SUPPLEMENTAL MATERIAL

Supplement to:

Prospective Validation of the 0/1h-Algorithm for Early Diagnosis of Myocardial Infarction

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Table of contents

I. Supplemental Methods

Adjudication of the final diagnosis

MI was defined and cTn levels were interpreted as recommended in current guidelines.(1-4) In brief, MI was diagnosed when there was evidence of myocardial necrosis with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Patients with MI were further subdivided into Type 1 MI (primary coronary events) and Type 2 MI (ischemia due to increased demand or decreased supply, for example tachyarrhythmias or hypertensive crisis).(1,2)

The adjudication of final diagnoses was performed centrally in the core lab of the respective study for all patients incorporating serial measurements of different hs-(cTn) levels obtained as part of routine clinical care and in addition serial measurements of hs-cTnT from study blood draws in APACE (see test characteristics above). In BACC, the adjudication was based on serial measurements of hs-cTnT obtained as part of routine clinical care. More specifically, two independent cardiologists not directly involved in patient care reviewed all available medical records (including patient history, physical examination, results of laboratory testing including hs-cTnT levels, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, discharge summary) pertaining to the patient from the time of ED presentation to 90-day follow-up. Late samples were available for adjudication of final diagnosis in all patients. In general, serial sampling was performed until at least 3h after presentation to the ED or onset of chest.[5] In situations of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third cardiologist. While discharge diagnoses often were correct and in agreement with the final adjudicated diagnosis, there were also cases where those diagnoses needed to be revised, most often because more

information became available from medical testing during early follow-up, and more rarely, because the discharge diagnosis was not in agreement with the Universal Definition of MI.

The 99th percentile of the hs-cTnT assay (14ng/L) was used as cut-off for myocardial necrosis. In the APACE-study, absolute hs-cTnT changes were used to determine significant changes based on the diagnostic superiority of absolute over relative changes.(5,6) Based on studies of the biological variation of hs-cTnT(7,8) as well as on data from previous chest pain cohort studies,(9,10) a significant absolute change was defined as a rise or fall of at least 10ng/L within six hours, or, in an assumption of linearity, as an absolute change of 6ng/L within three hours. In the BACC-study, relative hs-cTnT changes were used to determine significant changes.(4) A significant relative change was defined as a rise in hs-cTnT of >50% within 3 hours in patients presenting with a normal (≤99th percentile) hs-cTnT level at baseline or as a rise in hs-cTnT of >20% within 3 hours in patients presenting with an elevated (>99th percentile) hs-cTnT level. All other patients were classified in the categories of unstable angina (UA), stable angina, non-cardiac chest pain (NCCP), cardiac but non-coronary disease (e.g. tachyarrhythmias, perimyocarditis), and symptoms of unknown origin with normal levels of hs-cTnT.

The decision limits applied for the additional (hs)-cTn assays measured as part of routine clinical care in APACE were as follows: Routine clinical care comprised five different cTn assays at the different hospitals and at the different recruitment periods. The cTn assays used clinically in most of the participating institutions changed during the study from a conventional cTn assay to the hs-cTnT assay. In patients in whom clinically a conventional cTn assay was used, the conventional cTn values and the hs-cTnT values were available for the adjudication. In patients in whom hs-cTnT was

also used clinically, two sets of serial hs-cTnT measurements were available for the adjudication.

The following conventional cTn assays were used: For the Roche cTnT 4th generation assay, the 10% CV level is 0.035µg/l. The laboratories of the participating sites reported only two decimals; therefore 0.04µg/l was used as a cut-off for myocardial necrosis. In order to fulfil the criteria of a significant change (30% of 99th percentile or 10% CV level), a patient would e.g. need to have a level of <0.01µg/l at presentation and 0.04µg/l at 6h. A patient would also qualify if the first level is 0.02µg/l and the second 0.04µg/l. A patient would not fulfil the criteria if the first level is 0.03µg/l and the second is 0.04µg/l. If the first level is 0.04µg/l, the second level needs to be at least 0.06ug/l. For the Abbott Axsym cTnI ADV, the 10% CV level is 0.16µg/l. A patient having 0.16µg/l at presentation would meet the criteria for significant change if the second was ≥0.21µg/l. A patient having <0.12µg/l at presentation (limit of detection) would qualify if the second is >0.16µg/l. For the Beckmann Coulter Accu cTnI, the 10% CV level is 0.06µg/l. A patient having 0.06µg/l at presentation would qualify if the second is ≥0.08µg/l. A patient having 0.05µ/l at presentation would qualify if the second is 0.07µg/l, but not 0.06µg/l. A patient having undetectable cTnI (cTnI<0.01µg/l) at presentation would qualify if the second is ≥0.06µg/l. For the Siemens Dimension Vista s-cTnI, the 10% CV level is 0.04µg/l. The limit of detection is 0.015µg/l and the 99th percentile is 0.045µg/l. An absolute change of 0.02µg/l or more within 3-6 hours was considered significant.

Measurements of hs-cTnT and hs-cTnI

Blood samples were collected into tubes containing plasma or serum at the time of the patient's presentation to the ED and after one hour. All measurements performed as part of routine clinical care were performed from fresh samples directly in the central laboratory (APACE: different (hs)-cTn including hs-cTnT; BACC: hs-cTnT). Measurements performed specifically for study purposes (hs-cTnT and hs-cTnI) were performed in part from fresh samples and in part in batches from samples that were frozen at -80°C in a dedicated core laboratory. Levels of hs-cTnT were determined on the Elecsys (Roche Diagnostics, Rotkreuz, Switzerland) and levels of hs-cTnI on the ARCHITECT (STAT high-sensitivity troponin I, Abbott Laboratories, IL, USA)**.** According to the manufacturer, the hs-cTnT assay has a 99th percentile concentration of 14ng/L, fulfilling a co-efficient of variation (CV) of <10% at 13ng/L and a limit of detection (LOD) at 5ng/L.(11,12) This study did not include measurements with hs-cTnT lots that required the revision of the calibration curve.(13-15) According to the manufacturer, the hs-cTnI assay has a 99th percentile concentration of 26.2ng/L with a corresponding CV of <5% and a LOD of 1.9ng/L.(16-18)

Statistical analysis

As the European Society of Cardiology 0/1h-Algorithm does not reflect a simple binary test (positive/negative) but contains three triage categories (rule-out/observe/rule-in), no classical 2x2 tables but 2x3s tables were constructed to assess its diagnostic performance. All diagnostic performance measures (proportions, predictive values and LR for NSTEMI in the three triage categories) were derived from these 2x3 tables as described in detail in **Online Figure 3.** Interaction p-values were calculated using Chi-square test to compare the ESC 0/1h-algorithm's diagnostic performance in the predefined subgroups.

Mortality and MACE during follow-up was analyzed using Kaplan-Meier survival curves. Categorical net reclassification improvement analysis was used to compare the ability of the ESC hs-cTnT and the hs-cTnI 0/1h-algorithms to correctly classify patients to rule-out, observe and rule-in according to risk of one-year mortality and incidence of MACE. The incremental prognostic value of triage by the ESC 0/1h-algorithm on top of the NSTEMI diagnosis was assessed using univariable and multivariable COX-regression analyses.

