Misdiagnosis of Myocardial Infarction Related to Limitations of the Current Regulatory Approach to Define Clinical Decision Values for Cardiac Troponin

Running title: *Wildi et al.; Misdiagnosis of myocardial infarction*

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Journal Subject Codes: Diagnostic testing:[33] Other diagnostic testing
Abstract

Background—Misdiagnosis of acute myocardial infarction (AMI) may significantly harm patients and may result from inappropriate clinical decision values (CDV) for cardiac troponin (cTn) due to limitations in the current regulatory process.

Methods and Results—In an international prospective multicenter study we quantified the incidence of inconsistencies in the diagnosis of AMI using fully characterized and clinically available high-sensitivity cTn assays (hs-cTnI Abbott and hs-cTnT Roche) among 2300 consecutive patients with suspected AMI. We hypothesized that the approved CDV for the two assays are not biologically equivalent and might therefore contribute to inconsistencies in the diagnosis of AMI. Findings were validated using gender-specific CDV as well as with parallel measurements of other hs-cTn assays. AMI was the adjudicated diagnosis in 473 patients (21%). Among these, 86 patients (18.2%) had inconsistent diagnoses using the approved uniform CDV. Using gender-specific CDV, 14.1% of female and 22.7% of male AMI patients had inconsistent diagnoses. Using biologically equivalent CDV reduced inconsistencies to 10% (p<0.001). These findings were confirmed with parallel measurements of other hs-cTn assays. The incidence of inconsistencies was only 7.0% for assays with CDV that were nearly biologically equivalent. Patients with inconsistent AMI had comparable long-term mortality as compared to patients with consistent diagnoses (p=ns), and a trend to higher long-term mortality than patients diagnosed with unstable angina (p=0.05).

Conclusions—Currently approved CDV are not biologically equivalent and contribute to major inconsistencies in the diagnosis of AMI. One out of five AMI patients will receive a diagnosis other than AMI if managed with the alternative hs-cTn assay.


Key words: acute cardiac care, myocardial infarction, biomarker
Introduction

Acute myocardial infarction (AMI) is a major cause of death and disability worldwide. Patients with symptoms suggestive of AMI account for about 10% of all emergency department (ED) consultations, even though only 10-20% of them are diagnosed as suffering from AMI. Rapid identification of AMI is of paramount clinical importance for early treatment and management.1-3

Misdiagnosis of AMI and inconsistencies in the diagnosis of AMI may significantly harm patients. First and of most importance, withholding evidence-based therapies such as rhythm monitoring for 24-48h, antiplatelet therapy, high-dose statins, intense lifestyle modifications, and early revascularization may increase morbidity and mortality in patients with AMI. Second, therapies approved for AMI may be even harmful for patients with other diagnoses such as peptic ulcer. Accordingly, misdiagnosis of AMI is a common cause for malpractice claims.4

The clinical introduction of the universal definition of AMI has led to a harmonization worldwide in the diagnosis of AMI and thereby contributed to a reduction in diagnostic inconsistencies.2,3,5,6 Cardiac troponins (cTn) I and T are two proteins unique to the heart and specific and sensitive biomarkers of cardiomyocyte damage.2,3,6 According to the universal definition of AMI, a cTnI or cTnT level above the 99th percentile of a healthy reference population is a “conditio sine qua non” for the diagnosis of AMI.5 There are limitations to the current regulatory approach to define clinical decision values (CDV) for cardiac troponin. Manufacturers are asked to establish the 99th percentile in a healthy reference population. As there is a lack of consensus on how to define “healthy” and as possible effects of age and gender on levels of cTnI and cTnT have recently been identified, the current regulatory process has come under scrutiny.7-11 Apparently, differences in these cohorts of healthy individuals are substantial and may lead to major differences in resulting 99th percentiles and therefore
biological non-equivalent CDV. The younger the reference population and the more stringent the criteria to define cardiac health, the lower the resulting 99\textsuperscript{th} percentile\textsuperscript{7,12–14}. The clinical availability of fully developed high-sensitivity assays for both cTnI and cTnT in Europe and other countries now for the first time provides the methodological requirement to quantify the clinical consequences of the limitations of the current regulatory approach to define the CDV’s. The aim of this large multicenter study was to explore remaining sources for misdiagnosis of AMI after the introduction of the universal definition of AMI\textsuperscript{6} and to quantify inconsistencies in the diagnosis of AMI related to the limitations of the current regulatory process on how to define CDV for cTn.\textsuperscript{7–9,13}

Methods

Study Design and Population

Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) is an ongoing prospective international multicenter study designed to advance the early diagnosis of AMI\textsuperscript{15,16–19}. From April 2006 to September 2012, consecutive adult patients presenting to the ED with symptoms suggestive of AMI with an onset or peak within the last 12 hours were recruited, after written informed consent was obtained.

Patients were enrolled irrespective of their renal function, only patients with terminal kidney failure requiring regular dialysis were excluded. For this analysis patients were also excluded if A) hs-cTnI (Abbott) or hs-cTnT (Roche) levels were not available or B) the final diagnosis remained unclear after adjudication and at least one cTn level was elevated (possibly indicating presence of AMI) (\textbf{Figure 1S}). The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees.
Routine Clinical Assessment

All patients underwent a clinical assessment that included medical history, physical examination, 12-lead ECG, continuous ECG monitoring, pulse oximetry, standard blood test, and chest radiography. Levels of cTn were measured at presentation and serially thereafter as long as clinically indicated. Treatment of patients was left to discretion of the attending physician.

