Review Article

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Overview of COVID-19's relationship with thrombophilia proteins

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Abstract: COVID-19 is the most devastating pandemic situation we have experienced in our age, affecting all systems. Although it affects all systems, it shows its most important effect through thrombophilia. Therefore, the possible cause of sudden death due to COVID-19 may be embolism caused by thrombophilia. D-dimer amounts increase due to COVID-19. The thrombosis is associated with sudden death in COVID-19 disease in populations. Since individuals with thrombophilia will be more prone to death due to COVID-19, it may be appropriate to administer low doses of Clexane (*Enoxaparin sodium*) or low-weight heparin for prophylactic purposes in order to consider these individuals at high risk and to prevent deaths. Moreover, in

order not to risk the lives of healthcare professionals with thrombophilia, it would be appropriate to keep them away from individuals with COVID-19 disease and to employ them in different healthcare services according to their fields of expertise. It should also not be forgotten that different symptoms related to COVID-19 appear day by day, these different symptoms probably show that the virus has undergone mutations in order to survive, but no matter what, its effect on thrombophilia has not been eliminated yet. This compilation aims to present the reasons and causes of death due to COVID-19, possible treatment options, and thrombophilia panel tests and new parameters that may have a place in the meticulous interpretation of these tests and possible etiopathology in the light of current information. Therefore, presenting this information in a rational manner and keeping the parameters of the thrombophilia panel under strict control predict that the deaths due to the virus will be partially reduced.

Keywords: anticoagulant; COVID-19; thrombophilia; thrombosis.

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Introduction

Since the last month of 2019, mankind has been making great efforts to control severe acute respiratory syndrome (COVID-19) caused by the new coronavirus [1]. The most important of these efforts are the efforts to produce a vaccine, efforts to reveal the symptoms caused by the virus, efforts to treat severe acute respiratory syndrome by relying on drugs previously marketed to treat different diseases, and efforts to stop the cytokine storm [2]. Despite all these efforts, the disease could not be brought under control worldwide and deaths could not be prevented. Sudden cardiac death is one of the most common complications in this disease [3]. The human body over-activates the immune system in the fight against the virus and causes a cytokine storm [4]. Furthermore, one of the most serious complications that can occur in patients affected by COVID-19 is thrombosis. The probable cause of sudden death due to

COVID-19 observed by humans to date is embolism-related deaths [5]. Therefore, this study was compiled to help prevent sudden deaths by associating the subject of thrombophilia (increased tendency to form thrombus) with COVID-19 in the light of current information.

Thrombophilia

Thrombophilia is the increased tendency of blood to thrombose, that is, it causes hypercoagulability [6]. Another cause of hypercoagulability is hypofibrinolysis (decreased ability to break down thrombin). Whatever the cause is, stasis and coagulation system disorders in the venous system cause thrombosis, while in the arterial system, endothelial damage and functional disorders of platelets play a role. This pathological condition causes decreased compliance and vasomotion in the blood vessels of major organ systems [7]. Moreover, due to thrombophilia, clots form in organs such as the heart, lungs, and brain, and this formation leads to sudden death [8]. Thrombophilia presents with two different mechanisms: hereditary and acquired. While hereditary and acquired factors may be the sole cause of thrombophilia, in most cases, both hereditary and acquired causes coexist [9]. In the next part of the article, hereditary and acquired causes of thrombophilia will be discussed in detail, respectively.

Hereditary thrombophilia and its causes

Three factors called Virchow triad (venous stasis, hypercoagulability and endothelial damage) for the preservation

of haemostatic balance; (1) the vessel wall should be intact, (2) the blood's coagulation ability should be normal, (3) the elements of the haemostasis system (vascular endothelial cells, platelets, von Willebrand factor, tissue factor, other coagulation proteins, fibrinolytic system and anticoagulant proteins) should be flawless [10]. In other words, to ensure haemostasis, the coagulation system, natural anticoagulants and fibrinolytic system must be in balance. Therefore, a defect that may occur in the coagulation system, natural anticoagulant and fibrinolytic system causes deterioration of haemostasis. The clinical manifestation of this condition is generally pulmonary embolism (PE) or deep vein thrombosis (DVT) [11]. In this context, many thrombophilic factors leading to hereditary thrombophilia have been described so far [12]. Hereditary factors leading to thrombophilia are summarized in Table 1 [12].

