

DOUBLE-STRANDED DNA AND NETs COMPONENTS IN RELATION TO CLINICAL OUTCOME AFTER ST-ELEVATION MYOCARDIAL INFARCTION

SUPPLEMENTARY MATERIAL

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Reference: Figures S2 and S3 were created using software from IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Available from <https://www.ibm.com/se-en/products/spss-statistics>.

Figure S1. Study flow chart.

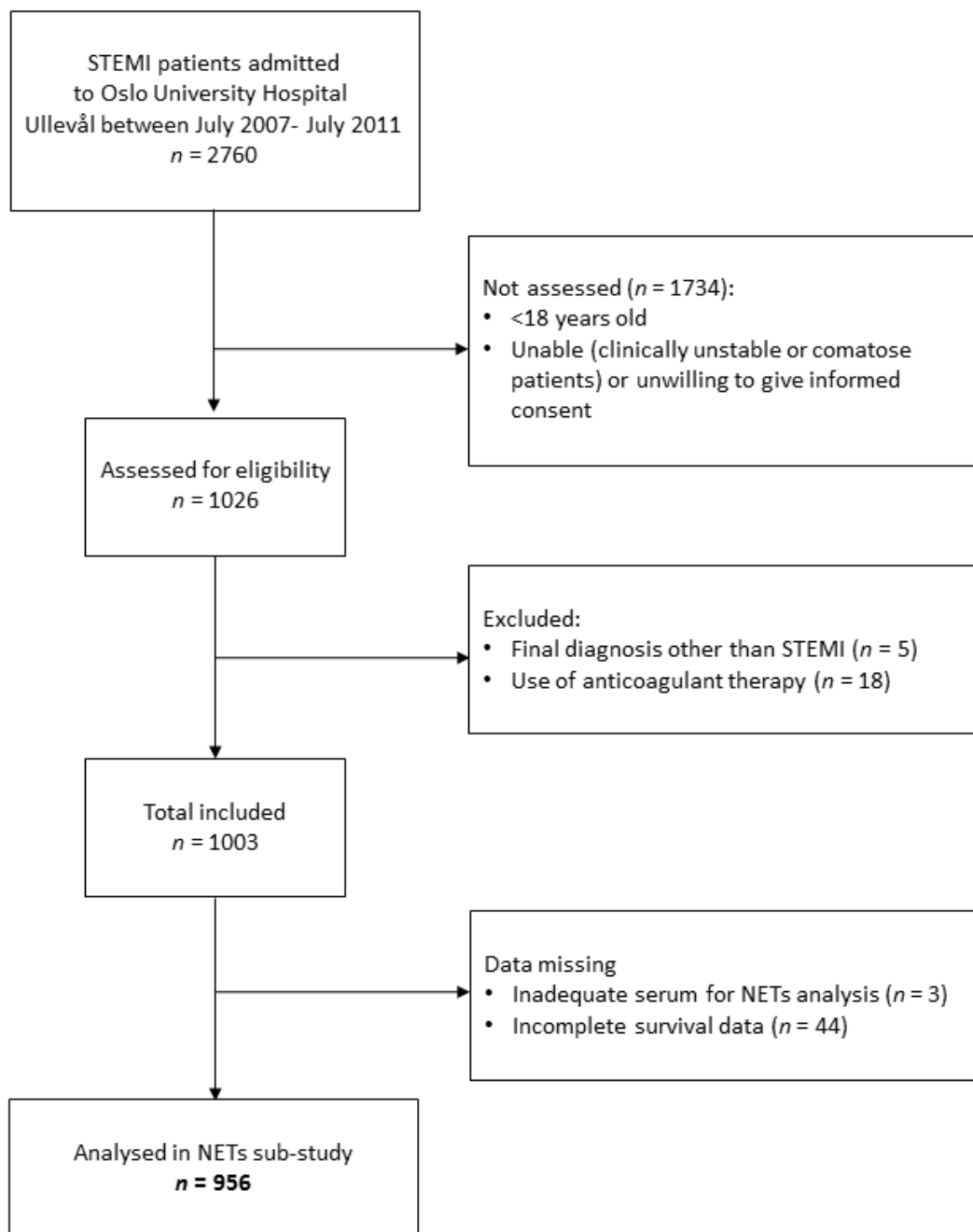


Figure S2. Scatter-plots demonstrating the relationship between potential NETs-related markers.

