Evidence of SARS-CoV-2 Spike protein on retrieved thrombi from COVID-19 patients

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Abstract

The pathophysiology of COVID-19-associated coagulopathy is complex and not fully understood. SARS-CoV-2 spike protein (SP) may activate platelets and interacts with fibrin(ogen). We aimed to investigate the possible evidence of isolated SP in clots retrieved in COVID-19 patients with acute ischemic stroke (by mechanical thrombectomy) and myocardial infarction.

In this pilot study we could detect SP, but not nucleocapsid protein, on platelets of COVID-19 + patients' thrombi. In addition, in all the three COVID-19 + thrombi analyzed for molecular biology, no SARS-CoV-2 RNA could be detected by real time-polymerase chain reaction. These data confirm the hypothesis that free SP besides the whole virus, may be the trigger of platelet activation and clot formation in COVID-19.

Full Text

To the Editor,

Thrombotic complications are common features of coronavirus disease 2019 (COVID-19) but the underlying pathogenesis is not fully elucidated yet. It has been observed that the spike protein (SP), namely the protruding membrane protein of SARS-CoV-2, may activate the coagulation cascade by binding Angiotensin-converting enzyme 2 (ACE2) directly on platelets and/or endothelial cells.[1] Additionally, the isolated circulating SP may induce an hypercoagulability status by directly interacting with fibrin/fibrinogen.[2] Noteworthy, free SP fragments have been found in plasma of COVID-19 patients.[3] SARS-CoV-2 has been detected rarely in thrombi retrieved from brain arteries of acute ischemic stroke (AIS) patients [4] and more frequently in those retrieved from coronary arteries of acute myocardial infarct (AMI) patients.[5] A few data on SP detection in retrieved thrombi from stroke patients have been reported.[6.] We aimed to investigate the possible evidence of isolated SP in clots retrieved by mechanical thrombectomy of COVID-19 patients with AIS and AMI.

The study was conducted on patients admitted to the Emergency Department of Policlinico Umberto I hospital, University of Rome La Sapienza, from March 2020 to April 2021. Among a series of consecutive adult patients with large vessel occlusion (LVO) related AIS or with AMI and a concomitant diagnosis of COVID-19, we retrospectively selected patients with retrieved thrombus available after endovascular treatment for histological analysis. The diagnosis of COVID-19 was based on the positive results of SARS-CoV-2 on real-time reverse-transcription polymerase chain reaction (RT-PCR) analysis of nasopharyngeal swab specimens. We used as control, thrombi retrieved from patients with LVO-AIS not affected by COVID-19. The collected thrombi were immediately fixed in 10% formalin and embedded in paraffin. Sections were stained with Hematoxylin and Eosin. Immunohistochemical staining was performed using two different antibodies: SARS-CoV-2 SP (rabbit polyclonal anti-SARS-CoV-2 SP - Cell Signaling Technology, Boston, MA, USA, cat. #56996, dil. 1:100) and Nucleocapsid protein (NP) (monoclonal anti SARS/SARS CoV-2 (B46F) – Invitrogen, Rockford USA, MA1-7404, dil. 1:200). The positive control consisted of a COVID-19 lung section. A double immunofluorescence was performed to

co-localize platelets with SARS-CoV-2 SP, using the primary antibodies, anti-CD61 (Monoclonal Mouse Anti-Human CD61, Platelet Glycoprotein IIIa/APC, Clone Y2/51, dil. 1:100) and anti-SARS-CoV-2 SP, visualized, respectively, with Goat anti-Mouse Alexa Fluor 594 (dil. 1:300) and donkey anti-rabbit Alexa Fluor 488 (dil. 1:300) (Thermo Fisher). The nuclei were stained with DAPI. Morphologic and immunohistochemical findings were assessed by two of the authors (GD & ML).

SARS-CoV-2 RNA extraction from clots was carried out with using Total purification RNA kit (Norgen Biotek Corp.), according to the manufacturer's instructions. Viral RNA was amplified using a real time RT-PCR system (FTD SARS-CoV-2 test, Siemens Healthineers) for the qualitative detection of SARS-CoV-2 RNA, as previously described.[7]

We enrolled four COVID-19 positive (COVID-19+) patients: three with LVO-AIS (mean age: 67 [± 11]; 3 males) (one out of three patients was also treated with intravenous thrombolysis) and one affected by AMI (43 years old, male). All COVID-19+ patients had lung ground-glass opacity on pulmonary CT scan. We included a control group of four LVO-AIS without SARS-CoV-2 infection (COVID-19-) (mean age: 69 [± 11]; 3 males), three out of whom received intravenous thrombolysis.

The relative amount of platelets and fibrin/red blood cells did not significantly differ from COVID-19+ thrombi and controls. COVID-19+ thrombi retrieved from cerebral arteries showed mild positivity for SP whereas SP immunostaining was more marked in the COVID-19+ thrombus retrieved from anterior descending coronary artery (Figure, Panel 1 A,C). Neither cerebral nor coronary artery thrombi showed positive cells for NP (Figure, Panel 1 B,D). As for comparison, Figure Panel 2 reports representative immunohistochemical staining for SP and NP which was positive for both (E and F, respectively) in the lung of a patient affected by COVID-19 (positive control) and negative (G and H, respectively) in a thrombus retrieved from the middle cerebral artery of a patient not affected by COVID-19 (negative control).

