Elevated factor V activity and antigen levels in patients with Covid-19 are related to disease severity and 30-day mortality

To the Editor:
We were very interested to read the recent manuscript by Stefely and coworkers who reported markedly increased factor V (FV) activity levels in critically ill patients with Covid-19.1 In this report, high FV activity levels were shown to be associated with thrombotic events, whereas declining levels were associated with poor outcome. A paper by Voicu and coworkers confirmed elevated FV activity levels in critically ill Covid-19 patients.2 We have recently reported on the hemostatic profile of a Covid-19 patient cohort.3 These patients had much milder disease in comparison to the cohort studied by Stefely. We were interested in investigating if elevated FV activity levels also occur in patients with milder disease. In addition, we wondered if the increase in FV activity reflects an increase in FV protein (antigen) level or whether these unusually elevated FV activity levels could be explained by an increase in specific activity rather than an increase in FV antigen.

Patient characteristics have been described previously.3 In short, we included consecutive patients with Covid-19 in a single hospital in Sweden, and drew blood within 7 days of hospital admission. We measured FV activity using an automated coagulation analyzer (STACcompact 3, Stago, Breda, the Netherlands) and FV antigen using a commercially available enzyme-linked immunosorbent assay (Stago, Breda, The Netherlands) in platelet poor plasma of 97 patients with Covid-19 and 28 healthy controls. Both FV activity and antigen were higher in Covid-19 patients compared to controls, although the difference in antigen levels did not reach statistical significance (p = .072). Patients on medium or intensive care units had higher FV antigen levels compared to patients on general wards (Table 1). The FV activity levels were similar when patients were stratified according to their level of respiratory support, but FV antigen levels were higher in those patients with a higher level of respiratory support, although this difference did not reach statistical significance (Table 1). The FV activity and antigen levels were lower in those patients that died within 30 days of admission (Table 1). The specific FV activity (ie, the FV activity to antigen ratio) was similar between patients and controls, although patients admitted to higher levels of care had decreased specific activity (healthy controls vs high care patients p = .013). The correlation (performed by simple linear regression) between FV antigen and activity was less pronounced in patients (r² = 0.10, p < .001) compared to controls (r² = 0.20, p = .028).

Our data confirm and extend data by Stefely and coworkers. Hospitalized patients with Covid-19 have elevated FV activity levels, with more pronounced increases in patients receiving higher levels of care. Nevertheless, our data show that even those patients admitted to general wards and patients that do not require respiratory support have elevated FV activity levels. Interestingly, we find decreased FV activity levels in the first week of admission in patients that died within 30 days of admission, which is also in line with the observation of Stefely and coworkers that declining FV activity levels appear associated with a poor prognosis. No thrombotic events occurred in our cohort during a 30-day follow up, and we were therefore unable to confirm the findings by Stefely of elevated FV activity levels as a risk factor for Covid-19-associated venous thrombosis. Elevated levels of FV activity were in part related to increased FV antigen, although the correlation between FV activity and antigen was modest in both patients and controls. Besides fibrinogen, FV is the only liver-derived coagulation factor that shows elevated levels in Covid-19 patients, and the reason for this selective FV increase remains unclear. Part of the FV in patients with Covid-19 may be released from platelets, which are known to be activated in Covid-19.4 Megakaryocytes endocytose factor V from plasma, and proteolytic modification of FV results in a factor V molecule with procoagulant properties that are distinct from and more thrombogenic than plasma FV.5 The more pronounced increase in FV activity compared to FV antigen in patients, suggests FV in patients with Covid-19 to be hyperactive, but the specific activity of FV was not different between patients and controls. Interestingly, the specific activity of FV was slightly (but not significantly) higher in patients on general wards compared to controls, whereas in patients receiving higher levels of care, the specific activity of FV was decreased compared to controls. We thus hypothesize that part of the FV in patients with Covid-19 is platelet-derived, but is in part inactivated in sicker patients.

In conclusion, FV displays unusual behavior in patients with Covid-19 and we concur with Stefely and coworkers that studies on the value of FV as thrombosis biomarker and prognostic indicator are warranted.