The r - and p -values refer to Spearman's rho.

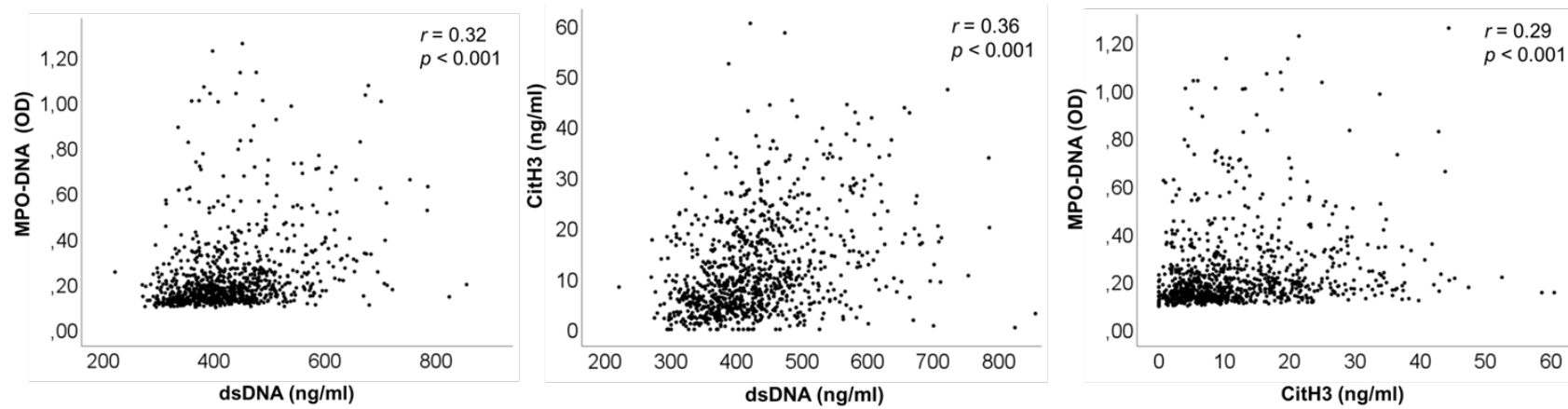


Figure S3. Levels of D-dimer and prothrombin fragment 1+2 (F1+2), according to below- or above-median levels of dsDNA. The p -values refer to Mann Whitney U tests. The height of each box represents the interquartile range (IQR), the horizontal line within each box is the median, and whiskers indicate 1.5 IQR.

