Are TFPI and β-TG indicators of severity in COVID-19?

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Research Article

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Abstract

Background: Thromboembolic complications have been reported as a life-theratening major pathologic event in severe coronavirus disease 2019 (COVID-19) affecting the lung as evidenced by autopsy reports of alveolar damage and pulmonary intravascular microthrombi. The new coronavirus (CoV) does not appear to have intrinsic procoagulant effects itself. The coagulation changes in COVID-19 are likely a result of the inflammatory response. Significant inflammation is present in COVID-19, based on elevated interleukin-6 (IL-6). This inflammation associated with COVID-19 results in coagulopathy, based on elevated D-dimer (DD). An endotheliopathy appears to contribute to microvascular thrombosis in COVID-19. The aim of this study is to confirm the coagulation abnormalities in 100 severe COVID-19 patients having lung involvement and their association with the severity and prognosis.

Methods: Inflammation, endothelial and coagulation indicators were performed and compared between severe and mild disease.

Results: IL-6 and TNF-a, and TF and VWF, exceeded in severe COVID-19 patients as well as D-dimer, TAT, and Fibrinogen. As PF4 has a rapid removal from plasma, we also measured b-TG levels which exceeded the plasma levels of PF4 in severe COVID-19 patients as well as increased platelet adhesion was observed. Shortened CT and CFT, high MCF and low LY at 30 minutes were present in 100% of severe COVID-19 patients compared with mild COVID-19 patients. **Conclusions:** It is reported that TFPI is a natural anticoagulant that lowes inflammation and coagulation. Therefore, we measured TFPI levels which exceeded without shutdown of the inflammation and coagulation documenting the clinical severity of severe COVID-19 patients.

Introduction

According to the China there are four severity levels of COVID-19 based on the clinical manifestations. The criteria used for classification are respiratory factors such as respiratory rate, oxygen saturation, and lesion progression in pulmonary imaging. [1]

In clinical practice thrombotic complications have been reported, including microvasular thrombosis, ischemic limbs, strokes, venous and arterial thromboembolism, and acute arterial thrombosis. [2] The observed pulmonary vessels occlusions that have been described in reports on COVID-19 patients are exclusively caused by pulmonary embolism. [3] However, in the experience of Cattaneo M et al [3] and in some reports [3, 4] filling defects of pulmonary vessels that are detected by computed tomography angiography (CTA) scans are in many instances more reminiscent of pulmonary thrombi rather than emboli, because they are not fully occlusive. This observation is compatible with postmortem description of presence of manifestations of thrombotic microangiopathy, enlarged pulmonary blood vessels containing microthrombi, presence of fibrin thrombin within distended small vessels and capillaries and extensive extracellular fibrin deposition. [3, 6] Diffuse thrombotic material is observed also in other organs, compatibly with the development of clinical signs of multiorgan failure. [5] Therefore, local thrombi, rather

than emboli from peripheral veins, appear to be the hallmark, which are responsible for the severe ischemic clinical manifestations. [3] Pulmonary thrombi in COVID-19 probably develop as a consequence of vascular damage associated with viral infection and severe inflammation, with the pathogenic contribution of platelets interacting with the vascular wall and leukocytes, factor XIIa with other components of the contact phase of coagulation, von Willebrand factor, complement, and other players in thromboinflammation, some of which have been shown to be implicated in the pathogenesis of acute respiratory distress syndrome already many years ago. [3]

Soluble markers of thrombosis including D-dimer, von Willebrand antigen levels and soluble platelet-derived factors including platelet factor 4 (PF4) levels were significantly elevated in COVID-19 patients leading to the COVID-19 hyercoagulability and thrombosis that are observed clinically. [6] Importantly, D-dimer positively correlates with PF4 and monocyte tissue factor (TF) expression. [7] Increased D-dimer, a early predictor of outcome in severe, is associated with high mortality. [8–10]

