Supplementary Online Content

Keller T, Zeller T, Ojeda F, et al. Serial changes in highly sensitive troponin I assays and early diagnosis of myocardial infarction. *JAMA*. 2011;306(24):2684-2693.

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eMethods.

Study population

Between January 2007 and December 2008, 1,818 patients consecutively presenting with acute chest pain at the respective chest pain units of the three recruitment centers (1) Johannes Gutenberg-University Medical Center Mainz, (2) Federal Armed Forces Hospital Koblenz or (3) University Hospital Hamburg-Eppendorf were enrolled in the present prospective biomarker assessment registry as described earlier[1].

To accomplish a real life scenario, all patients between 18 and 85 years of age presenting with acute angina pectoris or equivalent symptoms were eligible to participate. Exclusion criteria were major surgery or trauma within the previous four weeks, pregnancy, obvious intravenous drug abuse and anaemia with haemoglobin level <10 g/dL. All patients enrolled were Caucasian with central European descent.

Time of first onset of chest pain symptoms was carefully assessed by independent research staff. Traditional cardiovascular risk factors have been defined as follows: previous diagnosis of hypertension or treatment with antihypertensive drugs was considered as hypertension. Smoking status was categorized as currently smoking, formerly smoking (if stopped 4 to 40 years prior) and never having smoked (if stopped >40 years ago or never smoked). Receiving medication for diabetes was considered as diabetes mellitus. Previous diagnosis of hyperlipidaemia or total cholesterol >200 mg/dL at admission was considered as hyperlipidemic.

Blood was drawn for routine blood work and sample storage on admission and after three and six hours. A 12-lead electrocardiogram was obtained on the same time points.

Because one criteria for the diagnosis of MI was based on conventional, less sensitive troponin determination, the diagnostic performance of the new high sensitive troponin assay may not be adequately appreciated. Therefore, as additional end point, mortality and MI at 30 days after index event were documented

The study was approved by the local ethics committee of Rhineland-Palatinate, Germany and Hamburg, Germany. Participation was voluntary, each patient gave written, informed consent.

Laboratory measures

Routine laboratory parameters including C-reactive protein and serum creatinine were measured immediately after blood withdrawal on admission by standardized methods. Additionally, EDTA plasma, citrate plasma and serum samples were collected at each time point, centrifuged, aliquoted and stored at -80°C.

Conventional troponin assays used for the adjudication of the final diagnosis based on all serial troponin measurements were: cardiac troponin T in Mainz and Hamburg and cardiac troponin I in Koblenz.

In-house troponin was measured immediately after blood withdrawal on admission. In-house troponin was represented at two study centers by cardiac troponin T measurement (4^{th} generation Elecsys 2010 TnT assay, Roche Diagnostics, Germany). Detection limit of the assay is 0.01ng/mL with measuring range of 0.01-25ng/mL. Reference limit based on the 99^{th} percentile for a healthy population is 0.01ng/mL and 10% coefficient of variation (CV) is represented by 0.03ng/mL used as diagnostic cut-off. At one study center in-house troponin is represented by cardiac troponin I measurement (Dimension RxL TnI, Siemens Healthcare Diagnostics, Germany). This assay has a detection limit of 0.04ng/mL with measuring range of 0.04-40ng/mL. The 99^{th} percentile is 0.07ng/mL and the 10% CV used as diagnostic cut-off is 0.14ng/mL.

As contemporary sensitive troponin I, defined by a coefficient of variation (CV) of 10% at or below the 99^{th} percentile the Architect STAT troponin I system (Abbott Diagnostics, Abbott Park, USA) was used. Limit of detection of this assay is 0.01ng/mL with measuring range of 0 - 50ng/mL. The 99^{th} percentile is 0.32ng/mL[3] as well as the 10% CV, which was used as diagnostic cut-off. The optimised diagnostic threshold for myocardial infarction according to the *World Health Organisation* (WHO) definition is given with 0.3 ng/mL by the manufacturer.

As high sensitive cardiac troponin I defined as detectability in at least 50% of healthy individuals a prototype of a cardiac troponin I assay (ARCHITECT STAT High Sensitive Troponin, Abbott Diagnostics, Abbott Park, USA) was

used. Limit of detection of this sensitive assay is 3.4pg/mL with measuring range of 0 – 50 ng/mL and 10% CV at a concentration of 5.2pg/mL. The concentration representing the 99^{th} percentile of a reference population was determined

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with 29.885pg/mL (diagnostic cut-off used 30pg/mL) along the CLSI guideline C28-A3 in 4,139 individuals with available measurement out of 5,000 individuals of the population-based *Gutenberg Health Study*. The highest measured troponin value was treated as outlier[4] and was excluded from the further analyses. Additionally a biomarker-guided approach was used to describe the diagnostic cut-off based on a healthy reference population regarding cardiovascular diseases. As suggested[5] individuals with elevated N-terminal B-type natriuretic peptide (above the 95th percentile) representing individuals with structural heart changes were excluded. In this population the concentration representing the 99th percentile was determined with 23.878pg/mL used as cut-off of 24pg/mL.

Characteristics of the *Gutenberg Health Study* individuals and histogram of the sensitive troponin I distribution are given as *eTable 1 and eFigure 1.*

The individual diagnostic threshold used for the various other early evaluated biomarkers is also based on the 99th percentile of a reference population as recommended by the universal definition of MI[2]. If no other reference is given this cut-off was determined as described above in 5,000 individuals of the *Gutenberg Health Study*. Distribution of the individual biomarker as well as characteristics of the used reference populations are given as eTable 1 and eFigure 1.
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Creatine kinase (CK) was measured in serum using a commercially available assay (Abbott Diagnostics, Delkenheim, Germany) on the Architect System. Limit of detection of the assay is 2.7 U/L with 99th percentile determined in 4993 individuals of the *Gutenberg Health Study* at 549.47 U/L.

Creatine kinase – myocardial band (CK-MB) was measured in serum using a commercially available assay (Abbott Diagnostics, Delkenheim, Wiesbaden) on the Architect System. Limit of detection of the assay is of 0.1ng/mL; the lowest value exhibiting a 10% CV is 4.6 ng/mL and 99th percentile is 6.6 ng/m (manufacturer's information).

Myoglobin was measured in serum using a commercially available assay (Abbott Diagnostics, Delkenheim, Wiesbaden) on the Architect System. Limit of detection of the assay is 1.5ng/mL. Lowest value exhibiting a 10% CV is 1.6ng/mL, intra CV was 4.6 %, the inter CV was 4.12% and 99th percentile is 140.1ng/mL (manufacturer's information).

