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Pro-coagulant imbalance in patients with community acquired pneumonia assessed on admission and one month after hospital discharge

https://doi.org/10.1515/cclm-2021-0538
Received May 6, 2021; accepted June 7, 2021;
published online July 1, 2021

Abstract

Objectives: Patients hospitalized because of community-acquired-pneumonia (CAP) are at risk of cardiovascular diseases. Although plasma procoagulant imbalance play a role, mechanisms are not completely understood. We aimed to investigate whether there is a measurable state of procoagulant imbalance following inflammation determined by CAP.

Methods: We analyzed blood from 51 CAP patients at admission and 51 healthy subjects (HS) for (i) pro and anticoagulants, (ii) thrombin generation (TG) with or without thrombomodulin (TM), which is the physiologic activator of the protein C anticoagulant pathway and(iii) by assessing the ratio between von Willebrand-factor (VWF) and its protease ADAMTS13. Thirty patients were re-analyzed one month after discharge when CAP was resolved.

Results: Median levels of TG parameters, including the endogenous thrombin potential (ETP), the ETP-TM-ratio (with/without TM), peak-thrombin and velocity index were higher in patients at baseline than HS. In particular, the median (IQR) ETP-TM-ratio in patients vs. HS was 0.88 (0.83–0.91) vs. 0.63 (0.48–0.71), p<0.001. Factor (F)VIII, a potent procoagulant involved in TG was higher in patients at baseline than HS [195 U/dL (100–388) vs. 127(108–145)], p<0.001]. The ratio of VWF/ADAMTS13 was higher at baseline than HS. Cumulatively, the findings indicate a state of pro-coagulant imbalance, which (although reduced), remained high [i.e., ETP-TM-ratio, 0.80 (0.74–0.84); FVIII, 152 U/dL (122–190)] one month after discharge when the infection was resolved.

Conclusions: Patients with CAP possess a state of pro-coagulant imbalance, which remains substantially high, even when the infection is resolved. The findings suggest CAP patients as candidates for antithrombotic prophylaxis even after the resolution of infection. Clinical trials are warranted to assess the benefit/risk ratio of prophylaxis extension.

Keywords: ADAMTS13; endothelial cells; factor VIII; respiratory infections; thrombin generation; von Willebrand factor.

Introduction

Blood coagulation is a tightly regulated mechanism triggered by the formation of the complex between tissue factor (TF) and activated factor (F)VII which leads to thrombin generation and conversion of fibrinogen to fibrin. There are *in vivo* mechanisms that in normal conditions limit excessive thrombin formation that work mainly but not exclusively through the balance between pro- and anticoagulants. Platelets and monocytes play in fact a regulatory role in physiologic conditions and endothelial cells, platelets, and leucocytes play their role in pathological conditions. For example, activated endothelial cells, platelets and monocytes may produce TF and procoagulant microparticles [1–3], which are disseminated into the circulation and contribute to thrombin production. Finally, activated neutrophils may release their nucleic

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content, composed of chromatin substances (e.g., DNA, histones, myeloperoxidase and elastase), collectively known as neutrophil external traps (NETs) [4]. NETs, which are commonly found in patients with inflammation and respiratory diseases [5] can trigger coagulation leading to thrombin production [6], which may result in fibrin deposition and vessel occlusion, as shown by animal models [7] or studies in humans [8–11]. The excessive production of thrombin following one or more of the above events leads to procoagulant imbalance (i.e., hypercoagulability), which is one of the Virchow's triad that, alone or in combination with the other two (i.e., decreased blood flow and endothelial perturbation), may trigger cardiovascular diseases such as venous thromboembolism (VTE). Endothelial perturbation can be investigated by measuring von Willebrand factor (VWF), which is normally stored in endothelial cells and is released into the systemic circulation when endothelial perturbation occurs [12]. Coagulation on the other hand, has been historically assessed by the traditional global tests prothrombin and activated partial thromboplastin times (PT, APTT). Other laboratory tools to investigate coagulation and hypercoagulability are the measurement of individual pro- and anticoagulants. However, neither of the above are completely suitable to represent the process that presumably occurs *in vivo* [13]. For example, PT/APTT are static tests, which are responsive to the procoagulants but not to the anticoagulant counterpart. On the other hand, the measurement of the individual pro- or anticoagulants when taken in isolation are not representative of the balance between the two players that occurs *in vivo*. The above limitations hampered for many years the investigation of coagulation in clinical conditions characterized by acquired coagulopathy, due to the concomitant reduction/increase of the components of the hemostatic system. One of such conditions is the inflammatory state following infections that has for long time been associated with the risk of cardiovascular diseases [14–17]. In this study, we elected community-acquired pneumonia (CAP) as a model of infection to be investigated for hypercoagulability and endothelial perturbation at the time hospitalization and one month after discharge by means of last generation hemostasis procedures.

