Validation of the Clinical Chemistry Score at emergency department presentation to detect 30-day adverse cardiac events in patients with possible acute coronary syndrome

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Abstract:	Background: The clinical chemistry score (CCS), incorporating high-sensitivity cardiac troponin I (hs-cTnI), glucose, and estimated glomerular filtration rate (eGRF) demonstrated superior clinical sensitivity (>99%) for myocardial infarction (MI) as compared to hs-cTnI alone in patients with suspected symptoms of acute coronary syndrome (ACS). To ensure the validity of these findings, we sought to assess the CCS using different hs-cTnI tests, in different emergency department populations for 30-day major adverse cardiac events (MACE). Methods: Two different emergency department (ED) cohorts with possible ACS were evaluated: cohort 1 (n=1058 patients) with retrospective measurement of the Ortho Clinical Diagnostics hs-cTnI assay; cohort 2 (n=5974 patients) who had an ED cardiac presentation

	blood test panel performed with the Abbott Diagnostics hs-cTnI assay. The sensitivity/specificity of the CCS (i.e., CCS≥1) versus hs-cTnI alone (using published cutoffs) was determined for MACE (composite of death/MI/unstable angina/revascularization) at 30-days for both cohorts and 90-days for cohort 2. Results: MACE rate at 30-days was higher in cohort 1 (19.4%; 95%CI: 16.8-22.2) versus cohort 2 (14.6%; 95%CI:13.6-15.6). In cohort 1, a CCS≥1 yielded a sensitivity/specificity of 99.5% (95%CI: 97.3-99.9)/12.8% (95%CI: 10.6-15.2) as compared to 91.2% (95%CI: 86.5-95.7%)/39.2% (95%CI: 35.9-42.5) using the Ortho hs-cTnI ≥1ng/L cutoff. A similar trend was observed in cohort 2 at 30-days and persisted at 90-days [CCS≥1 sensitivity=99.2% (95%CI: 98.5-99.6) versus Abbott hs-cTnI ≥5ng/L sensitivity= 87.7% (95%CI: 85.6-89.6)]. Interpretation: A CCS≥1 derived with different hs-cTnI assays and in different ED populations yielded a sensitivity above 99% and is suitable to rule-out MACE within 30-days of ED presentation.
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Running Title: Clinical chemistry score and ACS

Validation of the Clinical Chemistry Score at emergency department presentation to detect 30-day adverse cardiac events in patients with possible acute coronary syndrome

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Keywords: death; myocardial infarction; high-sensitivity cardiac troponin; emergency department; low-risk, high-risk

Abstract

Background: The clinical chemistry score (CCS), incorporating high-sensitivity cardiac troponin I (hs-cTnI), glucose, and estimated glomerular filtration rate (eGRF) demonstrated superior clinical sensitivity (>99%) for myocardial infarction (MI) as compared to hs-cTnI alone in patients with suspected symptoms of acute coronary syndrome (ACS). To ensure the validity of these findings, we sought to assess the CCS using different hs-cTnI tests, in different emergency department populations for 30-day major adverse cardiac events (MACE).

Methods: Two different emergency department (ED) cohorts with possible ACS were evaluated: cohort 1 (n=1058 patients) with retrospective measurement of the Ortho Clinical Diagnostics hs-cTnI assay; cohort 2 (n=5974 patients) who had an ED cardiac presentation blood test panel performed with the Abbott Diagnostics hs-cTnI assay. The sensitivity/specificity of the CCS (i.e., CCS≥1) versus hs-cTnI alone (using published cutoffs) was determined for MACE (composite of death/MI/unstable angina/revascularization) at 30-days for both cohorts and 90-days for cohort 2.

Results: MACE rate at 30-days was higher in cohort 1 (19.4%; 95%CI: 16.8-22.2) versus cohort 2 (14.6%; 95%CI:13.6-15.6). In cohort 1, a CCS≥1 yielded a sensitivity/specificity of 99.5% (95%CI: 97.3-99.9)/12.8% (95%CI: 10.6-15.2) as compared to 91.2% (95%CI: 86.5-95.7%)/39.2% (95%CI: 35.9-42.5) using the Ortho hs-cTnI ≥1ng/L cutoff. A similar trend was observed in cohort 2 at 30-days and persisted at 90-days [CCS≥1 sensitivity=99.2% (95%CI: 98.5-99.6) versus Abbott hs-cTnI ≥5ng/L sensitivity= 87.7% (95%CI: 85.6-89.6)].