Continuous variables are described as median with interquartile range [IQR], categorical variables by numbers and percentages. Differences in baseline characteristics were assessed using the Mann-Whitney-U test for continuous variables and the Pearson Chi-square test or Fisher's exact test for categorical variables, as appropriate.

All hypothesis testing was two-tailed and p-values <0.05 were considered statistically significant. No adjustment for cohort origin was performed. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0. (IBM Corp., Armonk, NY) and MedCalc for Windows, version 18.2.1 (MedCalc Software, Ostend, Belgium).

II. Supplemental Tables

Online Table 1: STARD checklist for studies of diagnostic accuracy

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	**3**
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	**3**
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	**5**
	4	Study objectives and hypotheses	**5**
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	**6**
Participants	**6**	Eligibility criteria	**6-7**
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	**6**
	8	Where and when potentially eligible participants were identified (setting, location and dates)	**6**
	9	Whether participants formed a consecutive, random or convenience series	**6**
Test methods	**10a**	Index test, in sufficient detail to allow replication	**8, Suppl. 5**
	10b	Reference standard, in sufficient detail to allow replication	**7-8, Suppl. 2-4**
	11	Rationale for choosing the reference standard (if alternatives exist)	**7-8**
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	**8, Suppl. 5**
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	**7-8, Suppl. 2-4**
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	**7**
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	**7-8, Suppl. 2-4**
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	**9-10, Suppl. 5-6**
	15	How indeterminate index test or reference standard results were handled	**6, Supp. Fig. 1**
	16	How missing data on the index test and reference standard were handled	**6, Supp. Fig. 1**
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	**Suppl. 5-6**
	18	Intended sample size and how it was determined	**n.a.**
RESULTS			
Participants	**19**	Flow of participants, using a diagram	**Suppl. Figure 1**
	20	Baseline demographic and clinical characteristics of participants	**11, Table 1**
	21a	Distribution of severity of disease in those with the target condition	**11**
	21b	Distribution of alternative diagnoses in those without the target condition	**11**
	22	Time interval and any clinical interventions between index test and reference standard	**n.a.**
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	**Figures 1-3, Table 2**
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	**12-14**
	25	Any adverse events from performing the index test or the reference standard	**n.a.**
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalizability	**19**
	27	Implications for practice, including the intended use and clinical role of the index test	**19-20**
OTHER INFORMATION			
	28	Registration number and name of registry	**3**
	29	Where the full study protocol can be accessed	**3**
	30	Sources of funding and other support; role of funders	**1-2**

Online Table 2: Baseline Characteristics of Patients in the Diagnostic Dataset A According to Recruiting Study Population

	All patients (n=4368)	APACE study (n=2859)	BACC study (n=1509)	p-value
Age – years	62 [50,74]	61 [49,74]	65 [51,75]	<0.001
Male gender	2921 (67)	905 (32)	542 (36)	0.004
Risk factors				
Hypertension	2739 (63)	1717 (60)	1022 (68)	<0.001
Hypercholesterolemia	1988 (46)	1397 (49)	591 (39)	<0.001
Current smoking	1069 (24)	720 (25)	349 (23)	0.133
History of smoking	1541 (35)	1088 (38)	453 (30)	<0.001
History				
Coronary artery disease	1459 (33)	945 (33)	514 (34)	0.501
Previous MI	919 (21)	676 (24)	243 (16)	<0.001
Peripheral artery disease	236 (5)	148 (5)	88 (6)	0.363
Previous stroke	258 (6)	158 (6)	100 (7)	0.141
Chest pain characteristics				
Early presenters (<3h after CPO)	1322 (30)	803 (28)	519 (34)	<0.001
ECG findings				
ST-segment depression	339 (8)	220 (8)	119 (8)	0.822
T-wave inversion	373 (9)	228 (8)	145 (10)	0.066
No significant ECG-changes	3539 (81)	2362 (83)	1177 (78)	<0.001
Vital signs				
Heart frequency – bpm	77 [66,89]	76 [66,89]	78 [67,90]	0.012
Systolic blood pressure – mmHg	143 [128,159]	141 [126,157]	146 [130,161]	<0.001
Diastolic blood pressure - mmHg	81 [72,91]	81 [71,91]	83 [75,92]	<0.001
Body mass index - kg/m^2	26 [23,29]	26 [24,30]	25[22,29]	<0.001
Creatinine clearance - $mL/min/1.73m^2$	85 [66,99]	89 [72,102]	77 [59,93]	<0.001
Chronic medication				
ASA	1561 (36)	1022 (36)	539 (36)	0.985
Anticoagulants	526 (12)	287 (10)	239 (16)	<0.001
B-blockers	1577 (36)	964 (34)	613 (41)	<0.001
Statins	1498 (34)	1010 (35)	488 (32)	0.048
ACEIs/ARBs	1789 (41)	1095 (38)	694 (46)	<0.001
Calcium antagonists	660 (15)	422 (15)	238 (16)	0.375
Nitrates	390 (9)	309 (11)	81 (5)	<0.001

Numbers are presented as median [q1, q3] or numbers (%). ASA = acetylsalicylic acid; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CPO = chest pain onset; ECG = electrocardiogram; NSTEMI = Non-ST-Segment-Elevation Myocardial Infarction; Creatinine clearance was calculated using CKD-EPI (chronic kidney disease epidemiology collaboration) formula.

Online Table 3: Baseline Characteristics of Patients in the Diagnostic Dataset A Triaged by the ESC 0/1h-Algorithm using hs-cTnT

	Rule-out (n=2493)	Observe (n=1107)	Rule-in (n=768)	p-value
Age – years	54 [44,65]	74 [64,80]	71 [59,79]	<0.001
Male gender	1576 (63)	816 (74)	529 (69)	<0.001
Risk factors				
Hypertension	1252 (50)	919 (83)	568 (74)	<0.001
Hypercholesterolemia	904 (36)	671 (61)	413 (54)	<0.001
Current smoking	722 (29)	178 (16)	169 (22)	<0.001
History of smoking	796 (32)	430 (39)	315 (41)	<0.001
History				
Coronary artery disease	575 (23)	573 (52)	311 (40)	<0.001
Previous MI	355 (14)	355 (32)	209 (27)	<0.001
Peripheral artery disease	55 (2)	107 (10)	74 (10)	<0.001
Previous stroke	98 (4)	102 (9)	58 (8)	<0.001
Chest pain characteristics				
Early presenters (<3h after CPO)	780 (31)	301 (27)	241 (31)	0.036
Hours since chest pain onset*	5.0 [2.0,15.0]	5.0 [3.0,12.0]	5.0 [2.0,12.0]	0.293
Hours since chest pain peak*	3.0 [1.5,6.0]	3.0 [2.0,6.0]	3.0 [2.0,8.0]	0.006
Pressurelike chest pain*	1127 (64)	465 (70)	332 (75)	<0.001
Radiating chest pain*	1068 (61)	353 (53)	273 (61)	0.001
Duration > 30 minutes*	1105 (63)	396 (60)	281 (63)	0.240
ECG findings				
ST-segment depression	67 (3)	108 (10)	164 (21)	<0.001
T-wave inversion	122 (5)	153 (14)	98 (13)	<0.001
No significant ECG-changes	2248 (90)	813 (73)	478 (62)	<0.001
Vital signs				
Heart frequency – bpm	76 [66,88]	77 [65,90]	80 [69,94]	<0.001
Systolic blood pressure – mmHg	142 [128,157]	144 [127,161]	142 [124,161]	0.247
Diastolic blood pressure - mmHg	83 [74,92]	80 [70,89]	80 [71,92]	<0.001
Body mass index - kg/m^2	26 [23,29]	27 [24,30]	26 [24,29]	<0.001
Creatinine clearance - $mL/min/1.73m^2$	94 [80,105]	69 [52,85]	71 [51,90]	<0.001
Chronic medication				
ASA	664 (27)	568 (51)	329 (43)	<0.001
Anticoagulants	164 (7)	249 (22)	113 (15)	<0.001
B-blockers	651 (26)	596 (54)	330 (43)	<0.001
Statins	636 (26)	557 (50)	305 (40)	<0.001
ACEIs/ARBs	744 (30)	668 (60)	377 (49)	<0.001
Calcium antagonists	264 (119)	254 (23)	142 (18)	<0.001
Nitrates	126 (5)	179 (16)	85 (11)	<0.001

