Is the Current Threshold for Diagnosis of "Abnormality", including Non ST Elevation Myocardial Infarction, using Raised High Sensitivity Troponin Appropriate for a Hospital Population? The CHARIOT Study

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The CHARIOT Study

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Abstract

Objective

Clinicians use the cardiac troponin (cTn) assay to aid in the diagnosis of an acute myocardial infarction (AMI). Each assay manufacturer provides the 99th percentile for cTn levels in a group of healthy individuals, and this level is taken as the upper limit of normal (ULN). The objective of this study was to determine the distribution, and specifically the true 99th percentile, for the whole hospital population, using the cTn assay currently employed routinely at our institution.

Design

Prospective study of 20,000 consecutive patients undergoing blood sampling for any reason at a large teaching hospital. Hs-cTnI concentrations (Beckman Coulter Access AccuTnI+3 assay) were nested for analysis in all cases except those in whom the supervising physician had requested hs-cTnI for clinical reasons.

Setting

University Hospital Southampton NHS Trust (UHS).

Participants

20,000 consecutive individuals, inpatient or outpatient, undergoing blood tests at UHS for any clinical reason.

Main outcome measures

Distribution of hs-cTnI concentrations of all study patients, and specifically the 99th percentile.

Results

The 99th percentile of hs-cTnI for the whole population (n=20,000) was 296 ng/L, compared to a manufacturer quoted 99th percentile of 40 ng/L (currently used clinically as the ULN). In 1 in 20 (5.4%, n=1080) of the total population hs-cTnI concentrations were above 40 ng/L. After exclusion of individuals diagnosed with an acute myocardial infarction (AMI) (n=122), or those in whom troponin was requested (n=1707), the 99th percentile for the remainder (n=18,171) was 189 ng/L. The 99th percentile for inpatients (n=4759) and outpatients (n=9280) was 563 ng/L and 65 ng/L, respectively. Patients from the emergency department (n=3706) had a 99th percentile of 215 ng/L, with 6.1% (n=225) above the quoted ULN. 39.02% (n=48) of all individuals from the critical care units (n=123) and 14.16% (n=67) of all medical inpatients had a hs-cTnI concentration above the quoted ULN.

Conclusions

In 20,000 consecutive patients undergoing a blood test for any reason at this hospital 1 in 20 have a hs-cTnI above the supplied ULN. These data highlight the need for clinical staff to interpret hs-cTnI concentrations carefully, particularly when applying the supplied ULN to diagnose AMI. The use of hs-cTnI to diagnose AMI in any patient could lead to misdiagnosis in the absence of an appropriate clinical presentation.

Introduction

The use of increasingly sensitive troponin assays for the exclusion or diagnosis of acute myocardial infarction (AMI) has become universal. The diagnosis of AMI is now defined by a rise and/or fall of cardiac troponin (cTn) concentration, now the gold standard biomarker(1), with at least one value above the 99th percentile derived from a reference population of healthy individuals in the context of an appropriate clinical presentation (3-5).

Under most circumstances, the troponin assay is requested by front line clinical staff to determine whether or not a patient is experiencing a Type 1 myocardial infarction (T1MI), which is due to coronary plaque rupture or erosion, since robust evidence has demonstrated symptomatic and prognostic benefit from the application of early pharmacological and interventional treatment strategies in such patients. However, particularly with the advent of newer assays, this strategy has 2 potential challenges.

Firstly, elevated cTn concentrations, particularly in patients not presenting with a typical history of cardiac pain, are often due to myocardial injury or Type 2 myocardial infarction (T2MI)(6, 7), which is secondary to ischaemia due to either increased oxygen demand or decreased supply rather than a plaque erosion event (8-10). This is not well recognized when the troponin test is requested, or the result interpreted, and is especially important because the majority of patients with T2MI have not been shown to benefit from the same aggressive pharmacotherapy and invasive investigation and treatment that is offered as standard in cases of T1MI(11), with some exceptions including spontaneous coronary dissection, coronary embolism and coronary spasm (10, 12). In fact, such misinterpretation may lead to inappropriate management, including prolonged antiplatelet therapy and invasive coronary angiography, with or without revascularization.

Secondly, the assay-specific 99th centile (ULN) is generally applied as a binary "rule in" or "rule out" threshold for AMI. Whilst recent trial data confirm the veracity of the use of early cTn concentrations to confidently exclude the diagnosis of AMI (13-16), the assumption that a concentration above that level implies AMI (and in particular a T1MI) is often inappropriate. Both of these potential issues may be compounded in clinical practice by the increasing sensitivity of the available assays that are able to detect troponin at much lower concentrations than previously (5). Consequently, new highly sensitive cardiac troponin (hs-cTn) assays (17-21) allow for rapid exclusion of AMI, and thereby facilitate the early discharge of patients from hospital. Furthermore, modern hs-cTn assays can detect troponin in more than 50% of the general population, with some assays able to detect troponin in everyone(22). The appropriate interpretation of the "elevated" hs-cTn, particularly in relation to the diagnosis of T1MI, is therefore dependent upon a clinical presentation consistent with this diagnosis, and in particular, a history of cardiac-sounding chest pain, according to the guidelines.

The International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers (IFCC TF-CB) currently recommends that the 99th percentile for any assay can be calculated using 300 'healthy' men and 300 'healthy' women(23). Given the number of factors that are well known to affect an individual's troponin (23), including age(24), gender(25), glomerular filtration rate(26), left ventricular function(27), and the presence of significant inflammatory conditions (28), the appropriateness of the clinically applied concept of an ULN for the hs-cTn assay requires closer scrutiny, particularly when it was derived from a limited number of healthy individuals. Importantly, the approaches to determining the supplied 99th percentile are also variable (29-31).

The aims of this study were to determine (a) the true distribution of hs-cTnI concentration in an unselected all comer hospital population, both inpatient and outpatient, and, more specifically, (b) the 99th percentile for this population using 20,000 consecutive patients. Our hypothesis was that the true distribution of hs-cTnI in this population would differ from the supplied ULN for this assay,

thereby highlighting the potential for misinterpretation of a value above this level in routine clinical practice, particularly the validity of applying the latter as the binary arbiter of the diagnosis of AMI, especially T1MI.

Methods

Study population

This was a prospective, observational study that included 20,000 consecutive patients aged at least 18 years in whom a biochemistry blood investigation was requested for clinical reasons determined by their supervising physician at our institution, a large University teaching hospital in the United Kingdom. Patients were included regardless of the setting in which the blood test was requested, so that the study population included outpatients and inpatients, emergency department attendees, elective and emergency admissions, and every specialty within the hospital. For each patient included in the study only one troponin measurement was performed on the first biochemistry blood sample that became available during the study period. That individual was then excluded from further sampling, in order that a consecutive series of 20,000 different patients were included. For some of the study analysis, patients who were discharged from hospital with a diagnosis of AMI or in whom a hs-cTnI level was requested by the clinical team, which was determined through a review of the electronic blood request forms submitted to the biochemistry department and via electronic discharge summaries, were excluded.

Ethics & Regulatory Approval

This research project was undertaken according to the principles of Good Clinical Practice and the Declaration of Helsinki. The study was approved by the local ethical committee who then referred it

to the Health Research Authority (HRA) UK and its independent Confidentiality Advisory Group (CAG) for further approval (Rec reference: 17/SC/0042, IRAS project ID: 215262). The CAG approval was required based upon 2 unusual aspects of the methodology. Firstly, the method did not require knowledge or consent from patients that an extra blood assay was being performed. Secondly, apart from those in whom a hs-cTnI was requested as part of their routine clinical care by their supervising clinician, the result of the hs-cTnI test was nested and never revealed to either patient or their supervising clinical team, regardless of whether the result was above the supplied ULN. The study is registered with Clinicaltrials.gov, number NCT03047785.

Cardiac troponin I assay

The Beckman Coulter Access AccuTnI+3 assay (Beckman Coulter, Brea, CA, USA) is employed in routine clinical practice at our Trust and is employed in routine clinical practice at our Trust and was used to measure hs-cTnI concentrations in the study population. The supplied 99th percentile (ULN) is 40 ng/L, which is the level used in routine clinical practice at our institution. The coefficient of variation (CV) of the assay is <10% at 40ng/L, the limit of quantification (LOQ 10% CV) is 20ng/L; the limit of detection (LOD) is 8ng/L; the limit of blank is 5ng/L. For those patients in whom troponin had not been requested for clinical reasons, the hs-cTnI concentration was measured for every individual using serum which was surplus to clinical need. An automated, bespoke system was put in place in Biochemistry to ensure each individual was only included once in the study. Serum was collected into serum separator tubes and stored at room temperature for up to 24 hours before cTnI levels were measured through the use of the DxI800 platform (Beckman Coulter, Brea, CA, USA). Quality control of the assay was undertaken on a daily basis as is routine in clinical practice.

Data Collection

Baseline demographic data were limited to those derived from electronic request forms for blood tests and, for inpatients, from electronic discharge summary codes. These data, together with the troponin levels and other study data were collected on a bespoke database for later analysis.

Patient and public involvement

The British Cardiac Patients Association (BCPA) assisted the researchers in review of the study protocol, with particular reference to the lack of consent of participants. A letter of support for our methodology from the Chairman of the BCPA was submitted to the HRA/CAG as part of our study application.

Statistical Analysis

T The 99th percentile for the study population was defined using a non-parametric procedure based on frequency tables. Statistical analyses were performed using IBM SPSS V.22.0 (SPSS, IBM Corporation, Armonk, New York, USA). We used Stata 14.0 (College Station, USA) to perform multiple logistic regressions to identify factors associated with elevated highly sensitive troponin above 40 ng/L. Variables in the model included age, male sex, serum sodium, estimated glomerular filtration rate and location.

Results

A total of 20,000 consecutive patients were included in CHARIOT between 29/06/2017 to 24/08/2017. The median age was 61 (standard deviation 20) years and 52.9% were female, (n = 10,580).

The 99th percentile hs-cTnI concentration for the whole study population (n=20,000) was 296 ng/L, with 1 in 20 (5.4%; n=1080) of the patients having a hs-cTnI concentration above the supplied ULN (40 ng/L). Once all patients who had been diagnosed with an AMI on discharge or in whom a hs-cTnI level had been requested on the basis of a clinical suspicion of MI had been excluded, this left 18,171 patients in whom the 99th percentile was 189 ng/L, with 4.6% (n=836) above 40 ng/L (Figure 1). Baseline characteristics are shown in Table 1.

Of the 1707 patients in whom hs-cTnI concentrations were requested by the clinical team, 73% (n=1246) had presented with chest pain, with arrhythmia (n=52) and suspected blackouts (n=63) the next most common reason for the test.

Patient Location

Patients were stratified according to their location at the time the biochemistry test was requested.

Specifically, the study included 9280 (51.1%) hospital outpatients in whom the observed 99th percentile was 65 ng/L, with hs-cTnI concentrations above the supplied ULN in 2% (n=186). 4759 (26.2%) of the study population were patients admitted. The 99th percentile for this inpatient group was 563 ng/L, and the hs-cTnI concentrations were above the supplied ULN in 7.29% (n=347).

