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Acknowledgement

Table of Contents

Abstract

Background and Objectives: Further validation of the diagnostic performance of the relatively new Siemens ADVIA Centaur® High-Sensitivity Troponin I (TNIH) immunoassay might still yield useful information before possible implementation in clinical practice at St. Olav's University Hospital. We aimed to evaluate the diagnostic accuracy of the TNIH assay for non-ST elevation myocardial infarction (NSTEMI) in patients with suspected acute myocardial infarction (AMI) based on blood samples collected shortly after hospital admission.

Materials and Methods: We conducted a prospective, observational pilot study at St. Olav's University Hospital in Trondheim, Norway. Consecutive patients aged 18 years or older presenting to the emergency department (ED) with suspected AMI based on the ordering of an "infarction status" analysis package was enrolled. The blood sample for the index test was collected at the same time as standard blood samples including the reference standard, retrospectively requesting written informed consent. A final diagnosis of NSTEMI extracted from the discharge summary (diagnostics performed by treating clinicians based on clinical findings and serial samples of high-sensitivity (hs)-cardiac troponin T) was used as the reference standard. Data were collected without knowing the patient outcome. Diagnostic accuracy was evaluated by calculating area under the Receiver Operating Characteristic curve (ROC AUC), and sensitivity, negative predictive value (NPV), specificity and positive predictive value (PPV) at relevant decision limits.

Results: Of 124 included patients, NSTEMI was the final diagnosis in 14 (11.3 %). The overall ROC AUC for TNIH shortly after admission was 0.93 (95 % CI, 0.87–0.97). Using the 99th percentile suggested by the manufacturer of the TNIH assay, we found a sensitivity of 71.4 % (95 % CI, 41.9–91.6), NPV of 96.0 % (95 % CI, 91.4–98.2), specificity of 88.2 % (95 % CI, 80.6–93.6), and a PPV of 43.5 % (95 % CI, 29.5–58.6).

Discussion: Compared to similar studies conducted on this specific assay this pilot study shows a tendency of lower overall diagnostic accuracy as quantified by the ROC AUC of the TNIH assay for NSTEMI. Because of the small population size, and especially the low number of patients with NSTEMI, the point estimates are too uncertain to draw definite conclusion. Larger studies are therefore needed to confirm or refute these tendencies before possible implementation in clinical practice at St. Olav's University Hospital.

Introduction

The detection of biomarkers of cardiomyocyte injury is essential in diagnosing acute myocardial infarction (AMI) and to distinguish between the different types of acute coronary syndromes (ACS). Cardiac troponin I (cTnI) or T (cTnT) are the preferred biomarkers, where both plasma concentration and concentration dynamics in terms of rise and/or fall play a role in the interpretation of results. It is especially of importance when faced with a possible non-ST elevation myocardial infarction (NSTEMI) (1). ACS includes both ST-elevation myocardial infarction (STEMI) and non-ST-elevation ACS (NSTE-ACS). NSTE-ACS is further divided in two, in which the pathological correlate at myocardial level is either cardiomyocyte necrosis (NSTEMI) or myocardial ischaemia without cell loss (unstable angina).

While detection of an elevated cTn value above the 99th percentile upper reference limit (URL) is defined as myocardial injury, the myocardial injury is considered acute if there is a rise and/or fall of cTn values. If the acute myocardial injury occurs with clinical evidence of acute myocardial ischaemia, it is defined as an AMI (2).

Cardiac troponins can be measured by several available assays, whereof high-sensitivity (hs)-cTn assays are recommended. The assays for cTn are not well standardized (2), and values may also be different between assay generations and on different instruments. Clinicians are therefore dependent on each individual assay being thoroughly researched and validated with respect to analytical and diagnostic characteristics before implementation in clinical practice. This includes consideration of important factors such as analytical precision, limit of quantification and diagnostic accuracy in relevant populations. For hs-cTn assays both analytical and biological variation must be estimated in order to be able to assess the difference between two consecutive results from the same patient (3). Ideally, each lab should ensure the quality of their analytical instruments and method, using their own proven variation as grounds for decision-making. With high analytical precision, i.e. low grade of random analytical variation, small changes can be detected with high reliability. For hs-cTn assays, an imprecision of ≤ 10 % coefficient of variation (CV) at the 99th percentile URL is required, but assays with CV between 10–20 % are acceptable for clinical use (2).

Furthermore, the 99th percentile URL must also be determined for each specific assay and is relevant as a limit of decision for AMI. The limit of detection (LoD) also varies between assays (4), and a requirement for the hs-cTn assays is an ability to measure cTn values above

the assay's LoD in ≥ 50 % in healthy individuals. It is important to know that there is no consensus about specific criteria for how the 99th percentile URL should be defined, and different 99th percentile URLs are available.

At St. Olav's University Hospital, the current biochemical approach used in clinical practice in patients with suspected AMI is measurements of plasma concentration of hs-cTnT with the Roche Elecsys Troponin T hs immunoassay on a Cobas 8000 instrument. The hospital is considering changing to the novel assay for hs-cTnI, Siemens ADVIA Centaur® High-Sensitivity Troponin I immunoassay (TNIH). This assay has promising analytical properties, including measuring the concentration of cTnI very precisely at low concentrations (5).

The 99th percentile URL for TNIH is available from the manufacturer (6) and through conducted studies (7, 8), but only few clinical studies have described the diagnostic accuracy of this specific assay and how the assay works in clinical practice compared to other troponin assays (5, 9, 10).

Current recommendations for use of hs-cTn assays from ESC is either the 0 h/1 h algorithm or the 0 h/2 h algorithm with assay specific cut-off levels (1). However, according to the Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) study, combining hs-cTnI at presentation with results at later time points did not increase the diagnostic accuracy significantly (Supplemental Table 3A in (9)). Also, the Fourth universal definition of myocardial infarction states that "Strategies employing either very low levels of hs-cTn on presentation or the lack of any change and persistently normal hs-cTn values over a 1–2 h period after presentation have been advocated to exclude acute myocardial injury, and MI as well." (2). In turn, this may facilitate a quicker discharge from the hospital, provided that other serious conditions are excluded. The introduction of more sensitive assays has resulted in this use of shorter time intervals in algorithms. This is possible because smaller changes in concentration can be detected, even introducing the option of single test rule-out strategies. Thus, the quality of time registrations is of importance. With exact information about time of relevant events such as symptom onset and time of blood sampling, one can better indicate where in the cycle of kinetic changes of troponins the patient is. A recent study reported that the single test rule-out approach had high diagnostic sensitivity and negative predictive value (NPV) for TNIH to rule out AMI (10). This might be a promising approach to shorten the hospital stays and avoiding potential crowding in the emergency department (ED). To safely trust the use of this approach, the registered times of relevant events must be reliable.

In order to implement the new TNIH assay, more knowledge is needed on how it performs in clinical practice and how it may help to exclude and diagnose AMI. This pilot study aims to evaluate the diagnostic accuracy of the TNIH assay for NSTEMI in patients acutely admitted to St. Olav's University Hospital with suspected AMI based on blood samples collected shortly after hospital admission. The objective is to obtain an indication of what can be found in a following larger study – The BIOMAK project. For St. Olav's University Hospital, the knowledge gained from BIOMAK will be an important basis for possibly deciding to change from the currently used assay, as it is potentially associated with significant financial savings due to reduced analysis costs. Moreover, it provides opportunities for faster analysis of blood samples, which can provide faster clarification, possibly better treatment, and earlier discharge of patients from the hospital.

Thus, the hypothesis of this pilot study was that the diagnostic accuracy of TNIH for NSTEMI is comparable to similar studies using the same and other cTn assays.

Materials and Methods

Study Design and Population

The recruitment of patients took place in the time period 2 February to 15 March 2019, as a planned initial phase of the bigger BIOMAK project already approved by the Regional Committees for Medical and Health Research Ethics (REK), project number 2018/648. Blood samples were collected from patients aged 18 years or older where a pre-defined "Infarction status" analysis package was ordered either by the ED or hospital ward. This is mainly done by nurses or medical doctors if there is suspicion of symptoms suggestive of AMI. Written informed consent was requested retrospectively from 424 patients after the hospital stay sending a questionnaire by mail, and one reminder was also sent by mail to non-responders. A total of 226 patients gave their written informed consent and were included in the study. These patients constitute the primary study population used in this pilot study conducted as a prospective observational study. Only the first hospital admission with suspected AMI or ACS was included if a patient had multiple admissions within the inclusion period.