Among these hereditary factors, prothrombin G20210A (alone), and Factor V Leiden and prothrombin G20210A gene mutation coexistence are the most common hereditary risk factors for venous thromboembolism [13]. In addition to the above-mentioned hereditary risk factors for venous thromboembolism, protein C, protein S and antithrombin-III deficiency are the causes of thrombophilia in many cases [14]. In this part of our article, PC, PS, AT deficiencies and FVL and PT G20210A gene mutations, which are clinically significant only in terms of VTE risk, are discussed in detail in the light of current literature [15, 16], while other parameters (dysfibrinogenemia, hyperhomocysteinemia, cystathionine-beta synthetase, methionine synthetase and methylene tetrahydrofolate reductase hereditary deficiency plasminogen deficiency, high lipoprotein a, tissue plasminogen activator, heparin cofactor II, Histidine-rich glycoprotein, platelet glycoprotein, plasminogen activator, thrombomodulin, Factor VIII elevation,

Table 1: The most common inherited causes of thrombophilia that can cause thrombosis.

Increases	Abnormalities – mutations	Decreases
– APCR (factor V)	– Dysfibrinogenemia	– AT III (thrombin neutralization)
– CBS gene mutation (hyperhomocysteinemia)	– Fibrinolytic system abnormalities	– HC II
– Factor VII	– GP VI gene mutation	– HRG (also high levels)
– Factor VIII	– JAK2 gene mutation	– Plasminogen (Hypo-dysplasminogenemia)
– Factor IX	– KNG1 gene mutation	– PC
– Factor XI		– PS
– Factor XII		– VWF (G1691A)
– Lipoprotein A		
– MTHFR gene mutation (C677T) homozygosity		
– PAI-1		
– Prothrombin (G20210A) gene mutation		

APCR, active protein C resistance; AT III, Antithrombin III; CBS, cystathionine beta-synthase; GP VI, Glycoprotein VI; HC II, heparin cofactor II; HRG, histidine-rich glycoprotein; JAK2, Janus kinase 2; KNG1, Kininogen one gene mutation; MTHFR, methylene tetrahydrofolate reductase; PAI-1, plasminogen activator inhibitor-1; PC, protein C; PS, Protein S; VWF, von Willebrand factor.

Factor IX elevation, Factor XI elevation, Factor XII and Factor) will be briefly mentioned [17, 18].

Antithrombin (AT) deficiency

The presence of AT deficiency was first described by Egeberg in 1965 [19]. AT (heparin cofactor or Antithrombin III; AT III) inhibits thrombin and is inherited in an autosomal dominant manner [20]. So, it is a protein synthesized in the liver and released into the blood, consisting of 432 amino acids, which naturally prevents the formation of blood clots (thrombus). Antithrombin-III exists in plasma in two states as latent and active [21]. Temperature and pH affect the equilibria of these two phases against each other [22]. In addition to the thrombin inhibition of this protein (inhibition of fibrin formation), it also inhibits Xa, XIa, XIIa and kallikrein serine proteases [23]. It has been reported that the effect of AT increases approximately 1,000 times in the presence of heparin or heparin-like molecules. Therefore, if antithrombin-heparin cofactor measurement is to be performed, if heparin is included in the treatment, it should be done after at least two weeks of heparin discontinuation [24].

Two types of protein AT deficiency are known. (A) Type-I deficiency: Low synthesis of the AT molecule. Functional tests are impaired due to low AT synthesis. (B) Type II deficiency; there is no problem in synthesis. That is, the amount is normal, but the function is defective. Type II deficiency also has three subtypes. (1) “reactive site” defect of AT, (2) Type II RLS: “Heparin binding site” defect; and (3) Type II PE: “pleiotropic effect” with a multifunctional defect. The frequency of VTE events is reported to be 6% in heterozygous individuals with defects in the heparin-binding domain [25]. However, more severe VTE events may occur in homozygous individuals. Antithrombin-III deficiency has been reported in around 50% of those with VTE [26, 27]. Therefore, AT deficiency should be considered in order to prevent thrombus-related deaths in those who have COVID-19 disease.

Protein S (PS) deficiency

The presence of PS deficiency was first reported in 1984 [28]. PS is a vitamin K-dependent glycoprotein and is inherited in an autosomal dominant manner. This deficiency is found in 0.5% of the general population. Protein S is synthesized mainly from the liver. It has also been reported to be expressed in endothelium, megakaryocytes and testicular

Leydig cells [29, 30]. Protein S is a nonenzymatic cofactor in the inactivation of FVa and FVIIIa by activated protein C (APC). PS itself can also inhibit tenase and prothrombinase complexes; this reaction is independent of APC [31, 32]. In protein S deficiency, the factor Va, which causes thrombophilia, is excessively increased. Therefore, suppression of Va factor activity is associated with protein S. There are both heterozygous and homozygous conditions, and most of the patients are heterozygous [32].

