**Cardiac troponin assays with improved analytical quality – a trade-off between enhanced diagnostic performance and reduced long-term prognostic value**

**Running title:** *Tjora et al.; Troponin algorithms; diagnosis and risk prediction*

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# ABSTRACT

**Background:** Cardiac troponin (cTn) permits early rule-out/rule-in of patients admitted with possible non–ST-elevation myocardial infarction (NSTE[MI]). In this study, we developed an admission and a 0/1 hour rule-out/rule-in algorithm for a troponin assay with measurable results in >99% of healthy individuals. We then compared its diagnostic and long-term prognostic properties with other protocols.

**Methods and Results:** Blood samples were collected at 0, 1, 3, and 8–12 hours from patients admitted with possible NSTEMI. cTnT (Roche Diagnostics), $cTnI_{(Abbott)}$ (Abbott Diagnostics), and $cTnI_{(sgx)}$ (Singulex Clarity System) were measured in 971 admission and 465 1-hour samples. An admission and a 0/1 hour rule-out/rule-in algorithm were developed for the $cTnI_{(sgx)}$ assay and compared with $cTnT_{ESC}$, $cTnI_{(Abbott)ESC}$, and two earlier $cTnI_{(sgx)}$ algorithms. The prognostic composite endpoint was all-cause mortality and future nonfatal MI during a median follow-up of 723 days. NSTEMI prevalence was 13%. The novel $cTnI_{(sgx)}$ algorithms showed similar performance regardless of time from symptom onset, and area under the curve was significantly better than comparators. The $cTnI_{(sgx)0/1hour}$ algorithm classified 92% of patients to rule-in or rule-out compared with ≤78% of comparators. Patients allocated to rule-out by the prior published 0/1 hour algorithms had significantly fewer long-term events compared to the rule-in and observation groups. The novel $cTnI_{(sgx)0/1hour}$ algorithm used a higher troponin baseline concentration for rule-out and did not allow for prognostication.

**Conclusions:** Increasingly sensitive troponin assays may improve identification of NSTEMI but could rule-out patients with subclinical chronic myocardial injury. Separate protocols for diagnosis and risk prediction seem appropriate.

**Key words**: chest pain, 0/1 hour algorithm, myocardial infarction, chronic myocardial injury

## Non- standard Abbreviations and Acronyms

| | |
|---|---|
| CMI | Chronic myocardial injury |
| ESC | European Society of Cardiology |
| CDK-EPI | Chronic Kidney Disease Epidemiology Collaboration |
| HEART Score | History, ECG, Age, Risk factors, Troponin |
| LOB | Limit of blank |
| LOD | Limit of detection |
| REK Ethics | The Regional Committees for Medical and Health Research |
| Sgx | Singulex Clarity System |

**Clinical Perspective**

**What is new**

- Troponin assays with analytical sensitivity and precision beyond the current high sensitivity troponin assays are likely to show improved diagnostic performance for NSTEMI.

- Diagnostic algorithms that are very precise for identification of NSTEMI will to a lesser extent identify patients with subclinical or overt chronic myocardial injury, and consequently show lower long-term prognostic power compared to less precise algorithms.

**What are the clinical implications?**

- Troponin assays with improved analytical sensitivity have a high ability for early identification of NSTEMI, making early presenters patients with low admission troponin concentrations eligible for rule-out.

- Development of efficient diagnostic follow-up schemes allocating > 90% of patients presenting with chest pain to rule-in or rule-out for NSTEMI should improve the logistics in the emergency room.

- Long term cardiovascular  risk should be considered even in patients who are ruled out for NSTEMI

Clinical suspicion of non–ST-elevation myocardial infarction (NSTE[MI]) is a frequent cause of hospital admission,[1] and cardiac troponin (cTn, T or I) measurement is a cornerstone in evaluation of these patients.[2] Approximately 40% of patients are "early presenters,"[3, 4] and accurate detection of low cTn concentrations for immediate rule-out of NSTEMI may therefore have clinical utility. The European Society of Cardiology (ESC) recommends that patients with a detectable baseline cTn concentration undergo serial testing. Based on the baseline and delta concentrations obtained, such cases can be classified as rule-out, observation, or rule-in for NSTEMI.[2] A few limitations apply when troponin based algorithms are used for rule-out of NSTEMI, e.g. the suboptimal analytical sensitivity (unavailability to provide measurable concentrations in all healthy individuals) and large analytical imprecision (reproducibility of sequential measurements) at low troponin concentrations, which could produce a false low delta value leading to an inappropriate rule-out. Improved analytical sensitivity and precision of these assays might facilitate algorithms with higher specificity allowing for admission rule-out in all individuals with low concentrations regardless of time from symptom onset and greater reliability of deltas allowing for rule-out in subjects with high normal or increased baseline concentrations.

Stable troponin concentrations above the 99th percentile is considered to indicate chronic myocardial injury (CMI) and are associated with poor long-term outcomes.[5-7] Studies have shown that risk rises continuously with troponin concentrations below the 99th percentile.[8] Some studies have identified this association even at concentrations lying between the limit of detection (LOD) and limit of blank (LOB) of current assays.[9] Thus, identifying subclinical myocardial injury (i.e., myocardial injury with stable troponin concentration <99th percentile) may be of clinical relevance because it could indicate increased cardiovascular risk in patients with acute chest pain.[10, 11 12, 13] Whether assays with

improved analytical performance could allow for further improvement in long-term risk prediction is not known.

Two previous reports[14, 15] described findings with a cTn assay (Singulex Clarity System) (cTnI$_{(sgx)}$) with improved analytical sensitivity, providing measurable results in >99% of healthy individuals (vs corresponding values of 72% for Roche Diagnostics and 85% for Abbott Diagnostics[16]). This assay also provided 10% analytical variation at concentrations below 1 ng/L.[16] Neither study, however, directly addressed whether this increased analytical quality translated into improved clinical utility. Although this assay is currently unavailable because the company stopped trading in 2019, the data derived from it are highly relevant to understanding the possible benefits and drawbacks of improved analytical quality with troponin or other cardiac injury biomarker assays.

In this cross-sectional observational study, we hypothesized that compared to currently used or suggested cTnT and cTnI algorithms, cTnI$_{(sgx)}$ could offer better performance, with a greater rate of correct rule-out and rule-in of patients presenting with possible NSTEMI. For its development, the admission sample algorithm should include all patients regardless of time between sampling and symptom onset, and the 0/1 hour algorithm should allow rule-out in patients with increased baseline concentration, given low delta values. Because algorithms using high baseline troponin concentration can rule out patients with both subclinical myocardial injury and CMI, we also analyzed data from a prospective follow-up period to evaluate the relative long-term risk-prediction ability of these algorithms.

## Methods

*Study design*

The data that support the current findings are available from the corresponding author upon reasonable request. The WESTCOR-study (Clinical Trials number NCT02620202) is a two-

center, cross-sectional, prospective observational study described in detail earlier.[17] The cross-sectional study design was used to investigate the accuracy of different algorithms. Patients were then prospectively followed to determine if the different algorithms could predict future cardiovascular outcomes.[18]

The current article reports data from the WESTCOR derivation cohort (WESTCOR-D) including 985 patients admitted to Haukeland University Hospital, Norway, with suspected non–ST-elevation (NSTE) acute coronary syndrome (ACS). The inclusion period lasted from September 2015 to February 2017. The study and biobank were approved by the Regional Committees for Medical and Health Research Ethics (2014/1365 REK West and 2014/1905 REK West).

*Study enrollment and biobanking*

Patients age >18 years admitted with chest pain or symptoms suggesting NSTE-ACS and who did not have a short life expectancy (e.g., advanced cancer) and could provide informed consent were eligible for inclusion.[17] Patients had serum samples drawn on arrival to the emergency department and after 3 and 8–12 hours. Samples were centrifuged after 30 minutes, and material for the biobank was aliquoted and frozen. High-sensitivity cTnT was measured in fresh samples, and the results were reported to the attending clinician. After an initial period of fine-tuning of the study, an additional biobank sample was drawn 1 hour after admission, and the results were not reported to the attending clinician. This adjustment was planned a priori as a part of the study.[17] Biobank admission samples were available from 971 patients, and a 1-hour sample was available for 465 patients.

*Biochemical analyses*

Routine and 1-hour samples were measured for cTnT (Roche Diagnostics) with LOB 3 ng/L, LOD 5 ng/L, 99th percentile 14 ng/L, and analytical within-series coefficient of variation (CV_A) 10% at 4.5 ng/L. For cTnI (biobanked samples), measured using the Abbott Diagnostics assay (cTnI_(Abbott)), these values were LOB 0.9 ng/L, LOD 1.7 ng/L, 99th percentile 26 ng/L, and 10% CV_A 4.6 ng/L. For the cTnI measured used the Singulex Clarity System, these values were LOB 0.02 ng/L, LOD 0.08 ng/L, 99th percentile 8.67 ng/L, and 10% CV_A 0.53 ng/L.[16] All other clinical chemistry tests were measured using Cobas e602 or Cobas 8000 from Roche Diagnostics. The glomerular filtration rate was estimated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula and an enzymatic isotope dilution mass spectrometry traceable creatinine assay (Roche Diagnostics).

*Diagnosis*

The diagnostic endpoint was NSTEMI during the index hospitalization. The adjudicating process has been described earlier,[17] but briefly, two independent cardiologists adjudicated the final diagnosis based on all available clinical, routine laboratory (including cTnT at admission and at 3 and 8–12 hours from admission), electrocardiogram (ECG), ultrasound, and imaging findings, including cardiac computed tomographic angiography and conventional angiography. A third adjudicator resolved disagreements. Specific diagnostic criteria were predefined for 22 different medical conditions based on guidelines that were available during planning of the study (see Data S1). NSTEMI was defined according to the third universal definition for MI, including a significant rise and fall of cTn with at least one value above the 99th percentile combined with symptoms of ischemia, ECG changes, and image evidence of loss of viable myocardium or intracoronary thrombus.[19] Delta values of 20% (baseline cTnT concentration >14 ng/L) or 50% (baseline cTnT concentration ≤14 ng/L) in serial cTnT measures were regarded as significant, as suggested by the ESC in 2012.[17, 19]

Since 2012, several studies have found a significantly lower 99th percentile concentration of cTn for women compared with men,[20-22] but knowledge of sex-specific cutoffs for women regarding diagnosing NSTEMI is partial because of a lack of data on pathophysiology.[23] Consequently, we chose to apply a common cutoff for all patients.