Interpretation: A CCS≥1 derived with different hs-cTnI assays and in different ED populations yielded a sensitivity above 99% and is suitable to rule-out MACE within 30-days of ED presentation.

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Introduction

Evidence and guidelines have indicated that an undetectable or low high-sensitivity cardiac troponin (hs-cTn) concentration at emergency department (ED) presentation may be suitable in certain patients to rule-out myocardial infarction (MI) and possibly discharge patients home.[1-4] The basis for this is the high clinical sensitivity and negative predictive value (NPV) which can be attained when using an hs-cTn cutoff close to 5 ng/L.[2,3,5,6] However, the ability to rule-out MI does not necessarily equate to low-risk for a subsequent cardiovascular event. An international survey of ED physicians indicated that an acceptable miss-rate of major adverse cardiac events (MACE) would be ≤1% within 30-days.[7] Accordingly, the sensitivity required for MACE at 30-days should be ≥99% to achieve this threshold and to allow discharge and cessation of investigations in patients presenting with possible acute coronary syndrome (ACS) to the ED.[7] Therefore, uncertainty exists on whether a single low hs-cTn cutoff at presentation provides sufficient sensitivity to exclude 30-day MACE, with the American College of Emergency Physicians latest clinical policy document issuing the following statement "a single high-sensitivity troponin may not have adequate sensitivity for MACE".[8] A recent study in patients with suspected ACS in the United States for 30-day death, MI and myocardial revascularization identified that two measurements (0 and 3 hours) <6 ng/L by a hs-cTn assay achieved a NPV of 99.3%, but no data were provided for the sensitivity and NPV for the presentation (0 hour) measurement alone using this low cutoff.[9]

We have previously demonstrated in over 4000 patients enrolled in clinical studies assessing hs-cTn in patients with suspected ACS that a simple clinical chemistry score (CCS)

test provided superior sensitivity for index MI and 30-day MI or death over the first two hs-cTn assays approved by Health Canada, namely Roche hs-cTnT and Abbott hs-cTnI.[10] Since this publication, three additional companies (Beckman Coulter, Siemens Healthcare Diagnostics, and Ortho Clinical Diagnostics) have gained regulatory approval for hs-cTnI assays by Health Canada.[11-14] There is no standardization between hs-cTnI assays, so the same sample tested with different hs-cTnI assays may give very different results (not relevant for hs-cTnT, as Roche is the sole manufacturer)[15,16]. The goal of this study was to determine if the CCS, using different hs-cTnI assays, will achieve the same diagnostic performance ≥99% sensitivity , and superiority over hs-cTnI alone, for 30-day MACE, outside the clinical study setting and in a general ED population being worked-up for possible acute cardiac injury.

Methods

Study Design and Participants

After research ethics board approval, two ED cohorts that had parallel hs-cTn and conventional troponin measurements performed were selected for this study; hs-cTn results remained unreported to the treating ED physician. This was accomplished in accordance with the Standards for the Reporting of Diagnostic Accuracy Studies guideline.

Cohort 1 was the Canadian cohort used in the initial publication on the CCS.[10] Briefly, this was an observational cohort study (NCT01994577) which enrolled patients (May 2013 to August 2013) presenting to the ED from three adult hospitals within Hamilton, Ontario. Eligible patients were 18 years of age and older, not transferred from a different hospital, and had a cardiac troponin ordered by an ED physician. Patients were excluded if their symptoms were

non-ACS; or had ST-segment elevation MI (STEMI) at presentation; or had chest trauma, cardiac surgery or manipulation within 30-days of presentation; a MI (STEMI or non-STEMI) or pulmonary embolus confirmed within the previous month; known active cancer or non-cardiac fatal illness; sepsis; ventricular fibrillation or sustained ventricular tachycardia.[10,17-19] Ethylenediaminetetraacetic acid (EDTA) plasma samples were collected and stored below - 70^0 C. Further selection for the cohort was dependent of the availability of a presentation sample with sufficient volume to obtain a result for the Ortho VITROS® hs-cTnI assay (Ortho Clinical Diagnostics, Pencoed, UK) (Figure 1).

