Received: 26 October 2022 | Revised: 20 September 2023 | Accepted: 2 October 2023

https://doi.org/10.1016/j.rpth.2023.102289

ORIGINAL ARTICLE

Coagulopathy and adverse outcomes in hospitalized patients with COVID-19: results from the NOR-Solidarity trial

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Handling Editor: Dr Vânia Morelli

Abstract

Background: Several studies have examined parameters of increased thrombogenicity in COVID-19, but studies examining their association with long-term outcome and potential effects of antiviral agents in hospitalized patients with COVID-19 are scarce.

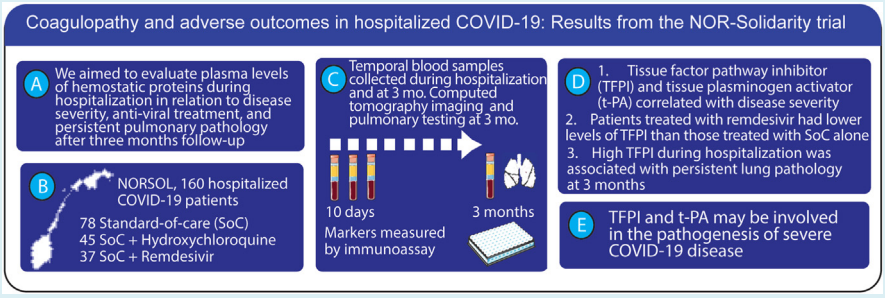
Objectives: To evaluate plasma levels of hemostatic proteins during hospitalization in relation to disease severity, treatment modalities, and persistent pulmonary pathology after 3 months.

Methods: In 165 patients with COVID-19 recruited into the NOR-Solidarity trial (NCT04321616) and randomized to treatment with hydroxychloroquine, remdesivir, or standard of care, we analyzed plasma levels of hemostatic proteins during the first 10 days of hospitalization (n = 160) and at 3 months of follow-up (n = 100) by enzyme immunoassay.

Results: Our main findings were as follows: (i) tissue plasminogen activator (tPA) and

tissue factor pathway inhibitor (TFPI) were increased in patients with severe disease (ie, the combined endpoint of respiratory failure [Po_2-to-FiO_2 ratio, <26.6 kPa] or need for treatment at an intensive care unit) during hospitalization. Compared to patients without severe disease, tPA levels were a median of 42% ($P < .001$), 29% ($P = .002$), and 36% ($P = .015$) higher at baseline, 3 to 5 days, and 7 to 10 days, respectively. For TFPI, median levels were 37% ($P = .003$), 25% ($P < .001$), and 10% ($P = .13$) higher in patients with severe disease at these time points, respectively. No changes in thrombin-antithrombin complex; alpha 2-antiplasmin; a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; or antithrombin were observed in relation to severe disease. (ii) Patients treated with remdesivir had lower levels of TFPI than those in patients treated with standard of care alone. (iii) TFPI levels during hospitalization, but not at 3 months of follow-up, were higher in those with persistent pathology on chest computed tomography imaging 3 months after hospital admission than in those without such pathology. No consistent changes in thrombin-antithrombin complex, alpha 2-antiplasmin, ADAMTS-13, tPA, or antithrombin were observed in relation to pulmonary pathology at 3 months of follow-up.

Conclusion: TFPI and tPA are associated with severe disease in hospitalized patients with COVID-19. For TFPI, high levels measured during the first 10 days of hospitalization were also associated with persistent pulmonary pathology even 3 months after hospital admittance.



KEYWORDS
COVID-19, hospitalization, remdesivir, tissue factor pathway inhibitor, tissue plasminogen activator

Essentials

- Blood clotting laboratory tests were performed in hospital and 3 months later in severe COVID-19.
- Tissue factor pathway inhibitor correlated with severity and 3-month lung pathology.
- Tissue plasminogen activator correlated with disease severity.
- Tissue factor pathway inhibitor and tissue plasminogen activator may be involved in the pathogenesis of severe COVID-19 disease.

1 | INTRODUCTION

Similar to other severe infections, COVID-19 disease is associated with increased risk of venous thromboembolism, particularly in patients with severe disease [1,2]. Indeed, markers of coagulation and fibrinolysis, such as D-dimer and prothrombin time, have been associated with adverse outcome in hospitalized patients with COVID-19 [3–6]. The interaction between SARS-CoV-2 and signaling through the angiotensin-converting enzyme 2 receptor could potentially contribute to the increased venous thromboembolism risk in COVID-19 disease, at least partly through activation of endothelial cells and platelets [7–9]. Moreover, whereas inflammation could enhance thrombophilia, several of the mediators of thrombus formation may enhance inflammation. In addition to their direct role in thrombus

formation, several of these factors have numerous other effects that could further promote COVID-19 disease severity, including their proinflammatory potential [10–12].