*Follow-up and prognostic endpoints*

Follow-up data were collected through the Norwegian Patient Register and Norwegian Cause of Death Registry. The prognostic endpoint was a composite of all-cause mortality and subsequent non-fatal MI (all MIs after the index NSTEMI). Patients were followed until an endpoint occurred or until a median follow-up time of 723 days after inclusion (ranging from 4 to 900 days).

*Comparator algorithms*

According to current recommendations, patients are eligible for early discharge or further investigations for ACS based on the troponin results.[2] The algorithms encompass an initial review of the admission sample in patients who present more than 3 hours after symptom onset. If the concentration is below the LOD of the troponin assay, the patient is eligible for "rule-out" and may be discharged if the ECG and/or the clinical symptoms suggest a lower likelihood of ACS. The remaining patients undergo serial sampling at 1-hour intervals. Based on the baseline and delta values obtained, patient status is established as "rule-out," "observation," or "rule-in" for NSTEMI. Again, ruled-out patients may be discharged if the clinical suspicion of ACS is low, those who are ruled in may go directly to cardiac angiography and eventually invasive treatment, and those in the observation group undergo diagnostic follow-up. We compared the recommended ESC (cTnT and cTnI$_{(Abbott)}$)

algorithms[2] and those suggested by Body et al.[14] and Neumann et al.[15] to the novel algorithms described in Tables 1 and 2.

*Development of novel algorithms*

The novel $cTnI_{(sgx)}$ rule-out algorithms were defined based on the following hierarchy of criteria: 1) diagnostic sensitivity for NSTEMI ≥99.0%, as previously described,[24] and the maximum possible specificity. Sensitivity was preferred over negative predictive value (NPV) as the criterion because sensitivity is independent of disease prevalence and applicable in chest pain cohorts with higher and lower prevalences compared to our cohort. Applicable concentrations for the rule-in algorithms were based on the following criteria: 1) diagnostic specificity for NSTEMI ≥95% (less than 5% rule-in of non-NSTEMI patients) and a simultaneously maximized sensitivity for NSTEMI. Specificity was considered preferable to positive predictive value (PPV) because specificity is independent of prevalence.

We chose the preferred algorithms based on the number of ruled-out and ruled-in patients that would give a sensitivity and specificity corresponding to the pre-specified criteria. "Direct rule-out" was defined as rule-out regardless of time since symptom onset. Diagnostic performance was calculated with and without early presenters, defined as patients with <3 hours since symptom onset.

*Statistical analysis*

The baseline characteristics are reported as medians with interquartile ranges for continuous data and percentages for categorical data. The data were analyzed using the non-parametric Kruskal–Wallis test for continuous variables and the Chi-square and Fisher's exact test for categorical variables, as appropriate. Statistical analyses included calculation of sensitivity, specificity, NPV, PPV, and likelihood ratios, receiver operating characteristic (ROC) curve

analysis, and calculation of area under the curve (AUC) for all algorithms. Significant differences in AUC were evaluated using the Delong test, and efficiency (defined as percentage of patients ruled out plus percentage of patients ruled in) was calculated for all algorithms. Kaplan–Meier curves were drawn for the composite endpoint stratified according to categories, and the number of events was calculated. Cox proportional hazards regression analysis was used to calculate the unadjusted hazard ratio for the composite endpoint, and adjusted analysis was undertaken using age, sex, current or previous smoking, chronic kidney disease, hypertension, hyperlipidemia, diabetes mellitus, and previous MI as covariates. Definitions of the different risk factors are given in Data S1. We used SPSS Statistics 24 and MedCalc for the statistical analyses.

## Results

*Baseline characteristics and troponin concentrations*

The characteristics of patients according to diagnostic category are presented in Table S1 (total cohort, n=971) and Table S2 (0/1 hour cohort, n=465). The prevalence of NSTEMI was 13%, and the prevalence of unstable angina pectoris was 11%. Figure 1 shows the median (25 and 75 percentiles) troponin concentrations at admission and 1 hour for the three different assays. cTnI$_{(sgx)}$ was measurable in 99.9%, cTnT in 74%, and cTnI$_{(Abbott)}$ in 87% of samples obtained at admission.

*Derivation of a direct rule-out and a 0/1 hour rule-out and rule-in algorithm*

The number of NSTEMIs that would be ruled out at different admission sample cTnI$_{(Sgx)}$ concentrations was calculated (see Table S3). A direct rule-out algorithm using <2 ng/L as the cutoff showed a diagnostic sensitivity >99%, in accordance with the pre-specified criteria (Table 1). The 0/1 hour algorithm was developed in a similar way by calculating the number

of NSTEMIs ruled out at different admission and delta value concentrations combined (see Table S4 and Table S5). The optimal rule-out algorithm was a baseline $cTnI_{(Sgx)}$ concentration <10 ng/L and a delta value of <3 ng/L (Table 2). This algorithm did not rule out any NSTEMI patients and consequently had a sensitivity of 100%, with a corresponding specificity of 89%. Of note, this decision threshold is higher than the 99th percentile of the assay.

If 8.67 ng/L was used as the baseline concentration in the algorithm, the resulting sensitivity was 100% and specificity was 87%. Regarding rule-in, the optimal algorithm showed a specificity of 97% using a baseline concentration of ≥70 ng/L or a delta value of ≥5 ng/L (Table S5 and Table 2) as cutoffs. An alternative algorithm using a lower baseline concentration of ≥30 ng/L or a delta value of ≥5 ng/L showed similar (within 95% confidence intervals [CIs]) specificity and sensitivity.

*Comparing diagnostic performance of direct rule-out algorithms*

Baseline concentrations analyzed as a continuous variable showed a higher AUC for $cTnI_{(Sgx)}$ compared to the other assays (Figure 2; Delong test, $P \leq 0.004$). Direct rule-out by $cTnI_{(Sgx)}$ was the only direct rule-out algorithm that fulfilled the criteria of sensitivity >99% (Figure 3 and Table 1), and only one patient was falsely ruled out. A similar sensitivity was achieved for $cTnT_{ESC}$ when a time lag of 3 hours between testing and symptom onset was applied.

This NSTEMI patient was inappropriately ruled out by all admission algorithms. She was a 65-year-old woman admitted with chest pain lasting more than 3 days. A few years earlier, she had been treated with percutaneous coronary intervention in all three coronary vessels because of unstable angina pectoris. Upon admission, her EGC showed non-specific T changes, and she had a high clinical risk for ACS (e.g., a HEART score of 7). At 72 hours after symptom onset, the baseline troponin samples were cTnT 4 ng/L, $cTnI_{(Abbott)}$ 1.5 ng/L,

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and cTnI$_{(sgx)}$ 1.3 ng/L, which increased significantly to cTnT 71 ng/L/, cTnI$_{(Abbott)}$ 80 ng/L, and cTnI$_{(sgx)}$ 56 ng/L after 3 hours. The coronary angiogram revealed a thrombus in a small vessel that was not available for percutaneous coronary intervention.

The cTnI$_{(Abbott)ESC}$ algorithm had the lowest sensitivity, missing a second patient, a 73-year-old woman with one previous MI. She was admitted to the hospital with a history of chest pain related to defecation and baseline blood samples taken 3.5 hours after symptom onset showing cTnT 11 ng/L, cTnI$_{(Abbott)}$ 2 ng/L, and cTnI$_{(sgx)}$ 9.25 ng/L. After 3 hours, values for cTnI$_{(Abbott)}$ increased to 5 ng/L, but cTnT and cTnI$_{(sgx)}$ showed stable values. At 18 hours after admission, the cTnT increased to 52 ng/L and the cTnI to 29 ng/L. Unfortunately, the coronary angiography failed because of difficult arterial access.

*Comparing diagnostic performance between 0/1 hour rule-out and rule-in algorithms*

None of the algorithms ruled out any patient with NSTEMI, so that the sensitivity was 100% (Figure 3 and Table 2). The novel cTnI$_{(sgx)}$ 0/1 hour rule-out algorithm had a higher rule-out rate of non-NSTEMI patients (higher specificity) and an overall higher AUC (Delong test, $P<0.001$) than comparators.

Concerning the rule-in algorithms, results were quite similar for all algorithms: a few patients without NSTEMI were ruled in (false positive), for a specificity of 95%–97%. However, the cTnT$_{ESC}$ 0/1 hour algorithm showed a lower AUC (Delong test, $P<0.05$) compared to the cTnI algorithms because of a slightly higher number of NSTEMI patients allocated to the observation group (lower sensitivity). More than 90% of NSTEMIs were ruled in using the cTnI algorithms, whereas 78% were ruled in by cTnT$_{ESC}$ (Table S6).

*Efficiency of the algorithms*

The novel $cTnI_{(sgx)}$ direct rule-out and 0/1 hour algorithms were more efficient than the comparators (Figure 3). The algorithm classified 37% of admitted patients as candidates for early discharge, compared to 31% with $cTnT_{ESC}$, 23% with $cTnI_{(Abbott)ESC)}$, 24% with $cTnI_{(sgx)Body}$, and 9% with $cTnI_{(sgx)Neumann}$. When the early presenters were excluded for the ESC algorithms in accordance with the guideline, the direct rule-out rate dropped to 24% with $cTnT_{ESC}$ and 18% with $cTnI_{(Abbott)ESC}$ (Figure 3).

Concerning 0/1 hour serial sampling algorithms, the novel $cTnI_{(sgx)}$ rule-out algorithm would have suggested discharge for 77% of patients, compared with 64% with $cTnT_{ESC}$ and 57% with $cTnI_{(Abbott)ESC}$ (Table S4). Only 40% would be eligible for discharge if the $cTnI_{(sgx)Neumann}$ 0/1 hour algorithm were applied. Rule-in would be recommended for 13%–17% (Table S5). Total efficiency values showed that the novel $cTnI_{(sgx)}$ 0/1 hour algorithms would allocate 92% (95% CI, 89%–94%) of the patients to either rule-out or rule-in. Corresponding numbers for the $cTnT_{ESC}$, $cTnI_{(Abbott)\ ESC}$, and $cTnI_{(Neumann)}$ 0/1 hour algorithms were 78% (95% CI, 74%–82%), 74% (95% CI, 70%–78%), and 56% (95% CI, 51%–60%), respectively.