Patients included on the wrong basis, i.e. analysis package ordered without suspected AMI or ACS, patients where the pre-defined analysis package was taken more than four hours after hospital admission, patients with confirmed STEMI, and patients with missing cTnT or cTnI results were excluded. In addition, patients with recent admission with confirmed AMI diagnosis, patients where cTn were obtained after witnessed cardiac arrest and patients admitted to the hospital seven days after symptom onset were also excluded. Of 226 patients, 30 patients were excluded *before* review of medical records and 72 patients were excluded *after* review of medical records. Thus, the final study population consisted of 124 included patients. The flow of patients is explained in detail in figure 1.
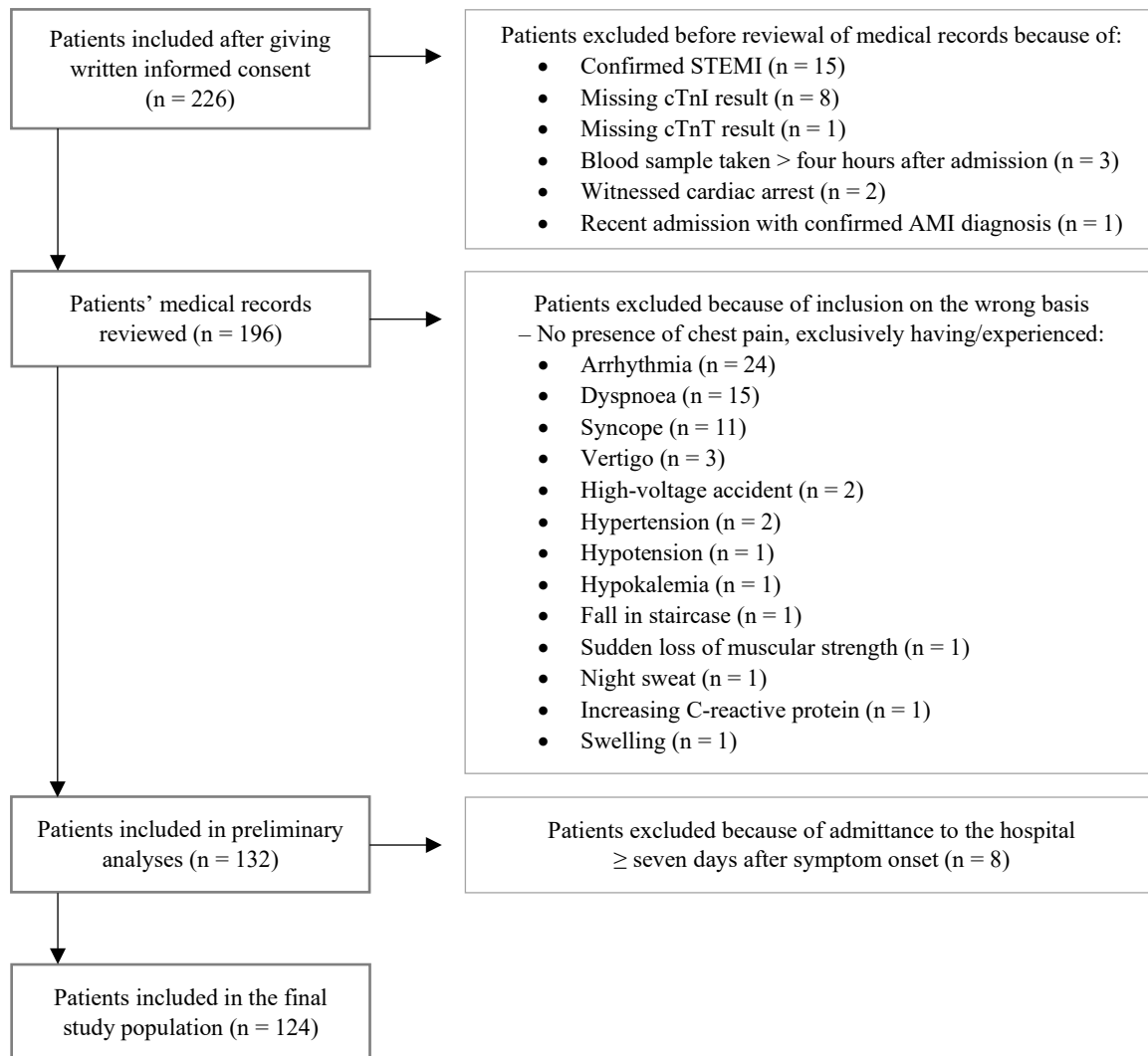
Figure 1: Flow Chart Explaining the Inclusion and Exclusion Process.

Sample Collection

After nurses or medical doctors ordered the pre-defined "Infarction status" analysis package, labels for plasma and serum sample tubes were generated. For these patients, the index test (TNIH in plasma and serum) was automatically ordered and analysed in addition to regular tests including cTnT. Thus, blood samples for cTnI were collected shortly after admission to the ED or hospital ward at the same time as standard blood samples including the first cTnT sample were collected, only subjecting the patients to an additional 5 mL of blood draw. Two serial samples of cTnT were acquired for 75 % of the population, and three samples for 30 %. Only serial samples collected before angiography were included in the analyses to exclude

high troponin values because of rise caused by the procedure. In this pilot study, no serial samples were acquired for cTnI.

While cTnT was analysed and results reported to the treating health care workers, cTnI was analysed with the TNIH assay with results stored in the laboratory data system only available to laboratory staff, and no results were reported to health care workers. Thus, the treating health care workers were blinded to the cTnI values and patients received treatment and follow-up in accordance with current procedures based on cTnT values.

Obtaining Patient Information

Clinical information on the patients were obtained from a paper-based questionnaire completed at the same time as the written informed consent, the patient administration system (PAS), the patients' medical records including the medical chart, the hospital discharge summary, prehospital medical records and ED observation form, as well as the emergency database at St. Olav's University Hospital.

Before reviewing the mentioned sources of information, relevant variables were defined by a group consisting of a senior consultant at the Department of Medical Biochemistry, a senior consultant at the Clinic of Cardiology, a senior consultant at the Clinic of Emergency Medicine and Prehospital Care, and a 5th year medical student. A pilot review of 18 patients were conducted before the variables were revised into the final list of variables. Collection of patient information took place in the time period 17 September to 6 October 2020.

Important information collected included time registrations used in estimation of times of relevant events (time of symptom onset, hospital admission and blood sampling) and lab results (troponin values, plasma cholesterol and plasma creatinine). Time of symptom onset was based on information collected from one or more sources. A "best guess" category was made to comprise those with especially vague estimations of symptom onset, such as "the last two days" etc. Certainty levels for these times were assessed based on whether a precise time was mentioned or not. At St. Olav's University Hospital the following times are registered with respect to admission and blood sampling: Emergency database "time in" (time registered in the ED by the co-ordinating nurse once the patient arrives at the ED), emergency database "blood sampling" (time of blood sampling in the ED (if performed there) registered by the nurse responsible for the patient), PAS "time in" (time when the patient arrives at the hospital, registered by the responsible nurse – based on the emergency database "time in"), date and time when the blood sample was ordered or (preferably) collected (can and should be adjusted

in the requisition by the responsible health care worker once the blood sample is collected), and date and time when the sample tube is received and scanned at the laboratory.

Quality of registered times were assessed by investigating all available registrations except emergency database "blood sampling" as this was already missing for 67 % of the patients after finishing the pilot review. This time registration was therefore considered insufficient to use as the estimate of true time of blood sampling. Assessment included calculating percentages of the population where the times were available and looking for evident associated errors. Because of revealed uncertainty associated with the remaining option for time of blood sampling, for some patients the time of this event was set to the time of arrival of the sample tube at the laboratory. This was only done for those where time of blood sampling seemingly was not adjusted by the responsible health care worker, with blood samples registered as collected *before* time of hospital admission.

Creatinine was used to calculate estimated GFR (eGFR) (11). It is important to note that the laboratory does not report eGFR values > 90 as these values are unreliable and is reported to the clinicians as "> 90", i.e. censored.

Additionally, information on symptoms, ECG findings, image findings (echocardiography (both conventional and portable hand-held Vscan), MR, CT), ejection fraction (EF) and angiography findings were collected in order to confirm the AMI diagnosis. However, registered EF was not used in any analyses, as it was not recorded in a systematic fashion in the echocardiography report or medical record.

Risk factors for cardiovascular disease including hypertension, hypercholesterolemia, diabetes mellitus, smoking, family history, history of atherosclerotic disease and body mass index was collected where this was explicitly stated in the medical records.