Measurement of sensitive and high-sensitive cTn

Blood samples for determination of hs-cTnI (Abbott) and hs-cTnT (Roche) were collected at presentation to the ED, as well as serially at 1, 2, 3 and 6 hours. When treatment required transferring the patient to the catheter laboratory or coronary care unit, serial sampling was interrupted. After centrifugation, samples were frozen at -80°C until assayed in a blinded fashion in a dedicated core laboratory. The Abbott hs-cTnI assay used was the final pre-commercial release version of the ARCHITECT High Sensitive STAT Troponin I assay (Abbott Laboratories, IL). Samples were thawed, mixed, and centrifuged prior to analysis and according to manufacturer’s instructions. The hs-cTnI assay has a 99th percentile concentration of 26.2ng/L with a corresponding co-efficient of variation (CV) of <5% and a limit of detection (LoD) of 1.9ng/L. The gender-specific 99th percentile has been defined as 15.6ng/L in women and 34.2ng/L in men. Long-term stability of TnI and very high correlation between plasma and serum has been demonstrated. The limit of blank (LoB) and LoD of the Roche hs-cTnT assay were determined to be 3ng/l and 5ng/l respectively. The 99th-percentile of a healthy reference population was reported at 14ng/l with an imprecision corresponding to 10% coefficient of variation (CV) at 13ng/l. The gender-specific 99th percentile has been defined as 8.9ng/L in women and 15.5ng/L in men.

The Siemens Ultra s-cTnI assay was performed with the use of the ADVIA Centaur
immunoassay system (Siemens), with a LoD of 6 ng/l, a 99th percentile cut-off point of 40 ng/l, and a CV of less than 10% at 30 ng/l\textsuperscript{23}. The Siemens hs-cTnI assay, an experimental prototype assay, was performed with the use of the Dimension Vista\textsuperscript{®} 1500 immunoassay system (Siemens), with a LoD of 0.5 ng/l, a 99th-percentile cut-off point of 9 ng/l, and a CV of less than 10% at 3 ng/l\textsuperscript{24}. The Beckman-Coulter hs-cTnI assay was measured on the Access 2 analyzer using an investigational prototype assay. According to the manufacturer, LoD is 2 ng/l, the 99th-percentile of a healthy reference population is 9 ng/l with a 10% CV lower than the 99th percentile\textsuperscript{20}.

Calculation of the glomerular filtration rate was performed using the abbreviated Modification of Diet in Renal disease formula\textsuperscript{25}.

**Adjudicated Final Diagnosis**

Two independent cardiologists reviewed all available medical records - patient history, physical examination, results of laboratory testing, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography - pertaining to the patient from the time of ED presentation to 90-day follow up. In situations of disagreement about the diagnosis, cases were reviewed and adjudicated with a third cardiologist. For all patients recruited from all sites the adjudication of the final diagnosis was performed centrally in the core lab (University Hospital Basel) using hs-cTnT Roche levels with CDV as recommended by the manufacturer and is described in detail in the Methods section in the Online Supplement.

AMI was defined and cTn levels interpreted as recommended in current guidelines\textsuperscript{2,6,9}. In brief, AMI was diagnosed when there was evidence of myocardial necrosis in association with a clinical setting consistent with myocardial ischemia. Myocardial necrosis was diagnosed by at least one hs-cTnT value above the 99th percentile together with a significant rise and/or fall\textsuperscript{9}. For
hs-cTnT, the 99th percentile (14ng/l) was used as cut-off for myocardial necrosis\textsuperscript{26,27}. Absolute changes in hs-cTnT were used to determine significant changes based on the diagnostic superiority of absolute over relative changes\textsuperscript{17,28}. Based on studies of the biological variation of cTn\textsuperscript{29,30} as well as on data from previous chest pain cohort studies\textsuperscript{23,31}, a significant absolute change was defined as a rise or fall of at least 10ng/l within six hours. In patients, in whom a 6 hour hs-cTnT level was not available, changes were assessed at earlier time points. In an assumption of linearity, an absolute change of 6ng/l within three hours was considered. All other patients were classified as “no AMI” for this analysis.

**Follow-up**

After hospital discharge, patients were followed at 3, 12 and 24 months by telephone or in written form, performed by trained researchers.

**Statistical analysis**

The primary outcome measure was the percentage of patients with an adjudicated diagnosis of AMI inconsistently assigned a diagnosis of AMI or non-AMI at presentation using the approved CDV of the hs-cTnI and the hs-cTnT assay. The selection of these assays for the primary analysis is further supported by their overall comparable diagnostic accuracy for the early diagnosis of AMI\textsuperscript{32}. Therefore, inconsistencies in the diagnosis of AMI cannot be attributed to different diagnostic performance of the assays. Among several criteria\textsuperscript{2,3,6,33}, the diagnosis of AMI invariably requires a cTn level above the CDV. Therefore, we quantified inconsistencies regarding the diagnosis of AMI by the position of level-pairs (troponin values of different assays at the same time point taken) according to quadrants defined by the CDV for each assay. This analysis was done twice: once using a uniform CDV for both genders and once using the gender-specific CDV for each assay. As the hs-cTnT was used for the adjudication, the CDV of the hs-
cTnT assay (14ng/L) was used as the reference to determine the biologically equivalent CDV for the other assays by linear regression analysis. Subgroup analysis was predefined in order to explore potential contributing causes for inconsistencies: in order to explore whether and to what extent pre-analytical aspects (e.g. small differences in time the sample was frozen at -80°C until measurements were performed) could have played a role, we selected the subgroup of patients in whom the measurements of hs-cTnT and cTnI-ultra (Siemens) were performed from the same tube and on the same day. Patients with AMI were further classified as either MI type I or MI type II\(^a\). MI type 4B (related to stent thrombosis) is rare event in patients presenting to the ED and has a lot in common with other type 1 MIs. Therefore MI type 4B events were classified with the MI type 1 for this analysis.

