University of Southampton

Academic Thesis: Declaration Of Authorship

I, Mark Mariathas, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The Utility of High Sensitivity Troponins in Clinical Practice

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

3. Where I have consulted the published work of others, this is always clearly attributed;

4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

5. I have acknowledged all main sources of help;

6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

7. Either none of this work has been published before submission, or parts of this work have been published as: [please list references below]:

Mariathas M, Gemmell C, Olechowski B, Nicholas Z, Mahmoudi M, Curzen N. High sensitivity troponin in the management of tachyarrhythmias. Cardiovasc Revasc Med. 2018 Jul;19(5 Pt A):487-492. Epub 2017 Nov 28. PMID: 29352700.

Mariathas M, Allan R, Ramamoorthy S, Olechowski B, Hinton J, Azor M, Nicholas Z, Calver A, Corbett S, Mahmoudi M, Rawlins J, Simpson I, Wilkinson J, Kwok CS, Cook P, Mamas MA, Curzen N. True 99th centile of high sensitivity cardiac troponin for hospital patients: prospective, observational cohort study. BMJ. 2019 Mar 13;364:l729

Mariathas M, Olechowski B, Mahmoudi M, Curzen N. High sensitivity troponins in contemporary cardiology practice: are we turning a corner? Expert Rev Cardiovasc Ther. 2018 Jan;16(1):49-57. doi: 10.1080/14779072.2018.1419063. Epub 2017 Dec 28. PMID: 29260921**.**

Mariathas M, Gemmell C, Olechowski B, Nicholas Z, Mahmoudi M, Curzen N. The Association Between HsTrop and Tachyarrhythmias: Type 1 or Type 2 MI? The STRIPE-MI Study. J Am Coll Cardiol. 2017 Oct, 70 (18_Supplement) B278–B279

Mariathas M, Curzen N. Troponin assays: developing indications. Lancet. 2018 Jun 16;391(10138):2398-2399.

Mariathas M, Curzen N. Use of troponins in clinical practice: Evidence against the use of troponins in clinical practice. Heart. 2020 Feb;106(4):251-252.

Signed:

Date:

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE

Human Development & Health Academic Unit

Thesis for the degree of Doctor of Medicine

THE UTILITY OF HIGH SENSITIVITY TROPONINS IN CLINICAL PRACTICE

Mark Mariathas

Cardiac troponins (cTn) are the gold standard biomarker of myocardial injury. The evidence for the use of cTn in clinical practice is well validated and used globally in the management of patients with suspected acute coronary syndromes (ACS). As a rule out test, the evidence for the use of cTn is unrivalled. When first utilised in clinical practice a common issue with cTn assays was the need for clinicians to wait 10-12 hours post suspected infarction before clinical decisions based on the cTn result. This period of waiting has been the driver for the improvement of the assays. To help improve efficiency within different health care systems for suspected ACS patients, high sensitivity cardiac troponin (hs-cTn) assays have been developed. These assays are now able to be detected 1 hour post infarction, thus allowing clinicians to make decisions on management at a much quicker pace than under the early cTn assays. The improvements in sensitivity thus allow clinicians to safely rule out ACS and discharge patients. However, as a 'rule in' test there are flaws in how clinicians interpret the hs-cTn levels. We are now aware that there are many different types of MI, however, in only type 1 myocardial infarction (T1MI) are there proven treatments, such as antiplatelet therapy and percutaneous coronary intervention (PCI), which improve prognosis. Despite this, a common flaw is to treat all patients with a raised cTn as a T1MI.

Despite the descriptions and prevalence of the different types of MI throughout the literature, in clinical practice most patients diagnosed as MI are treated and labelled as T1MI. Current data suggests 20-50% of MI patients are in fact type 2 MI (T2MI). The Fourth Universal Definition of MI recommends the use of the 99th percentile as the correct cut off to diagnose MI. The 99th percentile is defined by manufacturer's data derived as part of the internal validation for the assay: subsequently this level is quoted and usually used as a clinical "upper limit of normal". There are many variables that can affect an individual's troponin level. This has a significant effect on the definition of the ULN for any assay. *This is particularly important as each individual manufacturer will have a different inclusion and exclusion criteria when defining the reference population used to quantify the 99th centile*. For example, it has been shown that the younger the reference population is, and the stricter the criteria that are used to define cardiac health are, the lower the 99th centile will be. This raises important questions. Firstly, is it appropriate to use hs-cTn as a binary marker to 'rule in/rule out' MI? Secondly, how should abnormal hs-cTn levels be defined; is it appropriate to use the 99th percentile from a young healthy population and apply the marker of abnormality for this population to the older heterogeneous population that presents to hospitals throughout the world?

The objectives of the studies presented in this thesis are as follows. Firstly, the prevalence of patients presenting with a tachyarrhythmia and associated hs-cTn rise will be assessed. The management of these patients will be assessed. The short and mid-term outcome in these patients will also be described. Secondly, the

99th percentile of high sensitivity cardiac troponin I concentration for an entire hospital population will be defined.

In chapter 3, I undertook retrospective analysis of 704 consecutive emergency admissions to UHS FT with either a diagnosis of MI or tachyarrhythmia. The clinical management of these patients was analysed. Furthermore, the tracked mortality for these patients was analysed. The study found the mortality rate of patients with a tachyarrhythmia and raised hs-cTn level is similar to that of T1MI. Furthermore, only one patient in the study population (0.14%) was diagnosed with T2MI, highlighting that T2MI is rarely diagnosed in clinical practice.

In chapter 4, I set out to define the 99th percentile for the hospital population. I undertook an observational study of hs-cTnI levels in 20,000 consecutive patients who utilised the hospital services. This was an all comers study that had never been undertaken before. I found that the 99th percentile for the population studied was 296 ng/L, more than 7 times the ULN quoted by the manufacturer. The study also showed 1 in 20 of all patients included had a raised hs-cTnI level.

