marked increase of D-dimer (DD) levels as well as of fibrin/fibrinogen degradation products; at the same

time, changes of prothrombin time (PTT), partially activated thromboplastin time (PATPT), and platelets quantity are insufficient; such features are not common for the usual DIC syndrome. The hemostasis damage described for the COVID-19 infection includes the increase of DD and fibrinogen levels [9], changes of platelets quantities and of their activation levels, increase of the von Willebrand factor (VWF) level [10] as well as changes of other coagulation parameters. A constantly increased risk of thromboembolism events being observed even at the beginning of prophylactic anticoagulation therapy testifies the presence of hypo-fibrinolysis process in addition to hyper-coagulation found in SARS-CoV-2-infected patients [11]. All researchers agree the coagulopathy manifestations in these patients do not completely correspond to the DIC-syndrome determination; however, the dynamical monitoring of C-reactive protein (CRP) levels as well as of fibrinogen (FIB) and DD ones and of their ratios in sera (CRP/FIB, FIB/DD) are to optimize the timely disease diagnostics, management, and disease course prognosis [12].

Summing up all the data obtained it is possible to approve that micro- and macro-vascular thromboses are rather common; they are COVID-19-associated and are markers of poor prognosis; these thromboses possess their own etiopathogenetic properties being yet not completely studied and needing further investigations in the field of their role in disease course and prognosis as well as for justification of fibrinolytic therapy approaches in COVID-19 cases.

THE AIM

To determine the peculiarities of laboratory data concerning blood coagulation and systemic inflammation in COVID-19 patients in three months after discharge and recovery.

The state of coagulation, anticoagulation, and fibrinolytic systems as well as their prognostic value having been well studied in hospitalized COVID-19 patients, their state in three months after hospitalization are not yet well understood.

In this regard, in the course of the study it is planned to study the role of thrombosis in the course and complications of COVID-19, as well as to substantiate the strategies of fibrinolytic therapy of this condition, to evaluate the changes in prothrombin time, partially activated thromboplastin time, the number of platelets, fibrinogen, D-dimer and PCT, to identify their relationship relationship with systemic inflammation, i.e. IL-6, IL-10, IgA, CIC, cortisol and CRP levels.

In addition, it is necessary to detect and evaluate rheological and serological changes in patients three months after the transfer of COVID-19.

MATERIALS AND METHODS

During 2020-2021, 20 patients were examined in 3 months after their treatment at the hospital because of the COVID-19, the studies having been realized in the Center of Primary Health Care N3 of the Sviatoshyn region of Kyiv-City. The SARS-CoV-19 was diagnosed according to the recommendations of the World Health Organization [13], clinical guidelines «Clinical Management of COVID-19 Patients» [14], Order of the Ministry of Health Care of Ukraine № 762 (02.04.2020) (the version of the Order of the Ministry of Health Care of Ukraine N 358, 22.02.2022) «Protocol for the provision of medical care for the treatment of coronavirus disease (COVID-19)» [15], Order of the Ministry of Health Care of Ukraine N 771 (20.04.2021) «Protocol for the provision of rehabilitative aid to patients with the diagnosis of coronavirus disease (COVID-19) and convalescents" [16]. The COVID-19 diagnosis in our patients has been earlier confirmed in clinical laboratories by the detection of the SARS-CoV-2 RNA [16].

All the patients received all the necessary information for patients and signed their "Voluntary informed consent for the realization of diagnostics, management, carrying out of operation and anesthesia" (form №003-6/o) and "Voluntary informed consent for the treatment of patient's data" according to the Order of the Ministry of Health Care of Ukraine № 110 (14.02.2012) [17]. The research protocol was prepared according to the Helsinki Declaration. According to the Order "Protocol for the provision of medical care for the treatment of coronavirus disease (COVID-19)" [18] was used after the patients' voluntary informed consent. The inclusion criterion was the following: patients aged 45-65 years after laboratory confirmed COVID-19 infection who have been under the supervision of family doctors during 3 months. The exclusion criteria were the following: patients with comorbidities, aged > 65 years with habits and states able to change their clinical parameters during the natural COVID-19 development.