TF is normally present in the circulation at very low levels. [11] An increase in TF expression caused by inflammation tends to shift the haemostatic balance in favor coagulation/thrombosis, generating thrombin. [12] Thrombin has a variety of activities on cells that result in augmentation of the inflammatory response. Its ability to augment leukocyte adhesion and activation likely contributes to amplification of the inflammatory response. Thrombin activation of the endothelium results in high levels of platelet-activating factor formation, which works as a potent neutrophil agonist, especially when neutrophils are tethered to selectins. [13, 14] In addition, thrombin activation of platelets releases CD40 ligand which in turn can induce TF formation [15, 16] and increase inflammatory cytokines, including inteleukin-6 (IL-6) and IL-8. [17, 18] Increased levels of IL-6 have been shown in vivo to increase platelet reactivity increasing their thrombogenic potential, thus further linking inflammation and thrombosis. [19] Inflammation also elevates fibrinogen synthesis. [20] Fibrinogen levels rise under these conditions unless a consumptive coagulopathy occurs. The natural anticoagulant and anti-inflammatory properties of endothelial cells are critically important to limit microvascular thrombosis, inflammation and organ injury. [21] TFPI is a endogenous serine protease inhibitor producted by the endothelium that directly inhibits FXa and the FVIIa/TF complex and a significant percentage of this seems to be stored in endothelial cell granules that can be released by thrombin treatment. [21, 22] Preclinical trials have demonstrated that recombinant TFPI (rTFPI) attenuates cytokine responses (tumor-necrosis factor-α and IL-8) in a pig peritonitis-induced bacteriemia model without improving survival. [23] However, rTFPI reduced bacterial sepsis. [24] and it was associated with lower levels of inflammatory and coagulation biomarkers (IL-6 and TAT, respectively). [25] rTFPI has been tested and demonstrated to be safe in healthy humans following bolus IV injection of endotoxin. rTFPI attenuated endotoxin-induced α-thrombin generation with complete blockade of coagulation by high-dose rTFPI. Interestingly, rTFPI did not affect endotoxin-induced changes. [26] rTFPI has also been trialed in phase 2 studies on patients with severe sepsis and it appeared to be safe and effective, with reduction in TAT and IL-6 levels [27] and reduced mortality. However, results from trail failed to demonstrated an effect of rTFPI (tifacogin). [27]

Here, we investigated the coagulopathy in severe and mild COVID-19. We confirm that severe COVID-19 patients exhibit increased inflammation including IL-6 and TNF-α, endotheliopathy including TF and von Willebrand factor, and coagulopathy including D-dimer, TAT, and Fibrinogen. As PF4 has a rapid removal from plasma, [28] we also measured β-TG levels which exceeded the PF4 and increased platelet adhesion was observed as well as shortened CT and CFT, high MCF and low LY at 30 minutes. It is reported that TFPI is a natural anticoagulant that lowes inflammation and coagulation. Therefore, we measured TFPI levels which exceeded without shutdown documenting the clinical severity.

Material And Methods

We enrolled 100 severe hospitalized having fever, respiratory symptoms and requiring mechanical ventilation and sixteen presenting mild-self-limiting disease and 16 asymptomatic. 40 SARS-CoV-2$^-$ controls included subjects of age-, and sex-matched. (Table 1) Each study participant gave written informed consent for study enrollment in accordance with the Declaration of Helsinki.

All severe patients received empirical antimicrobial treatment (amoxifloxacin and/or cephalosporin) and antiviral therapy (oseltamivir and/or ganciclovir). In addition, all severe cases were administered corticosteroid (methylprednisolone) during the course of hospitalization.

Laboratory mesaurements

Cytokines

Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) were measured using multiplex bead array (Millipore Sigma) and analyzed on a Luminex 200 machine.

Endothelium

Tissue factor (TF), von Willebrand Factor antigen (VWF) were measured using ELISA kits (R&D Systems; Abcam).

Coagulation

D-dimer and thrombin antithrombin complex (TAT) were measured using ELISA kit (Diagnostic Stago, Boehringer Mannheim, Mannheim, Germany; Dade Berhing Marburg GmbH, Marburg, Germany), Fibrinogen (Fib) and plasminogen activator inhibitor-1 (PAI-1) were detected using Clauss method (Giesse Diagnostics, Italy), and ELISA kit (American Diagnostica Inc., Greenwich, CT), respectively.

Platelets

PF4 and β-TG were assessed using ELISA kit (R&D Systems).

Whole blood viscoelastic analysis

The viscoelastic properties of blood were analyzed using Thromboelastometry method (Rotem delta System - Pentapharm GmbH, Germany).

Tissue Factor Pathway Inhibitor (TFPI)

TFPI was measured using ELISA kit (American Diagnostica Inc., Greenwich, CT).

Statistical analysis

The results were using the Student's t-test and Pearson or Spearman test. A p-value of < .05 wassignificant (SPSS 21.0 for Windows (SPSS Inc.)