The data are expressed as medians ± interquartile range (IQR) for continuous variables, and for categorical variables as numbers and percentages. Continuous variables were compared with Mann-Whitney-U test, and categorical variables using Pearson chi-square test. All hypothesis testing was two-tailed and p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows 22.0 (SPSS Inc) and MedCalc 9.6.4.0 (MedCalc software).

Results

Patient characteristics

The baseline characteristics of 2300 patients with suspected AMI are shown in Table 1. The adjudicated final diagnosis was AMI in 21% of patients, unstable angina in 9%, cardiac but non-coronary artery symptoms in 13%, non-cardiac cause in 52% and symptoms of unknown origin in 5%. Among the 473 AMI patients, 74 (16%) had STEMI, 399 (84%) had NSTEMI. In the
group of patients with NSTEMI, 85% were classified as type 1 and 15% as type 2 AMI. Baseline levels of hs-cTnT Roche and hs-cTnI Abbott according to final diagnosis are shown in Figure 2S (Online Supplement).

Correlation between hs-cTnI and hs-cTnT

For hs-cTn levels measured at presentation (2300 pairs) as well as for all time points (6559 pairs), hs-cTnI and hs-cTnT levels correlated closely (r=0.813 and r=0.790, both p<0.001; Figure 1A). The correlation was comparable in patients with an adjudicated diagnosis of AMI (r=0.797 and r=0.770, both p<0.001; Figure 1B and 1C) and in patients with other diagnosis (r=0.799 and r=0.808, both p<0.001, respectively). Distribution of hs-TnI and T values in quadrants according to the approved uniform CDV in patients with an adjudicated diagnosis of AMI was comparable to that in the overall cohort irrespective of the adjudicated diagnosis.

Detectable levels (higher or equal the LoD) of hs-cTnI and hs-cTnT were observed in 87.7% and 75.5% of all samples (p<0.001; n=6559). The scatter between hs-cTnI and hs-cTnT seemed rather uniform throughout the entire range of hs-cTn values.

Diagnostic inconsistencies for AMI

AMI was the adjudicated diagnosis in 473 patients (21%). Among these, 86 patients (18.2%) had inconsistent diagnoses using the uniform CDV at presentation. The incidence was 17.1% for the analysis of all level-pairs obtained during serial sampling. These inconsistencies seemed to be at least in part due to the fact that the approved CDV for hs-cTnI is not biologically equivalent to the approved CDV for hs-cTnT (Figure 2). The biologically equivalent hs-cTnI value corresponding to the CDV for hs-cTnT was less than half the approved CDV for hs-cTnI. Therefore, nearly all of these inconsistencies were related to under-diagnosis of AMI with hs-cTnI. Diagnostic accuracy for AMI of the different assays is shown in Table 1S (Online
Supplement). At the approved CDV hs-cTnT had higher sensitivity, while hs-cTnI had higher specificity and PPV.

Hs-cTnI Using gender-specific CDV, 14.1% of women and 22.7% of men with AMI had inconsistent diagnoses at presentation, again nearly all of them related to under-diagnosis of AMI with hs-cTnI (Figure 3A and 3B). Median c-Tn values in patients with inconsistent diagnoses of AMI were lower than in patients with consistent diagnoses indicating smaller infarct size (Table 2S, Online Supplement).

These findings were confirmed with parallel measurements using other sensitive and hs-cTn assays showing that most did not have biologically equivalent CDV (Figure 4A-F). The incidence of inconsistencies for level-pairs obtained at presentation in patients with AMI was as low as 7.0% for assays with CDV that were nearly biologically equivalent.

**Diagnostic inconsistency using biologically equivalent CDV**

Using the hs-cTnI (Abbott) biologically equivalent CDV for hs-cTnT (8.7 ng/l instead of 26.2 ng/l) reduced inconsistencies regarding the diagnosis of AMI from 86 of 473 AMIs (18.2%; 83 underdiagnosis with hs-cTnI, 3 underdiagnosis with hs-cTnI) to 47 of 473 (22 underdiagnosis with Abbott, 25 underdiagnosis with Roche) (9.9%, p<0.001).

These findings were replicated using other hs-cTn assays, e.g. using the sensitive cTnI Ultra (Siemens) biologically equivalent CDV for hs-cTnT (9.4 ng/l instead of 40 ng/l) reduced inconsistencies from 17% to 10.3% (p<0.001).

**Subgroup analysis regarding potential pre-analytical contributors**

Pre-analytical aspects did not seem relevant contributors to the observed inconsistencies, as findings in the subgroup with identical pre-analytical conditions and in fresh samples were similar to the overall cohort (Online Supplement and Figure 3S).
Long-term mortality in patients with inconsistent AMI diagnoses

Patients with inconsistent AMI diagnoses (e.g. Roche+/Abbott- or Roche-/Abbott+) due to biologically non-equivalent clinical decision values for different pairs of cTn assays had comparable long-term mortality (12 deaths among 86 patients) as compared to patients with consistent AMI diagnoses (Roche+/Abbott+; 66 deaths among 387 patients; p=ns for all comparisons; Figure 5A and B). Patients with inconsistent AMI diagnoses for Roche/Abbott did have higher mortality as compared to patients consistently diagnosed as unstable angina (15 deaths in 216 patients; p=0.05).

