Chapter

DNA Polymorphisms as Potential Biomarkers of Thrombophilic Prognosis for COVID-19 Patients

Tatyanny Paula Pinto da Costa Santos Fucci, Rubens Pitliuk and Ane Claudia Fernandes Nunes

Abstract

Coronavirus disease 2019 (COVID-19) is a major issue of our times. Many aspects and features of this new and complex disease are being described on a daily basis. Major endpoints are systemic inflammation, markedly characterized by the cytokine storm, respiratory failure, and coagulation disorders, such as thrombophilia. In its terms, thrombophilia has a major impact on the COVID-19 prognosis. With regard to this, paying attention on molecular variants, such as DNA polymorphisms, epigenetic factors, and other biomarkers, could be an important approach to optimizing and personalizing the treatment of patients according to their inherited thrombotic features. This chapter brings an overview on the three major DNA polymorphisms associated with thrombophilia and proposes that these same biomarkers could be used in pretreatment screenings of patients with COVID-19 to seek the most appropriate therapy for each individual molecular profile.

Keywords: COVID-19, coagulation, thrombophilia, biomarkers, DNA polymorphisms

1. Introduction

One of the major issues of the 21st century, without any doubt, is the viral respiratory disease discovered at the end of 2019, the COVID-19. Using next-generation sequencing, the pathogen related to COVID-19 was described as a novel coronavirus, which is related to the SARS-coronavirus described in 2003, mainly in Asia, from the molecular and phylogenetic aspects [1].

A severe respiratory disease was reported in Wuhan, Hubei province, China. Epidemiological investigations have suggested that the outbreak was associated with a seafood market in Wuhan [2].

The first case reported in the medical literature was that of a 41-year-old man who was hospitalized in the Central Hospital of Wuhan. The patient was admitted to the hospital reporting an extensive set of symptoms since one week before his admission on December 26, 2019, which included dry cough without sputum, fever, weakness, chest tightness, and widespread pain. Normal signs were observed at physical examination on abdominal, cardiovascular, and neurological features.

2. General aspects on COVID-19 diagnosis

Among the biomarkers analyzed, one of the most important findings was the raised C-reactive protein (CRP) level of 41.4 mg/L (standard at 0–6 mg/L) whose circulating concentrations rise in response to inflammation. Followed by the higher CRP, the biochemistry cardiac panel also showed raised myocardial infarction markers, such as lactic dehydrogenase (LDH), aspartate aminotransferase (AST), and creatine kinase (CK). Taken together, these biomarkers strongly indicated a severe acute inflammatory phase with the ongoing cardiac effect to be controlled. Mild lymphopenia with less than 9 × 10e5 cells per mL with normal platelet counts completes the first patient's overview [2].

Pulmonary function and lung aspects were investigated in this patient in order to do the etiological diagnosis. Hypoxemia was observed according to oxygen levels of 67 mm Hg. Lungs' aspect was set by chest radiographs on day 1 of hospital admission, which was the 6th day of disease progression. The images showed abnormal features with focal and patchy consolidation in both lungs, beyond air-space shadowing such as ground-glass like opacities [2].

Image examination of the chest shows a consolidation pattern at computed-tomography (CT) scans: bilateral focal consolidation, lobar consolidation, and patchy consolidation, especially in the lower lung. Five days after admission, at the 11th day of disease progression, a chest radiograph revealed a bilateral diffuse patchy and fuzzy shadow [2].

With the advancement of the pandemic, what was seen as a respiratory disease became a more complex disease and new studies were set up to list other complications and associated risk factors.

3. Potential impact of thrombotic complications on COVID-19 prognosis

Many factors can contribute to increasing the risk for severe COVID-19, in some cases followed to death. The main comorbidities described are high age, obesity, diabetes, and hypertension. Beyond the inflammation and impaired coagulation, focal damage in some tissues/organs is also related to the COVID-19 spectrum, such as liver, kidney, and heart [3].