Results

Increased IL-6 and TNF-α (64 ± 12pg/ml, 55 ± 5 pg/ml) compared with mild/asymptomatic COVID-19 (5 ± 1pg/ml, 7 ± 2 pg/ml) and healthy subjects (4 ± 2 pg/ml, 10 ± 2 pg/ml) confirmed inflammation.

TF and VWF and TFPI (2100 ± 500 pg/ml and 310 ± 50% and 170 ± 69 ng/ml) were higher than mild/asymptomatic COVID-19 (19 ± 2 pg/ml and 40 ± 7% and 69 ± 10 ng/ml) and healthy subjects (22 ± 5 pg/ml and 59 ± 10% and 80 ± 12 ng/ml), confirming endotheliopathy. IL-6 and TNF-α correlated with TF, VWF, and TFPI confirming the interrelation between inflammation and endotheliopathy. (Table 2)

DD, TAT, and Fib (555 ± 100 µg/l, 75 ± 10 µg/l, 620 ± 20mg/dl) were elevated compared with mild/asymptomatic COVID-19 (60 ± 4 µg/l, 2 ± 1 µg/l, 151 ± 10 mg/l) and healthy subjects (70 ± 4 µg/l, 3 ± 1 µg/l, 180 ± 10 mg/dl) (Table 2), confirming activation of coagulation. TF, VWF, and TFPI correlated with DD and TAT and Fib, confirming the interrelation between endotheliopathy and coagulopathy.

PF4 and β-TG (160±64 IU/ml and 250±16 IU/ml) were higher in severe COVID-19 than mild/asymptomatic COVID-19 (4±1 UI/ml and 15±5 IU/ml) and healthy subjects (5 ± 1 IU/ml and 16 ± 5 IU/ml) (Table 2), confirming the platelet activation. PF4 and β-TG correlated with DD, TAT, and Fib confirming the interrelation between coagulopation activation and platelet activation.

Platelet adhesion on C/ADP and C/EPI (C/ADP 45 ± 10 s and C/EPI 40 ± 5 s) compared with mild/asymptomatic (C/ADP 75 ± 10 s and 95 ± 10 s) and controls (80 ± 15 s and 110 ± 20 s) (Table 3), showed that circulating platelets are hyperadhesive.

Shorter CT (CT, unit: s. n.v. 100–240 s) (35 ± 20 s), shorter CFT (CFT, unit: s, n.v. 30–160 s (10 ± 10 s), longer MCF (MCF, unit: mm, n.v. 50–72 mm (125 ± 10 mm) and lower LY-30 (LY-30, %: v.n. 15% (0.7%) were observed in severe COVID-19 than mild/asymptomatic COVID-19 (CT 110 ± 10 s and CFT 45 ± 5 s and MCF 60 ± 10 mm and LY-30 (15%) and healthy subjects (CT 115 ± 10 s and CFT 35 ± 5 and MCF 65 ± 10 mm and LY-30 (15%) (Table 4), confirming coagulation activation.

Discussion

Thrombosis in COVID-19 is an clinical picture that needs to be considered. [29]

COVID-19-associated coagulopathy (CAC) is emerged as prominent feature of severe or critically ill patients. [30]

Multiple retrospective and prospective clinical trials have evaluated the thrombo-inflammatory biomarkers linking them to poor prognosis among patients with COVID-19 infection [8] and several reports and meta-analysis have evaluated the link between elevated D-dimer levels and severity and mortality of COVID-19 infection. [31] However, several concerns have been raised with the use of D-dimer as a biomarker among COVID-19 infected patients. D-dimer has low specificity and elevated levels are often seen with advanced age, African American race, female sex, active malignancy, surgery, pregnancy, immobility, cocaine use, connective tissue disorders, end-stage renal disease, and prior thromboembolic disease. [32] Also, D-dimer reflects a later stage in the haemostatic process and is released when a clot is degraded by the fibrinolytic processes. Other standard laboratory tests including PT and aPTT measure the clotting activity from the plasma and ignore other components of the coagulation such as the platelets and fibrinolysis. The platelet count and fibrinogen concentration also have the caveat of providing static measures with no information regarding their functionality. [32]

Our resuts confirm that inflammation and endoteliopathy lead to coagulation and platelet activation in severe COVID-19. Infact, we found a positive correlation between high levels of IL-6 and TNF-α and high levels of TF and VWF. High D-dimer, TAT and Fg originated from thrombin-activated fibrinolysis positively correlated with TF and VWF reflecting a endothelial hypercoagulability state. High TFPI correlated with high IL-6 and TNF-α and high D-dimer, TAT and Fg suggesting a severe inflammation and coagulation.

