Appendix 2 (as supplied by the authors): Supplemental Methods

Description of Study Populations

Hamilton population: The details of this population have been previously described.[11,16,19] Briefly, the Hamilton population was an observational cohort study (NCT01994577) enrolled patients presenting to the ED from three tertiary care hospitals within Hamilton, Ontario from May 2013 to August 2013. Eligible patients were ≥18 years, who were not transferred from another hospital, and for whom the ED physician ordered cardiac troponin. Patients were excluded if their symptoms were non-ACS; or they had chest trauma, cardiac surgery or manipulation within 30 days of presentation; a MI (STEMI or NSTEMI) or pulmonary embolus confirmed within the previous month; known active malignancy or non-cardiac fatal illness; sepsis; ventricular fibrillation or sustained ventricular tachycardia; or STEMI at presentation.

Brisbane population: Data were from the Brisbane cohort of the ADAPT (2-hour Accelerated Diagnostic Protocol to Assess Patients with Chest Pain Symptoms using Contemporary Troponins as the Only Biomarker) study.[20] This was a prospective observational study of 983 patients presenting to the ED of an Australian tertiary care hospital between November 2008 and January 2011. Eligible patients were those aged 18 years or older who presented to the ED with at least five minutes of chest pain suggestive of ACS. Patients were excluded if there was a clear non–ACS cause for symptoms, they were unwilling or unable to provide informed consent, staff considered that recruitment was inappropriate, they were transferred from another hospital, were pregnant, were recruited to the study within the previous 45-days, had a STEMI at presentation, or were unable or unwilling to be contacted after discharge.

Christchurch population: Christchurch data consisted of patients from the New Zealand cohort of the ADAPT observational study[20] and the Christchurch EDACS-ADP randomized controlled trial (Emergency Department Assessment of Chest Pain Score - Accelerated Diagnostic Pathway).[21] Eligible patients were adults (≥18 years), who presented with symptoms consistent with ACS in which the attending physician planned to do serial cardiac troponin measurements. Patients were excluded for STEMI, a clear cause other than ACS for the symptoms, transfer from another hospital, pregnancy, previous enrollment, or no consent.

Hamburg population: The Biomarkers in Acute Cardiac Care (BACC) Study included patients with suspected acute MI presenting to the ED of the University Hospital Hamburg-Eppendorf, Germany. The BACC study is an ongoing, prospective cohort study including patients presenting with suspected myocardial infarction to the emergency department. Patients were included if they were above 18 years of age and provided written informed consent; and excluded if they had STEMI (NCT02355457).[22,23]

Storage and sample type for hs-cTn testing

In the North American (i.e., Hamilton) cohort two EDTA plasma samples collected at presentation were tested for hs-cTnI and hs-cTnT (samples not frozen, results not reported clinically). In the Australian (i.e., Brisbane) cohort, two samples were also collected from the same draw with the plasma frozen below -70^{0}C for subsequent analyses for hs-cTnI and hs-cTnT. In the New Zealand (i.e., Christchurch) cohort 15% of the lithium heparin plasma samples were measured for hs-cTnI (non-frozen) with the remaining samples from the same draw frozen below -70^{0}C and subsequently tested for hs-cTnT and hs-cTnI. In the Europe (i.e.,

Hamburg) cohort, hs-cTnT was measured as part of routine care (non-frozen) with hs-cTnI measured in these serum samples for 74% of the cohort. The remainder of hs-cTnI testing occurred in serum samples that were stored frozen (below -70^{0}C). The time (in exact hours) from chest-pain onset to presentation to the ED was not captured in the Hamilton and Hamburg cohorts but was for the Brisbane cohort (median 5.6h) and Christchurch cohort (median 5.5h).

Description of hs-cTn in CCS

In addition to the low normal hs-cTn concentration, two additional cut-points for each hs-cTn assay were selected as prior studies have demonstrated a concentration-dependent effect on health outcomes in both stable patients with cardiovascular disease and in the emergency setting.[26-28] Briefly, the second cut-point was selected based on the observed analytical variation (±10 ng/L) from the low-risk cut-points: 15ng/L for hs-cTnI and 19ng/L for hs-cTnT.[29,30] The third cut-point was selected based on the general population 99th percentile for both assays which was 30ng/L. [31,32] Points were assigned based on the hs-cTn concentration: 1-point for hs-cTnI between 4-14ng/L or hs-cTnT 8-18 ng/L, 2-points for hs-cTnI between 15-30ng/L or hs-cTnT 19-30ng/L and 3-points for hs-cTnI or hs-cTnT >30ng/L; with the scoring to reflect the increased risk of an event per hs-cTn concentration.[26-28] This graded approached was supported by our previous study assessing risk (hazard ratio, HR) for myocardial infarction, stroke, or cardiovascular death in the HOPE study population, where as compared to hs-cTnT <8 ng/L (referent), for hs-cTnT concentrations 8-21 ng/L the HR=1.5, hs-cTnT 21-31 ng/L the HR= 2.0, and for hs-cTnT >31 ng/L the HR=3.2 ng/L.[26]