Although several studies have addressed the association between coagulation parameters and disease severity in the acute phase of COVID-19, data on the relationship of these parameters to long-term outcome are scarcer. Moreover, while the effects of various anticoagulants have been reported in several studies [13,14], the impact of antiviral agents on these variables is not known.

In the present study, we examined the associations between several hemostatic markers and disease severity (ie, the degree of respiratory failure [RF] and/or the need for treatment in the intensive care unit [ICU]) during hospitalization. We also examined whether these variables differed from those in healthy controls (HCs) 3 months after hospital admission and whether abnormalities in coagulation markers at 3 months were related to pulmonary pathology assessed by pulmonary function tests and chest computed tomography (CT). As the study population was part of a randomized trial (NOR-Solidarity trial) investigating the use of remdesivir and hydroxychloroquine (HCQ), we additionally examined whether the use of these medications modulated the coagulation markers as compared with standard of care (SoC) [15].

We examined a wide range of mediators attenuating thrombin formation (ie, antithrombin and tissue factor pathway inhibitor [TFPI]), inhibiting (alpha 2-antiplasmin [α2AP]) and promoting (tissue plasminogen activator [tPA]) fibrinolysis, and enhancing endothelial/platelet interaction (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 [ADAMTS-13]) as well as soluble levels of thrombin-antithrombin (TAT) complex as a marker of net activation of coagulation.

2 | METHODS

2.1 | Study design and participants

Plasma samples were obtained from the NOR-Solidarity trial, an open-label, multicenter, adaptive, randomized controlled trial evaluating the effect of antiviral drugs on hospitalized patients with COVID-19 admitted to 23 Norwegian hospitals (NCT04321616) [15]. The study served as an add-on to the World Health Organization (WHO) Solidarity trial [16]. Participants were included from March 28, 2020, until October 5, 2020, encompassing the first 2 COVID-19 waves. Adult patients aged ≥18 years admitted to hospital with polymerase chain reaction–confirmed SARS-2-CoV-2 infection were eligible for inclusion. Key exclusion criteria were severe comorbid conditions with life expectancy <3 months, level of aspartate aminotransferase or alanine aminotransferase >5 times the upper limit of normal, rate-corrected QT interval greater than 470 milliseconds, pregnancy, breastfeeding, acute occurrence of a comorbid condition in a 7-day period before inclusion, known intolerance to study drugs, participation in a potentially confounding trial, or concomitant medications interfering with the study drugs.

Between 1 and 3 blood samples were obtained from each patient within the first 48 hours of admission and up to 10 days during hospitalization, and in subgroups, samples were also obtained at 3 months of follow-up. As previously described, reverse-transcription polymerase chain reaction analysis determined viral load in oropharyngeal specimens [15]. All participants consented to the study before inclusion, either themselves or via a legally authorized representative. The study was approved by the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (reference number 118684) and the Norwegian Medicines Agency (20/04950-23).

For comparison, we compared levels of hemostatic markers with those in age- and sex-matched HCs (n = 21). All HCs had a C-reactive protein (CRP) level of <5 mg/L and were hospital workers recruited from the same area as 57% of the patients (Oslo area). Mean age of patients during hospitalization (n = 160) was 58.8 ± 15 (SD) years and 102 (64%) were men, compared to HCs, who were aged 57.2 ± 10.7 years and 13 (62%) of them were men.

Differences in the number of included patients between this substudy and the main NOR-Solidarity [15] study were based on the following: (i) not all study sites had the ability to perform biobanking (both during hospitalization and at 3 months) (ie, 160 of all 180 patients had biobanking during hospitalization); (ii) in addition, pulmonary function tests and/or chest CT was not performed in all patients at all study sites (performed in 108 patients) (ie, in this substudy, 100 with both examination for pulmonary pathology and biobanking [5 of these had biobanking only at 3 months of follow-up] out of the 149 who attended the 3-month follow-up).

2.2 | Intervention and outcome

Participants (n = 165, of whom 5 had biobanking only at 3 months of follow-up) were randomized and allocated to 3 different treatment arms: (i) SoC; (ii) SoC plus 800 mg of HCQ (oral) twice daily on day 1 and then 400 mg daily twice up to 9 days; or (iii) SoC plus 200 mg of remdesivir (intravenous) on day 1 and then 100 mg daily up to 9 days. All treatments were stopped at discharge from hospital or if contraindicated during the study. The interventions did not affect clinical outcome, viral clearance, or systemic inflammation [15]. Severe outcome was defined as the combination of having either acute RF, defined as a Po_2-to-FiO_2 ratio of <26.6 kPa (<200 mmHg), during hospitalization or the need for treatment at the ICU during hospitalization.

2.3 | Follow-up

In total, 100 patients attended a follow-up visit 3 months after hospital discharge, including 5 patients without blood sampling during hospitalization. At follow-up, blood sampling for routine clinical biochemistry and biobanking, pulmonary function assessment, and/or CT scan were performed.