Materials and methods

Study subjects

Consecutive patients with symptoms of acute respiratory failure referred to the emergency department (IRCCS Maggiore Hospital, Milan, Italy) were evaluated by the attending physicians. The evaluation was pragmatically made on clinical criteria [18] based on the presence of raised respiratory rate (>25 breaths/min), low blood pressure and age. Suspected pneumonia was confirmed by chest radiography [19] or CT scan (when required) and patients were admitted to the Divisione Medicina Generale Alta Intensità di Cura (IRCCS Maggiore Hospital) and enrolled in this observational case-control study. Patients underwent transthoracic ultrasound at both admission and one month after discharge. The enrollment started on December 2018 and terminated on December 2019 well before the COVID-19 outbreak in Northern Italy (i.e., February 2020). The study was approved the local Ethics Committee. Upon informed consent blood was collected into vacuum tubes (Vacutainer, Becton Dickinson, Plymouth, UK) containing 1/10 volumes of trisodium citrate 0.109 M; plasma obtained after centrifugation for 20 min at 3,000*g* was harvested, aliquoted, snap frozen by immersion in liquid nitrogen and stored at −70° until testing. One month after discharge, patients were recalled, and an additional blood sample was collected and processed as above. Healthy subjects (HS) were enrolled in the study and tested along with patients' samples. HS were randomly selected from a population of healthy volunteers and laboratory staff, free from respiratory infections, personal or family history of hemorrhagic or thrombotic diseases based on validated questionnaire [20]. Patients and HS at the time of blood sampling were free from anticoagulants, including heparin, vitamin K antagonists or direct oral anticoagulants.

Methods

Biochemical parameters: Biochemical parameters required for patient's management were obtained from the hospital records.

Thrombin generation (TG): TG was performed by a homemade method, according to Hemker et al. [21] as described [22]. Coagulation in the test plasma was triggered by small amounts of TF (RecombiplasTin 2G, Werfen, Orangeburg, NY, USA) at a concentration of 1pM and a blend (1:1:1 phosphatidylcholine, phospatidylethanolamine and phosphatidylserine) of 1 μM synthetic phospholipids (Avanti Polar, Alabaster, AL) in the presence or absence of 2 nM rabbit thrombomodulin (TM) (Hematologic Technologies, Essex, VT, USA). The generated thrombin was continuously monitored with a fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem, Bubendorf, Switzerland) (617 μM) by a fluorometer (Fluoroskan Ascent®, ThermoLabsystem, Helsinki, Finland). Signals from the fluorometer were recorded and analyzed with a dedicated software (ThrombinoscopeTM, Thrombinoscope BV, Maastricht, Netherlands), which displays the TG curve and calculates the following parameters. The lag-time, defined as the time (minutes) between the addition of triggers and the initiation of TG. The thrombin peak (nM). The time needed to reach the peak (minutes). The area under the curve, defined as endogenous thrombin potential (ETP) (nM × min). ETP represents the net amount of thrombin that can be generated under the experimental conditions and under the driving forces of the procoagulants opposed by the anticoagulants operating in plasma. The velocity index, defined as [Peak/(time-to-peak − lag time)] (nM/min) represents the velocity of thrombin generation. Results were also expressed as ETP-TM ratio, i.e., the ratio of ETP measured in the presence of TM to the ETP measured in its absence. The ETP-TM ratio represents the resistance to the anticoagulant action of TM and depends mainly on the ratio of FVIII to protein C.

It is considered as an index of hypercoagulability; the higher the ETP-TM ratio the greater the hypercoagulability [23].

To minimize the analytical variability, appropriate numbers of samples from HS, patients at baseline and one month from discharge were analyzed within the same working session.

Other parameters: Fibrinogen was measured according to the Clauss method (Werfen). PT and APTT were measured by recombinant tissue factor (RecombiplasTin 2G, Werfen) and SynthASil APTT (Werfen) and results expressed as ratio (patient-to-normal clotting time). FVIII and FII activities were measured with one-stage clotting assays. Antithrombin and protein C, were measured by specific chromogenic assays (Werfen). VWF antigen was measured by a homemade ELISA and the VWF-cleaving plasma protease ADAMTS13 (a Disintegrin and Metalloprotease with ThromboSpondin 1 repeats, number 13) was measured by the FRETS-VWF73 activity assay as previously described [24]. Results for the above parameters were expressed as U/dL with reference to a pooled normal plasma arbitrarily assigned the potency of 100 U/dL. Myeloperoxidase and elastase were measured by ELISA methods according to the respective manufacturers' specifications (Cayman Chemical, Ann Arbor, MI; Elabscience, Houston, TX, USA).