The results from the studies described in this thesis highlight that there are many factors which can raise an individual's cTn level. The evidence for the hs-cTn assays as a rule out test is robust, however, as a rule in tests questions remain. The work presented here demonstrates the use of hs-cTn ULN as a binary 'rule in/rule out' as a flawed concept. This has the potential for patients to be managed inappropriately and could lead to issues with patient safety. The work presented here is the stimulus for

further work in this field to establish more optimal hs-cTn cut off levels. Finally, the work presented here shows that hs-cTn can be a marker of risk.

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PUBLICATIONS IN PEER REVIEWED JOURNALS AND PRESENTATIONS AT MEDICAL CONFERENCES

Original Articles

1. **Mariathas M**, Gemmell C, Olechowski B, Nicholas Z, Mahmoudi M, Curzen N. High sensitivity troponin in the management of tachyarrhythmias. Cardiovasc Revasc Med. 2018 Jul;19(5 Pt A):487-492. Epub 2017 Nov 28. PMID: 29352700.

2. **Mariathas M**, Allan R, Ramamoorthy S, Olechowski B, Hinton J, Azor M, Nicholas Z, Calver A, Corbett S, Mahmoudi M, Rawlins J, Simpson I, Wilkinson J, Kwok CS, Cook P, Mamas MA, Curzen N. True 99th centile of high sensitivity cardiac troponin for hospital patients: prospective, observational cohort study. BMJ. 2019 Mar 13;364:l729.

Please note most of the text in the results chapters is lifted directly from the above original research papers. I was the first author for both papers, with the first draft of the papers written by me.

Review

Mariathas M, Olechowski B, Mahmoudi M, Curzen N. High sensitivity troponins in contemporary cardiology practice: are we turning a corner? Expert Rev Cardiovasc Ther. 2018 Jan;16(1):49-57. doi: 10.1080/14779072.2018.1419063. Epub 2017 Dec 28. PMID: 29260921**.**

Commentary/Responses

1. **Mariathas M**, Curzen N. Troponin assays: developing indications. Lancet. 2018 Jun 16;391(10138):2398-2399.
2. **Mariathas M**, Curzen N. Use of troponins in clinical practice: Evidence against the use of troponins in clinical practice. Heart. 2020 Feb;106(4):251-252.

Presentations

1. **Mariathas M**, Allan R, Ramamoorthy S, Olechowski B, Hinton J, Azor M, Nicholas Z, Calver A, Corbett S, Mahmoudi M, Rawlins J, Simpson I, Wilkinson J, Kwok CS, Cook P, Mamas MA, Curzen N. 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.** British Cardiovascular Intervention Society, Royal College of Physicians London – BCIS Young Investigators Award, London, December 2018.

2. **Mariathas M**, Gemmell C, Olechowski B, Nicholas Z, Mahmoudi M, Curzen N. The Association Between HsTrop and Tachyarrhythmias: Type 1 or Type 2 MI? The STRIPE-MI Study. TCT, Denver, USA, October 2017.

Abstracts

Mariathas M, Gemmell C, Olechowski B, Nicholas Z, Mahmoudi M, Curzen N. The Association Between HsTrop and Tachyarrhythmias: Type 1 or Type 2 MI? The STRIPE-MI Study. J Am Coll Cardiol. 2017 Oct, 70 (18_Supplement) B278–B279

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Finally, I would like to thank my wife, Dr Onoshua Mariathas, who offered me unconditional support throughout this process whilst ensuring the needs of our two young children were met. I dedicate this work to her, Loki and Tiya.

Abbreviations

ACC	American College of Cardiology
ACS	Acute Coronary Syndrome
AF	Atrial Fibrilliation
AHA	American Heart Association
AHF	Acute Heart Failure
AMI	Acute Myocardial Infarction
BCPA	British Cardiac Patients Association
BNP	Brain Natriuretic Peptide
CA	Coronary angiography
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
CAG	Confidentiality Advisory Group
CKD	Chronic Kidney Disease
CK-MB	Creatinine Kinase Myocardial Band
CRG	Coronary Research Group
cTn	Cardiac Troponin
CV	Coefficient of Variation
DAPT	Dual Antiplatelet Therapy
ED	Emergency Department
ELISA	Enzyme-Linked Immunosorbent Assay
ESC	European Society of Cardiology
FFR	Flow Fractional Reserve
GFR	Glomerular Filtration Rate

GRACE	Global Registry of Acute Coronary Events
HF	Heart Failure
HRA	Health Research Authority
Hs-cTn	High Sensitivity Cardiac Troponin
ICD	International Classification of Diseases
IFCC TF-CB	International Federation of Clinical Chemistry and Laboratory Medicine Task Force On Clinical Applications of Cardiac Bio-Markers
IHD	Ischaemic Heart Disease
IQR	Interquartile range
IVUS	Intravascular ultrasound
LOD	Limit of Detection
LOQ	Limit of Quantification
LV	Left Ventricle
LVSD	Left Ventricular Systolic Dysfunction
MI	Myocardial Infarction
MOP	Medicine for Older Peoples
NIIT	Non-Invasive Ischaemia Tests
NIMI	Non-Ischaemic Myocardial Injury
NPV	Negative Predictive Value
NSTEMI	Non ST-Elevated Myocardial Infarction
OAT	Occluded Artery Trial
OR	Odds Ratio
PCI	Percutaneous Coronary Intervention
PDS	Patient Demographic Service
REC	Research Ethics Committee

