Supplementary Appendix

Table 1: Analytical characteristics of the biomarkers assays considered for inclusion in the final model

Biomarker	Manufacturer/assay	Analytical characteristics	Medium	AUC (95% CI) for MACE (derivation study)
Troponin T	Roche Diagnostics 4th generation Elecsys	LOD 10ng/L 10% CV 35ng/L 99th percentile 10ng/L	Serum	0.81 (0.76 – 0.86)
High sensitivity troponin T	Roche Diagnostics 5th generation Elecsys	LOB 3ng/L LOD 5ng/L 10% CV 13ng/L 99th percentile 14ng/L	Serum	0.89 (0.86 – 0.93)
Heart-type fatty acid binding protein (H-FABP)	Randox Laboratories County Antrim, Northern Ireland Cardiac Plus Array, Evidence Investigator	Measuring range 0-100ng/L Sensitivity 0.15ng/L 95th percentile 2.5ng/ml CV 9.1% at 3.1ng/ml	Serum	0.86 (0.83 – 0.90)
Creatine kinase-MB	Randox Laboratories County Antrim, Northern Ireland Cardiac Plus Array, Evidence Investigator	Measuring range 0-100ng/ml Sensitivity 0.4ng/ml 95th percentile 1.92ng/ml CV 5.7% at 3.8ng/ml	Serum	0.79 (0.74 – 0.83)
Myoglobin	Randox Laboratories County Antrim, Northern Ireland Cardiac Plus Array, Evidence Investigator	Measuring range 0-700ng/ml Sensitivity 1.8ng/ml 95th percentile 66ng/ml CV 8.8% at 83ng/ml	Serum	0.76 (0.72 – 0.81)
P-selectin	R&D Systems (Abingdon, UK) DuoSet ELISA	Dynamic range 0-5ng/ml Analytical sensitivity 0.026ng/ml	Plasma	0.66 (0.61 – 0.70)
E-selectin	R&D Systems (Abingdon, UK) DuoSet ELISA	Dynamic range 0-6ng/ml Analytical sensitivity 0.10ng/ml	Plasma	0.54 (0.49 – 0.60)
Pregnancy-associated plasma protein A (PAPP-A)	Demeditec Diagnostics (Kiel, Germany) Ultra-sensitive ELISA	Dynamic range 0 – 450ng/ml Analytical sensitivity 0.023ng/ml	Serum	0.55 (0.49 – 0.60)
Thrombospondin-1 (TSP-1)	R&D Systems (Abingdon, UK) Quantikine ELISA	Dynamic range 0 – 500ng/ml Analytical sensitivity 0.095ng/ml	CTAD (citrate, theophylline, adenosine, dipyridamole) plasma	0.54 (0.49 – 0.60)
Soluble CD40 ligand	R&D Systems	Dynamic range 0-	CTAD plasma	0.51 (0.45

(CD40L)	(Abingdon, UK) DuoSet ELISA	300pg/ml Analytical sensitivity 1.07pg/ml		– 0.56)
NT-pro-BNP	Roche Diagnostics Elecsys	LOD 1pg/ml Range 0 – 25000pg/ml Functional sensitivity (20% CV) 10pg/ml	Serum	0.72 (0.68 – 0.77)

(Analytical characteristics provided by the manufacturers)

Table 2: Summary of univariate analyses demonstrating the predictive value of individual variables recorded at the time of presentation for the primary outcome of major adverse cardiac events (MACE) at 30 days (derivation study). In univariate analyses, all available was data was utilised, thus data from patients without an available serum sample were included. (n=796, of whom 186 had MACE). Statistically significant predictors (p<0.05) are in bold type

