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ORIGINAL ARTICLE

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

Jean-Philippe Desilles[1,2] | Mialitiana Solo Nomenjanahary[1] | Arturo Consoli[3] | Véronique Ollivier[1] | Dorothée Faille[1] | Marie-Charlotte Bourrienne[1] | Mylène Hamdani[2] | Sébastien Dupont[1] | Lucas Di Meglio[1] | Simon Escalard[2] | Benjamin Maier[2] | Raphael Blanc[1,2] | Michel Piotin[1,2] | Bertrand Lapergue[3] | Nadine Ajzenberg[1] | Marc Vasse[4] | Mikael Mazighi[1,2] | Benoît Ho-Tin-Noé[1] | the compoCLOT study group

[1]Université de Paris and Université Sorbonne Paris Nord, INSERM, LVTS, Paris, France

[2]Interventional Neuroradiology Department, Biological Resource Center, Rothschild Foundation Hospital, Paris, France

[3]Department of Stroke Centre and Diagnostic and Interventional Neuroradiology, University of Versailles and Saint Quentin en Yvelines, Foch Hospital, Suresnes, France

[4]Biology Department, UMR-S 1176, Foch Hospital, Suresnes, France

Correspondence
Benoît Ho-Tin-Noé, Laboratory of Vascular Translational Science, U1148 INSERM, 46 rue Henri Huchard 75018 Paris, France.
Email: benoit.ho-tin-noe@inserm.fr

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Abstract

Background: Resistance to fibrinolysis, levels of procoagulant/antifibrinolytic neutrophil extracellular traps (NETs), and the severity of acute ischemic stroke (AIS) are increased by COVID-19. Whether NETs are components of AIS thrombi from COVID-19 patients and whether COVID-19 impacts the susceptibility of these thrombi to thrombolytic treatments remain unknown, however.

Objectives: We aimed to characterize AIS thrombi from COVID-19 patients by immunohistology and to compare their response to thrombolysis to that of AIS thrombi from non-COVID-19 patients.

Patients/Methods: For this monocentric cohort study, 14 thrombi from COVID-19 AIS patients and 16 thrombi from non-COVID-19 patients, all recovered by endovascular therapy, were analyzed by immunohistology or subjected to *ex vivo* thrombolysis by tissue-type plasminogen (tPA)/plasminogen.

Results: COVID-19 AIS thrombi were rich in neutrophils and contained NETs, but not spike protein. Thrombolysis assays revealed a mean resistance profile to tPA/plasminogen of COVID-19 AIS thrombi similar to that of non-COVID-19 AIS thrombi. The addition of DNase 1 successfully improved thrombolysis by potentiating fibrinolysis irrespective of COVID-19 status. Levels of neutrophil, NETs, and platelet markers in lysis supernatants were comparable between AIS thrombi from non-COVID-19 and COVID-19 patients.

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Trial Registration: This trial was registered at www.clinicaltrials.gov as # NCT03268668.

Conclusions: These results show that COVID-19 does not impact NETs content or worsen fibrinolysis resistance of AIS thrombi, a therapeutic hurdle that could be overcome by DNase 1 even in the context of SARS-CoV-2 infection.

KEYWORDS
COVID-19, ischemic stroke, neutrophil extracellular traps, SARS-CoV-2, thrombolysis, thrombus

1 | INTRODUCTION

In patients with severe COVID-19, systemic thrombotic complications have been described, including cerebrovascular diseases, in 5.7%–23% of cases.[1,2] We and others have studied COVID-19 patients treated for an acute ischemic stroke (AIS) with endovascular therapy (EVT).[3,4] These patients shared as the main common feature a poor prognosis with higher severity large vessel occlusion strokes and larger thrombus burden with higher rate of multiple vessel occlusion compared to non-COVID-19 patients.[5] Although COVID-19 worsens the prognosis of AIS, it remains unknown whether it impacts AIS thrombus composition or its response to thrombolysis, but there is evidence suggesting that it might.

Studies based on viscoelastic assays have reported that critically ill COVID-19 patients have an increased resistance to fibrinolysis, a mechanism that could contribute to COVID-19 coagulopathy and impair the efficacy of thrombolytic therapy.[6–11] Several hemostatic abnormalities that may contribute to fibrinolysis resistance in COVID-19 have been reported. These notably include higher plasma levels of plasminogen activator inhibitor-1[12–14] fibrinogen,[11,14,15] and von Willebrand factor (VWF).[16,17] Indeed, plasminogen activator inhibitor-1 is one of the main natural inhibitors of tissue-type and urokinase-type plasminogen activators (tPA and uPA) and hyperfibrinogenemia has been shown to be associated with increased resistance to fibrinolysis.[18,19] The effect of VWF on fibrinolysis is more controversial, with reports of a positive[20] or negative impact.[19,21] In addition to these factors, neutrophil extracellular traps (NETs), which impair tPA-mediated thrombolysis, are elevated in COVID-19[22] and were found in both pulmonary microthrombi and coronary thrombi of COVID-19 patients.[23–25]

Here, to determine whether COVID-19 impacts the susceptibility of AIS thrombi to fibrinolysis, we investigated the histopathologic features of AIS thrombi from COVID-19 patients and compared their response to *ex vivo* thrombolysis with that of non-COVID-19 AIS thrombi.

Essentials

- There is evidence that COVID-19 may impact thrombus composition and resistance to thrombolysis.
- Stroke thrombi from COVID-19 patients were rich in NETs but contained no spike protein.
- COVID-19 did not affect *ex vivo* thrombolysis of stroke thrombi, nor its potentiation by DNase 1.
- COVID-19 does not increase thrombolysis resistance in stroke, which can be overcome by DNase 1.