Numbers are presented as median [q1, q3] or numbers (%). *detailed chest pain characteristics only available in the APACE study. ASA = acetylsalicylic acid; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CPO = chest pain onset; ECG = electrocardiogram; NSTEMI = Non-ST-Segment-Elevation Myocardial Infarction; Creatinine clearance was calculated using CKD-EPI (chronic kidney disease epidemiology collaboration) formula.

Online Table 4: Treatment Characteristics of the Patients Triaged by the ESC 0/1h-Algorithm Using High-Sensitivity Cardiac Troponin T

	Rule-out (n=2493)	Observe (n=1107)	Rule-in (n=768)	p-value
Cardiac examinations within 30 days following index presentation				
Cardiac stress test	361 (19%)	137 (16%)	65 (11%)	<0.001
Coronary angiography	174 (9%)	219 (26%)	383 (64%)	<0.001
Myocardial revascularisation	79 (4%)	124 (15%)	264 (44%)	<0.001
Discharge medication*				
ASA	385 (34%)	261 (61%)	216 (75%)	<0.001
DAPT	116 (10%)	103 (24%)	170 (59%)	<0.001
Anticoagulants	102 (9%)	111 (26%)	55 (19%)	<0.001
B-blockers	325 (28%)	270 (63%)	204 (71%)	<0.001
Statins	354 (31%)	293 (69%)	221 (77%)	<0.001
ACEIs/ARBs	362 (32%)	280 (66%)	217 (76%)	<0.001
Calcium antagonists	149 (13%)	130 (31%)	57 (20%)	<0.001
Nitrates	90 (8%)	101 (24%)	53 (18%)	<0.001

Numbers are presented as numbers (%). * data on discharge medication only available in the APACE study. ASA = acetylsalicylic acid; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; DAPT = dual antiplatelet therapy.

It is important to highlight that although the vast majority of patients triaged towards rule-in will ultimately be found to have MI, the rule-in group also includes patients with myocarditis, takotsubo cardiomyopathy, and heart failure. In addition, particularly in the initial phase of enrollment (2006-2010) several of the institutions contributing to patient enrollment used a less sensitive cardiac troponin assay for routine clinical care. Accordingly, some patients adjudicated as MI by the independent cardiologists based on all information available including serial hs-cTnT sampling, were missed by the clinical team (due to the sensitivity deficit of the clinical cTn assay) and did not receive the appropriate MI-treatment.

Online Table 5: Patients with an Adjudicated Diagnosis of Myocardial Infarction Missed by the hs-cTnT 0/1h-Algorithm (n=5)

Age	Sex	Time from CPO to first study blood draw, h	History of CAD	hs-cTnT (ng/L; peak value underlined)				hs-cTnI (ng/L; peak value underlined)				ST-depression	T-inversion	Clinical discharge diagnosis	CABG performed	PCI performed	NSTEMI type
				0h	1h	2h	3-24h	0h	1h	2h	3-24h						
74	female	1	no	10	12	17	<u>17</u>	3.1	7.3	9.5	<u>10.8</u>	no	no	unknown cause of chest pain	no	yes	1
67	female	1	no	6	7	12	<u>106</u>	4.0	8.0	<u>18.0</u>	-	no	no	NSTEMI	no	yes	1
86	female	17	yes	8	7	7	<u>79</u>	27	24	27	<u>29</u>	no	no	NSTEMI	no	no	1
65	female	3	yes	5	7	-	<u>12</u>	44.3	44.4	-	<u>56.0</u>	no	yes	NSTEMI	no	no	1
58	male	3	no	7	7	-	<u>19</u>	3.6	3.3	-	<u>18.9</u>	yes	no	arrhythmia	no	no	2

CPO = chest pain onset; CAD = coronary artery disease; CABG = coronary artery bypass grafting; PCI = percutaneous coronary intervention.

Online Table 6: Baseline Characteristics of Patients Triaged by the ESC 0/1h-Algorithm Using High-Sensitivity Cardiac Troponin I

	Rule-out (n=1533)	Observe (n=1167)	Rule-in (n=800)	p-value
Age – years	53 [43,64]	70 [59,78]	69 [57,78]	<0.001
Male gender	981 (64)	804 (69)	546 (68)	0.015
Risk factors				
Hypertension	739 (48)	916 (79)	592 (74)	<0.001
Hypercholesterolemia	520 (34)	622 (53)	428 (54)	<0.001
Current smoking	442 (29)	195 (17)	197 (25)	<0.001
History of smoking	479 (31)	476 (41)	302 (38)	<0.001
History				
Coronary artery disease	327 (21)	536 (46)	324 (41)	<0.001
Previous MI	189 (12)	324 (28)	214 (27)	<0.001
Peripheral artery disease	33 (2)	87 (7)	69 (9)	<0.001
Previous stroke	58 (4)	101 (9)	53 (7)	<0.001
Chest pain characteristics				
Early presenters (<3h after CPO)	488 (32)	347 (30)	229 (29)	0.232
Hours since chest pain onset*	5.0 [2.0,15.5]	6.0 [3.0,16.0]	6.0 [2.0,16.0]	0.190
Hours since chest pain peak*	3.0 [1.5,6.0]	3.0 [2.0,7.0]	3.0 [1.5,8.0]	0.012
Pressurelike chest pain*	662 (64)	388 (68)	325 (72)	0.004
Radiating chest pain*	636 (61)	314 (55)	261 (58)	0.062
Duration >30 minutes*	667 (64)	333 (59)	268 (60)	0.052
ECG findings				
ST-segment depression	32 (2)	91 (8)	135 (17)	<0.001
T-wave inversion	57 (4)	132 (11)	112 (14)	<0.001
No significant ECG-changes	1411 (92(	913 (78)	5147 (65)	<0.001
Vital signs				
Heart frequency – bpm	77 [67,88]	76 [65,89]	80 [70,93]	<0.001
Systolic blood pressure – mmHg	141 [128,155]	145 [129,162]	145 [128,161]	<0.001
Diastolic blood pressure - mmHg	83 [74,91]	80 [70,91]	82 [72,92]	0.006
Body mass index - kg/m^2	26 [23,29]	26 [34,30]	27 [24,30]	<0.001
Creatinine clearance - $mL/min/1.73m^2$	94 [80,106]	75 [57,90]	74 [55,91]	<0.001
Chronic medication				
ASA	383 (25)	546 (47)	349 (44)	<0.001
Anticoagulants	110 (7)	230 (20)	114 (14)	<0.001
B-blockers	373 (24)	575 (49)	342 (43)	<0.001
Statins	360 (23)	523 (45)	306 (38)	<0.001
ACEIs/ARBs	434 (28)	655 (56)	392 (49)	<0.001
Calcium antagonists	151 (10)	259 (22)	143 (18)	<0.001
Nitrates	70 (5)	151 (13)	79 (10)	<0.001

Numbers are presented as median [q1, q3] or numbers (%). *detailed chest pain characteristics only available in the APACE study. ASA = acetylsalicylic acid; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CPO = chest pain onset; ECG = electrocardiogram; NSTEMI = Non-ST-Segment-Elevation Myocardial Infarction; Creatinine clearance was calculated using CKD-EPI (chronic kidney disease epidemiology collaboration) formula.