*Long-term prognostic value*

A total of 82 events occurred among the 971 patients included in the admission sample cohort. Table S7 shows the number of endpoints stratified according to the different algorithms. With the exception of $cTnI_{(sgx)Neumann}$ (which allocated only 9% of patients to rule-out), the direct rule-out algorithms showed a significant ability to predict long-term endpoints. The discrimination power of the rule-out algorithms was confirmed in a Cox regression analysis (Table 3), after adjustment for well-established risk factors.

The 465 patients included in the 0/1 hour cohort experienced 32 events. Patients who were ruled out by the novel $cTnI_{(sgx)}$ 0/1 hour algorithm had an event rate of 5.3% (Table S7),

which was not significantly different from the event rate in the observation and rule-in groups of 10%−13% (Figure 4 and Table 3). In contrast, those allocated to rule-out by the ESC or the Neumann 0/1 hour algorithms had significantly lower event rates (1.6%−3.4%) compared to the corresponding observation and rule-in groups. Cox regression analysis results confirmed the discrimination power of rule-out by these algorithms (Table 3).

## Discussion

This study yielded three main findings. First a cardiac injury marker with improved analytical sensitivity and precision beyond the current high-sensitivity assays could improve logistics and categorizing of patients investigated for NSTEMI. Second, the cost of this screening could be higher rates of rule-out of patients with increased long-term risk because of subclinical myocardial injury or CMI. Third, our data are in agreement with the proposed ESC algorithms but only partly correspond to two earlier reports for the $cTnI_{(sgx)}$ assay. This pattern highlights a need for robust validation before rule-in and rule-out algorithms are implemented for any particular assay.

Our data suggest that the improved analytical sensitivity of the $cTnI_{(sxg)}$ assay translates into a better "signal-to-noise ratio" compared to the other high sensitivity assays and reduces the time window required for reliably detecting myocardial injury. Body et al.[14] reported similar outcomes using a slightly lower cutoff than ours (1.5 ng/L). The very low direct rule-out cut off suggested by Neumann et al.[15] was 100% sensitive but proved clinically unsuitable in our cohort because of the low number of patients eligible for rule-out (9%). Using a $cTnI_{(sgx)}$ cutoff of <2 ng/L would lead to allocation of more patients to direct rule-out than the comparator algorithms suggested by ESC and Body et al.[4, 14, 25, 26]

We used a 0/1 rule-out algorithm that depended more on delta values and allowed for rule-out at high baseline concentrations (>99th percentile of the assay), without compromising

sensitivity. This approach resulted in a highly specific algorithm, ruling out large numbers of patients without NSTEMI. It is noteworthy that the novel $cTnI_{(sgx)}$ 0/1 hour algorithm could allocate more than 90% of patients to either rule-out or rule-in, with a similar or higher diagnostic accuracy compared with the other algorithms.

The second important finding in our study is that the ability to predict long-term MI and all-cause mortality seems to depend on the algorithm used for rule-out. The direct rule-out algorithms that used low troponin concentrations as cutoffs showed an excellent prognostic ability. This finding was robust for the $cTnI_{(sgx)}$ and $cTnT_{ESC}$ algorithms after adjusting for well-known risk factors, and borderline significant for $cTnI_{(Abbott)ESC}$.[11, 27, 28] That the direct rule-out according to Neumann et al. did not predict long-term prognosis may be explained by the low rule-out frequency of 9%.

Concerning the 0/1 hour algorithms, our data suggest that the $cTnI_{(sgx)}$ algorithm could not predict long-term risk. This algorithm had twice as many endpoints compared with the others. The higher baseline concentration used for rule-out included more patients with subclinical myocardial injury (high-normal troponin concentrations) and even CMI,[5] which could explain this observation because these patients have increased long-term risk.[11, 27, 28] For all algorithms, we observed a similar event rate in patients allocated to observation and to rule-in. The prevalence of NSTEMI and CMI was reciprocal in the two groups, with high NSTEMI frequency in the rule-in group and high CMI frequency in the observation group (Table S6 and Table S7).[27, 29] These findings highlight that NSTEMI and CMI are both serious conditions with increased long-term risk. Future studies should target identifying more accurate diagnostic and treatment options for CMI patients.

The last important observation is the discrepancy between our findings and the data reported by Neumann et al.[15] The reason could be related to coincidence, as the cutoffs in both studies were based on the few NSTEMI patients with low baseline concentration. Also,

both studies were single center, and differences in health care systems could have affected the patient cohort that was recruited. The fact that Neumann et al. also ruled out fewer patients for the $cTnI_{(ESC)}$ algorithm suggests that the cohorts likely were different. Furthermore, analytical issues such as reagent and calibrator lot variations are highly likely to influence the performance of cutoffs in the low range of an assay.[30] This assumption is strengthened by the observation that the diagnostic performance for the different rule-in algorithms showed better alignment, given that lot-to-lot differences are usually less prominent at higher cTn concentrations. Our data demonstrate the need for large sample sets, validation in several different patient cohorts, and knowledge about long-term analytical performance before rule-in and rule-out algorithms are implemented into practice.

An obvious strength of our study is the comparison of the $cTnI_{(sgx)}$ algorithm to well-validated algorithms from ESC, including cTnT and another cTnI assay. Other strengths are a long observation time during the index hospitalization, during which the patients were observed for at least 8 hours, ensuring the validity of the adjudicated diagnosis. The study closely mirrored clinical practice by not excluding patients with end-stage renal disease or with more than a 12-hour history of symptoms suggestive of ACS. The last strength is a long follow-up period registering endpoints after the index NSTEMI/hospitalization, allowing for prognostication.

*Study limitations*

The main limitation of the study is that the $cTnI_{(sgx)}$ assay currently is no longer available on the market because of bankruptcy. The baseline concentrations used in the rule-out and rule-in algorithms were chosen based on sensitivity, specificity, and efficiency for diagnosing NSTEMI, and more studies are necessary to confirm those concentrations that can indicate subclinical myocardial injury and increased long-term risk. The suggested algorithms should

therefore be taken as an example of possibilities and limitations that might be expected from high-precision cardiac injury markers with measurable concentrations in almost all healthy participants. Other limitations are the relatively low number of patients used for development of the 0/1 hour algorithm, the single-center inclusion, the lack of a validation cohort, and the relatively low number of early presenters. As we have noted, our data should be seen as hypothesis-generating and as offering examples, and all new high-sensitivity biomarkers and algorithms need extensive validation in multiple cohorts before they can be ready for clinical use. Another limitation is that cTnT was used as part of the adjudication process. This use could have introduced a positive bias for the cTnT algorithms and underestimation of the performance of cTnI algorithms. Finally, this study involved a long inclusion period, which is a common problem in similar studies; however, the broad inclusion criteria should ensure a broad and representative inclusion. In addition, the NSTEMI rate and patient characteristics in this cohort are similar to those from comparable studies.[31, 32]

*Conclusion*

In this study, we show that cardiac injury markers with improved analytical performance may improve emergency department efficiency by ruling out and ultimately categorizing more patients compared to current recommendations and algorithms. Our data indicate that patients with increased baseline troponin concentrations who might suffer from subclinical myocardial injury should not be deemed low risk even if they are ruled out for NSTEMI. Future studies should aim at simultaneous development of dedicated algorithms identifying both patients with NSTEMI and those with increased long-term risk. Our final observation is that the inter-cohort variability in algorithm performance should not be underestimated, and validation including several different cohorts and clinical settings is necessary for all suggested emergency department algorithms.

**Supplemental Material:** Data S1, Tables S1-S7, References 33–40.

# References:

1. Goodacre S, Cross E, Arnold J, Angelini K, Capewell S, Nicholl J. The health care burden of acute chest pain. *Heart*. 2005;91:229-230

2. Roffi M, Patrono C, Collet J-P, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP et al Group ESCSD. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent st-segment elevationtask force for the management of acute coronary syndromes in

patients presenting without persistent st-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2016;37:267-315

3.  Boeddinghaus J, Nestelberger T, Twerenbold R, Wildi K, Badertscher P, Cupa J, Burge T, Machler P, Corbiere S, Grimm K et al. Direct comparison of 4 very early rule-out strategies for acute myocardial infarction using high-sensitivity cardiac troponin i. *Circ*. 2017;135:1597-1611

4.  Twerenbold R, Costabel JP, Nestelberger T, Campos R, Wussler D, Arbucci R, Cortes M, Boeddinghaus J, Baumgartner B, Nickel CH et al. Outcome of applying the esc 0/1-hour algorithm in patients with suspected myocardial infarction. *JACC*. 2019;74:483-494

5.  Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: From myocardial infarction to chronic disease. *Cardiovasc. res*. 2017;113:1708-1718

6.  Bardají A, Bonet G, Carrasquer A, González-Del Hoyo M, Vásquez-Nuñez K, Ali S, Boqué C, Cediel G. Clinical features and prognosis of patients with acute and chronic myocardial injury admitted to the emergency department. *Am J Med*. 2019;132:614-621

7.  Kadesjö E, Roos A, Siddiqui A, Desta L, Lundbäck M, Holzmann MJ. Acute versus chronic myocardial injury and long-term outcomes. *Heart*. 2019;105:1905-1912

8.  Collinson P. The role of cardiac biomarkers in cardiovascular disease risk assessment. *Curr Opin Cardiol*. 2014;29:366-371

9.  Parikh RH, Seliger SL, de Lemos J, Nambi V, Christenson R, Ayers C, Sun W, Gottdiener JS, Kuller LH, Ballantyne C et al. Prognostic significance of high-sensitivity cardiac troponin t concentrations between the limit of blank and limit of detection in community-dwelling adults: A metaanalysis. *Clin Chem*. 2015;61:1524-1531

10.     de Lemos JA, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, Hashim I, Berry JD, Das SR, Morrow DA et at. Association of troponin t detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. *Jama*. 2010;304:2503-2512

11.     Willeit P, Welsh P, Evans JDW, Tschiderer L, Boachie C, Jukema JW, Ford I, Trompet S, Stott DJ, Kearney PM et al. High-sensitivity cardiac troponin concentration and risk of first-ever cardiovascular outcomes in 154,052 participants. *JACC*. 2017;70:558-568

12.     Nestelberger T, Wildi K, Boeddinghaus J, Twerenbold R, Reichlin T, Gimenez MR, Puelacher C, Jaeger C, Grimm K, Sabti Z et al. Characterization of the observe zone of the ESC 2015 high-sensitivity cardiac troponin 0h/1h-algorithm for the early diagnosis of acute myocardial infarction. *Int J Cardiol*. 2016;207:238-245

13.     Bularga A, Lee KK, Stewart S, Ferry AV, Chapman AR, Marshall L, Strachan FE, Cruickshank A, Maguire D, Berry C et al. High-sensitivity troponin and the application of risk stratification thresholds in patients with suspected acute coronary syndrome. *Circ*. 2019;140:1557-1568

14.     Body R, Twerenbold R, Austin C, Boeddinghaus J, Almashali M, Nestelberger T, Morris N, Badertscher P, McDowell G, Wildi K et al, Freemont A. Diagnostic accuracy of a high-sensitivity cardiac troponin assay with a single serum test in the emergency department. *Clin Chem*. 2019;65:1006-1014

15.     Neumann JT, Sorensen NA, Rubsamen N, Ojeda F, Schock A, Seddighizadeh P, Zeller T, Westermann D, Blankenberg S. Evaluation of a new ultra-sensitivity troponin i assay in patients with suspected myocardial infarction. *Int J Cardiol*. 2019;283:35-40

16.     Apple F, Kavsak P, Hammarsten O, Saenger A, Body R, Lam SPC, Collinson P, Jaffe A, Ordoñez-Llanos J, Omland T. Committee on Clinical Applications of Cardiac Bio-Markers (C-CB). https://www.ifcc.org/media/478231/high-sensitivity-cardiac-troponin-i-and-t-assay-analytical-characteristics-designated-by-manufacturer-v122019.pdf.   Assessed September 2020.