ROC	Receiver Operating Characteristic
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
STEMI	ST-Elevated Myocardial Infarction
SVT	Supraventricular Tachycardia
T1MI	Type 1 Myocardial Infarction
T2MI	Type 2 Myocardial Infarction
T3MI	Type 3 Myocardial Infarction
T4MI	Type 4 Myocardial Infarction
T5MI	Type 5 Myocardial Infarction
Tn	Troponin
TRELAS	The Troponin Elevation in Acute Ischaemic Stroke Study
UHS	University Hospitals Southampton
ULN	Upper Limit of Normal
VT	Ventricular Tachycardia

1.0 Introduction

1.1 What is troponin?

Troponin is the gold standard biomarker currently used in clinical practice to help establish the diagnosis of myocardial injury(1). Commonly, clinicians request a troponin concentration on patients when they are attempting to rule in or rule out a myocardial infarction (MI). MI has been defined as a rise in cardiac troponin (cTn) above the 99th percentile derived from a reference population associated with an appropriate clinical context.(2) The 99th percentile of a reference population of healthy individuals is regarded as the upper limit of normal (ULN) for troponin values.(3, 4)

1.2 Troponin structure and function

Myofibrils are the contractile apparatus of myocytes. The contractile unit of each myofibril is made up of the sarcomere, which regularly repeats throughout myofibrils(5). The sarcomere is in control of converting chemical energy into mechanical energy, and in doing so this enables cardiomyocyte contraction(6). The sarcomere is composed of both thick and thin filaments. The thick filament is made up of myosin, whereas the thin filament contains both actin and tropomyosin/troponin regulatory complex (7-9). Actin monomers form a double-helical structure that wrap around myosin(10).

Troponin is a complex of three regulatory proteins, which is able to modulate the interaction between actin and myosin. The three regulatory proteins are troponin c (TnC), troponin I (TnI), and troponin T (TnT). TnT, is the 35-kD subunit which binds troponin to tropomyosin and the thin filament of myofibrils(11). The function of tropomyosin is to block the binding site for myosin on actin filaments. TnI, the 23-kD subunit, is inhibitory and precludes the actin

activated myosin ATPase activity. The binding of TnI to actin ensures that the troponin-tropomyosin complex is held in place. Troponin plays a crucial role in the regulation of excitation-contraction coupling in the heart. This interaction is calcium-mediated. TnC is the 18-kD calcium binding subunit that is able to bind calcium and regulate the activation of the thin filament. In 1883, Ringer first described the role calcium has on contraction of the heart(12). Furthermore, Heilbrunn(13) revealed that calcium acts as the intracellular trigger for contractility. Calcium has subsequently been shown to initiate the activation of contractile proteins, with the sarcoplasmic reticulum regulating the intracellular movement of calcium in muscle (14-16). A rise in intracellular calcium, which is instigated by an action potential, results in the binding of calcium to TnC. As a result of this, TnI is dislodged and this ultimately causes tropomyosin to be removed from the myosin binding site on actin filaments. The binding of actin and myosin results in muscle contraction(17). Cardiac TnI (cTnI) is cardiac-specific, in contrast to cardiac TnT (cTnT), which can also be found in skeletal muscle(18).

For each cardiac cycle the activity of cTnI is dependent on the intracellular calcium levels(19). In diastole, low intracellular concentrations of calcium alter the structure of cTnI resulting in the inhibition of the actin-myosin interaction, which is achieved via the location of the cTnI inhibitory region on actin(20). During systole, intracellular calcium levels rise from a 100 nmol/L in diastole to 1mmol/L. This rise initiates contraction(21). Specifically, the rise in calcium causes the bond between cTnI and actin to weaken through the binding of calcium to the N-terminal domain of cTnC(22).

cTnC, after binding to calcium, is able regulate both muscle contraction and relaxation. The C-terminal domain of cTnC interacts with the thin filament and binds to calcium with a high affinity. The N-terminal domain of cTnC has two binding sites for calcium. The first site is

inactive. The combination of the binding of calcium to the second site and the interaction between cTnC and cTnI triggers contraction (23, 24).

In response to cardiomyocyte damage there are rises in both cTnI and cTnT concentrations in systemic blood, hence making these proteins candidates as biomarkers of cardiomyocyte damage and necrosis. Although cTnT is found in skeletal muscle, this subtype of cTnT is not usually detected in currently available assays (25).

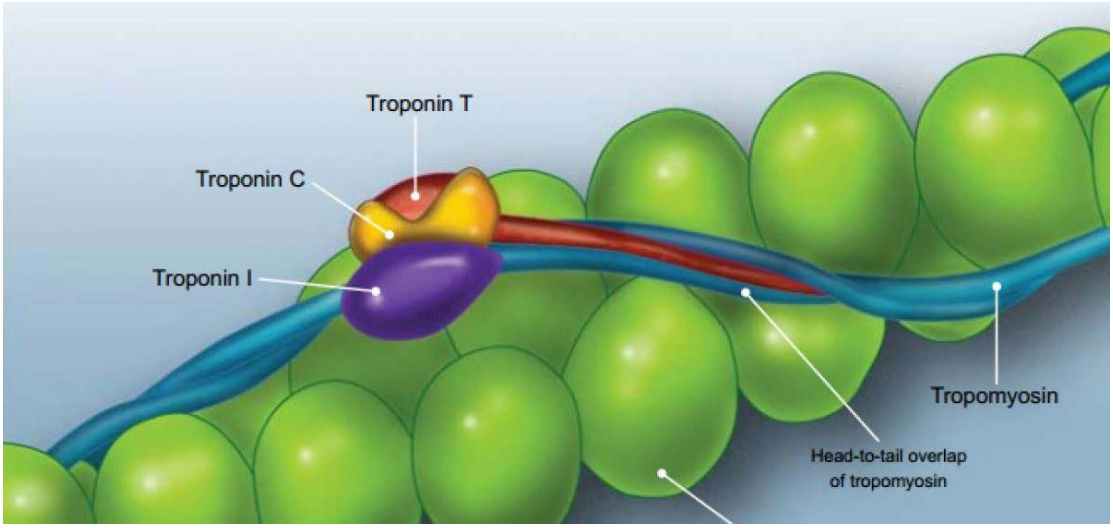

Figure 1: Structure of the troponin complex ©Creative Biolabs, Inc. Original image available at www.creative-biolabs.com/drug-discovery/diagnostics/ivd-antibodies-for-troponin-marker.htm.

