Original Article

Complement activation is associated with right ventricular dysfunction and the severity of pulmonary embolism: links with prothrombotic state

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Background: Little is known about the role of complement activation in acute pulmonary embolism (PE). We investigated whether complement activation is associated with the severity of acute PE, along with the associated prothrombotic state, systemic inflammation and neutrophil extracellular traps (NETs) formation.

Methods: We studied 109 normotensive, non-cancer PE patients (aged 58.1±15.0 years). On admission prior to initiation of anticoagulation, plasma soluble complement components, i.e., C3a and sC5b-9, were measured with enzyme-linked immunosorbent assay (ELISA), along with thrombin generation, fibrinolysis proteins (plasminogen, antiplasmin, plasminogen activator inhibitor-1), factor VIII (FVIII) activity, and fibrin clot properties, including clot permeability (K_s, a measure of clot density) and clot lysis time (CLT). Moreover, we determined inflammatory markers and citrullinated histone H3, a specific marker of NETs formation.

Results: Patients in the lower tertile of C3a (≤1.45 ng/mL, n=37) had lower simplified Pulmonary Embolism Severity Index (sPESI) values and were less likely to have right ventricular (RV) dysfunction compared to the remaining subjects. The former subgroup also had 13% lower FVIII activity, but not fibrinogen, interleukin-6, fibrinolysis proteins, and thrombin generation. Plasma C3a levels correlated inversely with K_s and positively with CLT indicating formation of denser and poorly lysable clots in subjects with elevated C3a. Despite a positive association between C3a and sC5b-9, the latter parameter was solely associated with higher FVIII, but not with other variables.

Conclusions: We showed that in acute PE enhanced complement activation characterizes patients with poorer short-term prognosis who display prothrombotic fibrin clot properties and elevated FVIII, which supports the involvement of complement proteins in acute thromboembolism.

Keywords: Complement; prothrombotic state; fibrin clot; pulmonary embolism (PE)

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Introduction

The complement system, which encompasses over 40 proteins, is a key effector mechanism of the innate immune system involved in the rapid first-line recognition and clearing pathogens, apoptotic cells, and cellular debris (1,2). There are three pathways of complement activation i.e., classical, lectin and alternative, which converge to form C3 convertases that cleave C3 to the biologically active split products, C3a and C3b (3-5). C3b acts as an opsonin and also incorporates into the C3 convertase complexes to form C5 convertase complexes, which cleave C5 to C5a and C5b, the initiator of the terminal pathway of complement and formation of the C5b-9 complex, the terminal complement complex (TCC), which exists in two forms: surface-bound lytic C5b-9 forming the membrane attack complex (MAC) and a soluble form (sC5b-9) that binds to vitronectin and clusterin (3-5). It has been demonstrated that C5a and sC5b-9 are pro-coagulant molecules in several noninfectious diseases driven by complement dysregulation, e.g., paroxysmal nocturnal hemoglobinuria as shown *in vitro* and in animal models (3,4).

Complement activation, which can be determined using plasma C3a and sC5b-9 levels, is closely, evolutionarily related to blood coagulation and fibrinolysis, may be involved in venous thromboembolism (VTE), encompassing acute pulmonary embolism (PE) and deep vein thrombosis (DVT) (6-8). Several complement components, including C1s, C3, C3a, C4, C5a, C7s and C8 α-chains, are incorporated into the thrombus and may modulate its stability and local inflammation (3,4,6,9). In a large population-based cohort, Nørgaard *et al.* demonstrated that individuals with plasma C3 levels in the top tertile had a 58% higher risk of VTE than those in the lowest tertile (10). C3 levels above the 90th percentile have been found to increase the odds of pregnancy-related VTE by 1.8 times, whereas C4 levels above the 90th percentile doubled this risk (11). Høiland *et al.* found that high plasma sC5b-9 concentrations were associated with a substantially higher VTE risk, especially unprovoked events (8).

Although growing evidence supports the interplay between complement activation and blood coagulation, fibrinolysis, inflammation, and neutrophil extracellular traps (NETs) formation (i.e., NETosis) (7,9,12-14), the role of these links in acute PE and its sequelae is unclear. Therefore, we investigated whether increased complement activation is associated with the severity of acute PE, along with the associated prothrombotic state, systemic inflammation and NETs formation. We present this article in accordance with the STROBE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-171/rc).

Highlight box

Key findings

- Enhanced complement activation characterizes patients with more severe pulmonary embolism (PE).
- There is a link between complement activation and right ventricular (RV) dysfunction in acute PE.
- Increased complement activation is associated with higher factor VIII and the prothrombotic fibrin clot phenotype.
- There is no relationship between complement activation and fibrinogen, neutrophil extracellular traps (NETs) formation, or thrombin generation.

What is known and what is new?

- Complement activation, which is closely related to blood coagulation and fibrinolysis, may be involved in the pathogenesis of venous thromboembolism (VTE), including acute PE. Several complement components, e.g., C1s, C3, C3a, C4, C5a, C7s and C8 α-chains, are incorporated into the thrombus and may modulate its stability and local inflammation. Clinical studies have confirmed the link between complement activation and VTE risk.
- This study is the first to show that enhanced complement activation characterizes patients with more severe PE, who developed RV dysfunction. Increased complement activation was associated with higher factor VIII and the prothrombotic fibrin clot phenotype, but not with fibrinogen, thrombin generation, or NETs formation. Our findings demonstrate that complement activation is observed in acute PE patients especially at higher early mortality risk, and this phenomenon is linked with thrombotic and inflammatory mechanisms in this disease.