Final Diagnosis, Reference Standard and Adjudication

The final diagnosis was extracted from PAS or the hospital discharge summary. A final diagnosis of NSTEMI (diagnostics performed by treating clinicians based on clinical findings and serial samples of hs-cTnT) was used as the reference standard when evaluating the diagnostic accuracy. AMI was defined and diagnosed according to the Fourth universal definition of myocardial infarction (2). Criteria for AMI included the presence of acute myocardial injury with clinical evidence of acute myocardial ischaemia with specified changes in blood levels of cTnT (rise and/or fall with at least one value above the 99th

percentile URL) and at least one of 1) certain ischaemic symptoms according to case history information, 2) new ischaemic ECG changes, 3) imaging evidence of coronary ischaemia by echocardiography, MR or CT, or 4) identification of a coronary thrombus or stenosis by angiography. The initial baseline value of cTnT determined the magnitude of the required relative change. Baseline values below or equal to the 99th percentile URL required serial changes > 50 %, while values above the 99th percentile URL required serial changes > 20 %. The accuracy of the discharge diagnoses in PAS has previously been validated (12), and the quality is considered sufficient regarding using this information for classification of patients according to relevant end points including confirmed NSTEMI. A retrospective adjudication of the discharge diagnosis into an alternative reference standard was also performed. This was done both to study the correspondence between discharge diagnosis and diagnosis based on retrospective assessment, and the possible effect on diagnostic accuracy for NSTEMI using the TNIH assay. Adjudication into the alternative reference standard was done based on a retrospective evaluation of the patient's medical record by a trained 5th year medical student alone and, when evaluating more complicated cases where there was doubt, in cooperation with a cardiologist. Both clinical information and results for cTnI and cTnT were available for the student and cardiologist, but only the cTnT values were used in the adjudication.

The primary end point was NSTEMI based on discharge diagnoses specified using relevant ICD-10 codes (I21.4 and I21.9) in PAS/the hospital discharge summary. Secondary end point was a major adverse cardiac event (MACE) within 30 days of discharge, with MACE defined as the combination of all-cause mortality, nonfatal AMI or coronary revascularization (percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG)), based on data from PAS or the patient's medical records.

Limit of Decision: 99th Percentiles of the URL

The limit of decision for the presence of AMI (positive test result) was the 99th percentile of the URL for both the reference standard and the index test.

The overall 99th percentile for the Roche Elecsys Troponin T hs immunoassay is, according to the manufacturer, 14 ng/L with a corresponding CV of 10 % at 13 ng/L (13). The 99th percentiles for men and women are 16.8 ng/L and 9.0 ng/L, respectively. Limit of blank (LoB), limit of detection (LoD) and limit of quantification (LoQ) have been determined to be 3 ng/L, 5 ng/L, and 13 ng/L, respectively.

According to the manufacturer, the overall 99th percentile in healthy men and women for the TNIH assay is 47 ng/L with a corresponding CV of < 5 % (6). The 99th percentiles for men and women are 57 ng/L and 37 ng/L, respectively. LoB, LoD and LoQ have been determined to be 0.90 ng/L, 2.21 ng/L, and 2.50 ng/L, respectively.

Assays for cTn measurements are not well standardized, and therefore each laboratory should ideally establish the 99th percentile concentration relevant for its assay and population. In a recent study this was done for the TNIH assay at St. Olav's University Hospital (8), suggesting different levels than proposed by both the manufacturer and a recent Italian study (7). The suggested 99th percentile by Odsæter et. al in healthy men and women is 96 ng/L with a corresponding CV of < 5 %. The 99th percentiles for men and women are 144 ng/L and 59 ng/L, respectively.

Statistical Analyses

For categorical variables frequencies and percentages are presented. For continuous variables median and interquartile range (IQR) are presented. Assessing for normality of distributions were done for continuous variables utilizing a test of normality (Kolmogorov-Smirnov statistic) combined with visual evaluation of histograms and/or simple dot plots. Scatter plots were made to study potential associations between different continuous variables. Spearman's Rank Order Correlation (rho) were performed on variables showing a monotone relationship and thus meeting the required assumptions for the analysis. Mann-Whitney U Test was used to test for differences between two independent groups of continuous variables. Kappa Measure of Agreement was used to assess agreement between two different reference standards for the presence of AMI. An Independent-Samples Median Test was conducted to compare medians of time intervals.

All the above-mentioned statistical analyses were performed using IBM® SPSS® Statistics 25.0.

Diagnostic accuracy was evaluated using Receiver Operating Characteristic (ROC) curve analysis as well as comparison of area under the ROC curves (ROC AUC) of independent ROC curves. ROC curves are used for diagnostic test evaluation, with the true positive rate (sensitivity) plotted in function of the false positive rate (100 % minus specificity) for different cut-offs of a parameter. Each point on the ROC curve represent a sensitivity/specificity pair corresponding to a particular decision threshold. The ROC AUC express how well a diagnostic test can distinguish between healthy and sick patients. Passing

and Bablok regression analysis and Bland-Altman plot were conducted to compare plasma and serum cTnI values. A filter using only serum cTnI values < 200 ng/L was applied for both analyses, as this is the most interesting level regarding limits of decision for AMI.

All ROC analyses, the Passing and Bablok regression analysis and Bland-Altman plot were performed using MedCalc version 17.5.5.

Censored data from the TNIH assay (cTnI < 2.5 ng/L) were assigned the value "0" and included when calculating median and IQR, making plots and in ROC analyses. Censored values were not included when conducting Sperman's rho, Passing and Bablok regression analysis and Bland-Altman plot.

The laboratory does not report cTnT values < 10 ng/L from the Roche Elecsys Troponin T hs immunoassay and are reported to the clinicians as "< 10", i.e. censored. However, exact values were available in the laboratory data system and used in analyses on troponin values.

Results

Patient Characteristics

Baseline characteristics of the study population are shown in Table 1. This shows an overall balanced distribution in terms of sex. The median age is approximately the same in the "NSTEMI" and "No NSTEMI" group, with the highest peak seen between 70 and 80 years when looking at all patients. Time from symptom onset to first blood sampling was available for all patients, but 93 and 37 patients underwent two and three serial samples of cTnT, respectively. Time intervals between serial sampling is therefore based on fewer patients and explicitly stated in the table. The point estimates and IQRs show a tendency of less time from symptom onset to first blood sample in the NSTEMI group (table 1). It also indicates that the differences are caused by the time from symptom onset to hospital admission. A Mann-Whitney U Test revealed no statistically significant differences in age or eGFR between patients with and without NSTEMI, $p > 0.14$ for both.

	All patients (n = 124)	NSTEMI (n = 14)	No NSTEMI (n = 110)
Age, years	68 (56–75)	74 (62–79)	67 (55–75)
Male sex, n (%)	63 (51)	8 (57)	55 (50)
Female sex, n (%)	61 (49)	6 (43)	55 (50)
eGFR, mL/min/1.73 m²	85.5 (69–100.5)	82 (51–97)	86 (69–101)
Time from SO to first blood sample, h:min	6:16 (3:26–13:20)	4:43 (3:24–6:41)	6:46 (3:28–15:08)
Time from SO to admission, h:min	5:07 (2:44–12:20)	3:44 (2:58–5:33)	6:16 (2:37–14:28)
Time from admission to first blood sample, h:min	0:44 (0:25–1:00)	0:47 (0:31–0:55)	0:43 (0:24–1:00)
Time between 1st and 2nd plasma cTnT sample, h:min	3:52 (3:19–5:23) n = 93	3:35 (2:56–4:27) n = 12	4:00 (3:20–5:44) n = 81
Time between 2nd and 3rd plasma cTnT sample, h:min	9:51 (5:58–13:21) n = 37	7:54 (5:12–10:26) n = 10	10:32 (7:11–14:46) n = 27
RISK FACTORS, n (%)			
Hypertension:			
Yes	61 (49)	8 (57)	53 (48)
Unknown	54 (44)	5 (36)	49 (45)
Hypercholesterolemia:			
Yes	21 (17)	2 (14)	19 (17)
Unknown	94 (76)	10 (71)	84 (76)
Diabetes:			
Yes	15 (12)	4 (29)	11 (10)
Unknown	100 (81)	8 (57)	92 (84)

Smoking:			
Current	18 (15)	2 (14)	16 (15)
Recent cessation	3 (2)	0 (0)	3 (3)
Former	40 (32)	5 (36)	35 (32)
Unknown	8 (7)	0 (0)	8 (7)
Atherosclerotic disease:			
Yes	54 (44)	8 (57)	46 (42)
Unknown	48 (39)	2 (14)	46 (42)

Table 1: Baseline Patient Characteristics. Values are median (IQR) or n (%). SO, symptom onset. For analyses with available results only for a proportion of the population, total numbers (n) of included patients are explicitly stated.