1.3 Troponin assays

The first cTnI radioimmunoassay was developed in 1987(26). It took 2 days to perform and had a limit of detection of 10µg/l. In this assay the cTnI levels were detectable 4-6 hours after myocardial infarction and levels remained elevated for 6-8 days(26). Furthermore, peak cTnI concentrations correlated with peak levels of CK-MB, the biomarker used in contemporary clinical practice at that time. Soon after this, the first fully automated cTnT ELISA was developed, with a limit of detection of 0.1µg/l and results available in 90 minutes(27). The TnT assay has undergone several improvements which have led to a lower level of skeletal muscle cross-reactivity(28), reduced processing time to 9 minutes(29), higher linearity(30) and detection at lower concentrations(31). In 2009, the fifth generation of TnT assays were developed to be high sensitivity (hs), with a limit of detection of 2ng/l (32, 33). Although the sensitivity of the hs-TnT assays is significantly improved compared to the first generation assays, the issue with skeletal muscle cross-reactivity still exists and this can lead to false positive results, particularly in patients with skeletal muscle disease(18). Importantly, in healthy skeletal muscle cross-reactivity with TnT assays has been shown to be insignificant(34).

Autoantibodies for both the TnI and TnT have been described, with the prevalence in healthy individuals reported as 12.7% for TnI and 9.9% for TnT(35). The presence of autoantibodies can cause false results via two routes. Firstly, when the binding site for the immunoassay is blocked by the antibody, thereby potentially providing a false negative result(36). Second, through the binding of the antibody to troponin itself, preventing the breakdown of troponin, which ultimately can lead to a false positive result(37). The overall effect the presence of autoantibodies has on clinical practice is yet to be fully determined.

The first TnI ELISA was developed in 1992, an assay that had a limit of detection of 1.9µg/l with a processing time of 3.5 hours(38). Like the TnT assay the TnI assay has undergone several improvements, with primary focus in improving the sensitivity and processing time, thus allowing clinicians to make decisions on patients suspected of MI in a timely fashion. Cardiac Tn (cTn) can now be reliably detected 1 hour post infarction, a significant improvement on the 10-12 hours with the first assays used in clinical practice (39-43). The hs-cTn assays are also now able to detect troponin at much lower concentrations than the previous assays(4).This is in keeping with the universal definition of MI, which recommends that a troponin assay used to diagnose MI should have a coefficient of variation of ≤ 10% at the threshold concentration representing the 99th percentile upper limit of a "normal reference" population (ULN). *Modern hs-cTn assays can detect troponin in more than 50% of the general population with some assays able to detect troponin in everyone*(44). The implications for interpretation of the results of the assay by front line staff are important: no longer is the presence of absence of troponin a binary indicator of MI/Acute coronary syndrome (ACS). This may well not be fully appreciated(45): see below. The International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Cardiac Bio-Markers (IFCC TF-CB) has proposed that for an assay to be defined as high-sensitivity (hs) the following criteria need to be met (46):

1) The percentage of the coefficient of variation (CV) at the 99th percentile value should be ≤10%.
2) The ability to measure levels above the limit of detection (LoD) in at least 50% of normal individuals (both males and females).

Examples of currently available Hs-cTn assays are shown in table 1(47). Interestingly, the hs-cTnT assay (Roche) has shown lower than recommended rates of measurable concentrations according to the IFCC TF-CB criteria (47).

Table 1: Hs-cTn assays (LoD = Limit of detection, ULN = 99th percentile, CV = Coefficient of variation)

Assay	LoD(ng/L)	ULN M/F (ng/L)	%CV at ULN	10% CV ng/L
Abbott ARCHITECT hs-cTnI	1.2/1.9	34/16	5	3
Roche E1 70 hs-cTnT	5	20/13	8	13
Beckman Coulter Access hs-cTnI	2.5	52/23	<10	8

1.4 The classification of MI

The Fourth Universal Definition of MI (48) is a classification, achieved by expert consensus that yields: type 1 MI (T1MI), type 2 MI (T2MI), type 3 MI (T3MI), type 4 MI (T4MI), type 5 MI (T5MI) and myocardial injury. This has been driven by the availability of Hs-cTn assays. Table 2 shows the different types of MI and their causes.

Table 2: Type of MI and causes (CABG = Coronary artery bypass grafting)

TYPE OF MI	AETIOLOGY
TYPE 1	CLASSICAL MI CAUSED BY SPONTANEOUS PLAQUE RUPTURE/ EROSION.
TYPE 2	ISCHAEMIC INJURY CAUSED BY SUPPLY-DEMAND MISMATCH OF OXYGEN.
TYPE 3	LIKELY MI IN THE ABSENCE OF BIOMARKERS, EITHER DEAD OR PRESENTED BEFORE BIOMARKER RISE.
TYPE 4A	MI RELATED TO PCI PROCEDURE, INDEX PROCEDURE WITHIN 48 HOURS.
TYPE 4B	MI RELATED TO STENT THROMBOSIS.
TYPE 4C	MI RELATED TO STENT RESTENOSIS.
TYPE 5	MI RELATED TO CABG

T1MI has been defined as a troponin elevation related to an acute plaque rupture in a suspected ACS (49, 50). According to the fourth universal definition (48) T1MI is diagnosed based on this criteria:

'Detection of a rise and/or fall of cTn values with at least one value above the 99th percentile URL and with at least one of the following:

- *Symptoms of acute myocardial ischaemia*
- *New ischaemic ECG changes;*
- *Development of pathological Q waves;*
- *Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology;*
- *Identification of a coronary thrombus by angiography including intracoronary imaging or by autopsy.'*

Figure 2 depicts the different presentations of a T1MI at a vascular level.