Thrombotic complications seem to emerge as an important issue in patients infected with COVID-19. Preliminary reports on COVID-19 patients' clinical and laboratory findings include thrombocytopenia, elevated D-dimer, prolonged prothrombin time, and disseminated intravascular coagulation.

In the course of the COVID-19 studies, a clear association with coagulation dysfunction was pointed in many cases. Intra-alveolar clots were prominent findings in COVID-19 patients who developed severe respiratory disease. The same findings have been described in both clinical and animal model studies. Apparently, an impaired response in the prothrombotic pathway is in charge of diffuse alveolar hemorrhage since it is related to overt clot formation [4].

In the recent publication "Should COVID-19 be branded to Viral Thrombotic Fever?," the authors intended to frame COVID-19 in more clinical terminology, making an analogy to Viral Hemorrhagic Fever (VHF). In this article, the authors reported: "We found irrefutable evidence in the current literature that COVID-19 is the first viral disease that can be marketed as a viral thrombotic fever" [5]. Although this is a very categorical statement, considering the small number of studies exclusively dedicated to the characterization of COVID-19 as a thrombotic fever, it is very important to consider this designation. Categorizing COVID-19 as a febrile variant of thromboembolism adds a series of procedures to be adopted in

patients' care. This approach can advance the treatment adequacy by many steps, making it as more personalized as possible.

4. DNA polymorhisms for a pharmacogenonic approach to COVID-19 treatment

The risk of thromboembolism in COVID-19 is documented in an article published in The Lancet [6]. This finding brings up an important issue to be screened on COVID-19 patients: the impact of inherited predisposition to thrombotic events in patients affected by COVID-19.

Given this, thrombophilic genetic abnormalities in variants were widely reported in the medical sciences such as Factor V Leiden (F5), Prothrombin (F2), and the polymorphism in methylenetetrahydrofolate reductase (MTHFR), among others [7]. These polymorphisms could put a patient's carriers of mutant alleles in the Risk Group, beyond the well-known factors, such as elderly patients, hypertension, cardiac and respiratory diseases, cancer, and diabetes [8].

In this chapter, we present a brief review of the three main DNA polymorphisms associated with thrombophilic events and suggest the inclusion of these, as well as the coagulation profiles of their carriers, as aggravating comorbidities of COVID-19.

Firstly, a brief review of the main molecular characteristics of these polymorphisms is as follows:

4.1 Factor V Leiden (FVL or F5)

It represents one of the main causes of resistance to protein C, as mutation increases the risk of thrombotic disease three to ten times for heterozygous carriers and eighty times for homozygous carriers [4, 9]. About 90% of cases of protein C resistance result from point mutation in the coagulation factor V gene. This mutation occurs in exon 10 of the factor V gene, causing a substitution of the G/A base (Guanine/Adenine) in nucleotide 1691, resulting in the exchange of Arg (Arginine) by Gln (Glutamine) at position 506 of the protein, one of the main cleavage sites for protein C activation [10]. FVL is the most common inherited cause of venous thrombosis.

In patients with increased protein C resistance, venous thrombosis without known etiology and familiar history of unexplained thrombosis, the FVL mutations' screening should be considered beyond a strong clinical investigation. The diagnosis for FVL mutations is based on well-known molecular biology approaches. The clotting time-based functional assays and genetic biomarkers' screening become together the basis for clinical decisions. It is a very important step to guide the clinical approach, balancing the long-term anticoagulation with its side effects and benefits [11].

Briefly, the mechanism of action of factor V could be described as follows. Factor V is cleaved by thrombin on its B domain at cleavage sites R709, R1018, and R1545, producing an amino-terminal heavy chain and a carboxy-terminal light chain, which binding themselves create a dimer called Factor Va (FVa). In turn, FVa binds with Factor Xa creating a prothrombinase complex which on the platelet surface converts prothrombin (II) to thrombin (IIa). FV can also be split by the action of activated protein C (APC) at the cleavage site R506 before it is cleaved by thrombin. It results in the inactivation of factor V to factor Vi and the generation of an imperfect peptide, the Factor Vac, which apparently has anticoagulant characteristics by stimulating APC- and protein S-mediated inactivation of factor VIIIa. A second mechanism of thrombosis observed with FVL is its diminished cofactor activity with APC and phospholipid in the inactivation of factor VIIIa to factor