Grouped by time from symptom onset to hospital admission, 34 patients (27 %) were admitted within the first three hours after symptom onset, 30 (24 %) between three and six hours, 28 (23 %) between six and 12 hours and 32 (26 %) more than 12 hours after symptom onset. The distribution of time from symptom onset to first blood sample grouped by confirmed NSTEMI status shows that all but one patient with confirmed NSTEMI had their first blood sample collected before 10 hours (figure 2). This female patient underwent first blood sample 124 hours after symptom onset, caused by an abnormally long case history of 121 hours from symptom onset to hospital admission. This was most likely due to diffuse symptoms, also resulting in an uncertain time of symptom onset. Both ECG changes and angiography with evidence of a stenosis were though found, resulting in an NSTEMI diagnosis.
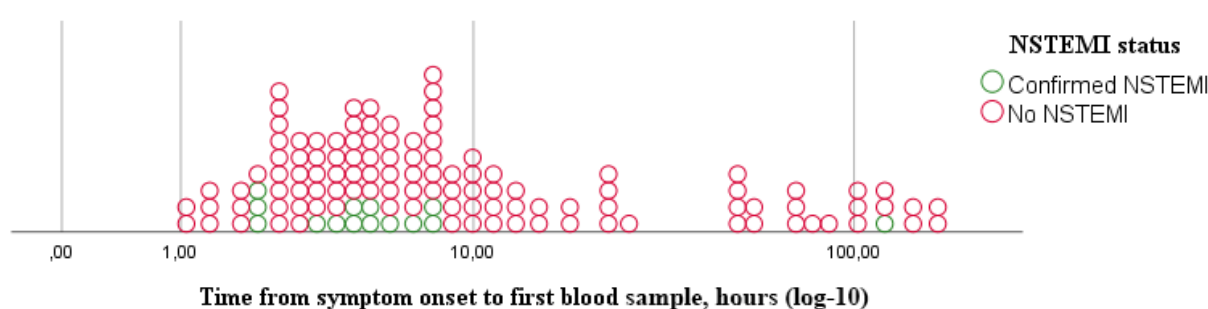
Figure 2: Distribution of time from symptom onset to time of first blood sample. Time presented on a logarithmic scale.

Troponin Values

Troponin values and plasma cTnT changes are higher in the NSTEMI group compared to those without NSTEMI (table 2). Median plasma cTnI values in male and female are both similar to the overall plasma cTnI value, being 12 (IQR 4–34, n = 63) and 11 (IQR 5–23, n = 61), respectively. The same is also true for serum cTnI values, being 13 (IQR 4–36, n = 63) and 11 (IQR 5–25, n = 60) for men and women, respectively.

	All patients	NSTEMI	No NSTEMI
Plasma cTnI, ng/L	11 (4–28) n = 124	135 (43–627) n = 14	9 (4–19) n = 110
Serum cTnI, ng/L	12 (5–32) n = 123	144 (58–696) n = 13	10 (4–20) n = 110
First plasma cTnT sample, ng/L	11 (6–21) n = 124	45 (24–84) n = 14	9 (6–18) n = 110
Absolute change from PcTnT1 to PcTnT2, ng/L	2 (1–4) n = 93	75 (18–116) n = 12	1 (1–3) n = 81
Relative change from PcTnT1 to PcTnT2, %	12.2 (6.0–26.3) n = 93	91.7 (27.5–325.5) n = 12	10.7 (5.8–22.9) n = 81
Absolute change from PcTnT2 to PcTnT3, ng/L	3 (1–22) n = 37	166 (35–253) n = 10	2 (1–4) n = 27
Relative change from PcTnT2 to PcTnT3, %	16.6 (8.0–56.8) n = 37	246.1 (47.1–734.0) n = 10	10.9 (5.9–33.0) n = 27

Table 2: Troponin values in the first blood sample collected after hospital admission and serial cTnT changes. Values are median (IQR). PcTnT1/-2/-3, plasma cTnT sample number 1, -2 and -3. Serum cTnI was missing for one patient. Some patients were also missing serial samples.

Figure 3 and 4 shows a tendency of greater dispersion of plasma cTnI values compared to plasma cTnT values.
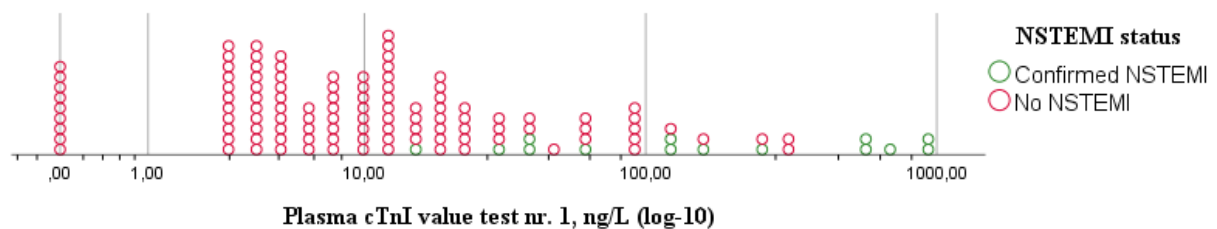
Figure 3: Distribution of plasma cTnI values in ng/L in the first blood sample after hospital admission. Troponin values presented on a logarithmic scale. "0" = < 2.5 ng/L.
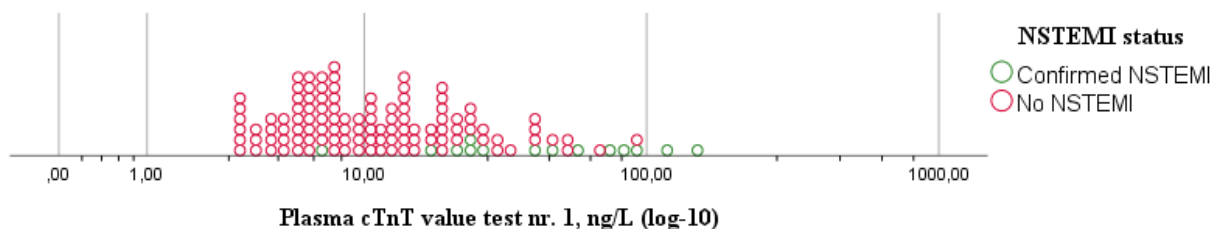
Figure 4: Distribution of plasma cTnT values in ng/L in the first blood sample after hospital admission. Troponin values presented on a logarithmic scale.

Final Diagnosis

Of 124 patients, the final diagnosis extracted from PAS or the hospital discharge summary was NSTEMI in 14 (11 %), unspecified chest pain in 39 (31 %), heart failure in 5 (4 %), arrhythmia in 23 (19 %), angina or atherosclerosis in 13 (10 %), other cardiovascular disease in 6 (5 %), lung disease including respiratory infection in 8 (7 %), other inflammation/infection in 4 (3 %), CNS/PNS related in 4 (3 %) and "other" in 8 (7 %).

In comparison, the adjudicated final diagnosis (the alternative reference standard) made retrospectively by an experienced cardiologist and trained student, was NSTEMI in 20 (16 %). Assessment of inter-rater agreement for NSTEMI vs. no NSTEMI between the two reference standards was evaluated using Kappa Measure of Agreement, showing an inter-rater agreement of $\kappa = 0.80$, $p < 0.001$, n = 124. The percentage of correspondence between the two diagnoses was 95.2 % (n = 124). Comparison of ROC AUCs for cTnI with the actual vs. alternative diagnosis as reference standard showed no statistically significant difference (difference 0.039, $p = 0.36$), confirming a non-significant difference in diagnostic accuracy.

Diagnostic Accuracy of the TNIH Assay for NSTEMI

The diagnostic accuracy of the TNIH assay for NSTEMI based on the first blood sample collected after hospital admission (designated 0 h) for men and women overall, as quantified by ROC AUC, was 0.93 (95 % confidence interval (CI), 0.87–0.97).
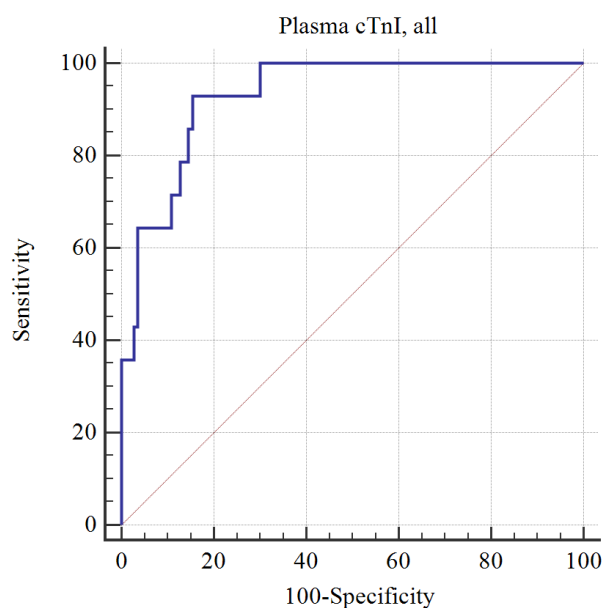
Figure 5: ROC curve showing the diagnostic accuracy of TNIH for NSTEMI for men and women overall.

