Enhanced complement consumption and histone H3 citrullination predict disease severity and early mortality risk in non-high-risk pulmonary embolism

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INTRODUCTION

Acute pulmonary embolism (APE), a life-threatening manifestation of venous thromboembolism (VTE), is one of the most common causes of cardiovascular morbidity and mortality worldwide [1–3]. Although antithrombotic treatment is the cornerstone of VTE therapy, anticoagulants do not completely prevent thrombotic events. Up to 50% of patients with deep vein thrombosis develop post-thrombotic syndrome, and up to 4% of APE patients develop chronic thromboembolic pulmonary hypertension [4, 5]. This therapeutic gap is related to the mechanism(s) not yet fully understood, among which thrombo-inflammation seems to play a central role [4–6]. It is plausible that this pathological interaction between blood coagulation and fibrinolysis, complement activation, inflammation, and neutrophil extracellular traps (NETs) formation (i.e., NETosis) may also influence clinical presentation and prognosis in APE [4-7].

Therefore, we investigated whether the links between complement consumption, expressed by the C3a-to-C3 ratio and histone H3 hypercitrullination, which is a feature of pathological NETosis, are associated with disease severity and early mortality risk in APE.

METHODS

This article is a *post hoc* analysis of APE patients recruited between 2016 and 2021 [8]. In brief, we included 109 adult, cancer-free, non-high-risk patients with APE diagnosed according to the European Society of Cardiology guidelines [1]. The exclusion criteria were recent arterial thromboembolism, chronic anticoagulation, and pregnancy.

The simplified Pulmonary Embolism Severity Index (sPESI) and the risk of early (in-hospital or 30-day) death were calculated based on European guidelines [1]. Right ventricular (RV) dilatation was defined as an RV/left ventricle diameter ratio of >1.0 obtained from the subcostal or apical 4-chamber view on transthoracic echocardiography.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee (No. 214/KBL/OIL/2022).

The antecubital vein was used to take venous blood on admission and before initiation of anticoagulation. Plasma C3 and C3a levels were measured by ELISA (BD Bioscience, San Jose, CA, US). The C3a-to-C3 ratio was used as a marker of complement consumption, as described previously [9]. Serum levels of citrullinated histone H3 (H3Cit) and 8-isoprostane were assayed by ELISA (Cayman Chemical, Ann Arbor, MI, US).

Factor VIII (FVIII) activity was determined using the Behring Coagulation System (Siemens Healthcare Diagnostics, Marburg, Germany). To assess the fibrinolysis efficiency, plasminogen activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI) (both, Hyphen-Biomed, Neuville-sur-Oise, France), plasminogen and α_2-antiplasmin (both, Siemens Healthcare Diagnostics, Marburg, Germany) were measured along with clot lysis time (CLT), assessed using the

assay of Pieters et al. [10]. Fibrin clot permeability was determined as described previously [7]. For details, see Supplementary material.

Statistical analysis

Variables were presented as numbers and percentages or medians and interquartile ranges (IQR). Intergroup differences in continuous variables were evaluated using the Kruskal–Wallis test. Categorical variables were compared using Pearson's χ^2 test. Spearman correlation coefficients were used to evaluate the relationship between the variables. Logistic regression analysis was performed to determine predictors of the C3a-to-C3 ratio and H3Cit above the established cut-offs. The best cut-off values of H3Cit and the C3a-to-C3 ratio for sPESI ≥1 were calculated using the receiver operating characteristics curves and the Youden index. A *P*-value <0.05 was considered statistically significant. All calculations were made using STATISTICA 13.3 software package (TIBCO Software Inc., Palo Alto, CA, US).

RESULTS AND DISCUSSION

We studied 109 non-high-risk, cancer-free APE patients (51.4% male) aged from 23 to 87 years (median 58.0, IQR 48.0–70.0).

The median C3 and C3a concentrations were 7.4 (IQR 3.7–9.8) mg/ml and 1.8 (IQR 1.3–2.9) µg/dl, respectively. The median C3a-to-C3 ratio was 0.22 (IQR 0.17–0.52). The median H3Cit was 2.7 (IQR 1.9–3.9) ng/ml. There was no correlation between H3Cit and the C3a-to-C3 ratio.