C-terminal proArgininvasopressin (CT-proAVP, Copeptin) was measured in EDTA plasma by a sandwich immunoluminometric assay (CT-proAVP LIA B.R.A.H.M.S AG, Hennigsdorf, Germany) [6, 7]. Limit of detection of the assay is of 0.4pmol/L with measuring range of 0.4-1.250 pmol/L and lowest concentration that can be determined with an inter-assay CV below 20% of 1.0 pmol/L. The used 99th percentile was determined in 4963 individuals of the *Gutenberg Health Study* as described earlier at 18.9 pmol/L[8].

Growth-differentiation factor 15 (GDF-15) was measured in Serum plasma using a research prototype GDF15 assay (Abbott Diagnostics, Abbott Park, USA). Limit of detection of the assay is 10pg/mL, intra CV is 5.2%, inter CV is 4.11% and the 99th percentile is 1424.0pg/mL (personal communication Abbott Diagnostics).

Glycogen phosphorylase BB (GP-BB) was measured in Serum using a commercially available assay (Diacordon-ELISA, Diagenics Int. Corp, Essen, Germany). Limit of detection of the assay is 3ng/mL, the intra CV is 8.2%, inter CV is 5.9% and the 99th percentile was determined in 520 apparently healthy individuals of the *Gutenberg Health Study* pilot phase with 43.64 ng/mL. Characteristics of this reference cohort are given in *eTable 1*.

Myeloperoxidase (MPO) was measured in EDTA plasma using an ELISA assay (CardioMPO, PrognostiX, Cleveland, USA). Limit of detection of the assay is 14pmol/L with measuring range of 0–8000pmol/L. The inter CV is 5.16%, intra CV is 5.19% and the 99th percentile was determined in 4899 individuals of the *Gutenberg Health Study* at 693 pmol/L.

Heart type fatty acid binding protein (htFABP) was measured in EDTA plasma using a commercially available assay on the Evidence EV180 system (Randox Laboratories Ltd., Crumlin, UK). The sensitivity of the assay is 0.35ng/mL, intra CV of the assay is 8.6% and inter CV is 11.6%. The 99th percentile used as diagnostic cut-off is represented by 5.3 and 5.8 mg/L for female and male as described by Bathia DP et al.[9].

Soluble vascular endothelial growth factor receptor-1 (sVEGFR-1/sFLT-1) was measured in EDTA plasma using a research prototype ARCHITECT sFlt-1 assay (Abbott Diagnostics, Abbott Park, USA). Limit of detection of the assay is 15pg/mL, intra CV is 4.08%, inter CV is 4.32% and the 99th percentile is 351.2 pg/mL (personal communication Abbott Diagnostics).

Placental growth factor (PlGF) was measured in EDTA plasma using a research prototype ARCHITECT PlGF assay (Abbott Diagnostics, Abbott Park, USA). Limit of detection of the assay is 2pg/mL, inter CV is 3.6%, the intra is CV 3.58% and the 99th percentile is 30.8pg/mL (personal communication Abbott Diagnostics).

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N-terminal B-type natriuretic peptide (BNP) was measured in the *Gutenberg Health Study* population by a sandwich immuno assay (Roche Elecsys proBNP II) with a measuring range of 5-35000pg/mL.

In-house imprecision was measured in all used biomarker assays with results congruent with the above claimed values. Reproducibility was determined by daily repeated measurements in validation samples with low, medium and high analyte concentrations resulting in CVs complying with the manufacturer's specifications.

Experienced technical assistants blinded to the patient's characteristics performed analytical determination of the evaluated markers for all study centers at the biomarker laboratory Mainz.

Treating physicians and research staff involved in enrollment or follow-up of study participants were unaware of the evaluated biomarker results including the contemporary and high sensitive troponin I measurements.

Statistical analysis

Continuous skewed variables were described by its quartiles whereas symmetric variables were characterized by its arithmetic mean and SD. Associations between biomarkers were assessed with Spearman rank correlation coefficient.

Locally weighted regression (loess)[10] was used for smoothing the scatterplot of each biomarker versus chest pain onset time for the patients with a diagnosis of acute MI. These loess curves were then plotted together (*eFigure 2*). Receiver-operating characteristic (ROC) curves based on continuous biomarker levels dependent on time since chest-pain onset were calculated and graphed. The area under the curve (AUC) was computed for single biomarkers and combinations of sensitive Troponin I with single markers. This combination was achieved via a logistic regression, after log-transforming the two variables. To compare AUCs the test of DeLong et al.[11] was used. The method described in the aforementioned reference estimates the covariance matrix of a vector of AUCs, which can then be used for testing AUC differences and also to compute confidence intervals for the AUCs.

The combinations of sensitive Troponin I and other markers described in the previous paragraph were also compared with the simple logistic model that has sensitive Troponin I, log-transformed, as its only independent variable, via the (relative) integrated discrimination improvement (IDI)[12]. For relative IDI bootstrap confidence intervals were computed. Sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) for the individual markers and combinations were computed for marker specific cut-off values. In this case a binary test combining sensitive Troponin I and other marker was obtained by declaring a proband positive if for any of the two biomarkers involved its value was above the respective diagnostic cut-off and negative otherwise.

To represent potentially relevant relative changes in troponin concentration, we included in addition to a receiver operator characteristic curve based threshold different cut-offs ranging from 20 to 250%. Additionally an optimized δ change was computed by finding the cut-off that maximizes the sum of specificity and sensitivity (or equivalently the Youden Index). In this context all analyses were done in patients with hsTnI values below the upper range of the used high sensitive assays of 50,000 pg/mL at admission and after 3 hours to gain a cohort in which an unbiased

increase or decreases could be tested.

The δ change for troponin was defined by the formula: 100*|hsTnI 3h after admission– hsTnI at admission|/(hsTnI at admission).

Histograms of the biomarkers in reference populations were produced in 2 different versions, the first one using the full range of the biomarker and Sturges method for determining the bin size. The second one was presented as an insert but only using the lower values of the given marker. In this case Scott's method was used to derive the bin size.

All analyses were carried out using R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria).

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Results

Biomarkers representing ischemia and necrosis had a moderate correlation among themselves like hsTnI and H-FABP with R of 0.62 ($p<0.001$), whereas biomarker with different pathophysiologic background showed only mild correlations to necrosis markers. Correlations among all determined biomarkers are provided as eTable 2.

The myocardial necrosis markers CK, CK-MB and the two troponin I assays showed a comparable kinetic with an initial rise reaching maximum values 10 to 20 hours after chest pain onset and a delayed subsequent decrease whereas the biomarkers copeptin, sFLT-1 and MPO had highest values directly after symptom onset with a rapid consecutive decline. Myoglobin and H-FABP showed similar kinetics with earlier peak than troponin or CK reaching near normal values within one day.

The time courses of both troponin I assays, the classical necrosis markers and the evaluated early biomarkers all determined on admission according to time after chest pain onset in patients with the discharge diagnosis MI are provided as eFigure 2.