What is the implication, and what should change now?

- Modulation of complement activation could be beneficial in acute PE. However, this requires further research.

Methods

We studied 109 white adults, normotensive, non-cancer patients with documented acute PE diagnosed according to the European guidelines (15). This group is a subset of the study population described previously (16). Briefly, exclusion criteria were: high-risk PE on admission, known active cancer, pregnancy, recent arterial thromboembolism, and current anticoagulant therapy. In patients in whom heparin was administered prior to hospital admission, anti-Xa activity ≥0.2 U/mL on admission [arbitrarily chosen as a cut-off value based on our previous studies (16,17)] was

another exclusion criterion.

The simplified Pulmonary Embolism Severity Index (sPESI) was calculated as previously described (15). Right ventricular (RV) dysfunction was defined as dilatation of the RV (a RV/left ventricle diameter ratio of >1.0 from the subcostal or apical view four-chamber view) combined with the absence of the inspiratory collapse of the inferior vena cava or an elevated systolic gradient through the tricuspid valve (>30 mmHg), in the absence of significant left ventricular disease.

The European Society of Cardiology (ESC) guidelines were used to diagnose coronary artery disease (CAD), myocardial infarction (MI) and congestive heart failure (CHF) (18-20). Hypertension was diagnosed when systolic blood pressure was ≥140 mmHg in the office and/or diastolic blood pressure ≥90 mmHg or if the patient was taking antihypertensive drugs. The diagnosis of stroke was based on clinical presentation and brain imaging. Diabetes was established based on either a fasting glycaemia ≥7 mmol/L on two separate occasions or the use of antidiabetic drugs. Hypercholesterolemia was diagnosed if a patient was on lipid-lowering therapy or total cholesterol was above 5.0 mmol/L or low-density lipoprotein (LDL) cholesterol above 2.6 mmol/L. Chronic kidney disease (CKD) was defined as kidney damage or glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for 3 months or more, irrespective of cause. Asthma and COPD were diagnosed based on the patient records.

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Bioethics Committee of the Regional Chamber of Physicians in Krakow, Poland (No. 214/KBL/OIL/2022). All study participants had provided written informed consent.

The antecubital vein was used to take fasting venous blood on admission. Blood drawn into citrated tubes was centrifuged at 2,500 g at 20 ℃ for 20 min. Blood drawn into serum tubes was centrifuged at 1,600 g at 4 ℃ for 10 min. To allow for batch analysis, aliquots were stored at –80 ℃.

Blood cell count, international normalized ratio (INR), activated partial thromboplastin time (aPTT), D-dimer, lipid profile, creatinine, glucose, N-terminal B-type natriuretic propeptide (NT-proBNP), and CRP were determined in the hospital laboratory using routine techniques, while high-sensitivity interleukin-6 (IL-6) was assayed by the immunoenzymatic test [enzyme-linked immunosorbent assay (ELISA); Quantikine, R&D Systems, Minneapolis, USA]. A cut-off value of 600 pg/mL for NT-proBNP was used e for risk stratification of normotensive PE patients. Plasma L(+)-lactate (Abcam, Cambridge, UK; detection range 0.02–10 mM) concentrations were measured by an ELISA test. Abnormal plasma lactate levels were defined as values of 2 mM or greater, based on the literature (21,22).

Fibrinogen was determined using the Clauss assay. Factor VIII (FVIII) activity (Siemens Healthcare Diagnostics, Marburg, Germany) was measured by the Behring Coagulation System (Siemens). A chromogenic assay was used to measure anti-Xa activity (BIOPHEN, Hyphen Biomed, Neuville-Sur-Oise, France) in patients who received rivaroxaban or apixaban, and the Hemoclot thrombin inhibitor assay (Hyphen BioMed) for dabigatran. To evaluate efficiency of fibrinolysis, plasminogen activator inhibitor-1 (PAI-1) antigen, thrombin activatable fibrinolysis inhibitor (TAFI) activity (both, Hyphen-Biomed, Neuville-Sur-Oise, France), plasminogen, and α2-antiplasmin activity were measured (both, Siemens Healthcare Diagnostics, Marburg, Germany). An ELISA assay (Cayman Chemical, Ann Arbor, MI, USA) was used to quantify citrullinated histone H3 (H3Cit), a specific marker of NETosis.

All measurements were performed by technicians blinded to the origin of the samples.

Plasma C3, C3a, and sC5b-9 concentrations were measured with ELISAs (BD Bioscience, San Jose, CA, USA), according to the manufacturer's instructions. We arbitrarily decided that plasma C3a and sC5b-9 concentrations in the top tertile defined enhanced complement activation.

Calibrated Automated Thrombography (CAT; Thrombinoscope BV, Maastricht, The Netherlands) was applied to determine of endogenous thrombin potential (ETP) (23,24). Using the fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland), the area under the curve at 37 ℃ was generated. We added tissue factor (TF)-based activator (PPP Reagent containing 5 μmol/L recombinant TF and 4 μmol/L phospholipid vesicles; Diagnostica Stago) and FluCa solution (Diagnostica Stago) to 80 μL platelet-poor plasma. The ETP values were calculated as the area under the thrombin generation curve.