The patients were divided in 2 groups by a randomization method, each group contained 10 persons. The patients' mean age for groups 1 and 2 were 58.16±1.32 and 60.37±1.03, respectively; their mean height was 171.24±1.23 and 172.50±1.35 cm, respectively. The patients' mean body mass reached 83.24±1.94 kg and 85.00±2.00 kg for groups 1 and 2, respectively, the Quetelet index reaching 28.35±0.54 and 28.42±0.53 kg/m^2, respectively. The group 1 included 50% of males and 50% of females, the group 2 contained 40% of males and 60% of 40% of females. Twenty per cents of patients examined were smoking abusers, 80.0% of them being non-smokers. Among 20 patients having survived COVID-19 infection, 60.0% were persons with higher and 40% – with secondary education. The group 1 contained 10 patients with proved COVID-19 course of moderate severity, the group 2 containing 10 patients with proved COVID-19 infection of severe course. Our investigation was carried out according to the "Protocol for the provision of rehabilitative aid to patients with the diagnosis of coronavirus disease (COVID-19) and convalescents" given in the Order of the Ministry of the Health Care of Ukraine N 771 [16]. The data concerning the disease progress were taken from medical documentation and from detailed interviews with our patients. According

to the Order mentioned, the following up of COVID-19 patients includes the main results of investigations obtained for persons having survived acute respiratory failure in 6-8 weeks after discharge as well as different measures aiming to improve breathing and physical exercises. The persons having survived COVID-19 with present/continuous lung function damage in 6-8 weeks after discharge underwent a complex lung rehabilitation program according to established international standards (Quality Standards for Pulmonary Rehabilitation in Adults, 2014 [19], British Thoracic Society Guidelines on Pulmonary Rehabilitation, 2013 [20]; American Thoracic Society, Assembly on Pulmonary Rehabilitation «Guidance for Re-opening Pulmonary Rehabilitation Programs», 2020 [21]). The program of lung rehabilitation used in our study included the assessment of patients' condition as well as individualized rehabilitation program including also physical exercises, elements of sanitary education, and psycho-correction. We tried to improve both physical and psychological condition of patients with chronic respiratory diseases, to increase their desire to become healthy, and to understand the significance of different risk factors [16].

All the patients were examined by a cardiologist using ECG; common clinical blood analyses were realized (count of blood elements and hematocrit determination) as well as biochemical urine and blood analyses (in order to determine kidney and liver functions as well as levels of serum creatine kinase, lactate dehydrogenase, and glucose, enzymes of myocardium and CRP) [according to the Order of the Ministry of Health Care of Ukraine N 771 (20.04.2021) "Protocol for the provision of rehabilitation aid to patients with the diagnosis of coronavirus disease (COVID-19) and convalescents [16]. We estimated such indices as PTT, PTPT, FIB, and DD values [16]. IL-6 and IL-10 values were measured using a standard of human cytokines 27-Plex. A panel of analyses and a system Bio-Plex 200 (Bio-Rad, Hercules, Ca, USA) were used according to the manufacturer's instructions [22].

The cortisol level determination in post-COVID patients' sera was carried out using solid phase immunoenzyme analysis and test kits "Cortisol-EIA" of the firm "Khema" («Хема»), Ukraine, and a photometer "Stat Fax 303" for registration of immunoenzyme analysis results [22].

The level of circulating immune complexes (CIC), mostly of IgG-binding C1q, was determined by a precipitation approach using a Stat Fax 303 photometer and taking into consideration the referent data for adults (0.025-0.045 IU/mL) [22].

The IgA concentration was determined by a solid immunoanalysis approach using a photometer Stat Fax 303 [22] and immunoenzyme kits "Common IgA – EIA" («Загальний IgA – ІФА») from the "Khema" firm (Ukraine).

