RESEARCH ARTICLE

HYPERCOAGULATION AND VIRAL INFECTION: RATIONALE OF THE USE OF ANTI-THROMBOTIC THERAPIES IN CORONAVIRUS-19 DISEASE:

Giampaolo Palma¹, Pasquale Imitazione², Michele Cibelli³, Giorgio Emanuele Polistina⁴ and Giuseppe Fiorentino⁴

1. MD Physician- Cardiologist, “Palma Cardiology Medical Center”, Nocera Inf. Azienda Sanitaria Salerno, Italy.
2. MD Physician- Pulmonologist, Department of Intensive Care, AO of Colli, PO Monaldi, Naples, Italy.
3. MD Physician- Cardiologist, Azienda Sanitaria Marche N. 4 - Fermo, Italy.
4. MD Physician - Pulmonologist, Department of Intensive Care, AO of Colli, PO Monaldi, Naples, Italy.

Abstract

The key-events in the clinical evolution of Covid-19 disease are the coagulation disorders. Starting from the evidences of the literature and analyzing the mechanisms involved in the coagulopative process following Covid-19 infection, it is confirmed that Antithrombotic Therapies, associated with Anti-Inflammatory Drugs commonly used in the hospital, often avoid the access of the patients in Intensive Care for assisted ventilation with improved disease prognosis. To avoid the progression of the disease in severe respiratory insufficiency it is of fundamental importance to protect the blood vessels with Antithrombotic Therapy. The administration of heparins (standard or low molecular weight heparin) improves the prognosis of the disease by reducing the damage of pulmonary thromboembolism, myocardial infarction, heart failure, ischemic stroke and kidney damage avoiding the poor prognosis of the infection.

Introduction:

Hypercoagulation patterns during Covid-19 disease are confirmed, in the majority of hospitalized patients, by high blood levels of D-dimer and PT, markers of thrombosis, and also by numerous thrombotic manifestations with highly variable phenotypes. Covid-19 viral infection occurs with episodes of acute pulmonary thromboembolism and evolution in severe respiratory failure and often with deep vein thrombosis in the lower limbs. In some patients it has manifested itself with ischemic strokes even in young people in the absence of comorbidity with involvement of the cerebral arteries. In all patients with damaged endothelium, in diabetics, in hypertensive patients and in patients with previous myocardial infarction, the infection manifests itself with heart failure, severe arrhythmias and acute coronary syndromes such as direct and indirect damage of the myocardium. In other patients, the disease manifests itself in less aggressive forms involving kidneys, intestines, liver and vasculitic dermatitis. In the most serious cases with poor prognosis, the disease evolves into paintings of Acute Pulmonary Thromboembolism, Disseminated Intravascular Coagulation (DIC) and Septic Shock, with imposing activation of the coagulation cascade, endothelial dysfunction and endotheitis, with characteristic micro- and macro-thrombotic phenomena both in localization pulmonary than extrapulmonary (1) (2) (3).

Corresponding Author: Giampaolo Palma
Address: MD Physician – Cardiologist, “Palma Cardiology Medical Center”, Nocera Inf. Azienda Sanitaria Salerno, Italy.
The correlation between infection and hyper-coagulation:
The correlation between infection and hyper-coagulation has been widely demonstrated (4). Numerous inflammatory cytokines such as IL-6, IL-8 and TNF-a (tumor necrosis factor alpha) determine a state of "hyper-coagulation" through the expression of the tissue factor with the movement of leukocytes, platelets, endothelial cells and pericytes (5). In many inflammatory diseases and in sepsis there is an increased release of histones and nucleosomes which are toxic both for the endothelial wall and for the endothelial protective function (6).

In response to infection and inflammation, neutrophils produce strips of extracellular DNA (NETs Neutrophil Extracellular Traps NETs) which should allow neutrophils to cage and destroy "invading" microorganisms. NETs cause deposits of fibrin and platelet aggregation and this has been seen through "in vitro" experiments (7). A fragment of fibrin degradation operated by plasmin is D-dimer. The increase in D-dimer is a recognized marker of disseminated intravascular hyper-coagulation (8). The same D-dimer promotes activation of IL-6, inflammatory storm and activation of neutrophils and monocytes (9).