2 | METHODS

2.1 | Standard protocol approvals, registrations, and patient consents

Thrombi were collected at the end of EVT. The EVT procedure was chosen at the interventionalist's discretion using a stent-retriever and/or a contact aspiration technique.

COVID-19 was confirmed by positive nasopharyngeal SARS-CoV-2 real-time RT-PCR.[26] Patient data were collected prospectively using a standardized questionnaire (Endovascular Treatment in Ischemic Stroke-ETIS-registry NCT03776877). All patients were provided with a written explanation of the study. The patients or their representatives were given the opportunity to refuse participation. The local Ethics Committee approved this research protocol (CPP Nord Ouest II, ID-RCB number: 2017-A01039-44). The STROBE Statement checklist is provided in Supplementary Material S1.

2.2 | Acute ischemic stroke thrombi collection and processing

Thirty thrombi, from 29 different patients (14 from consecutive COVID-19 positive patients, one of which had one AIS recurrence, and 16 from non-COVID-19 patients), were recovered by EVT from March 15, 2020, to May 30, 2021, during the 3 first COVID-19 outbreaks in the Paris area.

Of the 14 thrombi from COVID-19 patients, eight were used exclusively for *ex vivo* thrombolysis assays, three were fixed in paraformaldehyde and used exclusively for immunohistological analysis, and three provided sufficient material to be analyzed both in *ex vivo* thrombolysis assays and immunohistology.

All 16 thrombi from non-COVID-19 patients were used for *ex vivo* thrombolysis assays, and 11 of them were further analyzed for quantification of D-dimer release.

A schematic flowchart summarizing sample processing after collection is shown in Figure 1.

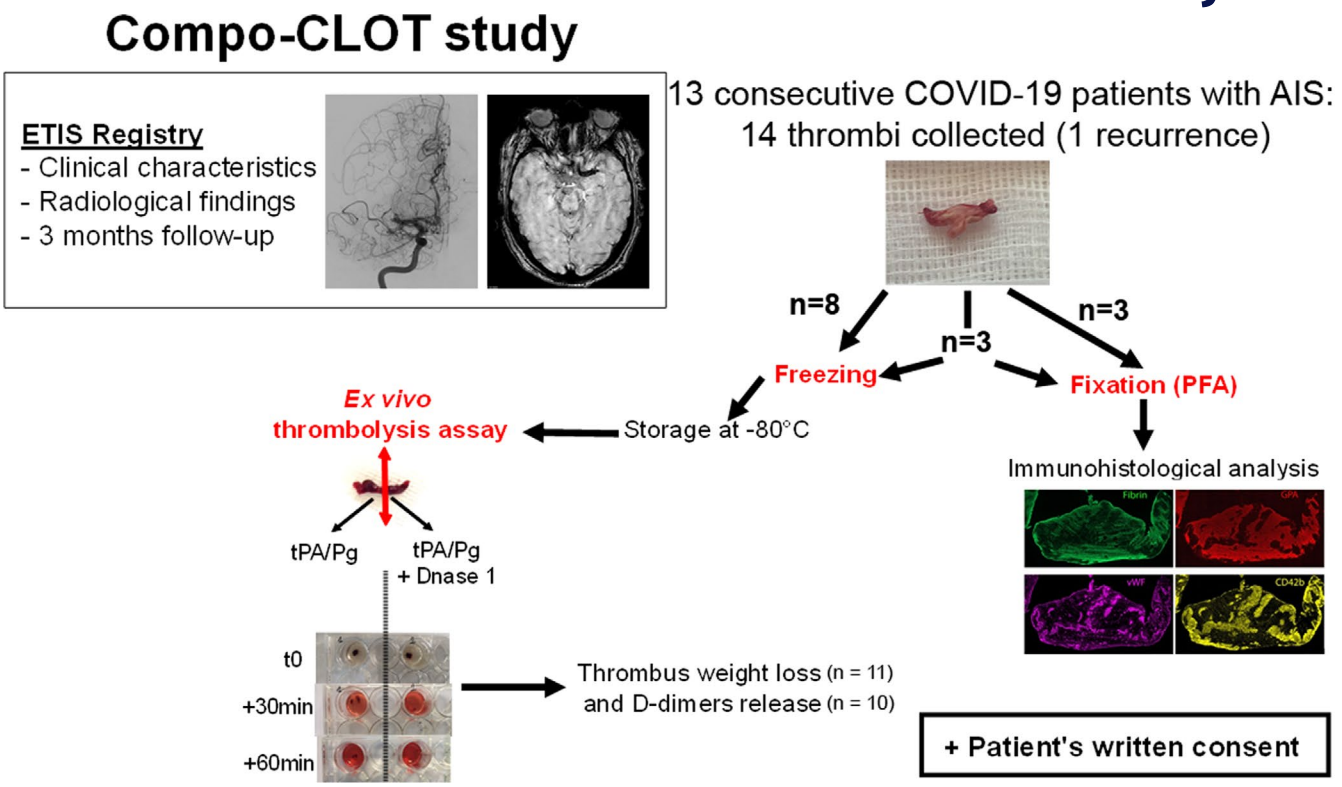

FIGURE 1 Schematic flowchart summarizing processing of endovascular therapy-retrieved thrombi from stroke patients during the COVID-19 pandemic

2.3 | Immunostaining

Freshly recovered thrombi were fixed in 3.7% paraformaldehyde for 48 h before being embedded longitudinally in paraffin and sectioned at 5 μm. Immunostainings were performed as described previously[27] using the following primary antibodies: anti-fibrinogen (20 μg/ml; Dako, ref A0080), anti-Von Willebrand factor (10 μg/ml; Abcam, ref ab11713), anti-CD42b (2 μg/ml; Beckman Coulter, ref IM0409), anti-platelet factor 4 (10 μg/ml; Peprotech, ref 500-P05), anti-myeloperoxidase (MPO, 10 μg/ml; Dako, ref A0398), anti-histone H4 citrulline 3 (H4Cit, 1/200; Millipore, ref 07-596), anti-angiotensin-converting enzyme 2 (ACE-2, 3 μg/ml, R&D Systems, ref AF933), and anti-SARS-CoV-2 Spike Protein (5 μg/ml, P06DHuRb and T01KHuRb from Invitrogen, 10 μg/ml 40150-R007 from Sino Biological). Red blood cells were identified by their autofluorescence (λex 440/9/λem > 570 nm).

