Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

eAppendix. Blood sampling and laboratory methods

Blood samples for the determination of diagnostic biomarkers were collected at presentation to the ED, and for some biomarker additionally at 1 and 2 hours after presentation. After centrifugation, samples were frozen at -80°C until assayed in a blinded fashion in a dedicated core laboratory blinded to all clinical information. Measurements were done after centrifugation from fresh samples for those biomarkers also used for routine clinical care. We selected biomarkers that had been shown in pilot studies to provide possible diagnostic value for MI in general and to have an established methodology with high precision for measurement from frozen samples obtained in APACE. In addition, measurement needed to be feasible in a blinded fashion either the central laboratory of the University Hospital Basel, one of the academic collaborating laboratories (Blood Bank, Basel, Cantonal Hospital Aarau), or in one of the laboratoyr facilities of the respective diagnostic companies developing the different biomarker assays.

For the determination of heart-type fatty acid binding protein (hFABP), we used the QuickSens hFABP assay (8sens.biognostic GmbH, Berlin, Germany), which is an immunochromatographic lateral-flow test applicable with whole blood, plasma or serum. The assay contains two different specific monoclonal antibodies for hFABP, one of which is gold-labelled and the other one biotinylated; results of the single measurements were evaluated and accurately quantified using the mobile optoe-lectronic reader system QuickSensΩ100 (8sens.biognostic GmbH), with an LoD of 0.6 ng/ml, a 99th percentile cut-off point of 5.76 ng/ml, measured in a population of healthy volunteers and a CV of less than 20% (20%CV) at 5 ng/ml.(1)

BNP was measured by amicroparticle enzyme immunoassay (AxSym and Architect; Abbott Laboratories, Abbott Park, Ill).(2)

Serum levels of NT-proBNP were determined with Elecsys proBNP (Roche Diagnostics, Zug, Switzerland), a quantitative electrochemiluminescence immunoassay.(3) The analytical detection limit of the assay was 5 pg/mL. The intra-assay coefficient of variation was 2.4% at 355 pg/mL and 1.8% at 4962 pg/mL; the inter-assay coefficients of variation were 2.9% at 355 pg/mL and 2.3% at 4962 pg/mL.

Cardiac Myosin binding protein C (cMyC) was measured using the previously established hs assay on the Erenna platform performed by Millipore Sigma. The assay has a limit of detection of 0.4ng/L and a lower limit of quantification of 1.2ng/L. The 99th percentile cutoff point determined previously (in patients without obstructive coronary artery disease on invasive angiography) is 87ng/L.(4)

MR-proANP was measured using a commercially available sandwich immunofluorescent assay (MR-proANP LIA, B.R.A.H.M.S. AG, Hennigsdorf, Germany); the functional assay sensitivity (interassay coefficient of variance 20%) is 20 pmol/L.(5)

Copeptin was measured in potassium EDTA using a novel commercial sandwich immunoluminometric assay (B.R.A.H.M.S. LUMItest CTproAVP, B.R.A.H.M.S. AG, Hennigsdorf/Berlin, Germany).(6) The lower limit of detection is 0.4 pmol/l, and the functional assay sensitivity (20% interassay CV) is 1 pmol/l. In healthy individuals, a copeptin level of 9.8 pmol/l corresponds to the 95th percentile and 13 pmol/l to the 97.5th percentile.(7)

GDF-15 was measured by using a precommercial sandwich immunoassay based on the enhanced chemiluminescence immunoassay principle. The assay used a

polyclonal biotinylated goat capture antibody and a monoclonal detection antibody linked to a ruthenium complex. According to the manufacturer, the lower limit of detection of the GDF-15 assay was 90 ng/L and the between-run imprecision of the assay was 2.8% (CV) at a concentration of 480 ng/L. The assay was standardized to the immunoradiometric GDF-15 assay described by Kempf et al., with reported median GDF-15 concentrations in healthy, elderly individuals of 762 ng/L [interquartile range (IQR) 600–959 ng/L].(8)

MPO was measured in EDTA plasma using a chemiluminescent automated microparticle immunoassay technology (Architect MPO, Abbott Diagnostics). According to the manufacturer, this assay had a dynamic range of 0 –10.000 pmol/L with an analytical sensitivity of 5 pmol/L and a functional sensitivity of 70 pmol/L (total CV of 10%).(9)

sFlt-1 was measured with precommercial sandwich enzyme electrochemiluminescence immunoassays (Roche Diagnostics, Germany). The limit of detection for sFlt-1 was 10 ng/L with a maximum detectable value of 85 000 ng/L. The intra-assay coefficient of variation was 1.6% (63 ng/L) and 0.8% (589 ng/L), and the inter-assay coefficient of variation 4.3% (63 ng/L) and 2.3% (589 ng/L; preliminary results provided by Research and Development of Roche Diagnostics, Germany).(10)