Evidence of hyper-coagulation in previous viral infections:
In viral infections, both hemorrhagic and non-hemorrhagic, in addition to the evidence of reduced platelet production and destruction of platelets (thrombocytopenia), a decrease in platelet function has also been observed. In the auto-immune body infection causes thrombocytopenia. Another important mechanism is represented by platelet hyper-aggregation and consequent increased consumption of platelets.

In the previous SARS-CoV2 infection both the presence of high levels of Von Willebrand factor in the blood (10) with activation of hyper-coagulation and thrombocytopenia caused by autoantibodies (11) (12) (13) were observed. Furthermore, fibrin clots in the alveoli were an important characteristic of the infection: perhaps the goal of this alveolar hyper-coagulation would be to protect the host by sealing the alveoli by preventing both alveolar edema and alveolar hemorrhages, but the consequence is certainly the limitation of pulmonary gas exchange (14).

Markers of impaired coagulation in viral infection:
A procoagulative state can be evidenced through an increase in the levels of coagulation proteins. Increased levels of fibrinogen, D-dimer, thrombin-antithrombin complex and plasmin-alpha-2-antiplasmin complexes and thrombomodulin have been reported in respiratory infections, influenza and SARS-COV infection. In addition, an increase in the levels of inhibitor of the plasminogen-1 activator, suggestive of impaired fibrinolysis, has also been shown (4).

A recent study by Tang and collaborators has revealed in 15 patients (71.4%) who died for Covid-19 an alteration of the laboratory parameters for DIC according to the diagnostic criteria of the International Society on Thrombosis and Haemostasis. In the advanced stage of the disease, high levels of D-dimer and fibrinogen degradation proteins have been observed (15).

Correlations between viral infection and coagulation disorders:
In several viral infections the clinical picture of the altered coagulation is manifested by bleeding, thrombosis or both. An exaggerated response can even lead to disseminated intravascular coagulation with the formation of microvascular thrombi in various organs (16). Respiratory tract infections increase the risk of deep vein thrombosis and pulmonary embolism (17). Both thrombotic and hemorrhagic complications such as deep vein thrombosis, acute pulmonary thromboembolism, pulmonary hemorrhage with hemoptysis, hematemeses, pet rash and sometimes diffuse petechial cerebral hemorrhage have been reported in the H1N1 swine flu epidemic (4). Avian influenza (H5N1) has been reported in numerous patients with disseminated intravascular coagulation, pulmonary hemorrhage and thrombocytopenia (18). In SARS-COV coronavirus infection, the clinical picture of coagulation consisted of vascular endothelial damage in medium and small-sized pulmonary vessels, disseminated intravascular coagulation, deep vein thrombosis and pulmonary thromboembolism (12) (13) (19). In Covid-19 disease these same clinical pictures have been reported by Tang and collaborators. According to Tang, in an advanced stage of pneumopathy, a consumption coagulopathy induced by sepsis and triggered by the release of cytokines following damage to the vascular endothelium and activation of monocytes would be established. thrombotic phenomena are tissue factor hyper-expression, von Willebrand factor secretion with excessive final fibrin production, platelet activation and fibrinolysis stimulation (15) (Fig.1).
Fig 1: SARS-COV 2 is a Systemic Vascular Inflammatory Disease.

Importance of the use of anticoagulation in SARS-COV2:
Both standard and low molecular weight heparins are anticoagulant substances used in the prophylaxis and therapy of venous thromboembolism (20). The heparin exerts its anticoagulant properties indirectly by binding reversibly to the anti-thrombin III (AT) by amplifying the inhibitory effect on activated X factor and on thrombin (Xa factor). (21). Heparin indirectly exerts its anticoagulant properties by reversibly binding to antithrombin III and amplifying its subsequent inhibitory effect on activated factor X and thrombin (factor Xa) (21) (22). For its action at the thrombin level, a heparin that contains at least 18 saccharide sequences is required, while the binding at ATIII level, which catalyzes the inhibitory action at the factor Xa level, takes place thanks to a peculiar saccharide sequence (23). This feature has been exploited by pharmacological research for the realization of low molecular weight heparins which are not able to bind to thrombin, but only to Factor Xa (24).

