

## Supplementary material

Rostoff P, Zabczyk M, Natorska J, et al. Enhanced complement consumption and histone H3 citrullination predict disease severity and early mortality risk in non-high-risk pulmonary embolism. *Pol Heart J.* 2024.

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**Table S1.** Correlation coefficients (Spearman's rho) of the C3a-to-C3 ratio and citrullinated histone H3 with disease severity, early mortality risk and parameters of the prothrombotic state and inflammation in the study patients

Parameter	Correlation with the C3a-to-C3 ratio		Correlation with H3Cit (ng/mL)	
	r	p	r	p
<b>Parameters of disease severity and early mortality risk</b>				
sPESI	0.34	<0.001	0.33	<0.001
NT-proBNP (pg/mL)	0.22	0.020	0.29	0.002
<b>Parameters of the prothrombotic state</b>				
Factor VIII (%)	0.26	0.007	0.12	0.231
ETP (nM*min)	0.18	0.063	0.29	0.002
CLT (min)	0.28	0.003	0.55	<0.001
K <sub>s</sub> (10 <sup>-9</sup> cm <sup>2</sup> )	- 0.24	0.011	- 0.11	0.238
TAFI (%)	- 0.21	0.032	0.01	0.917
PAI-1 (ng/mL)	0.12	0.213	0.44	<0.001
<b>Parameters of inflammation</b>				

<b>White blood cells (<math>10^9/L</math>)</b>	0.25	<b>0.009</b>	0.04	0.660
<b>Neutrophils (<math>10^9/L</math>)</b>	0.30	<b>0.002</b>	0.01	0.948
<b>hsCRP (mg/L)</b>	0.28	<b>0.003</b>	0.02	0.847
<b>Interleukin-6 (pg/mL)</b>	0.26	<b>0.011</b>	0.05	0.662

Abbreviations: CLT – clot lysis time, CRP – C-reactive protein, 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, RV – right ventricular, sPESI – simplified pulmonary embolism severity index, TAFI – thrombin-activatable fibrinolysis inhibitor

**Table S2.** Predictors of the C3a-to-C3 ratio  $>0.21$  and citrullinated histone H3  $>3.1$  ng/mL in the study patients\*

Parameter	<b>C3a-to-C3 ratio <math>&gt;0.21</math> and H3Cit <math>&gt;3.1</math> ng/mL</b>							
	<b>Unadjusted</b>		<b>Age-adjusted</b>		<b>Age- and sex-adjusted</b>		<b>Sex - and CKD-adjusted</b>	
	<b>OR (95% CI)</b>	<b>p</b>	<b>OR (95% CI)</b>	<b>p</b>	<b>OR (95% CI)</b>	<b>p</b>	<b>OR (95% CI)</b>	<b>p</b>
NT-proBNP $>300$ pg/mL	3.65 (1.40-9.51)	0.008	3.49 (1.33-9.14)	0.011	3.49 (1.33-9.16)	0.011	3.86 (1.45-10.30)	0.007
NT-proBNP $>600$ pg/mL	2.78 (1.16-6.65)	0.022	2.58 (1.05-6.30)	0.038	2.57 (1.05-6.29)	0.038	2.82 (1.17-6.82)	0.022
Intermediate-high risk APE	2.65 (1.04-6.76)	0.042	2.37 (0.87-6.44)	0.091	2.36 (0.86-6.46)	0.095	2.66 (1.02-6.90)	0.045
sPESI	2.21 (1.31-3.72)	0.003	2.13 (1.25-3.63)	0.005	2.14 (1.25-3.66)	0.005	2.25 (1.32-3.85)	0.003
PAI-1, ng/mL	1.05 (1.01-1.09)	0.009	1.05 (1.01-1.09)	0.009	1.05 (1.01-1.09)	0.009	1.05 (1.01-1.09)	0.008
CLT, min	1.03 (1.01-1.05)	0.002	1.03 (1.01-1.05)	0.004	1.03 (1.01-1.05)	0.004	1.03 (1.01-1.05)	0.002

\* The cut-off values of H3Cit and the C3a-to-C3 ratio were determined by ROC curve analysis for sPESI  $\geq 1$

Abbreviations: APE – acute pulmonary embolism, CI – confidence interval, H3Cit – citrullinated histone H3, CKD – chronic kidney disease, CLT – clot lysis time, NT-proBNP – N-terminal pro-B-type natriuretic peptide, OR – odds ratio, PAI-1 – plasminogen activator inhibitor-1, sPESI – simplified pulmonary embolism severity index

**Table S3.** Diagnostic value of the C3a-to-C3 ratio >0.21 and citrullinated histone H3 >3.1 ng/mL in the study patients\*

<b>Diagnostic value</b>	<b>C3a-to-C3 ratio &gt; 0.21 and H3Cit &gt; 3.1 ng/mL</b>	<b>C3a-to-C3 ratio &gt; 0.21</b>	<b>H3Cit &gt; 3.1 ng/mL</b>
<b>Intermediate-high risk APE</b>			
<b>Sensitivity, %</b>	42.3 (23.4-63.1)	76.9 (56.4-91.0)	61.5 (40.6-79.8)
<b>Specificity, %</b>	78.3 (67.9-86.6)	50.6 (39.4-61.8)	63.9 (52.6-74.1)
<b>Accuracy, %</b>	69.7 (60.2-78.2)	56.9 (47.1-66.3)	63.3 (53.5-72.3)
<b>Positive predictive value, %</b>	37.9 (25.0-52.9)	32.8 (26.5-39.8)	34.8 (26.0-44.7)
<b>Negative predictive value, %</b>	81.3 (75.4-86.0)	87.5 (77.1-93.6)	84.1 (76.1-89.9)
<b>sPESI ≥ 1</b>			
<b>Sensitivity, %</b>	31.2 (22.0-41.6)	64.5 (53.9-74.2)	49.5 (38.9-60.0)
<b>Specificity, %</b>	100.0 (79.4-100.0)	93.8 (69.8-99.8)	100.0 (79.4-100.0)
<b>Accuracy, %</b>	41.3 (31.9-51.1)	68.8 (59.2-77.3)	56.9 (47.1-66.3)
<b>Positive predictive value, %</b>	100.0 (88.1-100.0)	98.4 (89.9-99.8)	100.0 (92.3-100.0)
<b>Negative predictive value, %</b>	20.0 (17.9-22.3)	31.3 (25.2-38.1)	25.4 (21.8-29.4)
<b>Right ventricular dilatation</b>			
<b>Sensitivity, %</b>	37.5 (22.7-54.2)	72.5 (56.1-85.4)	50.0 (33.8-66.2)
<b>Specificity, %</b>	79.7 (68.3-88.4)	53.6 (41.2-65.7)	62.3 (49.8-73.7)
<b>Accuracy, %</b>	64.2 (54.5-73.2)	60.6 (50.7-69.8)	57.8 (48.0-67.2)
<b>Positive predictive value, %</b>	51.7 (36.7-66.5)	47.5 (39.8-55.5)	43.5 (33.3-54.3)
<b>Negative predictive value, %</b>	68.8 (62.7-74.2)	77.1 (66.0-85.4)	68.3 (60.0-75.5)

\* The cut-off values of H3Cit and the C3a-to-C3 ratio were determined by ROC curve analysis for sPESI ≥1

Abbreviations: APE – acute pulmonary embolism, H3Cit – citrullinated histone H3, sPESI – simplified pulmonary embolism severity index