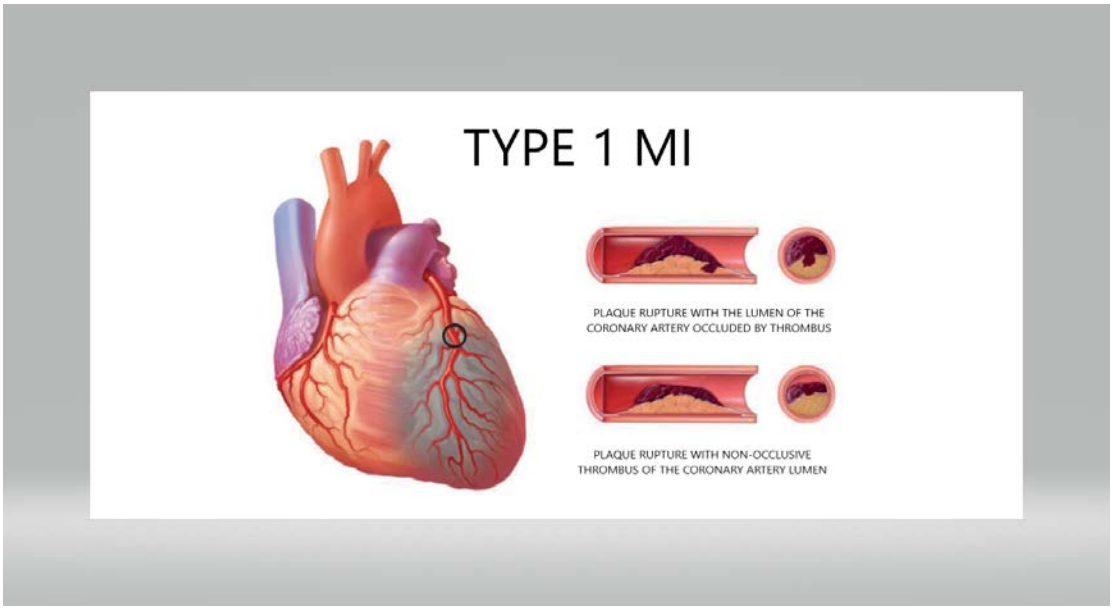

Figure 2: The different presentations of T1MI (adapted from Thygesen et al [48]).

The second form of MI or type 2 MI (T2MI) has been defined as MI secondary to ischaemia due to increased oxygen demand or reduced oxygen supply. Examples of clinical scenarios giving rise to T2MI include anaemia, arrhythmias, hypertension, hypotension, coronary artery spasm and embolism (50). Below is the criteria defined in the fourth universal definition (48) to diagnose T2MI:

'Detection of a rise and/or fall of cTn values with at least one value above the 99th percentile URL, and evidence of an imbalance between myocardial oxygen supply and demand unrelated to coronary thrombosis, requiring at least one of the following:

- *Symptoms of acute myocardial ischaemia*
- *New ischaemic ECG changes;*
- *Development of pathological Q waves;*
- *Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology.'*

Figure 3 depicts the different presentations of T2MI at the coronary artery level.

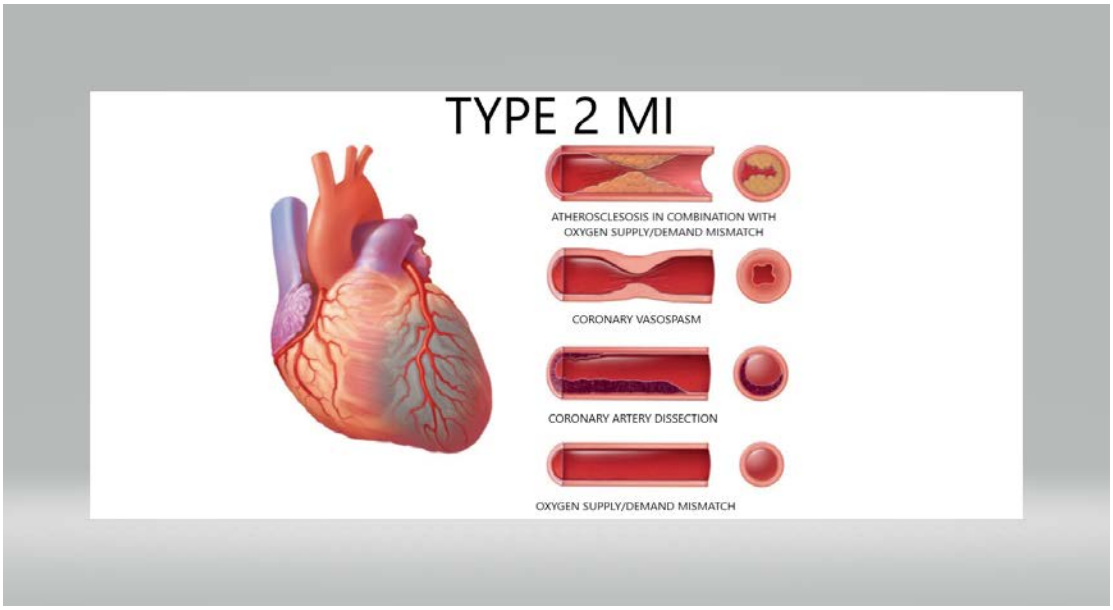

Figure 3: The different presentations of a T2MI at the coronary artery level (adapted from Thygesen et al [48]).

T3MI applies to those patients who are likely to have suffered an acute MI but have unfortunately died but with no biomarker evidence of an acute MI: i.e. death occurs before hospitalization or blood samples taken before a rise in the relevant biomarkers could be detected.