17.     Tjora HL, Steiro OT, Langorgen J, Bjorneklett R, Nygard OK, Renstrom R, Skadberg O, Bonarjee VVS, Lindahl B, Collinson P, Omland T et al. Aiming towards evidence based interpretation of cardiac biomarkers in patients presenting with chest pain-the westcor study: Study design. Scand. Cardiovasc J. 2019:1-6

18.     Thiese MS. Observational and interventional study design types; an overview. *Biochem Med*. 2014;24:199-210

19.     Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Thygesen K, Alpert JS, White HD, Jaffe AS et al. Third universal definition of myocardial infarction. *Eur Heart J*. 2012;33:2551-2567

20.     Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin t assay. *Clin Chem*. 2010;56:254-261

21.     Saenger AK, Beyrau R, Braun S, Cooray R, Dolci A, Freidank H, Giannitsis E, Gustafson S, Handy B, Katus H et al. Multicenter analytical evaluation of a high-sensitivity troponin t assay. *Clin Chim Acta*. 2011;412:748-754

22.     Gore MO, Seliger SL, Defilippi CR, Nambi V, Christenson RH, Hashim IA, Hoogeveen RC, Ayers CR, Sun W, McGuire DK et al. Age- and sex-dependent upper reference limits for the high-sensitivity cardiac troponin t assay. *JACC*. 2014;63:1441-1448

23.	Romiti GF, Cangemi R, Toriello F, Ruscio E, Sciomer S, Moscucci F, Vincenti M, Crescioli C, Proietti M, Basili S et al. Sex-specific cut-offs for high-sensitivity cardiac troponin: Is less more? *Cardiovasc Ther.* 2019;2019:9546931

24.	Than M, Herbert M, Flaws D, Cullen L, Hess E, Hollander JE, Diercks D, Ardagh MW, Kline JA, Munro Z et al. What is an acceptable risk of major adverse cardiac event in chest pain patients soon after discharge from the emergency department?: A clinical survey. *Int J Cardiol.* 2013;166:752-754

25.	Reichlin T, Twerenbold R, Wildi K, Rubini Gimenez M, Bergsma N, Haaf P, Druey S, Puelacher C, Moehring B, Freese M et al. Prospective validation of a 1-hour algorithm to rule-out and rule-in acute myocardial infarction using a high-sensitivity cardiac troponin t assay. *Can Med Assoc J.* 2015;187:E243-252

26.	Wildi K, Nelles B, Twerenbold R, Rubini Gimenez M, Reichlin T, Singeisen H, Druey S, Haaf P, Sabti Z, Hillinger P et al. Safety and efficacy of the 0 h/3 h protocol for rapid rule out of myocardial infarction. *Am Heart J.* 2016;181:16-25

27.	Sorensen NA, Ludwig S, Makarova N, Neumann JT, Lehmacher J, Hartikainen TS, Haller PM, Keller T, Blankenberg S, Westermann D et al. Prognostic value of a novel and established high-sensitivity troponin i assay in patients presenting with suspected myocardial infarction. *Biomolecules.* 2019;9:469.

28.	Neumann JT, Sorensen NA, Schwemer T, Ojeda F, Bourry R, Sciacca V, Schaefer S, Waldeyer C, Sinning C, Renne T et al. Diagnosis of myocardial infarction using a high-sensitivity troponin i 1-hour algorithm. *JAMA Cardiol.* 2016;1:397-404

29.	Roos A, Bandstein N, Lundbäck M, Hammarsten O, Ljung R, Holzmann MJ. Stable high-sensitivity cardiac troponin t levels and outcomes in patients with chest pain. *JACC.* 2017;70:2226-2236

30. Haagensen K, Collinson P, Åsberg A, Aakre KM. How does the analytical quality of the high-sensitivity cardiac troponin t assay affect the ESC rule out algorithm for NSTEMI? *Clin Chem*. 2019;65:494-496

31. Chapman AR, Hesse K, Andrews J, Ken Lee K, Anand A, Shah ASV, Sandeman D, Ferry AV, Jameson J, Piya S et al. High-sensitivity cardiac troponin i and clinical risk scores in patients with suspected acute coronary syndrome. *Circ*. 2018;138:1654-1665

32. Reichlin T, Twerenbold R, Reiter M, Steuer S, Bassetti S, Balmelli C, Winkler K, Kurz S, Stelzig C, Freese M et al. Introduction of high-sensitivity troponin assays: Impact on myocardial infarction incidence and prognosis. *Am J Med*. 2012;125:1205-1213.e1201

33. Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, Bugiardini R, Crea F, Cuisset T, Di Mario C et al. 2013 ESC guidelines on the management of stable coronary artery disease: The task force on the management of stable coronary artery disease of the European Society of Cardiology. *Eur Heart J*. 2013;34:2949-3003

34. Adler Y, Charron P, Imazio M, Badano L, Baron-Esquivias G, Bogaert J, Brucato A, Gueret P, Klingel K, Lionis C et al. 2015 ESC guidelines for the diagnosis and management of pericardial diseases: The task force for the diagnosis and management of pericardial diseases of the European Society of Cardiology (ESC) endorsed by: The European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2015;36:2921-2964

35. Caforio AL, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, Fu M, Helio T, Heymans S, Jahns R et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: A position statement of the

European Society of Cardiology working group on myocardial and pericardial diseases. *Eur Heart J*. 2013;34:2636-2648, 2648a-2648d

36.    Prasad A, Lerman A, Rihal CS. Apical ballooning syndrome (tako-tsubo or stress cardiomyopathy): A mimic of acute myocardial infarction. *Am Heart J*. 2008;155:408-417

37.    Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA et al. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: The task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129-2200

38.    Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ, Iung B, Lancellotti P, Lansac E, Rodriguez Munoz D et al. 2017 ESC/EACTS guidelines for the management of valvular heart disease.  *Eur Heart J*. 2017;38:2739-2791

39.    Hirota M, Takada T, Kawarada Y, Nimura Y, Miura F, Hirata K, Mayumi T, Yoshida M, Strasberg S, Pitt H et al. Diagnostic criteria and severity assessment of acute cholecystitis: Tokyo guidelines. *J Hepatobiliary Pancreat Surg*. 2007;14:78-82

40.    Adamson PD, Anderson JA, Brook RD, Calverley PMA, Celli BR, Cowans NJ, Crim C, Dixon IJ, Martinez FJ, Newby DE et al. Cardiac troponin i and cardiovascular risk in patients with chronic obstructive pulmonary disease. *JACC.* 2018;72:1126-1137

**Table 1. Single sample rule-out of NSTEMI.**

| Direct rule-out (N=971) | Sensitivity | NPV | NLR | Specificity | PPV | PLR | AUC |
|---|---|---|---|---|---|---|---|
| cTnT$_{ESC}$ < 5 ng/L | 98.4 (94.4 – 99.8) | 99.3 (97.4 – 99.8) | 0.04 (0.01 – 0.18) | 35.1 (31.8 – 38.4) | 15.5 (17.7 – 19.3) | 1.52 (1.44 – 1.60) | 0.67 (0.64 – 0.70) |
| cTnI$_{(Abbott)ESC}$ < 2 ng/L | 97.6 (93.3 – 99.5) | 98.7 (96.0 – 99.6) | 0.09 (0.03 - 0.28) | 26.0 (23.0 – 29.1) | 16.6 (15.9 – 17.2) | 1.32 (1.26 - 1.38) | 0.62 (0.59 – 0.65) |
| cTnI$_{(sgx)Neumann}$ < 1.0 ng/L | 100 (97.1-100) | 100 (NC) | 0.0 (NC) | 10,3 (8.3-12.6) | 14.4 (14.1-14.7) | 1.11 (1.09-1.14) | 0.55 (0.52-0.58) |
| cTnI$_{(sgx)Body}$ < 1.5 ng/L ng/L | 99.2 (95.7-99.9) | 99.6 (97.1-99.9) | 0.03 (0.00-0.20) | 28.0 (25.0-31.1) | 17.2 (16.5-17.8) | 1.38 (1.32-1.44) | 0.64 (0.60-0.67) |
| **cTnI$_{(sgx)}$ < 2 ng/L** | **99.2 (95.7 – 100.0)** | **99.7 (98.1 – 100.0)** | **0.02 (0 – 0.13)** | **42.5 (39.2 – 46.0)** | **20.6 (20.0 – 21.6)** | **1.73 (1.63 – 1.83)** | **0.71 (0.68 – 0.74)** |
| **Admission sample rule-out and history ≥ 3 hours (N= 772)** | Sensitivity | NPV | NLR | Specificity | PPV | PLR | AUC |
| cTnT$_{ESC}$ < 5 ng/L | 98.9 (94.2 – 100.0) | 99.5 (96.8 – 99.9) | 0.03 (0 – 0.22) | 34.5 (30.9 – 38.2) | 18.4 (17.5 - 19.3) | 1.51 (1.42 - 1.60) | 0.67 (0.63 – 0.70) |
| cTnI$_{(Abbott)ESC}$ < 2 ng/L | 97.9 (92.5 – 99.7) | 98.8 (95.2 – 99.7) | 0.08 (0.02 – 0.34) | 25.4 (22.2 – 28.9) | 16.4 (15.7 – 17.1) | 1.31 (1.24 – 1.38) | 0.62 (0.58 - 0.65) |
| **cTnI$_{(sgx)}$ < 2 ng/L** | **98.9 (94.2 – 100.0)** | **99.6 (97.4 – 100.0)** | **0.03 (0 - 0.18)** | **42.0 (38.2 – 45.8)** | **20.3 (19.2 - 21.4)** | **1.70 (1.59 – 1.82)** | **0.70 (0.67 – 0.74)** |

Values are n (%) with 95% confidence intervals.

