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## Full Length Article



# Thromboembolic events in deceased patients with proven SARS-CoV-2 infection: Frequency, characteristics and risk factors

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## ABSTRACT

**Background:** Infection with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) results in respiratory syndromes but also in vascular complications such as thromboembolism (TE). In this regard, immunothrombosis, resulting from inflammation in SARS-CoV-2 infected tissues, has been described. Data on TE in COVID-19 are mainly based on clinical observational and/or incomplete autopsy studies. The true burden of TE and the relevance of genetic predisposition, however, have not been resolved.

**Objectives:** Here, we report on a consecutive cohort of 100 fully autopsied patients deceased by SARS-CoV-2 infections during the first wave of the pandemic (March to April 2020). We investigated the localization of TE, potential clinical risk factors, and the prothrombotic gene mutations, factor V Leiden and prothrombin G20210A, in postmortem blood or tissue samples.

**Results:** TE was found in 43/100 autopsies. 93 % of TE events were venous occlusions, with 23 patients having pulmonary thromboembolism (PT) with or without lower-extremity deep vein thrombosis. Of these, 70 % showed PT restricted to (sub)segmental arteries, consistent with in situ immunothrombosis. Patients with TE had a significantly higher BMI and died more frequently at an intensive care unit. Hereditary thrombophilia factors were not associated with TE.

**Conclusions:** Our autopsy results show that a significant proportion of SARS-CoV-2 infected patients suffer from TE, affecting predominantly the venous system. Orthotopic peripheral PT was the most frequent finding. Hereditary thrombophilia appears not to be a determinant for TE in COVID-19. However, obesity and the need for intensive care increase the risk of TE in these patients.

## 1. Introduction

Since its first isolation in China in late 2019 the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has caused a pandemic with >6.2 million deaths and >520 million infections (as of

16 May 2022) worldwide [1]. Like other human pathologic coronaviruses, SARS-CoV-2 has initially been recognized as a pathogen causing respiratory tract infections, which are referred to as coronavirus disease 2019 (COVID-19) [2]. In the meantime, however, evidence has accumulated according to which COVID-19 is more appropriately described

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as a multiorgan disease [3], which might contribute to the higher mortality rate compared to seasonal influenza. In particular, direct infection of endothelial cells in a variety of microvascular beds has been shown to evoke an endothelial cell activation with massive liberation of thrombogenic mediators, such as coagulation factor VIII or von Willebrand factor [4]. In this regard, earlier case studies on SARS-CoV-2 associated thromboembolism (TE), either venous thromboembolism (VTE), a composite of deep vein thrombosis (DVT) and pulmonary thromboembolism (PT), or arterial thromboembolism (ATE), were followed by more systematic (autoptic) analyses on the frequency of TE in patients with COVID-19 [5,6] (Suppl. Table 1).

With regard to venous thrombotic manifestations in the lungs of deceased COVID-19 patients, embolic vessel occlusions, mainly arising from lower-extremity DVT, must be differentiated from orthotopic thrombus formation. Here, endothelial cell activation results in complex interactions between procoagulant and proinflammatory pathways aimed at containing the spread of invading pathogens, a process referred to as immunothrombosis [7]. A previous study of seven COVID-19 pulmonary autopsies has shown that more than half of the patients suffered from pulmonary thrombi with a diameter of 1–2 mm, and alveolar capillary microthrombi occur nine times more frequent in patients with COVID-19 associated acute respiratory distress syndrome (ARDS) compared to patients with severe influenza A pneumonia [8]. In another postmortem study on eleven COVID-19 patients, orthotopic thrombosis of small and mid-sized pulmonary arteries was confirmed in all cases [9]. Furthermore, Menter and colleagues performed macro- and microscopic analyses of COVID-19 associated deaths and found PT or signs of pulmonary thrombotic microangiopathy in 4/21 and 5/11 patients, respectively [10].

Moreover, elevated plasma levels of D-dimer, a global hemostatic activation marker, have been linked to a more severe course of COVID-19 with higher likelihoods for intensive care unit (ICU) admittance, need of mechanical ventilation, and death [11,12]. A retrospective study from Wuhan, China, indicated that prophylactic anticoagulation is associated with a survival benefit in patients with severe COVID-19 who had either septic coagulopathy or significantly elevated baseline D-dimers [13]. Although national and international clinical practice guidelines and expert recommendations highlight the need for safe and efficacious thromboprophylaxis in hospitalized patients with COVID-19 [14,15], the true burden of TE in patients with proven SARS-CoV-2 infection and the underlying risk factors remain poorly understood [16].