Cohort 2 was also from an observational study (November 2012 to February 2013) where consecutive ED patients who visited the ED from the three adult hospitals within Hamilton, Ontario and had a cardiac troponin I ordered also had a hs-cTnI measurement performed on the same tube (Abbott ARCHITECT® hs-cTnI measured but not reported).[20] Patients were excluded if age or sex was missing in the registered persons database (RPDB), were not an Ontario resident or Ontario Health Insurance Plan (OHIP)-eligible, or did not have glucose and creatinine results at presentation (note, in Hamilton there is an order-set: ED cardiac laboratory order-set where in addition to troponin, glucose, creatinine and other blood tests are measured) (Figure 1).

Health Outcomes

The outcome for this analysis was MACE that occurred after the presentation blood work up to 30-days in both cohorts and 90-days in cohort 2. MACE was defined as the composite outcome of MI, unstable angina, revascularization [e.g., percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CAGB)] or all-cause death. In cohort 1, an emergency physician

led an adjudication panel with the outcomes independently adjudicated by at least two members with disagreements not resolved by consensus referred to a third blinded reviewer.[10]

In cohort 2, adjudication was not performed; rather administrative and clinical databases linked at ICES (Toronto, Ontario) were used via unique encrypted patient identifiers to obtain past medical history and outcomes (all-cause death, or hospitalization from MI or unstable angina, or PCI/CABG).[21] Briefly, the Ontario RPDB contained all information on patient demographics and death date. All inpatient hospital discharges and same day surgeries were captured in the CIHI Discharge Abstract Database (DAD) and Same Day Surgery Database (SDS), respectively. The OHIP database captured all physician billings and outpatient visits (see Supplemental Methods for additional details regarding health outcomes).

Laboratory methods and Cutoffs

The hs-cTnI assay selected for measurement in cohort 1 was from Ortho Clinical Diagnostics. As compared to other companies' hs-cTnI assays, the Ortho hs-cTnI assay generally yields the lowest cTnI concentrations.[12-14,22] This was supported by testing performed on patient pool quality control material used to validate hs-cTnI assays (published mean concentrations on this material were Beckman hs-cTnI=3.4 ng/L; Abbott hs-cTnI=5.1 ng/L; Siemens Centaur hs-cTnI=5.0 ng/L and Siemens EXL hs-cTnI=8.6 ng/L)[11,13,23,24] where measurement of this material by the Ortho hs-cTnI assay yielded the lowest concentration (Ortho hs-cTnI mean=1.1 ng/L with precision <10%). Suitability of frozen samples (below -70^{0}C for over 15 years) has also been demonstrated for the Ortho hs-cTnI assay, consistent with other assays.[14,25] In addition to conventional cardiac troponin I which was reported to the treating ED physician for the standard of care diagnosis, Abbott hs-cTnI testing was also performed on cohort 2 but remained

unreported.[20] All cardiac troponin testing performed in both cohort 1 and cohort 2 are consistent with laboratory quality recommendations published in 2018.[15] Glucose and creatinine measurements were from standard acceptable laboratory methods (Abbott ARCHITECT analyzer), with the CKD-EPI equation used to derive the estimated glomerular filtration rate (eGFR)[26], only after the studies were completed (i.e., eGFR result by this equation was not clinically reported during the enrolment).

The cutoffs selected were a CCS ≥1 and a hs-cTnI ≥5 ng/L alone as both of these cutoffs have been reported in the literature for ruling-out MI, with 5 ng/L proposed as a common cutoff for hs-cTn assays.[3,6,10,27] A second cutoff for the Ortho hs-cTnI assay ≥1 ng/L was also evaluated as this cutoff has recently been reported for the European Society of Cardiology 0/1 h algorithm.[28] Briefly, the CCS calculation includes glucose, eGFR and hs-cTnI with the cutoffs chosen selected based on accepted normal levels and low-risk clinical cut-points and has a range of 0-5 points.[10] For example, a CCS <1 would be patients with an eGFR ≥90 mL/min/1.73m^2, a glucose <5.6 mmol/L and an hs-cTnI <4 ng/L (a normal concentration by all hs-cTnI assays)[29], and would represent a biochemically healthy individual.[10]