Fibrin clot permeability was assessed according to established protocols (16,17). In brief, 20 mM calcium chloride and 1 U/mL human thrombin (Sigma-Aldrich, St. Louis, MO) were mixed with citrated plasma. The clot-containing tube was connected to a Tris-buffer (0.05 mol/L Tris-HCl, 0.15 mol/L NaCl, pH 7.5) reservoir, where the volume that flowed through the gels was measured within 1 h. The permeation coefficient (K_s) was calculated using

the formula: $K_s = Q \times L \times \eta/t \times A \times \Delta p$, where Q represents the flow rate during percolating time (t), L is the length of the fibrin gel, η is the liquid viscosity, A is the cross-sectional area (cm^2), and Δp is the differential pressure (in $dyne/cm^2$). Lower K_s values corresponded to denser fibrin networks, reflecting smaller pore sizes. The inter-assay coefficients of variation were less than 7%.

Clot lysis time (CLT) was used to assess fibrinolysis capacity (25). Citrated plasma was mixed with 15 mM calcium chloride, 0.5 U/mL human thrombin (Merck), 15 µM phospholipid vesicles (Rossix, Mölndal, Sweden), and subsequently with 20 ng/mL recombinant tissue-type plasminogen activator (Boehringer Ingelheim, Germany). This mixture was transferred to a microtiter plate and its absorbance was evaluated at 405 nm at 37 ℃. CLT was defined as the time from both midpoints of the clear-to-maximum-turbid transition to the maximum-turbid-to-clear transition. The intra- and inter-assay coefficients of variation were maintained at 5–7%.

Statistical analysis

Variables were presented as numbers and percentages or median and interquartile range (IQR), as appropriate. Normality was assessed using the Shapiro-Wilk test. Differences among three groups were compared by the one-way ANOVA or Kruskal-Wallis test depending on the equality of variances and data distribution. Categorical variables were compared using Pearson's chi-squared test or Fisher's exact test. Spearman's correlation coefficients were computed to measure the association between complement component concentrations and markers of prothrombotic state, inflammation and NETosis. Stepwise logistic regression analysis was performed for determining the significant predictors of increased complement activation defined as C3a and sC5b-9 levels in the top tertile. The best cut-off value of predictors that maximizes sensitivity and specificity and differentiates PE patients who had complement alterations was calculated with the use of the receiver operating characteristics (ROC) curve. A P value <0.05 was considered statistically significant. Calculations were made using the STATISTICA 13.3 software package (TIBCO Software Inc., Palo Alto, CA, USA).

Results

A total of 109 normotensive non-cancer acute PE patients (51.4% male) aged from 23 to 87 years were studied (*Table 1*). Fifty-six (51.4%) of subjects had concomitant DVT. sPESI score ≥1 was found in 93 (85.3%) of the study participants. NT-proBNP levels ≥600 pg/mL were detected in 44 (40.4%) subjects. RV dysfunction was diagnosed in 40 (36.7%) patients.

A median C3 concentration of C3 was 7.38 (IQR, 3.71–9.80) mg/mL, C3a was 1.84 (IQR, 1.29–2.86) µg/dL and sC5b-9 was 711 (IQR, 540–850) ng/mL. There was a positive correlation between plasma concentrations of C3a and sC5b-9 (r=0.43; P<0.001).

Analysis of C3a and sC5b-9 tertiles showed that except for age and the prevalence of CKD, there were no differences in demographic characteristics and comorbidities (*Table 1*), but those in the lower tertile of sC5b-9 were older compared to the remaining subjects (P=0.001). The prevalence of CKD in the lower tertile of C3a was 18.9% and was 1.8 times lower than in patients from the middle C3a tertile and 2.3 times higher compared to those in the top C3a tertile (P=0.03). There were 26 (70.3%) high-risk patients (sPESI score ≥1) in the lower tertile of C3a, 32 (88.9%) in the middle tertile and 35 (97.2%) in the top tertile (P=0.004). High-risk PE patients had higher C3a levels compared to low-risk patients [1.91 (IQR, 1.32–3.01) *vs.* 1.38 (IQR, 0.99–1.55) µg/dL; P=0.004]. The logistic analysis showed that with each tertile of C3a, the odds of sPESI score ≥1 increased almost 3.7 times in patients with acute PE (OR 3.67, 95% CI: 1.55–8.67; P=0.003). No such relationship was observed for sC5b-9. There was no association between both C3a and sC5b-9 concentrations and thrombus location in the pulmonary arteries, as well as with the incidence of provoked *vs.* unprovoked PE episodes.

Importantly, patients in the lower tertile of C3a, but not sC5b-9, were less likely to have RV dysfunction on echocardiography (P=0.04) and had lower NT-proBNP levels (P=0.03), as compared to the remaining subjects (*Table 1*). PE patients with RV dysfunction had higher C3a levels compared to the remaining subjects [2.13 (IQR, 1.68–3.84) *vs.* 1.55 (IQR, 1.23–2.33) µg/dL; P=0.003], but not sC5b-9. With each tertile of C3a, the odds of RV dysfunction increased almost 1.9-fold (OR 1.88, 95% CI: 1.14–3.11; P=0.01).

There was a weak positive association of C3a and NT-proBNP concentrations (r=0.20; P=0.04). Despite a positive association between plasma C3a and sC5b-9, the latter parameter was not correlated with sPESI, RV dysfunction, and NT-proBNP.