ESC and earlier published Singulex Clarity System (sgx) algorithms are referenced in the table, and the novel algorithm in bold. A minor non-significant change in the diagnostic performance, e.g., sensitivity, is seen when early presenters are excluded because of a change in the nominator.

AUC=area under the receiver operating characteristic curve; cTnI$_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); cTnI$_{(sgx)}$=cardiac troponin I (Singulex Clarity System); cTnT=cardiac

troponin T; ESC=European Society of Cardiology; NLR=negative likelihood ratio;

NPV=negative predictive value; NSTEMI=non–ST-elevation myocardial infarction;

PLR=positive likelihood ratio; PPV=positive predictive value.

**Table 2. Serial 0/1 hour algorithms for rule-out and rule-in of NSTEMI.**

| 0/1 hour rule-out (N=465) | Sensitivity | NPV | NLR | Specificity | PPV | PLR | AUC |
|---|---|---|---|---|---|---|---|
| $cTnT_{ESC} < 12$ ng/L and $\Delta_{0-1} < 3$ ng/L | 100.0 (94.1 – 100.0) | 100 (NC) | 0 (NC) | 74.3 (69.7 – 78.5) | 37.0 (33.2 – 40.9) | 3.88 (3.29 – 4.58) | 0.87 (0.84 – 0.90) |
| $cTnI_{(Abbott)ESC} < 5$ ng/L and $\Delta_{0-1} < 2$ ng/L | 100.0 (94.1 – 100.0) | 100 (NC) | 0 (NC) | 65.4 (60.5 – 70.0) | 30.4 (27.6 – 33.3) | 2.89 (2.52 – 3.30) | 0.83 (0.79 – 0.86) |
| $cTnI_{(sgx)Neumann} < 2$ ng/L and $\Delta_{0-1} < 1$ ng/L | 100 (94.1-100.0) | 100 | 0 | 46.0 (41.1-51.0) | 21.9 (20.4-23.4) | 1.85 (1.69-2.03) | 0.73 (0.69-0.77) |
| $cTnI_{(sgx)} < 8.67$ ng/L and $\Delta_{0-1} < 3$ ng/L | 100 (94.1 – 100.0) | 100 (NC) | 0 (NC) | 87.1 (83.5 – 90.2) | 53.7 (47.4 - 59.9) | 7.77 (6.03 -10.01) | 0.94 (0.91 – 0.96) |
| **$cTnI_{(sgx)} < 10$ ng/L and $\Delta_{0-1} < 3$ ng/L** | **100.0 (94.1 – 100.0)** | **100 (NC)** | **0 (NC)** | **88.6 (85.1 -91.5)** | **57.0 (50.3 - 63.5)** | **8.78 (6.69 -11.53)** | **0.94 (0.92 – 0.96)** |
| 0/1 hour rule-in (N=465) | Sensitivity | NPV | NLR | Specificity | PPV | PLR | AUC |
| $cTnT_{ESC} \geq 52$ ng/L or $\Delta_{0-1} \geq 5$ ng/L | 78.7 (66.3 – 88.1) | 96.8 (94.9 – 98.0) | 0.22 (0.14 – 0.36) | 96.5 (94.3 – 98.1) | 77.4 (66.8 – 85.4) | 22.7 (13.4 – 38.6) | 0.88 (0.84 – 0.90) |
| $cTnI_{(Abbott)ESC} \geq 52$ ng/L or $\Delta_{0-1} \geq 6$ng/L | 91.8 (81.9 - 97.3) | 98.7 (97.1 - 99.4) | 0.09 (0.04 – 0.20) | 94.6 (91.9 – 96.6) | 71.8 (62.7 – 79.4) | 16.9 (11.2 – 25.5) | *0.93 (0.90 – 0.95) |
| $cTnI_{(sgx)Neumann} \geq 25$ ng/L or $\Delta_{0-1} \geq 6$ ng/L | 90.2 (79.8-96.3) | 98.5 (96.8-99.3) | 0.10 (0.05-0.22) | 95.3 (92.8-97.2) | 74.3 (64.9-81.9) | 19.2 (12.3-30.0) | 0.93 (0.90-0.95) |
| **$cTnI_{(sgx)0} \geq 70$ ng/L or $\Delta_{0-1} \geq 5$ ng/L** | **90.5 (80.4 - 96.4)** | **98.7 (97.2 – 99.4)** | **0.10 (0.05 – 0.21)** | **97.0 (94.9 – 98.5)** | **80.6 (70.3 – 87.9)** | **30.5 (17.4 – 53.5)** | **0.94 (0.91 - 0.96)** |
| **$cTnI_{(sgx)0} \geq 30$ ng/L or $\Delta_{0-1} \geq 5$ ng/L** | **93.4 (84.1 – 98.2)** | **99.1 (97.7 –** | **0.07 (0.03 -** | **96.3 (94.0 – 97.9)** | **77.4 (67.5 - 85.0)** | **25.2 (15.3 – 41.5)** | **0.95 (0.92 – 0.97)** |

| | | | 99.6) | 0.18) | | | | |
|---|---|---|---|---|---|---|---|---|

Values are n (%) with 95% confidence intervals.

ESC and earlier published Singulex Clarity System (sgx) algorithms are referred in the table, the novel algorithm in bold.

AUC=area under the receiver operating characteristic curve; $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac Troponin I (Singulex Clarity System); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NLR=negative likelihood ratio; NPV=negative predictive value; NSTEMI=non–ST-elevation myocardial infarction; PLR=positive likelihood ratio; PPV=positive predictive value.

**Table 3. Cox regression analysis.**

| | Univariable | | Multivariable* | |
|---|---|---|---|---|
| | HR (95% CI) | P value | HR (95% CI) | P value |
| Direct rule-out (n=971) | | | | |
| $cTnT_{ESC}$ < 5 ng/L vs. observation and rule-in | 9.361 (3.423-25.583) | <0.001 | 3.421 (1.170-10.000) | 0.025 |
| $cTnI_{(Abbott)\ ESC}$ < 2 ng/L vs. observation and rule-in | 8.179 (2.582-25.909) | <0.001 | 3.050 (0.917-10.146) | 0.069 |
| $cTnI_{(sgx)Neumann}$ < 1 ng/L vs. observation and rule in | 8.296 (1.155-59.609) | 0.035 | 2.365 (0.318-17.583) | 0.400 |
| **$cTnI_{(sgx)}$ < 2 ng/L vs. observation and rule-in** | **7.769 (3.363-17.840)** | **<0.001** | **3.286 (1.359-7.946)** | **0.008** |
| 0/1 hour rule-out (n=465) | | | | |
| $cTnT_{ESC}$ < 12 ng/L and $\Delta_{0-1}$ < 3 ng/L vs. observation and rule-in | 4.959 (2.294-10.718) | <0.001 | 3.190(1.345-7.562) | 0.008 |
| $cTnI_{(Abbott)\ ESC}$ < 5 ng/L and $\Delta_{0-1}$ < 2 ng vs. observation and rule-in | 3.456 (1.599-7.469) | 0.002 | 2.227 (0.972-5.105) | 0.058 |
| $cTnI_{(sgx)Neumann}$ < 2 ng/L and $\Delta_{0-1}$ < 1 ng/L, vs observation and rule in | 6.537 (1.991-21.461) | 0.002 | 3.671 (1.046-12.885) | 0.042 |
| **$cTnI_{(sgx)}$ < 10 ng/L and $\Delta_{0-1}$ < 3 ng/L, vs observation and rule-in** | **2.373 (1.172-4.805)** | **0.016** | **1.603 (0.754-3.406)** | **0.220** |

95% confidence interval in brackets.

Unadjusted and adjusted hazard ratios for the composite endpoint of future non-fatal MI and all-cause mortality. Patients are dichotomized based on the admission sample algorithm, i.e., rule-out (reference category) vs. observation/rule-in.

$cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity System); cTnT=cardiac troponin T; eGFR=estimated glomerular filtration rate; ESC=European Society of Cardiology; MI=myocardial infarction; HR=hazard ratio.

*Included in the multivariable model: algorithm as applicable, age, sex, current or previous smoking, eGFR above vs below 60 mL/min/1.73 m$^2$, diabetes, hypertension, hyperlipidemia, and previous MI.

**Figure Legends:**


**Figure 1. Cardiac troponin concentrations; median, (interquartile range) stratified according to diagnosis. A**, Cardiac troponin concentrations at admission. **B**, Cardiac troponin concentrations after 1 hour. ACS=acute coronary syndrome; cTnI$_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); cTnI$_{(sgx)}$=cardiac troponin I (Singulex Clarity System); cTnT=cardiac troponin T; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; UAP=unstable angina.


**Figure 2. AUC-ROC for admission troponin concentrations as a continuous variable in NSTEMI patients versus non-NSTEMI patients.** cTnI$_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); cTnI$_{(sgx)}$=cardiac troponin I (Singulex Clarity System); cTnT=cardiac troponin T; ROC=receiver operating characteristic curve.


**Figure 3. Main diagnostic performance measures and efficiency of the different algorithms.** Diagnostic performance of the admission ESC rule-out algorithm was calculated based on late presenters (n=772) because the ESC does not recommend direct rule-out until >3 hours after onset of symptoms. The cardiac troponin I from the Singulex Clarity System (cTnI$_{(sgx)}$) data is based on all participants (N=971). Efficiency was calculated as the percentage eligible for rule-out from the total cohort (all algorithms). cTnI$_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); cTnI$_{(sgx)}$=cardiac troponin I (Singulex Clarity System); cTnT=cardiac troponin T; ESC=European Society of Cardiology.