Statistical Analyses

The diagnostic test parameters: sensitivity, specificity, NPV, and positive predictive value (PPV) with 95% confidence intervals (CIs) were calculated using CCS ≥1 and hs-cTnI ≥5 ng/L as the cutoffs in both cohorts. Additionally, in cohort 1 a hs-cTnI ≥1ng/L cutoff was also evaluated. Common variables between both cohorts were calculated (mean, standard deviation, or percentages). Clinical significance of the test would be ascertained if the point estimate of the

sensitivity was ≥99%.[7,8] In cohort 2, counts ≤5 were not reported to protect privacy per ICES protocols.[21] Accordingly, if counts ≤5 were obtained at 30-days, an additional analysis would be performed at 90-days to obtained numbers to calculate the 95%CIs. Statistical analyses were performed using SAS 9.1.3 software (SAS Institute Inc, Cary, NC) and MedCalc Statistical Software version 19.1.6 (MedCalc Software Ltd, Ostend, Belgium).

Results

In both cohort 1(n = 1058) and cohort 2 (n = 5974) the average age exceeded 65 years (67 years and 68 years, respectively) with over half of the population being women (53% and 51%, respectively) (Table 1). The presentation hs-cTnI concentration was normal (i.e., below the 99th percentile cutoff which is designated as the upper limit of normal) in over three quartiles of the population (80% in cohort 1 and 79% in cohort 2). The MACE rate at 30-days was higher in cohort 1 (19.4%; 95%CI: 16.8 to 22.2) as compared to cohort 2 (14.6%; 95%CI: 13.6 to 15.6) ($p<0.01$).

In cohort 1, there were 110 patients with a CCS <1 (10.4% of total population), with 1 of these patients having an event at 30-days (0.9%) (Table 2). Applying a CCS ≥1 cutoff yielded a sensitivity of 99.5% (95%CI: 97.3 to 99.9) and NPV of 99.1% (95%CI: 93.9 to 99.9). In contrast, there were 722 patients with Ortho hs-cTnI concentrations <5 ng/L (68.3% of total population), with 53 of these patients having an event at 30-days (7.3%). Applying a hs-cTnI ≥5 ng/L cutoff yielded a sensitivity of 74.2% (95%CI: 67.6 to 80.0) and NPV of 92.7% (95%CI: 90.9 to 94.1). Lowering the Ortho hs-cTnI cutoff to ≥1 ng/L (352 patients with 18 outcomes (5.1%) for hs-cTnI <1 ng/L) yielded a sensitivity of 91.2% (95%CI: 86.5 to 94.7) and NPV of 94.9% (95%CI: 92.2

to 96.7). The specificity was lower for CCS ≥1 as compared to hs-cTnI ≥1 ng/L [12.8% (95%CI: 10.6 to 14.2) versus 39.2% (95%CI: 35.9 to 42.5)].

In cohort 2, there were 399 patients with a CCS <1 (6.7% of the total population), with ≤5 patients having an event at 30-days. The point estimates at 30-days for sensitivity/NPV for CCS ≥1 were ≥99%. By comparison, there were 103 outcomes in 2374 patients (4.3%) with Abbott ARCHITECT hs-cTnI concentrations <5 ng/L. Applying the hs-cTnI ≥5 ng/L cutoff yielded a sensitivity of 88.1% (95%CI: 85.8 to 90.2) and NPV of 95.6% (95%CI: 94.7 to 96.4). At 90-days, the MACE rate was lower in the CCS <1 group (2.0%; 95%CI: 0.9 to 4.0) versus those with Abbott ARCHITECT hs-cTnI concentrations <5 ng/L (5.7%; 95%CI: 4.8 to 6.7) ($p<0.01$). The sensitivity of CCS ≥1 was 99.2% (95%CI: 98.5 to 99.6) as compared to hs-cTnI ≥5 ng/L sensitivity of 87.7% (95%CI: 85.6 to 89.6). The specificity was lower for CCS ≥1 as compared to hs-cTnI ≥5 ng/L [12.8% (95%CI: 10.6 to 14.2) versus 39.2% (95%CI: 35.9 to 42.5)] at 90-days (Table 3). Combining the CCS ≥1 from both cohorts, the 30-day MACE rate would be ≤1% (≤6 from 509 patients).