Fondaparinux is a synthetic analogue of the pentasaccharide sequence and compared to heparin it has a longer half-life and does not interact with platelets (25). Fondaparinux binds selectively and irreversibly to antithrombin III. This occurs in a neutralization of Factor Xa, which ultimately inhibits the formation of thrombin and the formation of thrombus. Fondaparinux is also indicated in the prophylaxis and treatment of venous thrombo-embolism (26).

Heparin, used clinically as an anti-coagulant, also has anti-inflammatory properties (27). The proposed mechanisms, although not fully clarified, are: inhibition of neutrophil chemotaxis and leukocyte migration, neutralization of complement factor C5a, sequestration of acute inflammatory phase proteins such as selectin-P and selectin-L, induction of cell apoptosis through the TNF-alpha and NF-kb pathways; also another mechanism proposed to block inflammation is the link with inflammatory cytokines (28) (29).

Viral infection damages endothelial cells that are ubiquitous in the body causing their dysfunction. Furthermore, the histones released by the damaged cells themselves cause endothelial damage (30). Another mechanism is through its effects on histone methylation and on the pathways on the MAPK and NF-Kb signal (31).
For all these reasons, heparin can affect microcirculatory dysfunction and can decrease endothelial damage.

In addition, heparin also has an antiviral role, which is being studied in experimental models. The polyanionic nature of heparin allows it to bind to different proteins and therefore to act as an effective inhibitor of the viral adhesion (32). An example is that of herpes simplex infections in which heparin competes with the virus for the surface glycoproteins of the host cell to limit the infection; in addition, in Zika virus infection, heparin prevents virus-induced cell death of human neural progenitor cells (32) (33). In addition, the use of heparin at a concentration of 100 mcg / mL halved the infection in experimental cells contaminated with sputum from patients with SARS-CoV (34).

In recent work, heparin has been shown to interact with the Spike S1 protein receptor of SARS CoV-2 (35). In the study of Tang et al. a favorable course has been highlighted in severe patients with Covid-19 who meet the criteria for coagulopathy induced by sepsis and with markedly high d-dimer through the use of LMWH (36). Out of 99 patients treated with heparin for at least 7 days, in almost all patients (n = 94), an LMWH s.c. dosage of 4000/6000 UI was used while in 5 patients non-fractionated heparin was administered (10000-15000 UI/day).

Therapeutic results:
Increasing evidence confirms an involvement of the coagulation spectrum on an inflammatory basis in patients with COVID-19 disease. Although the data are contradictory on therapeutic dosage, it is believed that both the use of standard heparin and the use of low molecular weight heparin can have a positive impact in the progression of Covid-19 disease.

The data confirm that the use of heparin is not necessary in asymptomatic patients. However, in the event of the onset and persistence of respiratory symptoms, even in patients in isolation at home, it is considered useful to start a prophylaxis with low molecular weight heparin (LMWH) or with Fondaparinux, the latter characterized by therapeutic coverage in the 24 hours and non-interference with platelets. If the patient develops a clinical picture with worsening respiratory symptoms in association with the increase in hyper-coagulation markers, heparin should be administered at therapeutic/sub-therapeutic dosages based on the patient's characteristics and pharmacological kinetics. In the most advanced stage of the disease there may be a role for unfractionated heparin since a powerful intravascular formation of thrombin and thrombosis occurs. Furthermore, careful monitoring of coagulation parameters is necessary in these patients given the possible progression in intravascular coagulation disseminated in the final phase of the disease (15).

In addition to the antiviral therapies that block the entry and replication of the virus in the cell and the anti-inflammatory therapies in the initial pulmonary and advanced inflammatory phase, in Covid-19 disease it is of fundamental importance to protect the endothelium and vessels to avoid the progressive coagulation and cytokine storm that characterize the serious phases of the illness. (37) (Fig. 2).
Conclusions and Future Directions:-
The COVID-19 disease is characterized by a wide variability of phenotypes that have viral attack on the endothelium via ACE2 receptors as their common denominator. The disease manifests itself as a coagulopathy with widespread endothelial involvement, from the lungs, to the heart, brain, kidneys, intestines and liver. The pathogenesis of coagulopathy in Covid-19 disease seems to follow the Virchow-Triad and includes anomalies of the blood vessel wall or endothelial surface, alteration of blood flow and prothrombotic components within the circulating blood. In Covid-19 coagulopathy there are inflammation and dysfunction of endothelial cells on a large scale, dynamics of abnormal flow and activated platelets, high concentrations of von Willebrand Factor, cell-free DNA, histones and viral RNA that together cause both activation of Factor XI, both Thrombin generation and Fibrin formation. For this reason, in addition to antiviral and anti-inflammatory therapies, in Coronavirus-19 infection it is of fundamental importance to protect the vascular endothelium and blood circulation with Antithrombotic Drugs for the improvement of the prognosis of the disease.