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eTable 1. Characteristics of the reference cohorts used for determination of the biomarkers diagnostic cut-off values

Supplementary Table 1		
	Reference Cohort 1 n=5,000	**Reference Cohort 2** n=520
Age [years]	55.5 ± 10.9	51.9 ± 12.1
Gender [% males]	2540 (50.8)	261 (50.2)
Body-mass-index [kg/m^2]	27.2 ± 4.8	26.4 ± 4.82
Diabetes mellitus [%]	374 (7.5)	19 (3.7)
Hypertension [%]	2564 (51.3)	249 (48.7)
Dyslipidemia [%]	1462 (29.3)	109 (21.0)
Smoking Status, current [%]	959 (19.2)	100 (19.5)
Family history of AMI [%]	886 (17.7)	135 (26.4)
Prevalent CAD [%]	226 (4.6)	27 (5.3)
Atrial Fibrillation [%]	136 (2.7)	13 (2.6)

Characteristics of reference population 1, based of 5,000 individuals of the *Gutenberg Health Study* which was used to determine the concentration representing the 99th percentile of high sensitive troponin I (with 4,139 individuals with available measurement), copeptin (with 4963 individuals), myeloperoxidase (with 4899 individuals) and creatine kinase (with 4993 individuals). Given numbers based on the dataset of the *Gutenberg Health Study* as of September 2011.

Reference population 2 with 520 individuals was used to determine the concentration representing the 99th percentile of glycogen phosphorylase BB. Data presented as percentage of patients and mean ± standard deviation for symmetric continuous variables. CAD denotes coronary artery disease; AMI, acute myocardial infarction.

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eTable 2. Correlations of biomarkers

	Cont. TnI	hs TnI	CK	CK-MB	Myo	Crea	CRP	sFLT1	GDF15	PIGF	htFABP	MPO	GPBB	Copeptin
Cont. Troponin I		0.79	0.30	0.55	0.45	0.14	0.23	0.15	0.24	0.16	0.55	0.15	0.14	0.28
hs Troponin I	< .0001		0.29	0.58	0.55	0.22	0.28	0.15	0.39	0.23	0.62	0.14	0.16	0.33
CK	< .0001	< .0001		0.66	0.52	0.15	-0.02	-0.02	-0.02	-0.02	0.30	-0.03	0.11	0.05
CK-MB	< .0001	< .0001	< .0001		0.62	0.14	0.09	0.07	0.22	0.11	0.55	0.04	0.12	0.19
Myoglobin	< .0001	< .0001	< .0001	< .0001		0.39	0.13	0.17	0.41	0.19	0.76	0.13	0.17	0.35
Creatinine	< .0001	< .0001	< .0001	< .0001	< .0001		0.09	0.09	0.37	0.16	0.36	0.05	0.05	0.36
CRP	< .0001	< .0001	.50	.00042	< .0001	.00023		0.06	0.30	0.32	0.22	0.16	0.15	0.18
sFLT1	< .0001	< .0001	.35	.0054	< .0001	.00021	.015		0.16	0.02	0.28	0.75	0.14	0.31
GDF15	< .0001	< .0001	.42	< .0001	< .0001	< .0001	< .0001	< .0001		0.35	0.52	0.15	0.15	0.38
PIGF	< .0001	< .0001	.35	< .0001	< .0001	< .0001	< .0001	.31	< .0001		0.24	0.08	0.09	0.15
htFABP	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001		0.24	0.18	0.43
MPO	< .0001	< .0001	.25	.12	< .0001	.022	< .0001	< .0001	< .0001	.0013	< .0001		0.15	0.23
GPBB	< .0001	< .0001	< .0001	< .0001	< .0001	.050	< .0001	< .0001	< .0001	.00037	< .0001	< .0001		0.18
Copeptin	< .0001	< .0001	.085	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	

Spearman correlation coefficients are given above, corresponding p-values below the diagonal. Cont. TnI denotes contemporary sensitive troponin I, hs TnI denotes high sensitive troponin I, sFLT1 denotes soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), GDF15 denotes growth differentiation factor 15, PIGF denotes placental growth factor, htFABP denotes heart type fatty acid binding protein, MPO denotes myeloperoxidase and GPBB denotes glycogen phosphorylase BB, CRP denotes C-reactive protein, Crea denotes creatinine

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eTable 3. Diagnostic performance for identification of an acute myocardial infarction of the individual biomarkers and the combination with a high sensitive troponin I according to time after chest pain onset, gender and age

Suppl. Table 3A	**Overall cohort** n=1818			**Chest pain onset <3h** n=696		
	AUC (CI) Individual Marker	**AUC (CI)** Combination with hs Troponin I	**p_{ROC}-Value** Combination vs. hs Troponin I	**AUC (CI)** Individual Marker	**AUC (CI)** Combination with hs Troponin I	**p_{ROC}-Value** Combination vs. hs Troponin I
cont. Troponin I	0.92 (0.90,0.94)	-	-	0.88 (0.85,0.91)	-	-
hs Troponin I						
On admission	0.96 (0.95,0.97)	-	-	0.96 (0.94,0.97)	-	-
δ change 0 to 3 h	0.66 (0.62,0.70)	0.99 (0.98,0.99)	< 0.001	0.82 (0.77,0.87)	0.98 (0.97,1.00)	0.003
Creatine kinase	0.71 (0.68,0.74)	0.96 (0.95,0.97)	0.90	0.64 (0.58,0.69)	0.96 (0.94,0.97)	0.80
Creatine kinase-MB	0.85 (0.82,0.87)	0.96 (0.95,0.97)	0.90	0.78 (0.74,0.83)	0.96 (0.94,0.97)	0.64
Myoglobin	0.83 (0.80,0.85)	0.96 (0.95,0.97)	0.82	0.84 (0.81,0.88)	0.96 (0.95,0.98)	0.92
sFLT1	0.65 (0.62,0.68)	0.97 (0.96,0.97)	0.03	0.66 (0.61,0.70)	0.96 (0.95,0.98)	0.24
GDF15	0.64 (0.61,0.68)	0.96 (0.95,0.97)	0.12	0.69 (0.64,0.74)	0.96 (0.94,0.97)	0.22
PIGF	0.59 (0.56,0.63)	0.96 (0.95,0.97)	0.11	0.58 (0.53,0.63)	0.96 (0.94,0.97)	0.68
H-FABP	0.89 (0.87,0.91)	0.97 (0.96,0.98)	0.02	0.91 (0.87,0.94)	0.96 (0.95,0.98)	0.35
MPO	0.63 (0.60,0.66)	0.96 (0.95,0.97)	0.39	0.63 (0.58,0.68)	0.96 (0.94,0.97)	0.83
GPBB	0.63 (0.59,0.66)	0.96 (0.95,0.97)	0.99	0.65 (0.59,0.70)	0.96 (0.94,0.97)	0.996
Copeptin	0.74 (0.70,0.77)	0.97 (0.96,0.98)	0.01	0.79 (0.75,0.84)	0.97 (0.96,0.98)	0.06