As different 99th percentiles are established and suggested we chose two alternative cut-offs as the limit of decision. Parameters describing the diagnostic accuracy overall and in subgroups are presented in table 3.

|	Sensitivity	NPV	Specificity	PPV
MANUFACTURER:				
Overall, 99th percentile, 47 ng/L (closest cut-off = 45 ng/L)	71.4 (41.9–91.6)	96.0 (91.4–98.2)	88.2 (80.6–93.6)	43.5 (29.5–58.6)
Men, 99th percentile, 57 ng/L (closest cut-off = 43 ng/L)	75.0 (34.9–96.8)	96.1 (88.0–98.8)	89.1 (77.8–95.9)	50.0 (29.8–70.2)
Women, 99th percentile, 37 ng/L (closest cut-off = 29 ng/L)	66.7 (22.3–95.7)	95.8 (88.1–98.6)	83.6 (71.2–92.2)	30.8 (16.3–50.3)
ODSÆTER ET. AL:				
Overall, 99th percentile, 96 ng/L (closest cut-off = 94 ng/L)	64.3 (35.1–87.2)	95.4 (91.1–97.7)	94.6 (88.5–98.0)	60.0 (38.6–78.2)
Men, 99th percentile, 144 ng/L (closest cut-off = 127 ng/L)	50.0 (15.7–84.3)	93.1 (87.1–96.4)	98.2 (90.3–100.0)	80.0 (33.7–96.9)
Women, 99th percentile, 59 ng/L (closest cut-off = 57 ng/L)	66.7 (22.3–95.7)	96.1 (88.7–98.7)	89.1 (77.8–95.9)	40.0 (20.6–63.1)

Table 3: Diagnostic accuracy of overall and sex-specific cut-offs. *Values are percentages (95 % CI). The cut-off from our analyses closest to and lower than the suggested cut-offs were used to extract the results, as the low number of included patients resulted in a lack of registrations at the exact, suggested cut-offs.*

When exploring different criteria from the ROC curve analysis, one criterion (> 15.3 ng/L) resulted in a sensitivity of 100.0 % (95 % CI, 76.8–100.0) and a specificity of 70.0 % (95 % CI, 60.5–78.4).

When using the suggested cut-off value (≥ 5 ng/L) presented by Shah et al. (14) in a single test rule-out strategy, we found a sensitivity of 100.0 % (95 % CI, 76.8–100.0), NPV of 100 % (95 % CI, N.A.), specificity of 36.4 % (95 % CI, 27.4–46.1), and PPV of 16.7 % (14.8–18.7), ruling out n = 40 (32.3 %), of which none was diagnosed with AMI or developed MACE.

Subgroup Analyses

Diagnostic accuracy as quantified by ROC AUC of TNIH, based on the first blood sample collected after hospital admission, were 0.96 (95 % CI, 0.88–1.00) for men and 0.91 (95 % CI, 0.81–0.97) for women. Comparison of AUCs showed no statistically significant difference (difference 0.06, p = 0.329). Performance of sex specific 99th percentiles are presented in table 3.
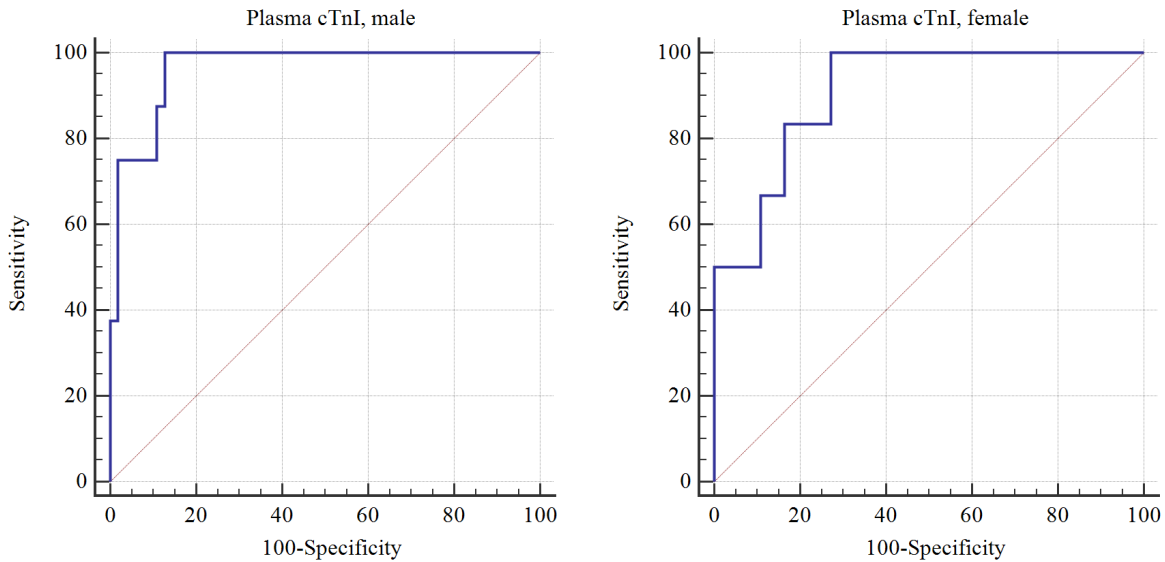
Figure 6: ROC curves showing diagnostic accuracy of TNIH for NSTEMI for men and women respectively.

Diagnostic accuracy was also evaluated according to time since symptom onset. Because of few included patients, diagnostic accuracy was evaluated for those admitted before and after the median time from symptom onset to hospital admission (median 5:07 h:min, IQR 2:44–12:20) at 0 h, and AUC was 0.96 (95 % CI, 0.88–0.99), and 0.89 (95 % CI, 0.79–0.96), respectively.

For patients with eGFR $\geq$ 60 mL/min/1.73 m^2 (n = 105) the AUC of TNIH at 0 h was 0.91 (95 % CI, 0.84–0.96). All patients with eGFR < 60 mL/min/1.73 m^2 (n = 19 of which four had NSTEMI) were correctly classified with a diagnostic accuracy of 1.00 (95 % CI, 0.82–1.00).

Predictive Ability of cTn for MACE

Due to few patients, it was not possible to evaluate the predictive ability of cTn for MACE within 30 days of discharge because only one patient suffered MACE. This patient was first admitted to the hospital and diagnosed with NSTEMI, then suffered a nonfatal re-infarction within 30 days of discharge.

Relationship Between Troponin Values

The relationship between plasma cTnT and -cTnI was investigated based on the first blood sample collected after hospital admission using Spearman's Rank Order Correlation (rho) with rho = 0.82, n = 115, *p* < 0.001. A scatter plot displaying the relationship shows a tendency of plasma cTnI values being lower at low levels and higher at high levels compared to plasma cTnT (figure 7).
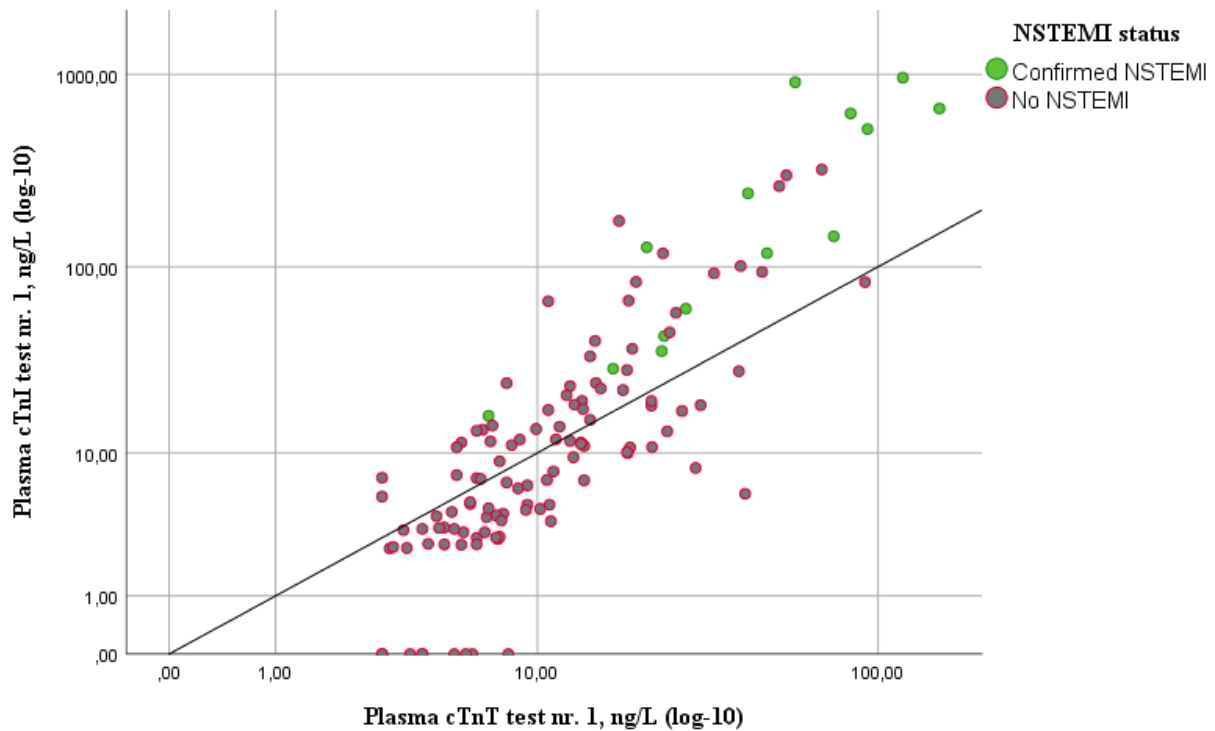
Figure 7: Scatter Plot showing the correlation between plasma cTnI and cTnT. Grey, solid line is the line of equality. Censored data (cTnI < 2.5 ng/L) are given the value "0". Both axes presented on a logarithmic scale.