CONFLICT OF INTEREST
The authors have no conflicts of interest to disclose.
TABLE 1  Factor V activity and antigen levels in Covid-19 patients related to severity of disease and mortality

|                     | Factor V activity (%) | Factor V antigen (%) | Factor V specific activitya |
|---------------------|-----------------------|----------------------|-----------------------------|
| Healthy controls (n = 28) | 107 [84–124]         | 81 [66–98]           | 1.26 [1.12–1.48]            |
| Covid-19 patients (n = 97) | 125 [102–145]         | 93 [67–137]           | 1.33 [0.87–1.86]            |
| P-value             | .002                  | .072                 | .868                        |

Covid-19 patients stratified by location

|                     | Factor V activity (%) | Factor V antigen (%) | Factor V specific activitya |
|---------------------|-----------------------|----------------------|-----------------------------|
| General ward (n = 85) | 124 [100–144]         | 92 [67–126]           | 1.39 [0.98–1.91]            |
| High careb (n = 12) | 142 [124–149]         | 158 [106–184]         | 0.93 [0.66–1.16]            |
| P value             | .103                  | .007                 | .007                        |

Covid-19 patients stratified by level of respiratory support

|                     | Factor V activity (%) | Factor V antigen (%) | Factor V specific activitya |
|---------------------|-----------------------|----------------------|-----------------------------|
| No respiratory support (n = 36) | 124 [103–141]         | 92 [63–126]           | 1.37 [0.94–1.91]            |
| Nasal cannula/mask ≤5 Liter O2 (n = 45) | 125 [96–148]         | 92 [68–128]           | 1.39 [0.98–1.89]            |
| Higher respiratory supportc (n = 17) | 128 [103–150]         | 126 [61–183]          | 1.16 [0.83–1.60]            |
| P value             | .472                  | .173                 | .446                        |

Covid-19 patients stratified by 30-day survival

|                     | Factor V activity (%) | Factor V antigen (%) | Factor V specific activitya |
|---------------------|-----------------------|----------------------|-----------------------------|
| Survivors (n = 87)  | 126 [61–183]          | 97 [68–130]          | 1.35 [0.92–1.89]            |
| Non-survivors (n = 10) | 97 [78–123]          | 59 [51–166]          | 1.32 [0.86–1.77]            |
| P value             | .030                  | .380                 | .752                        |

Note: The results are presented as median [interquartile range]. Comparisons were made using the Mann–Whitney U test or Kruskal-Wallis test, as appropriate. P values <.05 were considered statistically significant.

aFactor V specific activity is defined as the FV activity to antigen ratio.
bThree patients were admitted to the intensive care unit and nine patients were admitted to the intermediate care unit.
cRespiratory support in this group comprised >5 L O2 by nasal cannula/mask (n = 13), non-invasive ventilation (n = 2), and intubation (n = 2).

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ETHICS APPROVAL AND PATIENT CONSENT STATEMENT
The study complied with the declaration of Helsinki, and informed consent was obtained from all healthy individuals and patients, or in the case of incapacity, their next-of-kin. The protocol was approved by the Stockholm Ethical Review Board (COMMUNITY study dnr 2020–01653).

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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REFERENCES
1. Stefely JA, Christensen BB, Gogakos T, et al. Marked factor V activity elevation in severe COVID-19 is associated with venous thromboembolism. Am J Hematol. 2020;95:1522–1530.
2. Voicu S, Delrue M, Chousterman BG, et al. Imbalance between procoagulant factors and natural coagulation inhibitors contributes to hypercoagulability in the critically ill COVID-19 patient: clinical implications. Eur Rev Med Pharmacol Sci. 2020;24:9161–9168.
Sudden death due to massive bone marrow sequestration crisis in a patient with sickle cell disease

To the Editor:

Sickle cell disease (SCD) is a severe hemoglobinopathy characterized by an increased susceptibility to infections, chronic hemolytic anemia and continuous micro-vascular occlusion with acute exacerbations leading to accumulating organ damage and a decreased life expectancy. One of the most acutely life-threatening complications is the splenic sequestration crisis. Sluggish blood flow through splenic sinusoïds with low oxygen tension and low pH favor the sickling process. Acute splenic pooling of blood can lead to hypovolemic shock and death. This occurs mostly in children with sickle cell anemia (HbSS) prior to the occurrence of splenic infarction with ensuing atrophy. In sickle cell patients with compound heterozygous states or hereditary persistence of fetal hemoglobin (HPFH) (or drug induced elevated fetal hemoglobin [HbF]) the spleen remains anatomically intact and thus these patients remain at risk for this complication. Chronic sequestration of sickle erythrocytes can occur in the liver, leading to hepatomegaly which can also, in severe forms, lead to rapid liver enlargement, extreme jaundice and circulatory collapse. Here we present, to our knowledge, the first case of sudden death in a sickle cell patient due to massive sequestration of blood in the bone marrow.