**Figure 4. Kaplan–Meier curves for the prognostic composite endpoint of future myocardial infarction (MI) and all-cause mortality stratified according to the**

**algorithms. A**, 0/1 hour $cTnT_{ESC}$. **B**, 0/1 hour $cTnI_{(Abbott)ESC}$. **C**, 0/1 hour $cTnT_{(sgx)Neumann}$. **D**, 0/1 hour $cTnI_{(sgx)}$. $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity System); cTnT=cardiac troponin T; ESC=European Society of Cardiology.
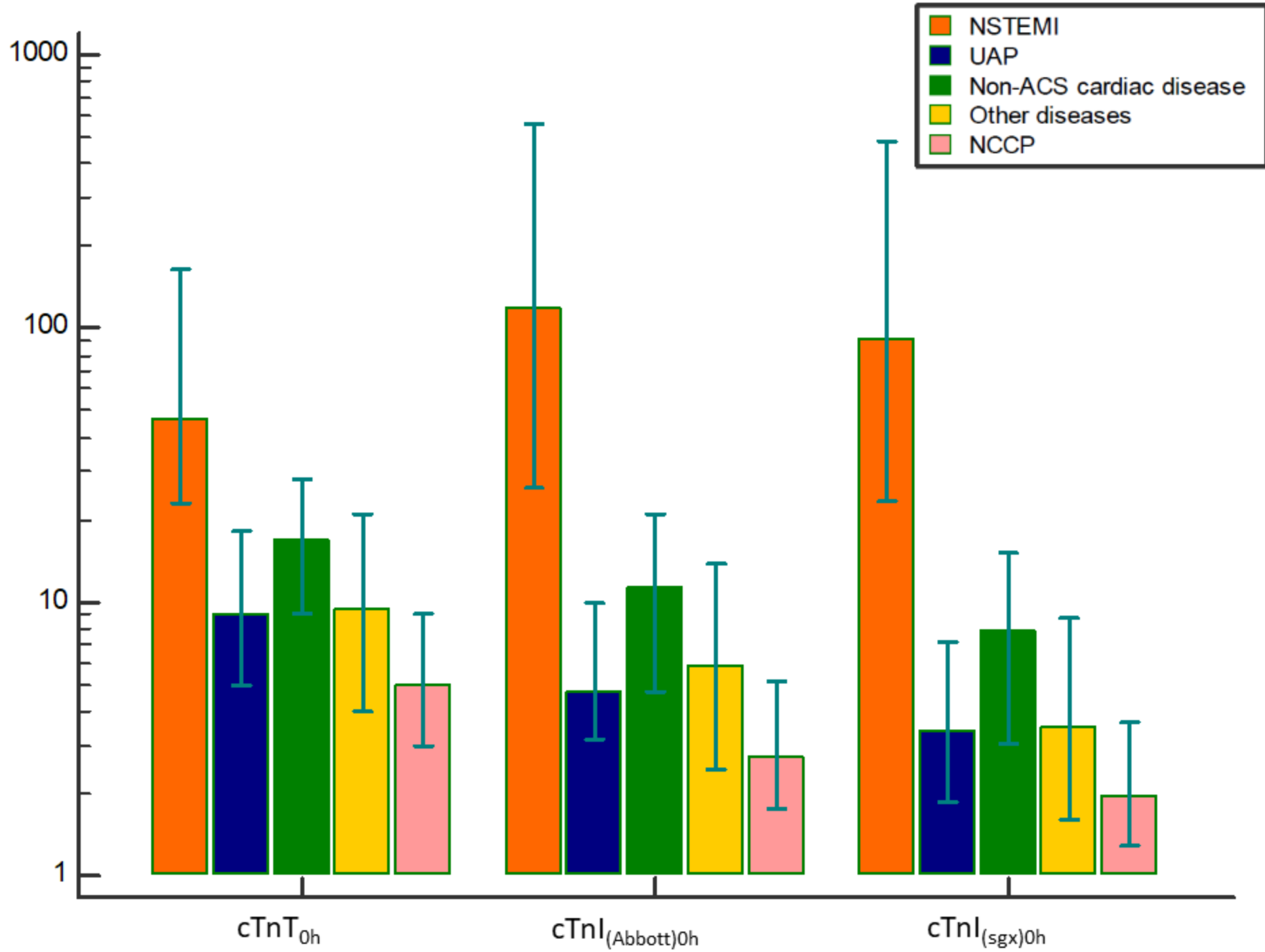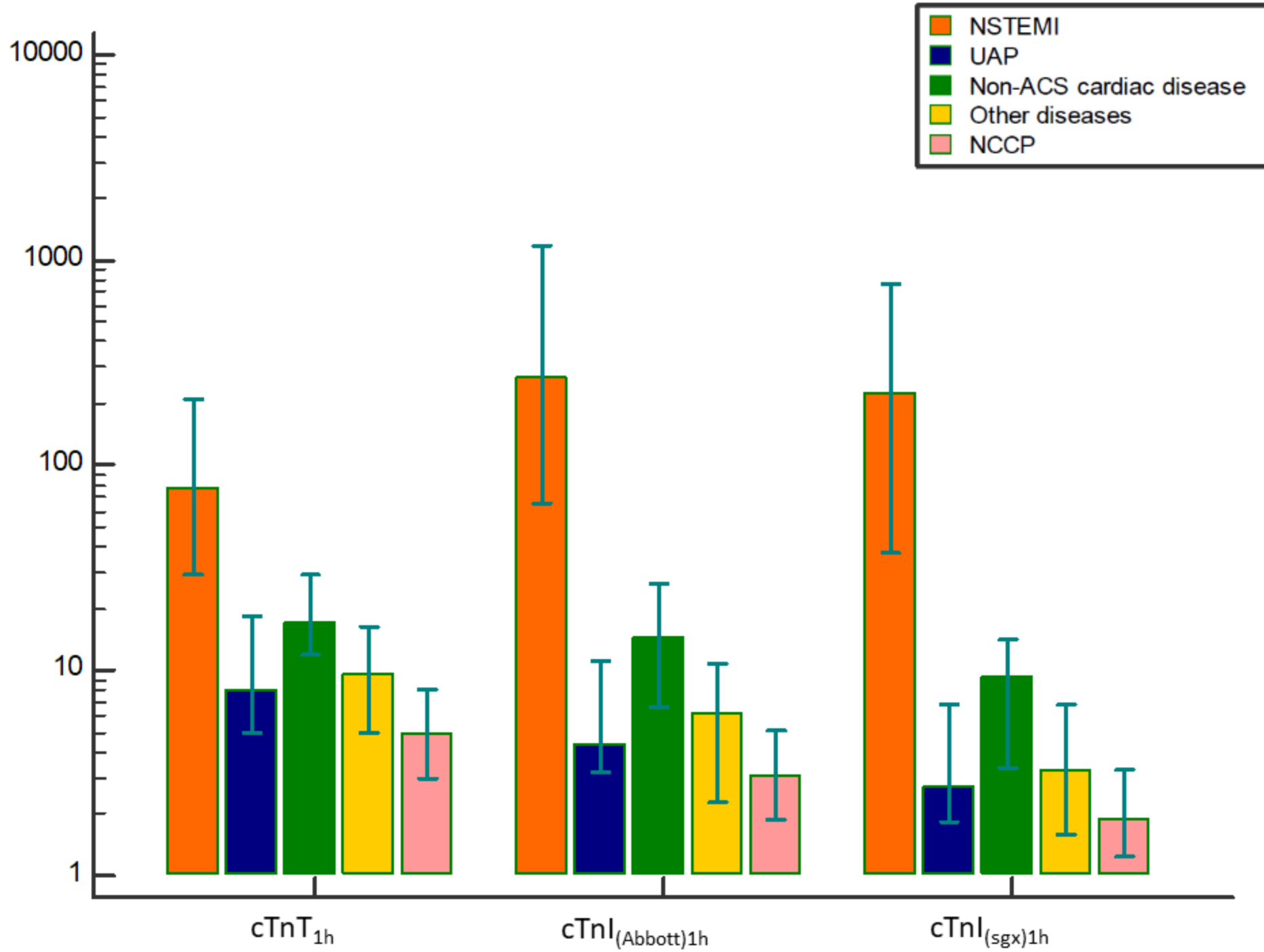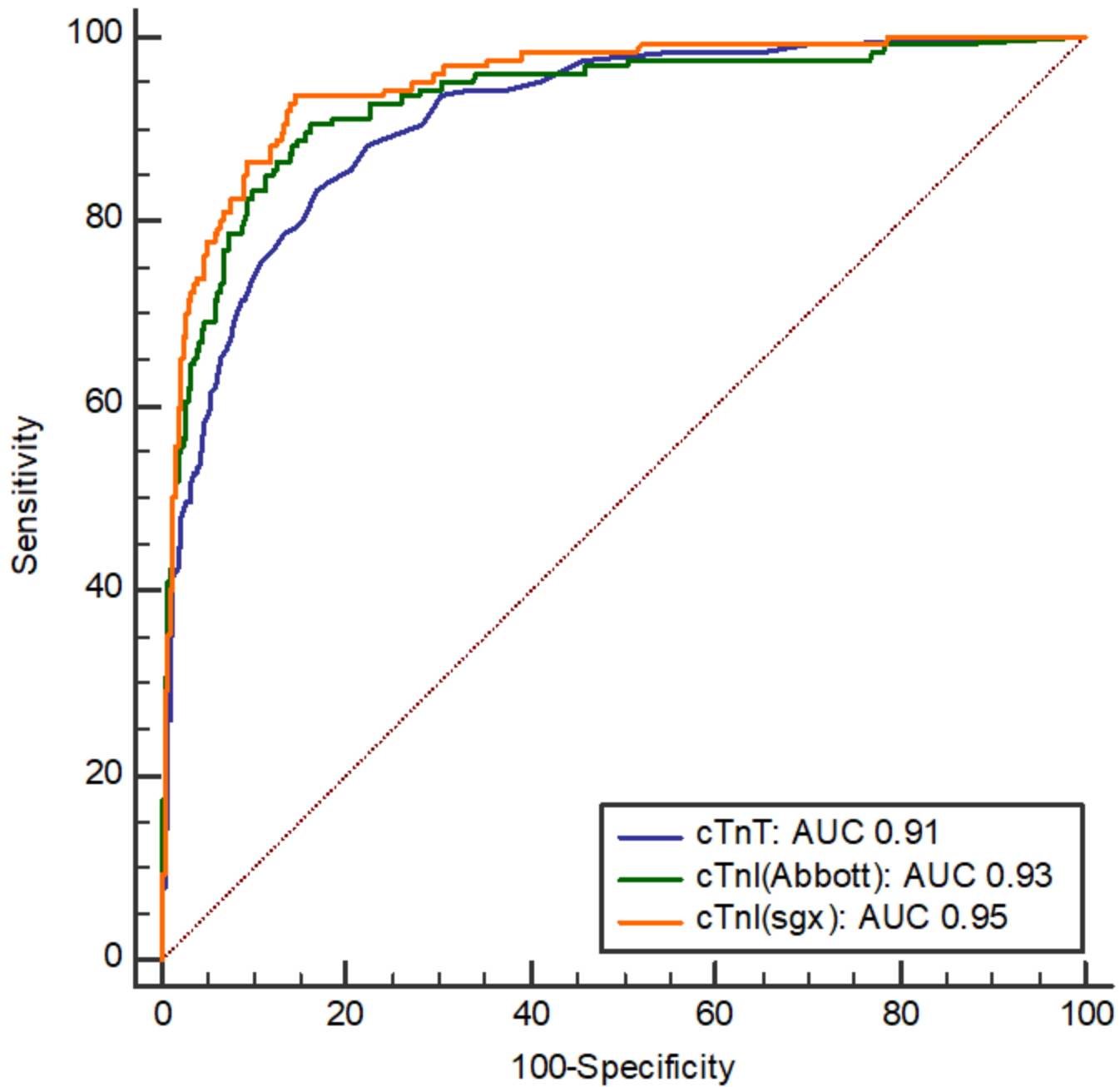
## Admission single sample rule-out