PlGF was measured with precommercial sandwich enzyme electrochemiluminescence immunoassays (Roche Diagnostics, Germany). For PlGF, the limit of detection was 3 pg/mL with a maximum detectable value of 10 000 ng/L. The intra-assay coefficient of variation was 1.1% (107 ng/L) and 1.2% (563 ng/L), and the inter-assay coefficient of variation 2.7% (107 ng/L) and 2.6% (563 ng/L).(10)

PAPP-A was measured in serum using a 1-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics). This assay measures all forms of PAPP-A, the heterodimer as well as the free form. The lower limit of detection is 4 mIU/L and the 95th percentile in healthy individuals has been reported at 7.15 mIU/L.(11)

Anti-apoA-1 IgG antibodies were sampled and measured as published before.(12) Briefly, Maxisorp plates (Nunc, Roskilde, Denmark) were coated with purified, human-derived delipidated ApoA-1 (20 mg/mL; 50 lL/ well) for 1 h at 37 °C. After washing, the wells were blocked for 1 h with phosphate buffered saline (PBS) and 2% bovine serum albumin (BSA) at 37 °C. Then, serum samples diluted 1⁄50 were incubated for 1 h at 37 °C. Patient serum samples were also added to a noncoated well to assess the individual nonspecific binding. After washing six times, 100 lL/well) alkaline phosphatase-conjugated anti-human IgG (Sigma-Aldrich, St Louis, MO, USA) diluted 1⁄1000 in PBS⁄BSA solution was incubated for 1 h at 37 °C. After washing again six times, the phosphatase substrate p-nitrophenylphosphate disodium (Sigma-Aldrich) dissolved in diethanolamine buffer (pH 98) was added. Each sample was tested in duplicate, and absorbance in optical density units (OD) was determined at 405 nm, after incubation for 20 min at 37 °C using a plate reader (Molecular Devices Versa Max; Molecular Device, Sunnyvale, CA, USA). The corresponding nonspecific binding was subtracted from the mean absorbance for each sample. The cut-off value for positivity was prospectively defined and set at 06 OD and 37% of the positive control value, as previously described.(12) At the cut-off level, the intra- and interassay coefficients of variation (CV) were 16% (n = 10) and 12% (n = 8), respectively.

Anti-PC IgM levels were assessed using a commercially available enzyme-linked immunosorbent assay kit (CVDefine; Athera Biotechnologies, Uppsala, Sweden), with

purified PC as antigen and performed according to the manufacturer's instructions. Results are expressed in U/mL, based on a standard curve build on six points. Samples were run in duplicate. Interassay CV were 24% at 125 U/ml and 20% at 25 U/mL (n = 4). The intra-assay coefficient of variation at 65 U/mL was 5% (n = 8). For anti-PC IgM, we used the prespecified cut-off set of 29 U/mL as previously described.(13)

CT-proET-1 was detected with a novel chemiluminescence sandwich immunoassay with coated tubes (BRAHMS GmbH, Thermo Fisher Scientific).(14) The reference values of healthy individuals were reported as mean (SD) of 44.3 (10.6) pmol/L [95% confidence interval (CI) of the mean, 43.1– 45.4 pmol/L] and a range of 10.5–77.4 pmol/L. The 99th percentile of a healthy population is 72.8 pmol/L. For CT-proET-1 the lower limit of detection (limit of the blank) is 0.4 pmol/L.

MR-proADM was collected in EDTA plasma and detected with a sandwich imunoluminometric assay (MR-proADM, BRAHMS AG, Hennigsdorf/Berlin, Germany), as described elsewhere.(15) The 95th percentile of a normal reference population as reported by the manufacturer amounts to 0.52 nmol/l.