Schechter et al. showed a correlation between proinflammatory cytokines and thrombin contributing to TF expression in monocytes. [33] We showed a correlation between proinflammatory cytokines and TAT contributing to TF expression in serum of severe COVID-19.

Middleton et al. showed high levels of PF4 in COVID-19 patients. [6] We showed high levels of β-TG, a more sensitive marker of platelet activation due to its more slow removal from the plasma level, in severe COVID-19 patients. Manne et al observed increased platelet aggregation in COVID-19 patients. [34] We observed increased palatelet adhesion in severe COVID-19 patients.

As aspirin reduces platelet hyperactivity in COVID-19 patients, [33] our finding of platelet activation marked by increased β-TG suggests that antiplatelet therapy may be warranted in treating COVID-19 patients.

In this study, coagulation and platelet activation were further studied by rotational thromboelastometry (ROTEM) confirming the severity of coagulopathy marked by TFPI and β-TG.

Conclusion

This study has a worldwide, clinical, and scientific relevance as it faces a disease spread all over the world that affects all age groups and that is aimed at a large scientific community such as infectious disease specialists, pulmonologists, internists, and hematologists. The morbidity and mortality of COVID-19 is afffected by the occurrence of thrombosis whose pathogenesis lies in inflammation that causes platelet and coagultaion activation. To date, DD, PT, PTT, platelet count, fibrinogen, and PF4 have some limitations. Therefore, it is important to identify more reliable indicators of thrombosis. In this study, TFPI and β-TG provide more specificity in predicting the severity of coagulopathy and can serve to guide treatment and to reduce the mortality.

Declarations

Declarations

-Ethycal Approval and Consent to participate “Not applicable”

-Consent for publication “Not applicable”

-Availability of Supporting data “Not applicable”

-Competing interests “The authors declare no competing financial interests”

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Abbreviations

COVID-19, coronavirus disease 2019; CoV, coronavirus; IL-6, interleukin-6; DD, D-dimer; TNF-α, Tumor Necrosis Factor-α; TF, Tissue Factor; VWF, Von Willebrand Factor; TFPI, Tissue Factor Pathway Inhibitor; TAT, thrombin antithrombin complex; Fib, Fibrinogen; PF4, Platelet Factor 4; β-TG, β-Thromboglobulin; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; LY-30, clot lysis at 30 minutes; computed tomography angiography (CTA); FXII, factor XII; IL-8, Interleukin-8; rTFPI, recombinant TFPI; CAC, COVID-19-associated coagulopathy; IL-1, interleukin-1; TM, thrombomodulin

References

1. Liao D, Zhou F, Luo L, Xu M, Wang H, et al. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. Lancet Haematol 2020; 7:e671-678.
2. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 2020; 191:145–147.
3. Cattaneo M, Bertinato EM, Birocchi S, Brizzio C, Malavolta D, et al. Pulmonary embolism or pulmonary thrombosis in COVID-19? Is the Recommendation to use High-Dose Heparin for Thromboprophylaxis justified? Thromb Haemost 2020; 120:1230–1232.
4. Fox SE, Akmatbekov A, Harbert JL, Li G, Brown JQ, Heide RSV. Pulmonary and cardiac pathology in African American patients with COVID-10: an autopsy series from New Orleans (published online ahead of print 27 May 2020). Lancet Respir Med.
5. Du Y, Tu L, Zhu P, Mu M, Wang R, et al. Clinical features of 85 fatal cases of COVID-19 from Wuhan: a retrospective observational study. Am J Respir Crit Care Med 2020; 201(11):1372–1379.
6. Middleton EA, HE X-Y, Denorme F, Campbell RA, Ng D, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 2020; 136(10):1169–1179.
7. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. Blood 2020, 136(11):1330–1341.
8. 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(4):844–847.
9. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe 2020; 27(6):992–1000. e3
10. Zhou F, Yu T, Du R, Fan G, Liu Y, et al. Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a restrospective cohort study. Lancet 2020; 395(10229):1054–1062.
11. Giesen PLA, Rauch U, Bohmann B, Kling D, Roquè M, et al. Blood-borne tissue factor: Another view of thrombosis. Proc. Natl Acad Sci USA 1999; 96:2311-5.
12. Esmon CT. Inflammation and thrombosis. J Thromb Haemost 2003; 1:1343–8.
13. Bar-Shavit R, Kahn AJ, Mann KG, Wilner GD. Identifiaction of a thrombin sequence with growth factor activity in on macrophages. Proc Natl Acad Sci USA 1986; 83:976–80.
14. Lorant DE, Patel KD, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. J Cell Biol 1991; 115:223–34.
15. Pendurthi UR, Alok D, Rao LVM. Binding of factor VIIa to tissue factor induces altetations in gene expression in human fibroblast cells: upregulation of poly (A) polymerase. Proc Natl Acad Sci USA