Discussion

Misdiagnosis of AMI may occur in patients with or without ST-segment elevation and may significantly harm patients5. This large multicenter study reports eight major findings regarding misdiagnosis of AMI related to the limitations of the current regulatory process on how to define CDV for cTn. First, among level-pairs of hs-cTnI and hs-cTnT the correlation was high and similar for levels obtained at presentation and during serial sampling, as well as in the overall diagnostic cohort and in patients adjudicated to have AMI. Second, and of most importance, using the approved uniform CDV -the 99th percentile of healthy individuals- almost one out of five AMI patients had inconsistent diagnoses using hs-cTnI versus hs-cTnT. This means that of 100 patients diagnosed as AMI in institution A with hs-cTnT, 20 patients would receive a diagnosis other than AMI if treated in institution B using hs-cTnI. It is important to highlight that these assays overall have comparable diagnostic accuracy for the early diagnosis of AMI32. Therefore, the observed inconsistencies cannot be attributed to differences in diagnostic accuracy. Inconsistencies seemed to large extent due to the fact that the approved CDV for hs-
cTnI is not biologically equivalent to the approved CDV for hs-cTnT. In fact, the biologically equivalent hs-cTnI value corresponding to the CDV for hs-cTnT was less than half the approved CDV for hs-cTnI. This finding is supported by a recent observation that the 99th percentile of hs-cTnI in an Australian cohort of healthy individuals was also less than half the approved CDV for this assay. The observation that most of the consistencies were related to under-diagnosis of AMI with hs-cTnI does not indicate that the hs-cTnI assay would have lower sensitivity. In fact, our data confirmed previous investigations documenting even higher analytical sensitivity for hs-cTnI versus hs-cTnT with a substantially higher percentage of patients with detectable hs-cTnI versus hs-cTnT levels. Therefore, among two excellent hs-cTn assays, that overall have very high and comparable diagnostic accuracy in the early diagnosis of AMI, the test with even higher analytical sensitivity applied clinically as approved in many countries worldwide results in a substantial under-diagnosis of AMI, because the approved CDV is much higher as the biologically equivalent CDV of hs-cTnT. It is important to highlight that the use of hs-cTnT as reference and for the adjudication does not affect the main findings of the study. If we would have used hs-cTnI as the reference, the percentage of inconsistencies would have been identical, merely with the majority of inconsistencies due to over-diagnosis of AMI with hs-cTnT. Also, it is key to emphasize that our findings do not and should not be used to criticize manufacturers or regulators, but rather to quantify the limitations of the current process and to encourage them to pursue new roads for the definition of CDV. In order to put the implications of these inconsistencies into perspective, it might help to highlight that the change in AMI incidence resulting from these inconsistencies is of similar magnitude to the change in AMI incidence occurring when switching clinically from a conventional cTn assay to a hs-cTn assay. Third, the discrepancies due to biologically non-equivalent CDV affected all patients irrespective of
their final diagnosis and therefore are also independent of the details of the adjudication. E.g. they result in discrepancies in similar magnitude between the diagnoses pericarditis versus perimyocarditis, with the respective differences in patient management. Fourth, the incidence of inconsistencies could not be significantly reduced when using gender-specific CDV, as also the gender-specific CDV for hs-cTnI is not biologically equivalent to the gender-specific CDV for hs-cTnT in both women and men. Fifth, these findings were confirmed in parallel measurements with several other hs-cTnI assays. Sixth, the incidence of inconsistencies was as low as 7.0% for level-pairs of hs-cTnI assays with CDV that were nearly biologically equivalent, further supporting the hypothesis that more than half of the observed inconsistencies in the diagnosis of AMI could be avoided by a new regulatory process that ensures that CDV for cTn are biologically equivalent. Seventh, pre-analytical issues did not seem to be relevant contributors to our findings. Among 1355 patients with parallel measurements of hs-cTnT and s-cTnI-ultra performed from the same tube and on the same day as well as hs-cTnI and hs-cTnT in fresh samples, findings were similar to that of the overall cohort. Eighth, patients with inconsistent AMI diagnoses had comparable long-term mortality as compared to patients with consistent AMI diagnosis. This observation supports the conclusion that these events are clinically meaningful, and require detection and appropriate treatment.

The findings of this large multicenter study corroborate and extend other recent observations that had begun to challenge the current regulatory process to define CDV for cTn - the 99th percentile of healthy individuals- by documenting the effect of population selection. In the current regulatory process each manufacturer defines the 99th percentile in a separate cohort of healthy individuals. Apparently, differences in these cohorts of healthy individuals are substantial and lead to major differences in resulting 99th percentiles (≡CDV).
The younger the reference population and the more stringent the criteria to define cardiac health, the lower the resulting 99th percentile7,12–14.

Our findings also impact on clinical trials that use AMI as the primary endpoint and highlight the potential under- or over-reporting of AMI events depending on the assay used. That could substantially impact reporting of AMI rates that test efficacy or safety of drug compounds being considered for approval in case of imbalance in the assay type. In some trials, the ratio of the peak cTn to the 99th percentile is expressed as a multiple to provide a gross estimate of AMI size. This measure is based on the assumption that the 99th percentiles of different cTn assays are biological identical. The findings of this study clearly highlight that this assumption does seem incorrect and accordingly raise serious concerns regarding the use of this measure in clinical trials.