The Roche hs-cTnT assays used the Elecsys 2010 system (Roche Diagnostics, Rotkreuz, Switzerland), with a limit of detection (LoD) of 5 ng/L, a 99th-percentile cut-off point of 14 ng/L, and a coefficient of variation (CV) of less than 10% at 13 ng/L.(16)(17)(18)

The Abbott-Architect hs-cTnI assay used was the final pre-commercial release version of the ARCHITECT High Sensitive STAT Troponin I assay (Abbott Laboratories, Abbott Park, USA). The Abbott-Architect hs-cTnI assay was performed with the use of the Architect system with a LoD of 1.9ng/L and a 99th percentile cut-off point of 26.2ng/L with a corresponding CV of <5%.(19)

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eTable 1.				
Diagnostic performance for differentiation between AMI (n = 1106) and no AMI (n=4781)				
			ROC AUC	
	MI	no MI	(95% CI)	
Cardiomyocyte injury				
0h Hs-cTnT	1097	4744	0.93 (0.92 to 0.94)	
1h Hs-cTnT	895	3922	0.95 (0.94 to 0.96)	
2h Hs-cTnT	695	3116	0.96 (0.95 to 0.96)	
0h Hs-cTnI	1089	4703	0.92 (0.92 to 0.93)	
1h Hs-cTnI	868	3827	0.94 (0.94 to 0.95)	
2h Hs-cTnI	670	3025	0.95 (0.94 to 0.95)	
CK-MB	458	1400	0.78 (0.75 to 0.80)	
HFABP	220	849	0.80 (0.76 to 0.83)	
0h cMyC	408	1600	0.92 (0.91 to 0.94)	
1h cMyC	339	1306	0.93 (0.92 to 0.95)	
2h cMyC	264	1052	0.95 (0.93 to 0.96)	
Hemodynamic cardiac stress				
BNP	517	2341	0.53 (0.50 to 0.55)	
NT pro BNP	190	850	0.77 (0.74 to 0.81)	
MR pro ANP	134	490	0.76 (0.71 to 0.81)	
Endogenous stress				
0h Copeptin	410	1517	0.67 (0.64 to 0.70)	
1h Copeptin	318	1160	0.67 (0.63 to 0.70)	
2h Copeptin	238	939	0.66 (0.62 to 0.70)	
Glucose	1073	4679	0.61 (0.59 to 0.63)	
Plaque instability and angiogenesis				
MPO	130	480	0.60 (0.55 to 0.65)	
sVEGFr/sFLT-1	132	482	0.65 (0.60 to 0.71)	
PlGF	89	324	0.64 (0.58 to 0.71)	
PAPP-A	89	323	0.61 (0.54 to 0.68)	
Anti APO1 IgG OD	218	880	0.49 (0.44 to 0.53)	
Anti PC IgM	218	880	0.51 (0.47 to 0.55)	
Endothelial dysfunction				
CT pro ET	134	490	0.68 (0.62 to 0.73)	
MR pro ADM	408	1513	0.67 (0.64 to 0.70)	
Inflammation				
CRP	873	4074	0.58 (0.56 to 0.60)	
Leucocytes	1075	4746	0.59 (0.57 to 0.61)	
Combination of inflammation, hemodynamic stress and vascular aging				
GDF 15	128	470	0.69 (0.64 to 0.74)	

Abbreviations: AMI, acute myocardial infarction; ROC AUC, receiver operating area under the

curve; PPV, positive predictive value; CI, confidence interval; Hs-cTnT/I, high sensitivity-cardiac troponin T/I; H-FABP, heart-type fatty acid binding protein; cMyC, cardiac myosin binding protein C; BNP, B-type natriuretic peptide; CK-MB, creatine kinase myocardial band;	
NTproBNP, N-terminal pro-B-type natriuretic peptide; MR-proANP, midregional pro–A-type	
natriuretic peptide; MPO, myeloperoxidase; sVEGFr/sFLT-1, soluble vascular endothelial growth factor	
receptor; PlGF, placental growth factor; PAPP-A, pregnancy-associated plasma protein-A;	
anti-apoA-1 IgG, autoantibodies to apolipoprotein A-1; anti-PC IgM, antibodies to phosphorylcholine;	
CT-proET-1, c-terminal-pro-endothelin-1; MR-proADM, midregional pro adrenomedullin; CRP, C reactive protein; GDF-15, growth differentiation factor 15	
If not mentioned otherwise blood samples were collected immediately after presentation to the emergency department	

eTable 2.
Direct comparison of hs-cTnI with other biomarkers

	number	ROC Biomarker AUC	95% CI		ROC hs-cTnI AUC	95% CI		p-value
0h cMyC	404	0.67	0.62	0.73	0.73	0.64	0.76	0.12
CT pro ET	132	0.72	0.62	0.82	0.73	0.64	0.82	0.88
MR pro ANP	132	0.76	0.67	0.86	0.72	0.67	0.77	0.65
MR pro ADM	404	0.67	0.60	0.73	0.70	0.64	0.76	0.25
CK-MB	449	0.68	0.62	0.74	0.75	0.66	0.84	0.55
GDF 15	127	0.68	0.57	0.79	0.71	0.67	0.74	0.44