Data analyses

Results are reported as median and interquartile range (IQR) defined as the interval from the 25th to the 75th centiles of results distribution and analyzed with non-parametric tests. Briefly, median values for each of the investigated parameters for patients at baseline were compared with the HS values with the Mann Whitney test (non-paired data) and median values for patients at baseline were compared with those obtained for the same subjects one month from discharge with the Wilcoxon test (paired data). p-Values <0.05 were considered as statistically significant. Analyses were performed with the SPSS statistical package (SPSS, IL).

Results

Fifty-one patients diagnosed with CAP were available for the laboratory investigation. Twenty-five (49%) were males and the median (IQR) age was 70 years (49–80). Arterial hypertension, diabetes or dyslipidemia was present in 46%, 12 or 24%, respectively. Of the 51 patients investigated at baseline, plasma samples were available for 30 after one month from hospital discharge. Plasma samples from 51 HS [24 (47%) females; median (IQR) age of 58 years (50–65)] were concomitantly tested with the patients' plasma for all parameters, except VWF, ADAMTS13 and C-reactive protein.

Conventional coagulation parameters

PT and FII, although significantly different for all comparisons were still within the reference range (Table 1). Conversely, FVIII levels were significantly higher in patients at baseline than HS ($p<0.001$); at one month from discharge, they decreased ($p<0.001$) but did not reach levels of HS ($p<0.05$) (Table 1). Antithrombin and protein C were significantly lower in patients at baseline than HS ($p<0.001$) and reached levels of HS one month after discharge (Table 1). Patients at baseline had median FVIII/protein C ratio higher than HS ($p<0.001$); at one month from discharge, this ratio was reduced ($p<0.001$) but was still higher than those of HS [i.e., 1.37 (0.97–1.73) vs. 1.15 (0.89–1.35), $p<0.05$] (Table 1 and Figure 1).

Myeloperoxidase and elastase

The median values of the two parameters representing neutrophil activation were higher in patients at baseline than HS [96 ng/mL (64–121) vs. 26 ng/mL (16–26), $p<0.001$] myeloperoxidase; [180 ng/mL (84–329) vs. 49 ng/mL (33–67), $p<0.001$] elastase. Both decreased one month from discharge [41 ng/mL (30–57) myeloperoxidase and 87 ng/mL (53–123) elastase, $p<0.001$] but remained substantially higher than HS ($p<0.05$) (Table 1).

Inflammatory parameters

Fibrinogen levels were significantly higher in patients at baseline than HS ($p<0.001$); one month after discharge they decreased but did not reach HS levels ($p<0.001$) (Table 1). Patients at baseline had median levels of C-reactive protein [i.e., 17 mg/dL (11.2–31.8)], which was significantly higher when compared to the upper limit of the reference range; results at one month from discharge were available only for 13 patients, and the median value [i.e., 1.1 (0.6–2.0) mg/dL] was significantly different from that of the patients at baseline ($p<0.01$) (Table 1).

Von Willebrand factor (VWF) and ADAMTS13

The median VWF value at baseline was 283 U/dL (211–364) and 100% of the patients had levels which were higher than the mean value of the laboratory reference range (i.e., 108 U/dL); the median value one month from discharge decreased to some extent [i.e., 145 (109–197), $p<0.001$] but remained higher than the mean value of the laboratory reference range in 77% of the patients.

The median ADAMTS13 at baseline was 56 U/dL (43–78) and 96% of the patients had levels which were smaller than the mean value of the reference range (i.e., 91 U/dL);

Table 1: Median (IQR) values of parameters of coagulation, neutrophil activation, endothelial perturbation and inflammation for healthy subjects and patients at baseline and one month after discharge (post-discharge).