A total of 5708 patients had their blood sampling in the emergency department (ED). Of this group, 1551 (27.2%) had hs-cTnI concentrations requested by the ED clinicians. The 99th percentile for the remaining ED population (n= 3706) was 215 ng/L, with 6.07% (n=225) of these having hs-cTnI

concentrations above the supplied ULN. Patients managed in the resuscitation room (n=426) of the ED had hs-cTnI concentrations above the ULN in 19.48% (n=83).

For patients managed in the critical care environment (3 intensive care and 2 high dependency units) (n= 123), 39.02% (n=48) had hs-cTnI concentrations above the ULN.

Once all patients who had either been diagnosed with MI or hs-cTnI requested by the clinical team were excluded, a total of 14.16% (n=67) of all medical inpatients (excluding cardiac) had hs-cTnI concentrations above the supplied ULN. 20.8% of patients from the medicine for older people (MOP) (n= 20) also had hs-cTnI concentrations above the supplied ULN. 4.62% (n=16) of patients managed on the acute surgical unit had hs-cTnI above the ULN. For orthopaedic patients 5.24% (n=13) had hs-cTnI concentrations above the ULN. In none of these patients was an acute MI suspected or diagnosed (Table 2; Figure 2).

Age

There was an association between increasing age and distribution of troponin concentration. Percentiles (25^{th}, 50^{th}, 75^{th}, and 99^{th}) and proportion of patients with hs-cTnI above the ULN according to age is shown in supplementary Table 1 and Figure 3.

Gender

The 99^{th} percentiles for males and females were 373 ng/L and 236 ng/L, respectively. 6.6% (n=622) of male and 4.38% (n=463) of females had hs-cTnI concentrations above the ULN. Significant differences were seen in mean hs-cTnI levels when comparing males to females (62 vs 31 ng/L, p=0.021).

Multivariable analysis

Once all patients who had either been diagnosed with MI or had hs-cTnI concentrations requested by the clinical team (n=1829) were excluded, a multivariable analysis was undertaken to assess the independent predictors of an individual having a hs-cTnI concentration above the supplied ULN (40 ng/L). Advancing age (odds ratio (OR) 1.03(1.03-1.04), p<0.001), male gender (OR 1.33, (1.14-1.54), p<0.001) and reducing estimated glomerular filtration (OR 0.98(0.97-0.98), p<0.001) were shown to be independent predictors. Furthermore, when compared to the outpatient population, location in the ED (OR 2.79 (2.26-3.43), p<0.001), resuscitation room(OR 9.91 (7.3-13.46), p<0.001), critical care units (OR 36.62(23.86-56.2), p<0.001), cardiac wards (OR 9.08, (6.44-12.81), p<0.001), acute surgical unit (OR 2.52(1.47-4.33), p<0.001), medical wards (OR 4.74(3.45-6.50), p<0.001), MOP wards (OR 3.70 (2.16-6.34), p<0.001) and orthopaedic wards (OR 2.24 (1.23-4.05), p=0.008) were independent predictors for hs-cTnI concentration above the ULN (table 3). Independent predictors for the full cohort (n=20,000) are shown in the Supplementary table 3.

Discussion

This study, which is to our knowledge the largest of its kind, has shown that 1 in 20 consecutive all comer patients at a large UK hospital have a troponin level that is greater than the supplied 99th centile (ULN) for the assay. Our data also demonstrate that the 99th centile varies according to the clinical setting, age and gender, and location, with a range of 2% of outpatients and 39% of patients in critical care settings having a cTnI greater than the supplied ULN.

These results have important clinical implications that are almost certainly relevant to the application of all modern hs-cTn assays. Firstly, they confirm our original hypothesis that the true 99th centile for a general hospital population is not consistent with the supplied ULN. Secondly, these data raise

important questions about the applicability of the quoted ULN as an arbiter of Type 1 AMI in patients who do not give a typical history consistent with this diagnosis. The previous evidence for the use of cTnI levels to rule out AMI is clear cut and robust (14-16, 32). The Fourth Universal Definition(3) recommends the diagnosis of AMI when there is clinical evidence of acute myocardial ischaemia and with detection of a rise and/or fall of cTn values. However, the utility of the supplied ULN as a "rule in" test for AMI in patients presenting with atypical symptoms and other comorbidities, such as in the context of ED or acute medicine and surgical patients, is flawed and potentially exposes such patients to inappropriate pharmacological and invasive treatment that has only been shown to be beneficial in true T1MI populations. This study highlights the importance of interpreting hs-cTnI results with caution in an individual patient. The risk of potential systematic misdiagnosis of AMI is particularly illustrated by the observed 99th centile for hs-cTnI in our subpopulations of ED (215 ng/L) and acute medical admissions (1459 ng/L), and that close to 40% of patients in some clinical settings have hs-cTnI levels above the supplied ULN. It is particularly important for frontline clinical staff to understand that using a single cutoff of hs-cTnI to diagnose AMI may be inappropriate and that the ULN of the assay will depend on the clinical environment as well as clinical characteristics of patients. We would advocate that clinical staff are aware of the current guidelines in diagnosing AMI, which are not always adhered to, and also that they have a very clear indication for requesting the test.

Our analysis highlights a number of factors that are associated with "elevated" hs-cTnI results as judged by the supplied reference, including mode of presentation. Thus, 7.29% of all inpatients in this study had an "elevated" hs-cTnI concentration, including 6.07% of ED patients and 19.48% of those admitted to the resuscitation room. It is more predictable that nearly 40% of patients admitted to a critical care setting have an elevated concentration. However, the finding that our observed 99th centile for hs-cTnI concentrations was 65ng/L in outpatients, and that 2% of these patients who attended the hospital only for a clinic appointment had a concentration above the supplied ULN, highlights the need for a review of quoted distribution of hs-cTn assay in a hospital setting. Further

research is now required to understand whether there is an association between absolute troponin concentration and outcome in such populations.

Other factors that were clearly associated with increasing hs-cTn concentrations were age and gender. Specifically, almost double the proportion of patients in the 7th decade of life have hs-cTnI concentrations above the ULN when compared patients in their 6th decade of life. Together with the tendency for higher levels in males compared to females in our study, these observations lend weight to the concept that there should be age- and gender-specific quoted levels for ULN.

Strengths of this study

Previous literature in this field has confirmed the utility of the newer hs-cTn assays for early exclusion of AMI in a robust and safe manner (14-16, 32). However, interpretation of a single hs-cTnI concentration above the supplied ULN as being an indicator of AMI, and, more specifically, a T1MI, by front line clinicians has the potential to lead misdiagnosis and inappropriate investigations and treatment. The data presented here indicate that the prevalence of troponin levels above the supplied ULN in an important proportion of patients in whom there is no clinical suspicion of acute MI should raise a cautionary note.

The current findings also raise the important and interesting question about the potential implications of our observed distribution of hs-cTnI in the hospital population. Specifically, are the levels that we observe in these patients, for whom the suspicion of AMI is low (for example outpatients), actually abnormal? Do the levels indicate myocardial injury in their own right, and, if so, are they associated with adverse outcome, perhaps as biomarkers for future cardiovascular risk? There is an accumulating body of evidence that suggests that hs-cTn concentrations in populations of stable patients with chronic disease states, of both cardiac and non-cardiac origin, are indeed associated

with risk of cardiovascular events(33-42). Notably, in the outpatient population it has been reported that cTnI has indeed been shown to be associated with an increased risk of vascular events and all-cause mortality(43, 44). It is conceivable that the "elevated" hs-cTn concentrations in a stable patient always indicates myocardial injury or unwellness: the so called "never means nothing" hypothesis(45).

Implications of this study

The results of this study have significant implications for patient care. The notion of using a single binary value above the ULN of any assay to diagnose whether a patient has suffered an acute MI is flawed. This is highlighted by the observed 99th percentile in the CHARIOT study population which is over seven times higher than the ULN supplied by the manufacturer. Further, the observed frequency of hs-cTnI above the supplied ULN in our study, regardless of location, in patients in whom there was no clinical suspicion of acute MI or myocardial injury raises concerns about the utility of a 99th percentile value from a 'healthy population'. In particular, applying this supplied 99th centile value to determine the management of patients who are typically older, have more comorbidities, higher incidence of subclinical cardiac disease and in a worse physical condition than the reference healthy population may be flawed.

The results of this study should highlight to front line clinicians that whilst hs-cTnI can contribute to the diagnosis of AMI, this should only be when used in conjunction with other key factors such as the clinical history and other investigations(9, 24, 25, 29, 46-50). At present, the use of the 99th percentile to help rule out a diagnosis of AMI is clear and this is based on using a 'healthy' reference population. However, the use of this threshold level and its application to patients presenting to hospital to rule in AMI is problematic, particularly where the degree of suspicion is low and there are other factors that will contribute to the cTn concentration obtained in an individual. Currently, the implications of detecting a hs-cTnI above the supplied ULN, in terms of outcome and management, are unclear in

patients in whom there is low clinical suspicion of AMI. A more considered approach to application of cTnI concentrations would be a more tailored ULN according to the patient's baseline characteristics and comorbidities. The feasibility of this approach, however, remains unanswered. Further data regarding the potential association between hs-cTnI level and CV risk are required.

Limitations of this study

There are a number of limitations. Firstly, this is an observational study of a large number of consecutive patients. Necessarily, therefore, the level of detail with regard to management and diagnoses can only be obtained from the best records available for each patient, which included any electronic blood request or discharge summary data and formalised coding record. Secondly, this study has not looked at clinical outcomes since this was not part of our objective. Thirdly, in our analysis we have used discharge codes for diagnosis of AMI, but have not independently verified these final diagnoses. Finally, this study has looked at hs-cTnI concentrations in 20,000 patients based on a single sample for each patient, as a result this study cannot differentiate between acute and chronic myocardial injury.

Conclusions

This study has shown that the 99th percentile of the hospital population is substantially higher than the supplied ULN used in clinical practice according to the manufacturer provided 99th centile for a healthy population. Furthermore, the 99th percentile for the hospital population varies depending on the clinical acuity, location, age and gender of the individual, but in all subgroups there is a proportion of the patients in whom the hs-cTnI concentrations are above the clinically applied ULN. This is the largest study to date to evaluate hs-cTnI levels in an unselected cohort of 20,000 consecutive patients

and the observations from this study highlight the need for clinicians to interpret hs-cTnI concentrations carefully and systematically when attempting to diagnose AMI, particularly Type 1 MI.

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Transparency declaration

Professor Nick Curzen affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Author Contributions

M. Mariathas – Literature search, figures, study design, data collection, data analysis, data interpretation, writing.

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B. Olechowski – Data collection, data analysis, data interpretation and writing.

J. Hinton – Data collection, data analysis, data interpretation and writing.

S. Ramamoorthy – Data collection, data analysis, data interpretation and writing.

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References

1. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ. 2005;173(10):1191-202.

2. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. J Am Coll Cardiol. 2012;60(16):1581-98.

3. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol. 2018;72(18):2231-64.

4. Saenger AK, Jaffe AS. Requiem for a heavyweight: the demise of creatine kinase-MB. Circulation. 2008;118(21):2200-6.

5. Apple FS, Collinson PO, Biomarkers ITFoCAoC. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem. 2012;58(1):54-61.