Pulmonary function testing (n = 90) consisted of diffusion capacity of the lungs for carbon monoxide (DL_{CO}), performed as described previously [17]. DL_{CO} is defined as the primary analysis of pulmonary function in our study as it is frequently affected after hospitalization for COVID-19 [18]. DL_{CO} in percentage of predicted value and the lower limit of normal (LLN) were estimated according to the Global Lung Function Initiative Network [17]. Persistent respiratory dysfunction was defined as having a DL_{CO} lower than the LLN.

Low-dose, thin-section chest CT images (n = 91) were acquired in supine and prone positions during breath-holding in deep inspiration, as described previously [17]. For our study, we assessed the prevalence of any ground-glass opacities, where ground-glass opacity ≥10% in 1 or more of the 4 lung zones or mosaic pattern was considered a potentially reversible change, and any consolidations, reticular patterns, parenchymal bands, interlobular septal thickening, or bronchiectasis were taken as potentially irreversible changes. CT changes classified as potentially reversible changes were further interpreted to reflect inflammation, and changes classified as irreversible were interpreted to reflect fibrosis [17].

2.4 | Blood sampling protocol and biochemical analyses

Peripheral venous blood was drawn into pyrogen-free blood collection tubes with EDTA, immediately immersed in melting ice, and centrifuged at 2500g for 20 minutes within 30 minutes to obtain platelet-poor plasma. All samples were stored at −80 °C and thawed <3 times. A similar blood sampling protocol was used for both patients and HCs.

Soluble levels of TAT complex, α2AP, ADAMTS-13, tPA, TFPI, and antithrombin were analyzed (in duplicate) by enzyme immunoassays using antibodies from R&D Systems in a 384-well format using a SELMA (Analytik Jena Logo) pipetting robot in combination with a BioTek (Agilent Technologies) dispenser/washer. Absorption was read at 450 nm with a wavelength correction set to 540 nm using a plate reader (Bio-Rad). The intra-assay coefficient of variation as a percentage was <10%.

Routine laboratory variables (CRP, D-dimer, ferritin, and total leukocyte, neutrophil, lymphocyte, and monocyte counts) were measured at the biochemical laboratories at the participating hospitals. More information on the different D-dimer assays is given in the methods section of the Supplementary material.

2.5 | Statistical analysis

For demographic variables, normally distributed continuous variables were compared with Student's t-test and are presented as mean ± SD, while nonnormally distributed variables are presented as median (25th/75th percentile) and were compared with the Mann–Whitney U-test. Categorical data were compared using the chi-square test.

As shown in Supplementary Figure S1, levels of TAT, α2AP, ADAMTS-13, tPA, TFPI, and antithrombin were nonnormally distributed, and nonparametric statistics were used when comparing levels of these between groups. For each time point, a comparison between groups was assessed with the Mann–Whitney U-test. When comparing 3 groups (ie, treatment modalities), the Kruskal–Wallis H test was used, followed by pairwise comparison with Mann–Whitney U-test if significant.

For body mass index, we lacked data from 5 patients, and for biochemical measurements, we lacked 4 to 6 measurements for hemoglobin and estimated glomerular filtration rate (eGFR) and 9 to 12 measurements for CRP, D-dimer, white blood count, and neutrophil counts. No strategy was used to replace missing data.

Associations between variables were assessed by Spearman correlation. The P values are 2-sided and considered significant when <.05.

3 | RESULTS

As shown in the Table comparing demographics between patients with COVID-19 with (n = 53) or without (n = 107) severe outcome (ie, RF/ICU) during hospitalization, patients with severe outcome were older, received more oxygen therapy, and had higher levels of CRP, D-dimer, ferritin, neutrophil count, and lower eGFR, and P_{O_2}-to-FiO_2 ratio.

For patients with severe outcome, 59% had RF, and 98% were admitted to the ICU. During the study, 8 patients died (5%), 4 during the 10-day hospitalization period where blood samples were obtained, and 4 before 60 days follow-up. All patients who died were admitted to the ICU and in the severe outcome group.

3.1 | Hemostatic markers in relation to the combined endpoint of having RF and/or ICU admission during hospitalization

Figure 1 shows the temporal profile of hemostatic markers in patients with COVID-19 during the first 10 days of hospitalization and HCs for comparison. Patients with COVID-19 had higher levels of TAT, α2AP, tPA, and TFPI but not ADAMTS-13 or antithrombin compared to those in HCs throughout the observation period, except TFPI, which was only higher at 7 to 10 days in patients without severe outcome.