Logistic regression analysis showed that sPESI and RV dysfunction were independently associated with increased

Table 1 Baseline characteristics of the study population

Variables	Value (n=109)	Tertiles of C3 (mg/mL)				Tertiles of C3a (μg/dL)				Tertiles of sC5b-9 (ng/mL)			
		≤5.70 (n=38)	5.71–8.80 (n=35)	>8.80 (n=36)	P	≤1.45 (n=37)	1.46–2.33 (n=36)	>2.33 (n=36)	P	≤600 (n=37)	601–812 (n=36)	>812 (n=36)	P
Age (years)	58.0 (48.0, 70.0)	58.5 (48.0, 71.0)	60.0 (50.0, 69.0)	56.0 (45.5, 69.0)	0.65	57.0 (48.0, 64.0)	61.0 (47.5, 70.0)	62.5 (47.5, 74.5)	0.28	69.0 (56.0, 74.0)	53.5 (44.0, 66.0)	57.0 (46.0, 64.5)	0.001
Men	56 (51.4)	18 (47.4)	20 (57.1)	18 (50.0)	0.69	19 (51.4)	23 (63.9)	14 (38.9)	0.11	15 (40.5)	20 (55.6)	21 (58.3)	0.26
BMI (kg/m^2)	27.0 (25.0, 31.1)	27.6 (25.0, 32.5)	27.0 (24.0, 28.0)	27.5 (26.0, 32.7)	0.17	27.0 (25.4, 33.2)	27.1 (24.5, 30.2)	27.0 (24.0, 31.1)	0.82	27.1 (25.4, 33.2)	28.0 (26.0, 31.9)	26.0 (24.0, 29.0)	0.10
Pulmonary embolism characteristics													
sPESI score	2.0 (1.0, 2.0)	2.0 (1.0, 2.0)	1.0 (0.0, 2.0)	2.0 (1.0, 2.0)	0.15	1.0 (0.0, 2.0)	2.0 (1.0, 2.0)	2.0 (1.5, 3.0)	<0.001	2.0 (1.0, 2.0)	1.5 (1.0, 2.0)	2.0 (1.0, 2.0)	0.44
Central PE	61 (56.0)	19 (50.0)	20 (57.1)	22 (61.1)	0.62	19 (51.4)	24 (66.7)	18 (50.0)	0.29	21 (56.8)	21 (58.3)	19 (52.8)	0.89
Unprovoked PE	71 (65.1)	24 (63.2)	23 (65.7)	24 (66.7)	0.95	23 (62.2)	20 (55.6)	28 (77.8)	0.13	22 (59.5)	25 (69.4)	24 (66.7)	0.65
Concomitant DVT	56 (51.4)	15 (39.5)	24 (68.6)	17 (47.2)	0.04	24 (64.9)	13 (36.1)	19 (52.8)	0.048	16 (43.2)	21 (58.3)	19 (52.8)	0.43
RV dysfunction	40 (36.7)	18 (47.4)	9 (25.7)	13 (36.1)	0.16	9 (24.3)	12 (33.3)	19 (52.8)	0.04	15 (40.5)	8 (22.2)	17 (47.2)	0.07
Comorbidities													
CAD	43 (39.4)	12 (31.6)	13 (37.1)	18 (50.0)	0.25	13 (35.1)	19 (52.8)	11 (30.6)	0.13	13 (35.1)	16 (44.4)	14 (38.9)	0.72
Hypertension	58 (53.2)	24 (63.2)	15 (42.9)	19 (52.8)	0.22	19 (51.4)	19 (52.8)	20 (55.6)	0.94	23 (62.2)	17 (47.2)	18 (50.0)	0.39
Heart failure	18 (16.5)	3 (7.9)	7 (20.0)	8 (22.2)	0.20	7 (18.9)	7 (19.4)	4 (11.1)	0.57	2 (5.4)	7 (19.4)	9 (25.0)	0.07
Diabetes mellitus	37 (33.9)	9 (23.7)	12 (34.3)	16 (44.4)	0.17	15 (40.5)	11 (30.6)	11 (30.6)	0.58	12 (32.4)	15 (41.7)	10 (27.8)	0.45
Asthma or COPD	12 (11.0)	5 (13.2)	5 (14.3)	2 (5.6)	0.44	4 (10.8)	4 (11.1)	4 (11.1)	0.99	2 (5.4)	4 (11.1)	6 (16.7)	0.31
Hypercholesterolemia	76 (69.7)	18 (47.4)	31 (88.6)	27 (75.0)	<0.001	29 (78.4)	24 (66.7)	23 (63.9)	0.36	22 (59.5)	26 (72.2)	28 (77.8)	0.22
CKD	22 (20.2)	2 (5.3)	8 (22.9)	12 (33.3)	0.01	7 (18.9)	12 (33.3)	3 (8.3)	0.03	6 (16.2)	8 (22.2)	8 (22.2)	0.76
Previous stroke	10 (9.2)	2 (5.3)	3 (8.6)	5 (13.9)	0.43	3 (8.1)	5 (13.9)	2 (5.6)	0.46	3 (8.1)	5 (13.9)	2 (5.6)	0.46