VIIIi. Taken together, FVL is a prothrombotic mutation due to a combination of a gain of function, with higher prothrombin activation, and loss of function due to low cofactor activity with APC in the inactivation of factor VIIIa. Normally, patients with defective FVL have a variable thrombophilia phenotype, have increased thrombin generation, have a longer factor Va half-life in plasma, and are resistant to factor Va inactivation [11]. A larger C-terminal peptide results if factor V is cleaved by APC before it is cleaved by thrombin [12].

4.2 Prothrombin (PTB, factor 2 or F2)

The G20210A mutation of prothrombin causes a G to A transition at the nucleotide position 20,210. This mutation increases circulating prothrombin activity and levels [13]. PTB is a vitamin K-dependent coagulation factor, which in its active form is cleaved, forming in this way the thrombin. The thrombin catalyzes many other coagulation-related reactions and acts as a serine protease that converts fibrinogen to fibrin. PTB mutations are the second-most common inherited thrombophilia. In the United States, the heterozygous carrier frequency is about 1–2%, accounting for approximately 6–18% of VTE cases. Hyperthrombinemia has been associated with a mutation in the 3′ termination of the PTB gene, called c.∗97G > A, which results in increased production, due to the increased PTB mRNA expression and stabilization. An increased amount of circling prothrombin can lead to higher thrombin generation in the plasma, followed by coagulation activation and thrombosis. This mutation is also more common in the Caucasian population and is rare in other ethnic groups. Homozygosity, for this mutation, is found in about 1 in 10,000 individuals. Transheterozygosity for FVL and prothrombin c.*97G > A affects about 1 in 1000 individuals. Additional variations identified in the 3′-untranslated region of the prothrombin gene include changes at positions 20,207, 20,209, 20,218, and 20,221. High PTB levels also inhibit APC-mediated inactivation of activated FV and factor VIII. The prevalence of prothrombin G20210A mutation varies in different countries and ethnic groups, being highest in Caucasians, especially those in Southern Europe, and in the Mediterranean region [14].

4.3 Polymorphism in the MTHFR enzyme

Hotoleanu, in his article Genetic Risk Factors in Venous Thromboembolism, described that MTHFR acts on homocysteine metabolism, reducing 5.10-Methylenetetrahydrofolate to 5-methylenetetrahydrofolate. The enzyme polymorphisms generally occur at two sites, at position C677T, which characterizes the substitution of alanine for valine at codon 222, and at position A1298C, which occurs due to the substitution of glutamine for alanine at codon 429, the second mutation being less aggressive than C677T, which is homozygous and in the presence of low levels of folate decreases enzyme activity leading to hyperhomocysteinemia, a risk factor for thrombophilia [15].

Simoni et al. corroborate this theory, when they describe that mutations in the MTHFR enzyme reduce its activity leading to hyperhomocysteinemia. Increase in homocysteine levels is a risk factor for thromboembolism [16].

Considering patients with COVID-19, especially those seriously ill, there are several potential risk factors for venous thromboembolism, including infection, immobilization, respiratory failure, mechanical ventilation, and use of a central venous catheter [17, 18]. Wang et al. reported in their Lancet article that patients at a high risk for venous thromboembolism had worse results with COVID-19 than patients at a low risk for venous thromboembolism, suggesting that these patients may require more attention in the event of rapid deterioration [6].

Overall venous thromboembolism (TE): 21% (95% CI: 17–26%) ICU: 31% (95% CI: 23–39%)
Overall deep vein thrombosis rate: 20% (95% CI: 13–28%) ICU: 28% (95% CI: 16–41%) Postmortem: 35% (95% CI: 15–57%)
Overall pulmonary embolism rate: 13% (95% CI: 11–16%) ICU: 19% (95% CI: 14–25%) Postmortem: 22% (95% CI: 16–28%)
Overall arterial TE rate: 2% (95% CI: 1–4%) ICU: 5% (95% CI: 3–7%)
Pooled mortality rate among patients with TE: 23% (95% CI: 14–32%) and Pooled mortality rate among patients without TE: 13% (95% CI: 6–22%)
The pooled odds of mortality among patients who developed TE was 4% higher compared to those who did not (OR: 1.74; 95% CI: 1.01–2.98; $P = 0.04$)

Table 1.
Summary of the data set described by Malas et al. [19].