T4MI relates to periprocedural troponin rise in the context of percutaneous coronary intervention (PCI) and T5MI relates to periprocedural troponin rise in the context of CABG.

The Fourth Universal Definition makes a clear distinction between MI and myocardial injury. Myocardial injury is defined as the detection of an elevated cTn value above the 99th percentile URL. Where there is a rise and fall in cTn the injury is defined acute, where there is no dynamic change in cTn concentrations the injury is defined as chronic. Myocardial injury occurs both in the setting of ischaemia causing infarction but also in the absence of no ischaemia. Non-ischaemic causes of myocardial injury, for example, include myocarditis,

chronic kidney disease and heart failure. Table 3 shows both cardiac and non-cardiac causes of myocardial injury.

Table 3: Causes of myocardial injury

CARDIAC CAUSES	NON-CARDIAC CAUSES
Cardiac contusion	Pulmonary Embolism
Cardiac surgery	Pulmonary Hypertension
Cardioversion	Renal Failure
Acute and Chronic Heart Failure	Cerebrovascular disease
Aortic dissection	Sepsis
Hypertrophic cardiomyopathy	Drugs
Arrhythmias	Extreme exertion
Post PCI	Burns
Hypertension	Critical illness
Myopericarditis	Chronic obstructive pulmonary disease

Importantly, it is often very difficult to ascertain the exact mechanism of cTn rise in some patients, particularly if their presentation and clinical context are complex. There is considerable overlap between T2MI and myocardial injury, and, in fact, both conditions can occur due to a common cause such as infection. This ambiguity is highlighted by the fact that infection is sometimes cited as a cause for T2MI (51-53), whereas in other references it is classified as myocardial injury(54). Not surprisingly, this can lead to variability and confusion when the reported incidences of T2MI and myocardial injury are evaluated. Sarkisian et al (55) have reported in a prospective study of patients who had a cTnI requested as part of their clinical management (n=3762) , a total of 1577 patients (41.2%) had a raised cTnI level. Of these patients with an elevated cTnI level, 1089 (69.1%) were diagnosed with myocardial

injury and only 119 (7.5%) were ultimately diagnosed with a T2MI. This is in contrast to Shah et al(52) where the incidence of myocardial injury is 24% and T2MI 20%.

For most patients presenting acutely, the intention of clinical staff is to use Hs-cTn assays to accurately and rapidly diagnose or exclude T1MI. Unfortunately, for the reasons laid out above, many patients with raised Hs-cTn are actually in the T2MI or myocardial injury categories, and this has important implications for their management unless the general level of awareness of this potential diagnostic confusion is high. Both T2MI and myocardial injury, can be related due to a multitude of medical and surgical conditions. There is, however, a paucity of guidelines or diagnostic criteria available for clinicians to use to adjudicate whether patients have suffered a T2MI/injury.

As such, there is significant disparity in the literature, in particular, over the incidence of T2MI with the proportion of MI being attributed to T2MI ranging from 1.6% to 29.6% (51, 56-58). The registry published by Baron et al (51) (n=20,138) has reported the incidence of T2MI in the MI population to be 7.1% and T1MI to be 88.5%. It should be noted, however, that there was considerable variation in the incidence of T2MI between different centres (0-13%). There is also evidence to show that there is a greater increase in the diagnosis of T2MI in proportion to T1MI when Hs-cTn assays are used instead of cTnI assays (59, 60). Inevitably, and appropriately, this raises concerns about the prospect that some patients whose true diagnosis is T2MI are being treated as T1MI: in particular, being exposed to aggressive invasive investigation and treatment for which there is no evidence base in T2MI. (61-63). The study from Shah et al(52) has shown the utilization of lower thresholds and Hs-cTn assays reduces the risk of recurrent infarction and death in the T1MI population. By contrast, in the T2MI population, despite increasing the rates of detection and clinical investigations, there

was no improvement in outcomes. This is certainly at odds with some definitions of T2MI that suggest significant coronary artery disease is required to cause T2MI as opposed to myocardial injury (64, 65). Given this, clinicians are frequently in a position in which the result of the test can cause confusion. Specifically, conditions may be present that lead to a chronically elevated level, such as chronic renal impairment, or that are associated with an acute rise in hs-cTn, but outside the context of an ACS, such as an arrhythmia. In the absence of a clinical history of ACS, therefore, the unwary clinician may inappropriately diagnose an acute MI, and potentially thereby commit the patient to the wrong treatment pathway. It is this common clinical dilemma that is the main stimulus for the original research in this thesis.

It cannot be overlooked that patients diagnosed with T2MI are twice as likely to be readmitted at one year with a T1MI when compared to patients who have been diagnosed with myocardial injury(52). Irrespective of the issues relating to the classification of T2MI and myocardial injury and the considerable overlap, the outcome for both conditions is not benign. Stein et al(53) have shown that at 30 days and 1 year there is a higher mortality in patients with T2MI compared to T1MI (30 days; 13.9% vs 4.9%, n= 2818, $p<0.0001$: 1 year; 23.9% vs 8.6%, $p<0.0001$). In the myocardial injury population Sarkisian et al have shown a greater risk of all-cause mortality when compared to patients suffering an acute MI(59% vs 39%, $p<0.0001$). In this study, acute MI is defined as a cTn above the ULN alongside evidence of myocardial ischaemia, whereas myocardial injury is defined cTn above the ULN in the absence of myocardial ischaemia. Recent data from Chapman et al has reported that at 5 years, 60% of patients with T2MI and 75% of patients with myocardial injury were dead (66).