There are three types of protein S deficiency. In Type 1 PS deficiency (quantitative disorder); both total PS and free PS were decreased. In Type 2 PS deficiency, total PS and free PS are normal, but PS function is impaired. Type 3 PS deficiency; total PS amount is normal, free PS amount is decreased, functional test is also impaired [29, 30, 33]. 40% of Protein S, circulates free and 60% with C4b-binding protein (C4b-BP) in the plasma [34]. But free protein S activates protein C as a cofactor [35]. A decrease in free protein S has been reported in HIV infection [36]; a viral infection (possibly due to COVID-19-related declines is also possible). It has been reported that free PS is low as a result of urinary excretion in nephrotic syndrome. Free protein S is also decreased in inflammatory bowel disease [37]. Moreover, protein S levels decrease in the use of oestrogen, acute thrombosis, warfarin treatment, chronic liver disease and DIC development [9]. Since free protein S might decrease due to comorbidities that occur during COVID-19 disease (for example, in nephrotic syndrome, tables such as DIC) or individuals who have COVID-19, the tendency of thromboembolism increases in patients. Therefore, it is useful to consider these situations in COVID-19 infections.

Protein C (PC) deficiency

The anticoagulant effect of PC (autoprothrombin IIA and blood coagulation factor XIX) was first demonstrated in 1960 [38]. PC deficiency is detected in 0.5–6% of patients presenting with venous thromboembolism [39]. Consisting of two subunits linked by disulphide bonds, this protein is 62 kDa in size and is synthesized in the liver [40]. PC has a very short half-life of 8 h [41]. Vitamin K is required for its synthesis [42]. It is inherited in an autosomal dominant manner. Thrombin activates the PC. Binding of thrombin to endothelial thrombomodulin and endothelial protein C receptor (EPCR) makes PC 20,000 times more active. In other words, when thrombin binds to thrombomodulin (TM) on the endothelial surface, its ability to convert fibrinogen to fibrin is lost. Activated protein C (APC), active FV and FVIII causes its inactivation. In

addition to its anti-coagulant effects, PC also has different biological effects such as anti-inflammatory and cell-protective effects [39, 43].

Two types of protein C deficiency, Type 1 and Type 2, have been reported to date. (A) In Type 1PC deficiency, protein C amounts are 50% of the normal, and this type is the most common type. (B) In the absence of Type 2PC, the blood protein C level is normal, but it is not functionally active [43]. Thrombosis in PC abnormalities is usually observed in lower extremity veins, iliofemoral veins, mesenteric veins, and cerebral veins. VTE and pulmonary embolism may accompany these events. Furthermore, protein C deficiency in pregnancies causes fetal losses (spontaneous abortions) [44].

Resistance to activated protein C (APCR)

Dahlbäck reported in 1993 that the response obtained with activated partial thromboplastin was impaired in families with venous thrombosis of unknown origin and the presence of activated protein C resistance [45, 46]. APCR (decreased anticoagulant response) is the most common cause of inherited coagulation tendency and is autosomal recessive. The most common cause of this resistance is Factor V Leiden mutation [47]. The frequency of this mutation in the general population is around 2–10%. On the molecular basis of the active protein C-resistance state, as a result of the replacement of guanine at nucleotide 1,691 in the FV gene with adenine and the replacement of arginine at position 506 by glutamine, APC cannot inactivate the FVa protein, resulting in APCR [48]. It is responsible for 21–60% of patients with a history of thromboembolism [47]. It has been reported that the risk of thrombosis increases 5–10 times in heterozygotes and 50–100 times in homozygotes. Factor V Leiden causes hypercoagulopathy for two reasons; (i) Activated FV Leiden with reduced clearance in serum accelerates thrombin formation from prothrombin. (ii) Protein C-formed fragments of factor Va contribute to the degradation of FVIIIa by acting as a cofactor. As a result, the resistance of FVa to degradation indirectly prevents the degradation of FVIIIa by APC, and thus the anticoagulant effect is reduced [47, 49]. Presence of APCR is a risk factor for pulmonary embolism, deep vein thrombosis, cerebral, mesenteric and portal vein thrombosis. FV Leiden mutation in children causes cerebral infarcts more frequently than adults [47]. APCR has also been reported to have direct anti-inflammatory and antiapoptotic effects [50]. The most common cause of active protein C resistance is the FV Leiden mutation [51]. Now, in this part of the article, let's look at the FV Leiden mutation in detail.