The C3a-to-C3 ratio correlated with sPESI (r = 0.34; *P* <0.001) and N-terminal pro B-type natriuretic peptide (NT-proBNP) (r = 0.22; *P* = 0.020) (Supplementary material, *Table S1*). Similarly, H3Cit correlated with sPESI (r = 0.33; *P* <0.001) and NT-proBNP (r = 0.29; *P* = 0.002).

The cut-off value for the C3a-to-C3 ratio was 0.21 (sensitivity 64.5%, specificity 93.8%; area under the curve 0.793, *P* <0.001), while for H3Cit 3.1 ng/ml (sensitivity 50.5%, specificity 100.0%; area under the curve 0.690, *P* <0.001). Therefore, the C3a-to-C3(+) ratio was defined as >0.21, and H3Cit(+) as >3.1 ng/ml (Table 1).

Patients with higher H3Cit levels, regardless of the C3a-to-C3 ratio, had higher sPESI and NT-proBNP (Table 1). Moreover, individuals with a higher C3a-to-C3 ratio and/or H3Cit were more likely to have sPESI ≥1, intermediate-high-risk APE, NT-proBNP >125 pg/ml and NT-proBNP >300 pg/ml. In contrast, those in the C3a-to-C3(–)H3Cit(–) group had the lowest sPESI, early mortality risk, and prevalence of RV dilatation as well as the lowest NT-proBNP levels.

Regression analysis with adjustment for sex and chronic kidney disease showed that sPESI (OR, 2.25; 95% CI, 1.32–3.85; *P* = 0.003), intermediate-high-risk APE (OR, 2.66; 95% CI, 1.02–6.90; *P* = 0.045), NT-proBNP >300 pg/ml (OR, 3.86; 95% CI, 1.45–10.30; P = 0.007) and NT-proBNP >600 pg/ml (OR, 2.82; 95% CI, 1.17–6.82; *P* = 0.022) were predictors of the C3a-to-C3 ratio >0.21 and H3Cit >3.1 ng/ml (Supplementary material, *Table S2*).

The combined assessment of the C3a-to-C3 ratio and H3Cit predicted intermediate-high-risk APE and RV dilatation with higher specificity (78.3% and 79.7%, respectively) and accuracy (69.7% and 64.2%, respectively), compared to the evaluation of these parameters separately (Supplementary material, *Table S3*).

Furthermore, the C3a-to-C3 ratio correlated with FVIII (r = 0.26; *P* = 0.007), TAFI (r = -0.21; *P* = 0.032), K_s (r = -0.24; *P* = 0.011), and CLT (r = 0.28; *P* = 0.003). H3Cit correlated with PAI-1 (r = 0.44; *P* <0.001), CLT (r = 0.55; *P* <0.001), and endogenous thrombin potential (r = 0.29; *P* = 0.002).

Individuals in the C3a-to-C3(–)H3Cit(–) group had the lowest levels of FVIII, PAI-1, CLT, and thrombin generation. No differences were found in K_s (Table 1).

Regression analysis with adjustment for sex and chronic kidney disease showed that PAI-1 (OR, 1.05; 95% CI, 1.01–1.09; *P* = 0.008) and CLT (OR, 1.03; 95% CI, 1.01–1.05; *P* = 0.002) were predictors of the C3a-to-C3 ratio >0.21 and H3Cit >3.1 ng/ml.

There were no differences in inflammatory markers and 8-isoprostane (Table 1). However, the C3a-to-C3 ratio, but not H3Cit, correlated with white blood cells (r = 0.25; *P* = 0.009), neutrophils (r = 0.30; *P* = 0.002), C-reactive protein (r = 0.28; *P* = 0.003), and interleukin-6 (r = 0.26; *P* = 0.011).

To our knowledge, this study was the first to show that a higher C3a-to-C3 ratio, reflecting enhanced complement consumption, characterizes APE patients with more severe disease and higher early mortality risk. We also confirmed that higher H3Cit levels are associated with worse prognoses in APE, as previously reported [7]. Furthermore, the clinical value of the combined evaluation of the C3a-to-C3 ratio and H3Cit in diagnosing severe APE with higher early mortality risk was superior to assessing these parameters separately. Our results support the important role of complement activation and histone H3 citrullination in the pathogenesis of cancer-free, non-high-risk APE and indicate their prognostic significance in this entity.