A 19-year-old female with sickle cell anemia was admitted to our hospital with a painful crisis. Her high-performance liquid chromatography result was compatible with HPFH (HbF of 29.2%, a normal HbA2 and the remaining hemoglobin constituting HbS). She rarely suffered painful crises and experienced no other specific complications. Several weeks earlier she was diagnosed with IgG4-related hepatitis. She had persistently elevated ALAT and ASAT levels with normal liver function tests. Viral serology, ANA screen, anti-mitochondrial antibodies were all negative, with normal serum ceruloplasmin and antitrypsin concentrations. Ultrasound revealed a normal sized liver and spleen (respective length, width and height being 6.3 x 3.9 x 6.7 in and 2.3 x 3.2 x 3.7 in). Serum IgG4 levels were increased (7.16 g/L, ULN 1.40). A liver biopsy revealed marked chronic inflammation and fibrosis grade 2. She started oral corticosteroids (1 mg/kg) with rapid normalization of ALAT and ASAT concentrations. At admission she was on folic acid and a tapering dose of prednisone (40 mg daily) which were continued.

She had pain in both upper arms and lower back, recognizable as that of a painful crisis. Vital signs were normal (BP 118/65 mmHg, pulse rate 88/minute, oxygen saturation on room air 99%, temperature 98.1 °F) and there was no organomegaly. Her weight, length and BMI were 128 lb, 5.1 ft and 24 kg/m², respectively. Laboratory studies at admission (day 1) are shown in the Supplementary table 1. Kidney function and electrolytes were normal. She received parenteral hydration with 3 liters of saline (0.45%) per 24 hours, acetaminophen and morphine. On the 3rd day she was almost free of pain, blood work revealed no unexpected findings (Supplementary Table 1), and discharge was planned for the next day. In the morning of day 4 she felt nauseated and went to the bathroom where she vomited once. She was hypotensive (BP 77/56 mmHg, pulse rate 102/minute, respiration rate 16/minute, oxygen saturation on room air 99%, temperature 97.5 °F), erroneously interpreted by the attending nurse as a vagal reaction to vomiting and she was guided back to her bed. She was found unresponsive shortly thereafter and immediate cardiopulmonary resuscitation (CPR) was started. She was in asystole. There was no indication of pneumothorax, no organomegaly, capillary blood glucose excluded hypoglycemia. Despite absence of right heart strain on cardiac ultrasound the thrombolytic agent, alteplase was administered with a working diagnosis of pulmonary embolism. After several attempts finally blood could be drawn via the femoral artery and arterial blood gas analysis revealed severe lactic acidosis and severe anemia (Supplementary Table 1). CPR was stopped after 74 minutes, and she was pronounced dead. Permission for autopsy was granted.

Macroscopic examination ruled out acute myocardial infarction, pulmonary thromboembolism and stroke. The most striking feature was that during the organ dissection there was hardly any blood within the blood vessels, as seen in cases of complete exsanguination. There was no blood in the gastro-intestinal tract. For this reason, forensic pathology was consulted. There were no findings suggesting external or internal blood vessel trauma or hematoma. The respective weights of liver and spleen were 3.35 and 0.49 lb, ruling out massive acute splenic and/or hepatic sequestration of blood as the cause of the almost empty vascular bed. The weight of the lungs was normal. Microscopic findings of the liver and spleen revealed red cell entrapment (Figure 1A,B) without red cell entrapment in the lungs (Figure 1C). Examination of a lumbar vertebra revealed massive entrapment of sickled red blood cells (Figure 1D). Based on these findings it seems that circulatory arrest occurred largely due to acute blood sequestration in the bone marrow.

A bone marrow sequestration crisis has not, to our knowledge, been previously described. Normal bone marrow histology shows at