**$cTnT_{ESC} < 5$ ng/L**

Rule-out: 235 (24%)
Sensitivity: 98.9 %
NPV: 99.5 %

**$cTnI_{(Abbott)ESC} < 2$ ng/L**

Rule-out: 174 (18%)
Sensitivity: 97.9%
NPV: 98.8%

**$cTnI_{(sgx)Neumann} < 1$ ng/L**

Rule-out: 87 (9%)
Sensitivity: 100%
NPV: 100%

**$cTnI_{(sgx)Body} < 1.5$ ng/L**

Rule-out: 237 (24%)
Sensitivity: 99.2%
NPV: 99.6%

**$cTnI_{(sgx)} < 2$ ng/L**

Rule-out: 360 (37%)
Sensitivity: 99.2%
NPV: 99.7%

## 0/1 hour rule-out & rule-in protocol

**$cTnT_{ESC}$**

Rule out: $cTnT_0 < 12$ ng/L and $\Delta_{0-1} < 3$ ng/L
Rule in: $cTnT_0 \geq 52$ ng/L or $\Delta_{0-1} \geq 5$ ng/L

Rule-out: 300 (64%)
Sensitivity: 100%
NPV: 100%

Rule-in: 62 (13%)
Specificity: 96.5%
PPV: 77.4%

**$cTnI_{(Abbott)ESC}$**

Rule out: $cTnI_0 < 5$ ng/L and $\Delta_{0-1} < 2$ ng/L
Rule in: $cTnI_0 \geq 52$ ng/L or $\Delta_{0-1} \geq 6$ ng/L

Rule-out: 264 (57%)
Sensitivity: 100%
NPV: 100%

Rule-in: 78 (17%)
Specificity: 94.6%
PPV: 71.8%

**$cTnI_{(sgx)Neumann}$**

Rule out: $cTnI_0 < 2$ ng/L and $\Delta_{0-1} <$ ng/L
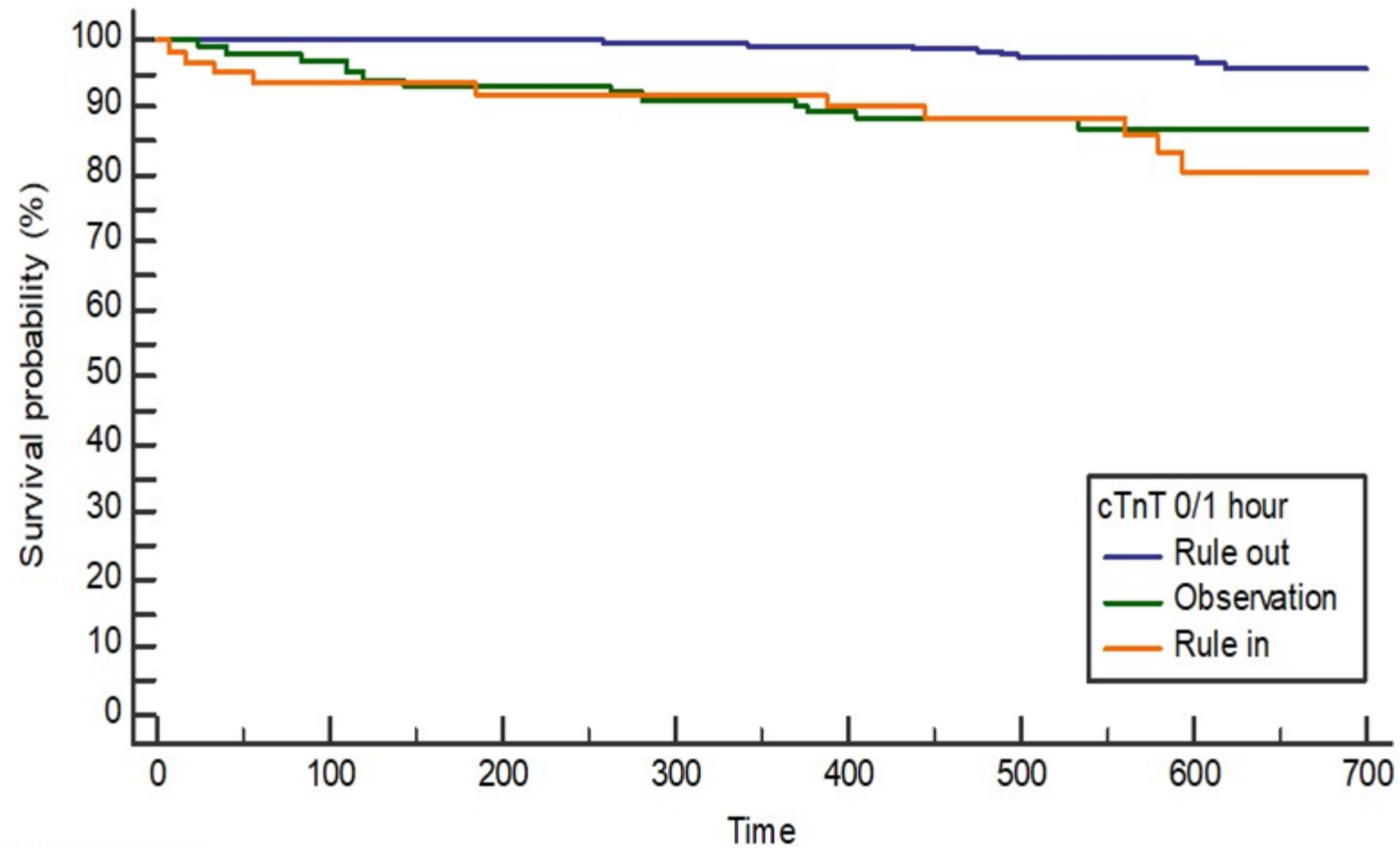Rule in: $cTnI_0 \geq 25$ ng/L or $\Delta_{0-1} \geq 6$ ng/L

Rule-out: 186 (40%)
Sensitivity: 100%
NPV: 100%

Rule-in: 74 (16%)
Specificity: 95.3%
PPV: 74.3%

**$cTnI_{(sgx)}$**

Rule out: $cTnI_0 < 10$ ng/L and $\Delta_{0-1} < 3$ ng/L
Rule in: $cTnI_0 \geq 70$ ng/L or $\Delta_{0-1} \geq 5$ ng/L

Rule-out: 358 (77%)
Sensitivity: 100 %
NPV: 100 %

Rule-in: 67 (14%)
Specificity: 97.0%
PPV: 80.6%

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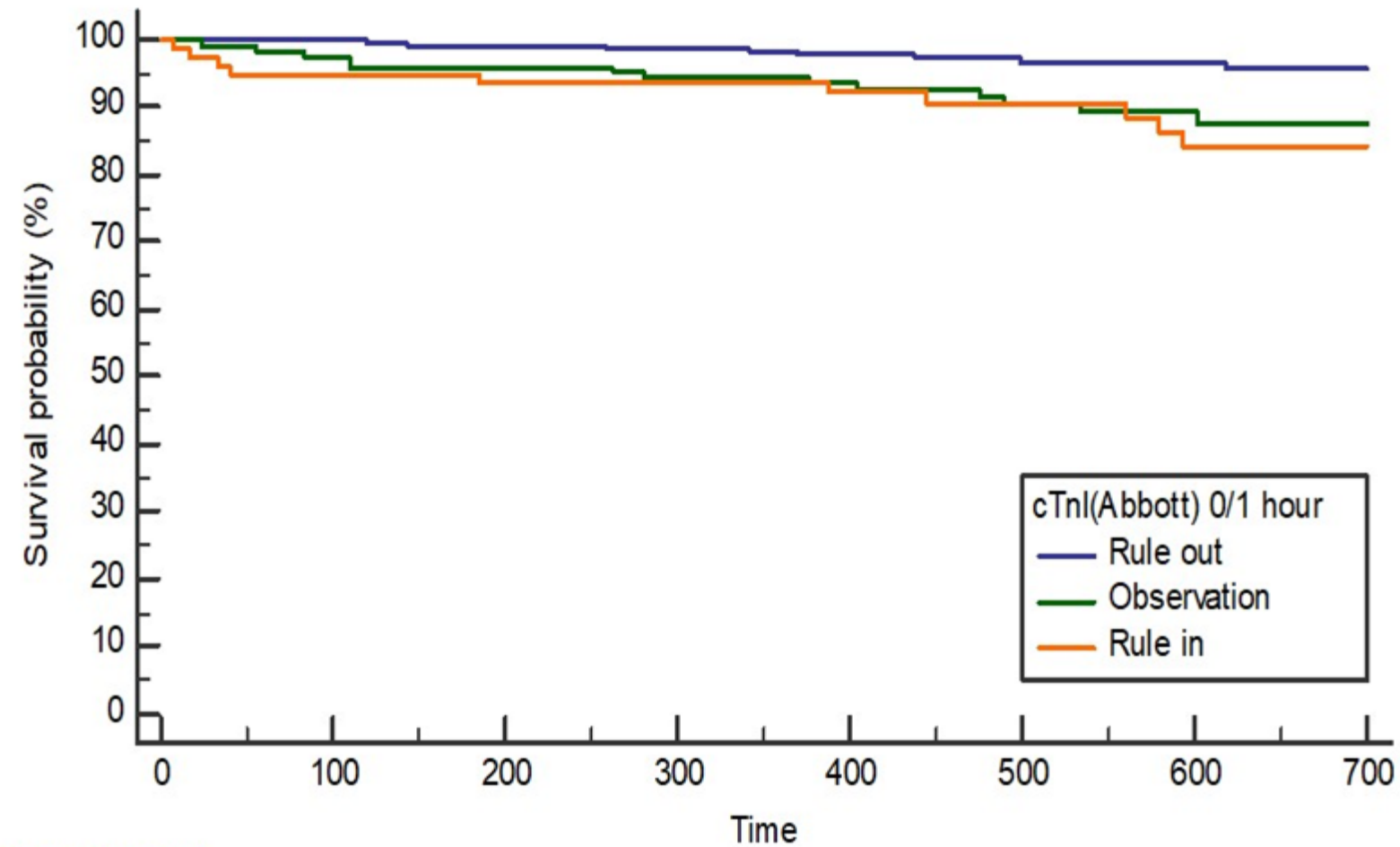| Number at risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group: Rule out | | | | | | | |
| 300 | 300 | 300 | 299 | 276 | 208 | 147 | 56 |
| Group: Observation | | | | | | | |
| 103 | 100 | 96 | 94 | 90 | 68 | 40 | 15 |
| Group: Rule in | | | | | | | |
| 62 | 58 | 57 | 57 | 53 | 40 | 29 | 15 |