1.5 Use in clinical practice

The primary role of cTn assays in ACS is risk stratification. The evidence for early and aggressive treatment, which includes pharmacological and invasive interventions, of ACS in medium to high risk individuals is robust, and this is particularly, and consistently, true for revascularization of patients who are troponin positive.(67, 68) The serum cTn level in these patients is therefore crucial as it plays a significant role in determining early how these patients are managed.(69-71) However, this is only of clinical relevance in patients in whom the clinical presentation otherwise fits with ACS, an important issue in the context of this thesis that will be discussed in detail later.

Perhaps the major advantage of the new Hs-cTn assays over previous biomarkers, and unquestionably their most robust value in frontline clinical practice, is the reduction in time to rule out a diagnosis of MI. In the UK, there are approximately 1 million attendances to the emergency department with chest pain(72). Importantly, many of these patients may be suitable for discharge directly from the emergency department(73). Historically, serial cTn measurements were required by clinicians before a diagnosis of ACS could be safely ruled out. The new hs-cTn assays have now given clinicians the opportunity to safely discharge patients with a single hs-cTn measurement (74). It has been suggested from previous studies that patients with undetectable cTn levels are low risk for MI (2, 75). In a prospective study of 6304 consecutively enrolled patients with suspected ACS, Shah et al (74) have shown that when using the Abbott ARCHITECT$_{STAT}$ hs-cTnI (LoD = 1.2ng/L, ULN (men) = 34 ng/L, ULN (women) = 16 ng/L) a level of <5ng/L confers a negative predictive value (NPV) of 99.6% (95% CI 99·3–99·8) for the primary outcome of index myocardial infarction, or subsequent myocardial infarction or cardiac death at 30 days. Furthermore, a study by Sandoval and colleagues looked specifically at the NPV of hs-cTnI levels below the LoD (n=3845). The LoD of hs-cTnI

assay used in the study was 1.9ng/L. In two separate cohorts the authors found 27% (n=448) in one cohort and 22% (n=447) in the other cohort had a hs-cTnI below the LoD. The NPV for AMI or cardiac death at 30 days was 99.6% (95% CI, 98.4 –100) and 99.1% (95% CI, 98.2–99.8) in both cohorts respectively. The data from these studies highlight how the development of the hs-cTn assays allow clinicians to now safely discharge a significant proportion of patients safely based on the result of a single hs-cTn measurement. This will ultimately allow greater efficiency within healthcare systems whilst not compromising safety.

This has had an important impact on the clinical guidelines algorithms that are available to guide the adjudication of a diagnosis of MI. In 2015 the European Society of Cardiology (ESC) produced guidelines on the management of ACS (76), this included two algorithms, the 0/1h and the 0/3h, which both have a class I recommendation.

Specifically, the 0/3h ESC algorithm recommends that MI can be ruled out if the concentrations of Hs-cTn are below the ULN in blood samples taken at presentation and 3 hours later, if they fulfill concurrent clinical criteria. Thus, patients should be pain free and be deemed “low risk” for in hospital mortality using the Global Registry of Acute Coronary Events (GRACE) score (76). Where the time from onset of symptoms to presentation to hospital is over 6 hours, a single Hs-cTn concentration below the ULN is considered sufficient to rule out a diagnosis of MI. By contrast, a diagnosis of MI can be made if patients have a highly abnormal Hs-cTn with clinical correlation (usually 5 fold above the ULN) or if there is a significant change, also known as the delta change, in the 3-hour blood sample, this is dependent on the assay used(76). It is important to also acknowledge that there is also a cohort of patients who will not have an elevated Hs-cTn concentration at 3 hours but may nevertheless have a later elevation and be diagnosed with MI. However, the safety of these rule out protocols is well established. When using the 0/3h algorithm, one study has shown that 56% of patients are

directed towards outpatient management once a diagnosis of MI is excluded, with a median time of stay in the emergency department (ED) of 4.5 hours for the patients deemed suitable for outpatient management (77). A recent meta-analysis (n=9241) has shown individuals presenting to the ED with a non-ischaemic ECG and a single low hs-cTnT level (<0.005µg/L) can be classified as low risk. The pooled sensitivity for acute MI in this group was 98.7% (95% CI, 96.6% to 99.5%) and for 30 day MACE 98.0% (CI, 94.7% to 99.3%). None of these patients labelled as low risk died. On this basis these low risk patients can safely be discharged from the ED and managed on an outpatient basis (78).

The 0/1h ESC algorithm (see figure 4) does not utilize scoring systems such as GRACE in the assessment of patients presenting with suspected MI (76). The use of the 0/1h algorithm allows clinicians to make safe early decisions about admission to hospital or discharge to outpatient management.