Finally, to characterize the cellular population expressing SP we performed a double-immunostaining with antibody against CD61 and we found that most of the SP co-localized with platelets (Figure, Panel 3).

No SARS-CoV-2 RNA could be identified in three COVID+ thrombi analyzed with RT-PCR.

In conclusion, present data confirm the hypothesis that free SP besides the whole virus, may be the trigger of platelet activation and clot formation in COVID-19. To best of our knowledge only one other group has recently looked at the presence of SP in retrieved thrombi from 6 AIS patients, however, with negative results.[6] The different kind of anti-SP antibodies used (monoclonal versus polyclonal in our study) as well as the different burden of COVID-19 on stroke pathogenesis may be plausible explanations. In addition, a possibly diverse genetically-determined ACE2 receptor expression on platelets and endothelial cells, could also justify the different chance to find SP on clots.

Abbreviations

SP: spike protein

RT-PCR: real time-polymerase chain reaction

ACE2: Angiotensin-converting enzyme 2

COVID-19: coronavirus disease 2019

AIS: acute ischemic stroke

AMI: acute myocardial infarct

LVO: large vessel occlusion

NP: Nucleocapsid protein

COVID-19+: COVID-19 positive

COVID-19-: COVID-19 negative

Declarations

Ethics approval and consent to participate:

The study was approved by the Policlinico Umberto I Hospital's Ethic Committee and informed consent was obtained from all participants.

Availability of data and materials

The data of this report are available from the corresponding author upon reasonable requests.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

M.D.M. conceived and designed the study, enrolled the patients, interpreted the results and prepared the original manuscript. G.D. and M.L. performed the histopathological examination and immunohistochemistry/immunofluorescence assay of retrieved thrombi and edited the Figure; M.I. is the neurointerventional radiologist who retrieved the thrombi of patients and reviewed the manuscript. I.B.

and O.G.S participated in patients' data collection; S.L. critically reviewed and edited the first draft of the manuscript. L.M. and O.T. performed the real time-polymerase chain reaction (RT-PCR) analysis on COVID+ thrombi; D.T. critically reviewed and edited the manuscript.

References

1. Zhang S, Liu Y, Wang X, Yang L, Li H, Wang Y et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol. 2020;13(1):120.
2. Grobbelaar LM, Venter C, Vlok M, Ngoepe M, Laubscher GJ, Lourens PJ et al. SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. Biosci Rep. 2021; 41(8).
3. Ogata AF, Maley AM, Wu C, Gilboa T, Norman M, Lazarovits R, et al. Ultra-Sensitive Serial Profiling of SARS-CoV-2 Antigens and Antibodies in Plasma to Understand Disease Progression in COVID-19 Patients with Severe Disease. Clin Chem. 2020; 66(12):1562–1572.
4. Genchi A, Semerano A, Schwarz G, Dell'Acqua B, Gullotta GS, Sampaolo M et al. Neutrophils predominate the immune signature of cerebral thrombi in COVID-19 stroke patients. Acta Neuropathol Commun. 2022; 10(1):14.
5. Marfella R, Paolisso P, Sardu C, Palomba L, D'Onofrio N, Cesaro A et al. SARS-COV-2 colonizes coronary thrombus and impairs heart microcirculation bed in asymptomatic SARS-CoV-2 positive subjects with acute myocardial infarction. Crit Care. 2021; 25(1):217.
6. Desilles JP, Solo Nomenjanahary M, Consoli A, Ollivier V, Faille D, Bourrienne MC et al; compoCLOT study group. Impact of COVID-19 on thrombus composition and response to thrombolysis: Insights from a monocentric cohort population of COVID-19 patients with acute ischemic stroke. J Thromb Haemost. 2022; 20(4):919–928.
7. Oliva A, Cancelli F, Brogi A, Curtolo A, Savelloni G, Siccardi G et al. Convalescent plasma for haematological patients with SARS-CoV-2 pneumonia and severe depletion of B-cell lymphocytes following anti-CD20 therapy: a single-centre experience and review of the literature. New Microbiol. 2022; 45(1):62–72.

Figures

Figure 1

Arterial thrombi from Covid-19+ patients contain Sars-CoV-2 SP but not N protein

Panel 1. Positive immunostaining for SARS-CoV-2 Spike protein (SP) (arrows) in representative thrombotic material from COVID19+ patients, retrieved from cerebral (A) and coronary (C) arteries. Immunohistochemistry for Nucleocapsid protein (NP) was negative in the same samples (B-D). **Panel 2**.

Representative positive immunohistochemical staining for SP (E) and NP (F) (arrows) in the lung of a patient affected by COVID-19 (positive control). Representative negative immunostaining for SP (G) and NP (H) in a thrombus retrieved from the middle cerebral artery of a patient not affected by COVID-19 (negative control). Original magnification 20X. **Panel 3.** Double immunofluorescence of thrombotic material retrieved from COVID19+ patient cerebral artery. Platelets are stained with antibodies CD61 (red) and SARS-CoV-2 Spike Protein (SP) (green). Overlap of SARS-CoV-2 SP and platelets is shown in yellow (merge).