We have previously reported an unexpectedly high rate of VTE in patients dying from COVID-19 [17,18]. Due to their clinical importance, preliminary autopsy findings of these first cases have been published and communicated to the World Health Organization as an international health warning. In this study, we conducted a more detailed analysis of the frequency and characteristics of TE in deceased COVID-19 patients and aimed to identify potential demographic and clinical risk factors. Moreover, we analyzed two common thrombogenic gene mutations, factor V Leiden (FVL) and prothrombin G20210A (PT2020), in available postmortem blood or tissue samples to investigate whether hereditary thrombophilia is associated with TE in this COVID-19 patient population. Considering that efficacious antiviral treatment strategies are still limited, optimizing the prevention and treatment of frequent and potentially severe complications continue to play an important role in reducing COVID-19 morbidity and mortality.

## 2. Methods

### 2.1. Study population, ethical aspects, and data collection

In this study, we included the first 100 consecutive autopsies of patients with proven SARS-CoV-2 infection carried out at the Institute of Legal Medicine at the University Medical Center Hamburg-Eppendorf. Patients had deceased within or outside the hospital setting between March 7 and April 18, 2020.

**Table 1**

Demographic and clinical characteristics of patients deceased with proven SARS-CoV-2 infection.

	Patients (n = 100)
Age (years), median (IQR)	82.9 (75.0–87.9)
Age category (years), no. (%)	
<65	12 (12)
65–75	15 (15)
>75	73 (73)
Gender, no. (%)	
Male	58 (58)
Female	42 (42)
BMI (kg/m <sup>2</sup> ), median (IQR) <sup>a</sup>	23.5 (20.5–29.7)
BMI category (kg/m <sup>2</sup> ), no. (%) <sup>a</sup>	
<25	53 (58.9)
25–30	15 (16.7)
>30	22 (24.4)
Comorbidities, no (%) <sup>b</sup>	
Hypertension	46 (56.1)
Diabetes mellitus	21 (25.6)
Cardiovascular disease	47 (57.3)
Cerebrovascular disease	13 (15.9)
Liver disease	4 (4.9)
Chronic lung disease	11 (12.2)
Chronic kidney disease	17 (20.7)
Nervous system disease	38 (46.3)
Malignancy	14 (17.1)
History of TE	4 (4.9)
Place of death, no. (%)	
Home or nursing home	38 (38)
Normal hospital ward	41 (41)
ICU	21 (21)
Duration of hospitalization (days), median (IQR) <sup>c</sup>	7 (4–12)
Cause of death, no. (%)	
Pulmonary thromboembolism	9 (9)
Viral pneumonia (ARDS)	81 (81)
Cardiac death	5 (5)
Bacterial sepsis	1 (1)
Other <sup>d</sup>	4 (4)
Postmortem diagnosis of thromboembolism, no. (%)	
Patients with any TE	43 (43) <sup>e</sup>
Patients without TE	57 (57)
Antithrombotic medication, no. (%) <sup>b</sup>	
None	29 (35.4)
Antiplatelet medication <sup>f</sup>	28 (34.1)
Oral anticoagulation <sup>f</sup>	24 (29.3)
Heparin <sup>f,g</sup>	1 (1.2)

ARDS, acute respiratory distress syndrome; BMI, body mass index; ICU, intensive care unit; IQR, interquartile range; n.a., not applicable; TE, thromboembolism.

<sup>a</sup> BMI was not available for 10 patients.

<sup>b</sup> Data on comorbidities and antithrombotic medication were not available for 18 patients.

<sup>c</sup> Duration of hospitalization (i.e., from the date of documented SARS-CoV-2 infection until death) was not available for 4/62 patients. In patients with hospital-acquired COVID-19, duration of hospitalization was defined as the time from documented SARS-CoV-2 infection until death.

<sup>d</sup> Other causes of death include Addison's disease (n = 1), hepatorenal syndrome in liver cirrhosis (n = 1), and unknown (n = 2).