Online Table 7: Treatment Characteristics of the Patients Triaged by the ESC 0/1h-Algorithm Using High-Sensitivity Cardiac Troponin I

	Rule-out (n=1533)	Observe (n=1167)	Rule-in (n=800)	p-value
Cardiac examinations within 30 days following index presentation				
Cardiac stress test	270 (19%)	185 (17%)	108 (14%)	0.035
Coronary angiography	118 (8%)	221 (20%)	437 (58%)	<0.001
Myocardial revascularisation	45 (3%)	120 (11%)	302 (40%)	<0.001
Discharge medication*				
ASA	292 (31%)	290 (57%)	280 (69%)	<0.001
DAPT	84 (9%)	108 (21%)	197 (49%)	<0.001
Anticoagulants	76 (8%)	113 (22%)	79 (20%)	<0.001
B-blockers	240 (25%)	293 (58%)	266 (66%)	<0.001
Statins	264 (28%)	317 (63%)	287 (71%)	<0.001
ACEIs/ARBs	263 (28%)	310 (61%)	286 (71%)	<0.001
Calcium antagonists	109 (11%)	144 (28%)	83 (21%)	<0.001
Nitrates	71 (7%)	104 (21%)	69 (17%)	<0.001

Numbers are presented as numbers (%). * data on discharge medication only available in the APACE study. ASA = acetylsalicylic acid; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; DAPT = dual antiplatelet therapy.

It is important to highlight that although the vast majority of patients triaged towards rule-in will ultimately be found to have MI, the rule-in group also includes patients with myocarditis, takotsubo cardiomyopathy, and heart failure. In addition, particularly in the initial phase of enrollment (2006-2010) several of the institutions contributing to patient enrollment used a less sensitive cardiac troponin assay for routine clinical care. Accordingly, some patients adjudicated as MI by the independent cardiologists based on all information available including serial hs-cTnT sampling, were missed by the clinical team (due to the sensitivity deficit of the clinical cTn assay) and did not receive the appropriate MI-treatment.

Online Table 8: Patients with an Adjudicated Diagnosis of Myocardial Infarction Missed by the ESC hs-cTnI 0/1h-Algorithm (n=5)

Age	Sex	Time from CPO to first study blood draw, h	History of CAD	hs-cTnT (ng/L; peak value underlined)				hs-cTnI (ng/L; peak value underlined)				ST-depression	T-inversion	Clinical discharge diagnosis	CABG performed	PCI performed	NSTEMI type
				0h	1h	2h	3-24h	0h	1h	2h	3-24h						
77	male	5	no	55	53	-	20	4.7	4.8	-	5.1	no	no	unknown cause of chest pain	no	yes	2
73	male	4	yes	33	32	28	9	3.4	3.9	-	-	no	no	unknown cause of chest pain	no	no	2
58	male	3	no	7	7	-	19	3.6	3.3	-	18.9	yes	no	arrhythmia	no	no	2
61	male	2	yes	25	23	-	24	3.2	1.9	-	8.5	no	no	NSTEMI	yes	no	1
75	male	2	no	22	20	-	14	4.9	4.5	-	5.8	no	yes	arrhythmia	no	no	2

CPO = chest pain onset; CAD = coronary artery disease; CABG = coronary artery bypass grafting;

PCI = percutaneous coronary interventions.

Online Table 9: Diagnostic Performance of the ESC 0/1h-Algorithm in the Total Cohort as well as in the two Contributing Studies

	N		Triage Group Rule-out	Observe	Rule-in	Proportion Rule-out	Proportion Direct Rule-out	Rule-out NPV	Rule-out LR	Proportion Rule-in	Proportion Direct Rule-In	Rule-in PPV	Rule-in LR	Proportion Rule-out and Rule-In
Hs-cTnT														
All Patients	4368	no NSTEMI	2488	949	196	57% (2493/4368)	16% (706/4368)	99.8% (2488/2493)	0.010 (5/735):(2488/3633)	18% (768/4368)	11% (482/4368)	74.5% (572/768)	14.43 (572/735):(196/3633)	75% (3261/4368)
		NSTEMI	5	158	572									
APACE study	2859	no NSTEMI	1746	567	100	61% (1749/2859)	18% (503/2859)	99.8% (1746/1749)	0.009 (3/446):(1746/2413)	16% (445/2859)	10% (291/2859)	77.5% (345/445)	18.67 (345/446):(100/2413)	77% (2194/2859)
		NSTEMI	3	98	345									
BACC study	1509	no NSTEMI	742	382	96	49% (744/1509)	13% (203/1509)	99.7% (742/744)	0.011 (2/289):(742/1220)	21% (323/1509)	13% (191/1509)	70.3% (227/323)	9.98 (227/289):(96/1220)	71% (1067/1509)
		NSTEMI	2	60	227									
Hs-cTnI														
All Patients	3500	no NSTEMI	1528	1082	302	44% (1533/3500)	10% (363/3500)	99.7% (1528/1533)	0.016 (5/588):(1528/2912)	23% (800/3500)	15% (523/3500)	62.3% (498/800)	8.17 (498/588):(302/2912)	67% (2333/3500)
		NSTEMI	5	85	498									
APACE study	2059	no NSTEMI	1038	525	182	51% (1040/2059)	11% (226/2059)	99.8% (1038/1040)	0.011 (2/314):(1038/1745)	22% (450/2059)	15% (319/2059)	59.6% (268/450)	8.18 (268/314):(182/1745)	72% (1490/2059)
		NSTEMI	2	44	268									
BACC study	1441	no NSTEMI	490	557	120	34% (493/1411)	10% (137/1441)	99.4% (490/493)	0.026 (3/274):(490/1167)	24% (350/1441)	14% (204/1441)	65.7% (230/350)	8.16 (230/274):(120/1167)	59% (843/1441)
		NSTEMI	3	41	230									