Previous VTE	7 (6.4)	2 (5.3)	1 (2.9)	4 (11.1)	0.34	4 (10.8)	1 (2.8)	2 (5.6)	0.36	3 (8.1)	4 (11.1)	0	0.14
Medications													
Acetylsalicylic acid	31 (28.4)	10 (26.3)	7 (20.0)	14 (38.9)	0.20	10 (27.0)	13 (36.1)	8 (22.2)	0.42	12 (32.4)	12 (33.3)	7 (19.4)	0.34
Statin	64 (58.7)	15 (39.5)	26 (74.3)	23 (63.9)	0.008	24 (64.9)	21 (58.3)	19 (52.8)	0.58	18 (48.7)	24 (66.7)	22 (61.1)	0.28
Laboratory investigations													
Hemoglobin (g/dL)	13.7 (12.7, 14.8)	13.7 (12.5, 14.9)	13.8 (13.2, 14.7)	13.8 (12.7, 14.8)	0.82	13.6 (12.8, 14.9)	13.8 (12.9, 14.7)	13.9 (12.6, 14.9)	0.99	13.6 (12.4, 14.7)	13.8 (12.9, 14.7)	14.0 (12.9, 15.1)	0.40
White blood cells (10^9/L)	7.3 (5.6, 9.5)	9.0 (6.7, 10.5)	6.1 (5.5, 8.5)	7.2 (5.2, 8.7)	0.005	6.7 (5.3, 9.2)	7.3 (5.6, 8.9)	8.4 (6.0, 9.9)	0.47	8.8 (6.7, 11.6)	6.7 (5.4, 8.7)	6.2 (5.1, 8.7)	0.005
Platelets (10^9/L)	216.0 (189.0, 269.0)	212.0 (170.0, 268.0)	234.0 (185.0, 268.0)	212.5 (198.5, 289.5)	0.77	215.0 (200.0, 254.0)	215.0 (180.0, 269.0)	217.5 (169.5, 314.0)	0.78	221.0 (189.0, 272.0)	211.5 (196.5, 248.0)	223.5 (184.5, 314.0)	0.64
Glucose (mmol/L)	5.9 (5.3, 6.9)	5.9 (5.3, 7.1)	6.2 (5.3, 6.7)	5.7 (5.3, 6.6)	0.85	5.5 (5.2, 6.3)	6.0 (5.3, 7.4)	6.2 (5.5, 7.0)	0.22	5.9 (5.3, 7.2)	5.9 (5.2, 6.6)	6.1 (5.3, 6.7)	0.63
LDL-cholesterol (mg/dL)	89.0 (75.0, 116.0)	98.0 (75.0, 118.0)	87.4 (75.0, 96.3)	94.2 (75.6, 129.9)	0.37	80.4 (74.1, 102.5)	91.8 (76.0, 129.7)	93.6 (80.1, 112.1)	0.27	94.0 (81.2, 115.0)	80.4 (73.3, 112.1)	92.0 (76.0, 123.4)	0.37
hs-troponin T (pg/mL)	8.7 (7.0, 22.2)	12.2 (7.2, 25.3)	7.8 (6.3, 22.2)	8.8 (7.3, 23.0)	0.52	8.5 (6.9, 25.3)	8.7 (7.1, 19.2)	9.3 (7.0, 26.2)	0.94	9.3 (6.9, 33.1)	8.2 (6.2, 17.9)	9.1 (7.4, 20.8)	0.30
NT-proBNP (pg/mL)	444.0 (103.0, 1,129.0)	335.5 (98.0, 1,493.0)	499.0 (126.0, 1,241.0)	318.5 (93.0, 1,020.5)	0.74	186.0 (71.0, 764.0)	537.0 (191.5, 1,203.5)	527.0 (157.5, 1,762.0)	0.03	292.0 (92.0, 3,169.0)	304.5 (105.0, 775.0)	557.5 (204.0, 1,270.5)	0.34
hs-CRP (mg/L)	4.2 (1.8, 15.7)	8.3 (2.8, 23.9)	1.9 (1.3, 5.0)	5.9 (2.1, 13.3)	<0.001	2.8 (1.9, 6.7)	3.0 (1.6, 18.7)	5.5 (2.4, 22.6)	0.30	14.1 (2.8, 34.0)	2.4 (1.7, 7.4)	2.8 (1.5, 7.5)	<0.001
IL-6 (pg/mL)	4.1 (3.4, 8.4)	6.5 (3.7, 17.7)	3.7 (3.3, 4.2)	4.1 (3.6, 8.1)	0.007	4.0 (3.4, 6.1)	3.8 (3.1, 6.4)	4.6 (3.9, 15.5)	0.05	7.5 (4.0, 23.3)	3.6 (3.2, 4.6)	4.1 (3.4, 4.7)	<0.001
Citrullinated H3 (ng/mL)	2.7 (1.9, 3.9)	3.0 (1.5, 3.9)	2.5 (1.9, 3.7)	3.1 (1.9, 4.0)	0.65	2.6 (1.9, 3.7)	2.7 (1.9, 3.8)	3.1 (1.3, 4.1)	0.93	2.6 (1.5, 3.8)	2.9 (2.1, 3.7)	2.6 (1.9, 4.1)	0.46
L-lactate (mM)	2.1 (1.6, 2.4)	2.0 (1.7, 2.3)	2.1 (1.6, 2.7)	2.1 (1.7, 2.5)	0.83	1.9 (1.6, 2.3)	2.2 (1.6, 2.4)	2.2 (1.7, 2.5)	0.54	2.1 (1.7, 2.3)	2.0 (1.7, 2.4)	2.3 (1.6, 2.9)	0.39