How could a new regulatory process be designed in order to provide biologically equivalent CDV for cTn? It is necessary to examine the problem from several perspectives in an effort to find a cutpoint or cutpoints that had high sensitivity and specificity in all circumstances. One might consider two novel approaches. First, all manufacturers could use the same cohort of healthy individuals for the derivation of the respective 99th percentile of all clinical assays. While this solution seems rather obvious and has been discussed among laboratory experts, regulators and diagnostic companies for many years, the lack of data quantifying the limitations of the current regulatory process might well have delayed implementation. Second, if this collaborative effort among different manufacturers fails, an alternative would be to appropriately define the CDV (99th percentile) of one hs-cTn assay in a very large well characterized cohort of healthy individuals and to do parallel measurements with all other clinical hs-cTn assays in a disease-cohort such as APACE in order to establish biologically equivalent CDV e.g. via linear
regression or ROC analysis. Our findings are supported by inconsistencies regarding AMI diagnosis observed with the use of two conventional cTn assay applying the 10% CV level as CDV, which resulted in 11.1% inconsistencies.

One cannot reiterate the clinical rule often enough that levels of cTn must always be used and interpreted in conjunction with all other clinical information. On the other hand, irrespective of the clinical presentation, irrespective of the ECG findings, and irrespective of the findings during coronary angiography, unless there is a rise in cTn above the 99th percentile indicating relevant cardiomyocyte damage, a nonfatal clinical event cannot be classified as AMI. Therefore, despite the clinical rule mentioned above, cTn has become such a crucial diagnostic tool that its regulatory aspects should fulfill the highest standards. Unfortunately, our data clearly show that currently this does not seem to be the case.

Several limitations of this study should be considered when interpreting our findings. First, we quantified inconsistencies exclusively in the diagnosis of AMI. It is important to highlight that also patients with other diagnoses possibly underlying acute chest pain (e.g. pericarditis versus permyocarditis) will receive inconsistent diagnosis due to biologically nonequivalent CDVs. However, we are unable to quantify their incidence with sufficient precision. Therefore, this analysis tends to underestimate the clinical implications of biologically nonequivalent CDVs of cTn. Second, our main findings were obtained by measuring in parallel the two hs-cTn assays that have been approved for clinical use in Europe and many other countries. The CDVs of these assays accordingly are well established. The CDV of some of the pre-commercial hs-cTnI assays used for the validation of our findings may be considered less well established. Third, while this study included patients with various types of chronic kidney disease and various degrees of renal dysfunction, patients with terminal kidney disease on
chronic hemodialysis were excluded. Therefore, we cannot comment on CDVs of cTnI or cTnT in those patients. Fourth, this study cannot quantify the increase in morbidity and/or mortality possibly associated with missing these 18% of AMI patients with discordant CDV, who usually have small AMIs. Given the substantial reduction in morbidity and mortality shown by currently available treatments for AMI including rhythm monitoring, antiplatelet therapy, high-dose statins, intense lifestyle modifications and early revascularization\textsuperscript{23,37}, we assume that overall the harm could be substantial.

In conclusion, currently approved CDVs for cTn are not biologically equivalent and therefore contribute to major inconsistencies in the diagnosis of AMI. One out of five AMI patients will receive a diagnosis other than AMI if managed with the alternative hs-cTn assay.

Acknowledgments: We thank the patients who participated in the study, the staff of the EDs, the research coordinators, and the laboratory technicians (particularly Esther Garrido, Irina Klimmeck, Kathrin Meissner, and Fausta Chiaverio) for their most valuable efforts.

Funding Sources: This study was supported by research grants from the Swiss National Science Foundation, the Swiss Heart Foundation, the European Union, the Cardiovascular Research Foundation Basel, the University Hospital Basel, Abbott, Roche, Nanosphere, Siemens, 8sense, Bühlmann and BRAHMS.

Conflict of Interest Disclosures: Professor Mueller has received research grants from the Swiss National Science Foundation and the Swiss Heart Foundation, the European Union, the Cardiovascular Research Foundation Basel, 8sense, Abbott, ALERE, Brahms, Critical Diagnostics, Nanosphere, Roche, Siemens, and the University Hospital Basel, as well as speaker or consulting honoraria from Abbott, ALERE, Brahms, Cardiorentis, Novartis, Roche, and Siemens. We disclose that Dr. Reichlin has received research grants from the Swiss National Science Foundation (PASMP3-136995), the Swiss Heart Foundation, the University of Basel, the Professor Max Cloetta Foundation and the Department of Internal Medicine, University Hospital
Basel as well as speakers honoraria from Brahms and Roche. All other authors declare that they have no conflict of interest with this study. The sponsors had no role in the design of the study, the analysis of the data, the preparation of the manuscript, or the decision to submit the manuscript for publication. Karin Wildi, Maria Rubini Gimenez and Christian Müller had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References:


24. Apple FS, Collinson PO, Biomarkers ITF on CA of C. Analytical characteristics of high-


Table 1. Baseline characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>all (n=2300)</th>
<th>AMI (n=473)</th>
<th>no AMI (n=1827)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, in years</td>
<td>62 (49-75)</td>
<td>72 (59-80)</td>
<td>60 (47-73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1432 (62)</td>
<td>368 (78)</td>
<td>1064 (58)</td>
<td>&lt;0.001</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>1147 (50)</td>
<td>309 (65)</td>
<td>838 (46)</td>
<td>&lt;0.001</td>
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<tr>
<td>Diabetes mellitus</td>
<td>401 (18)</td>
<td>125 (27)</td>
<td>276 (15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current or previous smoking</td>
<td>1415 (62)</td>
<td>302 (64)</td>
<td>1113 (61)</td>
<td>0.2</td>
</tr>
<tr>
<td>Family history</td>
<td>569 (27)</td>
<td>137 (32)</td>
<td>432 (26)</td>
<td>0.01</td>
</tr>
<tr>
<td>History, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>794 (35)</td>
<td>220 (47)</td>
<td>574 (31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous AMI</td>
<td>525 (23)</td>
<td>148 (31)</td>
<td>377 (21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous revascularization</td>
<td>621 (27)</td>
<td>156 (33)</td>
<td>465 (26)</td>
<td>0.001</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>145 (6)</td>
<td>60 (13)</td>
<td>85 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>126 (6)</td>
<td>43 (9)</td>
<td>83 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ECG findings, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left bundle branch block</td>
<td>69 (3)</td>
<td>25 (5)</td>
<td>44 (2)</td>
<td>0.001</td>
</tr>
<tr>
<td>ST-segment elevation</td>
<td>102 (5)</td>
<td>70 (15)</td>
<td>32 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ST-segment depression</td>
<td>266 (12)</td>
<td>155 (34)</td>
<td>111 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T-wave inversion</td>
<td>317 (14)</td>
<td>121 (26)</td>
<td>196 (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>26 (24-30)</td>
<td>26 (24-29)</td>
<td>26 (24-30)</td>
<td>0.3</td>
</tr>
<tr>
<td>eGFR</td>
<td>85 (69-101)</td>
<td>74 (57-94)</td>
<td>87 (71-103)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication at presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>828 (36.0)</td>
<td>219 (46)</td>
<td>609 (33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin-K antagonists</td>
<td>194 (8)</td>
<td>44 (9)</td>
<td>150 (8)</td>
<td>0.4</td>
</tr>
<tr>
<td>B-Blockers</td>
<td>790 (34)</td>
<td>194 (41)</td>
<td>596 (33)</td>
<td>0.001</td>
</tr>
<tr>
<td>Statins</td>
<td>798 (35)</td>
<td>199 (42)</td>
<td>599 (33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACEIs/ARBs</td>
<td>867 (38)</td>
<td>231 (50)</td>
<td>636 (35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>326 (14)</td>
<td>87 (18)</td>
<td>239 (13)</td>
<td>0.003</td>
</tr>
<tr>
<td>Nitrates</td>
<td>259 (11)</td>
<td>87 (18)</td>
<td>172 (9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

AMI indicates Acute Myocardial Infarction; ECG, Electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ASA: Acetyl Salicylic Acid; ACEI, Angiotensin Converting Enzyme Inhibitor; ARB, Angiotensin Receptor Blocker. Values are expressed in percentage or medians ± Inter Quartile Ranges (IQR).
Figure Legends:

**Figure 1.** Distribution of hs-TnI and T values in quadrants according to the approved uniform clinical decision values A) at any time during serial sampling in the overall cohort irrespective of the adjudicated diagnosis; B) in patients with an adjudicated diagnosis of acute myocardial infarction for levels at presentation and C) at any time during serial sampling.

**Figure 2.** Receiver-operating characteristics curve for acute myocardial infarction The approved clinical decision values for hs-cTnT and hs-cTnI are not biologically equivalent and therefore differ in their sensitivity and specificity for acute myocardial infarction.

**Figure 3.** Distribution of hs-cTnI and T values at presentation in quadrants according to the gender-specific clinical decision values in patients with an adjudicated diagnosis of acute myocardial infarction. A) in women; B) in men.

**Figure 4.** Distribution of sensitive (s-Tn) and high-sensitivity cardiac troponin (hs-Tn) I and T values at presentation in quadrants according to the approved uniform clinical decision values in patients with an adjudicated diagnosis of acute myocardial infarction. A) Pairs of hs-cTnT and sensitive cTnI-ultra (Siemens); B) Pairs of hs-cTnI (Abbott) and sensitive cTnI-ultra (Siemens); C) Pairs of hs-cTnI (Abbott) and hs-cTnI (Siemens); D) Pairs of hs-cTnI (Abbott) and hs-cTnI (Beckman Coulter); E) Pairs of hs-cTnT and hs-cTnI (Siemens); f) hs-cTnT and hs-cTnI (Beckman Coulter).
Figure 5. Kaplan-Meier Survival curves in patients with consistent diagnoses of AMI, inconsistent diagnoses due to biological non-equivalent clinical decision values for the different pairs of cardiac troponin assays, and patients with diagnoses of unstable angina. A) Pairs of hs-cTnT and high-sensitivity cTnI (Abbott); B) Pairs of hs-cTnT and sensitive cTnI-ultra (Siemens)
Figure 1

A) $r=0.813$, $p<0.001$

B) $r=0.797$, $p<0.001$

C) $r=0.770$, $p<0.001$
Figure 2
Figure 3
Figure 4
Misdiagnosis of Myocardial Infarction Related to Limitations of the Current Regulatory Approach to Define Clinical Decision Values for Cardiac Troponin

Karin Wildi, Maria Rubini Gimenez, Raphael Twerenbold, Tobias Reichlin, Cedric Jaeger, Amely Heinzelmann, Christiane Arnold, Berit Nelles, Sophie Druey, Philip Haaf, Petra Hillinger, Nicolas Schärli, Philipp Kreutzinger, Yunus Tanglay, Thomas Herrmann, Zoraida Moreno Weidmann, Lian Krivosheii, Michael Freese, Claudia Stelzig, Christian Puelacher, Katharina Rentsch, Stefan Osswald and Christian Mueller

_Circulation_. published online May 6, 2015;

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

1) Patient flow
2) Use of local conventional cTn values and hs-cTnT values for adjudication of final diagnoses
3) Assumption of linearity of absolute changes of hs-cTnT within the first hours
4) Mismatches in the adjudicated diagnosis requiring involvement of a third cardiologist
5) Short-term changes in cardiac troponin in patients with markedly elevated levels at presentation

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8) Baseline levels of hs-cTnT Roche and hs-cTnI Abbott according to final diagnosis
9) Subgroup analysis regarding potential pre-analytical contributors
10) Infarct size in patients with inconsistent AMI diagnoses

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL METHODS

1. Patient flow

A detailed patient selection is shown in Figure 1S.