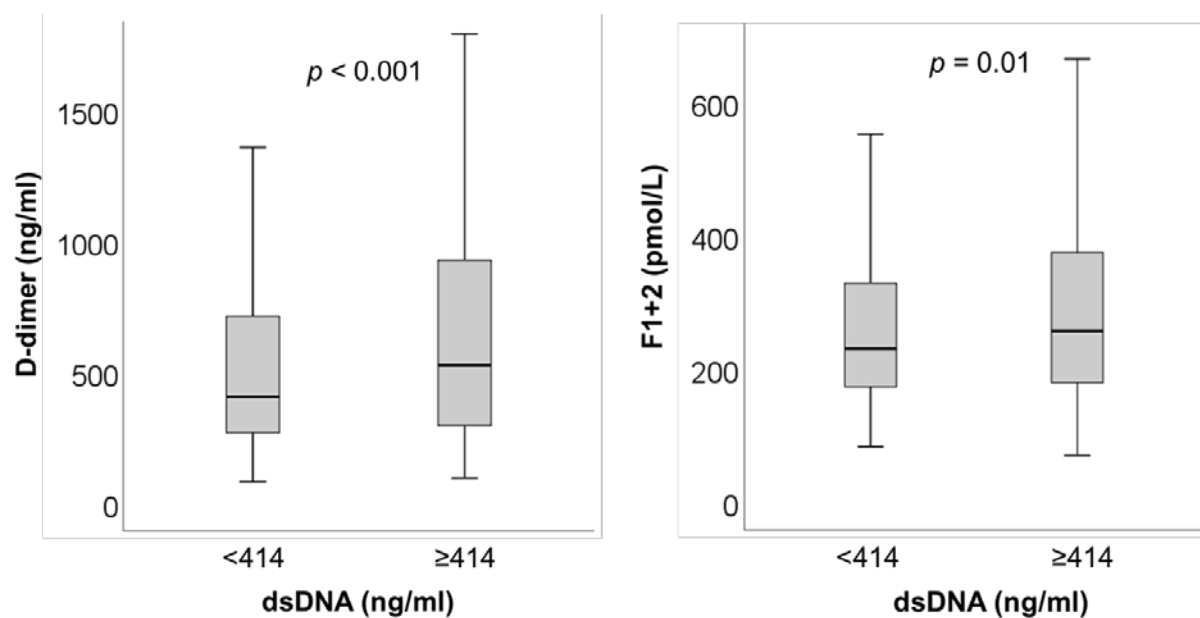


Table S1. Correlation table showing Spearman rho (r_s) analyses with 95% confidence intervals (CI) calculated using the Fisher Z transformation. Applying a Bonferroni correction for multiple testing, p-values <0.0024 are regarded as statistically significant.

		dsDNA	MPO-DNA	CitH3	Leukocyte count	Peak TnT	NT-proBNP	D-dimer	F1+2
dsDNA	r_s		0.32	0.36	0.22	0.17	0.19	0.17	0.08
	p	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.021
	n		956	955	952	956	926	950	950
	95% CI		0.26 to 0.38	0.30 to 0.41	0.16 to 0.28	0.11 to 0.23	0.13 to 0.25	0.11 to 0.23	0.01 to 0.14
MPO-DNA	r_s	0.32		0.29	0.18	0.12	-0.04	-0.02	-0.06
	p	<0.001	-	<0.001	<0.001	<0.001	0.255	0.552	0.068
	n	956		955	952	956	926	950	950
	95% CI	0.26 to 0.38		0.30 to 0.35	0.12 to 0.24	0.06 to 0.18	-0.10 to 0.03	-0.08 to 0.04	-0.12 to 0.00
CitH3	r_s	0.36	0.29		0.092	-0.02	0.10	-0.02	-0.05
	p	<0.001	<0.001	-	0.005	0.594	0.001	0.540	0.094
	n	955	955		951	955	925	949	949
	95% CI	0.30 to 0.41	0.30 to 0.35		0.03 to 0.15	-0.08 to 0.05	0.04 to 0.17	-0.08 to 0.04	-0.12 to 0.01

Table S2. Baseline characteristics of the study population according to above- or below-median levels of MPO-DNA and CitH3.