Interpretation

Patients with a CCS <1 with the ED presentation blood work have a rate of MACE at 30-days ≤1%, with the CCS ≥1 cutoff yielding a sensitivity ≥99%. Both of these metrics indicate that ED patients with possible ACS who have a CCS <1 are a low-risk group of patients which should enable ED physicians to consider discharge and the cessation of additional investigations in the ED for current and subsequent ACS over the short-term.

With the advent of hs-Tn testing there have been gains made in the ED for reducing the time between blood draws for testing when evaluating patients with possible ACS.[1,30] Also, with the hs-cTn assays, there is evidence to support a low hs-cTn concentration may be used to rule-out MI at ED presentation.[1-6] However, there are important caveats to using a low hs-cTnI concentration alone for decision-making in the ED: 1) patients who present early after pain onset may be misdiagnosed; 2) ruling-out MI does not equate patient discharge as patients may still be at risk for ischemic events; and 3) analytical imprecision at the low end for hs-cTn assays may result in patient misclassification.[4,31,32] Moreover, in an attempt to simplify interpretation of a low hs-cTn concentration some have advocated and studies have assessed a single cutoff of <5 ng/L.[2,3,5,27] This approach may be suitable for hs-cTnI assays if there is close agreement to the Abbott hs-cTnI assay at this cutoff; as this assay has been assessed in large multicenter studies assessing MI.[3,19]

However, our findings indicate that the 5 ng/L cutoff alone at presentation for the Ortho hs-cTnI assay would be unsafe with over 7% of patients with hs-cTnI concentrations <5 ng/L having MACE with a sensitivity below 75% at this cutoff. Lowering the hs-cTnI threshold to 1 ng/L as used in the 0/1h algorithm with the Ortho hs-cTnI assay yields sensitivity (91%) close to the Abbott hs-cTnI assay when using the 5 ng/L cutoff (88%). Importantly, both estimates by these different hs-cTnI assays would be too low to exclude MACE. Only the CCS ≥1 with both assays in two different populations obtained the required sensitivity (≥ 99%) to rule-out MACE at 30-days.

Limitations

As with any observational study there are limitations. First, ED physicians were not exposed to the hs-cTnI results or the CCS so the impact/intervention of applying either hs-cTnI ≥5 ng/L or the CCS ≥1 cannot be assessed. Second, these two different populations share a common variable in that both occurred at hospitals within the city of Hamilton; as such the impact of different geographic locations on the CCS cannot be assessed. However, previous studies across different countries and across different provinces within Canada have demonstrated close agreement of the CCS in different geographical locations and using different hs-cTn assays.[10,33] Third, as the Ortho hs-cTnI assay was measured in samples collected in 2013, there might have been some degradation in analyte; and as the latest hs-cTnI assay approved by Health Canada, real world analytical precision data for this assay is unknown. However, there are data to support the long-term stability of the cardiac troponin I analyte as measured by the Ortho VITROS hs-cTnI assay in samples stored frozen for over 15 years[14]; with the assay achieving a precision <10% at normal concentrations and thus fulling the analytical requirements for a hs-cTnI assay.[15,28] Finally, additional approaches, such as serial testing with hs-cTn may further identify patients at low-risk and suitable for discharge; however, ED patients with a CCS <1 at presentation have a lower mortality rate at 1-year as compared to ED patients with a hs-cTnI concentration <5 ng/L at presentation (2% versus 5%, respectively).[34]

Conclusion

A CCS <1 with different hs-cTnI assays identifies patients at low-risk for MACE at 30-days, which cannot be achieved when using hs-cTnI testing alone. An intervention study is needed to evaluate the impact of the CCS at both the patient and hospital level.