1997; 94:2598–603.

16. Miller DL, Yaron R, Yellin MJ. CD40L-CD40 interactions regulate endothelial cell surface tissue factor and thrombomodulin expression. J Leukoc Biol 1998; 63:373–9.
17. Andrè P, Srinivasa Prasad KS, Denis CV, He M, Papalia JM, et al. CD40L stabilizes arterial thrombi by a β3 integrin-dependent mechanism. Nature Med 2002; 8:247–52.
18. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998; 391:591–4.
19. Burstein SA. Cytokines, platelet productions and hemostasis. Platelets 1997; 8:93–104.
20. Taylor FB jr, Chang A, Esmon CT, Viagno-D'Angelo S, Blick KE. Protein C prevents the coagulopathic and lethal effects of E. coli infusion in the baboon. J Clin Invest 1987; Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. Blood 2019; 133(9):906–918.
21. Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. Blood 2019; 133(9):906–918.
22. Lupu C, Lupu F, Dennechy U, Kakkar B, Scully MF. Thrombin induces the redistribution and acute release of tissue factor pathway inhibitor from specific granules within human endothelial cells in culture. Arterioscler Thromb Vasc Biol 1995; 15:2055–62.
23. Goldfarb RD, Glock D, Johnson K, Creasey AA, Carr C, et al. Randomized, blinded, placebo-controlled trial of tissue factor pathway inhibitor in porcine septic shock. Shock 1998; 10(4):258–264.
24. Matyal R, Vin Y, Delude RL, Lee C, Creasey AA, Fink MP. Extremely low doses of tissue factor pathway inhibitor decrease mortality in a rabbit model of septic shock. Intensive Care Med 2001; 27(8):1274–1280.
25. Creasey AA, Chang AC, Feigen L, Wun TC, Taylor jr FB, Hinshaw LB. Tissue factor pathway inhibitor reduces mortality from Escherichia Coli septic shock. J Clin Invest 1993; 91(6):2850–2860.
26. de Jonge E, Dekkers PE, Creasey AA, Hack CE, Paulson SK, et al. Tissue factor pathaway inhibitor dose-dependently inhibits coagulation activation without influencing the fibrinolytic and cytokine response during human endotoxemia. Blood 2000; 95(4):1124–1129.
27. Abraham E, Reinhart K, Svoboda P, Seibert A, Olthoff D, et al. Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with severe sepsis: a multicenter, rabdomized, placebo-controlled, single-blind, dose escalation study. Crit Care Med 2001; 29(11):2081–2089.
28. Kaplan KL, Owen J. Plasma levels of β-Thromboglobulin and platelet factor 4 as indices of platelet activation in vivo. Blood 1981; 57(2):199–202.
29. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticaogulation. Blood 2020; 135(23):2033–2040.
30. Mucha SR, Dugar S, McCrae K, Douglas J, Bartholomew J, et al. Update to coagulopathy in COVID-19:Manifestations and management. Cleveland Clinic Journal of Medicine 2021; 1–10.

31. Tang N, Bai H, Chen X, Gong J, LI D, Sun Z. Anticoagulant treatmemt is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost 2020; 18(5):1094–1099.
32. Chaudhary R, Kreutz RP, Bliden KP, Tantry US, Gurbel PA. Pesonalizing antithrombotic therapy in COVID-19: Role of Thromboelastography and Thromboelastometry. Thromb Haemost 2020; 120:1594–1596.
33. Schechter ME, Andrade BB, He T, Haret Richter G, Tosh KW, et al. Inflammatory monocytes expressing tissue factor drive SIV and HIV coagulopathy. Sci Transl Med 2017; 9(405):eaam5441.
34. Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, et al. Platelet gene expression and function in patients with COVID-19. Blood 2020; 136(11):1317–1329.

Table

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