6. Cediel G, Gonzalez-Del-Hoyo M, Carrasquer A, Sanchez R, Boque C, Bardaji A. Outcomes with type 2 myocardial infarction compared with non-ischaemic myocardial injury. Heart. 2017;103(8):616-22.

7. Chapman AR, Adamson PD, Mills NL. Assessment and classification of patients with myocardial injury and infarction in clinical practice. Heart. 2017;103(1):10-8.

8. Melberg T, Burman R, Dickstein K. The impact of the 2007 ESC-ACC-AHA-WHF Universal definition on the incidence and classification of acute myocardial infarction: a retrospective cohort study. Int J Cardiol. 2010;139(3):228-33.

9. Shah AS, Griffiths M, Lee KK, McAllister DA, Hunter AL, Ferry AV, et al. High sensitivity cardiac troponin and the under-diagnosis of myocardial infarction in women: prospective cohort study. BMJ. 2015;350:g7873.

10. Baron T, Hambraeus K, Sundström J, Erlinge D, Jernberg T, Lindahl B, et al. Impact on Long-Term Mortality of Presence of Obstructive Coronary Artery Disease and Classification of Myocardial Infarction. Am J Med. 2016;129(4):398-406.

11. Shah AS, McAllister DA, Mills R, Lee KK, Churchhouse AM, Fleming KM, et al. Sensitive troponin assay and the classification of myocardial infarction. Am J Med. 2015;128(5):493-501.e3.

12. Chapman AR, Shah ASV, Lee KK, Anand A, Francis O, Adamson P, et al. Long-Term Outcomes in Patients With Type 2 Myocardial Infarction and Myocardial Injury. Circulation. 2018;137(12):1236-45.

13. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary

Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J. 2016;37(3):267-315.

14. Twerenbold R, Jaeger C, Rubini Gimenez M, Wildi K, Reichlin T, Nestelberger T, et al. Impact of high-sensitivity cardiac troponin on use of coronary angiography, cardiac stress testing, and time to discharge in suspected acute myocardial infarction. Eur Heart J. 2016;37(44):3324-32.

15. Boeddinghaus J, Nestelberger T, Twerenbold R, Wildi K, Badertscher P, Cupa J, et al. Direct Comparison of 4 Very Early Rule-Out Strategies for Acute Myocardial Infarction Using High-Sensitivity Cardiac Troponin I. Circulation. 2017;135(17):1597-611.

16. Sandoval Y, Smith SW, Love SA, Sexter A, Schulz K, Apple FS. Single High-Sensitivity Cardiac Troponin I to Rule Out Acute Myocardial Infarction. Am J Med. 2017;130(9):1076-83 e1.

17. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, et al. Sensitive troponin I assay in early diagnosis of acute myocardial infarction. The New England journal of medicine. 2009;361(9):868-77.

18. Keller T, Zeller T, Ojeda F, Tzikas S, Lillpopp L, Sinning C, et al. Serial changes in highly sensitive troponin I assay and early diagnosis of myocardial infarction. Jama. 2011;306(24):2684-93.

19. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. The New England journal of medicine. 2009;361(9):858-67.

20. Mueller M, Biener M, Vafaie M, Doerr S, Keller T, Blankenberg S, et al. Absolute and relative kinetic changes of high-sensitivity cardiac troponin T in acute coronary syndrome and in patients with increased troponin in the absence of acute coronary syndrome. Clin Chem. 2012;58(1):209-18.

21. Christ M, Bertsch T, Popp S, Bahrmann P, Heppner HJ, Muller C. High-sensitivity troponin assays in the evaluation of patients with acute chest pain in the emergency department. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2011;49(12):1955-63.

22. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem. 2009;55(7):1303-6.

23. Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, et al. Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem. 2018;64(4):645-55.

24. Eggers KM, Lind L, Venge P, Lindahl B. Factors influencing the 99th percentile of cardiac troponin I evaluated in community-dwelling individuals at 70 and 75 years of age. Clin Chem. 2013;59(7):1068-73.

25. Cullen L, Greenslade JH, Carlton EW, Than M, Pickering JW, Ho A, et al. Sex-specific versus overall cut points for a high sensitivity troponin I assay in predicting 1-year outcomes in emergency patients presenting with chest pain. Heart. 2016;102(2):120-6.

26. Kumar N, Michelis MF, DeVita MV, Panagopoulos G, Rosenstock JL. Troponin I levels in asymptomatic patients on haemodialysis using a high-sensitivity assay. Nephrol Dial Transplant. 2011;26(2):665-70.

27. Egstrup M, Schou M, Tuxen CD, Kistorp CN, Hildebrandt PR, Gustafsson F, et al. Prediction of outcome by highly sensitive troponin T in outpatients with chronic systolic left ventricular heart failure. Am J Cardiol. 2012;110(4):552-7.

28. Avouac J, Meune C, Chenevier-Gobeaux C, Dieudé P, Borderie D, Lefevre G, et al. Inflammation and disease activity are associated with high circulating cardiac markers in rheumatoid arthritis independently of traditional cardiovascular risk factors. J Rheumatol. 2014;41(2):248-55.

29. Collinson PO, Heung YM, Gaze D, Boa F, Senior R, Christenson R, et al. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. Clin Chem. 2012;58(1):219-25.

30. Sandoval Y, Apple FS. The global need to define normality: the 99th percentile value of cardiac troponin. Clin Chem. 2014;60(3):455-62.

31. Gore MO, Seliger SL, Defilippi CR, Nambi V, Christenson RH, Hashim IA, et al. Age- and sex-dependent upper reference limits for the high-sensitivity cardiac troponin T assay. J Am Coll Cardiol. 2014;63(14):1441-8.

32. Shah AS, Newby DE, Mills NL. High sensitivity cardiac troponin in patients with chest pain. BMJ. 2013;347:f4222.

33. Saunders JT, Nambi V, de Lemos JA, Chambless LE, Virani SS, Boerwinkle E, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. Circulation. 2011;123(13):1367-76.

34. Thorsteinsdottir I, Aspelund T, Gudmundsson E, Eiriksdottir G, Harris TB, Launer LJ, et al. High-Sensitivity Cardiac Troponin I Is a Strong Predictor of Cardiovascular Events and Mortality in the AGES-Reykjavik Community-Based Cohort of Older Individuals. Clin Chem. 2016;62(4):623-30.

35. Iribarren C, Chandra M, Rana JS, Hlatky MA, Fortmann SP, Quertermous T, et al. High-sensitivity cardiac troponin I and incident coronary heart disease among asymptomatic older adults. Heart. 2016;102(15):1177-82.

36. Melki D, Lugnegård J, Alfredsson J, Lind S, Eggers KM, Lindahl B, et al. Implications of Introducing High-Sensitivity Cardiac Troponin T Into Clinical Practice: Data From the SWEDEHEART Registry. J Am Coll Cardiol. 2015;65(16):1655-64.

37. Ford I, Shah AS, Zhang R, McAllister DA, Strachan FE, Caslake M, et al. High-Sensitivity Cardiac Troponin, Statin Therapy, and Risk of Coronary Heart Disease. J Am Coll Cardiol. 2016;68(25):2719-28.

38. Whelton SP, McEvoy JW, Lazo M, Coresh J, Ballantyne CM, Selvin E. High-Sensitivity Cardiac Troponin T (hs-cTnT) as a Predictor of Incident Diabetes in the Atherosclerosis Risk in Communities Study. Diabetes Care. 2017;40(2):261-9.

39. Cavender MA, White WB, Jarolim P, Bakris GL, Cushman WC, Kupfer S, et al. Serial Measurement of High Sensitivity Troponin I and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus in the EXAMINE Trial. Circulation. 2017.

40. Wrigley P, Khoury J, Eckerle B, Alwell K, Moomaw CJ, Woo D, et al. Prevalence of Positive Troponin and Echocardiogram Findings and Association With Mortality in Acute Ischemic Stroke. Stroke. 2017.

41. Neukamm A, Einvik G, Didrik Høiseth A, Søyseth V, Henrik Holmedahl N, Kononova N, et al. The prognostic value of measurement of high-sensitive cardiac troponin T for mortality in a cohort of stable chronic obstructive pulmonary disease patients. BMC Pulm Med. 2016;16(1):164.

42. Willeit P, Welsh P, Evans JDW, Tschiderer L, Boachie C, Jukema JW, et al. High-Sensitivity Cardiac Troponin Concentration and Risk of First-Ever Cardiovascular Outcomes in 154,052 Participants. J Am Coll Cardiol. 2017;70(5):558-68.

43. Everett BM, Zeller T, Glynn RJ, Ridker PM, Blankenberg S. High-sensitivity cardiac troponin I and B-type natriuretic Peptide as predictors of vascular events in primary prevention: impact of statin therapy. Circulation. 2015;131(21):1851-60.

44. Than MP, Aldous SJ, Troughton RW, Pemberton CJ, Richards AM, Frampton CMA, et al. Detectable High-Sensitivity Cardiac Troponin within the Population Reference Interval Conveys High 5-Year Cardiovascular Risk: An Observational Study. Clin Chem. 2018;64(7):1044-53.

45. Mariathas M, Curzen N. Troponin assays: developing indications. Lancet. 2018;391(10138):2398-9.

46. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. Clin Chem. 2012;58(11):1574-81.

47. Hammarsten O, Fu ML, Sigurjonsdottir R, Petzold M, Said L, Landin-Wilhelmsen K, et al. Troponin T percentiles from a random population sample, emergency room patients and patients with myocardial infarction. Clin Chem. 2012;58(3):628-37.

48. Aw TC, Phua SK, Tan SP. Measurement of cardiac troponin I in serum with a new high-sensitivity assay in a large multi-ethnic Asian cohort and the impact of gender. Clin Chim Acta. 2013;422:26-8.

49. Rubini Gimenez M, Reiter M, Twerenbold R, Reichlin T, Wildi K, Haaf P, et al. Sex-specific chest pain characteristics in the early diagnosis of acute myocardial infarction. JAMA Intern Med. 2014;174(2):241-9.

50. Koerbin G, Abhayaratna WP, Potter JM, Apple FS, Jaffe AS, Ravalico TH, et al. Effect of population selection on 99th percentile values for a high sensitivity cardiac troponin I and T assays. Clin Biochem. 2013;46(16-17):1636-43.