We have shown that higher values of the C3a-to-C3 ratio are associated not only with increased FVIII activity but also with a prothrombotic plasma fibrin clot phenotype and lower activity of TAFI, which, besides its anti-fibrinolytic effect by modifying fibrin, has anti-inflammatory functions through inactivating anaphylatoxins C3a and C5a [11]. Additionally, H3Cit levels were moderately positively correlated with CLT and PAI-1, which underlines the role of NETosis in prothrombotic and anti-fibrinolytic mechanisms in APE [12].

Recently, it has been suggested that thrombo-inflammation may be an attractive therapeutic target in the treatment of VTE, complementary to anticoagulant therapy [4, 5]. Our findings may suggest the potential usefulness of complement- and NET-targeted therapies, particularly in patients with a more severe course of APE and higher baseline risk of early mortality. Although preliminary results

Table 1. Baseline characteristics of the study population

	C3a-to-C3 (–) H3Cit (–) (n = 31)	C3a-to-C3 (–) H3Cit (+) (n = 17)	C3a-to-C3 (+) H3Cit (–) (n = 32)	C3a-to-C3 (+) H3Cit (+) (n = 29)	*P*-value
Age, years	53.0 (46.0, 64.0)	63.0 (53.0, 72.0)	60.0 (46.5, 71.5)	61.0 (55.0, 71.0)	0.07
Male sex	19 (61.3)	9 (52.9)	14 (43.8)	14 (48.3)	0.55
BMI, kg/m^2	27.0 (25.0, 33.0)	27.0 (25.0, 32.2)	27.0 (23.5, 31.3)	27.1 (26.0, 30.4)	0.85
Medications					
Aspirin, n (%)	6 (19.4)	8 (47.1)	6 (18.8)	11 (37.9)	0.07
Statin, n (%)	19 (61.3)	12 (70.6)	14 (43.8)	19 (65.5)	0.20
Pulmonary embolism characteristics					
sPESI score	1.0 (0.0, 1.0)	2.0 (2.0, 3.0)	1.0 (1.0, 2.0)	2.0 (2.0, 3.0)	<0.001
sPESI score ≥1, n (%)	16 (51.6)	17 (100.0)	31 (96.9)	29 (100.0)	<0.001
Early mortality risk, n (%):					
• low	15 (48.4)	0	1 (3.1)	0	<0.001
• intermediate-low	15 (48.4)	12 (70.6)	22 (68.8)	18 (62.1)	
• intermediate-high	1 (3.2)	5 (29.4)	9 (28.1)	11 (37.9)	
Central PE, n (%)	18 (58.1)	9 (52.9)	21 (65.6)	13 (44.8)	0.43
Saddle PE, n (%)	10 (32.3)	4 (23.5)	7 (21.9)	5 (17.2)	0.58
Unprovoked PE, n (%)	17 (54.8)	12 (70.6)	23 (71.9)	19 (65.5)	0.51
Concomitant DVT, n (%)	21 (67.7)	8 (47.1)	10 (31.3)	17 (58.6)	0.026
RV dilatation, n (%)	6 (19.4)	5 (29.4)	14 (43.8)	15 (51.7)	0.047
Laboratory investigations					
White blood cells, 10^9/l	6.2 (5.2, 8.0)	7.1 (5.3, 8.7)	8.4 (6.0, 10.1)	8.3 (6.0, 9.7)	0.20
Neutrophils, 10^9/l	3.6 (2.9, 4.3)	3.8 (2.8, 4.8)	5.0 (3.2, 6.1)	4.1 (3.5, 6.1)	0.24
Hs-CRP, mg/l	2.5 (1.6, 6.7)	2.3 (1.8, 14.1)	5.0 (1.8, 16.3)	10.2 (2.3, 28.3)	0.19
Interleukin-6, pg/ml	3.9 (3.3, 4.4)	5.3 (3.7, 23.3)	4.1 (3.6, 8.4)	4.6 (3.4, 17.1)	0.12
hs-troponin T, pg/ml	7.8 (6.7, 9.3)	8.7 (7.8, 59.7)	11.0 (7.2, 25.1)	16.4 (7.2, 40.0)	0.09
NT-proBNP, pg/ml	113.0 (67.0, 292.0)	767.0 (197.0, 1241.0)	495.5 (151.0, 995.5)	996.0 (425.0, 1992.0)	<0.001
NT-proBNP >125 pg/ml, n (%)	14 (45.2)	14 (82.4)	26 (81.3)	25 (86.2)	0.001
NT-proBNP >300 pg/ml, n (%)	7 (22.6)	11 (64.7)	19 (59.4)	22 (75.9)	<0.001
NT-proBNP >600 pg/ml, n (%)	6 (19.4)	10 (58.8)	11 (34.4)	17 (58.6)	0.006
Coagulation, fibrinolysis, and oxidative stress variables					
Fibrinogen, g/l	3.3 (2.8, 3.6)	3.2 (2.8, 4.4)	3.5 (3.0, 3.9)	3.4 (2.9, 4.2)	0.51
D-dimer, ng/ml	3750.0 (1989.0, 6210.0)	2479.0 (1622.0, 5307.0)	4218.5 (2133.0, 6448.3)	3271.0 (1760.0, 5293.0)	0.76
Factor VIII, %	132.0 (114.0, 150.0)	167.0 (136.0, 182.0)	164.0 (126.0, 185.5)	145.0 (132.0, 172.0)	0.008
Plasminogen, %	103.0 (98.0, 117.0)	106.0 (97.0, 111.0)	107.5 (98.0, 113.5)	103.0 (93.0, 116.0)	0.86
TAFI activity, %	108.0 (97.0, 119.0)	95.0 (90.6, 110.0)	99.4 (89.6, 105.5)	101.2 (94.4, 110.0)	0.08
PAI-1, ng/ml	19.9 (13.6, 28.0)	24.5 (17.9, 38.1)	22.0 (14.5, 27.8)	34.0 (20.0, 38.1)	0.002
α_2-antiplasmin, %	107.0 (94.0, 113.0)	107.0 (100.0, 111.0)	102.0 (93.5, 111.5)	100.0 (92.0, 111.0)	0.62
K_s, 10^{-9}cm^2	7.0 (6.1, 7.4)	6.1 (4.9, 7.0)	6.2 (4.5, 7.5)	6.2 (5.1, 6.9)	0.18
CLT, min	94.0 (82.0, 104.0)	127.0 (109.0, 149.0)	100.3 (93.0, 115.0)	121.0 (111.0, 132.0)	<0.001
ETP, nM × min	1568.0 (1436.0, 1749.0)	1865.0 (1683.0, 2111.3)	1641.5 (1496.0, 1903.4)	1774.0 (1638.0, 2088.0)	0.018
8-isoprostane, pg/ml	235.0 (234.0, 269.0)	435.0 (253.0, 471.0)	257.0 (215.0, 327.0)	384.5 (264.0, 524.0)	0.23