Table 1 (*continued*)

Table 1 (*continued*)

Variables	Value (n=109)	Tertiles of C3 (mg/mL)				Tertiles of C3a (μg/dL)				Tertiles of sC5b-9 (ng/mL)			
		≤5.70 (n=38)	5.71–8.80 (n=35)	>8.80 (n=36)	P	≤1.45 (n=37)	1.46–2.33 (n=36)	>2.33 (n=36)	P	≤600 (n=37)	601–812 (n=36)	>812 (n=36)	P
Coagulation variables													
Fibrinogen (g/L)	3.3 (2.9, 4.0)	3.4 (2.9, 4.4)	3.3 (2.7, 3.9)	3.4 (3.0, 3.8)	0.63	3.3 (2.7, 3.6)	3.4 (3.0, 4.1)	3.4 (3.0, 4.3)	0.23	3.4 (3.0, 3.7)	3.3 (2.8, 4.3)	3.4 (2.8, 4.0)	0.99
D-dimer (ng/mL)	3,738.0 (1,962.0, 5,940.0)	4,208.5 (2,013.0, 6,410.6)	2,860.0 (1,900.0, 5,940.0)	3,587.5 (1,617.6, 5,354.5)	0.45	3,296.0 (2,123.0, 5,293.0)	3,853.5 (1,818.7, 8,000.0)	3,692.0 (1,931.0, 5,354.5)	0.64	5,293.0 (2,718.0, 7,770.0)	3,338.5 (1,747.0, 5,260.5)	2,266.5 (1,641.5, 4,532.0)	0.004
PF4 (ng/mL)	69.1 (57.7, 80.3)	57.6 (45.6, 64.8)	73.7 (59.0, 85.4)	75.0 (69.4, 82.3)	<0.001	69.1 (55.4, 81.5)	72.7 (61.4, 84.2)	65.3 (58.1, 74.4)	0.24	68.7 (55.5, 79.1)	70.1 (59.1, 78.9)	71.6 (59.0, 83.0)	0.25
Factor VIII (%)	146.0 (125.0, 175.0)	153.5 (129.0, 172.0)	159.0 (131.0, 178.0)	136.0 (120.0, 167.0)	0.28	135.0 (114.0, 162.0)	157.5 (125.5, 175.5)	154.5 (132.5, 202.0)	0.02	142.0 (126.0, 170.0)	141.0 (120.5, 163.5)	164.0 (132.5, 192.5)	0.049
Plasminogen (%)	105.0 (96.0, 114.0)	102.0 (96.0, 113.0)	108.0 (99.0, 116.0)	104.0 (96.0, 113.5)	0.46	101.0 (97.0, 110.0)	109.0 (97.5, 117.0)	103.5 (96.0, 116.0)	0.19	105.0 (97.0, 117.0)	103.5 (96.0, 112.5)	107.0 (96.5, 116.0)	0.66
TAFI activity (%)	100.1 (92.0, 110.0)	95.1 (89.5, 102.0)	106.0 (97.0, 117.0)	102.5 (92.8, 114.5)	0.002	101.6 (94.4, 113.0)	100.0 (91.4, 111.5)	100.3 (89.8, 107.0)	0.57	95.8 (90.6, 102.8)	104.5 (92.2, 113.5)	101.6 (95.0, 113.5)	0.06
PAI-1 (ng/mL)	23.9 (16.7, 33.5)	20.8 (15.8, 35.6)	25.2 (17.0, 33.2)	22.4 (17.9, 30.0)	0.71	18.8 (16.1, 29.7)	23.7 (17.0, 35.7)	27.7 (17.2, 34.3)	0.32	18.4 (14.1, 27.4)	24.5 (18.2, 31.9)	27.9 (17.7, 35.1)	0.06
α2-antiplasmin (%)	104.0 (94.0, 112.0)	99.5 (92.0, 111.0)	107.0 (102.0, 112.0)	104.0 (90.0, 112.5)	0.13	105.0 (97.0, 113.0)	104.5 (90.0, 112.0)	101.5 (92.5, 111.0)	0.65	102.0 (93.0, 111.0)	101.5 (92.0, 111.0)	108.5 (98.0, 114.0)	0.11
ETP (nM·min)	1,688.0 (1,502.0, 1,943.0)	1,762.9 (1,612.1, 2,096.5)	1,599.0 (1481.0, 1,986.0)	1,650.5 (1,503.2, 1,818.5)	0.21	1,683.0 (1,521.0, 1,788.0)	1,690.1 (1,474.0, 2,097.7)	1,737.0 (1,510.0, 1,892.9)	0.93	1,578.0 (1,496.4, 2,096.5)	1,670.0 (1,484.5, 1,781.0)	1,797.0 (1,598.5, 2,035.0)	0.14
K_s (10^{-9} cm^2)	6.4 (5.3, 7.4)	5.9 (3.4, 7.2)	6.6 (5.9, 7.4)	6.8 (5.1, 7.4)	0.08	6.6 (5.8, 7.2)	6.8 (5.6, 7.4)	6.0 (3.9, 7.2)	0.17	6.0 (3.4, 7.2)	6.7 (5.6, 7.3)	6.5 (5.9, 7.5)	0.15
CLT (min)	108.0 (95.0, 124.0)	111.0 (99.0, 129.0)	101.0 (89.0, 121.0)	108.0 (96.0, 124.5)	0.10	102.0 (88.0, 115.0)	106.0 (96.5, 119.5)	115.0 (99.3, 126.0)	0.14	106.0 (92.0, 137.9)	106.5 (97.0, 121.0)	109.0 (98.0, 119.5)	0.99

Data are given as n (%) or medians (interquartile ranges). BMI, body mass index; sPESI, simplified pulmonary embolism severity index; PE, pulmonary embolism; DVT, deep vein thrombosis; RV, right ventricular; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; VTE, venous thromboembolism; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs, high sensitivity; CRP, C-reactive protein; IL-6, interleukin 6; PF4, platelet factor 4; TAFI, thrombin activatable fibrinolysis inhibitor; PAI-1, plasminogen activator inhibitor-1; ETP, endogenous thrombin potential; CLT, clot lysis time; K_s, permeation coefficient.