Summary Box

What is already known:

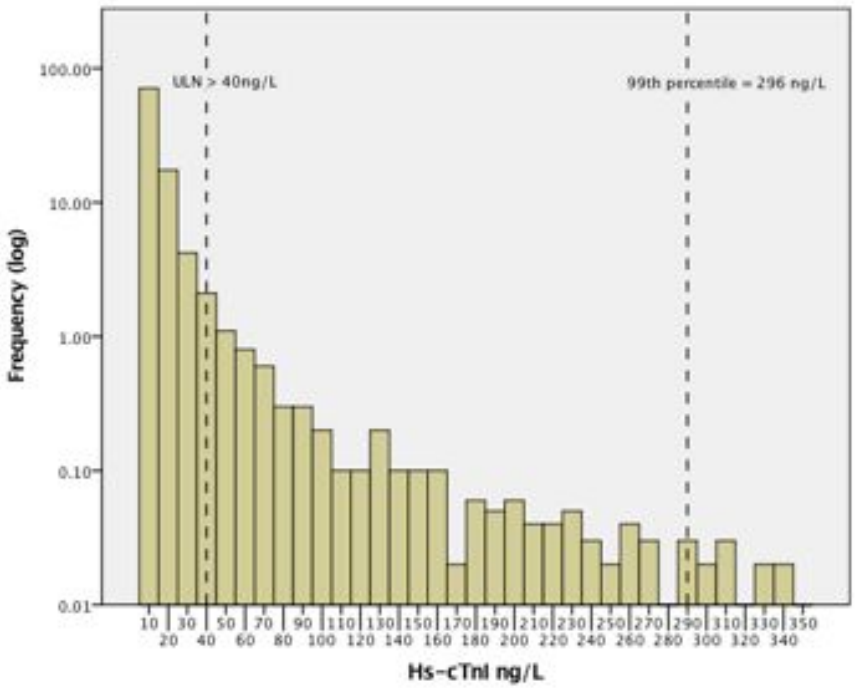
- Current guidelines recommend the use of troponin assays to aid in the exclusion or diagnosis of acute myocardial infarction.
- Manufacturers of troponin assays provide a recommended 99th percentile for the assay that is based upon a few hundred healthy subjects. This is often used as an upper limit of normal when applied to the hospital population.
- It is known that a variety of clinical factors affect the troponin level, such as age and renal function, but little is known about the true distribution of the troponin level in an all comers hospital population.

What this study adds:

- In a hospital population of 20,000 consecutive patients 1/20 of all patients have a high sensitivity troponin I concentration above the manufacturer's provided 99th percentile, in most of whom there was no clinical suspicion of acute MI.
- This highlights the importance of interpreting the troponin result in hospital patients according to (a) the individual patient and their clinical presentation and (b) the guideline recommendations for correct diagnosis of Type 1 & 2 MI.
- These results may help to avoid misdiagnosis and inappropriate treatment.

Figure 1: A log distribution of high sensitivity cardiac troponin I (Hs-cTnI) concentration (1a) in the whole population (n=20,00) and (1b) in the final study population (n=18,171). (ULN = Manufacturer's recommended upper limit of normal for Hs-cTnI concentration (>40 ng/L).

1a



1b

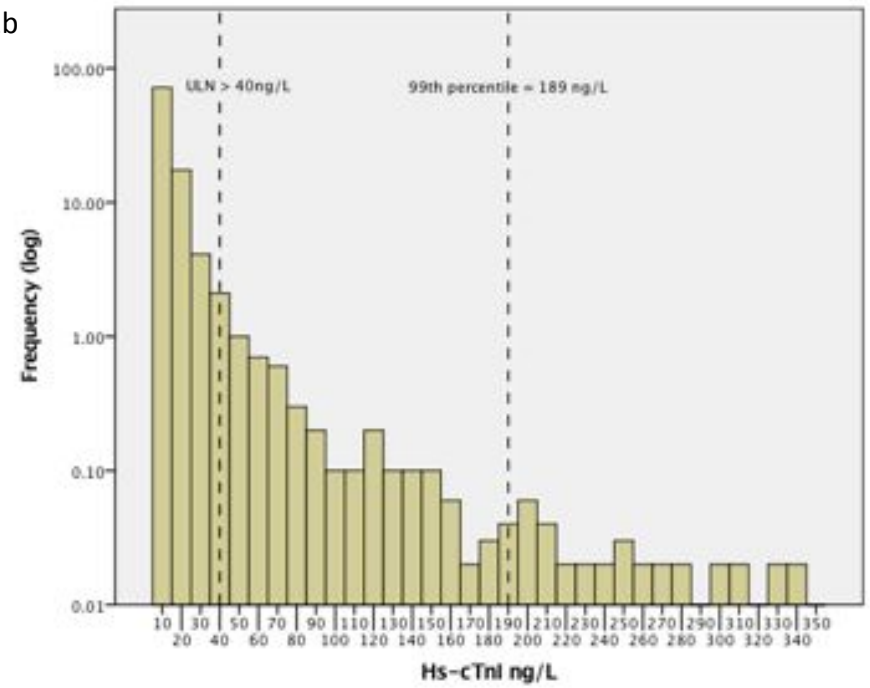


Figure 2: Proportion of patients with high sensitivity cardiac troponin I concentration above the upper limit of normal (ULN = 40ng/L) according to location

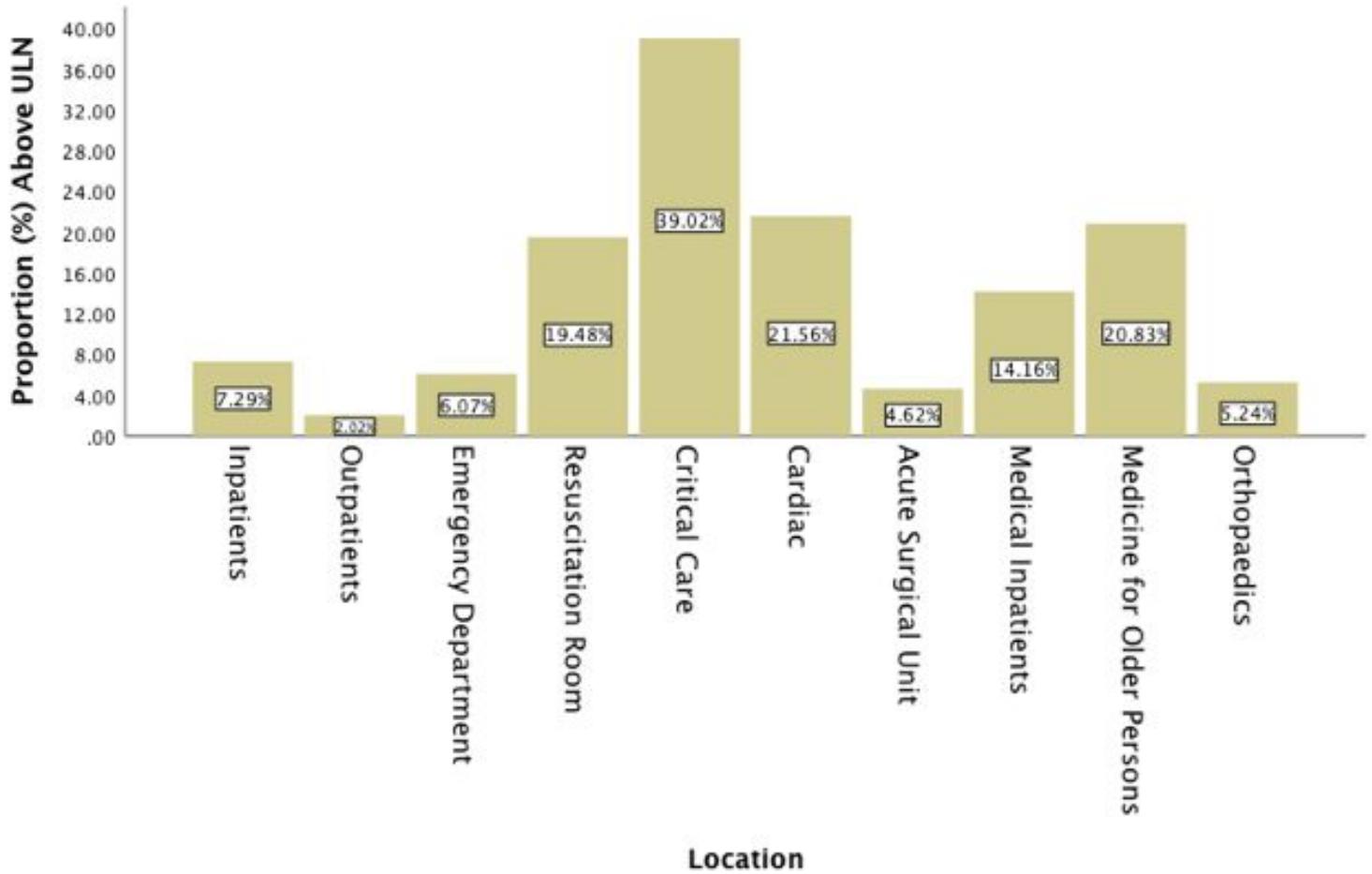


Figure 3: Upper limit of normal (ULN) high sensitivity cardiac troponin I concentration according to age

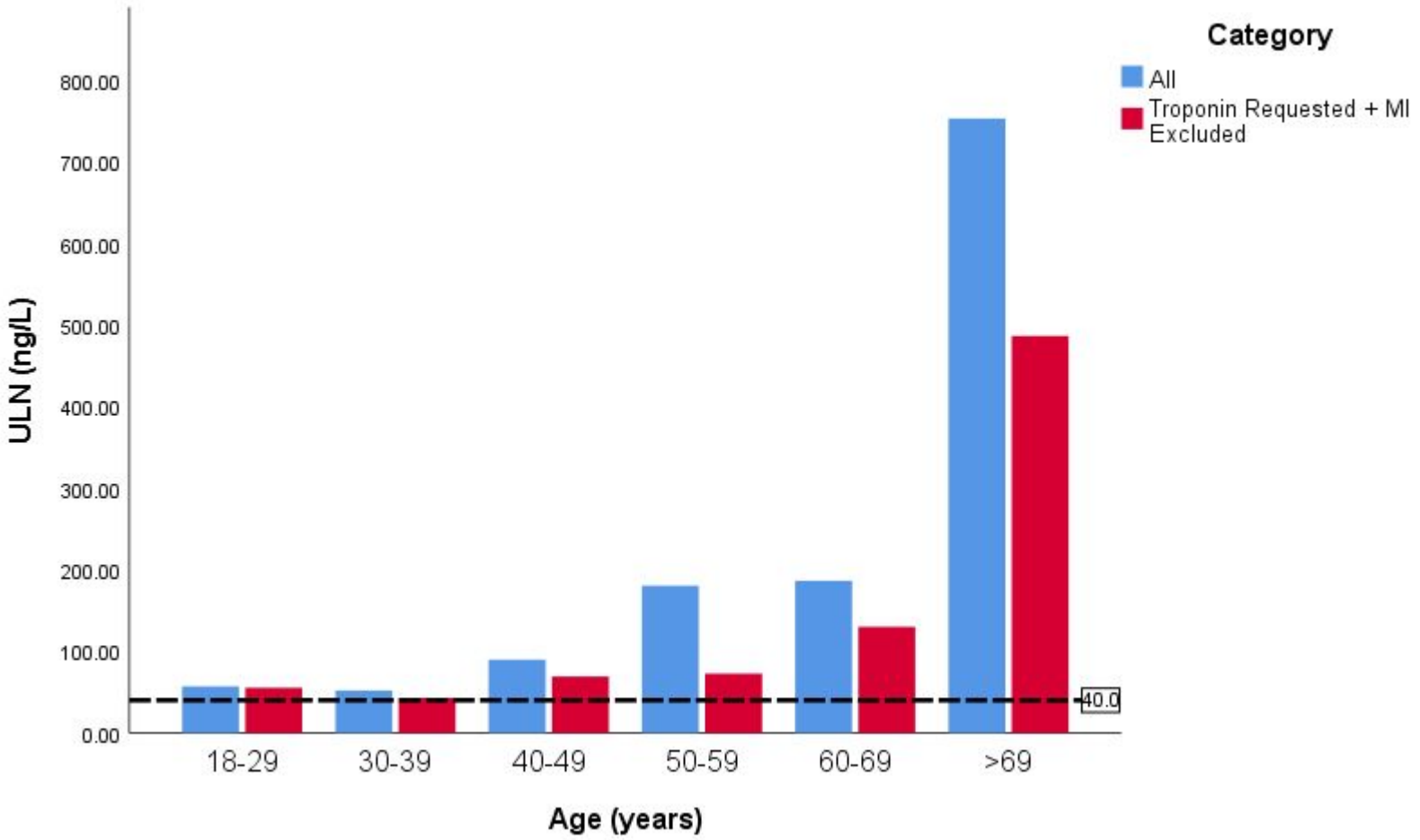


Table 1: Baseline characteristics stratified by hs-cTnI levels (ng/L) below or above the ULN (Upper limit of normal = 40ng/L)

	Hs-cTnI below ULN (n=18915)	Hs-cTnI above ULN (n=1085)	P value
Age (years)	57.4	74.2	<0.001
Male Gender (%)	46.5	57.3	0.005
eGFR	79.1	59.6	<0.001
Na^{2+}(mmol/L)	137.2	136	<0.001
Inpatients(%)	24.0	37.3	<0.001
Outpatients(%)	48.4	17.4	<0.001

Table 2: Distribution of hs-cTnI (ng/L) according to location. [ULN - Upper limit of normal = 40ng/L].