B

| Number at risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group: Rule out | | | | | | | |
| 264 | 264 | 262 | 261 | 239 | 184 | 123 | 45 |
| Group: Observation | | | | | | | |
| 123 | 120 | 118 | 116 | 113 | 81 | 59 | 24 |
| Group: Rule in | | | | | | | |
| 78 | 74 | 73 | 73 | 67 | 51 | 34 | 17 |

C

Survival probability (%)

cTnI(sgx)Neumann 0/1 hour
— Rule out
— Observation
— Rule in

Time
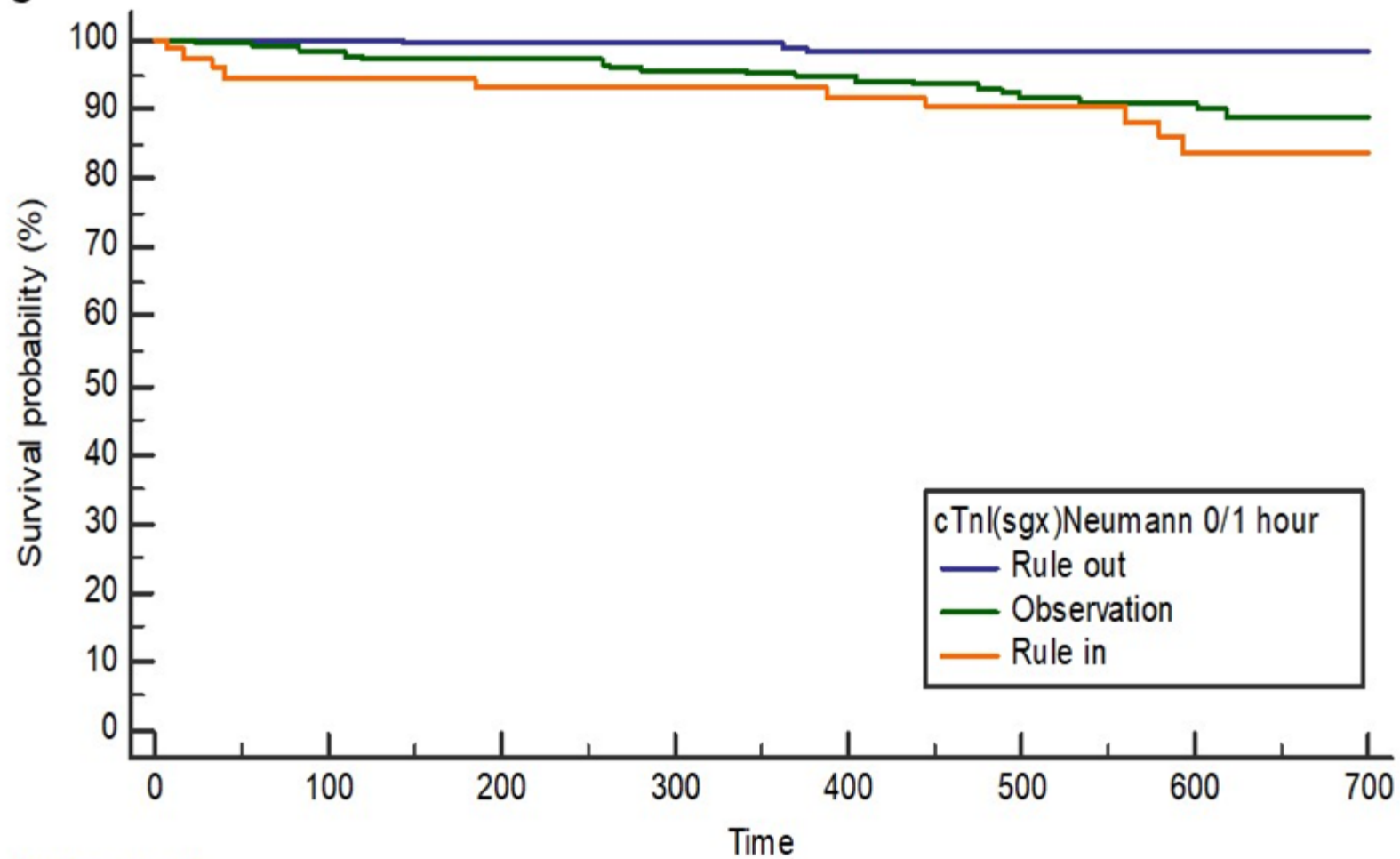
Number at risk
Group: Rule out

| 186 | 186 | 185 | 185 | 168 | 127 | 86 | 34 |

Group: Observation

| 205 | 202 | 199 | 196 | 186 | 140 | 96 | 35 |

Group: Rule in

| 74 | 70 | 69 | 69 | 65 | 49 | 34 | 17 |

D

Survival probability (%)

cTnI(sgx) 0/1 hour
— Rule out
— Observation
— Rule in

Time
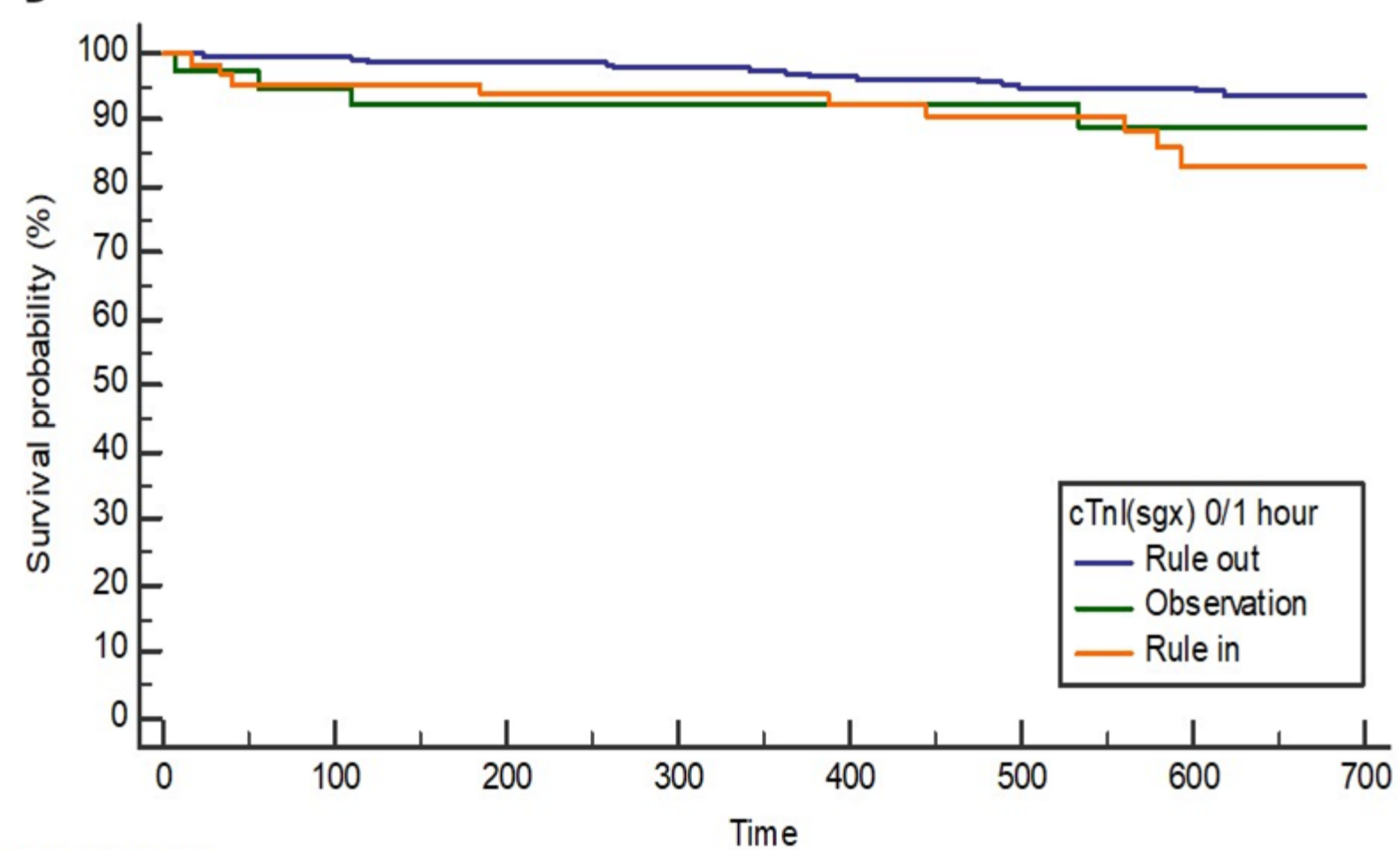
Number at risk
Group: Rule out

| 358 | 356 | 353 | 350 | 325 | 245 | 168 | 64 |

Group: Observation

| 40 | 38 | 37 | 37 | 35 | 26 | 19 | 6 |

Group: Rule in

| 67 | 64 | 63 | 63 | 59 | 45 | 29 | 16 |