Factor V (FV LEIDEN (FVL), A-506-G) mutation

The relationship between factor V and protein C is disrupted by mutation, and mutant factor V is named as Factor V Leiden (FVL) [52]. In factor V Leiden mutation, the inactivation of factor Va and VIIIa decreases 10 times, and thus the tendency towards thrombosis increases [53]. If a mutation occurs in factor V, activated protein C resistance occurs, and the tendency towards thrombosis increases. That is, APC resistance is often associated with deep vein thrombosis [54].

In the case of replacing guanine with adenine in the 1,691 nucleotide of the gene encoding factor V, glutamine replaces arginine at the 506th position in the structure of factor-V, and in this case, it is called the “A-506-G” mutation [55]. Furthermore, intracranial arterial and venous thrombosis occurs in people with Factor V Leiden mutations [56]. It has been reported that the rate of FVL mutation is 12% in patients with unknown cause of stroke. FVL is the most common hereditary cause of unexplained venous thrombosis. Its incidence in the general population is 4.8%. It has been reported that FVL heterozygous patients have a 10% lifetime risk of thrombosis [57].

As mentioned above, since the presence of FVL mutation increases the tendency towards thrombosis, COVID-19 patients should be questioned in terms of the presence of FVL mutation, and necessary precautions should be taken not to lose them from intracranial arterial and venous thrombosis events. This is because Factor V Leiden mutation may be a risk factor for intracranial arterial and venous thrombosis in COVID-19 patients regardless of age.

Possible causes of some important acquired natural anticoagulant deficiencies and COVID-19

Heparin-antithrombin III, protein C and tissue factor inhibitors (extrinsic pathway inhibitors) are natural anticoagulation mechanisms in the organism and keep the coagulation system under tight control [58]. Possible causes of acquired natural anticoagulant deficiencies are briefly summarized in Table 2.

Acquired antithrombin-III deficiency

Decreased AT-III syntheses in liver diseases and, in the case of nephrotic syndrome, an increased excretion of antithrombin-III cause antithrombin-III deficiency [59, 60].

Table 2: The most common causes of acquired thrombophilia that increase the tendency towards thrombosis.

Changing parameters		
– aB2GP1	– IBD	– Prolonged bad rest or immobilization
– aCL antibodies	– Inflammation	– Prothrombin complex concentrates L-asparaginase
– Air pollution	– Long haul travel (long-distance flights)	– Puerperium
– Apolipoprotein	– Lupus anticoagulants syndrome	– Renal failure
– APS	– Marked leukocytosis in acute leukemia	– SCA
– Cancer	– Medications: OCS, HRT, Tamoxifen	– Senilite
– (Malignancy)	– Bevacizumab, thalidomide, lenalidomide	– Smoking
– CHF	– Steroids	– Thrombophilia or coagulation protein regulation deficiency: combined thrombophilia. DIC, hyperestrogenemia (increased hepatic coagulation factors)
– DM	– MetS	– Tissue damage: Surgery (especially orthopedic). Fracture. Burns
– Drug induced: HIT	– MI – Prosthetic cardiac valves	– Trauma
– ET	– MM	– TIP
– Fractures	– Myeloproliferative diseases: PV.MPN	– Vines: SARS-CoV-1. SARS-CoV-2
– Hematologic	– Nephrotic syndrome (loss of small proteins including AT-III)	– VTE
– Diseases	– Obesity	– WM
– HIV/AIDS	– PNH	
– Hypertension	– pregnancy	
– Hyperviscosity	– Presence/indwelling of central venous catheter	

aB2GP1, anti-Beta two glycoprotein I; aCL, anticardiolipin; AIDS, acquired immunodeficiency syndrome; APS, antiphospholipid antibody syndrome; AT III, Antithrombin III; CHF, congestive heart failure; DIC, disseminated intravascular coagulation; DM, diabetes mellitus; ET, Essential thrombocythemia; HIT, heparin-induced thrombocytopenia; HIV, human immunodeficiency virus; HRT, hormone replacement treatments; IBD, Inflammatory bowel disease; MetS, metabolic syndrome; MI, myocardial infarction; MM, multiple myeloma; MPN, chronic myeloproliferative neoplasms; OCS, oral contraceptive drugs; PNH, paroxysmal nocturnal hemoglobinuria; PV, polycythemia vera; SARS-CoV, severe acute respiratory syndrome coronavirus; SCA, sickle cell anemia; TTP, thrombotic thrombocytopenic purpura; VTE, previous venous thromboembolism; WM, Waldenström's macroglobulinemia.

Antithrombin-III deficiency occurs due to increased AT-III consumption in preeclampsia, eclampsia, pregnancy and hypertension [61–63].