Location	Median (ng/L)	Interquartile Range (ng/L)	Range (ng/L)	Proportion above ULN (%)	99th Centile (ng/L)
Inpatients (n=4759)	7	10	14994	7.29 (n=347)	563
Outpatients(n=9280)	5	8	3255	2.02 (n=187)	65
Emergency Department (n=3706)	7	9	6106	6.07 (n=225)	215
Resuscitation Room (n=426)	11	24	10979	19.48 (n=83)	1839
Critical Care (n=123)	25	115	13086	39.02 (n=48)	12097
Cardiac (n=269)	14	28	14994	21.56 (n=58)	3967
Acute Surgical Unit (n=346)	6	9	2668	4.62 (n=16)	92
Medical Wards (n=473)	12	22	8807	14.16 (n=67)	1459
Medicine for Older People (n=96)	20	27	3508	20.83 (n=20)	-
Orthopaedics (n=248)	8	9	402	5.24 (n=13)	184

Table 3: Independent predictors of hs-cTnI levels [ULN - Upper limit of normal = 40ng/L].

Variable	Predictors of manufacturer troponin ULN >40 ng/L (n=18,171)	Predictors of non-parametric troponin ULN >189 ng/L (n=18,171)
Age (per year increase)	1.03 (1.03-1.04) (p<0.001)	1.03 (1.02-1.04) (p<0.001)
Male gender	1.33 (1.14-1.54) (p<0.001)	0.90 (0.66-123) (p=0.513)
Sodium (per unit increase)	0.99 (0.97-1.01) (p=0.336)	1.01 (0.97-1.04) (p=0.742)
eGFR (per unit increase)	0.98 (0.97-0.98) (p<0.001)	0.99 (0.98-1.00) (p=0.001)
Location vs outpatient		
Emergency Department	2.79 (2.26-3.43) (p<0.001)	3.46 (2.14-5.61) (p<0.001)
Resuscitation Room	9.91 (7.3-13.46) (p<0.001)	13.79(7.67-24.77)(p<0.001)
Critical Care	36.62 (23.86-56.2) (p<0.001)	99.27 (55.51-177.54) (p<0.001)
Cardiac	9.08 (6.44-12.81) (p<0.001)	14.91 (7.91-28.11) (p<0.001)
Acute surgical ward	2.52 (1.47-4.33) (p=0.001)	0.98 (0.13-7.21) (p=0.982)
Medical wards	4.74 (3.45-6.50) (p<0.001)	5.80 (2.95-11.42) (p<0.001)
Medicine for older people	3.70 (2.16-6.34) (p<0.001)	9.60 (4.00-23.00) (p<0.001)
Orthopaedics	2.24 (1.23-4.05) (p=0.008)	2.15 (0.51-9.14) (p=0.298)

Supplementary Table 1: hs-cTnI (ng/L) concentration and age

Cohort	Age group (n)	Centile			
		Centile 25	Centile 50	Centile 75	Centile 99
Full cohort (n=20,000)	18-29 (n=2,301)	0	3	6	56.94
	30-39 (n=2,046)	1	4	7	52
	40-49 (n=2,127)	1	4	8	89.88
	50-59 (n=3,079)	2	5	9	180.40
	60-69 (n=3,588)	3	6	11	186.55
	>69 (n=6,879)	6	11	20	752.60

Supplementary Table 2: hs-cTnI (ng/L) concentration and age with troponin requested and confirmed MI excluded

Cohort	Age group (n)	Centile			
		Centile 25	Centile 50	Centile 75	Centile 99
Sample excluding MI (n=18,171)	18-29 (n=2,050)	0	3	6	55.47
	30-39 (n=1,849)	1	4	7	43
	40-49 (n=1,898)	1	4	8	69.01
	50-59 (n=2,784)	2	5	9	72.60
	60-69 (n=3,322)	3	6	11	130
	>69 (n=6268)	6	11	19	486.34

Supplementary Table 3

Variable	Predictors of manufacturer troponin ULN >40 ng/L (n=20,000)	Predictors of non-parametric troponin ULN >296 ng/L (n=20,000)
Age (per year increase)	1.03 (1.03-1.04) (p<0.001)	1.03 (1.02-1.04) (p<0.001)
Male gender	1.38 (1.21-1.58) (p<0.001)	1.00 (0.75-1.34) (p=0.998)
Sodium (per unit increase)	0.99 (0.98-1.01) (p=0.538)	1.01 (0.97-1.05) (p=0.638)
eGFR (per unit increase)	0.979 (0.976-0.982) (p<0.001)	0.99 (0.99-1.00) (p=0.002)
Location vs outpatient		
Emergency Department	3.47 (2.88-4.19) (p<0.001)	7.46 (4.22-13.19) (p<0.001)
Resuscitation Room	11.84 (9.09-15.41) (p<0.001)	30.59(16.27-57.54)(p<0.001)
Critical Care	44.02 (29.81-65.01) (p<0.001)	190.86 (99.59-365.76) (p<0.001)
Cardiac	12.48 (9.28-16.77) (p<0.001)	31.30 (15.86-61.78) (p<0.001)
Acute surgical ward	2.62 (1.55-4.43) (p<0.001)	1.87 (0.25-14.26) (p=0.544)
Medical wards	4.85 (3.57-6.60) (p<0.001)	8.86 (3.92-20.05) (p<0.001)
Medicine for older people	3.83 (2.24-6.55) (p<0.001)	14.54 (5.08-41.56) (p<0.001)
Orthopaedics	2.22 (1.23-4.02) (p=0.008)	2.15 (0.28-16.42) (p=0.460)

REVISED MANUSCRIPT

Is the *C*urrent T*h*reshold for Diagnosis of "*A*bnormality", including Non-ST Elevation Myocardial Infarction, using *R*aised H*i*gh Sensitivity Tr*o*ponin Appropriate for a Hospi*t*al Population?

The CHARIOT Study

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High sensitivity troponin, 99th percentile, hospital population

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All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Abstract

Objective

~~Currently~~ Clinicians use the cardiac troponin (cTn) assay to ~~help determine if a patient has suffered~~ aaid in the diagnosis of an acute myocardial infarction (AMI). Abnormal cTn levels are predetermined by the assay manufacturer. ~~This abnormal level, which is also known as~~ Each manufacturer provides the 99th percentile for ~~or upper limit of normal (ULN), is traditionally based on~~ cTn levels in ~~300 healthy males and 300 healthy females~~a group of healthy individuals, and this level is taken as the upper limit of normal (ULN). The objective of this study was to determine ~~what is~~ the distribution, and specifically the true 99th percentile, for the whole hospital population, ~~when~~ using the cTn assay currently ~~in use~~employed routinely at our institution. ~~To define the 99th percentile of high sensitivity cardiac troponin I (hs-cTnI) concentration for a hospital population.~~

Design

Prospective study of 20,000 consecutive patients undergoing blood sampling for any reason at a large teaching hospital. Hs-cTnI concentrations (Beckman Coulter Access AccuTnI+3 assay) were nested for analysis in all cases except those in whom the supervising physician had requested hs-cTnI for clinical reasons.

Setting

University Hospital Southampton NHS Trust (UHS).

Participants

20,000 consecutive individuals, inpatient or outpatient, undergoing blood tests at UHS for any clinical reason. ~~Hs-cTnI concentrations (Beckman Coulter Access AccuTnI+3 assay) were nested for analysis in all cases except those in whom the supervising physician had requested hs-cTnI for clinical reasons.~~

Main outcome measures

Distribution of hs-cTnI concentrations of all study patients, and specifically the 99th percentile~~.~~

Results

The 99th percentile of hs-cTnI for the whole population (n=20,000) was 296 ng/L, compared to a manufacturer quoted 99th percentile of 40 ng/L (currently used clinically as the ~~"upper limit of normal",~~ ULN). In 1 in 20 (5.4%, n=1080) of the total population hs-cTnI concentrations were above 40 ng/L. After exclusion of individuals diagnosed with an acute myocardial infarction (AMI) (n=122), or those in whom troponin was requested (n=1707), the 99th percentile ~~of~~ for the remainder (n=18,171) was 189 ng/L. The 99th percentile for inpatients (n=4759) and outpatients (n=9280) was 563 ng/L and 65 ng/L, respectively. Patients from the emergency department (n=3706) had a 99th percentile of 215 ng/L, with 6.1% (n=~~491~~225) above the quoted ULN. 39.02% (n=48) of all individuals from the critical care units (n=123) and ~~15.7~~14.16% (n=~~87~~67) of all medical inpatients had a hs-cTnI concentration above the quoted ULN.

Conclusions

In 20,000 consecutive patients undergoing a blood test for any reason at this hospital 1 in 20 have a hs-cTnI above the supplied ULN. These data highlight the need for clinical staff to interpret hs-cTnI concentrations carefully, particularly when applying the ~~manufacturer's~~ supplied ULN to diagnose AMI. The sole use of hs-cTnI to diagnose AMI in any patient ~~without a typical history may be flawed~~could~~an~~ lead to misdiagnosis in the absence of an appropriate clinical presentation.

REVISED MANUSCRIPT

Introduction

The use of increasingly sensitive troponin assays for the exclusion or diagnosis of acute myocardial infarction (AMI) has become universal. The diagnosis of AMI is now defined by a rise and/or fall of~~in~~ cardiac troponin (cTn) concentration~~s~~, now the gold standard biomarker(1), with at least one value above the 99th percentile derived from a reference population of healthy individuals in the context of ~~associated with~~ an appropriate clinical ~~context, which includes the presence of clinical ischaemia~~presentation(2, 3) (3-5).

Under most circumstances, the troponin assay is ~~used~~ requested by ~~the~~ front line ~~clinician~~ clinical staff to determine whether or not ~~the~~ a patient is experiencing a Type 1 myocardial infarction (T1MI), which is due to coronary plaque rupture or erosion, since robust evidence has demonstrated symptomatic and prognostic benefit from the application of early pharmacological and interventional treatment strategies in such patients. However, particularly with the advent of newer assays, this strategy has 2 potential ~~flaws~~issueschallenges.

