

## **Supplementary material**

### **Appendix 1: Description of the clinical characteristic of the outlier**

The outlier identified in the analysis of NETs measurements is a 72 years old man undergoing aortic valve replacement with an EuroScore of 1.00%. He had severe symptomatic aortic stenosis with an impairment of exercise tolerance (NYHA class III). He was not known to have other medical comorbidities. He had a normal left ventricular ejection fraction before surgery (55%). Before surgery, he had a total leukocyte count of 7.9, a neutrophil count of 5.9 and a lymphocyte count of 0.3. He underwent aortic valve replacement with a cardiopulmonary bypass duration of 52 minutes, an aortic cross clamping time of 39 minutes and an intra-operative fluid balance of 2.2L. Pre-operative interleukin-6 levels and CRP levels were 2.29 and 0.59, respectively. MPO, H3Cit and PTX3 levels before CBP were 457 (ng/mL), 0.51 (ng/mL) and 980 (pg/mL). He did not develop complications after surgery and was discharged from the ICU after 24 hours. He was discharged from the hospital 7 days after surgery.

**Table S1: Comparison between markers in healthy controls and cardiac surgery patients before initiation of cardiopulmonary bypass.**

Marker	Healthy controls	Cardiac surgery: Low risk	Cardiac surgery: High risk	p-value
<b>IL-6 (pg/mL)</b>	1.26 (IQR: 1.17; 2.39)	2.30 (IQR: 1.78; 2.47)	5.38 (IQR: 3.82; 7.77)	0.004 <sup>a</sup>
<b>CRP (mg/L)</b>	0.66 (IQR: 0.97; 2.57)	1.58 (IQR: 0.97; 2.57)	1.86 (IQR: 0.86; 4.34)	0.012 <sup>b</sup>
<b>MPO (ng/mL)</b>	389.5 (IQR: 314.0; 513.0)	356.1 (IQR: 245.2; 470.1)	375.2 (IQR: 204.5; 712.3)	0.82
<b>H3Cit (ng/mL)</b>	0.88 (IQR: 0.36; 1.36)	0.64 (IQR: 0.49; 0.95)	0.63 (IQR: 0.25; 2.26)	0.89
<b>PTX3 (pg/mL)</b>	1033 (IQR: 749; 1448)	1040 (IQR: 669; 1287)	1022 (IQR: 501; 2479)	0.90

Legend: Results are presented in medians (interquartile range). Abbreviations: IL-6: Interleukin 6, CRP: C reactive protein, MPO: myeloperoxidase, H3Cit: Histone 3 Citrullinated, PTX3: Pentraxin related protein 3. P-value results from the comparison testing by the Kruskal-Wallis test. Multiple comparison testing for sub-group comparisons with Bonferroni correction: <sup>a</sup> Difference between Healthy controls and high risk is significant (p=0.001), <sup>b</sup> Difference between Healthy controls and high risk is significant (p=0.012).

**Table S2: Measurements of circulating interleukin 6 (IL-6; pg/mL)**

	All patients (n=24)	Low risk (n=12)	High risk (n=12)	p-value
<b>Before CPB</b>	3.05 (2.21; 6.02)	2.30 (1.78; 2.37)	5.38 (3.82; 7.77)	0.004
<b>3 hours after CPB</b>	249.4 (158.7; 587.2)	221.1 (125.2; 344.7)	333.8 (236.1; 681.1)	0.20
<b>Absolute change</b>	245.4 (153.4; 576.6)	219.3 (123.0; 343.3)	324.1 (233.5; 670.6)	0.24
<b>Fold-change</b>	90.1 (37.7; 196.6)	106.3 (41.7; 237.5)	62.4 (32.7; 165.1)	0.48

Legend: Results are presented in medians (interquartile range). Abbreviation: CPB: cardiopulmonary bypass. P-value results from the comparison testing by the Kruskal-Wallis test.

**Table S3: Measurements of C-reactive protein (CRP; mg/L)**

	All patients (n=24)	Low risk (n=12)	High risk (n=12)	p-value
<b>Before CPB</b>	1.68 (0.91; 3.28)	1.58 (0.97; 2.57)	1.86 (0.86; 4.34)	0.67
<b>3 hours after CPB</b>	2.30 (1.26; 3.74)	2.34 (1.30; 2.49)	2.24 (1.26; 5.56)	0.67
<b>Absolute change</b>	0.28 (0.04; 0.55)	0.16 (0.03; 0.44)	0.41 (0.11; 0.66)	0.20
<b>Fold change</b>	1.15 (1.02; 1.52)	1.08 (1.02; 1.28)	1.25 (1.03; 1.77)	0.35

Legend: Results are presented in medians (interquartile range). Abbreviation: CPB: cardiopulmonary bypass. P-value results from the comparison testing by the Kruskal-Wallis test.