Patients with severe outcome had higher levels of tPA (Figure 1D) and TFPI (Figure 1E) compared to the other patients. tPA levels were higher at all time points, while TFPI was significantly higher at baseline and 3 to 5 days but not at 7 to 10 days in patients with RF or those admitted to the ICU. Levels of antithrombin were lower in patients with RF or those admitted to the ICU at 3 to 5 days but largely within normal levels for HCs. As for levels of TAT complex,

TABLE Demographic, clinical, and biochemical characteristics of 160 patients hospitalized for COVID-19 according to severe outcome (intensive care unit admission and/or respiratory failure).

Parameter	No outcome, (*n* = 107)	ICU/RF (*n* = 53)
Age (y), mean ± SD	56.3 ± 15.2	63.9 ± 14.1
Male sex, *n* (%)	67 (63)	35 (66)
Body mass index (kg/m^2), mean ± SD	28.2 ± 4.7	29.1 ± 4.3
Treatment group		
SoC, *n* (%)	55 (51)	23 (43)
SoC + hydroxychloroquine, *n* (%)	29 (27)	16 (30.2)
SoC + remdesivir, *n* (%)	23 (22)	14 (26)
Dexamethasone, *n* (%)	11 (10)	9 (17)
LMWH prophylaxis, *n* (%)		
≤5000 IU daily	36 (34)	12 (23)
>5000 IU daily	4 (4)	3 (5)
Oxygen therapy, *n* (%)	46 (43)	46 (87)
Comorbidities, *n* (%)		
Chronic cardiac disease	13 (121)	10 (19)
Hypertension	32 (30)	18 (35)
Chronic pulmonary disease	6 (6)	3 (6)
Obesity	25 (23)	18 (34)
Diabetes	15 (14)	12 (23)
Current smoker	2 (2)	3 (6)
Outcomes, *n* (%)		
RF[a]	0 (0)	31 (59)
ICU admission	0 (0)	52 (98)
P/F ratio at admission (kPa), median (IQR)	45 (40-52)	29 (24-36)
Hemoglobin (g/dL), mean ± SD	13.2 ± 1.6	13.3 ± 1.4
C-reactive protein (mg/L), median (IQR)	54 (27-101)	127 (70-173)
D-dimer (mg/L FEU), median (IQR)	0.6 (0.4-0.9)	1.1 (0.5-1.6)
Ferritin (μg/L), median (IQR)	514 (271-938)	1040 (543-1518)
White blood cell count (×10^9/L), mean ± SD	6.7 ± 2.7	6.2 ± 3.1
Neutrophils (×10^9/L), mean ± SD	3.9 ± 2.1	6.7 ± 3.0
Lymphocytes (×10^9/L), mean ± SD	1.1 ± 0.5	1.3 ± 0.6
eGFR (mL/min/1.73 m^2), mean ± SD	92 ± 21	76 ± 29

eGFR, estimated glomerular filtration rate; FEU, fibrinogen equivalent unit; ICU, intensive care unit; LMWH, low-molecular-weight heparin; P/F ratio, Po_2/FiO_2 ratio; RF, respiratory failure; SoC, standard of care.
[a]Respiratory failure was defined as a Po_2/FiO_2 ratio of <26.6 kPa (<200 mmHg) during hospitalization.

α2AP, and ADAMTS-13 there were no relationship to outcome (Figure 1).

Based on these findings, further analysis mainly focused on tPA and TFPI. Supplementary Table S1 shows correlations between markers and demographics. Baseline levels of both tPA and TFPI correlated positively with body mass index but not age, sex, or kidney function.

3.2 | Temporal profile of tPA and TFPI in relation to treatment modalities

The study population was randomized to SoC alone or SoC + remdesivir or HCQ. There was no association between treatment modality and severe outcome (Table). As shown in Figure 2, tPA and TFPI levels were similar between treatment groups at baseline. However, at 3 to 5 and 7 to 10 days, TFPI levels were lower in patients receiving remdesivir compared with SoC alone. Furthermore, tPA levels were lower in HCQ-treated patients than in those treated with SoC alone at 7 to 10 days.

Next, we assessed the potential modifying effect of prophylaxis with low-molecular-weight heparin (LMWH) on the levels of coagulation biomarkers by comparing levels of TAT, AP2AP, ADAMTS-13, tPA, TFPI, and antithrombin during hospitalization between those who were using LMWH >5000 IU daily and those who were using LMWH ≤5000 IU daily or no LMWH. As shown in Supplementary Figure S2, the only significant differences that were noted for patients receiving >5000 IU daily LMWH (*n* = 38) were higher TFPI at baseline and higher antithrombin at 3 to 5 days.