When comparing plasma and serum cTnI values, the results showed great similarities. A Passing and Bablok regression analysis was conducted on all those who had their medical records reviewed with both plasma and serum values available (total n = 168). The analysis demonstrated good linearity with an intercept –0.62 (95 % CI, –0.81 to –0.42) and slope 0.979 (95 % CI, 0.963 to 0.995) (figure 8). Both the systematic and proportional differences were very small, although both the small intercept was statistically significantly different from zero and the slope statistically significantly different from one. Concentrations measured in serum was shown to be slightly higher than plasma, and a Bland-Altman analysis demonstrated a small mean difference of +1.5 ng/L (figure 9). The Bland-Altman plot shows four outliers with plasma levels clearly higher than serum levels, which may impact the mean. The difference between plasma and serum in these outliers are of such a magnitude that it may be of significance in clinical practice. The reason for the larger differences in these four patients was not explored as it is beyond what I looked at in the thesis. No statistically significant difference between AUCs was shown by comparison of the two dependent ROC curves (difference 0.0046, p = 0.13).
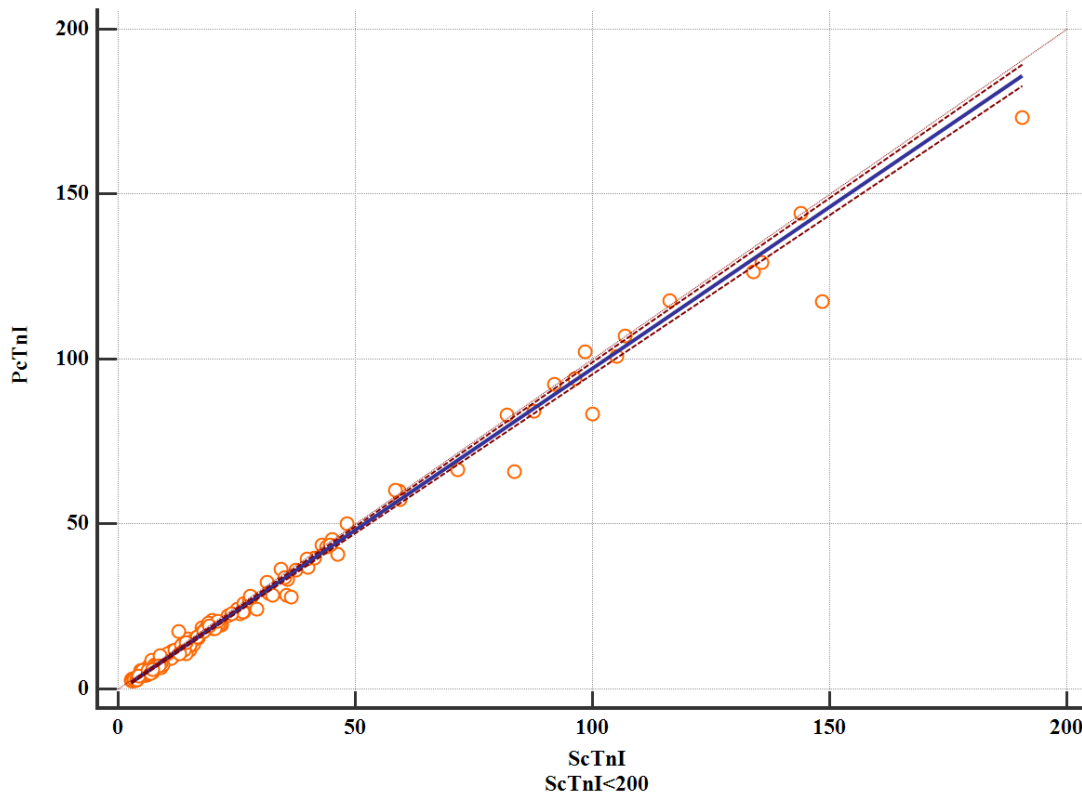
Figure 8: Scatter Plot showing the correlation between plasma and serum cTnI in ng/L. *Filtered by serum cTnI values < 200 ng/L. Thin, brown, solid line is the line of equality. Blue, solid line is the Passing and Bablok regression line. Dashed lines are the 95 % CIs.*
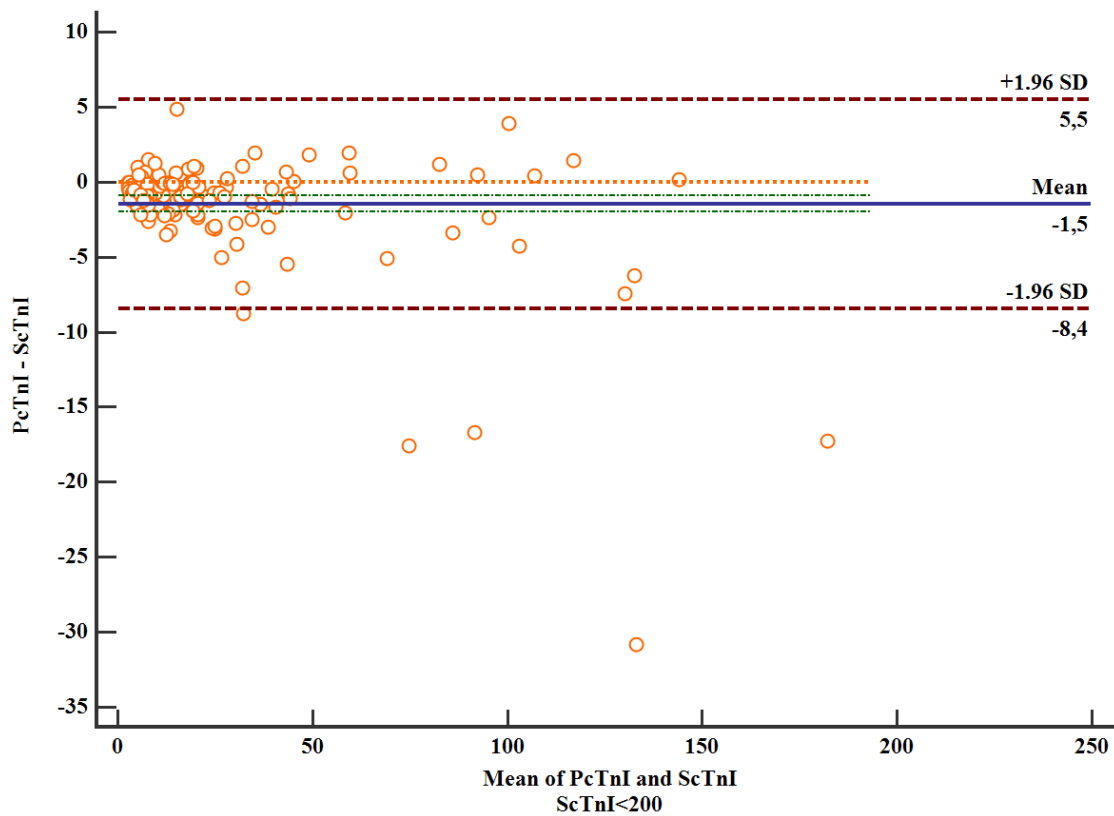
Figure 9: *Bland-Altman plot showing the differences between plasma and serum cTnI values in ng/L.*

22

Discussion

This pilot study was performed to evaluate the diagnostic accuracy of the TNIH assay for NSTEMI on basis of blood samples collected shortly after hospital admission, and gain insight into what can be expected findings in the BIOMAK project. Our main findings are that 1) TNIH shows a tendency of lower overall diagnostic accuracy as quantified by the ROC AUC compared to similar studies, with our study showing an AUC of 0.93, and 2) at cut-offs suggested by the manufacturer we find a moderate sensitivity and specificity of 71.4 % and 88.2 %, respectively, only reaching 100 % sensitivity at 15 ng/L – a much lower troponin level than suggested by the manufacturer.

Compared to similar studies conducted on the TNIH assay (5, 9, 10) this study shows a tendency of a lower overall diagnostic accuracy as quantified by the ROC AUC with a result of 0.93 (95 % CI, 0.87–0.97). As expected, the 95 % CI is wider for our point estimate. This is mainly explained by few included patients and a big variation in troponin values, making the point estimate less accurate. In the study from Kavsak et al., which presented the highest AUC (5), only the highest hs-cTn concentrations obtained per patient (over the first six hours in the ED) were used for ROC curve analysis. This might have affected the estimation of AUC, most likely in the direction of a higher AUC. Because of this, the results from that study cannot be directly compared to our results. The best estimates for comparison with previously conducted studies are therefore an AUC of 0.94 (95 % CI, 0.92–0.96) (9) and 0.96 (95 % CI 0.94–0.98) (10), with our result being near the first but lower than the latter. Both 95 % CIs overlap with the 95 % CI for our own estimate.