Oral contraceptives (OCS), L-asparaginase and heparin have also been reported to cause AT-III deficiency [64, 65]. Acute venous thromboembolism, disseminated intravascular coagulation (DIC) and sepsis are seen in COVID-19 disease [66–68]. This should be considered in the treatment of COVID-19 patients, as acquired antithrombin-III deficiency will occur due to increased AT-III consumption in cases of acute venous thromboembolism, disseminated intravascular coagulation (DIC) and sepsis.

Acquired protein C deficiency

Sepsis has an important place among the causes of acquired thrombophilia in relation to COVID-19. In other words, it has been reported that sepsis (causing acquired thrombophilia) develops in severe COVID-19 patients [69]. Fibrinolysis is also reduced in severe sepsis patients [70]. In other words, disseminated intravascular coagulation, microvascular thrombosis, organ failure and shock may develop in severe sepsis [71], because PC is consumed during this time (possibly PC is decreasing

due to vitamin K deficiency) [72]. Vitamin K deficiency may be caused by the fact that antibiotics given to prevent bacterial pneumonia in severe COVID-19 patients kill bacteria that synthesize vitamin K in the intestines, and the plasma level may decrease with liver disease and warfarin use [73]. Additionally, acute disseminated intravascular coagulation, extensive deep vein thrombosis, severe infections, sepsis, and chronic renal failure also cause PC deficiency [74]. Apart from all these, severe infections (meningococemia), cyclophosphamide, methotrexate, 5-fluorouracil and L-asparaginase treatment also cause acquired protein C deficiencies [75]. Protein C deficiencies or decrease of protein C increase the development of microvascular thrombosis and DIC [76]. In addition to decreasing the anticoagulant effect of PC, its anti-inflammatory effect is also reduced [77]. For all these reasons, the coagulation cascade should be followed strictly to prevent sudden death in severe COVID-19 patients.

Acquired protein S deficiency

Liver diseases, HIV infection, L-asparaginase treatment and recovery phase of chickenpox, acquired intravascular

coagulation, and acute thromboembolic events lead to protein S deficiency [78–80]. In addition to PC, PS levels also decrease in patients taking Warfarin [81]. Since protein S deficiency will probably occur during the recovery phase of COVID-19 disease, this should be considered in order to prevent deaths due to thromboembolism and venous thrombosis [82].

Causes of acquired APC resistance

Increased FVIII level [83], pregnancy [84], oral corticosteroids (OCS) use [85], systemic lupus erythematosus (SLE) [86], antiphospholipid antibodies, multiple myeloma [87] and cancers cause [88]. Also, *APCR* and factor *V Leiden* are associated with unexplained recurrent pregnancy loss and has been identified as the most common inherited risk factor for venous thrombosis [89]. Therefore, knowing the above-mentioned conditions, diseases and the presence of Factor V Leiden mutation in severe COVID-19 patients will bring a different perspective to the approach of COVID-19 treatment. Moreover, due to the anticoagulant and anti-inflammatory properties of APC, its use in the treatment of severe sepsis caused by COVID-19 may possibly be beneficial. Furthermore, bacterial infection, autoimmune diseases (e.g., lupus erythematosus), malignancies (hepatoma), or drugs used (corticosteroids) may cause hypercoagulation. Therefore, in these cases, it is inevitable that the use of anticoagulant molecules will be beneficial in order to prevent patient losses. It should not be forgotten that steroids increase the number of platelets in the blood elements in order to prevent the formation of fibrous bands in the lungs [90]. If the patient does not have fever and there is no agreement between CRP values and platelet values, it should be considered as steroid-related, and the condition should be followed up.

Acquired hyperhomocysteinemia

High homocysteine (hyperhomocysteinemia) is known to cause venous and arterial thrombosis [91]. It has been suggested that hyperhomocysteinemia develops due to cobalamin, pyridoxine and folate deficiency, and, in this case, it may cause thrombosis by affecting the vascular endothelium [92]. As it is known, under normal conditions, the endothelial surface has anti-thrombotic properties through molecules such as prostacyclin, NO (anti-aggregant), TFPI, thrombomodulin, heparan sulphate

(anticoagulant) and tissue plasminogen activator (fibrinolytic) [93].

Factor II (prothrombin G20210A) mutation

Prothrombin (G20210A) is a form of prothrombin and is the most common genetic origin point mutation of thrombosis [94]. If a patient has a Prothrombin G20210A mutation, it increases the level of prothrombin molecule in the blood. The increase in the level of prothrombin molecule in the blood increases the tendency of both vein and arterial thrombosis [16]. In particular, if there is an FVL mutation in these patients, the risk of thrombosis increases [95]. Prothrombin 20210A mutation is an autosomal dominant disorder [96]. The risk of thrombosis increases 3–10 times in heterozygotes. Although homozygosity is rare, there is a risk of thrombosis [97]. Also, hereditary deficiency of cystathionine -beta synthetase, methionine synthetase and methylene tetrahydrofolate reductase, Heparin cofactor II deficiency, Plasminogen deficiency, Dysfibrinogenemia Factor XII deficiency, and increase in factor VIII coagulant activity are among the factors that increase the tendency towards thrombosis [98–102].