Table 2 Predictors of plasma C3a in the top tertile (C3a >2.33 µg/dL) of the study patients

Variables	Unadjusted		Adjusted					
	OR (95% CI)	P	Age		Age and sex		Age and CKD	
			OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
RV dysfunction	2.77 (1.21–6.33)	0.02	2.60 (1.12–6.00)	0.03	2.51 (1.07–5.85)	0.03	2.58 (1.09–6.08)	0.03
sPESI	2.55 (1.52–4.25)	<0.001	2.45 (1.45–4.12)	0.001	2.40 (1.41–4.06)	0.001	2.39 (1.41–4.03)	0.001
Factor VIII, per 10%	1.16 (1.04–1.29)	0.009	1.16 (1.03–1.30)	0.01	1.15 (1.03–1.30)	0.02	1.21 (1.07–1.36)	0.002
K_s (10^{-9} cm^2)	0.77 (0.60–0.99)	0.04	0.78 (0.61–1.00)	0.054	0.81 (0.63–1.05)	0.11	0.82 (0.64–1.06)	0.14

RV, right ventricular; sPESI, simplified pulmonary embolism severity index; K_s, permeation coefficient; CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval.

Table 3 Predictors of plasma sC5b-9 in the top tertile (sC5b-9 >812 ng/mL) of the study patients

Variables	Unadjusted		Adjusted					
	OR (95% CI)	P	Age		Age and sex		Age and CKD	
			OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
L-lactate (mM)	2.13 (1.10–4.12)	0.02	2.35 (1.20–4.64)	0.01	2.34 (1.19–4.59)	0.01	2.35 (1.19–4.64)	0.01
Factor VIII, per 10%	1.11 (1.00–1.23)	0.04	1.12 (1.01–1.24)	0.03	1.13 (1.02–1.26)	0.02	1.12 (1.01–1.25)	0.03
α2-antiplasmin (%)	1.04 (1.01–1.08)	0.02	1.05 (1.01–1.09)	0.009	1.05 (1.01–1.09)	0.009	1.05 (1.01–1.09)	0.009

CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval.

complement activation defined as plasma C3a levels in the top tertile (*Table 2*). The ROC curve analysis demonstrated that a C3a cutoff value of 1.80 µg/dL indicated RV dysfunction with a sensitivity of 75.0%, specificity of 56.5%, and accuracy of 63.3% (AUC 0.670; 95% CI: 0.560–0.780; P=0.002).

There was association between plasma L(+)-lactate and sC5b-9 levels (*Table 3*). However, subjects with L(+)-lactate ≥2 mM did not have higher C3a and sC5b-9 levels, compared to the remaining patients.

There were no differences in troponin T levels between the C3a and sC5b-9 tertiles (*Table 1*). Both correlation and logistic regression analysis showed no association between complement activation and troponin T levels.

There were also no differences in D-dimer levels among C3a tertiles, but the lower tertile of sC5b-9 characterized by 84% higher D-dimer levels (P=0.001) as compared to the remainder.

There were no differences in thrombin generation and fibrin clot properties, as well as in fibrinolysis proteins among the tertiles of C3a and sC5b-9 (*Table 1*).

Interestingly, plasma C3a levels correlated with K_s (r=–0.21, P=0.03) and CLT (r=0.22; P=0.02), but not fibrinogen, fibrinolysis proteins, or thrombin generation, and these associations indicate formation of denser and poorly lysable clots in subjects with elevated C3a.

The patients in the lower tertile of C3a had lower FVIII activity by 13.2% (P=0.007), as compared to the remaining subjects. C3a correlated positively with FVIII (r=0.28; P=0.003) as confirmed by logistic regression analysis (*Table 2*).

The PE patients in the lower and middle tertile of sC5b-9 had lower FVIII activity by 13.4% (P=0.02), as compared to subjects in the top sC5b-9 tertile. Plasma sC5b-9 levels correlated with FVIII (r=0.20; P=0.04).

Logistic analysis with adjustment for age and sex showed that FVIII activity was a predictor of plasma sC5b-9 levels in the top tertile (OR 1.13, 95% CI: 1.02–1.26; P=0.02) (*Table 3*).

There were no differences in serum concentrations of inflammatory markers between the tertiles of C3a (*Table 1*). However, plasma C3a levels correlated weakly with CRP (r=0.23, P=0.02) and IL-6 (r=0.24; P=0.02).

The PE patients in the lower tertile of sC5b-9 had enhanced inflammatory state as reflected by 5.42-fold higher CRP (P<0.001), 97% higher IL-6 (P<0.001), and a higher white blood cell count by 34% (P=0.001), as

compared to the remaining subjects (*Table 1*).

In the study patients C3a and sC5b-9 were not associated with H3Cit (*Tables 1-3*).

Discussion

To our knowledge, this study is the first to show that enhanced complement activation characterizes patients with more severe PE, who developed RV dysfunction. Increased complement activation was associated with higher FVIII and the prothrombotic fibrin clot phenotype, but not with fibrinogen, NETs formation, or thrombin generation. We confirmed that also in acute PE enhanced complement activation is typical of subjects with increased IL-6-mediated inflammation. Our findings demonstrate that complement activation is observed in acute PE patients especially at higher early mortality risk, and this phenomenon is linked with thrombotic and inflammatory mechanisms in this disease. It might be speculated that suppression of complement activation could be beneficial in typical acute VTE patients, at least in part via attenuated blood coagulation activation, which might be of clinical importance.