2.4 | *Ex vivo* thrombolysis assay

Thrombi were cut in two equal parts and treated with or without DNase 1 (100 μg/ml, Dornase Alfa, Pulmozyme; Roche, Basel, Switzerland) at 37°C under agitation (500 rpm, Thermomixer) in phosphate buffered saline supplemented with Glu-plasminogen (1 μM, Technoclone; Vienna, Austria) and tPA (10 μg/ml Alteplase, Actilyse; Boehringer Ingelheim, Ingelheim am Rhein, Germany). The incubation volume was adjusted to the thrombus weight at a v/w ratio of 40 ml g^{-1}. Thrombus weight was measured before lysis and at 10, 30, and 60 minutes after lysis initiation. A 25-μl aliquot of lysis supernatant was collected at each of these time points for subsequent measurement of D-dimers by quantitative latex immunoassay (HemosIL D-dimer HS500, Werfen) on an ACL TOP 700 analyzer (Werfen). At the end of the *ex vivo* thrombolysis assays, lysis supernatants and residual thrombi were collected and kept at −80°C until analysis.

2.5 | Measurement of glycoprotein VI, myeloperoxidase, DNA, and citrullinated histones

Lysis supernatants and residual thrombi collected at the end of thrombolysis assays were centrifuged at 14,000*g* for 5 min for measurement of myeloperoxidase (MPO, Hycult Biotech), DNA (Quant iT Picogreen dsDNA Assay kit, Molecular Probes, Life Technologies), and citrullinated histone H3 (H3Cit, Cayman Chemical) using commercial kits. Glycoprotein VI (GPVI) was measured using a mesoscale-based immunoassay, as described previously.[28]

2.6 | Statistical analysis

Categorical variables were expressed as frequencies and percentages. Quantitative variables were expressed as median (interquartile range). The treatment or COVID-19 status effects in *ex vivo* thrombolysis assays were compared using the Wilcoxon signed-rank test for

paired samples or the Mann-Whitney-Wilcoxon test for independent samples. Results are represented as mean ± SEM. PrismGraph 4.0 software (GraphPad Software, San Diego, CA) was used. Values of $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics and demographics of COVID-19 stroke patients compared with non-COVID-19 patients

Patient characteristics are listed in Table S1. The characteristics of COVID-19 patients most notably included a higher proportion of male patients, markedly elevated CRP and D-dimer levels, an increased neutrophil to lymphocyte ratio, a more severe initial clinical deficit, and a higher rate of poor clinical outcome at 3 months compared with non-COVID-19 patients (Table S1). The mean time between COVID-19 symptom onset and AIS onset was of 10 days. The minimum and maximum delay between COVID symptom and AIS onset were of 4 and 18 days, respectively. COVID-19 patients were all symptomatic with clinical signs of respiratory distress, and their COVID-19 status was confirmed by both positive SARS-CoV-2 RT-PCR on nasopharyngeal samples collected at admission and suggestive signs on chest computed tomography scan performed within 24 h after their admission. Of the 13 COVID-19 patients, seven were previously hospitalized for COVID-19 symptoms at the time of AIS onset, including one in the intensive care unit, which led to immediate transfer to the stroke unit. The other six patients were directly admitted to the stroke unit without prior hospitalization. In the latter patients, COVID-19 status was established within 24 h after their admission.

3.2 | Acute ischemic stroke thrombi from COVID-19 patients contain neutrophils and neutrophil extracellular traps but no spike protein

Hematoxylin and eosin staining of AIS thrombi from six different COVID-19 patients showed that, with the exception of one thrombus in which erythrocyte distribution was widespread; all other thrombi were characterized by large erythrocyte-poor areas (Figure 2). We then analyzed the fibrillar and cell composition of these thrombi in more detail using multiple immunofluorescence labelling. Hoechst 33342 staining of DNA and anti-platelet immunostaining showed that all COVID-19 AIS thrombi contained numerous nucleated cells and platelets (Figure 3A). Platelets and nucleated cells were particularly abundant in red blood cell–poor areas (Figure 3A). In agreement with our previous studies in non-COVID-19 AIS patients,[27,29] platelets lined the thrombus periphery (Figure S1). With respect to thrombus fibrillar components, the extracellular scaffold of all six thrombi analyzed showed extensive positive labeling for both fibrin(ogen) and VWF (Figure 3B).

The vast majority of nucleated cells in COVID-19 AIS thrombi had a polymorphonuclear morphology indicating that they could be neutrophils, which was confirmed by their positivity in MPO immunostaining (Figure 4A,B). Remarkably, all thrombi contained abundant extracellular webs of decondensed DNA suggestive of NETs. The presence of NETs in AIS COVID-19 thrombi was confirmed by positive immunostaining for citrullinated histone H4 (H4Cit) (Figure 4C,D and Figure S1).

Several studies have indicated that neutrophils,[22,30] and possibly platelets,[31,32] express ACE-2, one of the main host proteins to which the SARS-CoV-2 spike glycoprotein binds to enter cells.[33] In all six COVID-19 AIS thrombi analyzed, ACE-2 was detected in subsets of leukocytes and platelets, but not red blood cells (Figure 5). No spike glycoprotein was found in AIS thrombi from COVID-19 patients using three different monoclonal antibodies directed against its RBD-containing S1 subunit (data not shown).