	MPO-DNA			CitH3		
	< median	≥ median	<i>p</i> *	< median	≥ median	<i>p</i> *
Age, mean (range)	62.0 (32-94)	59.5 (24-91)	0.001	60.9 (24-90)	60.6 (32-94)	0.675
Male sex	374 (77.9)	393 (82.6)	0.071	370 (77.4)	396 (83.0)	0.029
Current smoking	219 (45.6)	231 (48.5)	0.368	247 (51.7)	203 (42.6)	0.005
Hypertension	178 (37.1)	148 (31.1)	0.051	166 (34.7)	159 (33.3)	0.649
Diabetes	59 (12.3)	63 (13.2)	0.662	56 (11.7)	66 (13.8)	0.326
Previous CVD	117 (24.4)	111 (23.4)	0.715	104 (21.8)	123 (25.8)	0.139
Medication on admission						
- Single or DAPT	120 (25.0)	102 (21.4)	0.191	109 (22.8)	112 (23.5)	0.804
- Statins	125 (26.0)	90 (18.9)	0.008	110 (23.0)	105 (22.0)	0.711
- Beta-blockers	93 (19.4)	89 (18.7)	0.790	79 (16.5)	103 (21.6)	0.046
- ACEi/ARB	124 (25.8)	110 (23.1)	0.327	122 (25.5)	112 (23.5)	0.463
BMI, kg/m ²	26.7 (24.2, 29.2)	26.6 (24.3, 29.2)	0.850	26.9 (24.3, 29.2)	26.4 (24.3, 29.1)	0.466
Leukocyte count, x10 ⁹ /L	10.3 (8.4, 12.5)	11.3 (9.2, 14.1)	<0.001	10.4 (8.5, 12.9)	11.0 (8.9, 13.5)	0.028
Platelet count, x10 ⁹ /L	218 (187, 264)	222 (189, 266)	0.713	219 (185, 265)	222 (191, 265)	0.397
Total cholesterol, mmol/L	4.77 ±1.08	4.95 ±1.14	0.014	4.81 ±1.09	4.92 ±1.14	0.101
LDL-cholesterol, mmol/L	3.18 ±1.00	3.30 ±1.02	0.059	3.21 ±0.99	3.28 ±1.04	0.328
Fasting glucose, mmol/L	5.8 (5.2, 6.6)	5.8 (5.2, 6.7)	0.629	5.7 (5.2, 6.6)	5.8 (5.3, 6.7)	0.300
HbA _{1c} , %	6.0 (5.7, 6.3)	5.9 (5.6, 6.2)	0.054	5.9 (5.7, 6.2)	5.9 (5.6, 6.3)	0.304
NT-proBNP, ng/L	32 (12, 119)	28 (9, 118)	0.234	26 (10, 108)	39 (11, 129)	0.016
Peak TnT, ng/L	3390 (1575, 6538)	4400 (2018, 7840)	0.001	4055 (1715, 7538)	3760 (1720, 7110)	0.449
CRP, mg/L	12.5 (6.7, 29.2)	14.9 (7.5, 35.3)	0.128	12.4 (6.8, 27.3)	14.4 (7.5, 36.1)	0.080
LVEF ≤ 40 %	57 (15.6)	88 (23.6)	0.006	72 (19.6)	73 (19.7)	0.955
Prehospital thrombolysis	50 (10.4)	57 (12.0)	0.445	59 (12.3)	48 (10.1)	0.264
Anterior wall infarction	185 (38.5)	228 (47.9)	0.003	192 (40.2)	221 (46.3)	0.055
Time from symptom onset to blood sampling, hours	24 (19, 34)	23 (18, 32)	0.066	23 (18, 30)	24 (19, 37)	0.002

Values given as mean ($\pm$ SD) or (range), median (25th, 75th percentiles) or numbers (%) as appropriate.

**p*-value of Mann-Whitney U, Student's *t*, or Chi squared tests comparing groups with below- and above-median levels of MPO-DNA and CitH3.

DAPT: dual anti-platelet therapy

ACEi: angiotensin-converting enzyme inhibitor

ARB: angiotensin II receptor blocker

BMI: body mass index

LDL: low-density lipoprotein

HbA1c: hemoglobin A1c

Table S3. Baseline characteristics of the study population at inclusion according to composite endpoint and all-cause mortality groups.