A systematic review and meta-analysis done to analyze the thromboembolism risk on COVID-19 patients showed that its occurrence is high and associated with the worst clinical development. A total of 8271 patients from 425 eligible studies were included in the meta-analysis. In summary, the data set showed that COVID-19 patients had a higher risk of mortality, as described in **Table 1** [19].

Our suggestion of early detection and greater attention in COVID-19 patients with aggravating factors of thromboembolism may be addressed to the data found by Wang and colleagues [6]. Considering the correct prophylaxis, the majority of the venous thromboembolism occurrences could be prevented, mainly on patients with a higher risk for it. In spite of that, from the 140 patients investigated in the cohort, only 7% (10 patients) were maintained under anticoagulant therapy during their hospitalization. Among them, one received rivaroxaban and nine received heparin. It is a low proportion compared to the total number of patients with high risk to develop venous thromboembolism in their cohort. This finding possibly indicates that the prophylactic approach applied in the patients with COVID-19 was not adequate.

Other coagulation disorders observed in patients with COVID-19 also support the idea that a preliminary analysis of the genetic factors involved may better guide the therapeutic approach to be adopted. COVID-19-associated coagulopathy and disseminated intravascular coagulation (DIC) are being described as common findings in these patients. It is known that the pathophysiology of DIC associated with COVID-19 differs from that of septic DIC, and in this context both thrombotic and hemorrhagic pathologies must be observed. Thrombosis events in COVID-19 include macrothrombosis (MAT) and microthrombosis (MIT), and it is important to note that the diagnosis of MIT depends on coagulation and fibrinolysis markers. Consequently, molecular nuances can have a major impact on the worsening of the thrombohemorrhagic condition in different individuals [20, 21].

5. Conclusion

Screening and inclusion of COVID-19 patients with genetic abnormalities in thrombophilic conditions could guide the medical team to identify possible aggravating complication factors even if their patients are not in the group pre-determined risk, described by the World Health Organization (WHO) [8].

The treatment of COVID-19 is based on antiviral therapy, treatment to contain the cytokine storm, and treatment of thrombosis. Rather than providing uniform treatment, a method best suited for severity and stage should be selected. Considering the molecular profile of each individual can be an important tool in this race against time that characterizes care for patients with COVID-19. In this scenario, COVID-19 could be another exponent for a pharmacogenomics approach to the treatment of human diseases and it proved to be a challenge for humanity in the 21st century. The complications, sequels, and deaths took on catastrophic proportions. Despite the speed with which a significant range of vaccines were presented, comprehensive coverage worldwide is likely to face dares.

Author details

Tatyanny Paula Pinto da Costa Santos Fucci[1*], Rubens Pitliuk[2] and Ane Claudia Fernandes Nunes[3*]

1 Independent Scientist, São Paulo, SP, Brazil

2 Hospital Israelita Albert Einstein, São Paulo, SP, Brazil

3 University of California, Irvine, Irvine, CA, United States of America

*Address all correspondence to: nunes.acf@gmail.com and costafucci@gmail.com

References

[1] Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. The Lancet. 2020;**395**(10224):565-574. DOI: 10.1016/S0140-6736(20)30251-8

[2] Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;**579**(7798):265-269. DOI: 10.1038/s41586-020-2008-3

[3] Wolff D, Nee S, Hickey NS, Marschollek M. Risk factors for Covid-19 severity and fatality: A structured literature review. Infection. 2021;**49**(1):15-28. DOI: 10.1007/s15010-020-01509-1

[4] Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. Journal of Clinical Virology. 2020;**127**:104362. DOI: 10.1016/j.jcv.2020.104362