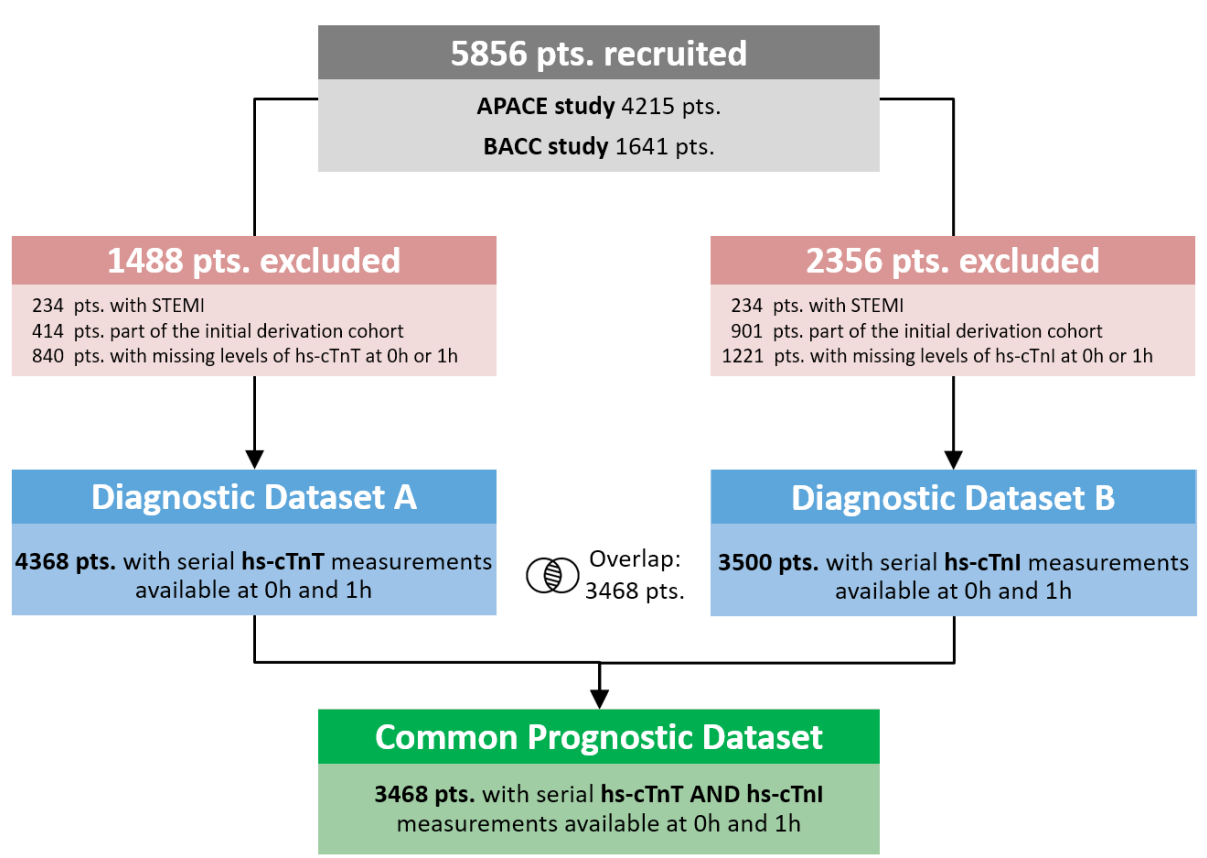
LR = likelihood ratio; NPV = negative predictive value; PPV = positive predictive value.

Online Table 10: Univariable and Multivariable Cox-Regression Models for the Prediction of one-year Mortality and MACE

	PREDICTION OF ONE-YEAR MORTALITY				PREDICTION OF ONE-YEAR MACE			
	Univariable		Multivariable		Univariable		Multivariable	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
Hs-cTnT								
NSTEMI	4.0 (2.8-5.6)	<0.001	1.3 (0.8-2.1)	<0.001	1789.0 (380.9-8402.6)	<0.001	1251.9 (175.0-8954.8)	<0.001
Triage group:								
○ Rule-out	Reference	-	Reference	-	Reference	-	Reference	-
○ Observe	9.6 (5.2-17.6)	<0.001	9.1 (5.0-16.9)	<0.001	14.5 (9.8-21.3)	<0.001	6.3 (4.2-9.6)	<0.001
○ Rule-in	14.9 (8.1-27.1)	<0.001	12.1 (6.0-24.2)	<0.001	64.3 (44.4-93.3)	<0.001	6.4 (4.1-9.9)	<0.001
Hs-cTnI								
NSTEMI	4.0 (2.8-5.6)	<0.001	2.5 (1.6-4.0)	<0.001	1789.0 (380.9-8402.6)	<0.001	1802.3 (251.8-12900.5)	<0.001
Triage group:								
○ Rule-out	Reference	-	Reference	-	Reference	-	Reference	-
○ Observe	7.2 (3.8-13.7)	<0.001	6.5 (3.4-12.4)	<0.001	7.1 (4.8-10.6)	<0.001	3.6 (2.4-5.5)	<0.001
○ Rule-in	10.3 (5.4-19.6)	<0.001	5.4 (2.6-11.4)	<0.001	41.2 (28.3-60.0)	<0.001	3.6 (2.3-5.6)	<0.001

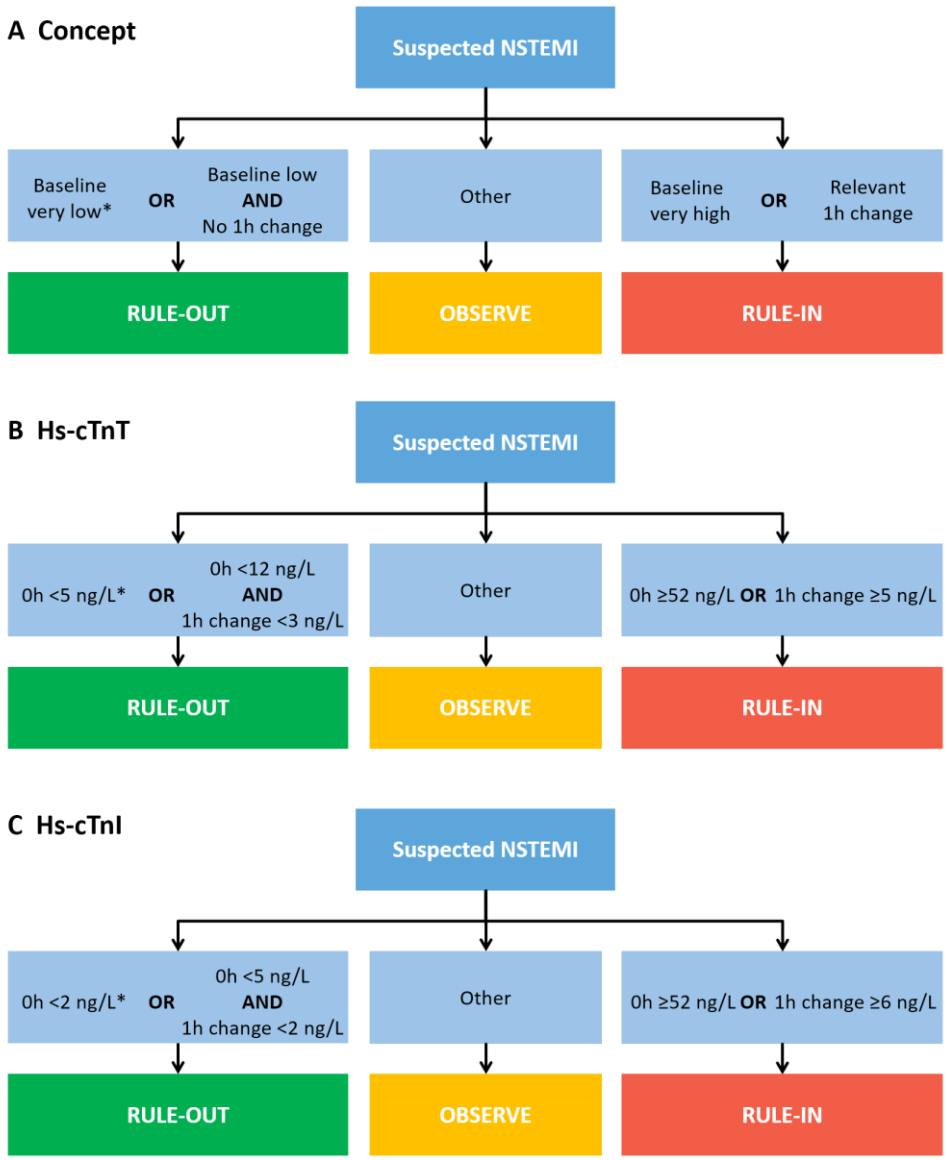
HR = Hazard ratio; Hs-cTn = High-sensitivity cardiac troponin; NSTEMI = Non-ST-segment-elevation myocardial infarction; 95%CI = 95% confidence interval.