	Composite endpoint			All-cause mortality		
	+	-	<i>p</i> *	+	-	<i>p</i> *
	(n = 190)	(n = 766)		(n = 76)	(n = 880)	
Age, mean (range)	63.9 (29-94)	60.0 (24-90)	<0.001	71.5 (34-94)	59.8 (24-90)	<0.001
Male gender	147 (77.4)	620 (80.9)	0.269	48 (63.2)	719 (81.7)	<0.001
Current smoking	87 (45.8)	363 (47.5)	0.670	33 (43.4)	417 (47.5)	0.495
Hypertension	74 (38.9)	252 (32.9)	0.115	38 (50.0)	288 (32.7)	0.002
Diabetes	32 (16.8)	90 (11.7)	0.060	18 (23.7)	104 (11.8)	0.003
Previous CVD	62 (32.6)	166 (21.7)	0.002	30 (39.5)	198 (22.5)	0.001
Medication on admission						
- Single or DAPT	63 (33.2)	159 (20.8)	<0.001	34 (44.7)	188 (21.4)	<0.001
- Statin	53 (27.9)	162 (21.1)	0.046	25 (32.9)	190 (21.6)	0.024
- Beta-blocker	47 (24.7)	135 (17.6)	0.025	26 (34.2)	156 (17.7)	<0.001
- ACEi/ARB	62 (32.6)	172 (22.5)	0.003	32 (42.1)	202 (23.0)	<0.001
BMI, kg/m ²	26.3 (24.2, 29.4)	26.6 (24.3, 29.1)	0.795	25.5 (23.9, 28.7)	26.7 (24.3, 29.2)	0.068
Leukocyte count, x 10 ⁹ /L	10.8 (8.4, 14.0)	10.7 (8.8, 13.1)	0.790	10.8 (8.4, 13.6)	10.7 (8.8, 13.2)	0.943
Platelet count, x 10 ⁹ /L	228 (191, 271)	219 (187, 263)	0.156	249 (200, 305)	219 (187, 263)	<0.001
Total cholesterol, mmol/L	4.71 ±1.21	4.90 ±1.09	0.041	4.40 ±1.27	4.90 ±1.10	<0.001
LDL-cholesterol, mmol/L	3.10 ±1.14	3.27 ±0.98	0.043	2.79 ±1.16	3.28 ±0.99	<0.001
Fasting glucose, mmol/L	5.9 (5.3, 6.9)	5.7 (5.2, 6.6)	0.030	6.1 (5.2, 7.5)	5.8 (5.2, 6.6)	0.017
HbA _{1c} , %	6.0 (5.7, 6.3)	5.9 (5.6, 6.2)	0.114	6.1 (5.8, 6.6)	5.9 (5.6, 6.2)	0.001
NT-proBNP, ng/L	50 (13, 201)	28 (10, 108)	0.003	102 (34, 336)	27 (10, 108)	<0.001
Peak TnT, ng/L	3790 (1410, 7458)	3875 (1810, 7183)	0.424	3960 (1485, 8515)	3845 (1753, 7140)	0.916
CRP, mg/L	14.8 (6.8, 35.5)	13.5 (7.1, 31.9)	0.968	16.4 (8.0, 74.0)	13.3 (7.0, 31.4)	0.243
LVEF ≤ 40 %	37 (26.4)	108 (18.1)	0.025	20 (35.1)	125 (18.4)	0.002
Prehospital thrombolysis	13 (6.8)	94 (12.3)	0.034	2 (2.6)	105 (11.9)	0.014
Anterior wall infarction	75 (39.5)	338 (44.1)	0.247	32 (42.1)	381 (43.3)	0.841
Time from symptom onset to blood sampling, hours	24 (19, 32)	24 (18, 33)	0.961	24.5 (20.0, 36.0)	24.0 (18.0, 32.0)	0.333

Values given as mean ($\pm$ SD) or (range), median (25th, 75th percentiles) or numbers (%) as appropriate.