Variable	Odds ratio (95% CI) for MACE within 30 days [primary outcome]	Odds ratio (95% CI) for AMI (prevalent)	Odds ratio (95% CI) for incident MACE within 30 days (excluding prevalent AMI)
Male sex*	**1.66 (1.17 – 2.36)**	**1.67 (1.15 – 2.45)**	**1.81 (1.21 – 2.71)**
Age (years)*	**1.03 (1.02 – 1.04)**	**1.03 (1.01 – 1.04)**	**1.02 (1.00 – 1.03)**
Rest pain	0.75 (0.47 – 1.19)	0.71 (0.43 – 1.15)	0.69 (0.41 – 1.13)
Previously identified as ischaemic pain	1.00 (0.70 – 1.44)	0.71 (0.47 – 1.07)	0.93 (0.62 – 1.40)
Worsening angina*	1.43 (0.94 – 2.17)	0.72 (0.43 – 1.20)	**1.64 (1.04 – 2.57)**
Any radiation of pain	1.17 (0.81 – 1.70)	1.05 (0.71 – 1.57)	1.31 (0.85 – 2.01)
Pain radiation to back	0.88 (0.54 – 1.43)	0.84 (0.49 – 1.42)	1.05 (0.62 – 1.78)
Pain radiation to epigastrium	0.65 (0.08 – 5.63)	0.84 (0.10 – 7.24)	0.00
Pain radiation to jaw, neck or throat	0.87 (0.56 – 1.36)	0.78 (0.48 – 1.28)	0.76 (0.56 – 1.51)
Pain radiation to left shoulder or arm	1.23 (0.88 – 1.73)	1.23 (0.86 – 1.77)	1.25 (0.86 – 1.82)
Pain radiation to right shoulder or arm*	**2.53 (1.57 – 4.07)**	**2.49 (1.52 – 4.10)**	**2.75 (1.66 – 4.56)**
Pain radiation to both shoulders or arms	**2.64 (1.50 – 4.65)**	**2.69 (1.50 – 4.83)**	**2.62 (1.44 – 4.76)**
Pain in central chest*	**1.75 (1.19 – 2.58)**	**2.49 (1.58 – 3.91)**	**1.70 (1.10 – 2.64)**
Pain in right anterior chest	0.85 (0.34 – 2.12)	0.87 (0.33 – 2.32)	0.76 (0.26 – 2.23)
Pain in left anterior chest*	**0.54 (0.36 – 0.81)**	**0.35 (0.21 – 0.57)**	**0.58 (0.37 – 0.91)**
Pain in left lateral chest	0.75 (0.21 – 2.67)	0.97 (0.27 – 3.44)	0.68 (0.15 – 3.04)
Pain character: heavy/pressure	1.25 (0.88 – 1.76)	1.32 (0.92 – 1.91)	1.32 (0.90 – 1.93)
Pain character: burning/indigestion-like	1.31 (0.68 – 2.55)	1.18 (0.57 – 2.43)	1.77 (0.89 – 3.50)
Pain character: tight/squeezing	1.23 (0.87 – 1.73)	1.00 (0.69 – 1.46)	1.22 (0.83 – 1.80)
Pain character: dull	0.81 (0.53 – 1.25)	0.90 (0.57 – 1.42)	0.75 (0.46 – 1.23)
Pain character: sharp/stabbing*	**0.53 (0.31 – 0.90)**	0.60 (0.34 – 1.05)	**0.48 (0.26 – 0.91)**
Reported sweating*	**1.68 (1.20 – 2.34)**	**1.70 (1.19 – 2.42)**	**1.76 (1.21 – 2.56)**
Reported dyspnoea*	**1.56 (1.12 – 2.18)**	1.38 (0.97 – 1.97)	1.43 (0.99 – 2.08)
Reported nausea	1.31 (0.93 – 1.83)	1.32 (0.92 – 1.89)	1.34 (0.92 – 1.95)
Reported vomiting*	**3.70 (2.15 – 6.38)**	**3.61 (2.08 – 6.28)**	**3.31 (1.88 – 5.83)**
Reported paraesthesiae	0.81 (0.54 – 1.22)	0.76 (0.49 – 1.19)	0.86 (0.55 – 1.35)
Pleuritic nature	0.79 (0.42 – 1.48)	0.73 (0.37 – 1.47)	0.64 (0.30 – 1.38)
Symptom – presentation time (h)	0.91 (0.87 – 0.95)	0.90 (0.85 – 0.95)	0.92 (0.87 – 0.97)
Previous myocardial infarction	1.22 (0.84 – 1.77)	0.86 (0.56 – 1.30)	1.23 (0.81 – 1.86)