Acknowledgments:-
1. The authors thank the Doctor Professor Aldo Palma, master of cardiology, at his great heart, culture and humanity.
2. The author Doctor Giampaolo Palma thank Gabriella Tagliamonte, for her great support and her great heart.
3. The authors thank the “Palma Cardiology Medical Center” www.centrocardiologicopalma.it.
4. The author Doctor Giampaolo Palma thank for their great support his children Emilia and Nicola Palma.
References:

1. Xie Y, Whang X, Yang P, Zhang S. Covid-19 complicated by acute pulmonary Embolism. Radiology: Cardiothoracic Imaging 2020; 2.
2. Danzi GB, Loffi M, Galeazzi G, Gherbasi E. Acute pulmonary embolism and Covid-19 pneumonia: a random association? Eur Heart J. 2020 Mar 30.
3. Chen, Jianpu and Wng, Xiang and Zhang, Shutong and Liu, Bin and Wu, Xiaoqing and Wang, Yanfang andWang, Xiaoqi and Yang, Ming and Sun, Jianqing and Xie, Yuanliang. Findings of acute pulmonary embolism in Covid-19 patients(3/1/2020). Available at SSRN: ssrn.com/abstract=3548771 or http://dx.doi.org/10.2139/ssrn.3548771.
4. Goejenbier M, van wissen M, van de weg C, Jong E, Gerdes VE, Meijers JC, Brandis DP, Van Gorp EC. Review: Viral infections and mechanisms of thrombosis and bleeding. J Med Virol 2012; 84:1680-96.
5. Branchford BR, Capenter SL. The role of inflammation in venous thromboembolism. Front Pediatr 2018; 6:142.
6. Xu J, Lupu F, Esmon CT. Inflammation, innate immunity and blood coagulation. Hamostaseologie 2010; 30:5-9.
7. Esmon CT, Xu J, Lupu F. Innate immunity and coagulation. J Thromb Haemost 2011; 9: 182-8.
8. Li J, Harra H, Wang Y, Esmon C, Cooper DKC, Iwase H. Evidence for the important role of inflammation in xenotransplantation. J Inflamm (Lond) 2019; 16:10.
9. Robson Sc, Shepard EG, Kirsch RE, et al. Fibrin degradation product D-dimer induces the synthesis and release of biologically active IL-1-beta, IL-6 and plasminogen activator inhibitors from monocytes in vitro. Br J Haematol 1994; 86:322-6.
10. Wu YP, Wei R, Liu ZH, Chen B, Lisman T, Ren DL, Han JJ, Xia ZL, Zhang FS, Xu WB, Pleissner KT, De Groot PG. Analysis of thrombotic factors in severe acute respiratory syndrome (SARS) patients. Thromb Haemost 2006; 96:100-101.
11. Wong RS, Wu A, To KF, Lee N, Lam CW, Wong CK, Chan PK, Ng MH, Yu LM, Hui DS, Tam JS, Cheng G, Sung J. Haematological manifestations in patients with severe acute respiratory syndrome: retrospective analysis. BMJ 2003; 326:1358-1362.
12. Chong PY, Chui P, Ling AE, Franks TJ, Tai DY, Leo YS, Kaw GJ, Wansaicheong G, Chan KP, Ean Oon LL, Teo ES, Tan KB, Nakajima N, Sata T, Travis WD. Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: Challenges in determining a SARS diagnosis. Arch Pathol Lab Med 2004; 128: 195-204.
13. Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, Ahuia A, Yung MY, Leung CB, To KF, Lui SF, Szeto CC, Chung S, Sung JJ. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003 348: 1986-1994.
14. Gralinski LE, Baric RA. Molecular pathology of emerging coronavirus infections. J Pathol 2015; 235: 185-95.
15. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020; 18:844-847 (a).
16. Levi M. Disseminated intravascular coagulation. Crit Care Med 2007; 35:2191-2195.
17. Smeeht L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P. Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. Lancet 2006.
18. Wiwanitkit V. Hemostatic disorders in bird flu infection. Blood Coagul Fibrinolysis 2008; 19:5-6.
19. Hwang DM, Chamberlain DW, Poutanen SW, Low DE, Asa SL, Butany J. Pulmonary pathology of severe acute respiratory syndrome in Toronto. Mod Pathol 2005: 18:1-10.
20. Alquwaizani M, Buckley L, Adams C, Fanikos J. Anticoagulants: a review of the pharmacology, dosing, and complications. Curr Emerg Med Rep 2013; 1: 83-97.
21. Brinkhous K, Smith H, Warner E, Seegeers W. The inhibition of blood clotting: an unidentified substance which acts in conjunction with heparin to prevent the conversion of prothrombin into thrombin. Am J Physiol 1939; 125: 683-687.
22. Lindhal U, Backstom G, Hook M, Thunberg L, Fransson LA, Linker A. Structure of the antithrombin - binding site in heparin. Proc Natl Acad Sci USA 1979, 76: 3198-3202.
23. Lane DA, denton J, Flynn AM, Thunberg L, Lindhal U. Anticoagulant activities of heparin oligosaccharides and their neutralization of platelet factor 4. Biochem J 1984; 218: 725-732.
24. Hirsh J, Warkentin TE, Shaughnessy SG, Anand SS, Halperin JL, Raschke R, Granger C, Ohman EM, Dalen JE. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy and safety. Chest 2001; 119: 645-945.
25. Zhang Y, Zhang M, Tan L, Pan N, Zhang L. The clinical use of Fondaparinux: a synthetic heparinepentasaccharide. Prog Mol Biol Transl Sci 2019; 163: 41-53.