Firstly, ~~elevations in~~elevated cTn concentrations, particularly in patients not presenting with a typical history of cardiac pain, are often due to myocardial injury or Type 2 myocardial infarction (T2MI)(6, 7), which is secondary to ischaemia due to either increased oxygen demand or decreased supply rather than a plaque erosion event (8-10). ~~Not only is T~~this is not well recognized when the troponin test is requested, or the result interpreted, ~~but~~ and is especially important because the majority of patients with T2MI have ~~never~~ not been shown to benefit from the same aggressive pharmacotherapy and invasive investigation and treatment that is offered as standard in cases of T1MI(11), with some exceptions including spontaneous coronary dissection, coronary embolism and coronary spasm. (10, 12). In fact, such ~~misdiagnosis~~ misinterpretation may lead to inappropriate management, including prolonged antiplatelet therapy and invasive coronary angiography, ~~+/-~~ with or without revascularization. ~~with the longer term risks that this entails, with the exception of some categories~~

of Type 2 MI. It should be noted,Whilst in some aetiologies of however, that in some T2MI with coronary aetiologies such as spontaneous coronary dissection, coronary embolism and coronary spasm., coronary angiography and further imaging may be useful, in the majority it may not be (10, 12) (Baron AM J Med/ Chapman circulation).

Secondly, the assay-specific manufacturer's 99th centile (ULN) is generally applied as a binary "rule in" or "rule out" threshold for AMI. Whilst recent trial data confirm the veracity of the use of early cTn concentrations to confidently exclude the diagnosis of AMI (13-16), the assumption that a concentration above that level implies AMI (and in particular a T1MI) is often inappropriate. Both of these potential flaws issues in our clinical practice may be compounded in clinical practice by the increasing sensitivity of the available assays, particularly with the introduction of hs-cTn assays that are able to detect troponin at much lower concentrations than previously assays (5). Consquently, nNew highly sensitive cardiac troponin (hs-cTn) assays (17-21) allow for rapid exclusion of AMI, in order to enableand thereby facilitate the early discharge of patients from hospital. Furthermore, modern hs-cTn assays can detect troponin in more than 50% of the general population, with some assays able to detect troponin in everyone(22). The appropriate interpretation of the "elevated" hs-cTn, particularly in relation to the diagnosis of T1MI, is therefore dependent upon a clinical presentation consistent with this diagnosis, and in particular, a history of cardiac-sounding chest pain, according to the guidelines. Currently, Tthe International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers (IFCC TF-CB) currently recommends that the 99th percentile for any assay can be calculated using 300 'healthy' men and 300 'healthy' women(23). Given the number of factors that are well known to affect an individual's troponin and therefore the ULN(23), including age(24), gender(25), glomerular filtration rate(26), left ventricular function(27), and the presence of significant inflammatory conditions (28), the appropriateness of the clinically applied concept of an ULN for the trop hs-cTn assay requires closer scrutiny, particularly when this binary cut-off levelit was derived from a limited number of healthy

individuals. Importantly, the approaches to determining the 99th percentile are ~~highly~~also variable ~~, with the more strict criteria used in determining healthy individuals to result in lower 99th percentile values~~(29-31).

The aims of this study were to determine (a) the true distribution of hs-cTnI concentration in an unselected all comer hospital population, both inpatient and outpatient, and (b) the 99th percentile for this population using 20,000 consecutive patients. Our hypothesis was that the true distribution of hs-cTnI in this population would differ from the ~~manufacturer's~~ supplied ULN for this assay, thereby highlighting the potential for misinterpretation of a value above this level in routine clinical practice, particularly ~~calling into question~~ the validity of applying the latter as the binary arbiter of the diagnosis of AMI, especially T1MI~~, in clinical practice~~.

Methods

Study population

This was a prospective, observational study that included 20,000 consecutive patients aged at least 18 years ~~who were undergoing~~in whom a biochemistry blood investigation~~s~~ was requested for clinical reasons determined by their supervising physician at our institution, a large University teaching hospital in the United Kingdom. Patients were included regardless of the setting in which the blood test was ~~taken~~requested, so that the study population included outpatients and inpatients, emergency department attendees, elective and emergency admissions, and every specialty within the hospital. For each patient included in the study~~,~~ only one troponin ~~assay~~ measurement was performed on the first biochemistry blood sample that became available during the study period. That individual

was then excluded from further sampling, in order that a consecutive series of 20,000 different patients were sampledincluded. For some of the study analysis, pPatients who were discharged from hospital with a diagnosis of AMI or in whom a hs-cTnI level was requestedwho had been suspected of AMI by the clinical team, which hadwas been determined through a review of the electronic blood request forms submitted to the biochemistry department and via electronic discharge summaries, had been were excluded. leaving a final study population of 18,171 individuals. A flow diagram of this is shown in figure 1.

Ethics & Regulatory Approval

This research project was undertaken according to the principles of Good Clinical Practice and the Declaration of Helsinki. The study was approved by the local ethical committee who then referred it to the Health Research Authority (HRA) UK and its independent Confidentiality Advisory Group (CAG) for further approval (Rec reference: 17/SC/0042, IRAS project ID: 215262). The latter CAG approval was required based upon 2 unusual aspects of the methodology. Firstly, the method did not require knowledge or consent from patients that an extra blood assay was being performed. Secondly, for all patients in our study, apart from those in whom a hs-cTnI was requested as part of their routine clinical care by their supervising clinician, the result of the hs-cTnI test was nested and never revealed to either patient or their supervising clinical team, regardless of whether the result was above the manufacturer's supplied ULN. The trialstudy is registered with Clinicaltrials.gov, number NCT03047785.

Cardiac troponin I assay

The Beckman Coulter Access AccuTnI+3 assay (Beckman Coulter, Brea, CA, USA) is employed in routine clinical practice at our Trust and is employed in routine clinical practice at our Trust and was used to measure hs-cTnI concentrations in the study population. The ~~manufacturer recommended~~ supplied 99th percentile (ULN) is 40 ng/L, which is the level used in routine clinical practice at our institution. The coefficient of variation (CV) of the assay is <10% at 40ng/L, the limit of quantification (LOQ 10% CV) is 20ng/L; the limit of detection (LOD) is 8ng/L; the limit of blank is 5ng/L. For those patients in whom troponin had not been requested for clinical reasons, the hs-cTnI concentration was measured for every individual using serum which was surplus to clinical need. An automated, bespoke system was put in place in Biochemistry to ensure each individual was only included once in the study. Serum was collected into serum separator tubes and stored at room temperature for up to 24 hours before cTnI levels were measured through the use of the DxI800 platform (Beckman Coulter, Brea, CA, USA). Quality control of the assay was undertaken on a daily basis as is routine in clinical practice.

Data Collection

Baseline demographic data were limited to those derived from electronic request forms for blood tests and, for in patients, from electronic discharge summary codes. These data, together with the troponin levels and other study data were collected on a bespoke database for later analysis.

Patient and public involvement

The British Cardiac Patients Association (BCPA) assisted the researchers in review of the study protocol, with particular reference to the lack of consent of participants. A letter of support for our methodology from the Chairman of the BCPA was submitted to the HRA/CAG as part of our study application.

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Statistical Analysis

Descriptive statistical methods were used Tto define Tthe 99th percentile for the study population was defined using a non-parametric procedure based on frequency tables. Statistical analyses were performed using IBM SPSS V.22.0 (SPSS, IBM Corporation, Armonk, New York, USA). We used Stata 14.0 (College Station, USA) to perform multiple logistic regressions to identify factors associated with elevated highly sensitive troponin above 40 ng/L. Variables in the model included age, male sex, serum sodium, estimated glomerular filtration rate and location.

Results

A total of 20,000 consecutive patients were included in CHARIOT betweenfrom 29/06/2017 to 24/08/2017. The median age was 61 (standard deviation of 20) years and 52.9% were female, (n = 10,580).

The 99th percentile hs-cTnI concentration for the whole study population (n=20,000) was 296 ng/L, with 1 in 20 (5.4%; n=1080) of the patients having a hs-cTnI concentration above the manufacturer's supplied ULN (40 ng/L). Once all patients who had been diagnosed with an AMI on discharge or in whom a troponin levelhs-cTnI had been requested on the basis of a clinical suspicion of -MI had been excluded, this left a group of 18,171 patients in whom -the 99th percentile -was 1899 ng/L, with 4.6% (n=836) above 40 ng/L (Figure 12). Baseline characteristics whether hs-cTnI concentrations were below or above the ULN are shown in table 1.

Of the 1707 patients ~~who had~~in whom hs-cTnI concentrations were requested by the clinical team, 73% (n=1246) had presented with chest pain, ~~both~~with arrhythmia (n=52) and suspected ~~cardiac~~ ~~syncope~~ blackouts(n=63) ~~were~~ the next most common reason for ~~referral~~the test.

Patient Location

Patients were stratified according to their location at the time the biochemistry test was requested.

Specifically, the study included 9280 (51.1%) hospital outpatients in whom the observed 99th percentile was 65 ng/L, with hs-cTnI concentrations above the ~~manufacturer's~~ supplied ULN in 2% (n=186). 4759 (26.2%) of the study population were patients admitted. The 99th percentile for this inpatient group was 563 ng/L, and the hs-cTnI concentrations were above the ~~manufacturer's quoted~~supplied ULN in 7.293% (n=~~1326~~347) ~~of these~~.

A total of 5708 patients had their blood ~~sampling~~ sampling ~~taken~~ in the emergency department (ED). Of this group, 1551 (27.2%) had hs-cTnI concentrations requested by the ED clinicians. ~~Once patients who had been diagnosed with MI or had hs-cTnI concentrations requested by the ED clinicians~~ Tthe 99th percentile for the remaining ED population (n= 3706) was 215 ng/L~~. This population did not include patients who had blood sampling undertaken on the resuscitation room.~~with 6.071% (n=2256) of ~~ED patients had~~these having hs-cTnI concentrations above the ~~manufacturer's~~ supplied ULN. Patients managed in the resuscitation room (n=426) of the ED had hs-cTnI concentrations above the ULN in 19.485% (n=83).

For patients managed in the critical care environment (3 intensive care and 2 high dependency units) ~~in the centre~~ (n= 123), 39.02% (n=48) had hs-cTnI concentrations above the ULN.

Once all patients who had either been diagnosed with MI or hs-cTnI requested by the clinical team were excluded, a total of ~~15.7~~14.16% (n=67~~87~~) of all medical inpatients (excluding cardiac) had hs-cTnI concentrations above the ~~manufacturer's~~ supplied ULN. 20.8% of patients from the medicine for older people (MOP) (n= 20) also had hs-cTnI concentrations above the ~~manufacturer's~~ supplied ULN. 4.62% (n=16) of patients managed on the acute surgical unit had hs-cTnI above the ULN. For orthopaedic patients ~~4.8~~5.24% (n=13~~2~~) had hs-cTnI concentrations above the ULN. In none of these patients was an acute MI suspected or diagnosed (Table 2; Figure 2).