The mathematical processing of results obtained was made using mathematical statistics approaches. The statistical description of investigation results was realized by methods of primary statistical analysis [23]. Having determined the arithmetic mean values (M) and arithmetic mean errors (m), we have found the distribution of indicators on normality using the Kolmogorov-Smirnov criterion. We have stated the distribution of the majority of indicators to be different from the normal one on the significance level 0.05. The distribution analysis was carried out for each criterion studied. The Student's t-test was taken for evaluation of scatter of random collections with "normal" distribution. In cases the collections with distributions being different from "normal" ones, the U-test according to the Mann-Whitney method was used. In cases of qualitative signs distribution different from the normal one median and interquartile range were determined (Me (25.0%; 75.0%)).

RESULTS

The PTT as an important blood coagulation index measures the time necessary for blood plasma coagulation. Although a routine PTT determination has been recommended for the evaluation of COVID-19 associated coagulopathy at the beginning of this epidemic, in the majority of COVID-19 patients this parameter is normal or near normal; there are sometimes mentions about elongated PTT in patients with severe disease course [24]. In all our patients as well as in patients of groups 1 and 2 the PTT values were 12.7, 12.6, and 12.8 s (Table), its level being below 11.5 s in 30.0%, 20.0%, and 10.0% of patients examined, respectively; 10% of all patients and 10% of them in each group showed the PTT value above 14.5 s.

Usually the index of PATPT in COVID-19 patients is normal and not associated with severity of this condition course. The PATPT elongation may be an indicator of blood coagulation factor, presence of specific coagulation inhibitors (antibodies to the factor VIII) or a laboratory artefact because of the presence of anti-phospholipid antibodies. In some COVID-19 patients the artefact PATPT elongation was found because of lupus erythematosus or increased heparin resistance because of high levels of fibrinogen or factor VIII [25]. In all our patients and in patients of groups 1 and 2 the PATPT values were 34.4, 34.1, and 34.6 s, respectively (Table).

The main platelets function is their participation in the system of blood coagulation and fibrinolysis processes. In all patients examined in our study as well as in patients of groups 1 and 2 the quantities of platelets were 206, 188 and 224×10^{9}/L, respectively (Table), their quantity below 100×10^{9}/L having been found only in 15.0 and 30.0% of all patients and of patients from the group 1; in 15.0% of all patients as well as in 10.0 and 20.0% of patients from groups 1 and 2 the platelet concentrations were above 300×10^{9}/L.

Fibrinogen is an important factor of blood coagulation system responsible for the final stage of thrombi formation, their stabilization, and stop of bleeding. In the interstitial tissue fibrinogen develops a background for fibroblasts and histiocytes growth; in any tissue being damaged fibrinogen and fibrin concentrations are increased; there they intensify the migration of granulocytes producing different growth factors and realizing necrosis products phagocytosis;

Table I. Indices of coagulation and systemic inflammation in patients after COVID-19

Indices of coagulation and systemic inflammation	Patients after COVID-19		
	All the patients	Group 1	Group 2
PTT, s	12.7 [11.3; 13.1]	12.6 [11.7; 13.1]	12.8 [12.2; 13.7]
PATPT, s	34,4 [29.5; 37.2]	34.1 [29.9; 37.6]	34.6 [29.0; 38.3]
Fibrinogen, g/L	4.1 [3.1; 4.63]	4.0 [3.03; 4.68]	4.2 [3.8; 5.28]
Platelets ×109 /L	206 [159; 235]	188 [152; 218]	224 [176; 277]
PCT, ng/mL	0.10 [0.05; 0.27]	0.12 [0.04; 0.26]	0.08 [0.04; 0.12]
DD, µg/L	271 [123; 282]	220 [135; 378]	321 [228; 560]
CRP, mg/L	6.5 [6.2; 6.7]	5.3 [5.0; 5.6]	7.7 [7.5; 7.95]
IL -6, pg/mL	4.3 [2.4; 5.8]	4.5 [2.2; 11.8]	4.1 [2.6;10.3]
IL -10, pg/mL	3.0 [0.9; 3.9]	2.5 [0.9; 3.8]	3.4 [1.9; 4.9]
Cortisol (nM/L)	209.3 [193.33; 233.05]	195.6 [191.38; 212.88]	223.0 [196.3; 269.9]
CIC (IU/mL)	0.037 [0.025; 0.047]	0.045 [0.033; 0.054]	0.028 [0.022; 0.036]
Ig A (g/L)	1.6 [1.3; 1.95]	1.8 [1.4; 2.2]	1.4 [1.1; 1.8]