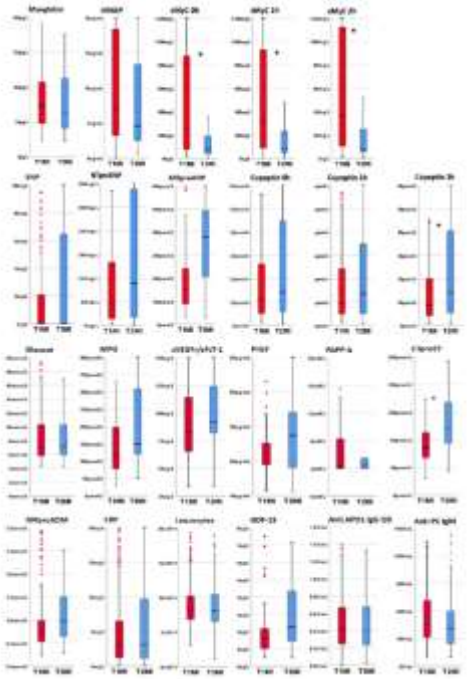
Abbreviations: ROC AUC, receiver operating area under the curve; CI, confidence interval; cMyC, cardiac myosin binding protein C; hs-cTnI, high sensitivity cardiac troponin I; CT-proET-1, c-terminal-pro-endothelin-1; MR-proADM, midregional pro adrenomedullin; MR-proANP, midregional pro–A-type natriuretic peptide; CK-MB, creatine kinase myocardial band; GDF-15, growth differentiation factor 15

p-value for direct comparison between hs-cTnI and other biomarkers in the respective subset of patients

eTable 3. **Sensitivity analysis**					
	number	ROC AUC	95%CI		p-value
0h hs-cTnT	93	0.65	0.53	0.76	0.03
0h hs-cTnI	93	0.72	0.60	0.83	-
0h cMyC	93	0.70	0.58	0.81	0.33
CT pro ET	93	0.74	0.62	0.86	0.81
MR pro ANP	93	0.78	0.67	0.89	0.47
MR pro ADM	93	0.69	0.55	0.83	0.80
CK-MB	93	0.65	0.53	0.77	0.32
GDF 15	93	0.69	0.56	0.82	0.77

Abbreviations: ROC AUC, receiver operating area under the curve; CI, confidence interval; cMyC, cardiac myosin binding protein C; hs-cTnT/I, high sensitivity cardiac troponin T/I; CT-proET-1, c-terminal-pro-Endothelin-1; MR-proADM, midregional pro adrenomedullin; MR-proANP, midregional pro–A-type natriuretic peptide; CK-MB, creatine kinase myocardial band; GDF-15, growth differentiation factor 15

p-value for comparison between hs-cTnI with other biomarkers



eFigure. Boxplots for other cardiac biomarkers

Concentrations of creatine kinase myocardial band (CK-MB), heart-type fatty acid binding protein (H-FABP; n=165/55), cardiac myosin binding protein C (cMyC; n=303/105), B-type natriuretic peptide (BNP; n=402/115), N-terminal pro-B-type natriuretic peptide (NT-proBNP; n=140/50), midregional pro–A-type natriuretic peptide (MR-proANP; n=97/37), copeptin, glucose, myeloperoxidase (MPO; n=94/36), soluble vascular endothelial growth factor receptor 1 or soluble FLT1 (VEGFr1-sFlt-1; n=96/36), placental growth factor (PlGF; n=65/24), pregnancy-associated plasma protein-A (PAPP-A; n=65/24), autoantibodies to apolipoprotein A-1 (anti-apoA-1 IgG; n=163/55), antibodies to phosphorylcholine (anti-PC IgM; n=163/55), c-terminal-pro-Endothelin-1 (CT-proET-1; n=97/37), midregional pro adrenomedullin (MR-proADM; n=305/103), C-reactive protein (CRP; n=669/204) leucocytes (n=850/245) and growth differentiation factor 15 (GDF-15; n=91/37) in patients with Type 1 myocardial infarction (red) versus patients with Type 2 myocardial infarction (blue). Boxes represent

medians and the interquartile ranges (IQRs) and stars represent statistical significant difference between groups ($p<0.05$)