**p*-value of Mann-Whitney U, Student's *t*, or Chi squared tests comparing groups with (+) or without (-) clinical endpoint as appropriate

DAPT: dual anti-platelet therapy

ACEi: angiotensin-converting enzyme inhibitor

ARB: angiotensin II receptor blocker

BMI: body mass index

LDL: low-density lipoprotein

HbA1c: hemoglobin A1c

Table S4. Circulating levels of potential NETs-related markers in patients with (+) and without (-) a clinical endpoint.

	Primary composite endpoint			All-cause mortality		
	+	-	<i>p</i> *	+	-	<i>p</i> *
	(n = 190)	(n = 766)		(n = 76)	(n = 880)	
dsDNA, ng/ml	429 (371, 481)	412 (372, 466)	0.255	460 (407, 508)	411 (370, 466)	<0.001
MPO-DNA, OD	0.166 (0.138, 0.244)	0.181 (0.139, 0.267)	0.329	0.167 (0.140, 0.255)	0.178 (0.138, 0.261)	0.909
CitH3, ng/ml	8.92 (4.48, 16.52)	9.32 (4.91, 17.30)	0.459	10.25 (4.96, 17.32)	9.07 (4.83, 17.24)	0.598

Values are given as median (25th, 75th percentiles).

**p*-value of Mann-Whitney U test, comparing groups with (+) or without (-) a clinical endpoint.

Table S5. Crude/unadjusted and adjusted Cox regression analysis (Model 2) of the association between dsDNA and all-cause mortality. The adjusted multivariable model included age, sex, smoking, leukocyte count, platelet count, NT-proBNP, fasting glucose, CRP, D-dimer, previous CVD, peak TnT, prehospital thrombolysis, and time from symptom onset to blood sampling as potential covariates.

	<i>n</i>	Univariable analysis			Multivariable analysis 2 (<i>n</i> = 907)		
		Unadjusted HR	95% CI	<i>p</i> *	Adjusted HR	95% CI	<i>p</i> *
dsDNA Q3 vs. Q1+2	956	2.03	1.12 to 3.66	0.019	1.88	1.01 to 3.49	0.045
dsDNA Q4 vs. Q1+2	956	3.36	1.95 to 5.78	<0.001	2.06	1.08 to 3.93	0.029
Age	956	1.09	1.07 to 1.12	<0.001	1.09	1.07 to 1.13	<0.001
Male vs. female sex	956	0.40	0.25 to 0.64	<0.001	0.73	0.45 to 1.32	0.252
Current smoking (+/-)	956	0.87	0.55 to 1.36	0.535	1.99	1.11 to 3.24	0.015
Leukocyte count	953	1.03	1.00 to 1.06	0.025	0.98	0.87 to 1.02	0.634
Platelet count	952	1.01	1.00 to 1.01	<0.001	1.00	1.00 to 1.01	0.019
NT-proBNP	926	1.00	1.00 to 1.00	<0.001	1.00	1.00 to 1.00	0.376
Fasting glucose	947	1.17	1.05 to 1.29	0.003	1.24	1.11 to 1.40	0.001
CRP	955	1.01	1.00 to 1.01	0.022	1.00	1.00 to 1.01	0.587
D-dimer	950	1.00	1.00 to 1.00	<0.001	1.00	1.00 to 1.00	0.038
Previous CVD (+/-)	955	2.25	1.42 to 3.56	0.001	2.04	1.23 to 3.39	0.006
Peak TnT	956	1.03	0.99 to 1.07	0.150	1.03	0.99 to 1.07	0.220
Prehospital thrombolysis (+/-)	956	0.21	0.05 to 0.86	0.030	0.00	0.00 to 0.00	0.956
Time (symptom onset to blood sampling)	951	1.00	0.99 to 1.01	0.722	0.99	0.97 to 1.00	0.146

Hazard ratios per one unit increase in the explanatory variables, except where otherwise stated, e.g. presence/absence of categorical variables (+/-).

* *p*-value corresponding to Wald test.