these processes are especially important for damaged tissue regeneration. Products of both fibrinogen and fibrin degradation possess anti-coagulant activity and are able to inhibit the process of fibrin formation. Fibrinogen is a valuable hemostasis index. A soluble fibrinogen precursor belongs to acute phase proteins, its concentration becoming higher in cases of inflammation processes of different etiologies. The fibrinogen level becomes quickly increased in cases of acute inflammation or tissue damage, its concentration being able to become 10 times higher. Fibrin takes part in adhesion processes as well as in trans-endothelial migration of monocytes and neutrophils; it stimulates the chemokines secretion by macrophages in extra-vascular space helping the strengthening of the immune response during the inflammation process. In all our patients and in patients of groups 1 and 2 the fibrinogen levels were 4.1, 4.0, and 4.2 g/L, respectively (Table I), this level being below 2 g/L only in 5% of all patients and in 10% of group 1 patients; the level above 4 g/L was found in 55.0% of all patients as well as in 50.0 and 60.0% of patients belonging to groups 1 and 2, respectively.

Our results are similar to data obtained in numerous investigations and confirm the opinion such a combination of anomalies to testify the presence of coagulopathy similar DIC, being, however, phenotypically different from DIC associated with other inflammation syndromes, such as hemophagocytic lymphohistiocytosis (HLH) accompanied by marked hypofibrinogenemia, and sepsis with significant thrombosis [26]; no significant deviations concerning fibrinogen level and quantity of platelets were found in patients with cases of severe and critically severe COVID-19. Besides, DIC-imitating coagulopathy in cases of COVID-19 does not meet the evident DIC criteria confirmed by the International Society of Thrombosis and Hemostasis (ISTH) [27].

DIC-syndrome is accompanied by the development of systemic or common vascular thrombosis leading to non-adequate blood delivery to different organs [28]. DIC-syndrome is a rather common, but fatal consequence of cytokine storm and a significant death precursor in cases of this pathology; however, it is not the main driving force for coagulopathy and critical illness seen in COVID-19 cases. Our results obtained for coagulation parameters suggest the coagulopathy to be associated with deep inflammation and to mediate the SARS-CoV-19-accompanying thrombotic complications.

COVID-19-associated coagulopathy is characterized by almost normal platelets level and PTT in the majority of patients, as well as by evenly high levels of DD and fibrinogen [24]. As a contrast, the DIC-syndrome seen rarely only in patients with severe COVID-19 cases is characterized by lowered platelets quantity, increased DD levels, PTT elongation and decreased fibrinogen level; it is associated with poor prognosis in COVID-19 patients [29]. Recent studies comparing the properties of coagulation in cases of severe COVID-19 due to pneumonia and cases of pneumonia due

to other factors show he quantity of platelets to be higher in SARS-CoV-2 infected persons, the relative levels of DD and PTT elongation being, however similar [30].

DD is the main fragment of the fibrinogen degradation used as a biomarker of coagulation and fibrinolysis [31]. During the SARS-CoV-2 pandemic the DD was widely investigated. Now it is known DD levels to be increased in the majority of patients hospitalized because of COVID-19. The DD levels reach the highest levels in 5 days after hospitalization and are significantly higher in critically-ill patients [32]. The extended study of the DD levels in 2,377 COVID-19 patients in hospitals has revealed an association between the initial and peak DD level – a situation associated with thromboses, acute kidney damage, and mortality due to all causes [33]. Another review demonstrates the DD levels may be used for identification of COVID-19 patients needing CT and lung angiography for diagnostics of lung artery thromboembolism, the boundary DD levels may reach ≥1000 μg/L [34]. In all our patients investigated and in patients of groups 1 and 2 the DD levels were 271, 220, and 312 μg/L, respectively (Table), its level above 300 μ/L having been found in 40.0% of patients; at the same time, 20% of all the patients examined in our clinic as well 20% of them belonging to groups 1 and 2 showed DD levels above 500 μg/L.