Suppl. Table 3B	**Females** n=610			**Males** n=1208		
	AUC (CI) Individual Marker	**AUC (CI)** Combination with hs Troponin I	**p_{ROC}-Value** Combination vs. hs Troponin I	**AUC (CI)** Individual Marker	**AUC (CI)** Combination with hs Troponin I	**p_{ROC}-Value** Combination vs. hs Troponin I
cont. Troponin I	0.97 (0.95,0.98)	-	-	0.96 (0.95,0.97)	-	-
hs Troponin I						
On admission	0.97 (0.95,0.98)	-	-	0.96 (0.95,0.97)	-	-
δ change 0 to 3 h	0.59 (0.50,0.68)	0.99 (0.98,0.99)	0.001	0.69 (0.64,0.74)	0.98 (0.98,0.99)	< 0.001
Creatine kinase	0.73 (0.67,0.79)	0.97 (0.96,0.98)	0.75	0.68 (0.64,0.72)	0.96 (0.95,0.97)	0.72
Creatine kinase-MB	0.87 (0.82,0.92)	0.97 (0.95,0.98)	0.47	0.83 (0.80,0.86)	0.96 (0.95,0.97)	0.86
Myoglobin	0.85 (0.80,0.89)	0.97 (0.95,0.98)	0.77	0.81 (0.78,0.84)	0.96 (0.95,0.97)	0.69
sFLT1	0.61 (0.55,0.67)	0.97 (0.96,0.99)	0.17	0.66 (0.63,0.70)	0.96 (0.95,0.97)	0.08
GDF15	0.66 (0.60,0.73)	0.97 (0.95,0.98)	0.50	0.64 (0.60,0.68)	0.96 (0.95,0.97)	0.11
PIGF	0.63 (0.57,0.69)	0.97 (0.95,0.98)	0.51	0.57 (0.53,0.61)	0.96 (0.95,0.97)	0.22

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H-FABP	0.90 (0.86,0.93)	0.97 (0.96,0.99)	0.06	0.89 (0.87,0.92)	0.97 (0.95,0.98)	0.08
MPO	0.61 (0.54,0.67)	0.97 (0.95,0.98)	0.41	0.64 (0.60,0.68)	0.96 (0.95,0.97)	0.55
GPBB	0.60 (0.54,0.67)	0.97 (0.95,0.98)	0.43	0.63 (0.59,0.66)	0.96 (0.95,0.97)	0.57
Copeptin	0.73 (0.67,0.80)	0.98 (0.97,0.99)	0.07	0.72 (0.69,0.76)	0.96 (0.95,0.97)	0.13

Suppl. Table 3C	**Patients > 70 years** n=524			**Patients ≤ 70 years** n=1294		
	AUC (CI) Individual Marker	**AUC (CI)** Combination with hs Troponin I	**p_{ROC}-Value** Combination vs. hs Troponin I	**AUC (CI)** Individual Marker	**AUC (CI)** Combination with hs Troponin I	**p_{ROC}-Value** Combination vs. hs Troponin I
cont. Troponin I	0.96 (0.94,0.97)	-	-	0.97 (0.96,0.98)	-	-
hs Troponin I						
On admission	0.96 (0.94,0.97)	-	-	0.97 (0.96,0.98)	-	-
δ change 0 to 3 h	0.69 (0.62,0.76)	0.99 (0.98,1.00)	< 0.001	0.66 (0.61,0.71)	0.98 (0.97,0.99)	< 0.001
Creatine kinase	0.79 (0.75,0.84)	0.96 (0.94,0.98)	0.53	0.68 (0.64,0.72)	0.97 (0.96,0.98)	0.55
Creatine kinase-MB	0.86 (0.82,0.90)	0.96 (0.94,0.98)	0.62	0.84 (0.81,0.87)	0.97 (0.96,0.98)	0.72
Myoglobin	0.86 (0.81,0.90)	0.96 (0.93,0.98)	0.95	0.82 (0.78,0.85)	0.97 (0.96,0.98)	0.74
sFLT1	0.59 (0.53,0.64)	0.96 (0.94,0.98)	0.27	0.67 (0.63,0.70)	0.97 (0.96,0.98)	0.11
GDF15	0.63 (0.57,0.68)	0.96 (0.94,0.97)	0.60	0.65 (0.61,0.68)	0.97 (0.96,0.98)	0.98
PIGF	0.57 (0.51,0.63)	0.96 (0.94,0.97)	0.86	0.59 (0.55,0.63)	0.97 (0.96,0.98)	0.72
H-FABP	0.89 (0.85,0.92)	0.97 (0.95,0.98)	0.01	0.90 (0.87,0.92)	0.97 (0.96,0.98)	0.13
MPO	0.57 (0.51,0.63)	0.96 (0.94,0.98)	0.41	0.65 (0.61,0.69)	0.97 (0.95,0.98)	0.85
GPBB	0.63 (0.57,0.69)	0.96 (0.94,0.98)	0.40	0.62 (0.58,0.66)	0.96 (0.95,0.97)	0.69
Copeptin	0.67 (0.61,0.74)	0.95 (0.93,0.97)	0.09	0.75 (0.71,0.79)	0.97 (0.96,0.98)	0.05

Presented area under the receiver operator characteristics (ROC) curve (AUC) for individual diagnostic biomarkers and their combination with a high sensitive troponin I determination (hs Troponin I) on admission as well as the diagnostic ability of the δ change between admission and 3 hours in hs troponin I concentration and its combination with the concentration on admission. Data is given individually for the overall cohort and patients presenting within 3 hours after symptom onset (A), female and male individuals (B) and for elderly patients (>70years) compared to patients 70 years or younger (C).

AUCs are given with corresponding 95% confidence interval (CI). sFLT1 denotes soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), GDF15 denotes growth differentiation factor 15, PIGF denotes placental growth factor, H-FABP denotes heart type fatty acid binding protein, MPO denotes myeloperoxidase and GPBB denotes glycogen phosphorylase BB.