<sup>e</sup> In 5/43 patients, TE was already diagnosed before death.

<sup>f</sup> A patient was defined as being on chronic antiplatelet or anticoagulant therapy, when intake of corresponding drugs had started >4 weeks before documentation of SARS-CoV-2 infection.

<sup>g</sup> One patient was on chronic anticoagulation with low-molecular-weight heparin due to history of cancer-associated thrombosis.

Based on the German Infection Protection Act §25(4) the authorities of the German federal state of Hamburg issued full autopsies in all patients dying with a diagnosis of SARS-CoV-2 infection confirmed by polymerase chain reaction (PCR). The Ethics Committee of the Hamburg Chamber of Physicians was informed about the study (no. WF-051/20). The study was approved by the local institutional review board and complied with the Declaration of Helsinki. In all fatalities, a full autopsy with subsequent histological investigation as well as a preparation of

both legs for detection of DVT were performed.

In addition, 47 deceased subjects with an autopsy-proven fatal pneumonia but without SARS-CoV-2 infection were included in the study. However, in these patients, as it is usual practice for autopsies, the legs were only examined in the presence of PT. Cases were identified using a keyword search (“pneumonia”) in the internal digital database of the Institute of Legal Medicine at the University Medical Center Hamburg-Eppendorf, which includes autopsies from 2017 to 2020.

## 2.2. DNA extraction and analysis of FVL and PT20210

DNA was extracted from postmortem blood samples or alternatively from (formalin-fixed) spleen or liver tissues using DNA IQ™ Casework Pro Kits for a Maxwell® 16 instrument (Promega). Formalin-fixed samples were washed in 99 % ethanol for three days, dried, and then ground to a powder using a ball mill (MM2000, Retsch) before being extracted. Human DNA content of all samples was quantified using a PowerQuant™ System (Promega) on an Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific). Genetic analysis for the detection of single-nucleotide mutations in the *F5* (c.1691G>A, factor V Leiden) and *F2* gene (c.\*97G>A, prothrombin G20210A) was performed by real-time polymerase chain reaction (RT-PCR) and fluorogenic target-specific hybridization using the LightMix® kit (TIB MOLBIOL, Berlin, Germany) on a LightCycler® Instrument (Roche, Rotkreuz, Switzerland) following established protocols.

## 2.3. Statistical analysis

Descriptive statistics were used to summarize the data and basic characteristics. Results are presented as count and relative frequency or median and 25–75 % interquartile ranges (IQR), as appropriate. Categorical variables were compared by chi-squared test. Continuous variables were compared by Student's *t*-test (if normally distributed) or by Mann-Whitney *U* test (if not normally distributed). In case of multiple comparisons, the Bonferroni correction was applied. Thereafter, data were evaluated by univariate and multivariate logistic regression analysis. If not otherwise indicated, the significance level was set at  $p < 0.05$ . Statistical analysis was conducted using IBM SPSS Statistics Version 25.0 (IBM Corp., Armonk, NY).

## 3. Results

This study included 100 consecutive patients with PCR-confirmed SARS-CoV-2 infection at the time of autopsy. Patients had deceased between March 7 and April 18, 2020.

Demographic and clinical patient characteristics are summarized in Table 1. Median age of study subjects was 82.9 years, and 58 % were men. Almost every fourth patient (24.4 %) was obese (body mass index [BMI],  $>30 \text{ kg/m}^2$ ). More than half of the patients suffered from hypertension and/or cardiovascular disease. Accordingly, only one third of patients did neither receive chronic antiplatelet nor anticoagulant medication. Almost half of patients showed nervous system disorders such as dementia or Parkinson's disease, and diabetes mellitus was known in one quarter of patients included. Most of the patients (81 %) died from ARDS due to viral pneumonia, and about two thirds of all patients (62 %) deceased inside the hospital. Median duration of hospitalization was one week.