PTC is a precursor of the hormone calcitonine being synthesized by thyroid C-cells. In some pathological conditions PCT may by produced by other tissues and organs including liver, kidneys, muscles, and fat tissue. We are talking about the cascade of extra-thyroid PCT synthesis being switched on as a response on the aggression of some microorganisms or toxins. The rapid increase of this biomarker level is seen in cases of systemic inflammation answer due to bacterial or fungal invasion as well as in cases of protozoan infections. The PCT levels are usually not changed if a human is infected only by any viral agent. In all our patients and in the patients of groups 1 and 2 the PCT levels were 0.10, 0.12, and 0.08 ng/mL, respectively (Table I).

Cytokines are known to be able to disturb the normal human hemostasis in some pathological conditions; they are incredibly among the main factors of thrombotic SARS-CoV-2 potential, contributing to disbalance in pro-thrombotic and anti-coagulating pathways including the loss of action of tissue factor pathway inhibitor (TFPI), decrease of the regulation of thrombomodulin expression in endothelial cells, and decrease of anti-thrombin (AT) III level in the blood serum. Such cytokines changes are associated with coagulopathy in cases of sepsis and lead to the process known as immunothrombosis. Different thrombotic triggers produced during this event including also cytokines, activated thrombocytes, extracellular traps of complement and neutrophils play a certain role in the inflammation process. The analysis having been carried by M. Ranucci shows 16 patients with COVID-19 accompanied by the acute respiratory distress-syndrome (ARDS) to have the IL-6 levels correlating with thrombosis and hyper-coagulation markers [34].

The IL-6 is produced by different cell types – by macrophages, T- and B-lymphocytes, fibroblasts, by endothelial, epidermal, and microglial cells as well as by chondrocytes and osteocytes. It promotes the production of acute phase proteins and corticotropin, induces fever development, terminal B-cell differentiation, and antibody production; in cooperation with other cytokines it accompanies the stem cells proliferation and differentiation as well as the activation of CD4+-, CD8+-, and T-lymphocytes. It is a pro-inflammation cytokine.

The cytokine IL-6 is a pleiotropic inflammation mediator and the central stimulus of the acute phase response. This cytokine plays an important role in the pathological response to inflammation leading to the severe COVID-19, high serum levels of the IL-6 being a marker of more severe consequences in hospitalized COVID-19 patients. The IL-6 is a biomarker of the severe COVID-19 infection as well as an important etiopathogenetic factor. Although IL-6 is a such marker, it cannot be used for differentiation of COVID-19 and other possible factors of severe disease. Meta-analysis of 19 investigations (1,245 patients) shows the increased IL-6 levels in critically ill COVID-19 patients to be, however, significantly lower comparing to its levels in cases of sepsis not associated with COVID-19 and in cases of ARDS [35]. The data obtained suggest the necessity of determination of the IL-6 levels as a prognostic marker in cases of severe COVID-19 course, its exact association with the thrombosis development being, however, not found [36].

IL-10 is a suppressive factor produced mostly by Th2. It inhibits the function of Thl, NK cells and monocytes decreasing the production of immunocytokines (gamma-IFN, PNP, IL-1, IL-8). IL-10 increases also the proliferation of B-lymphocytes and tissue basophiles. In such a way, IL-10 belongs to the most important regulatory cytokines determining the results of the immune response; the Th1-regulated cell response is inhibited under the IL-10 influence, the humoral response (Th2) becoming stimulated. IL-10 belongs to anti-inflammatory cytokines. In all our patients examined and in patients of groups 1 and 2 the IL-6 levels were 4.2, 4.4, and 4.0 pg/mL, respectively, the IL-10 levels in the same patients being 3.0, 2.5, and 3.3 pg/mL, respectively (Table I).

R. Bodnar, C. Yates, A. Wells [37] think IL-10 to activate the cell-medicated immune response of the T-helper type. This substance is produced by macrophages, endothelial cells, and fibroblasts; it acts as a chemoattractant for a lot of cells including, in particular, macrophages and T-cells. IL-10 inhibits proliferation, induces endothelial cells apoptosis and prevents the gaining of their mobility.