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eTable 4. Diagnostic information on identification of non-ST elevation acute coronary syndrome (NSTE-ACS) including myocardial infarction and unstable angina pectoris by use of a high sensitive troponin I determination (hsTnI) and the relative δ change in concentration within 3 hours after admission

Suppl. Table 4A

	hsTnI on admission		hsTnI after 3 hours	
	> LoD	> 99th percentile	> LoD%	> 99th percentile
Sensitivity (CI)	92.9 (90.0,95.2)	56.6 (51.7,61.5)	100.0 (98.6,100.0)	65.9 (61.1,70.5)
Specificity (CI)	37.3 (34.2,40.6)	93.7 (91.9,95.2)	2.0 (1.2,3.2)	92.2 (90.3,93.9)
PPV (CI)	40.5 (37.3,43.7)	80.5 (75.4,84.9)	31.9 (29.4,34.5)	79.6 (74.9,83.8)
NPV (CI)	92.0 (88.7,94.6)	82.5 (80.0,84.8)	100.0 (74.0,100.0)	85.5 (83.1,87.7)
N_{pos}/N_{all}	936/1297	287/1297	1279/1297	338/1297

Suppl. Table 4B

	hsTnI δ change 0 to 3 hours after admission								
	≥20%	≥30%	≥50%	≥75%	≥100%	≥150%	≥200%	≥250%	≥266%*
hsTnI δ change 0 to 3 hours									
Sensitivity (CI)	72.3 (67.7,76.6)	67.2 (62.4,71.7)	57.4 (52.4,62.2)	48.5 (43.6,53.5)	44.4 (39.5,49.3)	37.7 (33.0,42.6)	33.1 (28.5,37.9)	29.2 (24.8,33.8)	27.7 (23.4,32.3)
Specificity (CI)	24.7 (21.9,27.7)	32.1 (29.0,35.2)	44.9 (41.6,48.2)	53.7 (50.3,57.0)	57.8 (54.5,61.1)	67.5 (64.3,70.6)	83.0 (80.4,85.4)	92.1 (90.2,93.8)	93.6 (91.8,95.1)
PPV (CI)	30.6 (27.7,33.6)	31.2 (28.2,34.4)	32.3 (28.9,35.9)	32.5 (28.8,36.3)	32.6 (28.7,36.6)	34.8 (30.3,39.4)	47.2 (41.3,53.2)	63.0 (55.7,69.9)	66.5 (58.8,73.5)
NPV (CI)	66.1 (60.7,71.1)	68.0 (63.3,72.5)	69.6 (65.7,73.4)	69.4 (65.8,72.9)	69.4 (65.9,72.7)	70.3 (67.1,73.3)	73.0 (70.1,75.7)	73.9 (71.2,76.5)	73.8 (71.2,76.4)
N_{pos}/N_{all}	964/1297	878/1297	724/1297	610/1297	556/1297	443/1297	286/1297	189/1297	170/1297
hsTnI > LoD on admission AND hsTnI δ change 0 to 3 hours									
Sensitivity (CI)	65.2 (60.4-69.8)	60.0 (55.1-64.8)	50.2 (45.3-55.2)	41.4 (36.6-46.4)	37.3 (32.5-42.1)	31.9 (27.4-36.6)	28.7 (24.3-33.3)	26.5 (22.3-31.0)	26.2 (22.0-30.8)
Specificity (CI)	60.9 (57.6-64.1)	68.2 (65.0-71.2)	80.9 (78.1-83.4)	89.7 (87.5-91.6)	93.4 (91.5-94.9)	97.1 (95.7-98.1)	98.3 (97.2-99.1)	98.7 (97.7-99.3)	98.7 (97.7-99.3)
PPV (CI)	43.3 (39.4-47.3)	46.4 (42.1-50.8)	54.7 (49.5-59.8)	64.8 (58.6-70.5)	72.0 (65.5-78.0)	83.3 (76.5-88.8)	88.6 (82.0-93.5)	90.0 (83.2-94.7)	89.9 (83.0-94.7)
NPV (CI)	79.2 (76.0-82.2)	78.8 (75.7-81.6)	78.0 (75.2-80.6)	76.9 (74.2-79.5)	76.4 (73.8-78.9)	75.6 (73.0-78.1)	75.0 (72.4-77.5)	74.5 (71.9-77.0)	74.4 (71.9-76.9)
N_{pos}/N_{all}	708/1297	606/1297	434/1297	310/1297	254/1297	197/1297	171/1297	157/1297	156/1297
hsTnI > 99th percentile on admission AND hsTnI δ change 0 to 3 hours									
Sensitivity (CI)	39.0 (34.2-43.9)	35.5 (30.9-40.4)	30.9 (26.4-35.6)	26.5 (22.3-31.0)	24.8 (20.6-29.2)	21.3 (17.4-25.6)	19.6 (15.9-23.8)	18.4 (14.7-22.5)	18.1 (14.5-22.2)

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	≥20%	≥30%	≥50%	≥75%	≥100%	≥150%	≥200%	≥250%	≥266%*
Specificity (CI)	97.1 (95.7-98.1)	98.3 (97.2-99.1)	99.3 (98.5-99.8)	99.3 (98.5-99.8)	99.4 (98.7-99.8)	99.4 (98.7-99.8)	99.6 (98.9-99.9)	99.7 (99.0-99.9)	99.7 (99.0-99.9)
PPV (CI)	85.9 (80.1-90.6)	90.6 (85.0-94.7)	95.5 (90.4-98.3)	94.7 (88.9-98.0)	95.3 (89.3-98.5)	94.6 (87.8-98.2)	95.2 (88.3-98.7)	96.2 (89.2-99.2)	96.1 (89.0-99.2)
NPV (CI)	77.6 (75.0-80.0)	76.9 (74.3-79.3)	75.8 (73.2-78.2)	74.6 (72.1-77.1)	74.2 (71.6-76.7)	73.4 (70.8-75.8)	73.0 (70.4-75.4)	72.7 (70.1-75.2)	72.6 (70.0-75.1)
N_{pos}/N_{all}	221/1297	193/1297	161/1297	141/1297	131/1297	116/1297	107/1297	101/1297	100/1297
hsTnI δ change 0 to 3 hours AND hsTnI > 99th percentile after 3 hours									
Sensitivity (CI)	23.2 (17.2-30.1)	22.6 (16.7-29.5)	22.6 (16.7-29.5)	22.0 (16.2-28.9)	21.5 (15.7-28.3)	20.9 (15.2-27.6)	19.2 (13.7-25.8)	18.1 (12.7-24.6)	18.1 (12.7-24.6)
Specificity (CI)	97.8 (96.6-98.7)	97.8 (96.6-98.7)	98.1 (96.9-98.9)	98.1 (96.9-98.9)	98.3 (97.2-99.1)	98.3 (97.2-99.1)	98.8 (97.8-99.4)	99.0 (98.1-99.6)	99.0 (98.1-99.6)
PPV (CI)	69.5 (56.1-80.8)	69.0 (55.5-80.5)	71.4 (57.8-82.7)	70.9 (57.1-82.4)	73.1 (59.0-84.4)	72.5 (58.3-84.1)	77.3 (62.2-88.5)	80.0 (64.4-90.9)	80.0 (64.4-90.9)
NPV (CI)	85.7 (83.3-87.9)	85.6 (83.2-87.8)	85.6 (83.3-87.8)	85.5 (83.2-87.7)	85.5 (83.1-87.7)	85.4 (83.0-87.6)	85.2 (82.8-87.4)	85.1 (82.7-87.2)	85.1 (82.7-87.2)
N_{pos}/N_{all}	78/1010	76/1010	72/1010	71/1010	68/1010	67/1010	59/1010	54/1010	54/1010

Presented sensitivity, specificity, positive and negative predictive value (PPV and NPV) as percentage with 95% confidence interval (CI) for the high sensitive troponin I assay (hsTnI) on admission and after 3 hours by use of the 99th percentile with 30pg/mL and the level of detection (LoD) of the assay with 3.4pg/mL as cut-off. Additionally various relative troponin δ changes between admission and 3h and their combination with hsTnI are presented.