III. Supplemental Figures



Online Figure 1 **Patient Flow Diagram**

Flow diagram of all recruited patients from both study cohorts. A patient can be part of the diagnostic dataset A and B if both high-sensitivity cardiac troponin (hs-cTn) T and I is available at 0h and 1h. There is an overlap of 3468 patients between the diagnostic dataset A and B, which is considered the common prognostic dataset. APACE-study = Advantageous Predictors of Acute Coronary Syndrome Evaluation; BACC-study = Biomarkers in Acute Cardiac Care study; STEMI = ST-segment-elevation myocardial infarction. The enrolment period was April 2006 to December 2015 in APACE and July 2013 to April 2016 in BACC.



Online Figure 2 **European Society of Cardiology 0/1h-Algorithm**

A) Concept of the European Society of Cardiology 0/1h-algorithm for rapid triage of patients with suspected Non-ST-Segment-Elevation Myocardial Infarction (NSTEMI) towards rule-out, observe and rule-in according to high-sensitivity cardiac troponin blood concentrations obtained at presentation to the emergency department and their absolute changes within one hour.
B) Assay-specific cut-off levels for high-sensitivity cardiac troponin T (hs-cTnT, Elecsys®)
C) Assay-specific cut-off levels for high-sensitivity cardiac troponin I (hs-cTnI, Architect®)

1h change= absolute (unsigned) change of high-sensitivity cardiac troponin within the first hour. *if chest pain onset > 3 hours before presentation to the emergency department.

Disease \ Triage	Rule-out	Observe	Rule-in	Total
no NSTEMI	**A** *(a)*	**C**	**E** *(e)*	A+C+E
NSTEMI	**B** *(b)*	**D**	**F** *(f)*	B+D+F
Total	A+B *(a+b)*	C+D	E+F *(e+f)*	A+B+C+D+E+F

N = *number of patients triaged based on the 0h- and/or 1h-samples*
(n) = number of directly triaged patients based on the 0h-samples only

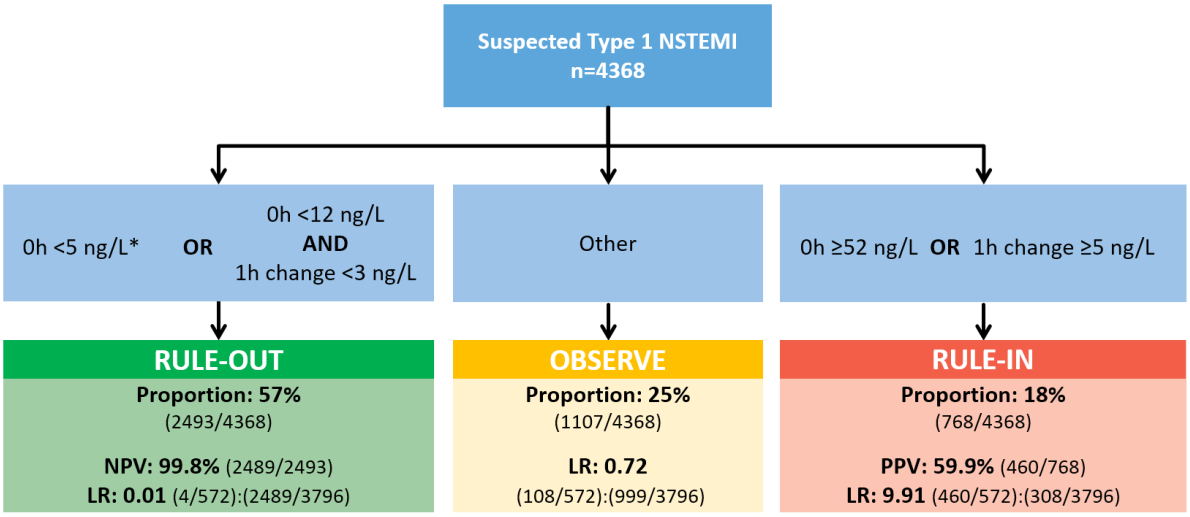
Measure	Formula
$Proportion_{\,rule-out} =$	$\frac{A+B}{A+B+C+D+E+F}$
$Proportion_{\,direct\ rule-out} =$	$\frac{a+b}{A+B+C+D+E+F}$
$Negative\ predictive\ value_{\,rule-out} =$	$\frac{A}{A+B}$
$Likelihood\ ratio_{\,rule-out} =$	$\frac{B/(B+D+F)}{A/(A+C+E)}$
$Proportion_{\,observe} =$	$\frac{C+D}{A+B+C+D+E+F}$
$Likelihood\ ratio_{\,observe} =$	$\frac{D/(B+D+F)}{C/(A+C+E)}$
$Proportion_{\,rule-in} =$	$\frac{E+F}{A+B+C+D+E+F}$
$Proportion_{\,direct\ rule-in} =$	$\frac{e+f}{A+B+C+D+E+F}$
$Positive\ predictive\ value_{\,rule-in} =$	$\frac{F}{E+F}$
$Likelihood\ ratio_{\,rule-in} =$	$\frac{F/(B+D+F)}{E/(A+C+E)}$
$Overall\ Efficacy =$	$\frac{A+B+E+F}{A+B+C+D+E+F}$

Online Figure 3 **2x3 Table for the Calculation of the European Society of Cardiology 0/1h-Algorithms' Diagnostic Performance Measures**

2x3 table systematically depicting patients' distribution according to i) the three triage categories of the European Society of Cardiology 0/1h-algorithm and ii) the absence or presence of Non-ST-segment-elevation myocardial infarction (NSTEMI). As the European Society of Cardiology 0/1h-Algorithm does not reflect a binary test (positive/negative) but contains three triage categories (rule-out/observe/rule-in), no classical 2x2 tables but 2x3 tables were constructed. Proportions, predictive values and likelihood ratios for NSTEMI in the three triage categories were derived from these 2x3 tables and calculated as expressed by the formulas in this figure.