An important factor is the synergism between IgA and non-specific defense mechanisms, such as complement, lysozyme, cells having been phagocytated and their enzymes; such synergism leads to higher antibacterial defense increasing its total efficacy. In all our investigated patients as well as in persons of groups 1 and 2 грyп the IgA levels were 1.6, 1.8, and 1.4 pg/mL.

One of the most important biological functions of immunoglobulins is antigen fixation and immune complexes

(IC) formation – a physiological process being constantly realized in the organism and directed on the support of its inner environment. The IC formation is one of normal immune response components. The most important IC ability is complement system activation determining the IC role in the inflammation development and regulation of functional immune system regulation. In all patients and persons from groups 1 and 2 the levels of CIC were 0.037, 0.045 and 0.028 IU/mL, the cortisol levels being 209.3, 195.6, and 223.0 nM/L, respectively.

CRP is a known marker of acute inflammation in a lot of diseases. It is produced by the liver as a response to inflammation-accompanying cytokines, especially to IL-6. The levels of both CRP and IL-6 in COVID-19 patients become higher and correlate positively with disease severity and mortality [36]. Meta-analysis of 20 studies including 4,843 COVID-19 patients found four times higher risk of complications and death for patients with increased CRP level [38]. A large analysis having been realized in the USA using data for 2,782 COVID-19 patients shows > 97% of these persons to have increased CRP levels at the moment of hospitalization; high initial CRP levels are associated with thromboses, acute kidney damage, and mortality due to different factors [11]. The data mentioned show a positive correlation between CRP levels and COVID-19 severity; contrary to IL-6, the CRP levels are a prognostic factor for thrombosis risk [39].

The CRP induces the increase of expression and activity of the tissue factor (TF) as well as the decrease of expression of tissue factor pathway inhibition (TFPI); it causes activation of inflammation and coagulation, disturbance of endogenic fibrinolytic ability as well as stimulation or increase of platelets adhesive activity and sensitivity. Epidemiological studies show the increased CRP concentrations to be associated with venous thromboembolism risk (VTR) [40]. In all patients examined in our study as well as in patients of groups 1 and 2 the CRP levels were 6.5, 5.3, and 7.7 mg/L (Table), its level above 10 mg/mL having been shown in 50.0, 40.0, and 60.0% of all the persons examined and patients of groups 1 and 2; at the same time the CRP level was above 30.0 mg/mL in 20.0% patients in each group.

DISCUSSION

At the moment, besides the treatment of the leading disease cause, the International Society of Thrombosis and Hemostasis (ISTH) recommends to treat thrombotic COVID-19 complications using prophylactic systematic introduction of low molecular weight heparin (LMWH) to all the hospitalized patients [27]. LMWH is a better choice than non-fractioned heparin, taking into consideration its use once per day and low risk of heparin-induced thrombocytopenia. Renewed scientific recommendations as well as standardized recommendations include the use of moderate doses of LMWH (enoxaparin, 40-60 mg daily); the drug dose is to be titrated especially for patients with obesity, severe thrombocytopenia or damaged renal function. Besides, because of the absence of specific data concerning COVID-19, the ISTH recommends to continue the thromboprophylaxis during 2-6 weeks using LMWH; the American Society of Hematology (ASH) and the American College of Cardiology [27] confirm these conclusions. The ASH proposes also an approach approved by the USA Food and Drug Administration (US FDA), i.e. the use of certain drugs (enoxaparin, dalteparin, tinzaparin) or peroral anticoagulants (rivaroxaban, betrixaban) after patient's stay in hospital, taking into consideration the low bleeding risk. However, in cases of significant BTE risk (advanced age, immediate resuscitation after hospitalization, malignant tumors, BTE in anamnesis, thrombophilia, severe immobility and increased DD level the patient management protocol is different. American schemas of prophylaxis after discharge include rivaroxaban (10 mg daily) during at least 31 days or betrixaban – 160 mg daily for the first day and the uptake of this drug during at least 35 days (80 mg daily) [21, 27].