Using the 99th percentile established by the manufacturer (47 ng/L) as the limit of decision we found an overall moderate sensitivity of 71.4 % and a high NPV of 96.0 %. The sensitivity was in accordance with our hypothesis of > 70 %, but the specificity of 88.2 % was lower than hypothesised (> 93 %) based on other studies on TNIH. The PPV of 43.5 % was also lower than anticipated. A study published in 2018 (9) shows an overall sensitivity, NPV, specificity and PPV of 70.4 % (95 % CI, 65.2–75.2), 93.4 % (95 % CI, 92.0–94.6), 93.3 % (95 % CI, 91.9–94.5) and 70.0 % (95 % CI, 64.8–74.8), respectively. Another newly published study (10) shows an even higher overall sensitivity, NPV, specificity and PPV of 74.6 % (95 % CI, 66.2–81.8), 96.2 % (95 % CI, 95.0–97.2), 96.5 % (95 % CI, 95.1–97.7) and 76.4 % (95 % CI, 69.2–82.3), respectively. In comparison, cut-offs suggested by Odsæter et. al (96 ng/L) elevated the specificity to the anticipated level, but consequently lowered the

sensitivity below what was expected. Thus, only the 99th percentile established by the manufacturer supported the hypothesis of a sensitivity of at least 70 %. The 95 % CIs are wider also for these estimates in our study compared to other studies, as expected due to few included patients.

To our knowledge, no further studies have numbers for sensitivity and NPV at the 99th percentile for the TNIH assay for further comparison. More studies have though been conducted on other hs-cTnI assays. Compared to one study on the Abbott ARCHITECT STAT hs-cTnI assay also using hs-cTnT as the reference standard, we found a higher sensitivity and NPV, but a lower specificity and PPV using the manufacturer's 99th percentile (15).

Exploration of different criteria in the overall ROC curve did show that the TNIH assay can achieve a high sensitivity while maintaining a moderate specificity, but at a much lower troponin level (15 ng/L) than the suggested overall cut-offs. This might indicate that the suggested cut-offs are too high in order to achieve an adequately sensitive analysis which is of importance when diagnosing AMI, as it is important not to miss a patient with this condition.

Sex-specific ROC AUCs showed a tendency of being higher for men and lower for women at 0 h when compared to overall AUC, but not statistically significant. They also provided higher sensitivity, NPV, specificity and PPV for men, while the opposite was shown for women, compared to overall cut-offs from the manufacturer. On the other hand, when compared to overall cut-offs suggested by Odsæter et. al, sex-specific cut-offs provided lower sensitivity and NPV but higher specificity and PPV in men, and higher sensitivity and NPV but lower specificity and PPV in women. This is the same finding as reported in (9), although with distinct differences in the actual percentages. It is also shown for most sub-group analyses presented in a study published in 2020 on another cTnI assay (table 5 in (16)). Contradictory results regarding sex-specific cut-offs might point in the direction of not implementing these in the classification of neither men nor women. These subgroup analyses are though even more uncertain than the overall estimations because of fewer patients when dividing the group, and definite conclusions cannot be drawn.

The same is also true for the subgroup analyses conducted on patients having eGFR < 60 mL/min/1.73 m^2. All patients in this group were correctly classified but only comprised 19 patients of which four had NSTEMI. Although the point estimate for this subgroup's ROC

AUC was 1.00, the 95 % CI was still wide. Precautions that this result is due to chance and few patients, especially with NSTEMI, must be made when interpreting the results.

Evaluating a single test rule-out at a 5 ng/L cut-off using cTnI showed high sensitivity and NPV, both of 100.0 %, to rule out AMI. However, the specificity of 36.4 % was lower than hypothesised in advance based on other studies (not less than 50 %). This is not yet a recommended algorithm in clinical practice but has been explored in multiple studies both alone and in combination with the requirement of a normal ECG. These studies have shown a variation of sensitivity ranging from 94.7 % to 99.2 % and an NPV from 98.9 % to 99.8 %. Three of the studies used the Abbott ARCHITECT STAT (17-19), one used the Siemens ATELLICA (20), one used both ATELLICA and ADVIA Centaur (21), and the most recent study used the ADVIA Centaur (10). The most recent study presented the highest sensitivity and NPV, as well as showing that accounting for ECG ischemia did not further improve sensitivity. Again, the associated 95 % CIs were wider in our study than in the mentioned studies.

Exploration of the relationship between plasma and serum cTnI values using Passing and Bablok regression analyses showed good linearity but statistically significant differences in both intercept and slope. However, these differences were very small, also shown by the Bland-Altman plot revealing only +1.5 ng/L in serum cTnI compared to plasma cTnI. Along with no statistically significant differences shown between ROC AUCs, this collectively suggests no need to use different cut-off values for plasma and serum respectively.

In addition to the lower number of patients in our study compared to the studies used for comparison, the baseline characteristics of the populations also varied to a certain extent. The median age in this pilot study was 6–12 years higher compared to similar studies from other countries (5, 9, 10, 22). However, compared to numbers from The Norwegian Myocardial Infarction Register's annual report from 2019 (table 20 in (23)) it is only four years lower, making it similar in age to the population of interest in Norway. A more apparent difference is the distribution of sex, where the population in our study consists of 51 % men, while both the referred studies and The Norwegian Myocardial Infarction Register reports a percentage of 63–69 % men. Proportions of patients with confirmed risk factors for cardiovascular disease also vary, with an overall tendency of a lower prevalence of risk factors such as hypertension, hypercholesterolemia, diabetes and current smoking in our population. How data were obtained in the other studies are not explained in detail, but two of them mention use of a research nurse and case report form, respectively. In this study, the presence of these risk

factors was potentially insufficiently registered because of a lack of systematicity in the recording at hospital admission. Therefore, the presence is unfortunately unknown for a big proportion of the patients (table 1). This might explain why our population has a lower certain prevalence of these risk factors compared to other conducted studies (9, 10, 22), although the possibility that our population is healthier might also be true. The proportion of patients with AMI was comparable to other small studies (5, 18) and one bigger study (21), although lower than in other bigger studies reporting AMI in $\geq$ 13 % (9, 10, 14-19, 24). Generally, there were fewer events of NSTEMI in our study, making the estimates unreliable. Only one of the studies stated median troponin values of patients with AMI, which showed a tendency of higher troponin values compared to our study with a median of 235 ng/L (IQR 39–1018), a point estimate 100 ng/L higher than in our study. Like our study, other studies also included patients aged 18 years or older, but with the cut-off of time from symptom onset to arrival to the ED set to either six or 12 hours. We had a cut-off of seven days, and originally included all patients with the "Infarction status" analysis package ordered. This is two apparent differences, as other studies included participants based on a decision from the treating clinician. Whether these identified differences explain the lower diagnostic accuracy in our study is uncertain.

Quality of time registrations of relevant events have not been thoroughly described in similar studies. The systematicity is though often explained, with times of blood sample collection being designated before inclusion, e.g. 0 h/1 h/3 h/6 h, according to which algorithms they wish to evaluate. In this pilot study no such definitions were made, with health care workers deciding when tests were ordered, i.e. based on clinical practice. We therefore tried to assess the certainty levels, consistency and reliability of registrations of times of relevant events, to ensure that this data was of good quality. The time intervals between the troponin tests (table 1) shows IQRs indicative of evident variations in these time intervals from patient to patient. Also, the number of consecutive serial samples is not consistent between patients. This might not be negative in terms of diagnosing AMI but may make it harder to compare results to other studies. A more similar systematic approach to serial sampling at St. Olav's University Hospital could be considered in future studies to increase the overall quality of comparison.

When it comes to the method of registration and their quality, time of symptom onset and time of blood sampling had the most associated uncertainty. How the time of symptom onset was registered greatly varied between the included patients, with a lack of systematics and possibility of misunderstanding for the patient. Patients received a questionnaire after the

hospital admission where they were, among other things, asked about symptom onset. Patients with more than one admittance sometimes based their answer on the wrong admission, making the time of symptom onset uncertain. At the same time many of the medical records lacked the exact time of symptom onset, thereby resulting in a best guess based on other information, further contributing to the uncertainty. Time of blood sampling had to be estimated based on two different time registrations. When exploring these, we found deviation in time of blood sampling reported in the requisition and the actual time of blood sampling in patients admitted to St. Olav's University Hospital with symptoms suggestive of AMI (see Appendix). The fact that 61 % of times of (presumable) blood sampling was registered as collected *before* time of hospital admission is an evident error, as blood sampling before admission is not routinely done. It also raises suspicion that for this proportion of the population, time of blood sampling was *not* adjusted by the health care workers who collected the blood sample, as is the preferred procedure. This makes the time of arrival of the sample tube at the laboratory the more reliable choice for these patients, but this time registration is not reported to the clinicians. Thus, this was not a limitation in our study as we had access to another time registration but is important to communicate to the clinicians. The only time registration reported to treating clinicians along with the cTn value is the (presumable) time of blood sampling stated in the requisition. In this study, this was revealed to possibly not be reliable for 61 % of the included patients, with the actual event of blood sampling happening later than what was reported. The effect this error has on troponin concentrations must be considered and might be of interest for the clinicians.