N_{pos}/N_{all} denotes number of patients with positive test criteria / number of patients with available data on criteria

* optimized δ change cut-off derived from receiver operator characteristics (ROC) analyses.

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eTable 5. 30-day outcome according to diagnosis and high sensitive troponin I level on admission

Suppl. Table 5	30-day follow-up		
	Myocardial Infarction n=10	**Death** n=20	**Myocardial Infarction and/or Death** n=26
According to gold standard diagnosis			
Non-coronary chest pain	3 (0.2%)	4 (0.2%)	5 (0.3%)
Unstable angina pectoris	1 (0.1%)	0 (0%)	1 (0.1%)
Acute Myocardial Infarction	6 (0.3%)	16 (0.9%)	20 (1.1%)
According to high sensitive troponin I on admission			
hsTnI > LoD	10 (0.6%)	16 (1.0%)	22 (1.4%)
hsTnI > 99th percentile	8 (0.5%)	14 (0.9%)	18 (1.1%)

Presented number and percentage within 30 days after enrollment for overall mortality and myocardial infarction after discharge (MI).

Follow-up information was available in 1783 patients.

HsTnI denotes high sensitive troponin determination on admission, LoD denotes level of detection of the high sensitive troponin I assay (3.4pg/mL), 99th percentile denotes the troponin I concentration representing the 99th percentile of a reference population (30pg/mL).

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eTable 6. Baseline characteristics of 1,176 low-risk patients presenting with chest pain

	N	NCCP n=875	UAP n=173	AMI n=128	All n=1176
Age (years)	1176	59.7 ± 13.9	65.2 ± 10.6	64.6 ± 12.0	61.0 ± 13.4
Male gender (%)	1176	539 (61.6)	117 (67.6)	100 (78.1)	756 (64.3)
Risk factors					
Body mass index (kg/m²)	1090	27.6 ± 4.7	28.0 ± 4.5	28.3 ± 4.6	27.8 ± 4.7
Hypertension (%)	1176	609 (69.6)	145 (83.8)	102 (79.7)	856 (72.8)
Diabetes mellitus (%)	1135	94 (11.1)	40 (24.2)	35 (27.8)	169 (14.9)
Smoking status					
Current smoker (%)	1169	198 (22.7)	31 (18.3)	40 (31.5)	269 (23.0)
Former smoker (%)	1149	240 (27.9)	51 (30.9)	38 (30.6)	329 (28.6)
Never smoker (%)	1147	422 (49.1)	81 (49.7)	46 (37.1)	549 (47.9)
Hyperlipidemia (%)	1176	620 (70.9)	138 (79.8)	95 (74.2)	853 (72.5)
Lipid status					
Total cholesterol (mg/dL)	1050	198.8 ± 49.9	200.3 ± 47.4	199.8 ± 56.4	199.1 ± 50.2
HDL cholesterol (mg/dL)	1048	52.2 ± 16.0	49.3 ± 14.8	46.6 ± 13.1	51.2 ± 15.6
LDL cholesterol (mg/dL)	1048	118.4 ± 41.4	121.2 ± 41.2	123.4 ± 49.8	119.3 ± 42.3
Parental CAD (%)	1137	290 (34.0)	55 (34.6)	41 (33.1)	386 (33.9)
Known CAD (%)	1147	258 (30.3)	96 (56.5)	51 (40.5)	405 (35.3)
Laboratory parameters at admission					
Cont. Troponin I (pg/mL)	1176	5.0 (5.0/5.0)	5.0 (5.0/5.0)	59.0 (12.0/133.5)	5.0 (5.0/5.0)
Hs Troponin I (pg/mL)	1176	4.5 (1.7/8.0)	7.7 (4.4/22.6)	65.2 (25.6/134.8)	5.6 (1.7/13.6)
Creatine kinase (U/L)	1166	74.000 (52.000/112.000)	71.000 (52.000/100.833)	91.000 (68.000/126.000)	76.000 (53.000/112.000)
Creatine kinase-MB (ng/mL)	1174	1.000 (0.600/1.400)	1.100 (0.700/1.600)	1.800 (1.200/3.200)	1.000 (0.700/1.600)
Myoglobin (ng/mL)	1172	46.200 (32.200/66.500)	48.700 (33.700/73.267)	113.100 (67.858/186.767)	49.450 (33.700/75.375)
C-reactive protein	1165	2.2 (1.1/5.1)	2.3 (1.3/4.3)	2.7 (1.3/6.0)	2.3 (1.2/5.0)
Creatinine	1172	0.93 (0.80/1.06)	0.93 (0.82/1.06)	0.98 (0.88/1.17)	0.93 (0.81/1.07)
eGFR (mL/min for 1.73m²)	1172	81.3 ± 20.5	79.8 ± 20.9	76.3 ± 21.0	80.6 ± 20.6
sFLT1 (pg/mL)	1152	303.900 (230.750/8047.450)	285.450 (222.017/7762.992)	5972.800 (285.617/10720.700)	322.650 (231.683/8419.292)
GDF15 (pg/mL)	1171	667.950 (497.150/1025.217)	777.900 (570.667/1118.367)	893.400 (592.017/1260.242)	712.900 (511.600/1069.483)
PIGF (pg/mL)	1158	16.300 (13.400/20.000)	17.800 (14.417/21.183)	16.800 (14.200/20.983)	16.600 (13.600/20.200)
H-FABP (ng/mL)	1114	2.030 (1.470/2.812)	2.160 (1.633/3.113)	6.960 (3.963/12.820)	2.180 (1.540/3.320)
MPO (pmol/L)	1152	561.930 (386.623/1258.617)	544.260 (352.452/1156.150)	1075.700 (460.463/1530.567)	583.515 (387.315/1266.025)
GPBB (ng/mL)	1112	4.614 (3.668/6.146)	4.859 (3.822/6.217)	5.552 (4.116/8.420)	4.716 (3.710/6.294)
Copeptin (pmol/L)	899	5.000 (2.800/11.333)	4.850 (2.792/11.300)	19.400 (7.967/48.667)	5.500 (3.000/13.183)
Time of chest pain onset (h)	1176	4.0 (1.9/10.7)	4.2 (2.0/14.5)	2.5 (1.5/5.5)	3.9 (1.9/10.1)

Only patients without ST-elevations or clearly elevated troponin I (contemporary troponin I determination on admission above WHO cut-off of 300pg/mL for MI) are presented.