Parameter	Healthy subjects (n=51)	Patients				
		Baseline (n=51)	p-Value[a]	Post-discharge (n=30)[d]	p-Value[b]	p-Value[c]
PT-ratio	0.93 (0.90–0.97)	1.10 (1.01–1.21)	<0.001	0.97 (0.92–1.03)	<0.001	<0.05
APTT-ratio	0.98 (0.93–1.03)	0.99 (0.93–1.07)	N.S.	1.03 (0.97–1.08)	N.S.	<0.05
Factor II, U/dL	102 (98–115)	93 (45–132)	<0.001	103 (91–115)	0.01	N.S.
Factor VIII, U/dL	127 (108–145)	195 (100–388)	<0.001	152 (122–190)	<0.001	<0.05
Antithrombin, U/dL	106 (98–114)	92 (80–103)	<0.001	105 (95–122)	<0.001	N.S.
Protein C, U/dL	114 (104–126)	93 (69–113)	<0.001	114 (98–140)	<0.01	N.S.
Factor VIII/protein C ratio	1.15 (0.89–1.35)	2.20 (1.74–2.87)	<0.001	1.37 (0.97–1.73)	<0.001	<0.05
Myeloperoxidase ng/mL	26 (16–26)	96 (64–121)	<0.001	41 (30–57)	<0.001	<0.001
Elastase ng/mL	49 (33–67)	180 (84–329)	<0.001	87 (53–123)	<0.001	<0.05
Fibrinogen, mg/dL	280 (246–313)	511 (387–600)	<0.001	340 (305–424)	<0.001	<0.001
C-reactive protein, mg/L	NA	17 (11.2–31.8)	NA	1.1 (0.6–2.0)[e]	<0.01	NA
VWF, U/dL	NA	283 (211–364)	NA	145 (109–197)	<0.001	NA
ADAMTS13, U/dL	NA	56 (43–78)	NA	81 (70–91)	<0.001	NA
VWF/ADAMTS13 ratio	NA	4.34 (3.16–6.81)	NA	1.90 (1.22–2.66)	<0.001	NA

PT-ratio, prothrombin time ratio (patient-to-normal); APTT-ratio, activated partial thromboplastin time ratio (patient-to-normal; ADAMTS13, A Disintegrin And Metalloprotease with ThromboSpondin 1 repeats, number 13.[a]Patients at baseline vs. controls. [b]Patients at baseline vs. post-discharge. [c]Patients at post-discharge vs. controls. [d]Number of patients for whom baseline, and post-discharge paired samples were available for analyses.[e]C-reactive protein results at post-discharge were available for 13 patients. N.S., not statistically significant

the median value at one month from discharge increased to some extent [i.e., 81 (70–91)], p<0.001, but remained smaller than the mean value of the reference range in 77% of the patients.

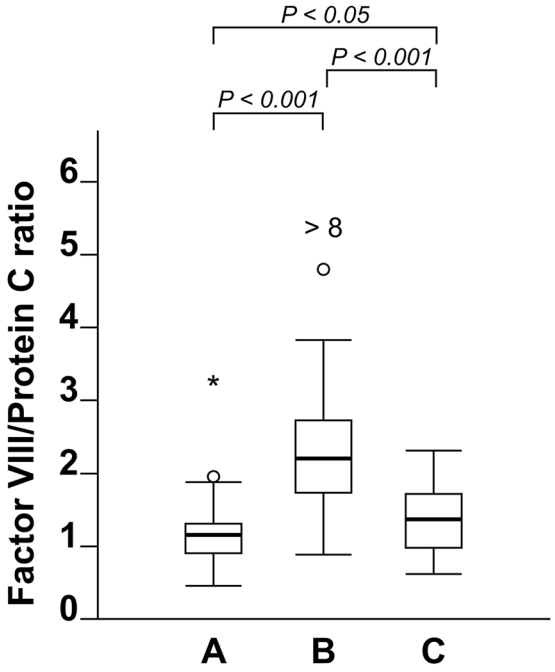

Figure 1: Box plots of factor VIII/protein C ratio for healthy subjects (A), patients at baseline (B) and one month after discharge (C).

The median VWF/ADAMTS13 ratio which was 4.34 (3.16–6.81) at baseline decreased to 1.90 (1.22–2.66) (p<0.001) one month from discharge but remained substantially higher the unity (Table 1 and Figure 2)

Thrombin generation (TG)

Endogenous thrombin potential (ETP)

The median ETP value was higher in patients at baseline than HS but reached statistical significance only in the presence of TM (p<0.001). One month from discharge, the median ETP decreased with respect to baseline but the degree of reduction was more pronounced when the ETP was measured in the presence of TM; the value was however still higher than HS [1,501 nM × min (1,228–1,774) vs. 1,227 (967–1,474), p<0.01] (Table 2 and Figure 3). The median ETP-TM ratio, which was higher in patients at baseline than HS (p<0.001), was slightly reduced one month from discharge (p<0.01) but was still higher than HS [0.80 (0.74–0.84) vs. 0.63 (0.48–0.71), p<0.001] (Table 2 and Figure 3).