3.3 | Acute ischemic stroke thrombi from COVID-19 and non-COVID-19 patients display similar response patterns to fibrinolysis- and DNA lysis-based thrombolytic treatments

The response of COVID-19 AIS thrombi to the gold standard thrombolytic treatment for AIS, recombinant tPA, was tested *ex vivo* and compared with that of non-COVID-19 thrombi. There was no difference in thrombus weight at any given time point after initiating lysis by addition of tPA and plasminogen (Figure 6A). When results were expressed relative to initial thrombus weight for normalization, non-COVID-19 and COVID-19 AIS thrombi displayed strictly identical mean lysis profiles (Figure 6B). After 60 min of lysis, the mean residual thrombus weight was of 52% of the initial weight in both groups ($p = .98$, Figure 6B).

Measurement of D-dimer release in lysis supernatants over time, however, revealed a possible delay in fibrinolysis initiation in COVID-19 thrombi as compared with non-COVID-19 AIS thrombi (Figure 6C). After 10 min of lysis, the mean level of released D-dimers had already reached 49.4 ± 9.3% of the maximal release obtained after 60 min of treatment in non-COVID-19 thrombi, whereas it was only of 7 ± 1.5% for COVID-19 thrombi ($p < .001$, Figure 6C). After 30 min of lysis, there was no more difference in D-dimer release between the two groups (Figure 6C).

NETs have been shown previously to impair tPA-mediated thrombolysis of arterial thrombi.[34–36] We thus tested and compared the efficacy of DNase 1 (dornase alfa) used as a thrombolytic adjuvant aimed at degrading antithrombolytic extracellular DNA in non-COVID-19 and COVID-19 AIS thrombi. DNase 1 significantly improved tPA/plasminogen-mediated thrombolysis of both non-COVID and COVID-19 AIS thrombi, leading to the almost complete lysis of both types of thrombi within 60 min (Figure 6B). Like in the absence of DNase 1, the mean thrombolysis response

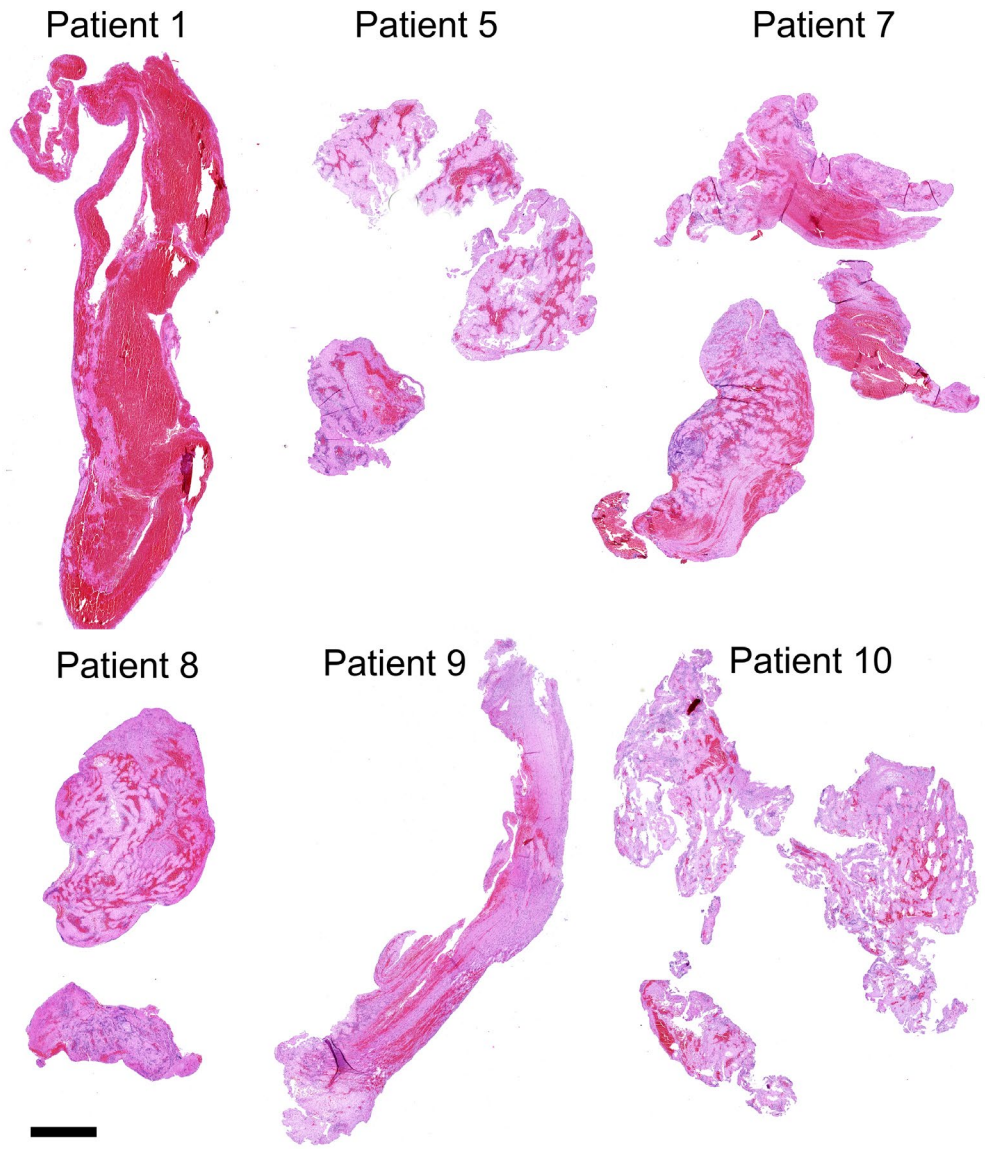

FIGURE 2 Histopathological aspect of acute ischemic stroke thrombi recovered from COVID-19 patients. Representative overviews showing whole tissue sections of thrombi from six different patients stained in hematoxylin and eosin. Note that with except for the thrombus from patient 1, all thrombi show vast red blood cell-poor areas

patterns of non-COVID and COVID-19 AIS thrombi in the presence of DNase 1 were very much alike (Figure 6B). Notably, however, despite their similar mean response patterns (Figure 6B), AIS thrombi from both COVID-19 and non-COVID-19 patients displayed an important individual variability in their response to thrombolysis, whether in the absence and presence of DNase 1 (Figures S2 and S3).