~~The distribution of hs-cTnI concentrations for each location is shown in figure 3table 21, with the proportion of patients with a hs-cTnI above the ULN displayed in figure 2.~~

Age

There was an association between increasing age and distribution of troponin concentration. Percentiles (25th,50th,75th, and 99th) and proportion of patients with hs-cTnI above the ULN according to age is shown in supplementary Ttable 1 and F~~f~~igure ~~4~~3.

Gender

The 99th percentiles for males and females ~~was~~ were 373 ng/L and 236 ng/L respectively. 6.6% (n=622) of male and 4.384% (n=46~~36~~) of females had hs-cTnI concentrations above the ULN. Significant differences were seen in mean hs-cTnI levels when comparing males to females (62 vs 31 ng/L, p=0.021).

Multivariate Multivariable analysis

Once all patients who had either been diagnosed with MI or had hs-cTnI concentrations requested by the clinical team (n=1829) were excluded, A a multivariablete analysis was undertaken to assess the independent predictors of an individual having a hs-cTnI concentration above the manufacturer's recommended supplied ULN (40 ng/L). Advancing age (odds ratio (OR) 1.036(1.031-1.041), p<0.001), male gender (OR 1.33449, (1.14253-1.54676), p<0.001) and reducing estimated glomerular filtration (OR 0.97798(0.974-0.980), p<0.001) were shown to be independent predictors. Furthermore, when compared to the outpatient population, patients who were fromlocation in the ED (OR 3.4512.79 (2.860-4.1652.26-3.43), p<0.001), resuscitation room(OR 11.8159.91 (9.062-15.4037.3-13.46), p<0.001), critical care units (OR 16.60136.62(9.252-29.78423.86-56.2), p<0.001), cardiac wards (OR 18.3899.08, (14.036-24.0926.44-12.81), p<0.001), acute surgical unit (OR 2.6222.52(1.549-4.4391.47-4.33), p<0.001), medical wards (OR 5.0084.74(3.693-6.7923.45-6.50), p<0.001), MOP wards (OR 3.6643.70 (2.135-6.28816-6.34), p<0.001) and orthopaedic wards (OR 2.24 5(1.24023-4.06405), p=0.008) were found to be independent predictors of an individual having afor hs-cTnI concentration above the ULN (Figure 5table 3). Independent predictors of an individual having a hs-cTnI concentration above the 99th centile for this population (hs-cTnI level of 189 (ng/L)) is also shown in table 3. Independent predictors for the full cohort (n=20,000) are shown in the Supplementary table 3.

Discussion

This study, which is to our knowledge the largest of its kind, has shown that 1 in 20 of consecutive all comer patients at a large UK University hospital have a troponin level that is greater than the manufacturer's supplied 99th centile (ULN) for the assay. These Our data also demonstrate that the 99th centile varies according to the clinical setting, age and gender, and location with a range of 2% of outpatients and 39% of patients in critical care settings having a cTnI greater than the manufacturer's supplied ULN depending upon their location and status.

These results have important clinical implications that are almost certainly relevant to the application of all modern hs-cTn assays. Firstly, they confirm our original hypothesis that the true 99th centile for a general hospital population is not consistent with the manufacturer's quotedsupplied ULN. Secondly, these data raise important questions about the appropriate applicability of the quoted ULN as an sole an arbiter of Type 1 AMI in patients who do not give a typical history consistent with this diagnosis. The previous evidence for the use of cTnI levels to rule out AMI is clear cut and justifiedrobust (14-16, 32)(10-12, 24). The Ffourth Uuniversal Ddefinition(3) recommends the diagnosis of AMI to be diagnosed when there is clinical evidence of acute myocardial ischaemia and with detection of a rise and/or fall of cTn values. SpecificallyHowever, the utility of the manufacturer's supplied ULN as a "rule in" test for AMI in patients presenting with atypical symptoms and other comorbidities, such as in the context of ED or acute medicine and surgical patients, is flawed and potentially exposes such patients to inappropriate pharmacological and invasive treatment that has only been shown to be beneficial in true T1MI populations. Although this management strategy is not recommended as per the Fourth Universal Definition(3), tThis study highlights the importance of interpreting hs-cTnI results with caution in an individual patientpitfalls of using this strategy. This The risk of potential systematic misdiagnosis of AMI is particularly highlighted illustrated by the observed 99th centile for hs-cTnI in our subpopulations of ED (215 ng/L) and acute medical admissions (1432

1459 ng/L), and that close to 40% of patients in some clinical settings have hs-cTnI levels above the ~~manufacturers~~ supplied ULN. It is particularly important for frontline clinical staff to ~~grasp and demonstrates~~understand that using a single cutoff of hs-cTnI to diagnose AMI may be~~is~~ inappropriate and that the ULN of the assay will depend on the clinical environment as well as clinical characteristics of patients. ~~that all~~ We would advocate that clinical staff ~~should be~~ are aware of the current guidelines in diagnosing AMI, which are ~~often~~ not always adhered to, and also that they have a very clear indication for requesting the test.

Our analysis highlights a number of factors that are associated with "elevated" hs-cTnI results as judged by the ~~manufacturer's~~ supplied reference, including mode of presentation. Thus, 7.~~3~~29% of all inpatients in this study had an "elevated" hs-cTnI concentration, including 6.~~1~~07% of ED patients and 19.~~5~~48% of those admitted to the resuscitation room. It is more predictable that nearly 40% of patients admitted to a critical care setting have an elevated concentration. However, the finding that our observed 99th centile for hs-cTnI concentrations was 65ng/L in outpatients, and that 2% of these patients who attended the hospital only for a clinic appointment had a concentration above the ~~manufacturer's~~ supplied ULN, highlights the need for a review of quoted distribution of ~~the~~ hs-cTn assay in a hospital setting ~~and its quoted distribution is used in a hospital setting~~. Further research is now required to understand whether there is an association between absolute troponin concentration and outcome in such populations.

Other factors that were clearly associated with increasing hs-cTn concentrations were age and gender. Specifically, almost double the proportion of patients in the 7th decade of life have hs-cTnI concentrations above the ULN when compared patients in their 6th decade of life. Together with the

tendency for higher levels in males compared to females in our study, these observations lend~~s~~ weight to the concept that there should be age- and gender-specific quoted levels for ULN.

Strengths of this study

Previous literature in this field has confirmed the utility of the newer hs-cTn assays for early exclusion of AMI in a robust and safe manner (14-16, 32). However, interpretation of a ~~the assumption that a~~if ~~it is assumed by clinicians that a~~ single hs-cTnI concentration ~~that is elevated~~ above the ~~manufacturer's~~ supplied ULN ~~is~~ as being an indicator of AMI, and, more specifically, a T1MI, ~~is called into question by our findings~~ by front line clinicians has the potential ~~is likely~~ to lead misdiagnosis and inappropriate investigations and treatment. The data presented here indicate that~~Certainly~~, the prevalence of troponin levels above the ~~manufacturer's~~ supplied ULN in an important proportion of patients in whom there is no clinical suspicion of acute MI should raise a cautionary note. ~~for front line clinical staff who request the troponin test in patients in whom the presentation is uncharacteristic of AMI.~~

The current findings also raise the important and interesting question about the potential implications of our observed distribution of hs-cTnI in the hospital population. Specifically, are the levels that we observe in these patients, for whom the suspicion of AMI is low (for example outpatients), actually abnormal? Do the levels indicate myocardial injury in their own right, and, if so, are they associated with adverse outcome, perhaps as ~~as such, carry importance as~~ biomarkers for future cardiovascular risk? There is an accumulating body of evidence that suggests that hs-cTn concentrations in populations of stable patients with chronic disease states, of both cardiac and non-cardiac origin, are indeed associated with ~~cardiovascular~~ risk of cardiovascular events(33-42). ~~It should be noted that~~Notably, in the outpatient population it has been ~~shown~~reported that cTnI has indeed been shown

to be associated with an increased risk of vascular events and all--cause mortality(43, 44) (Everett). Furthermore, patients with merely detectable hs-cTn levels have been shown to have a similar risk of adverse events as those who have presented with an ACS(44) (Than MP clinical chemistry 2018). How this adverse event risk can be modified is yet to be determined. It is conceivable that the "elevated" hs-cTn concentrations in a stable patient, always indicates myocardial injury or unwellness: the so called "never means nothing" hypothesis(45).

Implications of this study

The results of this study have significant implications for patient care. The notion of using a single binary value above the ULN of any assay to diagnose whether a patient has suffered an acute MI is flawed. This is reflected highlighted in by the observed 99th percentile ULN observed in theis CHARIOT study population which is over seven times higher than the currently recommended ULN by the manufacturersupplied by the manufacturer. Further, the observed frequency of In addition, the fact that only 1 in 7 of all patients with a hs-cTnI above the supplied ULN in our study, regardless of location, in patients in whom there was no clinical suspicion of acute MI or myocardial injury were diagnosed with MI also raises concerns over about the use utility of a 99th percentile value from a 'healthy population'. and, inIn particular, applying this supplied 99th centile value to direct determine the management of patients who are typically older, have more comorbidities, higher incidence of subclinical cardiac disease and in a worse physical condition than the reference healthy population may be flawed.

The results of this study should highlight to front line clinicians that whilst hs-cTnI can contribute to the diagnosis of AMI, this should only be when used in conjunction with other key factors such as the clinical history and other investigations(9, 24, 25, 29, 46-50). At present, the use of the 99th percentile to help rule out a diagnosis of AMI is clear and this is based on using a 'healthy' reference population.

However, the use of this threshold level and its apply~~ing~~ication to patients presenting to hospital to rule in AMI ~~can lead to issues~~is problematic, particularly where the degree of suspicion ~~of index~~ is low and there are other ~~conditions~~factors that will contribute to ~~an individual's~~the cTn concentration obtained in an individual. Currently, the implications of detecting a hs-cTnI above the supplied ULN, in terms of outcome and management, are unclear in patients in whom there is low clinical suspicion of AMI.~~where acute or chronic myocardial injury is diagnosed in the absence of AMI, as shown in this study, the optimal treatment strategy for this cohort of patients is unclear.~~ A more considered approach to application ~~using~~of cTnI concentrations would be a more tailored ULN according to the patient's baseline characteris~~i~~tics and comorbidities. ~~However, T~~the feasibility of this approach, however, ~~-~~remains unanswered. . Further data regarding the potential association between hs-cTnI level and CV risk are required.

Limitations of this study

There are a number of~~some~~ limitations ~~of this study~~. Firstly, this is an observational study of a large number of consecutive patients. Necessarily, therefore, the level of detail with regard to management and diagnoses can only be obtained from the best records available for each patient, which include~~s~~ any electronic blood request or discharge summary data and ~~otherwise the~~ formalised coding record. Secondly, this study has not looked at clinical outcomes since this was not part of our objective. Thirdly, in our analysis we have used discharge codes for diagnosis of AMI, ~~-~~but have not independently verified these final diagnoses. Finally, this study has looked at hs-cTnI concentrations in 20,000 patients based on a single sample for each patient, as a result this study cannot differentiate between acute and chronic myocardial injury.