In 43 patients, a postmortem diagnosis of any macroscopic TE, including VTE and ATE, was made. Characteristics of TE events are shown in Table 2. In 40 of 43 patients (93.0 %), a diagnosis of VTE was made. In two of these patients, concomitant ATE was present. The remaining three patients (7.0 %) showed ATE only. Of patients with VTE ( $n = 40$ ), almost 60 % had PT, mostly accompanied by DVT. All four patients with proximal lower-extremity DVT and PT showed bilateral central pulmonary manifestation. A representative finding during lung autopsy of one of these patients is shown in Fig. 1. In all three patients

**Table 2**  
Localization of TE events.

	Number of patients (%)
Any TE	43 (100)
VTE <sup>a</sup>	40 (93.0)
PT	23 (57.5)
PT with lower-extremity DVT	20 (87.0)
PT with proximal DVT ( $\pm$ distal) <sup>b</sup>	4 (20.0)
PT with isolated distal DVT <sup>c</sup>	16 (80.0)
PT without lower-extremity DVT <sup>d</sup>	3 (13.0)
PT in segmental and/or subsegmental arteries only	16 (70.0)
Isolated lower-extremity DVT	15 (37.5)
Proximal DVT ( $\pm$ distal)	1 (6.7)
Isolated distal DVT	14 (93.3)
Bilateral DVT (proximal and/or distal)	12 (80.0)
Isolated thrombosis in other location <sup>e</sup>	2 (5.0)
ATE <sup>f</sup>	3 (7.0)

ATE, arterial thromboembolism; DVT, deep vein thrombosis; PT, pulmonary thromboembolism; TE, thromboembolism; VTE, venous thromboembolism.

<sup>a</sup> Includes 2 patients with concomitant ATE (thrombosis in the carotid and popliteal artery, respectively).

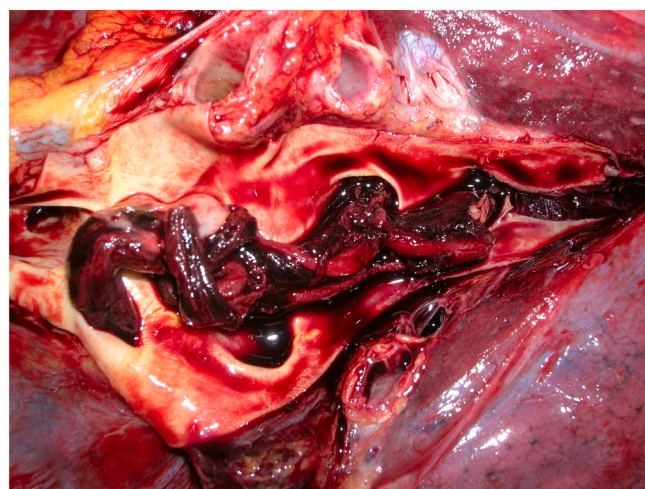
<sup>b</sup> All 4 patients showed bilateral central PT, i.e., thrombotic manifestations in the pulmonary trunk, main pulmonary arteries and/or lobar arteries.

<sup>c</sup> 13 patients had PT in segmental and/or subsegmental arteries only. The remaining 3 patients showed bilateral central PT.

<sup>d</sup> All 3 patients had bilateral PT in segmental and/or subsegmental arteries only.

<sup>e</sup> Thrombosis in the mesenteric vein ( $n = 1$ ) and prostatic veins ( $n = 1$ ).

<sup>f</sup> ATE localized in the coronary, femoral and iliac artery, respectively.



**Fig. 1.** Preparation of the lungs of a patient with bilateral proximal lower-extremity deep vein thrombosis (DVT) showed a massive thrombus in the right pulmonary artery. Due to its convoluted formation, embolism from proximal lower-extremity DVT can be assumed in this patient, rather than orthotopic thrombus formation in the pulmonary vessel.

with exclusive PT, pulmonary thrombi were found in segmental and/or subsegmental arteries only. Fifteen patients (37.5 %) had isolated lower-extremity DVT. Among these, most had blood clots restricted to distal leg veins (93.3 %). Of all patients with DVT ( $n = 35$ ), bilateral thrombosis was evident in 29 patients (82.9 %). In two further patients with VTE (5.0 %), venous thrombosis in the mesenteric vein or the prostatic vascular plexus was the only thrombotic manifestation.

In 5/43 patients, TE was diagnosed before death. Three of these patients suffered from PT, one from proximal DVT and one from mesenteric vein thrombosis. All of these events were symptomatic, one PT led to death. Two patients with PT were treated with systemic thrombolysis; the remaining three patients were treated with heparin. The maximum duration between diagnosis of TE and death was 11 days.