2. Use of local conventional cTn values and hs-cTnT values for adjudication of final diagnoses

The cTn assays used clinically in most of the participating institutions changed during the study from a conventional cTn assay to the hs-cTnT assay. In order to take advantage of the higher sensitivity and higher overall diagnostic accuracy offered by the hs-cTnT assay, patients were adjudicated using the hs-cTnT values in all patients. In patients in whom clinically a conventional cTn assay was used, the conventional cTn values and the hs-cTnT values were available for the adjudication. In patients in whom clinically the hs-cTnT assay was used, only the hs-cTnT values were available for the adjudication.

The following conventional cTn assays were used: For the Roche cTnT 4th generation assay, the 10% CV level is 0.035ug/l. The laboratories of the participating sites reported only two decimals; therefore 0.04ug/l was used as a cut-off for myocardial necrosis. In order to fulfil the criteria of a significant change (30% of 99th percentile or 10% CV level), a patient would e.g. need to have a level of <0.01ug/l at presentation and 0.04ug/l at 6h. A patient would also qualify if the first level is 0.02ug/l and the second 0.04ug/l. A patient would not fulfil the criteria if the first level is 0.03ug/l and the second is 0.04ug/l. If the first level is 0.04ug/l, the second level needs to be at least 0.06ug/l.

For the Abbott Axsym cTnI ADV, the 10% CV level is 0.16ug/l. A patient having 0.16ug/l at presentation would meet the criteria for significant change if the second was ≥0.21ug/l. A patient having <0.12ug/l at presentation (limit of detection) would qualify if the second is >0.16ug/l.

For the Beckmann Coulter Accu cTnI, the 10% CV level is 0.06ug/l. A patient having 0.06ug/l at presentation would qualify if the second is ≥0.08ug/l. A patient having 0.05
at presentation would qualify if the second is 0.07ug/l, but not 0.06ug/l. A patient having undetectable cTnI (cTnI<0.01ug/l) at presentation would qualify if the second is ≥0.06ug/l.

3. **Assumption of linearity of absolute changes of hs-cTnT within the first hours**

The assumption of linearity of absolute changes within the first hours is based on unpublished internal data as well as recent data from Ola Hammarsten et al. showing a near-linear increase in levels of hs-cTnT with increasing time from symptom onset in their NSTEMI cohort¹.

4. **Mismatches in the adjudicated diagnosis requiring involvement of a third cardiologist**

In our cohort of 2300 patients there were 95 (4%) mismatches in adjudicated diagnosis between the two cardiologists requiring involvement of a third cardiologist. Of these, 23 patients had the final diagnosis of AMI and 72 patients had another diagnosis. These cases were discussed in face-to-face meetings between the 3 cardiologists and a decision was reached by consensus. The same approach was followed in cases of 3 different diagnoses.

5. **Short-term changes in cardiac troponin in patients with markedly elevated levels at presentation**

Patients with a single markedly elevated cTn level and otherwise “clear” diagnosis of AMI including unequivocal ECG and coronary angiography findings were adjudicated to have AMI. This scenario fortunately was infrequent, but did exist. The adjudicating cardiologist at all times had the option to classify the patient to the category “chest pain of unknown cause” in case the available information was not sufficient to reliably
diagnose or exclude AMI. While the rise and/or fall criteria are important to
differentiate AMI from causes of chronic cardiomyocyte damage, it is of much less
importance in patients with markedly elevated cTn levels (e.g. 50-times the 99th
percentile), as the positive predictive value of the markedly elevated cTn level (e.g.
50-times the 99th percentile) on its own is already above 90%\(^2\) and approaches 100%
when combined with typical ECG and coronary angiography findings. The
documentation of changes of cTn is not mandatory in these patients, as the
differentiation from chronic cTn elevation is not a matter of concern. Therefore, as
also suggested by the ESC Biomarker study group\(^3\), a patient with a clear AMI would
still be classified AMI in case of only one markedly elevated cTn level available, as
long as all other information (ECG findings, coronary angiography findings, echo
findings) supported the diagnosis of AMI.

**SUPPLEMENTAL RESULTS**

6. **Disagreement in adjudicated final diagnosis in inconsistent diagnosis of AMI**

Mismatches in the adjudicated diagnosis requiring involvement of a third cardiologist
in patients who were now found to have inconsistent diagnosis of AMI related to
biological non-equivalent CDVs:

- hs-cTnT Roche and hs-cTnl Abbott: 11/86 (12.8%)
- hs-cTnT Roche and s-cTnI Siemens: 10/78 (12.8%)
- s-cTnI Siemens and hs-cTnI Abbott: 0/32 (0%)
- hs-cTnI Abbott and hs-cTnI Siemens: 7/46 (15.2%)
- hs-cTnI Abbott and hs-cTnI Beckmann: 1/32 (3.1%)
- hs-cTnT Roche and hs-cTnI Siemens: 1/31 (3.2%)
- hs-cTnT Roche and hs-cTnI Beckmann: 2/24 (8.3%)
7. **Diagnostic accuracy for the diagnosis acute myocardial infarction of the different assays**

Informations about diagnostic accuracy of all the used c-Tn assays provided in table 1S.