Peak thrombin

The median peak thrombin was higher in patients at baseline than HS irrespective of TM (p<0.001) and

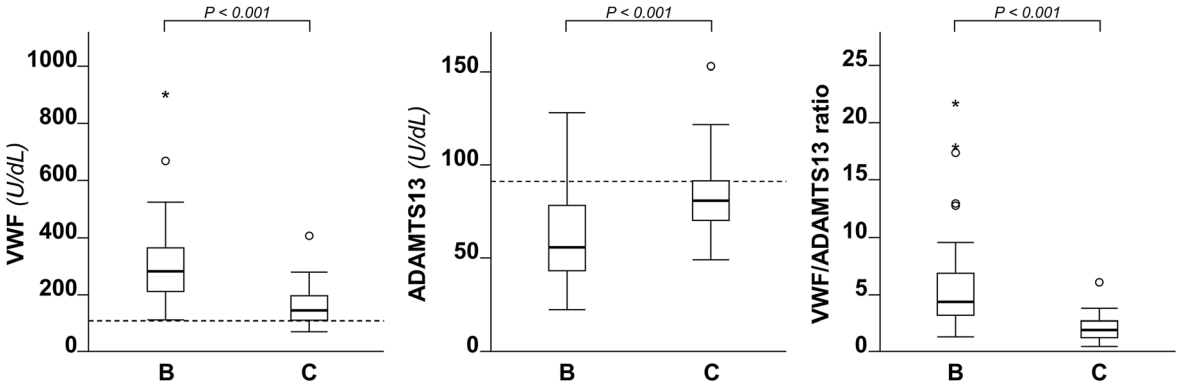

Figure 2: Box plots of von Willebrand factor (VWF), ADAMTS13 and their ratio for patients at baseline (B) and one month after discharge (C). The horizontal broken lines represent the mean value of the laboratory reference range.

Table 2: Median (IQR) values of thrombin generation parameters for healthy subjects and patients at baseline and one month after discharge (post-discharge) when measured with or without TM.

Parameter	Healthy subjects (n=51)	Patients				
		Baseline (n=51)	p-Value[a]	Post-discharge (n=30)[d]	p-Value[b]	p-Value[c]
ETP, nM × min No TM	1,962 (1,782–2,191)	2,070 (1,829–2,402)	N.S.	1,939 (1,624–2,197)	N.S.	N.S.
ETP, nM × min With TM	1,227 (967–1,474)	1,774 (1,591–2,131)	<0.001	1,501 (1,228–1,774)	N.S.	<0.01
ETP-TM ratio	0.63 (0.48–0.71)	0.88 (0.83–0.91)	<0.001	0.80 (0.74–0.84)	<0.01	<0.001
Peak, nM No TM	328 (302–364)	405 (363–450)	<0.001	389 (252–425)	<0.05	<0.001
Peak, nM With TM	243 (193–323)	413 (365–449)	<0.001	383 (339–441)	N.S.	<0.001
Velocity index, nM/min No TM	111 (97–128)	196 (156–241)	<0.001	182 (138–211)	<0.05	<0.001
Velocity index, nM/min With TM	101 (80–131)	212 (172–261)	<0.001	198 (146–231)	N.S.	<0.001

ETP, endogenous thrombin potential; TM, thrombomodulin; ETP-TM ratio, ETP with/without TM. [a]Patients at baseline vs. controls. [b]Patients at baseline vs. post-discharge. [c]Patients at post-discharge vs. controls. [d]Number of patients for whom baseline, and post-discharge paired samples were available for analyses. N.S., not statistically significant.