When comparing patients with and without TE, there was no significant difference in age and sex distribution between both groups (Table 3). In contrast, median BMI was significantly increased in the TE compared to the non-TE cohort (25.4 vs 22.0 kg/m<sup>2</sup>,  $p = 0.010$ ), with 32.6 % and 14.0 % of patients having a BMI of >30 kg/m<sup>2</sup>, respectively. Incidence of diabetes mellitus was >2-fold elevated in patients with TE as compared to controls (38.9 vs 15.2 %,  $p = 0.021$ ). According to the Bonferroni method, there were no significant differences in terms of comorbidities or chronic intake of antithrombotic medication. While the proportion of patients that had died outside the hospital was similar in both groups, significantly more patients in the TE than in the non-TE cohort had died on an ICU. In both groups, ARDS due to viral pneumonia was the predominant cause of death. PT was the leading cause of death in 9 of 43 TE patients (20.9 %). Consistent with the comparative analysis between patients with and without TE, patients with classical VTE only, i.e., patients with PT and/or DVT in the absence of concomitant ATE ( $n = 36$ ), showed a significantly increased BMI and died three times more often on an ICU compared to patients without any TE (Suppl. Table 2).

Variables with a  $p$  value <0.05 (as displayed in Table 3) were subjected to a univariate logistic regression analysis to calculate odds ratios (ORs) with corresponding 95 % confidence intervals (CIs) for a post-mortem diagnosis of any TE. Both, obesity (BMI >30 kg/m<sup>2</sup>) and death on an ICU conferred a significantly increased risk, with ORs of 3.21 (95 % CI, 1.18–8.73,  $p = 0.022$ ) and 3.45 (95 % CI, 1.25–9.53,  $p = 0.017$ ), respectively. However, in a multivariate logistic regression analysis including age, sex, BMI and place of death, none of these variables was significantly associated with a postmortem diagnosis of TE (Suppl. Table 3).

Postmortem whole blood for the analysis of thrombogenic gene mutations FVL and PT20210 was available for 54 individuals (Suppl. Fig. 1). In all of these patients, DNA extraction was feasible and RT PCR analysis led to unambiguous results. In 44 other patients, extraction of DNA from preserved organ tissues (spleen in 40 and liver in 4 patients, respectively) was insufficient in quantity and/or quality for gene analysis. Others have previously described similar difficulties in isolation and characterization of postmortem DNA [19]. Exclusive analysis of FVL or PT20210 was possible in 6 patients and 1 patient, respectively, while analysis completely failed for 2 patients. Thus, 77 patients were analyzed for FVL while 72 patients were tested for PT20210 (see Table 4). The prevalence of FVL in the patient cohort was 2.6 % (2/77), with both heterozygous FVL mutations identified in the non-TE cohort. The prevalence of PT20210 in the patient cohort was 1.4 % (1/72), with one patient in the TE cohort being affected by a heterozygous PT20210 mutation. No homozygous or compound heterozygous mutations were detected. There were no statistically significant differences between patients with and those without TE.

We also carried out a descriptive analysis of a cohort of 47 subjects who had died because of non-SARS-CoV-2 pneumonia with dates of death before the pandemic (Suppl. Table 4). Median age of these individuals was 68 years, and 70.2 % of subjects were male. One third of these patients suffered from obesity. Postmortem PT diagnosis was made in 4 patients only (8.5 %), ATE was not diagnosed in any of these cases.

#### 4. Discussion

To the best of our knowledge, we here report on the hitherto largest cohort of consecutive patients with full autopsy (including dissection of the veins of the legs) and proven SARS-CoV-2 infection at the time of death concerning TE events during the first wave of COVID-19. We found a rate of macroscopic TE as high as 43 %, with VTE being the predominant manifestation (40/100). Of patients with VTE, 57.5 % (23/40) had PT with or without lower-extremity DVT, and 37.5 % (15/40) had isolated lower-extremity DVT (Table 2). Our current study thus confirms earlier data from our institution, according to which post-mortem VTE was confirmed in 41 % of cases [18].