3.3 | Levels of tPA and TFPI in relation to pulmonary function and chest CT at 3 months of follow-up

Plasma samples were available from 100 patients at 3 months of follow-up after hospital admission with a mean age of 57.7 ± 13.9 (SD) years and 63 (63%) males compared to HCs, who were 57.2 ± 10.7 years and 13 (62%) males. Supplementary Table S2 compares baseline demographics in patients who attended and did not attend the 3-month follow-up, showing that patients who attended had less severe disease, as reflected by lower frequency of ICU admission/RF and oxygen therapy. In contrast, there were no differences in baseline levels of hemoglobin, CRP, D-dimer, ferritin, total leukocyte counts, neutrophil counts, lymphocyte counts, or eGFR between those who attended the 3-month follow-up and those who did not. Pulmonary function testing and/or chest CT were performed in 90 and 91 of these patients, respectively. We evaluated the relationship between tPA and TFPI levels, during hospitalization and at 3 months of follow-up, and the degree of (i) pulmonary function impairment, (ii) reversible changes on chest CT (thought to reflect inflammation), and (iii) possibly irreversible changes on chest CT (thought to reflect fibrosis). Supplementary Table S3 shows baseline demographics according to

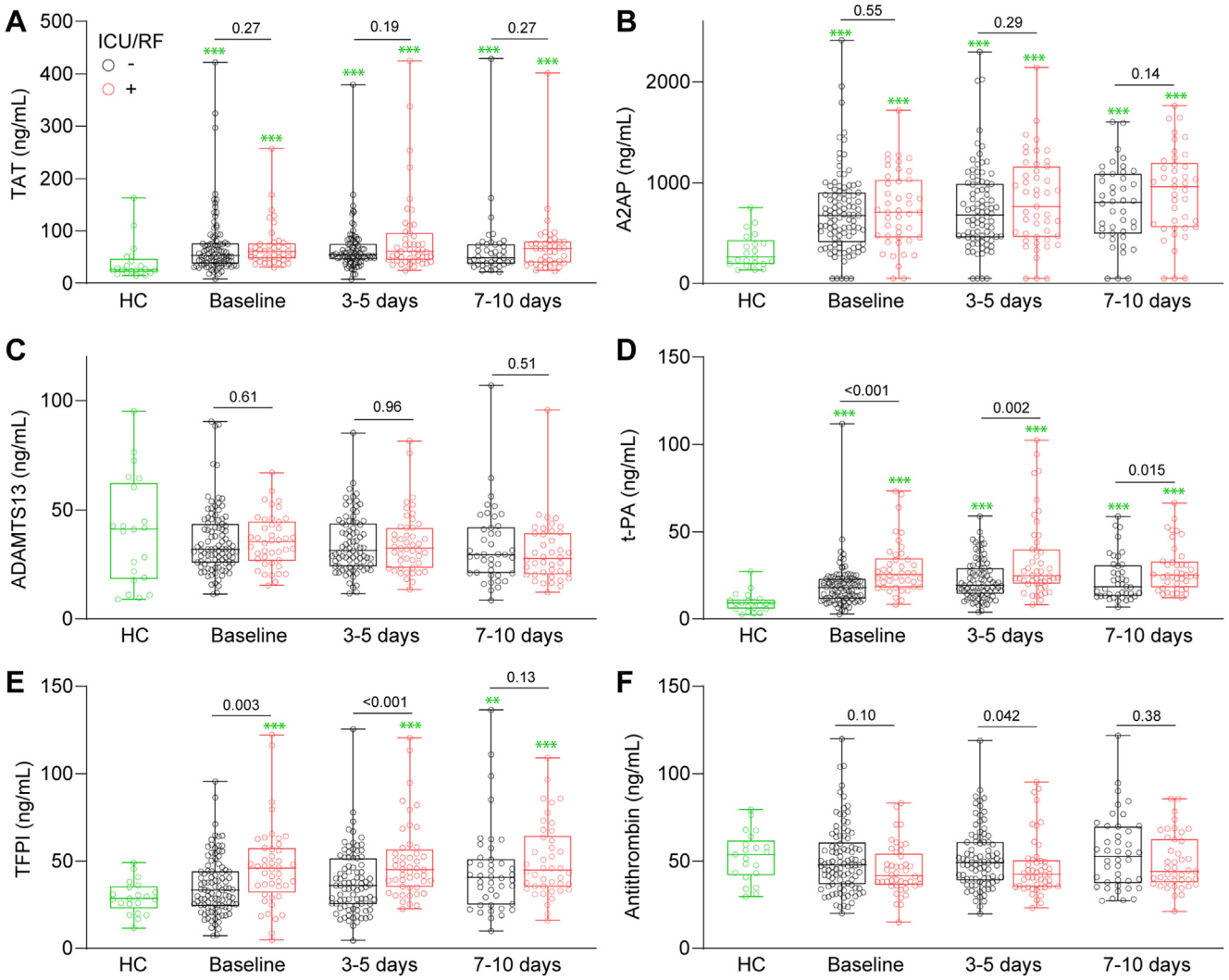


FIGURE 1 Hemostatic markers in relation to severe outcome (intensive care unit [ICU] admission during hospitalization and/or respiratory failure [RF]). Tukey plots (box represents median and 25th/75th percentile) showing the temporal profile of (A) thrombin-antithrombin (TAT) complex, (B) alpha 2-antiplasmin (α2AP), (C) ADAMTS-13, (D) tissue plasminogen activator (tPA), (E) tissue factor pathway inhibitor (TFPI), and (F) antithrombin according to having (red) or not having (black) severe outcome at different time points during the first 10 days after admission. Number of observations in ICU/RF−/ICU/RF+ at baseline (n = 97/44), 3 to 5 days (n = 86/48), and 7 to 10 days (n = 42/42). Twenty-one healthy controls (HCs) are shown in green. All comparisons between groups were performed with the Mann–Whitney U-test, and P values comparing severe outcome groups are given in the graph. Green asterisks are vs HC. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

pulmonary function impairment and total CT changes at 3 months of follow-up. Patients with impaired pulmonary function were older and had had more severe disease (ie, ICU/RF outcome), while patients with CT changes were older with a higher frequency of males.