Data presented as number (percentage) of patients, mean ± standard deviation for symmetric variables, or median and $25^{th}/75^{th}$ interquartile range for skewed variables.

HDL denotes high-density lipoprotein, LDL denotes low-density lipoprotein, CAD denotes coronary artery disease, cont. troponin I denotes contemporary sensitive troponin I, hs Troponin I denotes highly sensitive determined troponin I, sVEGFR-1/sFLT-1 denotes, soluble vascular endothelial growth factor receptor-1, GDF15 growth differentiation factor 15, PIGF denotes placental growth factor, H-FABP denotes heart type fatty acid binding protein, MPO denotes myeloperoxidase, GPBB denotes glycogen phosphorylase BB, NCCP denotes non-coronary chest pain, UAP denotes unstable angina pectoris, AMI denotes acute myocardial infarction, eGFR denotes estimated glomerular filtration rate.

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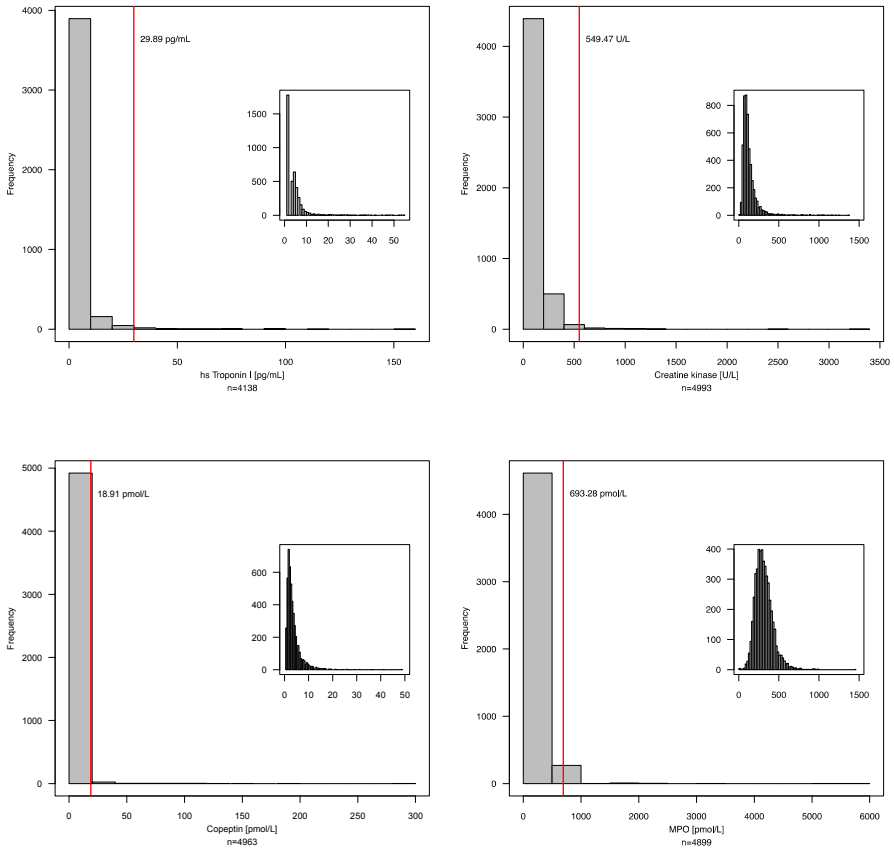
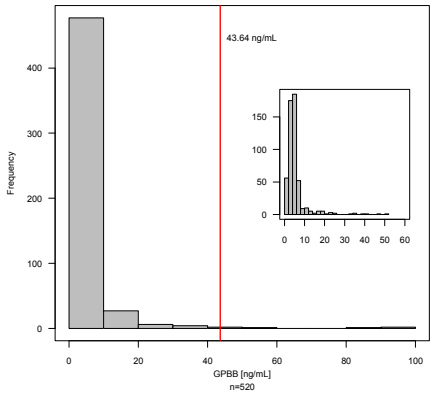
eFigure 1. Distribution of individual biomarkers in a reference population

A



B



Histogram and respective 99th percentile (red line) of high sensitive troponin I, creatine kinase, copeptin and myeloperoxidase determined in reference population 1, (*Gutenberg Health Study,* n=5,000, characteristics given in *supplementary table 1*)(A) and glycogen phosphorylase BB (GPBB) determined in reference population 2 (n=520, characteristics given in *supplementary table 1*)(B). As insert image the histograms are given with enlarged the x-axis around the biomarker concentration of interest for easier interpretation.

Bin size for the histogram of high sensitive troponin was 10 and 1 for the insert figure, of creatine kinase 200 and 20, of copeptin 20 and 0.5, of myeloperoxidase 500 and 20, and of glycogen phosphorylase BB (GPBB) 10 and 2.