**Table 3**  
Comparison of patients with and without postmortem diagnosis of TE.

	Patients with TE (n = 43)	Patients without TE (n = 57)	p value
Age (years), median (IQR)	82.4 (74.9–86.6)	84.2 (75.2–89.0)	0.313
Age category (years), no. (%)			0.351
<65	5 (11.6)	7 (12.3)	
65–75	9 (20.9)	6 (10.5)	
>75	29 (67.4)	44 (77.2)	
Gender, no. (%)			0.210
Male	28 (65.1)	30 (52.6)	
Female	15 (34.9)	27 (47.4)	
BMI (kg/m <sup>2</sup> ), median (IQR) <sup>a</sup>	25.4 (22.5–35.2)	22.0 (19.4–26.8)	<b>0.010</b>
BMI category (kg/m <sup>2</sup> ), no. (%) <sup>a</sup>			<b>0.039</b>
<25	17 (44.7)	36 (69.2)	
25–30	7 (18.4)	8 (15.4)	
>30	14 (36.8)	8 (15.4)	
Comorbidities, no (%) <sup>b</sup>			
Hypertension	21 (58.3)	25 (54.3)	0.824 <sup>c</sup>
Diabetes mellitus	14 (38.9)	7 (15.2)	0.021 <sup>c</sup>
Cardiovascular disease	17 (47.2)	30 (65.2)	0.120 <sup>c</sup>
Cerebrovascular disease	5 (13.9)	8 (17.4)	0.766 <sup>c</sup>
Liver disease	3 (8.3)	1 (2.2)	0.315 <sup>c</sup>
Chronic lung disease	4 (11.1)	6 (13.0)	1.000 <sup>c</sup>
Chronic kidney disease	6 (16.7)	11 (23.9)	0.584 <sup>c</sup>
Nervous system disease	15 (41.7)	23 (40.4)	0.508 <sup>c</sup>
Malignancy	4 (11.1)	10 (21.7)	0.248 <sup>c</sup>
History of TE	3 (8.3)	1 (2.2)	0.315 <sup>c</sup>
Place of death, no. (%)			<b>0.045</b>
Home or nursing home	17 (39.5)	21 (36.9)	
Normal hospital ward	12 (27.9)	29 (50.9)	
ICU	14 (32.6)	7 (12.3)	
Duration of hospitalization (days), median (IQR) <sup>d</sup>	9 (4.0–12.5)	6.5 (3.3–12.0)	0.638
Cause of death, no. (%)			n.a.
Pulmonary thromboembolism	9 (20.9)	0 (0)	
Viral pneumonia (ARDS)	32 (74.4)	49 (86.0)	
Cardiac death	1 (2.3)	4 (7.0)	
Bacterial sepsis	0 (0)	1 (1.8)	
Other <sup>e</sup>	1 (2.3)	3 (5.3)	
Antithrombotic medication, no. (%) <sup>b</sup>			
None	14 (38.9)	15 (32.6)	0.644
Antiplatelet medication <sup>f</sup>	14 (38.9)	14 (30.4)	0.486
Oral anticoagulation <sup>f</sup>	8 (22.2)	16 (34.8)	0.234
Heparin <sup>g,s</sup>	0 (0)	1 (2.2)	1.000

TE patients were compared with non-TE patients by Mann-Whitney  $U$  test for continuous variables and by chi-squared test for categorical variables.  $p$  values <0.05 were defined as statistically significant and were highlighted in bold. ARDS, acute respiratory distress syndrome; BMI, body mass index; ICU, intensive care unit; IQR, interquartile range; n.a., not applicable; TE, thromboembolism.

<sup>a</sup> BMI was not available for 10 patients.

<sup>b</sup> Data on comorbidities and antithrombotic medication were not available for 7/43 and 11/57 patients, respectively.

<sup>c</sup> According to the Bonferroni method, a  $p$  value of <0.005 was considered statistically significant.

<sup>d</sup> Duration of hospitalization (i.e., from the date of documented SARS-CoV-2 infection until death) was not available for 1/26 and 3/36 patients, respectively. In patients with hospital-acquired COVID-19, duration of hospitalization was defined as the time from documented SARS-CoV-2 infection until death.

<sup>e</sup> Other causes of death include Addison's disease ( $n = 1$ ), hepatorenal syndrome in liver cirrhosis ( $n = 1$ ), and unknown ( $n = 2$ ).