Word Count: 2491 (max 2500)

Contributors: Peter Kavsak had access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Peter Kavsak, Andrew Worster, and Dennis Ko were responsible for concept and design. Peter Kavsak, Joshua O Cerasuolo, Dennis T Ko, Jinhui Ma, Jonathan Sherbino, Shawn E Mondoux, Natasha Clayton, Stephen A. Hill, Matthew McQueen, Lauren Griffith, Shamir R. Mehta, Richard Perez, Hsien Seow, PJ Devereaux, and Andrew Worster acquired, analyzed or interpreted the data. Peter Kavsak and Andrew Worster drafted the manuscript. Joshua O Cerasuolo, Dennis T Ko, Jinhui Ma, Jonathan Sherbino, Shawn E Mondoux, Natasha Clayton, Stephen A. Hill, Matthew McQueen, Lauren Griffith, Shamir R. Mehta, Richard Perez, Hsien Seow, and PJ Devereaux critically revised the manuscript for important intellectual content. All of the authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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Conflict of Interest Disclosures: Dr. Kavsak has received grants/reagents/consultant/advisor/ honoraria from Abbott Laboratories, Abbott Point of Care, Beckman Coulter, Ortho Clinical Diagnostics, Randox Laboratories, Roche Diagnostics and Siemens Healthcare Diagnostics. McMaster University has filed patents with Drs. Kavsak and Worster listed as an inventor in the acute cardiovascular biomarker field, in particular, a patent has been filed on aspects related to the data presented in this study "A LABORATORY SCORE FOR RISK STRATIFICATION FOR PATIENTS WITH POSSIBLE CARDIAC INJURY". No other disclosures were reported.

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Figure 1. Flow diagram of the two different ED cohorts. Cohort 1 is from the ROMI-3 study and Cohort 2 are all consecutive ED patients that had an ED cardiac presentation blood panel resulted.

Table 1. Demographics of the two ED study cohorts with mean (SD) or number (percentages) provided.

Variables	Cohort 1 (n=1058)	Cohort 2 (n=5974)
Age in years, mean (SD)	67 (17)	68 (17)
Female sex, n (%)	558 (53%)	3025 (51%)
Hypertension, n (%)	744 (70%)	4045 (68%)
Diabetes mellitus, n (%)	312 (29%)	1961 (33%)
History of MI, n (%)	378 (36%)	674 (11%)
History of PCI or CABG, n (%)	236 (22%)	453 (8%)
History of stroke, n (%)	176 (17%)	186 (3%)
History of peripheral vascular disease, n (%)	75 (7%)	742 (12%)
Glucose mmol/L, mean (SD)	7.4 (3.6)	7.6 (4.4)
eGFR mL/min/1.73 m^2, mean (SD)	72 (27)	69 (27)
Presentation hs-cTnI <99th percentile cutoff*, n (%)	842 (80%)	4698 (79%)
30-day MACE, n (%)	205 (19.4%)	871(14.6%)

*99th percentile upper reference limit is 11 ng/L for Ortho hs-cTnI assay and 26 ng/L for Abbott hs-cTnI assay
PCI=percutaneous coronary intervention; CABG=coronary artery bypass grafting

Table 2. Sensitivity, specificity, NPV, PPV, and 30-day MACE with the clinical chemistry score (CCS) versus hs-cTnI alone in cohort 1 using the Ortho VITROS hs-cTnI assay

Method, number of patients in this group, (% from 1058)	30-day MACE, n (%)	Sensitivity % (95%CI)	Specificity % (95%CI)	NPV % (95%CI)	PPV % (95%CI)
CCS ≥1 cutoff [CSS <1 in n=110 (10.4%)]	1 (0.9%)	99.5% (97.3% to 99.9%)	12.8% (10.6% to 15.2%	99.1% (93.9% to 99.9%)	21.5% (21.1% to 21.9%)
hs-cTnI ≥1 ng/L cutoff [hsTnI <1 ng/L in n=352 (33.3%)]	18 (5.1%)	91.2% (86.5% to 94.7%)	39.2% (35.9% to 42.5%)	94.9% (92.2% to96.7%)	26.5% (25.1% to 27.8%)
hs-cTnI ≥5 ng/L cutoff [hsTnI <5 ng/L in n=722 (68.3%)]	53 (7.3%)	74.2% (67.6% to 80.0%	78.5% (75.6% to 81.2%)	92.7% (90.9% to 94.1%)	45.4% (41.6% to 49.2%)

Table 3. Sensitivity, specificity, NPV, PPV, and 90-day MACE with the clinical chemistry score (CCS) versus hs-cTnI alone in cohort 2 using the Abbott ARCHITECT hs-cTnI assay. Note at 30-days, MACE occurred in ≤5 patients with a CCS <1.