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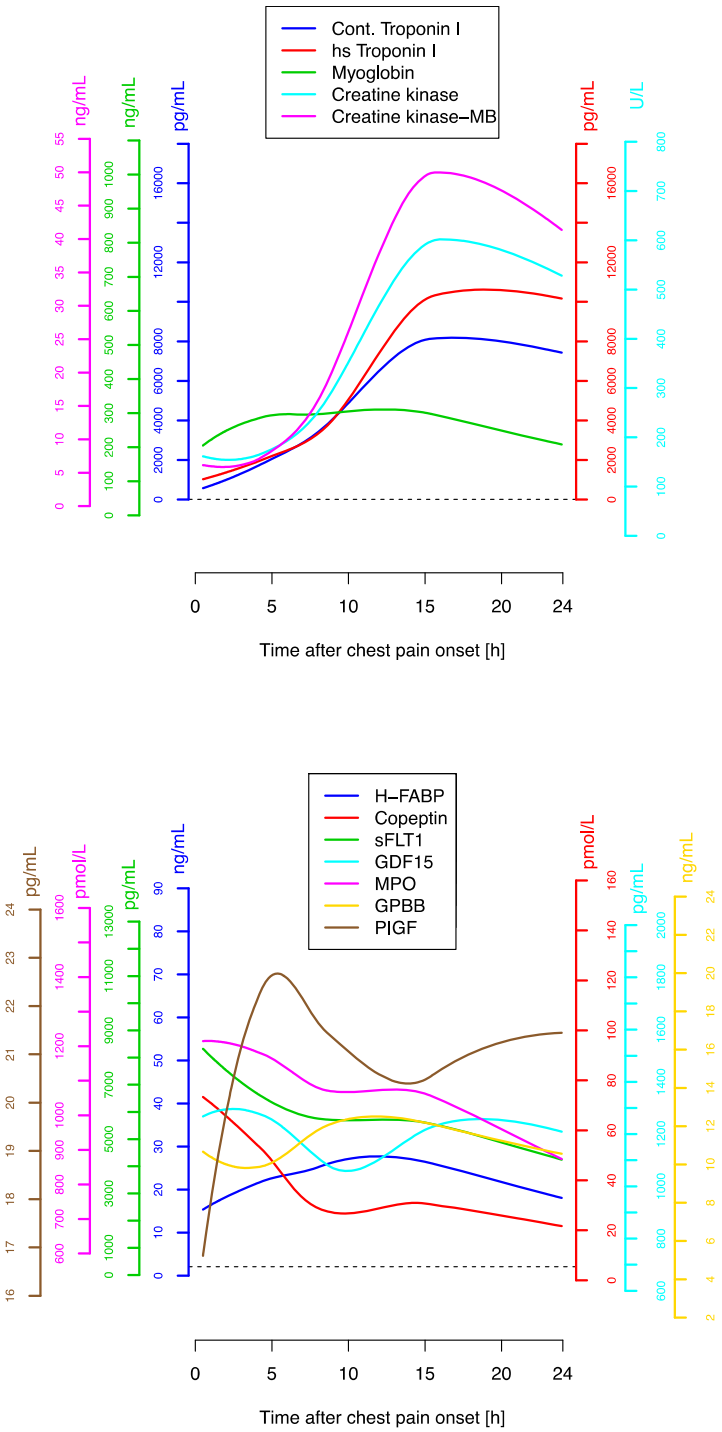
eFigure 2. Biomarker time course after acute myocardial infarction

A



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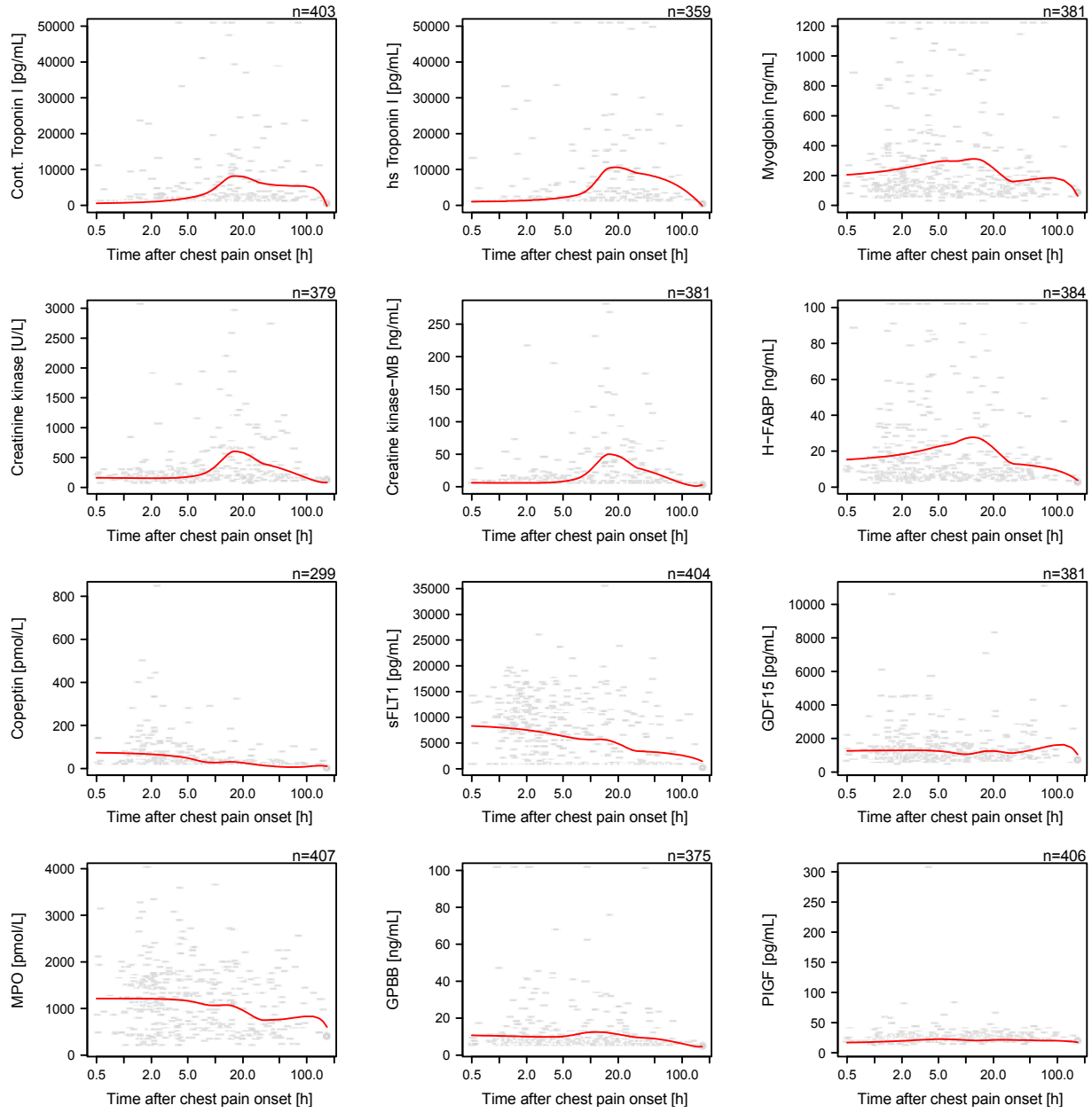
B.



Sensitivities, specificities (A) and positive and negative predictive values (PPV and NPV)(B) are graphed as percentage with 95% confidence interval for high sensitive troponin I (hsTnI) on admission and after 3 hours according to the 99th percentile as well as hsTnI above the 99th percentile in combination with the δ change in troponin concentration between admission and 3 hours or in combination with the evaluated individual early biomarkers.

As δ change cut-off 266% as optimized ROC derived cut-off, 82% as the reference change value (RCV) cut-off (*) and a 50% (**) cut-off were used. Error bars indicate 95% CIs

N denotes number of patients with positive test criteria / number of patients with available data on criteria.

sFLT1 denotes soluble vascular endothelial growth factor receptor-1 (sVEGFR-1); GDF15, growth differentiation factor 15; PlGF, placental growth factor; htFABP, heart type fatty acid binding protein; MPO, myeloperoxidase; GPBB, glycogen phosphorylase BB.

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