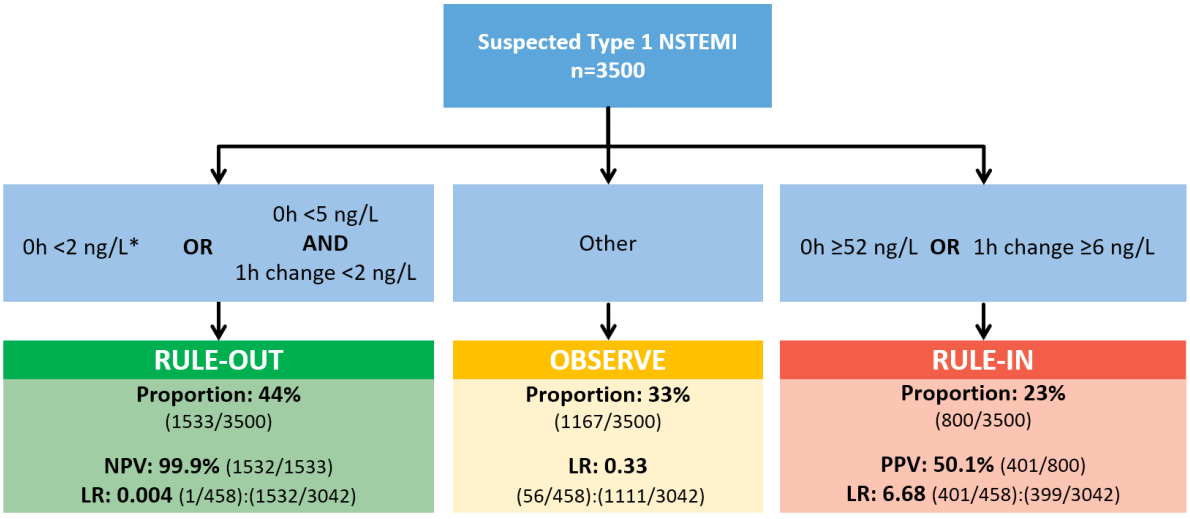
A Hs-cTnT

Disease \ Triage	RULE-OUT	OBSERVE	RULE-IN	Total
no Type 1 NSTEMI	2489	999	308	3796
Type 1 NSTEMI	4	108	460	572
Total	2493	1107	768	4368



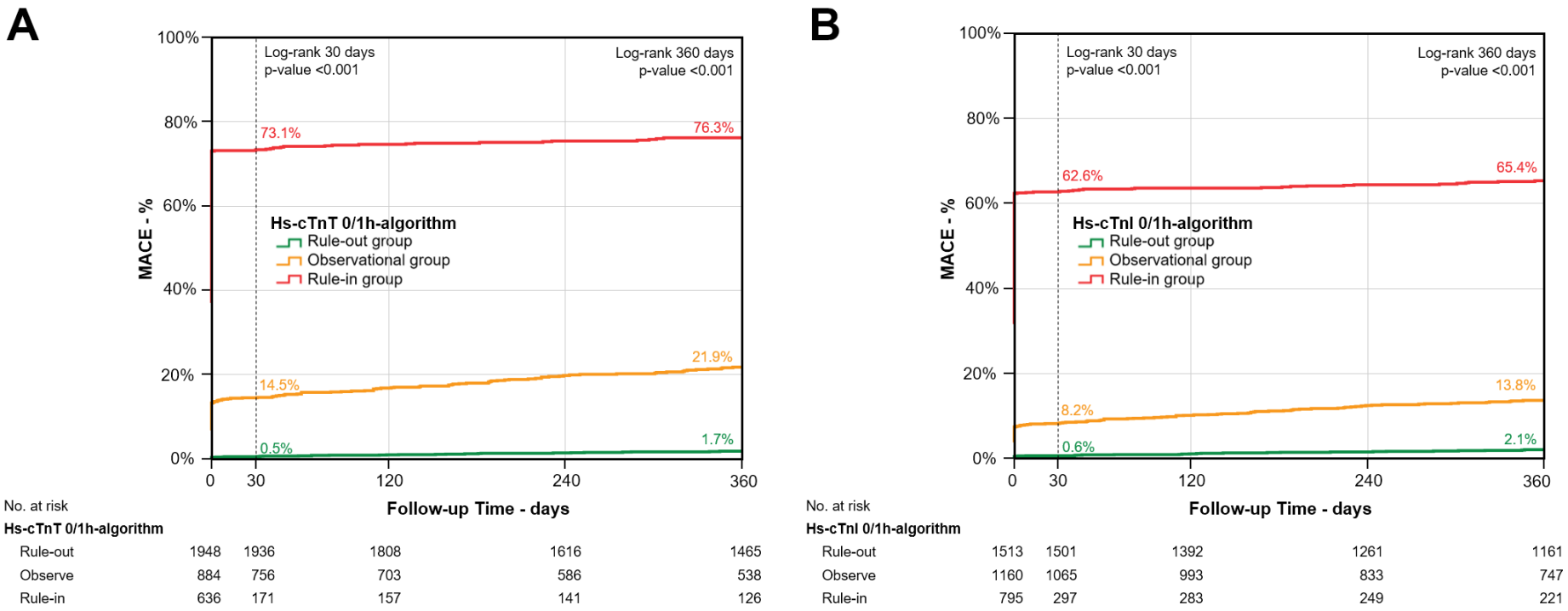
B Hs-cTnI

Disease \ Triage	RULE-OUT	OBSERVE	RULE-IN	Total
no Type 1 NSTEMI	1532	1111	39	3042
Type 1 NSTEMI	1	56	401	458
Total	1533	1167	800	3500



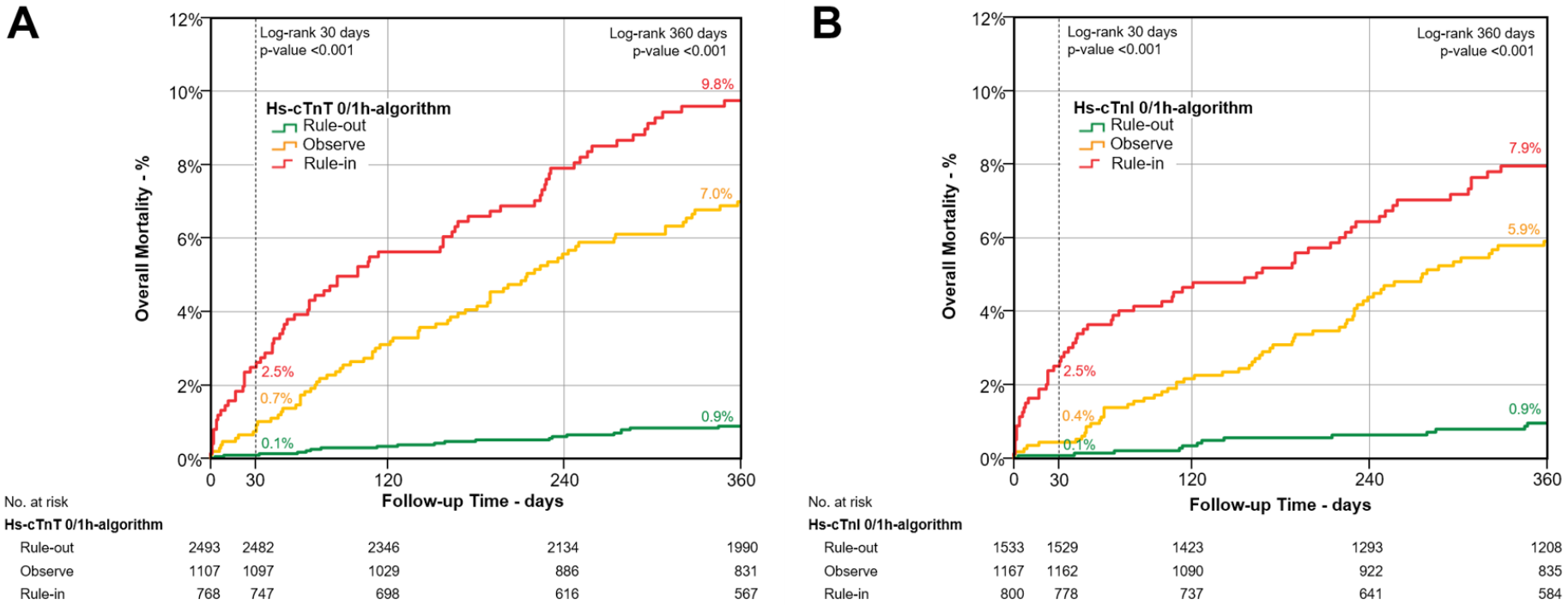
Online Figure 4 **Diagnostic Performance of the European Society of Cardiology 0/1h-Algorithm for the Diagnosis of Type 1 Myocardial Infarction Only**