It should be also taken into consideration disturbances of coagulation, anticoagulation, and thrombolytic blood systems to be often kept in former COVID-19 patients during three months after disease. In persons with severe COVID-19 course the mean PTT value was 12.8 s, its level being below 11.5 s and above 14.5 s in 10.0% of patients, respectively; the mean PATPT value in these patients reaches 34.6 s; their quantity of platelets was found to be 224×10^9/L, the values above 300×10^9/L having been seen in 20.0% of patients examined. The mean FAB content was 4.2 g/L, the values above 4 g/L having been found in 60.0% of persons examined; the mean DD level was 312 μg/L, this value being above 300 μg/L in 40.0% of patients and exceeding 500 μg/L in 20.0% of patients. The mean PCT level in these patients was 0.08 ng/mL.

Even in persons with COVID-19 course of moderate severity the mean PTT value was 12.6 s, its level being below 11.5 s in 20.0% of patients and above 14.5 s in 10.0% of patients. The mean PATPT value was 34.1 s and the platelets content reached 188×10^9/L; the platelets concentration above 300×10^9/L was seen in 10.0% of patients examined. The mean FAB content was 4.0 g/L, its level above 4 g/L was seen in 50.0% of patients. The DD level was 220 μg/L, its value being above 300 μg/L in 40.0% of patients examined and even above 500 μg/L in 20.0% of patients. The mean PCT level in these persons was 0.12 ng/mL.

As it has been underlined above cytokines disturb normal human hemostasis and belong to the main factors of SARS-CoV-2 thrombotic potential contributing to the disbalance in thrombotic and inner anti-coagulating processes. In persons with severe COVID-19 course the mean levels of IL-6, Il-10, IgA, CIC, and cortisol were the following: 4,0 pg/mL, 3,3 pg/mL, 1,4 pg/mL, 0.028 IU/mL, and 223.0nM/L, respectively. The mean CRP value was 7.7 mg/mL, its level being above 10 mg/mL in 60.0%, of patients and above 30 mg/mL in 20.0% of them. In patients having earlier had even moderate COVID-19 severity the mean IL-6 and IL-10 levels were 4.4 pg/mL and 2.5 pg/mL, respectively; the mean IgA level was 1.8 pg/mL; the mean levels of CIC, cortisol, and CRP were the following: 0.045 IU/mL, 195.6 nM/L, and 5.3 mg/mL, respectively. The CRP values above

10 mg/L were found in 40.0% of patients and above 30 mg/mL – in 20.0% of them.

The presence of certain rheological and serological changes in patients even in three months after survived COVID-19 needs further study in order to understand the necessity of anti-thrombolytic drugs uptake during a rather long time.

CONCLUSIONS

1. Summing up the results obtained it is possible to assert micro- and macro-vascular thromboses to be common in COVID-19 cases; they are associated with poor prognosis for diseased patients and are not completely investigated; the role of thromboses in COVID-19 course and complications are to be studied as well as the strategies of fibrinolytic therapies for such condition are to be justified.
2. In three months after the past COVID-19, the persons with severe disease course kept continuously unfavorable changes of PTT (12.8 s), PATPT (34.6 s), quantity of platelets (224×10^9 /L), FIB (4.2 g/L), DD (312 μg/L), and PCT (0,08 ng/mL); they are associated with systemic inflammation, i.e. with the levels of IL-6, IL-10, IgA, CIC, cortisol, and CRP reaching 4.0 pg/mL, 3.3 pg/mL, 1.4 pg/mL, 0.028 IU/mL, 223.0nM/L, and 7.7 mg/L, respectively.
3. In three months after the past COVID-19, in patients with the moderate disease course we have registered disturbances of PTT (12.6 s), PATPT (34.1 s), quantity of platelets (188×10^9 L), FIB (4.0 g/L), DD (220 μg/L), PCT (0.12 ng/mL); such indices were associated with systemic inflammation, the levels of IL-6, IL-10, IgA, CIC, cortisol, and CRP being 4.4 pg/mL, 2.5 pg/mL, 1.8 pg/mL, 0.045 IU/mL, 195.6nM/L, 5.3 mg/L.
4. The presence of certain rheological and serological changes in patients even in three months after survived COVID-19 needs further study in order to understand the necessity of anti-thrombolytic drugs uptake during a rather long time.

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