At 3 months of follow-up, tPA and TFPI levels remained higher compared to those in HCs (Figure 3). Also, tPA levels were modestly higher in patients with irreversible CT changes, while TFPI levels were increased in relation to impaired pulmonary function (ie, DL_{CO} < LLN), reversible, and irreversible CT changes. However, as shown in Supplementary Table S1, TFPI levels at 3 months of follow-up correlated with age, were higher in males, and were associated with lower eGFR, which could explain the higher levels in patients with impaired pulmonary function and CT changes. As for the other markers, as shown in Supplementary Figure S3, no differences in TAT, α2AP, ADAMTS-13, or antithrombin were observed compared to HCs or in relation to pulmonary pathology, except lower α2AP was observed in patients with irreversible CT changes.

Supplementary Figure S4 shows markers during hospitalization in relation to pulmonary pathology at 3 months of follow-up. Levels of TFPI were higher in patients with impaired pulmonary function and, in particular, reversible and irreversible CT changes toward the end of the 10-day hospitalization period. As TFPI was not associated with demographic differences such as age, sex, and kidney function during hospitalization (Supplementary Table S1), these data may suggest a sustained association between high TFPI levels during the acute phase and residual pulmonary pathology at 3 months of follow-up. No

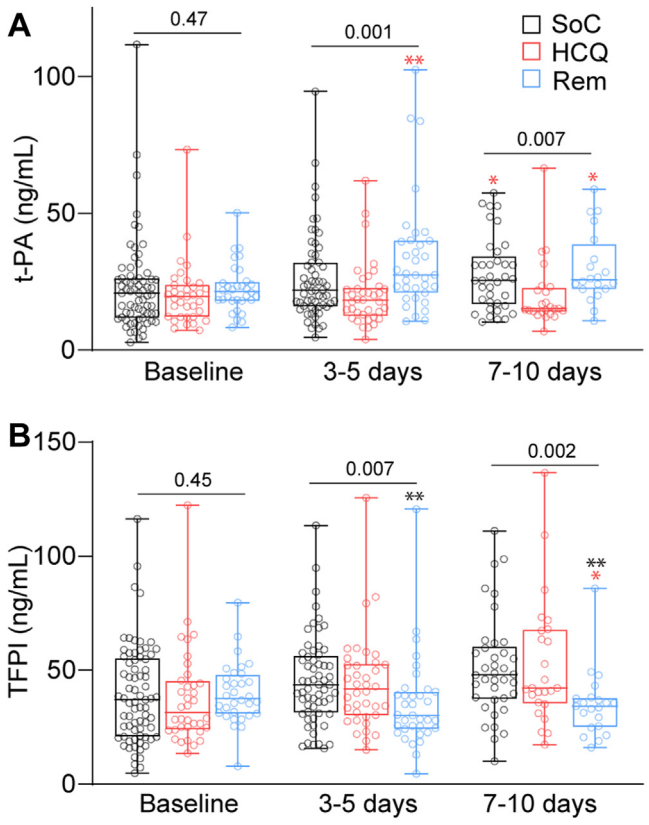


FIGURE 2 Temporal profile of tissue plasminogen activator (tPA) and tissue factor pathway inhibitor (TFPI) in relation to treatment modalities. Tukey plots (box represents median and 25th/75th percentile) showing the temporal profile of (A) tPA and (B) TFPI according to treatment with hydroxychloroquine (HCQ, red) or remdesivir (Rem, blue) compared with standard of care (SoC, black) during the first 10 days after admission. Number of observations in SoC/HCQ/Rem at baseline (n = 73/36/32), 3 to 5 days (n = 61/38/35), and 7 to 10 days (n = 38/25/21). Comparisons between 3 groups were performed with the Kruskal–Wallis H test (P values are given in the graph) and, if significant, between 2 groups with the Mann–Whitney U-test. The color of the asterisks indicates the comparison group and is independent of color. *P < .05, **P < .01, ***P < .001.

changes were observed in the other markers, except tPA, which was higher at 3 to 5 days in patients with impaired pulmonary function.