**Table S4: Measurements of Myeloperoxidase (MPO; ng/mL)**

	All patients (n=24)	Low risk (n=12)	High risk (n=12)	p-value
<b>Before CPB</b>	356.1 (228.1; 641.3)	356.1 (245.2; 470.1)	375.2 (204.5; 712.3)	0.76
<b>3 hours after CPB</b>	582.9 (452.4; 752.5)	597.9 (439.3; 668.1)	555.7 (452.4; 850.9)	0.71
<b>Absolute change</b>	180.5 (18.4; 343.4)	179.4 (56.9; 422.9)	223.4 (46.8; 323.2)	0.80
<b>Fold change</b>	1.47 (1.12; 2.64)	1.68 (1.12; 2.72)	1.43 (1.08; 2.44)	0.76

Legend: Results are presented in medians (interquartile range). Abbreviation: CPB: cardiopulmonary bypass. P-value results from the comparison testing by the Kruskal-Wallis test.

**Table S5: Measurements of citrullinated histone 3 (H3Cit; ng/mL)**

	<b>All patients (n=24)</b>	<b>Low risk (n=12)</b>	<b>High risk (n=12)</b>	<b>p-value</b>
<b>Before CPB</b>	0.64 (0.40; 1.15)	0.64 (0.49; 0.95)	0.63 (0.25; 2.26)	1.00
<b>3 hours after CPB</b>	1.91 (1.20; 2.96)	2.00 (1.33; 2.47)	1.71 (0.93; 3.75)	0.71
<b>Absolute change</b>	0.87 (0.46; 1.62)	1.49 (0.23; 1.98)	0.75 (0.46; 1.36)	0.63
<b>Fold change</b>	3.42 (1.45; 5.04)	3.76 (1.22; 5.04)	3.07 (1.63; 5.09)	0.89

Legend: Results are presented in medians (interquartile range). Abbreviation: CPB: cardiopulmonary bypass. P-value results from the comparison testing by the Kruskal-Wallis test.

**Table S6: Measurements of Pentraxin related protein 3 (PTX3; pg/mL)**

	<b>All patients (n=24)</b>	<b>Low risk (n=12)</b>	<b>High risk (n=12)</b>	<b>p-value</b>
<b>Before CPB</b>	1040 (593; 1349)	1041 (669; 1287)	1022 (501; 2479)	0.84
<b>3 hours after CPB</b>	2993 (1756; 3823)	2312 (1514; 3586)	3114 (2228; 4665)	0.32
<b>Absolute change</b>	1876 (992; 2309)	1100 (953; 2286)	2189 (1773; 2342)	0.16
<b>Fold change</b>	2.66 (1.93; 5.01)	2.27 (1.78; 6.07)	2.87 (2.12; 5.01)	0.51

Legend: Results are presented in medians (interquartile range). Abbreviation: CPB: cardiopulmonary bypass. P-value results from the comparison testing by the Kruskal-Wallis test.

**Table S7: Correlations between circulating NETs measurements and other inflammatory biomarkers during the study**

<b>Timepoint</b>	<b>Biomarker</b>	<b>Spearman correlation coefficient (r)</b>	<b>p-value</b>
<b>NETs measurements before CPB</b>	IL-6	0.02	0.93
	CRP	-0.06	0.79
	MPO	0.44	0.03
	H3Cit	0.20	0.36
	PTX3	0.00	0.99
<b>NETs measurements 3 hours after CPB separation</b>	IL-6	-0.04	0.86
	CRP	0.08	0.71
	MPO	0.62	0.001
	H3Cit	0.52	0.01
	PTX3	0.25	24
<b>Δ NETs measurements</b>	IL-6	0.00	1.00
	CRP	-0.19	0.37
	MPO	0.58	0.003
	H3Cit	0.67	<0.001
	PTX3	0.31	0.15

Abbreviations: CBP: cardiopulmonary bypass, CRP: C-related protein, H3Cit: Histone 3 Citrullinated, IL-6: Interleukin-6, MPO: myeloperoxidase, NETs: Neutrophil extracellular traps, PTX3: Pentraxin-related protein 3.

**Table S8: Change in circulating NETs measurements and complications in the post-operative period**

	<b>No complication</b>	<b>Complication</b>	<b>p-value</b>
<b>Acute kidney injury</b>	0.041 (0.010; 0.069)	0.023 (0.018; 0.038)	0.26
<b>Difficult CPB separation</b>	0.027 (-0.003; 0.038)	0.045 (0.038; 0.132)	0.20
<b>Right ventricular dysfunction after CPB separation</b>	0.032 (-0.016; 0.046)	0.021 (0.010; 0.038)	0.60

Abbreviations: CPB: cardiopulmonary bypass

**Table S9: Post-operative NETs measurements according to blood transfusion during surgery.**

	<b>No blood transfusions (n=18)</b>	<b>≥ 1 blood transfusions (n=6)</b>	<b>p-value</b>
<b>NETs measurements 3 hours after CPB</b>	0.090 (0.064; 0.157)	0.077 (0.067; 0.093)	0.68
<b>Difference in NETs measurements from baseline</b>	0.032 (0.014; 0.071)	0.041 (0.031; 0.045)	0.85

Abbreviation: CPB: cardiopulmonary bypass