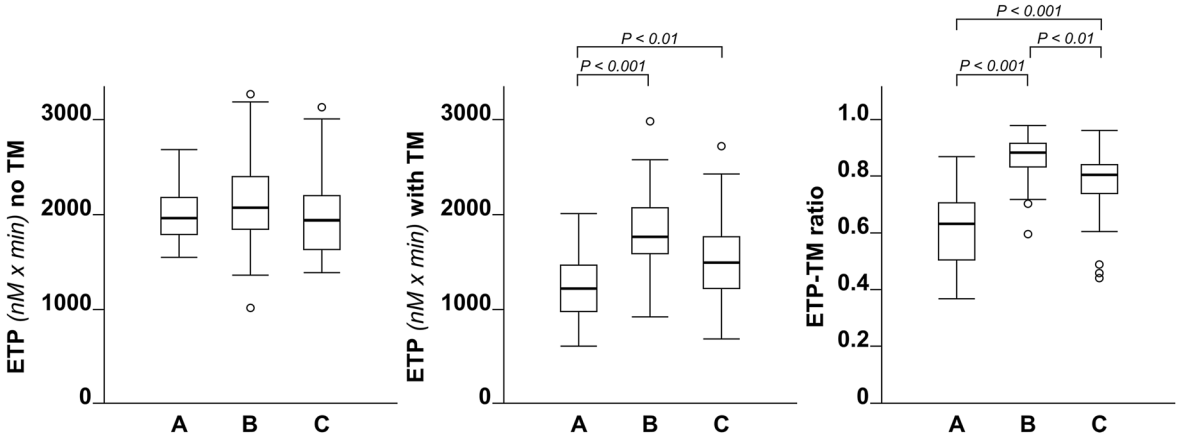

Figure 3: Box plots of endogenous thrombin potential (ETP) without or with thrombomodulin (TM) and ETP-TM ratio for healthy subjects (A), patients at baseline (B) and one month after discharge (C).

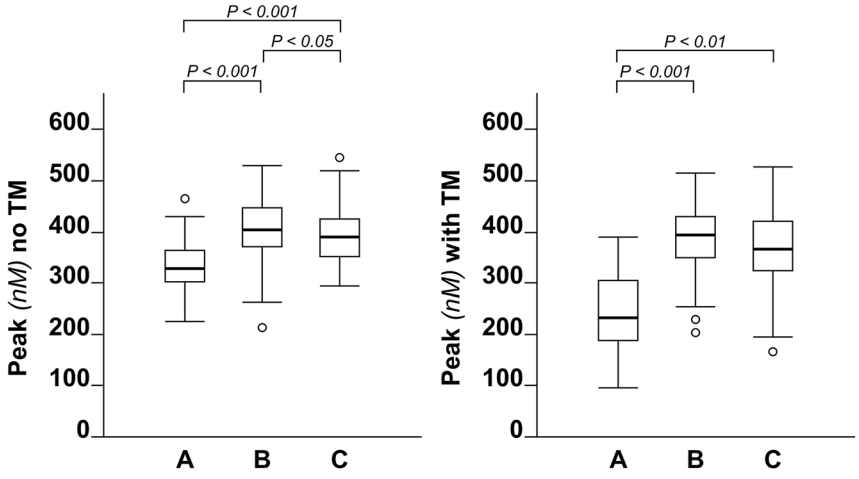

Figure 4: Box plots of peak thrombin without or with thrombomodulin (TM) for healthy subjects (A), patients at baseline (B) and one month after discharge (C).

decreased at one month from discharge but remained higher than HS, especially in the presence of TM [383 nM (339–441) vs. 243 (193–323), p<0.001] (Table 2 and Figure 4).

Velocity index

The median velocity index was higher in patients at baseline than HS irrespective of TM (p<0.001) and decreased one month from discharge but remained higher than HS, especially in the presence of TM [198 nM/min (146–231) vs. 101 (80–131), p<0.001] (Table 2 and Figure 5).

An adjusted linear regression model was used to assess whether age is a possible confounder on TG parameters: results did not change and the between group (i.e., patients at baseline vs. HS or patients at post-discharge vs. HS) statistical significance remained unchanged (data not shown).

Discussion

Cardiovascular diseases are commonly found in the general population and among them VTE is relatively frequent with a worldwide incidence of nearly two persons-years per 1,000 inhabitants in US and Europe [25]. VTE incidence is even higher in the elderly, reaching values of two persons-years per 200 inhabitants when one considers subjects aged 80 years or more [26]. Although, the causes for such high incidence are multifactorial, one of the major players is inflammation that in certain situations triggers at least two components of the Virchow's triad for thrombosis (i.e., hypercoagulability and endothelial perturbation). Both have been associated with inflammation through complex mechanisms that promote the release of inflammatory cytokines able to activate procoagulants and/or depress naturally occurring anticoagulants and endogenous fibrinolysis as well as endothelial perturbation [27].