Thrombophilia and viral infections

Although there are some antiviral drugs, viral infections do not have a complete drug treatment and they cause damage by affecting some organs (for example, in COVID-19 disease, lung, kidneys, muscle, eye, etc.) and lead to coagulation-related disorders [103–105]. It is beneficial to know the physiopathology of viral infections well, to eliminate bleeding and thrombotic complications associated with viral infections and to improve supportive care in treatment.

In cases of viral infection (including SARS-CoV-2), it has been reported that the amounts of coagulation molecules such as factor VIII, factor XI, soluble tissue factor and Von Willebrand factor, prothrombin fragment 1 β 2 and thrombin–antithrombin complexes, platelet activation, fibrin destruction and fibrinolysis (e.g., D-dimer and plasmin– α 2-antiplasmin complexes) increased [67, 106, 107]. On the other hand, it was reported that the amounts of natural anticoagulants (protein C, protein S, antithrombin and tissue factor pathway inhibitor) decreased [108, 109]. It has been reported that there is a link between the coagulation cascade and cytokine release in cases of viral infection [110, 111]. Namely, in cases of viral infection, the amounts of proinflammatory cytokines such as IL-6, IL-1,

IL-12, and TNF- α increase, and these increased proinflammatory cytokines cause an increase in the Von Willebrand factor responsible for platelet activation, triggering clot formation [112, 113]. Also, the amount of thrombomodulin on endothelial cell surfaces decreases, and accordingly, the amount of protein C required for protein C activation is reduced [109, 114, 115].

In many viral and bacterial infections (e.g., meningococcemia), which are severe and cause sepsis, damage on the inner surfaces of vessels and inflammation-induced coagulation occur [116–118]. In the H1N1 influenza (swine flu) epidemic, both thrombotic and hemorrhagic complications such as deep vein thrombosis, pulmonary embolism, and hemoptysis, as well as pulmonary hemorrhage, hematemesis, petechial rash, and petechial cerebral hemorrhage, have been reported [103, 119]. Intrarenal fibrin deposition has also been reported in some cases of influenza, resulting in renal failure [120]. Similarly, widespread intravascular coagulation, pulmonary hemorrhage and thrombocytopenia have been reported in some cases infected with H5N1 (bird flu) [121]. In the case of SARS disease, pulmonary thromboembolism resulting in intravascular coagulation, deep vein thrombosis and pulmonary infarction has also been reported [122]. It has been stated that coagulation disorders seen in viral infections may be associated with vascular endothelial damage disorders [123, 124].

Apart from all these, Hepatitis C infections (HCV) have been reported to cause thrombosis [125, 126]. As can be understood from the above information, there is a close connection between viral infections and the formation of coagulation [103]. Therefore, in other viral infections, including COVID-19, if hereditary thrombophilia is present, the coagulation cascade pathology will be more complex, and deaths due to thrombosis will become more common. For this reason, one of the main ways to prevent COVID-19-related deaths is to follow what is happening in the coagulation cascade.

Thrombophilia and COVID-19

Bleeding or bleeding disorders are the leading cause of death resulting from COVID-19 [118, 127, 128]. According to the reported findings, coronavirus causes hyperactivity in platelet cells, thus aggregating platelets, leading to clot formation. Hyperactivity in blood coagulation cells causes strokes and heart attacks [129, 130]. Also, it is known that the system responsible for the removal of blood clots in the disease shows excessive activity (hyperfibrinolysis) [131]. It has been reported that people with lung, diabetes, high blood pressure, and heart or kidney disease have a higher risk of developing COVID-19 [132].

It has been reported that plasminogen and plasmin levels are high in individuals with pre-existing lung, heart and kidney and diabetes. Plasminogen and plasmin levels higher than the normal lead to severe bleeding. In the blood, plasminogen is an inactive substance. When substances in the cells of blood vessels activate plasminogen, plasmin is produced from the blood, which dissolves blood clots [133, 134]. It has been reported that, in COVID-19 patients, who are associated with the dissolution of blood clots and the disease progresses severely, an increase in D-dimer levels and a bleeding picture similar to Disseminated Intravascular Coagulation (DIC) develops, and in parallel, there is a prolongation in INR/PT tests. D-dimer is a protein produced by the clot dissolving [115, 135]. It should not be forgotten that D-dimer amounts also increase due to different reasons such as severe infections, sepsis and trauma [136]. However, it has been reported that D-dimer levels decreased in survivors of COVID-19 and in cases that did not develop acute respiratory distress syndrome. The risk of disruption in coagulation mechanisms increases due to COVID-19 disease [137, 138].