Improved thrombolysis in the presence of DNase 1 was associated with increased release of D-dimers from non-COVID-19 and COVID-19 AIS thrombi (Figure 6C), indicating that DNase 1 effectively potentiated fibrinolysis irrespective of COVID-19 status. In both groups, after 60 min of lysis, the mean D-dimer release in the presence of DNase 1 was of 2.6-fold that obtained in the absence of DNase 1. The delay in fibrinolysis initiation observed in the absence of DNase 1 for COVID-19 compared with non-COVID-19 AIS thrombi (Figure 6C) was maintained in the presence of DNase 1 (Figure 6C).

At the end of *ex vivo* thrombolysis assays, lysis supernatants and residual thrombi were collected and centrifuged to obtain lysates for measurement of GPVI, MPO, DNA, and citrullinated histone H3 used as markers of platelet, neutrophil, and NET content. There was no difference in GPVI, MPO, DNA, or citrullinated histone H3 content between AIS thrombi from COVID-19 and non-COVID-19 patients (Figure 6D–G).

4 | DISCUSSION

In this study, we show that, whereas neutrophils and NETs are constitutive components of AIS thrombi from COVID-19 patients,

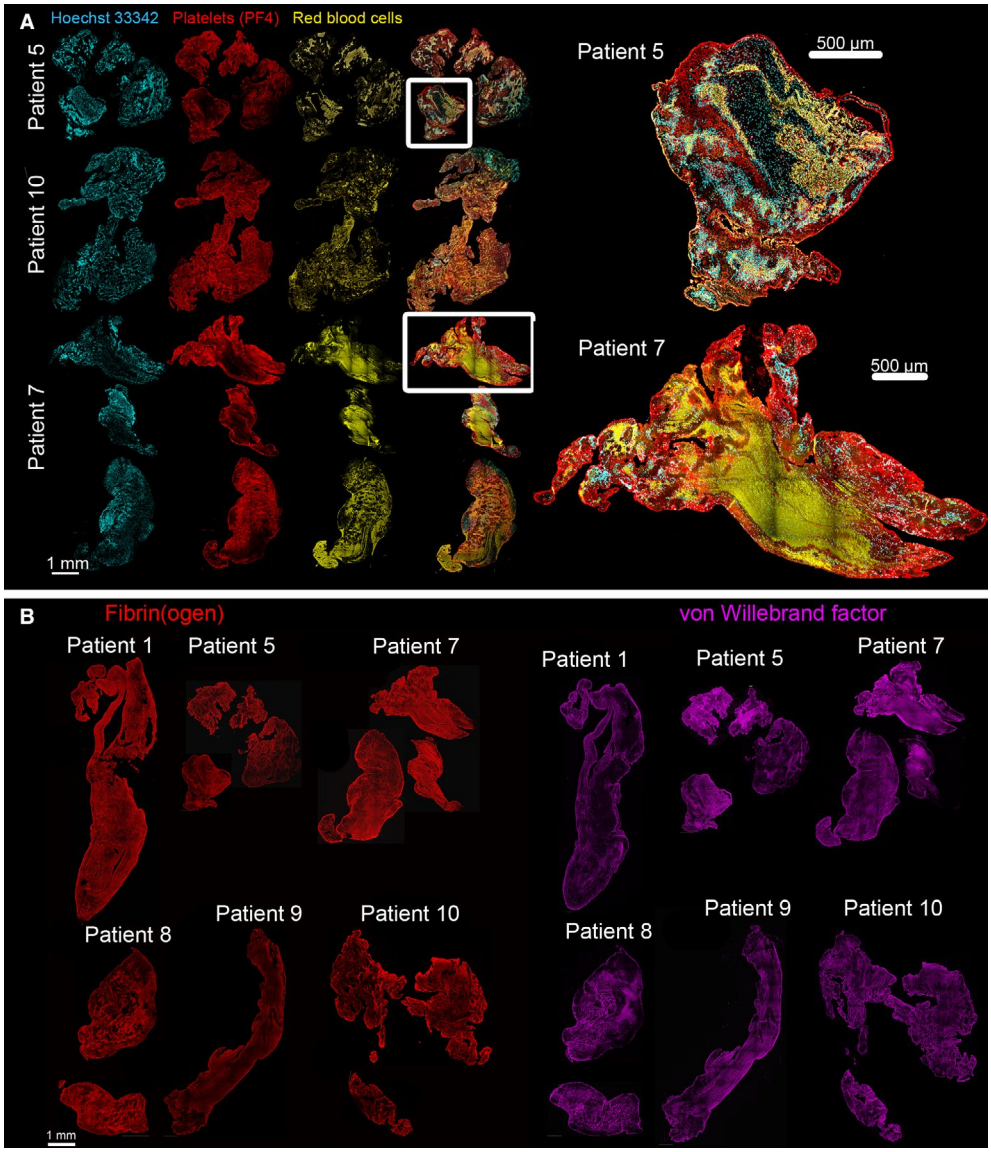

FIGURE 3 Cellular and fibrillar components of COVID-19 stroke thrombi. (A) Panoramic overviews (left panels) and higher magnification views (right panels) of whole tissue sections of thrombi from three different COVID-19 stroke patients stained for DNA (Hoechst 33342, blue), platelet factor 4 (PF4, red), and red blood cells (yellow). (B) Whole section images showing the presence and distribution of fibrin(ogen) and von Willebrand factor in acute ischemic stroke thrombi recovered from COVID-19 patients