2x3 Tables and flow-charts depicting the diagnostic performance of the European Society of Cardiology 0/1h-algorithm for rule-out and rule-in of type 1 myocardial infarction among patients presenting with suspected myocardial infarction using **(A)** high-sensitivity cardiac troponin T (hs-cTnT, Elecsys®) and **(B)** high-sensitivity cardiac troponin I (hs-cTnI, Architect®). NSTEMI = Non-ST-Segment-Elevation Myocardial Infarction; 1h change = absolute (unsigned) change of high-sensitivity cardiac troponin within the first hour; LR = likelihood ratio; NPV = negative predictive value; PPV = positive predictive value. *if chest pain onset > 3 hours before presentation to the emergency department.



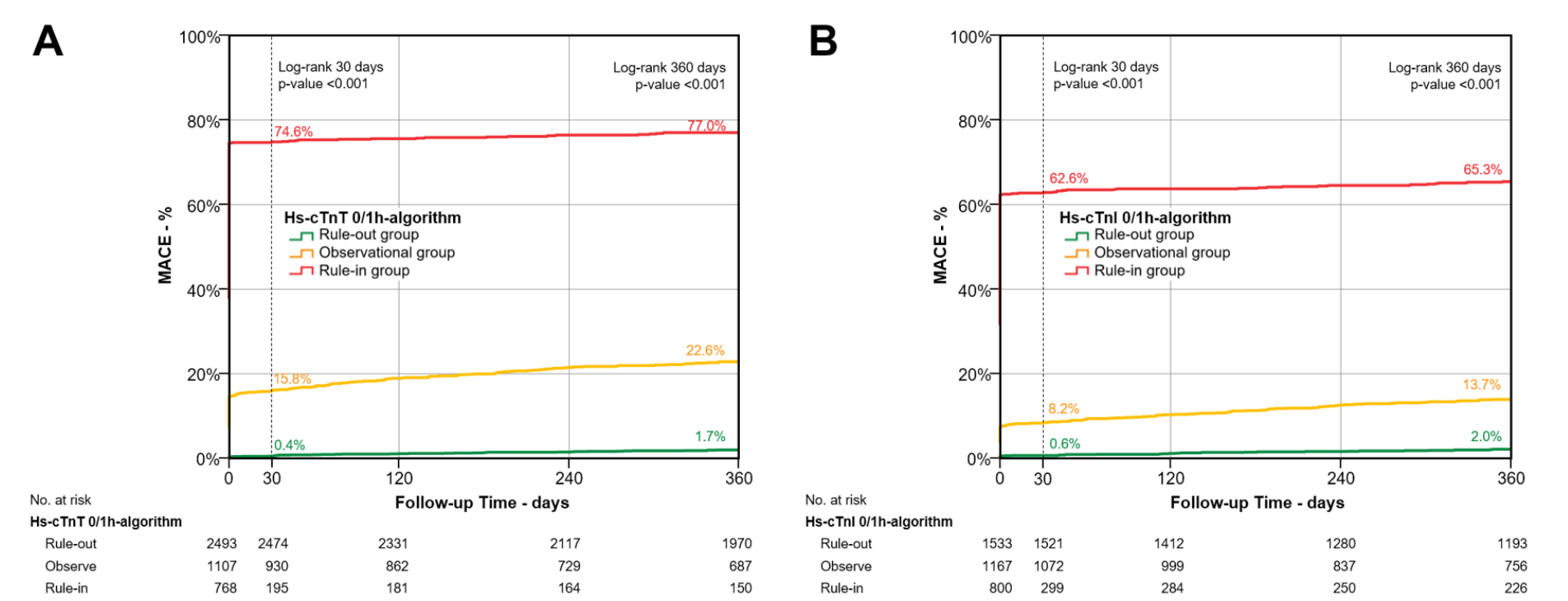
Online Figure 5 **Major Adverse Cardiac Events According to Triage Group by the European Society of Cardiology 0/1h-Algorithm**

Kaplan-Meier curves depicting incidence of major adverse cardiac events (MACE, the composite of overall mortality and myocardial infarction including the index events) within 30 days and one year for patients triaged to the rule-out (green lines), observational (orange lines) und rule-in (red lines) group by the European Society of Cardiology 0/1h-algorithm using **(A)** high-sensitivity cardiac troponin T (hs-cTnT, Elecsys®) and **(B)** high-sensitivity cardiac troponin I (hs-cTnI, Architect®).



Online Figure 6 **Overall Mortality According to Triage Group by the European Society of Cardiology 0/1h-Algorithm Assessed Separately in Diagnostic Dataset A and B**

Kaplan-Meier curves depicting overall mortality within 30 days and one year for patients triaged to the rule-out (green lines), observational (orange lines) und rule-in (red lines) group by the European Society of Cardiology 0/1h-algorithm using **(A)** high-sensitivity cardiac troponin T (hs-cTnT, Elecsys®) in the diagnostic dataset A with 4368 patients and **(B)** high-sensitivity cardiac troponin I (hs-cTnI, Architect®) in the diagnostic dataset B with 3500 patients.



Online Figure 7 **Major Adverse Cardiac Events According to Triage Group by the European Society of Cardiology 0/1h-Algorithm Assessed Separately in Diagnostic Dataset A and B**

Kaplan-Meier curves depicting incidence of major adverse cardiac events (MACE, the composite of overall mortality and myocardial infarction including the index events) within 30 days and one year for patients triaged to the rule-out (green lines), observational (orange lines) und rule-in (red lines) group by the European Society of Cardiology 0/1h-algorithm using **(A)** high-sensitivity cardiac troponin T (hs-cTnT, Elecsys®) in the diagnostic dataset A with 4368 patients and **(B)** high-sensitivity cardiac troponin I (hs-cTnI, Architect®) in the diagnostic dataset B with 3500 patients.

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