Considerations in the hereditary diagnostic tests of thrombophilia

There are reliable tests on the market to test for the presence of hereditary thrombophilia (activity of natural anticoagulants) [139, 140]. Among the thrombophilia tests, it is possible to work reliably with the Sanger Sequencing method (DNA; whole blood with EDTA) parameters of FII, FV Leiden, MTHFR (A1298C), MTHFR (C677T) [141, 142]. The blood sample must have been drawn in the correct test tube in the proper and required amount. Plasma is used to measure the activity of natural anticoagulants and should be a fresh sample if possible [143]. The instrument used in the measurement must be well calibrated. Blood samples that are hemolyzed, lipemic, clotted, or with a hematocrit value greater than 55% should not be used. These tests should be run by experienced laboratory personnel. The tests that should be requested in order of priority in COVID-19 patients with suspected thrombophilia are given in Table 3 [144].

General interpretation of hereditary thrombophilia panel tests

Factor V Leiden and prothrombin 20,210 mutations: These two parameters indicate the risk of developing venous thromboembolism (VTE) [145].

Table 3: Possible tests to be requested from laboratories in order of priority in case of suspected thrombophilia.

HIGH PRIORITY	MILD PRIORITY	LOW PRIORITY	UNKNOWN PRIORITY
<ul style="list-style-type: none"> • APCR / Factor V Leiden • Factor VIII • Homocystein • LAC • Prothrombin (G20210A) 	<ul style="list-style-type: none"> • Antiphospholipid antibodies • aB2GP1, aCL, LAC • AT III • PC • PS 	<ul style="list-style-type: none"> • Factor IX • Factor XI • Fibrinogen tests • MTHFR gene mutation (C677T) 	<ul style="list-style-type: none"> • CD55, CD59 (For PNH) • JAK2 gene mutation (For Myeloproliferative Disorders) • Plasma lipoprotein A (For CHD) • Plasminogen (For Acute Medically Illnesses) • TFPI (For Infection)

aB2GP1, anti-Beta 2 glycoprotein I; aCL, Anticardiolipin; APCR, Active Protein C Resistance; AT III, Antithrombin III; CD, cluster-of-differentiation-proteins; CHD, Congenital heart disease; JAK2, Janus kinase 2; LAC, Lupus anticoagulants; MTHFR, Methylene tetrahydrofolate reductase; PC, Protein C; PNH, Paroxysmal nocturnal hemoglobinuria; PS, Protein S; TFPI, Tissue factor pathway inhibitor.

AT-III deficiency: It shows the risk of occurrence of thrombotic images [146]. In other words, it can give information about venous thromboembolism (VTE), nephrotic syndrome, preeclampsia, eclampsia, pregnancy, hypertension, liver diseases, sepsis, DIC, oral contraceptives (OCS), L-asparaginase and heparin use. Considering Factor V Leiden, prothrombin gene mutation, protein C and protein S deficiencies, the frequency of AT-III deficiency in the first VTE events was found to be 0.5–1% [147–149].

Protein C deficiency: It is the main indicator of fetal losses (spontaneous abortions), VTE, purpura fulminans and warfarin-induced skin necrosis, pulmonary thromboembolism, and non-hemorrhagic cerebral strokes. Protein C deficiency can be hereditary, as well as acquired protein C deficiency due to DIC, ARDS, liver diseases, severe infections (meningococcemia), septic shock, postoperative period, cyclophosphamide, methotrexate, 5-fluorouracil and L-asparaginase treatment [44, 150–152]. It is possible that, leaving aside hereditary Protein C deficiency, the treatments received by individuals with co-morbidities in COVID-19 disease may have caused acquired protein C deficiency, leading to pulmonary thromboembolism and non-hemorrhagic cerebral strokes and heart attacks, leading to sudden death.

D-Dimer test: D-Dimer test for corona virus infection, diagnosis and follow-up may also give an idea [153], but it should be considered that the amount increases in sepsis and severe infections beside corona virus [154].