those thrombi do not display an increased resistance to thrombolysis compared with AIS thrombi from non-COVID-19 patients, whether in the presence or absence of DNase 1. These results indicate that, despite its impact on NET levels[22,23] and fibrinolysis in viscoelastic assays,[6–10] COVID-19 does not significantly alter the response of AIS thrombi to thrombolytic treatments. Although a slight delay in fibrinolysis initiation was observed in AIS thrombi from COVID-19 patients compared with non-COVID-19 patients, its biological and clinical significance remain unclear because the overall thrombolysis response patterns of COVID-19 and non-COVID-19 patients were identical. With respect to AIS thrombus composition, we show that AIS thrombi from COVID-19 and non-COVID-19 patients have similar platelets, neutrophils, and NETs content. Moreover, the histological features of AIS thrombi from COVID-19 patients are comparable to those reported previously for thrombi of non-COVID-19 AIS patients, which were also shown to contain large amounts of neutrophils, NETs, platelets, and VWF.[27,37]

We did not detect SARS-CoV-2 spike protein in COVID-19 AIS thrombi, although we did find one of its main host receptors, ACE-2, in association with subsets of leukocytes and platelets. Although ACE-2 can be expressed by neutrophils,[22,30] its expression by platelets remains controversial.[32] The patchy distribution of ACE-2 on subsets of platelets in AIS thrombi may suggest an uptake by platelets of ACE-2 released in the extracellular space.

In addition to being able to directly trigger NET formation, SARS-CoV-2 has been shown to prime platelets for activation, possibly via its glycoprotein spike.[31] Several groups have shown the ability of

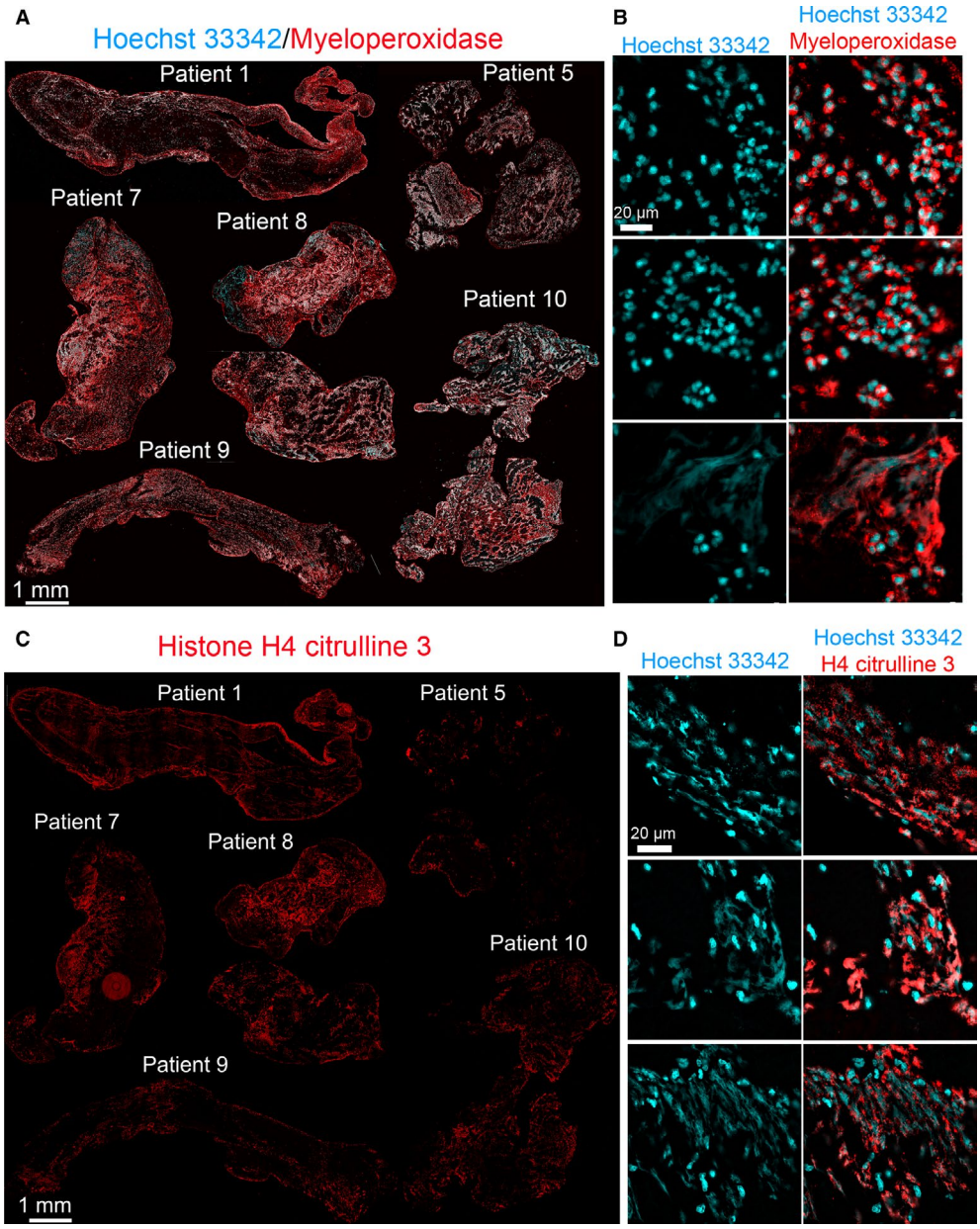

FIGURE 4 Acute ischemic stroke thrombi from COVID-19 patients contain neutrophils and neutrophil extracellular traps. (A) Panoramic overviews of myeloperoxidase and DNA immunostaining in COVID-19 stroke thrombi (left panels) and (B) higher magnification views showing that the majority of nucleated cells are neutrophils. (C) Panoramic overviews of citrulline histone H4 staining in COVID-19 stroke thrombi (left panels) and (D) higher magnification views of DNA and citrulline histone H4 immunostaining showing abundant extracellular webs of decondensed DNA with citrulline histone H4 costaining suggestive of neutrophil extracellular traps (NETs)