26. Johnston A, Hsieh SC, Carrier M, Kelly SE, Bai Z, Skidmore B, et al. A systematic review of clinical practice guidelines on the use of low molecular weight heparin and fondaparinux for the treatment and prevention of venous thromboembolism: implications for research and policy decision-making. PLoS One 2018, 13: e0207410.

27. Mousavi S, Moradi M, Khorshidahmad T, Motamedi M. Anti-inflammatory effects of heparin and hitsderivates: a systematic review. Adv Pharmacol Sci 2015; 507151. doi:

28. Oduah EI, Linhardt RJ, Sharfsttein ST. Heparin: past, present and future. Pharmaceuticals 2016; 9.

29. Thachil J. The versatile heparin in COVID-19. J Thromb Haemost 2020. (epub ahead of print).

30. Xu J, Lupu F, Esmon CT. Inflammation, innate immunity and blood coagulation. Hamostaseologie 2010; 30: 5-9.

31. Ma J, Bai J. Protective effects of heparin on endothelial cells in sepsis. Int J Clin Exp Med 2015; 8: 5547-5552.

32. Shukla D, Spear PG. Herpesviruses and heparinsulfate: an intimate relationship in aid of viral entry. J Clin Invest 2001; 108: 503-10.

33. Ghezzi s, Cooper L, Rubio a, Pagani I, Capobianchi MR, Ippolito G, et al. Heparin prevents Zika virusinduced-cytopathic effects in human neural progenitor cells. Antiviral Res 2017; 140: 13-17.

34. Vincenzi E, Canducci F, Pinna D, Mancini N, Carletti S, Lazzarin A, et al. Coronaviridae and Sars-associated coronavirus strain HSR1. Emerg Infect Dis 2004; 10: 413-18.

35. https://www.biorxiv.org/content/10.1101/2020.02.29.971093v1.full).

36. Tang N, Bai h, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J thrombi Haemost 2020; (Epub ahead of print).

37. Palma G., Imitazione P., Tarro G., Polistina GE., Fiorentino G. The Novel Coronavirus is a Coagulative Diseasewith Diffuse Thrombosis of the Vascular System: the fundamental role of the Antithrombotic Therapy. Intern Journ of Current Research. Vol. 12, Issue, 07, pp 12205-12212, July, 2020.