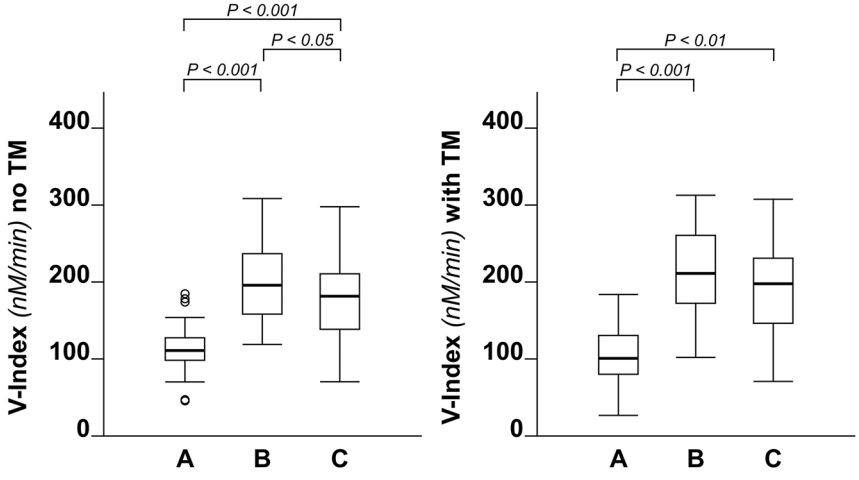

Figure 5: Box plots of velocity (V) index without or with thrombomodulin (TM) for healthy subjects (A), patients at baseline (B) and one month after discharge (C).

Acute infections are associated with inflammation and the ensuing hypercoagulability and endothelial perturbation are likely to increase the risk of VTE. We elected CAP as the prototype of acute infection as its burden is relatively high with an estimated overall incidence of 11 cases per 1,000 inhabitants per year [28]. CAP was investigated for hypercoagulability by a global coagulation procedure (i.e., thrombin generation, TG), which accounts for the balance between pro- and anticoagulants operating in plasma [29] and by the measurement of individual components of coagulation, and other molecular species associated with the risk of VTE.

The results show that patients with CAP possess at enrollment a genuine state of hypercoagulability as shown by increased TG parameters such as ETP. Other parameters, which indicate hypercoagulability (i.e., thrombin peak and velocity index) are increased; the former representing the highest amount of generated thrombin and the latter the velocity of its formation. Another important feature of our study indicating hypercoagulability in CAP is increased ETP-TM ratio. This ratio is a measure of the *in vitro* resistance to the anticoagulant action mediated by TM [23]. Protein C is one of the most important anticoagulants, which circulates in plasma as zymogen and must be converted into its active form (activated protein C) to act as an anticoagulant [30]. Protein C is activated *in vivo* by thrombin in combination with its endothelial receptor, TM. Activated protein C (in combination with protein S) is in turn able to inactivate the activated forms of the two key procoagulants (i.e., FVIIIa and Va), thus quenching TG [30]. The relatively high levels of ETP-TM ratio found in patients with CAP at enrollment indicate that the regulation of TG in the presence of TM is hampered and therefore define a state of hypercoagulability [23]. The main reason explaining high ETP-TM ratio rests on the FVIII-protein C axis. Protein C is the physiologic inhibitor to FVIII [30], hence the relatively high levels of FVIII combined with normal (or reduced levels) of protein C translate into a higher FVIII-to-protein C ratio (see Figure 1) and consequently to high ETP-TM ratio (see Figure 3). Interestingly, ETP-TM ratio was correlated with the FVIII/protein C ratio (rho=0.667, $p<0.001$). Additional reasons that explain the hypercoagulability are the relatively high levels of the parameters denoting neutrophil activation such as myeloperoxidase and elastase. Increased levels of these moieties have been associated with the risk of VTE both in animal models [7] and in humans [8–11].

Endothelial perturbation was assessed by measuring VWF, a biochemical marker released into the circulation when endothelial cells are perturbed [12]. VWF works by binding platelets [31] and its activity is tightly regulated by the plasma protease ADAMTS13, which degrades high molecular weight VWF multimers into smaller, less active size. Severe deficiency of ADAMTS13 (<10 U/dL) leads to the accumulation in plasma of hyperactive VWF multimers causing thrombotic thrombocytopenic purpura, a life-threatening thrombotic microangiopathy [32, 33]. In addition, low ADAMTS13 has been associated with increased risk of cardiovascular diseases [34, 35]. Finally, the imbalance between high VWF and low ADAMTS13 is likely to result into a prothrombotic state in inflammatory conditions such as sepsis and overt disseminated intravascular coagulation [36–38]. Hence, the VWF-ADAMTS13 axis gives the opportunity to assess for the contribution of endothelial perturbation and hypercoagulability in patients with CAP.

Another important finding of this study is that the hypercoagulability and endothelial perturbation observed during the acute phase of CAP are somewhat reduced one month after discharge (when patients recovered). However, they did not return to the levels observed in HS. These results indicate that hypercoagulability and endothelial perturbation sustained by CAP during the acute phase of the disease are long lasting and remain even after the infection has been resolved.