Cutoff, number of patients in this group, (% of 5974 total)	90-day MACE, n (%)	Sensitivity % (95%CI)	Specificity % (95%CI)	NPV % (95%CI)	PPV % (95%CI)
CCS ≥1 cutoff [CSS <1 in n=399 (6.7%)]	8 (2.0%)	99.2% (98.5% to 99.6%)	8.0% (7.2% to 8.8%)	97.9% (96.0% to 99.1%)	19.5% (18.5% to 20.6%)
hs-cTnI ≥5 ng/L cutoff [hsTnI < 5 ng/L in n=2374 (39.7%)]	135 (5.7%)	87.7% (85.6% to 89.6%)	45.9% (44.5% to 47.3%)	94.3% (93.3% to 95.2%)	26.8% (25.3% to 28.2%)

Supplemental Methods

Health Outcome Adjudication in Cohort 1

Death and Myocardial Infarction:

In the Hamilton cohort, an emergency physician led an adjudication panel with the outcomes independently adjudicated by at least two members with disagreements not resolved by consensus referred to a third blinded reviewer using the Third Universal Definition of Myocardial Infarction as the basis for the diagnosis of MI.[10] Participants were followed for at least 30-days for mortality status and MI. For the MI outcome, the contemporary Abbott cTnI (ug/L) assay was used with a cTnI concentration of >0.03 ug/L (>99th) with a significant rise/fall (absolute delta ≥0.03 ug/L for concentrations <0.10 ug/L or proportional changes of ≥20% for concentrations ≥0.10 ug/L, from n=1367 subsequent cTnI measurements with the median time (interquartile range) between 2nd and 1st samples = 3.03h (2.97-3.17)) , or new ST segment elevation or depression indicative of ischemia; new left bundle branch block; coronary artery intervention or pathologic findings of an acute MI.