4 | DISCUSSION

In the present study, we show that TFPI and tPA were associated with severe disease in patients with COVID-19 during hospitalization. Moreover, high TFPI levels during hospitalization were associated with the presence of residual pulmonary pathology as assessed by chest CT 3 months after hospital admission. No changes in TAT complex, α2AP, ADAMTS-13, or antithrombin were observed in relation to severe disease, and no consistent changes in these markers or tPA were observed in relation to pulmonary pathology at 3 months of follow-up.

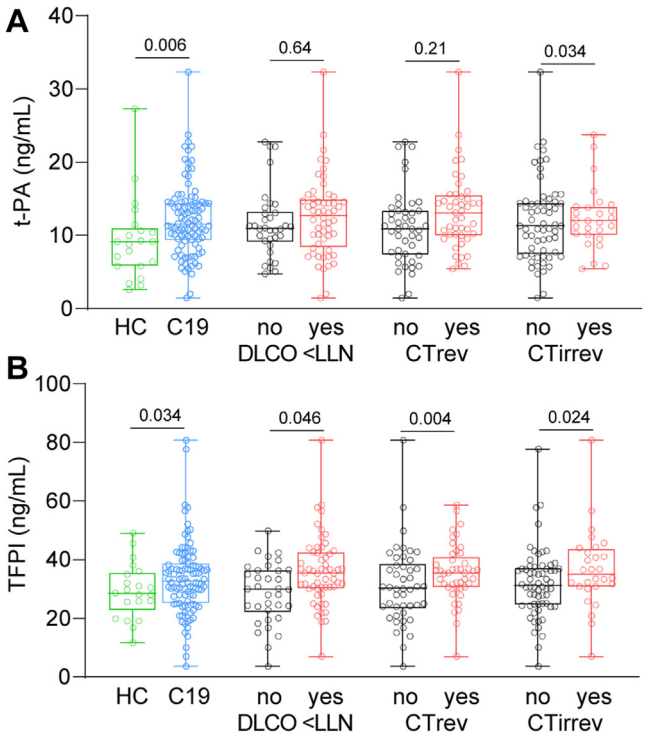


FIGURE 3 Levels of tissue plasminogen activator (tPA) and tissue factor pathway inhibitor (TFPI) in relation to pulmonary function and chest computed tomography (CT) at the 3-month follow-up. Tukey plots (box represents median and 25th/75th percentile) showing the temporal profile of (A) tPA and (B) TFPI in 100 patients (C19) and 21 healthy controls (HCs) at 3 months of follow-up after admission to the hospital and in relation to impaired pulmonary function test (diffusion capacity of the lungs for carbon monoxide (DL_{CO}) < lower limit of normal (LLN), no/yes n = 62/28), reversible CT (CTrev, no/yes n = 34/57), and irreversible CT changes (CTirrev, no/yes n = 46/45). All comparisons between groups were performed with the Mann–Whitney U-test, and P values comparing HCs with patients with COVID-19 or comparing groups with and without pulmonary pathology are given in the graph.

Compared with other coagulation-related variables in patients with COVID-19, data on TFPI expression are scarcer and conflicting. TFPI has been found to be lower [19] as well as higher [20,21] in COVID-19, with some relation to disease severity. Upregulation of TFPI in the lungs of patients with COVID-19 has also been reported [22], although these results have been debated [23]. Herein, we showed that levels of circulating TFPI were associated with severe COVID-19 disease as reflected by RF/ICU admittance. More intriguingly, higher levels measured during the first 10 days of hospitalization were also associated with pulmonary pathology even 3 months after hospital admittance. The reasons for these associations are not clear but could reflect counteracting mechanisms that, despite increased levels, do not manage to fully suppress TF-induced pathology. However, it is established that TFPI has properties beyond prohibiting coagulation and may modulate innate immunity, chemotaxis, angiogenesis, and extracellular matrix remodeling [10,11,24]. It could be hypothesized that such mechanisms may contribute to long-term

pulmonary sequelae of COVID-19. Reports of high levels of TFPI and tPA in chronic pulmonary disorders, such as idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease, may further support this notion [25–29].

Both TFPI and tPA levels have been reported to be independently associated with adverse outcomes during hospitalization of patients with COVID-19 [21,30], while some studies only show differences at discharge [31] or not at all [32]. Pharmacological treatment with tPA has been proposed as a therapeutic option in subgroups of severe COVID-19 disease with life-threatening thromboembolic episodes [33], illustrating that even high tPA levels observed in these patients may not be sufficient to counteract thrombus formation in severe cases. Moreover, similar to TFPI, growing evidence indicates that tPA has effects besides its proteolytic activity and may act as a cytokine to promote receptor-mediated signaling through NF-κB activation [34], leading to fibrosis and extracellular matrix remodeling [35]. Thus, the associations between TFPI and tPA and severe outcome may not merely reflect their effects on coagulation/fibrinolysis.