Ways to prevent sudden deaths from COVID-19 due to thrombophilia

Screening for thrombophilia in COVID-19 patients will possibly reduce casualties. In the physical examinations of

COVID-19 patients, it should be questioned whether there is thromboembolism, warfarin-induced skin necrosis, neonatal thrombosis, recurrent, migratory or massive thrombosis, cerebral, upper extremity, intra-abdominal thrombosis, and past thromboembolism attacks. There are no currently studies contain information concerning the prevalence of thrombophilia. Also, there are no studies present the association between thrombophilia's panel and COVID-19 mortality. However there are numerous studies present indicating that there are relationship between thrombophilia and COVID-19 [104, 155–159]. So that thrombophilia's panel and COVID-19 mortality relationship remains an important research topic.

As it is known, many health personnel around the world have lost their lives in the COVID-19 struggle. It is difficult to train health personnel (especially to train physicians), and it is extremely burdensome for countries' economies. Therefore, in order not to lose health personnel in this struggle, if the above-mentioned conditions are present in their history, thrombophilia panel screening should be performed. Health personnel whose thrombophilia panel is found to be problematic should be kept out of this struggle and employed in a different field.

Conclusions

Numerous studies have shown that viruses (including COVID-19) affect the coagulation cascade [160–162]. Undoubtedly, the coagulation cascade will be more affected in COVID-19 patients with hereditary thrombophilia [155]. In other words, thrombotic complications will come to the fore more. Therefore, it is useful to evaluate COVID-19 patients in terms of the Virchow triad [163]. At least 6% of Western population have a hypercoagulation, usually as an autosomal dominant inherited defect or mutation [12].

For this reason, special therapeutic interventions (such as low molecular weight heparin) may be required to restore the harmony of the haemostatic balance affected in COVID-19 disease, and thus, sudden deaths from COVID-19 can be prevented [164]. In addition, theoretically, anti-TNF, activated protein C may provide benefits in controlling the cytokine storm caused by COVID-19 [113]. Again, we also think that antihistamines, especially cetirizine, will provide benefits as soon as COVID-19 complaints arise [165]. This is because cetirizine has been found to inhibit the reproduction of the SARS-CoV-2 virus [166]. It has even been reported that anti-histamines inhibit the reproduction of the hepatitis C virus and provide the elimination of the liver damage of the virus [167]. Considering that both SARSCOV-2 and hepatitis C virus are RNA viruses [168], it is theoretically expected that antihistamines, especially cetirizine, may also be effective against SARS-CoV-2.

In the fight against viruses, especially during epidemic periods, the most effective way to protect against viral diseases is to follow the rules of hygiene, mask, distance, state authorities and health professionals. However, it is known that the best results in the fight against COVID-19 are obtained when antiviral drugs (for example, anti-virals such as Favipiravir) are given as soon as the patient starts to complain. Moreover, while the use of dexamethasone has no effect in patients with mild COVID-19 infection, it has been reported that it is effective and reduces mortality in severe COVID-19 patients who require oxygen or ventilator support. Genetic characteristics and age are important factors in COVID-19 related deaths [169]. We think that the biological age of the individual's tissues and cells is more important than the chronological age in age-related deaths from COVID-19. While many elderly patients recover from this disease and regain their health, it is known that many patients who are younger in chronological age die due to COVID-19. If desired, the biological ages of COVID-19 patients can be determined by measuring the amount of telomerase, and if there is a shortness of telomerase, we anticipate that deaths can be partially prevented by extending the anti-viral use periods in these patients. To sum up, viral infections lead to acquired coagulation cascade disorders [103]. It is predicted that the acquired coagulation disorder due to these viral infections will increase the severity even further in case of hereditary thrombophilia. Therefore, in order to prevent sudden death due to thrombosis, COVID-19 patients should also be questioned in terms of thrombophilia, and their treatment should be arranged accordingly.

Wishes and future recommendations

Screening of hereditary thrombophilia factors in every patient with a previous history of thrombosis (generally under the age of 40 years of VTE attack) and infected with COVID-19 may be beneficial in the follow-up of the disease and the survival of the patient. Hereditary thrombophilia tests can inform the physician about whether anticoagulant therapy should be initiated in the patient with COVID-19 or whether it should be discontinued if he or she is taking anticoagulants for any reason. Screening for PC and PS deficiency in patients with COVID-19 who also have Purpura fulminans may be useful for patient survival. If there is a family history of susceptibility to thrombosis (Thrombosis-prone lineages) in the patient and their relatives who are quarantined due to COVID-19, it may be useful to perform a thrombophilia panel screening. However, acquired causes should be excluded before screening a thrombophilia panel, because COVID-19 may be one of the new acquired causes leading to thrombophilia. In order to prevent sudden death due to this new acquired (COVID-19-related) thrombophilia, it may be more beneficial to use anticoagulant molecules by screening. Otherwise, it should not be forgotten that giving unnecessary anticoagulants will pose new risks.

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