platelets to internalize SARS-CoV-2 virion *in vitro*, and SARS-CoV-2 RNA and virion have been found in platelets from COVID-19 patients.[31,38–40] However, to date, no correlation has been established between platelet-associated SARS-CoV-2 and occurrence of thrombotic events in COVID-19 patients. The absence of SARS-CoV-2 spike protein in AIS thrombi from COVID-19 patients supports a model in which thrombotic complications of COVID-19 occur in an indirect manner through viral-induced immunothrombotic dysregulation, rather than through direct triggering of platelet activation by the virus or its spike glycoprotein.[25,41] This model is also consistent with the overrepresentation of pre-existing conditions known to prime the thromboinflammatory cascade (e.g., diabetes, hypertension) in patients with severe COVID-19.

Remarkably, although we and others have shown previously that DNase 1 enhances the thrombolytic efficacy of tPA toward arterial thrombi, we show here that this beneficial effect of DNase 1 occurs, at least in part, through potentiation of fibrinolysis. Our results are in agreement with those of several *in vitro* studies showing that NETs, extracellular DNA, and histones can interfere with plasminogen activation and plasmin activity.[42,43] We have also previously reported that DNase 1 alone did not have thrombolytic activity toward AIS thrombi.[35] This result suggested that the additional structural

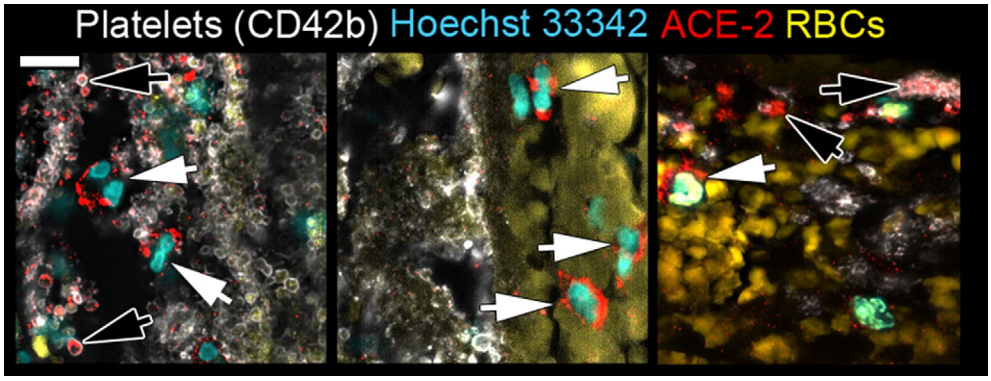

FIGURE 5 Angiotensin converting enzyme-2 (ACE-2) in COVID-19 stroke thrombi. Representative images from three different COVID-19 stroke thrombi showing the presence of ACE-2 (red) in association with subsets of platelets (black arrow) and leukocytes (white arrows). Bar = 10 μm