Health Outcome Adjudication

Briefly, all studies used the Third Universal Definition of Myocardial Infarction as the basis for the diagnosis of MI.[24]

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. 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. In the Brisbane cohort, at 30-days post-presentation, research nurses conducted telephone follow-up and reviewed medical records to obtain data on subsequent cardiac events or investigations performed (with data confirmed by the relevant healthcare providers). For the MI outcome, cTnI was measured using the Beckman Coulter second generation AccuTnI assay. The outcomes were adjudicated by local cardiologists according to predefined standardised guidelines, using clinical records, electrocardiogram (ECG) and cTnI results, and investigation results. Specifically for the MI diagnosis, there needed to be evidence of myocardial necrosis and ischaemia. Ischaemia included at least one of the following: ECG changes indicative of ischaemia; or positive imaging results including stress testing,

myocardial perfusion scan, stress echocardiography, computed tomographic coronary angiography or coronary angiography during catheterization. Evidence of necrosis included an increase or decrease in cTnI concentration during at least six hours, and where at least one value was above the 99th percentile (0.04 ug/L). All patients had serial cTnI sampling with 93% of patients having a 6h cTnI with a delta of 20% used to identify evolving injury. However a diagnosis of MI would also be made if the delta <20% and alternative causes for the cTnI elevation had been investigated and there was some objective evidence that MI was the most likely cause. In the Christchurch cohort, two cardiologists independently adjudicated outcomes. Where there were differences, a third cardiologist was used to break the deadlock. For the MI diagnosis, there needed to be evidence of myocardial necrosis together with clinical evidence of myocardial ischemia (ischemic symptoms, ECG changes, or imaging evidence). Necrosis was diagnosed on the basis of a rising or falling pattern (normally >20%) of the laboratory cTnI concentrations (Abbott contemporary cTnI for ADAPT, n=805; hs-cTnI for EDACS-ADP study, n=141), with at least 1 value above the decision cutpoint (99th percentile). If the cTnI concentration was elevated, but a <20% rise or fall was recorded, then other causes of a raised cTnI concentration were actively pursued by the adjudicators. If no clear alternative cause of the cTnI rise was evident, and if the clinical presentation was suggestive of an ACS, an adjudicated diagnosis of MI was decided. All patients had at least 2 measurements as per protocol and the adjudication was the same procedure for both cTnI and hs-cTnI. In the Hamburg cohort, two trained cardiologists adjudicated each final diagnosis separately, with a third cardiologist referred in cases of disagreement. The adjudication based on all available clinical and imaging parameters, as well as hs-cTnT (but not hs-cTnI) with the diagnosis of MI based on the Third Universal Definition. All patients were followed for at least 30-days to assess mortality or MI.

Myocardial infarction 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 cTn-value above the 99th percentile together with a significant rising and/or falling pattern. Relative hs-cTnT changes were used to determine significant changes. A significant relative change was defined as a rise in hs-cTnT >50% within 3h in patients presenting with a normal hs-cTnT concentration at baseline or as a rise in hs-cTnT >20% within 3h in patients presenting with an elevated (>99th percentile) hs-cTnT concentration. A second cardiac troponin result might not always be necessary (e.g., late presenters) for final adjudication.

Unstable Angina:

In the Hamilton cohort, 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)]. In the Brisbane cohort, diagnosis of unstable angina pectoris was based on ischemic symptoms, ECG changes, and objective investigations (exercise stress testing, stress echocardiography, computed tomographic coronary angiography, myocardial perfusion scan, and angiography), with normal biomarker levels. This definition included patients with new symptoms or a changing symptom pattern (i.e., from stable to unstable angina). Patients with equivocal ECG changes but clear positive changes on exercise tolerance testing or imaging evidence of critical coronary stenosis also were classified as having unstable angina pectoris. In the Christchurch cohort, UA was diagnosed if: 1. new cardiac symptoms and positive ECG findings with normal biomarkers 2. changing symptom pattern and

positive ECG findings with normal biomarkers. Patients with clinical history consistent with the diagnosis of unstable angina as described above, in whom ischaemia has been confirmed by the presence of ST-segment changes on the initial ECG or in association with recurrent rest pain, or by a positive objective test (e.g. stress test). In the Hamburg cohort, unstable angina is not available as follow-up variable.