No clinical effects of remdesivir on viral load in the oropharynx were observed in the NOR-Solidarity substudy or the first publication from the WHO Solidarity trial [15,16]. Further, in the report from the WHO Solidarity trial (meta-analysis), a moderate effect of remdesivir on survival was found in nonventilated patients, but potential harm was also noted in ventilated patients [36]. In the present study, we found increased tPA levels and decreased TFPI levels during hospitalization in patients receiving remdesivir compared with patients treated with HCQ and SoC alone. Whether the net effect of this perturbation is beneficial or harmful (ie, less counteracting mechanisms) is not clear, but it illustrates potential off-target effects of remdesivir, as highlighted by our recent report indicating a suppressive effect on interferon-γ [37].

The association of TFPI and tPA at 3 months of follow-up with abnormal pulmonary testing and chest CT pathology should be interpreted with caution, potentially reflecting confounding factors such as age and disease severity during hospitalization. However, the levels of tPA and TFPI at 3 months of follow-up were also higher than those in HCs, suggesting that the abnormal levels of markers of coagulation/fibrinolysis may persist after the acute phase. Indeed, persistent clotting protein pathology that includes increased levels of antiplasmin, D-dimer, and plasminogen activator inhibitor 1 has been suggested to be part of the long COVID syndrome [38,39]. However, we cannot exclude that these differences may be driven by pre-COVID pathology and not persistent pathology following severe COVID-19.

The present study has some limitations, including a relatively low number of patients, in particular for some subgroup analyses (eg, effects of remdesivir or higher dose LMWH), as well as a limited number of HCs. All-cause mortality (in-hospital) was 5.0% in our study, which was considerably lower than the overall mortality in the WHO Solidarity trial (11.8%), limiting the possibility to evaluate this outcome alone. Moreover, the data were obtained from the first part of the pandemic and may not be representative of perturbations seen for currently circulating SARS-CoV-2 variants. Also, we lack data on the occurrence of thromboembolic episodes, which would be needed to give our findings of higher tPA and TFPI during hospitalization a clinically useful context. Coagulation assays are classically performed in citrate plasma, and our use of EDTA plasma is a limitation, although of less impact, when analyzing antigen levels compared to activity. However, the use of antigen levels vs activity measurements is a further limitation of our study, as activity measurements of tPA and TFPI give more clinically relevant *in vivo* information. Also, we analyzed total TFPI rather than free TFPI, which can be differently regulated [40]. In addition, patients who attended follow-up had less severe disease, potentially underestimating residual pulmonary pathology at 3 months and relation to hemostatic markers. D-dimer was determined at the different centers using 3 different assays, and no strategy was used to harmonize levels, although the 2 major assays used seem to be quite comparable [41]. We did not have complete demographic data on all patients and no data on race/ethnicity, which could influence levels and effects of the measured parameters as it may impact inflammation and coagulation [42,43], and the lack of such data is a limitation to the study. While we use the phrase "associated with" frequently, this does not imply causality. Finally, we explored a large number of associations in a "post hoc" fashion, and these results should be considered exploratory.

Nonetheless, our data suggest that TFPI and tPA are associated with severe outcome in hospitalized patients with COVID-19. For TFPI, high levels measured during the first 10 days of hospitalization were also associated with pulmonary pathology even 3 months after hospital admittance.

FUNDING

This work was supported by the National Program of Clinical Therapy Research in the Specialist Health Services, Norway (grant number 2020201 to the NOR-Solidarity trial).

ETHICS STATEMENT

The study was approved by the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (reference number 118684) and the Norwegian Medicines Agency (20/04950-23). All participants consented to the study before inclusion either themselves or via a legally authorized representative.

AUTHOR CONTRIBUTIONS

T.U., T.B.D., A.M.D.-R., B.H., A.B.-D., and P.A. were responsible for the study conception and execution of the present substudy. T.U., A.A.T., T.K., A.-K.F., A.R.H., A.M.D.-R., A.M., A.B.-D., and M.T. were responsible for data collection and curation. A.M.D.-R., A.B.-D., K.N.H., M.T., and P.A. were responsible for the management, research activity planning, coordination, and execution of the NOR-Solidarity trial. O.H.S. and T.M.A. conducted the 3-month follow-up protocol for pulmonary function and computed tomography scan. A.M.D.-R., T.B.D., A.B.-D., B.H., T.K., A.E.M., and P.A. coordinated the collection and storage of

the biobank material. T.U., A.E.M., T.B.D., and B.H. were responsible for the biochemical analyses. T.U. and P.A. were responsible for writing the original draft. All authors revised and approved the final version of the manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.rpth.2023.102289