Previous coronary intervention	1.03 (0.68 – 1.54)	0.65 (0.40 – 1.06)	1.20 (0.77 – 1.87)
History of hyperlipidaemia	0.91 (0.65 – 1.26)	0.68 (0.47 – 0.97)	1.03 (0.71 – 1.49)
History of hypertension	1.18 (0.85 – 1.63)	0.98 (0.69 – 1.39)	1.01 (0.70 – 1.46)
History of diabetes mellitus*	**1.51 (1.00 – 2.26)**	0.99 (0.62 – 1.58)	1.54 (0.99 – 2.40)
History of angina	0.99 (0.70 – 1.41)	0.58 (0.39 – 0.88)	1.02 (0.69 – 1.52)
History of undiagnosed chest pain	**0.54 (0.34 – 0.85)**	**0.53 (0.32 – 0.88)**	**0.47 (0.27 – 0.81)**
History of cerebrovascular disease	1.28 (0.75 – 2.19)	0.94 (0.51 – 1.74)	1.21 (0.66 – 2.19)
History of peripheral vascular disease	1.20 (0.38 – 3.80)	1.05 (0.29 – 3.77)	1.21 (0.34 – 4.34)
Current tobacco smoker	**1.77 (1.26 – 2.50)**	**2.15 (1.49 – 3.08)**	**2.12 (1.45 – 3.09)**
Family history of coronary heart disease	0.79 (0.56 – 1.09)	0.72 (0.51 – 1.04)	0.92 (0.64 – 1.34)
Current aspirin use	0.83 (0.60 – 1.16)	**0.58 (0.40 – 0.84)**	0.80 (0.55 – 1.16)
Current clopidogrel use	0.96 (0.58 – 1.59)	0.74 (0.42 – 1.33)	0.87 (0.48- 1.55)
Current statin use	0.94 (0.67 – 1.30)	0.67 (0.47 – 0.97)	0.87 (0.60 – 1.26)
Current ACE inhibitor use	0.94 (0.64 – 1.40)	0.80 (0.52 – 1.23)	0.95 (0.61 – 1.48)
Current beta blocker use	0.94 (0.64 – 1.37)	0.65 (0.42 – 1.00)	0.81 (0.52 – 1.25)
Hypotension (systolic blood pressure <100mmHg) on arrival*	**5.26 (2.32 – 11.93)**	**4.88 (2.18 – 10.93)**	**4.02 (1.79 – 9.06)**
Bradycardia (heart rate <60 beats per minute) on arrival	1.47 (0.91 – 2.39)	1.23 (0.72 – 2.10)	**1.91 (1.15 – 3.17)**
Tachycardia (heart rate >100 beats per minute) on arrival*	**1.90 (1.17 – 3.09)**	**1.84 (1.10 – 3.07)**	**1.52 (0.94 – 2.78)**
Basal crepitations on auscultation of lung fields*	**1.94 (1.20 – 3.13)**	**1.78 (1.07 – 2.95)**	1.45 (0.84 – 2.50)
Abdominal tenderness	0.66 (0.38 – 1.14)	0.55 (0.29 – 1.04)	0.71 (0.38 – 1.31)
Sweating observed by clinician*	**7.49 (4.70 – 11.93)**	**8.93 (5.59 – 14.28)**	**6.52 (4.09 – 10.39)**
Chest wall tender	1.22 (0.84 – 1.78)	1.05 (0.69 – 1.58)	1.37 (0.91 – 2.07)
Elevated jugular venous pressure	0.69 (0.38 – 1.27)	0.83 (0.44 – 1.54)	0.68 (0.34 – 1.37)
ECG: Acute ischaemic features*	**9.50 (6.56 – 13.75)**	**10.14 (6.81 – 15.10)**	**8.65 (5.75 – 13.00)**
ECG: ST elevation myocardial infarction§	**290.00 (39.82 – 2112.02)**	**130.25 (40.01 – 424.00)**	26.80 (13.97 – **51.42)**
ECG: Left bundle branch block	2.25 (1.00 – 5.11)	2.03 (0.86 – 4.80)	1.54 (0.61 – 3.94)

* Considered for inclusion in the final model

§ Collinear with acute ischaemic features (which incorporates ST elevation MI) therefore not considered for inclusion in the final model

Table 3: Multivariate model detailing the components of the MACS rule with tobacco smoking included as a covariate. (Rounded values are presented).

Variable	Constant	Odds ratio (95% CI)	P value
High sensitivity troponin T*	0.070	1.1 (1.0 – 1.1)	<0.0001
Heart-type fatty acid binding protein*	0.17	1.2 (1.0 -1.4)	0.026
ECG ischaemia	1.83	6.3 (3.3 – 11.8)	<0.0001
Sweating observed	1.82	6.2 (2.9 – 13.0)	<0.0001
Vomiting	1.59	4.9 (1.7 – 14.4)	0.004
Systolic blood pressure <100mmHg	1.48	4.4 (1.2 – 16.0)	0.026
Worsening angina	1.00	2.7 (1.3 – 5.7)	0.007
Tobacco smoking (current)	0.78	2.2 (1.1 – 4.3)	0.03
Constant	-5.01	-	-

* Odds ratios are presented for a 1 unit increase (ng/L for hs-cTnT; ng/ml for H-FABP)

Table 4: Performance of the alternative MACS decision rule with tobacco smoking included

		Very low risk	Low risk	Moderate risk	High risk
Derivation study	Number with AMI (%)	0 (0.0)	4 (2.7)	50 (19.8)	76 (98.7)
	Number with MACE (%)	1 (0.5)	6 (4.0)	73 (28.9)	77 (100.0)
	Total number (%)	219 (31.4)	149 (21.3)	253 (36.2)	77 (11.0)
Validation study	Number with AMI (%)	0 (0.0)	0 (0.0)	37 (17.6)	42 (89.4)
	Number with MACE (%)	2 (1.7)*	4 (4.6)	48 (22.9)	44 (93.6)
	Total number (%)	119 (25.7)	87 (18.8)	210 (45.4)	47 (10.2)

Abbreviations: AMI, acute myocardial infarction; MACE, major adverse cardiac events

* Both coronary stenoses, identified on an outpatient basis. Neither patient underwent revascularisation within 30 days.