<sup>f</sup> A patient was defined as being on chronic antiplatelet or anticoagulant therapy, when intake of corresponding drugs had started >4 weeks before documentation of SARS-CoV-2 infection.

<sup>g</sup> One patient was on chronic anticoagulation with low-molecular-weight heparin due to history of cancer-associated thrombosis.

**Table 4**  
Analysis of common thrombogenic gene mutations.

	All patients (n = 100)	Patients with TE (n = 43)	Patients without TE (n = 57)	p value
Factor V Leiden, no. (%)				
Positive <sup>a</sup>	2 (2.6)	0 (0.0)	2 (4.3)	0.265
Negative	75 (97.4)	29 (100.0)	46 (95.7)	
Not evaluated	23 (n.a.)	14 (n.a.)	9 (n.a.)	
Prothrombin G20210A, no. (%)				
Positive <sup>a</sup>	1 (1.4)	1 (3.8)	0 (0.0)	0.176
Negative	72 (98.6)	25 (96.2)	47 (100.0)	
Not evaluated	27 (n.a.)	17 (n.a.)	10 (n.a.)	

TE patients were compared with non-TE patients by chi-squared test. p values <0.05 were defined as statistically significant.

n.a., not applicable; TE, thromboembolism.

<sup>a</sup> All positive patients had heterozygous factor V Leiden or heterozygous prothrombin G20210A mutation. There were no patients with homozygous or compound heterozygous mutations.

In our current study, we did not systematically investigate lungs or other organ tissues for signs of thrombotic microangiopathy. Instead, the diagnosis of TE was based on gross examination. In this regard, almost two thirds (23/38) of patients with VTE had PT with or without DVT, while only one third had isolated DVT. In contrast, recent clinical trials on the treatment of acute VTE report PT-to-DVT ratios of 1:2 [20]. The increased proportion of PT cases in our cohort supports the hypothesis that orthotopic pulmonary thrombosis explains this percentage switch: 1. The majority of patients with isolated distal lower-extremity DVT showed peripheral PT only. 2. On the contrary, all four patients with proximal lower-extremity DVT showed thrombi in central lung vessels, and due to its convoluted formation it is reasonable to assume “true” pulmonary embolism (PE). 3. In all three patients with exclusive PT, pulmonary thrombi were found solely in (sub)segmental arteries. Consistent with various autopsy studies of COVID-19 patients showing small to mid-sized pulmonary artery thrombosis only (Suppl. Table 1), a recent retrospective observational clinical study of patients with severe respiratory failure due to COVID-19 requiring extracorporeal membrane oxygenation (ECMO) found a large proportion of patients with immunothrombosis, i.e., image-proven segmental and subsegmental filling defects (27 % compared to 5 % in patients requiring ECMO due to influenza) [21]. Thus, both “true” PE (i.e., PE arising from lower-extremity DVT) and massive inflammation contributing to the formation of orthotopic pulmonary macrothrombi seem to be key drivers of morbidity and mortality in COVID-19.

It is important to mention that patients of our COVID-19 cohort all died during the first wave of the pandemic in early 2020 elicited by the ancestral SARS-CoV-2 Wuhan strain. Since different SARS-CoV-2 strains cause variable clinical phenotypes, one cannot draw conclusions regarding the thrombotic burden associated with the currently predominant omicron variant [22]. In this regard, a recent national autopsy registry study showed a decrease in PE as immediate cause of death from the first to the third wave [23]. Moreover, since all patients had been infected during the early pandemic, when evidence for the beneficial effect of disease-modifying drugs was still lacking, no anti-inflammatory treatment with corticosteroids and/or anti-IL-6 receptor monoclonal antibodies was routinely implemented.

Similar to earlier case series [24], ATE was found in a minority of our patients. In three patients (3 % of total patient cohort and 7 % of 43 patients with TE), ATE was the only thromboembolic manifestation at autopsy. Two further patients had concomitant VTE and ATE. In 3/5 patients (60 %) with ATE, thrombosis was restricted to lower limb arteries. Hence, our data are consistent with an earlier retrospective study of 12,630 hospitalized patients with COVID-19, in which ATE was found

in 49 patients (0.4 %), with 71 % suffering from lower-limb thrombosis and 16 % from concomitant VTE [25].