This showcases a potential room for improvement in the registration and reporting of times of relevant events used in cTn sampling when diagnosing AMI at St. Olav's University Hospital. Maybe also resulting in further optimization of patient care as well as overall quality of conducted studies.

Strengths and Limitations

Two of the main strengths of this pilot study are that patients were selected based on clinical symptoms (through ordering of an "Infarction status" analysis package) and that they were consecutively included in the study. Both aspects are important to avoid the possible effect of lowering or increasing the estimation of diagnostic accuracy (25, 26), and to avoid selection bias. Additionally, blood tests for both the index test and the reference standard were collected at the same time with predefined cut-offs for cTn. This eliminate potential variation in quantity of cTn in the blood tests caused by time differences, and any influence on the

following interpretation. Lastly, the same reference standard was used on all patients, eliminating the risk of verification bias.

Limitations important to address comprise the low participation rate, low number of included patients and thereby few patients with NSTEMI, the uncertainty of the reference standard, the possible uncertainty of the data quality with regards to times of relevant events (already discussed), and correctness of the final diagnosis.

The low number of included patients was anticipated because of the short inclusion period but is also partly explained by a low participation rate. Only 53.3 % of eligible patients gave their written informed consent. One therefore must think about the possibility of self-selection bias, the fact that volunteer participants often differ in characteristics from those who decline participation. A study published in 2009 (27) concluded that there were differences between participants and non-participants in prospective observational studies using data from medical records, possibly due to selection bias. Although not able to conclude the direction or magnitude of the possible effect, it is important to consider. Additionally, from some of the patients that died within 30 days after start of inclusion we did not receive written informed consent. These were therefore systematically excluded. This was due to a delay in the posting of the first batch of consent forms, resulting in fewer included patients during an already short inclusion period. The fact that the study population is small also makes it evident that estimates are unprecise with wide confidence intervals. Small changes in classification of patients with respect to AMI might therefore have great impact on the results (point estimates), also emphasizing the importance of discussing the uncertainty of the reference standard.

The choice of reference standard, i.e. diagnosis based on an hs-cTnT assay, is not the perfect reference standard for AMI as it is not 100 % sensitive nor specific and thus cannot detect AMI in 100 % of those admitted with the condition. The reference standard itself therefore most likely incorrectly classified some of the patient as "not AMI", i.e. false negative (and positive). This, in turn, is used as comparison for the classification from the studied assay, possibly resulting in an incorrect disagreement. Also, because of limited resources, we were not able to centrally adjudicate the final diagnosis using two independent cardiologists, which seems to be the preferred method in similar studies. The two cardiologists should ideally be blinded from the results of the index test while adjudicating the diagnosis, which is another big limitation as the 5th year medical student retrospectively evaluating the diagnosis in this study also was the one doing all the data registration. Although Kappa analysis showed fairly

good inter-rater agreement if we had chosen to use the alternative reference standard (the adjudicated diagnosis), this does not eliminate the influence of the lack of blinding and improper adjudication. This makes the choice of keeping the diagnosis registered in PAS as the final diagnosis and reference standard safer as the correctness of this has been verified (12).

In similar studies, the blood tests have been run on multiple available assays in addition to the studied assay, enabling comparison of diagnostic accuracy between assays. This was not done in our work as the only available assays was the assay for the index test and the assay used for the reference standard, thus not being eligible for comparison. The method used in similar studies are however not always thoroughly described (9), and it remains unclear to me whether they have compared the studied assay against their reference standard or not. If so, this should be questioned, with our approach being more correct.

Aspects of Interest for the Following BIOMAK Project

Because of few included patients and only one available cTnI value some analyses of interest could not be conducted. In the BIOMAK project it would therefore be interesting to explore 1) The possible relationship between time from symptom onset to first blood sample and the plasma cTnT and cTnI values of those with confirmed NSTEMI, 2) The use of absolute changes of cTnI in the diagnosis of AMI, and 3) The predictive ability of cTn for MACE.

Conclusion

This prospective, observational pilot study of TNIH for the diagnosis of NSTEMI in patients admitted to the ED shows a tendency of lower diagnostic accuracy than expected based on similar studies. However, the lowest presented overall ROC AUC in these studies is close to our overall AUC, indicating comparable overall diagnostic accuracy. Because of the small population size, and especially the low number of patients with NSTEMI, there is great uncertainty in our point estimates and no definite conclusions can be drawn. In order to do so and potentially demonstrate a diagnostic accuracy at the expected level, a larger study is needed before possible safe implementation of this new assay in clinical practice at St. Olav's University Hospital.

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Appendix

Time Registrations and Estimation of Time of Relevant Events

The most important time interval to describe in diagnostics of AMI is time from symptom onset to time of blood sample collection. Time of symptom onset was classified into certain (64 %, n = 79) or uncertain (36 %, n = 45). In the NSTEMI group 11 of 14 patients (79 %) was classified as having a certain time of symptom onset.

Time of blood sampling had to be estimated based on the time when the blood sample was ordered or (preferably) collected (can and should be adjusted in the requisition by the responsible health care worker once the blood sample is collected), and the time when the sample tube was received and scanned at the laboratory. Time of blood sampling stated in the requisition was recorded for all included patients, but for 61 % it was registered *before* the time of hospital admission (median 0:21 h:min before, IQR 0:10–0:40), thereby assumed unadjusted and considered unreliable. For these 61 %, time of blood sampling was set to the time when the sample tube was received and scanned at the laboratory. An Independent-Samples Median Test was conducted to compare the median time from (presumable) time of blood sampling to arrival of the sample tube at the laboratory between groups based on whether time of blood sampling was adjusted or assumed unadjusted. The median time in the group with the time of blood sampling adjusted was statistically significantly lower ($p <$ 0.001) compared to the group with time of blood sampling assumed unadjusted. The group with an adjusted time had a median time of 0:26 h:min (IQR 0:14–0:43, range 1:30) from blood sampling to arrival at the laboratory. The group with an assumed unadjusted time had a median time of 1:09 h:min (IQR 0:52–1:40, range 8:50) for the same time interval (figure 10). The most reliable estimate for the group with an assumed unadjusted time was therefore considered to be the time of the sample tube's arrival at the laboratory.
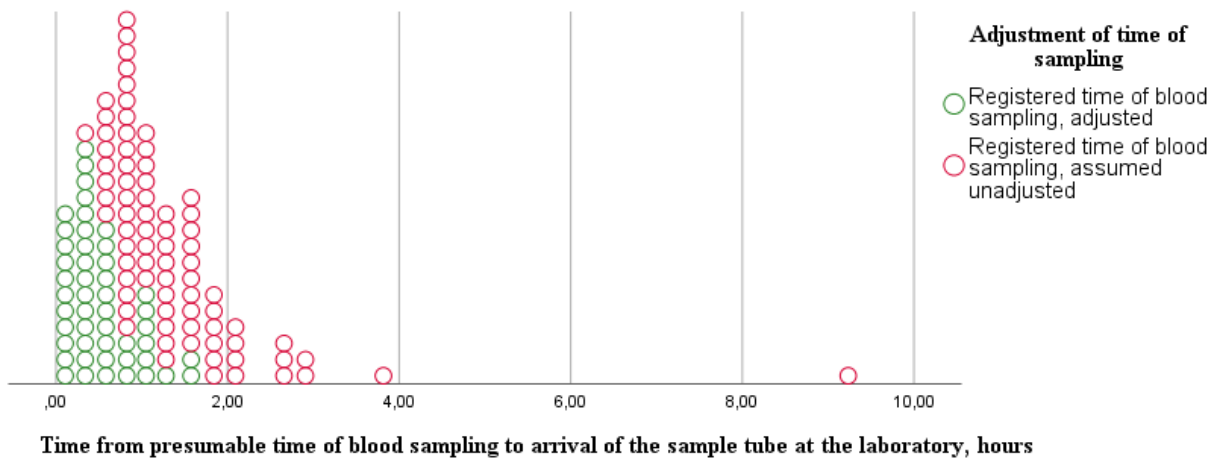
Figure 10: *Simple Dot Plot showing the time interval between the two time registrations used in estimation of time of blood sampling, grouped by adjustment status.*