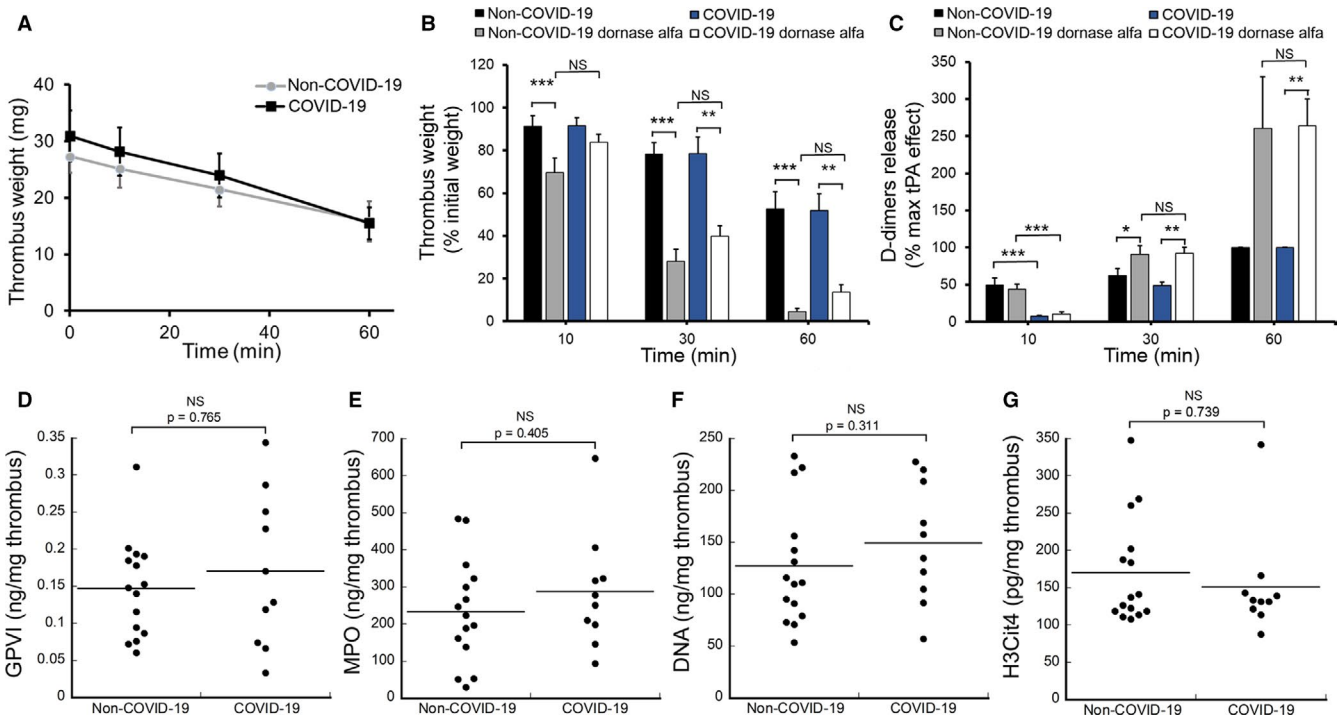

FIGURE 6 COVID-19 does not impact thrombolysis susceptibility nor cell and neutrophil extracellular traps (NETs) content of acute ischemic stroke thrombi. (A) Mean weight loss according to time of stroke thrombi recovered from COVID-19 and non-COVID-19 patients following the addition of tissue-type plasminogen (tPA) and plasminogen to promote fibrinolysis. $n = 16$ non-COVID-19 and 11 COVID-19 thrombi. (B) Mean weight loss of COVID-19 and non-COVID-19 thrombi in response to tPA/plasminogen, in the presence or absence of dornase alfa, as expressed as a percentage relative to initial thrombus weight. $n = 16$ non-COVID-19 and 11 COVID-19 thrombi. (C) Comparison of D-dimers release during tPA/plasminogen-mediated lysis, in the presence or absence of dornase alfa, between non-COVID-19 and COVID-19 thrombi. Results are expressed as a percentage relative to individual D-dimers release produced by tPA/plasminogen treatment after 60 min of lysis. $n = 11$ non-COVID-19 and 10 COVID-19 thrombi. (D-G) Comparison of glycoprotein VI (GPVI), myeloperoxidase (MPO), DNA, and citrullinated histone H3 levels in *ex vivo* thrombolysis supernatants of non-COVID-19 and COVID-19 thrombi. $n = 15$ non-COVID-19 and 10 COVID-19 thrombi. $^{*}p < .05$, $^{**}p < .005$, $^{***}p < .001$

scaffold provided by extracellular DNA to AIS thrombi was not central to the anti-thrombolytic effect of NETs. Our present findings showing that extracellular DNA in AIS thrombi has potent antifibrinolytic activity further supports this notion.

Our study has several limitations. Although we report no quantitative differences in cell type or NET content between AIS thrombi from COVID-19 and non-COVID-19 patients, this must be balanced by the relatively low number of patients included. In addition, because cell content does not necessarily equal cell activation, one cannot exclude differences in the level of platelet or neutrophil activation. In that regard, the addition of exogenous Glu-plasminogen in *ex vivo* thrombolysis assays may have masked potential differences in plasminogen

content but also in plasminogen degradation, which can be promoted by activated neutrophils.[43] Our analysis of EVT-recovered thrombi does not address possible endothelial alterations, which could contribute to AIS occurrence and severity in COVID-19. Finally, it should be noted that whereas our study aimed at determining whether COVID-19 was associated with modifications of AIS thrombi, it did not address or establish a link between COVID-19 and AIS onset.

In conclusion, our results show that COVID-19 does not impact AIS thrombus composition or resistance to thrombolysis, a therapeutic hurdle that can be overcome by DNase 1. Importantly, the preserved ability of DNase 1 to potentiate thrombolysis even in the context of SARS-CoV-2, a viral infection known to severely disrupt the immunothrombotic cascade, underscores the wide therapeutic potential of targeting NETs in the treatment of thrombotic disorders. Finally, that the increased severity of AIS[5] and the general thromboinflammatory dysregulation in COVID-19 patients[25,38,39] are not associated with major modifications of thrombi causing large vessel occlusion stroke suggests that worsening of AIS prognosis by COVID-19 might occur through alterations of ischemia-induced injury, which involves the thromboinflammatory cascade.[44]

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CONFLICT OF INTEREST

Dr. Mazighi has relevant financial activities outside the submitted work with the following companies: Acticor Biotech, Air liquide, Boerhinger Ingelheim, Medtronic, and Amgen. Dr. Lapergue has relevant financial activities outside the submitted work with the following companies: Microvention, Stryker, and Penumbra. The other authors report no conflicts.

AUTHOR CONTRIBUTIONS

Jean-Philippe Desilles, Mikael Mazighi, and Benoît Ho-Tin-Noé designed/conceptualized the study, collected data, analyzed/interpreted the data, and wrote the manuscript. Arturo Consoli, Simon Escalard, Benjamin Maier, Michel Piotin, Raphael Blanc, and Mialitiana Solo Nomenjanahary designed/conceptualized and initiated the study, supervised the study, collected data, analyzed/interpreted the data, revised the manuscript. Jean-Philippe Desilles and Benoît Ho-Tin-Noé performed the statistical analyses. All other authors collected data, analyzed/interpreted the data, and revised the manuscript. CompoCLOT Study Group (a complete membership list appears in "Nonauthor Collaborators Template"): All study group members have played an important role in the data collection and analysis of individual patients.

INFORMED CONSENT

Patient data were collected prospectively using a standardized questionnaire (Endovascular Treatment in Ischemic Stroke-ETIS-registry NCT03776877). All patients were provided with a written explanation of the study. The patients or their representatives were given the opportunity to refuse participation. The local Ethics Committee approved this research protocol (CPP Nord Ouest II, ID-RCB number: 2017-A01039-44).

ORCID

Jean-Philippe Desilles https://orcid.org/0000-0001-8395-6312
Marc Vasse https://orcid.org/0000-0002-8784-7209
Benoît Ho-Tin-Noé https://orcid.org/0000-0002-7428-1760

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SUPPORTING INFORMATION

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