The prevalence of RV dysfunction in our study patients was similar to that reported in other studies, from 40% to 70% (15,26). In a murine model, involvement of complement-related pathway genes, namely *Cfd* and *C3aR1*, in the pathophysiology of RV dysfunction has been suggested (27). Ito *et al.* (27) recently demonstrated that genes involved in the complement system (*Cfd*, *C3*, and *C3ar1* in mice and *CFD*, *C3*, and *C3AR1* in humans) were highly expressed in the RV under physiological conditions. Furthermore, *C3*-knockout mice with RV failure induced by pulmonary artery constriction show attenuation of RV dysfunction and fibrosis (27). Similar effects were found in *Cfd*-knockout mice (27). Interestingly, a C3a receptor (C3aR) antagonist significantly improved RV dysfunction in mice (27). The results of our study, particularly the observed association between C3a and RV dysfunction, seem to support the above observations. In addition, the study by Ito *et al.* showed that in patients with chronic RV failure, plasma levels of complement factor D (CFD, adipsin) correlated with the severity of RV dysfunction (27). This indicates the possible involvement of activation of the alternative complement pathway and the C3-Cfd-C3aR axis in the pathomechanisms of RV dysfunction, which remains a major cause of mortality associated with acute PE (27). The results of these studies and ours provide potential therapeutic targets for RV failure in acute PE. Importantly, complement activation has also been found in the myocardium also in other entities, such as arrhythmogenic right ventricular cardiomyopathy (ARVC), which appears to have significant clinical implications (28).

Since RV dysfunction is a well-established predictor of poor prognosis in acute PE (15), complement activation may have important clinical and prognostic significance. Thus, our novel findings expand the current knowledge on the role of the complement system in acute VTE by showing the links with PE severity.

For the first time we showed an association between C3a and sC5b-9 levels and increased FVIII, an acute phase reactant, in acute PE. Previous studies have reported that elevated FVIII is associated with an increased risk of recurrent VTE (29) and chronic thromboembolic pulmonary hypertension (CTEPH) in post-PE patients (26). High FVIII levels may persist over time after an acute VTE episode and therefore cannot be attributed to an acute phase reaction alone (29). The association between high FVIII levels and complement activation supports an important role of inflammation in acute PE.

In our study, a weak correlation between plasma C3a and both CRP and IL-6 was found. This observation might suggest that in acute PE classical complement pathway is activated, since based on previous data the complement activation by CRP is limited to C1, C4, C2 and C3 with only marginal consumption of C5b-9 (30). Moreover, surface bound CRP reduces generation and deposition of C5b-9 by the alternative pathway, due to CRP binding to factor H responsible for the activation of the complement alternative pathway (30).

We also demonstrated that enhanced complement activation, at least in part, contributed to prothrombotic plasma fibrin clot properties, involving formation of denser clots of reduced lysability tested *in vitro*, in patients with acute PE. This observation is novel and supports the data showing that complement components are present in fibrin clots generated from plasma of patients with VTE, as well as cardiometabolic diseases, including C3, that can bind to the β-chain of fibrinogen affecting CLT (9) and other complement components such as C1s, C3a, C4, C5a, C7s and C8 α-chains (4,6,7). In acute PE patients, concentrations of clot-bound C1s, C7s and C8 α-chains were negatively associated with the formation of more compact clots (6). Moreover, plasma C3 levels of >1.2 g/L in healthy individuals were associated with an increased risk of VTE during long-term follow-up (1).

Based on a logistic regression model, we showed that L(+)-lactate level was a predictor of plasma sC5b-9 in the top tertile. Previously, we demonstrated that L(+)-lactate concentrations ≥2 mM in acute PE patients were associated with impaired fibrinolysis accompanied by enhanced NET formation and increased thrombin generation, together with RV dysfunction (22,31).

It is worth noting that anticoagulant treatment, which is the cornerstone of pharmacotherapy of acute PE, may affect complement activity (32-36). Growing evidence suggests that anticoagulants, including unfractionated heparin (UFH), low-molecular-weight heparins (LMWHs) and direct factor Xa inhibitors (e.g., rivaroxaban) can modulate complement activity through a variety of mechanisms, primarily by affecting the cross-talk between the blood coagulation and complement cascades (34-36). However, in our study, all laboratory tests, including measurements of complement components, were performed on admission, before the initiation of anticoagulant treatment, which limited the impact of this confounder on the study results. Importantly, there is currently no evidence that the postulated effect of anticoagulant treatment on the complement system should determine the choice of anticoagulant and the duration of antithrombotic therapy in PE patients (15).

This study has several limitations. First, the sample size was relatively small; however, the group was representative for normotensive acute PE. High-risk PE patients and cancer patients were excluded, therefore our findings could not be extrapolated to these subsets. The associations presented here do not necessarily mean the cause-effect relationship, however they should be perceived a hypothesis generating observation, which deserves further investigation. The measurement of fibrin clot properties is poorly standardized, however our approach was successfully used to assess a prognostic role of these variables in several clinical settings (23,37). In addition, other echocardiographic modalities, such as 3D and strain imaging, were not available in all patients and were therefore not included. However, the main limitation of these technologies in acute PE patients, especially speckle tracking echocardiography, is their lack of standardization in this population and the use of different algorithms during analysis, which limits their value in daily practice. Our study does not provide mechanistic insights into the links between complement activation and the prothrombotic state in acute PE, and animal studies are needed to elucidate how complement components modulate the course of PE. Finally, it remains to be established whether complement activation can predict recurrent PE and post PE syndrome.

Conclusions

In conclusion, our findings suggest that enhanced complement activation might contribute to RV dysfunction and the fibrin related prothrombotic mechanisms in normotensive non-cancer patients with acute PE. It is tempting to speculate that modulation of complement activation could be beneficial in acute PE.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Bioethics

Committee of the Regional Chamber of Physicians in Krakow, Poland (No. 214/KBL/OIL/2022). All study participants had provided written informed consent.

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