These results are in line with the epidemiological observations of Smeeth et al. [14], who reported that patients with acute respiratory infections had a significantly raised risk of VTE, which was highest in the first two weeks, after the onset of infection and then gradually decreased, returning to baseline after one year. Berg et al. [39] reported an increased risk of cardiovascular diseases following hospital admission for sepsis or pneumonia, which remained high for more than five years after the infection. Our experimental observations highlight the contributory role played by plasma hypercoagulability and endothelial perturbation in determining and maintaining such a risk for long time after the infection has been resolved. Although the risk to which patients are exposed during hospitalization is recognized as shown by the common practice of providing appropriate antithrombotic prophylaxis [40], less attention has been paid to the fact that hypercoagulability and the ensuing risk may remain after the infection has been resolved. Currently, no recommendations on post discharge antithrombotic prophylaxis are in fact made [40] and most of these patients remain at risk of VTE and other cardiovascular diseases. Clinical trials are warranted to establish which kind of and how long for the antithrombotic prophylaxis should be given to these patients.

Recently, we reported on investigation of hemostatic derangement and endothelial perturbation in patients with pneumonia due to COVID-19 [41, 42]. In those studies,

TG was not measured as patients were on antithrombotic prophylaxis with standard, intermediate, or therapeutic doses of low molecular weight heparin. However, the other hemostasis parameters were measured and is therefore possible to make (albeit indirectly) a comparison between CAP and COVID-19 pneumonia. Hemostasis and endothelial perturbation although present in both conditions, are milder in CAP than in COVID-19. For example, FVIII in COVID-19 pneumonia was higher (median, 302 U/dL) than CAP (median 195 U/dL); similarly, median VWF was 466 U/dL in COVID-19 vs. 283 U/dL in CAP. These conclusions are in line with those of De Cristoforo et al. [43], who investigated head-to-head relatively small numbers of patients with pneumonia due or not to COVID-19.

Strengths and limitations of the present study should be recognized.

Strengths are the relatively large number of well characterized patients who have been investigated for hypercoagulability by procedures aimed to assess the dynamic potential of TG in a manner which mimics what presumably occurs *in vivo* [29] and the assessment of endothelial perturbation through the VWF-ADAMTS13 axis [42].

The limitations are the fact that we did not report on the association of clinical findings with hypercoagulability/endothelial perturbation. The relatively short period of observation from hospital discharge to recall (one month) combined with the fact that a certain number of patients were lost at follow up, precluded any assessment on the value of the association between hypercoagulability/endothelial perturbation and the occurrence of VTE and other cardiovascular diseases. However, earlier studies with appropriate prospective design, showed that enhanced TG is an established risk factor of both first and recurrent VTE [44, 45], myocardial infarction [46] or overall mortality [47]. Other studies have also provided evidence on the association between the imbalance of the VWF-ADAMTS13 and venous and/or arterial thrombosis [34–38]. In some of the above studies VWF was measured both as antigen and activity, whereas in our cohort as antigen only. However, it should be noted that we made the same measurement (i.e., VWF antigen) both for patients at baseline and one month after discharge. Hence, it is unlikely that our conclusion of the persisting imbalance between high VWF and low ADAMTS13 after recovery is modified by the measurement of the VWF activity.

In conclusions, the study shows that patients with acute respiratory infections such as CAP possess a state of hypercoagulability and endothelial perturbation, which remain even after the resolution of the infection. Clinical trials are warranted to establish the benefit of antithrombotic prophylaxis after hospital discharge.

Acknowledgments: We wish to thank the following: Drs. G. Cozzi, P. Colpani, M. Biganzoli, who helped with the measurement of von Willebrand factor and ADAMTS13, and G.L. Ghilardini, who helped with figures and graphical abstract.

Research funding: This work was also partially supported by the Italian Ministry of Health, Bando Ricerca Corrente 2020.

Author contributions: AT conceived the study, reviewed results, and wrote the manuscript. SCR, managed patients and collected clinical data. MC, GM, ES, IM, LB, made laboratory testing. MB, made statistical analysis. VM, FP supervised the study. All authors reviewed data and accepted the manuscript.

Competing interests: AT reports speaker's fees from Werfen, Stago and Sobi outside the submitted work. FP reports personal fees from Bioverativ, Grifols, Roche, Sanofi, Sobi, Spark, and Takeda, outside the submitted work. The other authors (SCR, MC, GM, ES, IM, LB, MB, VM) have nothing to disclose.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study was approved by the local Ethics Committee.

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