TE was confirmed in 14/21 patients (66.7 %) deceased on an ICU, while TE rates were 29.3 % (12/41) and 44.7 % (17/38) in patients deceased on a normal ward or outside the hospital, respectively (Table 3). Consequently, death on an ICU was associated with a >3-fold increased risk for a diagnosis of TE at autopsy. This finding is consistent with previous studies indicating that critically ill COVID-19 patients are at particularly high risk of experiencing TE [5,26]. Since only a small proportion of patients received an appropriate diagnosis for PT at the time of ICU admission, it is reasonable to consider this correlation as reverse causation: PT as cause of deterioration with negative outcome and not as “result” of ICU treatment. This association notwithstanding, TE rates of 30–65 % appear unacceptably high, considering that German guidelines recommend pharmacological VTE prophylaxis as a standard of care for all patients hospitalized for acute systemic infections, including COVID-19 [14,27,28].

Our study confirms that obesity is an established risk factor for TE. Obesity results in chronic inflammation and has been associated with enhanced platelet activity, hypercoagulability, and impaired fibrinolysis [29]. In this regard, it may be assumed that SARS-CoV-2 aggravates the inflammatory state and thus further increases the risk of TE in general [30]. Our autopsy data support current empirical guidance to consider weight adjusted pharmacological thromboprophylaxis in hospitalized COVID-19 patients with a BMI of >30–35 kg/m<sup>2</sup> [14,28].

In the general population, the prevalence of FVL and PT20210 is 2–7 % and 1–2 %, respectively, with heterozygous carriers having a 4- to 7-fold (FVL) and 2- to 4-fold (PT20210) increased risk of experiencing a first VTE [31]. Albeit limited by a comparatively small sample size, which was further diminished due to difficulties in DNA isolation from formalin-fixed samples [19] (Suppl. Fig. 1), we could not establish an association between postmortem detection of FVL or PT20210 and TE in our study cohort. In a recent nationwide Swedish matched cohort study, the risk of PE was increased about 140-fold and 290-fold in COVID-19 patients admitted to hospital and ICU, respectively [32]. In this light, we therefore conclude that two of the most common hereditary thrombophilic risk factors are of negligible relevance in the pathophysiology of TE in severely ill COVID-19 patients.

Several previous studies have shown that patients with non-COVID-19 pneumonia also have an increased risk of VTE [33]. In our cohort of 47 patients who had died from pneumonia due to other reasons than COVID-19, we found a prevalence of PT of 8.5 % only, as compared to 23 % in the COVID-19 cohort. However, since we cannot comment on the proportion of patients with non-COVID-19 pneumonia who had isolated lower-extremity DVT (legs of these patients had only been prepared in case of PT or sporadically due to other reasons) and who had died on an ICU, and since patients' characteristics differed between our two cohorts (i.e., in terms of age), one cannot draw reliable conclusions from a between group comparison here. A recent meta-analysis evaluated the risk of VTE in COVID-19 and non-COVID-19 patients, including seven studies with a total of >40,000 patients investigated during lifetime mainly based on symptomatic testing [34]. Keeping in mind the limitation of a meta-analysis including retrospective studies, there was no significant difference in VTE risk between both groups, except for the subgroup of patients who were treated on an ICU, when looking on clinical charts. This highlights the necessity of morphological-driven evaluation of clots and VTE in fatalities using consecutive autopsies.

Our study has several limitations. First, we could only include 100 patients deceased with COVID-19. Second, postmortem screening for thrombogenic gene mutations could only be performed in a subset of these cases. Third, although the Bonferroni method was used for multiple statistical calculations, our study only has exploratory character. Fourth, we can comment neither on the specific timing of TE development relative to death nor on accompanying clinical symptoms. Fifth, no systematic medical records on preexisting comorbidities, their durations during lifetime and antithrombotic drugs were available. Finally, due to

the paucity of available data and the heterogeneity of clinical courses following hospitalization, we did not consider laboratory findings and specific pharmacological or invasive interventions in close proximity to death for our analysis.

Despite these limitations, our study confirms a significant burden of TE in SARS-CoV-2 associated deaths and highlights that the risk of TE is particularly high in obese and ICU patients.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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