[5] Costa-Filho RC, Castro-Faria Neto HC, Mengel J, et al. Should COVID-19 be branded to viral thrombotic fever? Memórias do Instituto Oswaldo Cruz. 2021;**116**:e200552. DOI: 10.1590/0074-02760200552

[6] Wang T, Chen R, Liu C, et al. Attention should be paid to venous thromboembolism prophylaxis in the management of COVID-19. Lancet Haematology. 2020;**7**(5):e362-e363. DOI: 10.1016/S2352-3026(20)30109-5

[7] Franco RF. Trombofilias hereditárias. Medicina. 2001;**34**(3/4):248. DOI: 10.11606/issn.2176-7262.v34i3/4p248-257

[8] World Health Organization (WHO) Coronavirus. 2020. Available from: https://www.who.int/news-room/g-a-detail/q-a-coronaviruses [Accessed: April 2020]

[9] Van Cott EM, Soderberg BL, Laposata M. Activated protein C resistance, the Factor V Leiden mutation, and a laboratory testing algorithm. Archives of Pathology & Laboratory Medicine. 2002;**126**(5):577-582. DOI: 10.5858/2002-126-0577-APCRTF

[10] Norstrøm E, Thorelli E, Dahlbäck B. Functional characterization of recombinant FV Hong Kong and FV Cambridge. Blood. 2002;**100**(2):524-530. DOI: 10.1182/blood-2002-02-0343

[11] Rosendorff A, Dorfman DM. Activated protein C resistance and Factor V Leiden: A review. Archives of Pathology & Laboratory Medicine. 2007;**131**(6):866-871. DOI: 10.5858/2007-131-866-APCRAF

[12] Castoldi E, Rosing J. Factor V Leiden: A disorder of factor V anticoagulant function. Current Opinion in Hematology. 2004;**11**(3):176-181. DOI: 10.1097/01.moh.0000130315.41033.32

[13] Simioni P, Tormene D, Manfrin D, et al. Prothrombin antigen levels in symptomatic and asymptomatic carriers of the 20210A prothrombin variant: Prothrombin antigen and 20210A variant. British Journal of Haematology. 1998;**103**(4):1045-1050. DOI: 10.1046/j.1365-2141.1998.01112.x

[14] Jadaon MM. Epidemiology of prothrombin G20210A mutation in the Mediterranean region. Mediterranean Journal of Hematology and Infectious Diseases. 2011;**3**(1):e2011054. DOI: 10.4084/mjhid.2011.054

[15] Hotoleanu C. Genetic risk factors in venous thromboembolism. In: MDS I, editor. Thrombosis and Embolism: From Research to Clinical Practice. Vol. 906. Advances in Experimental Medicine and Biology. New York, USA: Springer

International Publishing; 2016. pp. 253-272. DOI: 10.1007/5584_2016_120

[16] Simoni RZ, Couto E, Barini R, et al. Malformações do sistema nervoso central e a presença da mutação C677T-MTHFR no sangue fetal. Revista Brasileira de Ginecologia e Obstetrícia. 2013;**35**(10):436-441. DOI: 10.1590/S0100-72032013001000002

[17] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. The Lancet. 2020;**395**(10223):507-513. DOI: 10.1016/S0140-6736(20)30211-7

[18] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. Journal of the American Medical Association. 2020;**323**(11):1061. DOI: 10.1001/jama.2020.1585

[19] Malas MB, Naazie IN, Elsayed N, Mathlouthi A, Marmor R, Clary B. Thromboembolism risk of COVID-19 is high and associated with a higher risk of mortality: A systematic review and meta-analysis. EClinicalMedicine. 2020;**29-30**:100639. DOI: 10.1016/j.eclinm.2020.100639

[20] Asakura H, Ogawa H. COVID-19-associated coagulopathy and disseminated intravascular coagulation. International Journal of Hematology. 2021;**113**(1):45-57. DOI: 10.1007/s12185-020-03029-y

[21] Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematology. 2020;**7**(6):e438-e440. DOI: 10.1016/S2352-3026(20)30145-9