8. **Baseline levels of hs-cTnT Roche and hs-cTnI Abbott according to final diagnosis**

In hs-cTnI Abbott, 15.4% of baseline values were below the LOD of 1.9ng/L, 57.9% were in the range between LOD and the 99th percentile of 26.2ng/L (73.3% below 99th P) (Figure 2S). Corresponding percentages in hs-cTnT Roche were 30.1% below LOD of 5ng/L and 35.3% between LOD and 99th percentile of 14ng/L (65.5% below 99th P). In conclusion, there were more of hs-cTnI Abbott values detectable, but in general the assay was less predictive for AMI than hs-cTnT Roche.

9. **Subgroup analysis regarding potential pre-analytical contributors**

In 1355 patients parallel measurements of hs-cTnT and s-cTnI-ultra (Siemens) were performed from the same tube and on the same day. In this subgroup findings were similar to that of the overall cohort. AMI was the adjudicated diagnosis in 294 patients (22%). Among patients with an adjudicated diagnosis of AMI levels of hs-cTnT and s-cTnI levels correlated closely (r=0.802, p<0.001). Among these, 53 patients (18%) had inconsistent diagnoses using the approved uniform CDV. Nearly all of these inconsistencies were related to under-diagnosis of AMI with s-cTnI-ultra. Using the s-cTnI-ultra biological-equivalent CDV for hs-cTnT (9.4ng/l instead of 40ng/l) reduced inconsistencies regarding the diagnosis of AMI from 19% to 9% (p<0.001). In 101
patients measurements of hs-cTnI and hs-cTnT were performed from fresh samples. Again, findings were similar to that in the overall cohort (Figure 3S).

10. Infarct size in patients with inconsistent AMI diagnoses

Median c-Tn values in patients with inconsistent diagnoses of AMI were lower than in patients with consistent AMI diagnoses, indicating that infarct size was smaller in patients with inconsistent diagnoses (Table 2S). This result was consistent in all comparisons between the different assays.

SUPPLEMENTAL REFERENCES


### Table 1S: Diagnostic accuracy for the diagnosis acute myocardial infarction of the different assays

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
<th>PPV (95%CI)</th>
<th>NPV (95%CI)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-cTnT Roche</td>
<td>89.9% (86.8-92.4)</td>
<td>79.8% (77.9-81.6)</td>
<td>53.5% (50.0-57.0)</td>
<td>96.8% (95.8-97.6)</td>
<td>81.9%</td>
</tr>
<tr>
<td>hs-cTnI Abbott</td>
<td>72.9% (68.7-76.9)</td>
<td>92.8% (91.5-93.9)</td>
<td>72.3% (68.1-76.3)</td>
<td>93.0% (91.7-94.1)</td>
<td>88.7%</td>
</tr>
<tr>
<td>hs-cTnI Siemens</td>
<td>92.4% (88.5-95.3)</td>
<td>74.1% (71.4-76.6)</td>
<td>45.2% (40.9-49.5)</td>
<td>97.7% (96.4-98.6)</td>
<td>77.5%</td>
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<tr>
<td>hs-cTnI Beckman</td>
<td>91.6% (86.9-95.1)</td>
<td>74.9% (71.8-77.7)</td>
<td>46.0% (41.1-51.0)</td>
<td>97.5% (95.9-98.5)</td>
<td>78.0%</td>
</tr>
<tr>
<td>s-cTnI Siemens</td>
<td>75.7% (71.5-79.5)</td>
<td>91.7% (90.4-93.0)</td>
<td>70.6% (66.3-74.6)</td>
<td>93.5% (92.2-94.6)</td>
<td>89.4%</td>
</tr>
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</table>

### Table 2S: median c-Tn values in ng/L during serial sampling in patients with consistent compared to inconsistent diagnosis of AMI

<table>
<thead>
<tr>
<th></th>
<th>hs-cTnT Roche</th>
<th>hs-cTnI Abbott</th>
<th>s-cTnI Siemens and hs-cTnT Roche</th>
<th>s-cTnI Siemens and hs-cTnI Abbott</th>
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<tr>
<td>consistent diagnosis</td>
<td>108.3</td>
<td>413.7</td>
<td>104.4</td>
<td>595.5</td>
<td>425.5</td>
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<tr>
<td>inconsistent diagnosis</td>
<td>28.9</td>
<td>19</td>
<td>28.4</td>
<td>29.3</td>
<td>61.5</td>
</tr>
<tr>
<td>p for comparison</td>
<td>&lt;0.001</td>
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<table>
<thead>
<tr>
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<th>hs-cTnT Roche and hs-cTnT Siemens</th>
<th>hs-cTnT Roche and hs-cTnI Beckman</th>
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<tr>
<td>consistent diagnosis</td>
<td>470</td>
<td>126.8</td>
<td>131.1</td>
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<tr>
<td>inconsistent diagnosis</td>
<td>27.7</td>
<td>29.8</td>
<td>29.4</td>
</tr>
<tr>
<td>p for comparison</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figures

Figure 1S: Selection of the study population

3030 patients included between April 2006 and September 2012

excluded

92 patients
final diagnosis remained unclear after adjudication and at least one cTn elevated

638 patients
hs-cTnI (Abbott) or hs-cTnT (Roche) levels were not available

n=2300

Figure 2S: Baseline levels of hs-cTnT Roche and hs-cTnI Abbott according to final diagnosis
Figure 3S: Measurements in fresh samples confirm that the approved clinical decision values (red lines) for the two assays are not biological-equivalent.