Conclusions

This study has shown that the 99th percentile of the hospital population is substantially higher than the supplied ULN used in clinical practice according to the manufacturer provided 99th centile for a healthy population. ~~according to the manufacturer's recommendation~~. Furthermore, the 99th percentile for the hospital population varies ~~greatly~~ depending on the clinical acuity, location, age and gender of the individual, but in all subgroups there is a proportion of the patients in whom the hs-cTnI concentrations are above the clinically applied ULN. This is the largest study to date to evaluate hs-cTnI levels in an unselected cohort of 20,000 consecutive patients and the observations from this study highlight the need for clinicians to interpret hs-cTnI concentrations carefully and systematically when attempting to diagnose AMI, particularly Type 1 MI.

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Transparency declaration

Professor Nick Curzen affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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References

1. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ. 2005;173(10):1191-202.
2. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. J Am Coll Cardiol. 2012;60(16):1581-98.
3. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol. 2018;72(18):2231-64.
4. Saenger AK, Jaffe AS. Requiem for a heavyweight: the demise of creatine kinase-MB. Circulation. 2008;118(21):2200-6.
5. Apple FS, Collinson PO, Biomarkers ITFoCAoC. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem. 2012;58(1):54-61.
6. Cediel G, Gonzalez-Del-Hoyo M, Carrasquer A, Sanchez R, Boque C, Bardaji A. Outcomes with type 2 myocardial infarction compared with non-ischaemic myocardial injury. Heart. 2017;103(8):616-22.
7. Chapman AR, Adamson PD, Mills NL. Assessment and classification of patients with myocardial injury and infarction in clinical practice. Heart. 2017;103(1):10-8.
8. Melberg T, Burman R, Dickstein K. The impact of the 2007 ESC-ACC-AHA-WHF Universal definition on the incidence and classification of acute myocardial infarction: a retrospective cohort study. Int J Cardiol. 2010;139(3):228-33.
9. Shah AS, Griffiths M, Lee KK, McAllister DA, Hunter AL, Ferry AV, et al. High sensitivity cardiac troponin and the under-diagnosis of myocardial infarction in women: prospective cohort study. BMJ. 2015;350:g7873.
10. Baron T, Hambraeus K, Sundström J, Erlinge D, Jernberg T, Lindahl B, et al. Impact on Long-Term Mortality of Presence of Obstructive Coronary Artery Disease and Classification of Myocardial Infarction. Am J Med. 2016;129(4):398-406.
11. Shah AS, McAllister DA, Mills R, Lee KK, Churchhouse AM, Fleming KM, et al. Sensitive troponin assay and the classification of myocardial infarction. Am J Med. 2015;128(5):493-501.e3.
12. Chapman AR, Shah ASV, Lee KK, Anand A, Francis O, Adamson P, et al. Long-Term Outcomes in Patients With Type 2 Myocardial Infarction and Myocardial Injury. Circulation. 2018;137(12):1236-45.
13. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J. 2016;37(3):267-315.
14. Twerenbold R, Jaeger C, Rubini Gimenez M, Wildi K, Reichlin T, Nestelberger T, et al. Impact of high-sensitivity cardiac troponin on use of coronary angiography, cardiac stress

testing, and time to discharge in suspected acute myocardial infarction. Eur Heart J. 2016;37(44):3324-32.
15. Boeddinghaus J, Nestelberger T, Twerenbold R, Wildi K, Badertscher P, Cupa J, et al. Direct Comparison of 4 Very Early Rule-Out Strategies for Acute Myocardial Infarction Using High-Sensitivity Cardiac Troponin I. Circulation. 2017;135(17):1597-611.
16. Sandoval Y, Smith SW, Love SA, Sexter A, Schulz K, Apple FS. Single High-Sensitivity Cardiac Troponin I to Rule Out Acute Myocardial Infarction. Am J Med. 2017;130(9):1076-83 e1.
17. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, et al. Sensitive troponin I assay in early diagnosis of acute myocardial infarction. The New England journal of medicine. 2009;361(9):868-77.
18. Keller T, Zeller T, Ojeda F, Tzikas S, Lillpopp L, Sinning C, et al. Serial changes in highly sensitive troponin I assay and early diagnosis of myocardial infarction. Jama. 2011;306(24):2684-93.
19. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. The New England journal of medicine. 2009;361(9):858-67.
20. Mueller M, Biener M, Vafaie M, Doerr S, Keller T, Blankenberg S, et al. Absolute and relative kinetic changes of high-sensitivity cardiac troponin T in acute coronary syndrome and in patients with increased troponin in the absence of acute coronary syndrome. Clin Chem. 2012;58(1):209-18.
21. Christ M, Bertsch T, Popp S, Bahrmann P, Heppner HJ, Muller C. High-sensitivity troponin assays in the evaluation of patients with acute chest pain in the emergency department. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2011;49(12):1955-63.
22. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem. 2009;55(7):1303-6.
23. Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, et al. Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem. 2018;64(4):645-55.
24. Eggers KM, Lind L, Venge P, Lindahl B. Factors influencing the 99th percentile of cardiac troponin I evaluated in community-dwelling individuals at 70 and 75 years of age. Clin Chem. 2013;59(7):1068-73.
25. Cullen L, Greenslade JH, Carlton EW, Than M, Pickering JW, Ho A, et al. Sex-specific versus overall cut points for a high sensitivity troponin I assay in predicting 1-year outcomes in emergency patients presenting with chest pain. Heart. 2016;102(2):120-6.
26. Kumar N, Michelis MF, DeVita MV, Panagopoulos G, Rosenstock JL. Troponin I levels in asymptomatic patients on haemodialysis using a high-sensitivity assay. Nephrol Dial Transplant. 2011;26(2):665-70.
27. Egstrup M, Schou M, Tuxen CD, Kistorp CN, Hildebrandt PR, Gustafsson F, et al. Prediction of outcome by highly sensitive troponin T in outpatients with chronic systolic left ventricular heart failure. Am J Cardiol. 2012;110(4):552-7.

28. Avouac J, Meune C, Chenevier-Gobeaux C, Dieudé P, Borderie D, Lefevre G, et al. Inflammation and disease activity are associated with high circulating cardiac markers in rheumatoid arthritis independently of traditional cardiovascular risk factors. J Rheumatol. 2014;41(2):248-55.
29. Collinson PO, Heung YM, Gaze D, Boa F, Senior R, Christenson R, et al. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. Clin Chem. 2012;58(1):219-25.
30. Sandoval Y, Apple FS. The global need to define normality: the 99th percentile value of cardiac troponin. Clin Chem. 2014;60(3):455-62.
31. Gore MO, Seliger SL, Defilippi CR, Nambi V, Christenson RH, Hashim IA, et al. Age- and sex-dependent upper reference limits for the high-sensitivity cardiac troponin T assay. J Am Coll Cardiol. 2014;63(14):1441-8.
32. Shah AS, Newby DE, Mills NL. High sensitivity cardiac troponin in patients with chest pain. BMJ. 2013;347:f4222.
33. Saunders JT, Nambi V, de Lemos JA, Chambless LE, Virani SS, Boerwinkle E, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. Circulation. 2011;123(13):1367-76.
34. Thorsteinsdottir I, Aspelund T, Gudmundsson E, Eiriksdottir G, Harris TB, Launer LJ, et al. High-Sensitivity Cardiac Troponin I Is a Strong Predictor of Cardiovascular Events and Mortality in the AGES-Reykjavik Community-Based Cohort of Older Individuals. Clin Chem. 2016;62(4):623-30.
35. Iribarren C, Chandra M, Rana JS, Hlatky MA, Fortmann SP, Quertermous T, et al. High-sensitivity cardiac troponin I and incident coronary heart disease among asymptomatic older adults. Heart. 2016;102(15):1177-82.
36. Melki D, Lugnegård J, Alfredsson J, Lind S, Eggers KM, Lindahl B, et al. Implications of Introducing High-Sensitivity Cardiac Troponin T Into Clinical Practice: Data From the SWEDEHEART Registry. J Am Coll Cardiol. 2015;65(16):1655-64.
37. Ford I, Shah AS, Zhang R, McAllister DA, Strachan FE, Caslake M, et al. High-Sensitivity Cardiac Troponin, Statin Therapy, and Risk of Coronary Heart Disease. J Am Coll Cardiol. 2016;68(25):2719-28.
38. Whelton SP, McEvoy JW, Lazo M, Coresh J, Ballantyne CM, Selvin E. High-Sensitivity Cardiac Troponin T (hs-cTnT) as a Predictor of Incident Diabetes in the Atherosclerosis Risk in Communities Study. Diabetes Care. 2017;40(2):261-9.
39. Cavender MA, White WB, Jarolim P, Bakris GL, Cushman WC, Kupfer S, et al. Serial Measurement of High Sensitivity Troponin I and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus in the EXAMINE Trial. Circulation. 2017.
40. Wrigley P, Khoury J, Eckerle B, Alwell K, Moomaw CJ, Woo D, et al. Prevalence of Positive Troponin and Echocardiogram Findings and Association With Mortality in Acute Ischemic Stroke. Stroke. 2017.
41. Neukamm A, Einvik G, Didrik Høiseth A, Søyseth V, Henrik Holmedahl N, Kononova N, et al. The prognostic value of measurement of high-sensitive cardiac troponin T for mortality in a cohort of stable chronic obstructive pulmonary disease patients. BMC Pulm Med. 2016;16(1):164.

42. Willeit P, Welsh P, Evans JDW, Tschiderer L, Boachie C, Jukema JW, et al. High-Sensitivity Cardiac Troponin Concentration and Risk of First-Ever Cardiovascular Outcomes in 154,052 Participants. J Am Coll Cardiol. 2017;70(5):558-68.
43. Everett BM, Zeller T, Glynn RJ, Ridker PM, Blankenberg S. High-sensitivity cardiac troponin I and B-type natriuretic Peptide as predictors of vascular events in primary prevention: impact of statin therapy. Circulation. 2015;131(21):1851-60.
44. Than MP, Aldous SJ, Troughton RW, Pemberton CJ, Richards AM, Frampton CMA, et al. Detectable High-Sensitivity Cardiac Troponin within the Population Reference Interval Conveys High 5-Year Cardiovascular Risk: An Observational Study. Clin Chem. 2018;64(7):1044-53.
45. Mariathas M, Curzen N. Troponin assays: developing indications. Lancet. 2018;391(10138):2398-9.
46. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. Clin Chem. 2012;58(11):1574-81.
47. Hammarsten O, Fu ML, Sigurjonsdottir R, Petzold M, Said L, Landin-Wilhelmsen K, et al. Troponin T percentiles from a random population sample, emergency room patients and patients with myocardial infarction. Clin Chem. 2012;58(3):628-37.
48. Aw TC, Phua SK, Tan SP. Measurement of cardiac troponin I in serum with a new high-sensitivity assay in a large multi-ethnic Asian cohort and the impact of gender. Clin Chim Acta. 2013;422:26-8.
49. Rubini Gimenez M, Reiter M, Twerenbold R, Reichlin T, Wildi K, Haaf P, et al. Sex-specific chest pain characteristics in the early diagnosis of acute myocardial infarction. JAMA Intern Med. 2014;174(2):241-9.
50. Koerbin G, Abhayaratna WP, Potter JM, Apple FS, Jaffe AS, Ravalico TH, et al. Effect of population selection on 99th percentile values for a high sensitivity cardiac troponin I and T assays. Clin Biochem. 2013;46(16-17):1636-43.