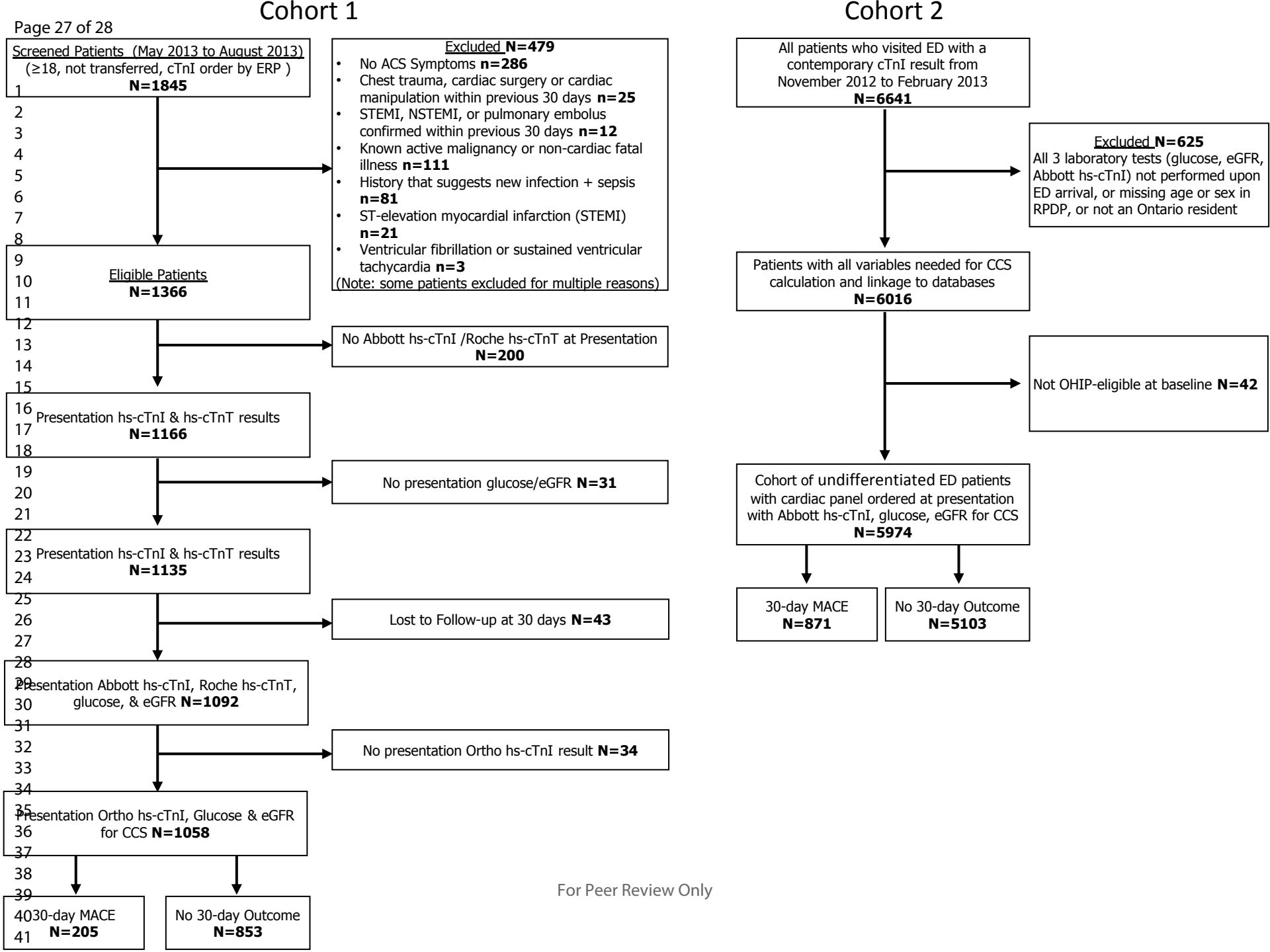
Unstable Angina and Revascularization:

Unstable angina (UA) was diagnosed when any of the following criteria were met: a discharge diagnosis of UA as per discharge summary and/or admission to hospital with ACS treatment [heparin or low molecular weight heparin, cardiac catheterization resulting in increased treatment (i.e., Plavix/ASA or revascularization)].

Health Outcome in Cohort 2

Diagnosis/intervention codes used for clinical endpoint (MACE)

Outcome	Data source	Codes
All-cause mortality	RPDB	Not applicable
Myocardial infarction	DAD	Diagnosis codes (ICD-10): I21 or I22
Angina	DAD	Diagnosis codes (ICD-10): I20, I2382, or I24
CABG	DAD	Intervention codes (CCI): 1IJ76 or 1IJ80
PCI	DAD or SDS	Intervention codes (CCI): 1IJ26, 1IJ50, 1IJ55, or 1IJ57



Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2,3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	4,5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5,6
Participants	**6**	Eligibility criteria	5,6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5,6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5,6
	9	Whether participants formed a consecutive, random or convenience series	5,6
Test methods	**10a**	Index test, in sufficient detail to allow replication	6,7
	10b	Reference standard, in sufficient detail to allow replication	6,7
	11	Rationale for choosing the reference standard (if alternatives exist)	6,7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	7,8
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7,8
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	5-8
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	5-8
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8,9
	15	How indeterminate index test or reference standard results were handled	8,9
	16	How missing data on the index test and reference standard were handled	5-9
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	5-9
	18	Intended sample size and how it was determined	5,6
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1
	20	Baseline demographic and clinical characteristics of participants	Table 1
	21a	Distribution of severity of disease in those with the target condition	9,10
	21b	Distribution of alternative diagnoses in those without the target condition	9,10
	22	Time interval and any clinical interventions between index test and reference standard	9,10
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9,10
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	9,10
	25	Any adverse events from performing the index test or the reference standard	12
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	11,12
	27	Implications for practice, including the intended use and clinical role of the index test	10-12
OTHER INFORMATION			
	28	Registration number and name of registry	5
	29	Where the full study protocol can be accessed	Not applicable
	30	Sources of funding and other support; role of funders	14

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.