Data were given as numbers and percentages or medians and interquartile ranges

The cut-off values of the C3a-to-C3 ratio and H3Cit were determined by receiver operating characteristics curve analysis. The C3a-to-C3 ratio (+) was defined as >0.21, while H3Cit (+) was defined as >3.1 ng/ml

Abbreviations: BMI, body mass index; CLT, clot lysis time; CRP, C-reactive protein; DVT, deep vein thrombosis; ETP, endogenous thrombin potential; H3Cit, citrullinated histone H3; hs, high-sensitivity; K_s, permeation coefficient; NT-proBNP, N-terminal pro B-type natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; PE, pulmonary embolism; sPESI, simplified pulmonary embolism severity index; RV, right ventricular; TAFI, thrombin-activatable fibrinolysis inhibitor

of such interventions are promising, further studies are needed in APE patients. However, due to their relatively low cost, easy testing, and quick turnaround time, both C3 and C3a, as well as H3Cit, could potentially be used in the future for risk stratification in APE, following standardization and validation of the methodology.

Study limitations

First, the sample size was limited, though well characterized and representative of non-high-risk noncancer APE. Second, H3Cit is not entirely specific to NETosis. Third, although specificity and diagnostic accuracy of the combined measurement of the C3a-to-C3 ratio and H3Cit were relatively high, sensitivity of this assessment was very low. Moreover, reported correlations were rather weak. Finally, the associations presented here do not necessarily mean the cause-effect relationship. However, they should be perceived as hypothesis-generating observations and deserve further studies.

CONCLUSIONS

Enhanced complement consumption and histone H3 citrullination can predict disease severity and early mortality risk in non-high-risk pulmonary embolism.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

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