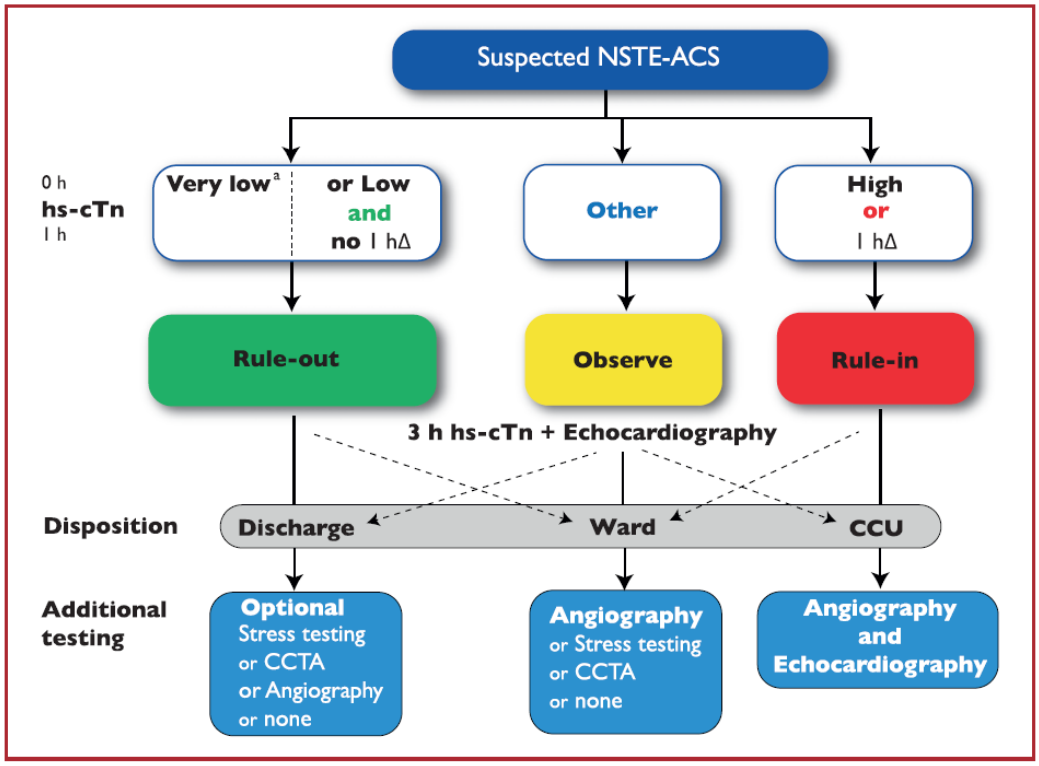

Figure 4: The European Society of Cardiology 0/1hr rule out and rule in algorithm (79). CCU = coronary care unit; CCTA = coronary computed tomography angiography; CPO = chest pain onset; hs-cTn = high-sensitivity cardiac troponin; NSTE-ACS = non-ST-segment elevation acute coronary syndrome; NSTEMI = non-ST-segment elevation myocardial infarction.

A diagnosis of MI can be safely ruled out by a very low Hs-cTn concentration, which is again assay dependent, or a low Hs-cTn concentration followed by a minimal change at 1 hour, the detail of which is again assay dependent. To illustrate this the definitive figures currently used for the Architect hs-cTnI assay (Abbott Laboratories) are a “very low” concentration of <2 ng/L or a “low” concentration of <5ng/L, followed by a change at 1 hour of <2ng/L. In contrast, the values for the Elecsys hs-cTnT (Roche Diagnostics) are 5ng/L for a very low concentration, 12ng/L for the low concentration, followed by a change at 1 hour of <3ng/L. To rule in a diagnosis of MI using either the Architect or Elecsys assay an admission value of ≥ 52ng/L or a change at 1 hour of ≥6ng/L (Architect) or ≥5ng/L (Elecsys) is recommended (76). Table 4 displays the assay specific cut offs as per the ESC 2020 NSTEMI guidelines (79).

Table 4: Assay specific cut-offs for the ESC 0/1hr algorithm.

Assay	Very Low (ng/L)	Low (ng/L)	No 1hΔ (ng/L)	High (ng/L)	1hΔ (ng/L)
hs-cTnT (Elecsys; Roche)	<5	<12	<3	>_52	>_5
hs-cTn I (Architect; Abbott)	<4	<5	<2	>_64	>_6
hs-cTn I (Centaur; Siemens)	<3	<6	<3	>_120	>_12
hs-cTn I (Access; Beckman Coulter)	<4	<5	<4	>_50	>_15
hs-cTn I (Clarity; Singulex)	<1	<2	<1	>_30	>_6
hs-cTn I (Vitros; Clinical Diagnostics)	<1	<2	<1	>_40	>_4
hs-cTn I (Pathfast; LSI Medience)	<3	<4	<3	>_90	>_20
hs-cTn I (TriageTrue; Quidel)	<4	<5	<3	>_60	>_8

Use of this algorithm has been very effective with decisions made on patient destination in 75% of cases, with 15% of patients diagnosed as MI and 60% of patients in whom MI was ruled out. However, Kavsak et al(80) have demonstrated that repeated testing on the same sample, at 0 hours, 1.5 hours and 3 hours post sampling, resulted in the reclassification of more than 10% patients when using the ESC 0/1h algorithm. This is in contrast to the reclassification of 2% of patients when using the 2h algorithm. Furthermore, Pickering et al (81) has shown that when the ESC 0/1h algorithm is utilized the sensitivity for the hs-cTnT algorithm is 96.9 % (91.5% to 100%) and for hs-cTnI 98.8 % (97.9% to 100%). This is obviously below the aspiration for 99% sensitivity, a safety level required by most physicians in the Emergency Department (ED) when investigating patients with suspected MI(82).

1.6 Troponin elevations in tachyarrhythmias

Tachyarrhythmias are known to be associated with elevated cTn levels, in addition to their historical association with T1MI they can be the driver behind T2MI and also myocardial injury. The predictive value of cTn in the setting of tachyarrhythmias for CAD is the source of much debate.

Parwani and colleagues undertook the first study evaluating the role of cTnI in the acute setting of atrial fibrilliation (AF)(83). A total of 354 consecutive patients presenting to an emergency department with a primary diagnosis of AF and signs and symptoms suggestive of myocardial ischaemia were included. 14.4% (n=51) of patients had a cTnI level above the ULN, 45% (n=23) of this cohort underwent coronary angiography with 26% (n=6) requiring PCI. Interestingly, 77 patients from the study population underwent coronary angiography despite a cTnI level below the ULN. 30% (n=23) underwent PCI. No significant difference was seen in the patients requiring PCI with a normal cTnI level compared to those with a raised cTnI level (p=0.75). The authors concluded that cTnI had a low predictive value with regards to significant CAD requiring PCI. Alghamry et al(84) undertook a retrospective study of 231 patients who presented with symptomatic AF (chest pain, dyspnoea or palpitations) and had serial cTnI measurements. The authors found that a cTnI level above the ULN was not predictive of CAD after the adjustment of other predictors (OR 1.62, 95% CI 0.79-3.32. p=0.19). A ROC curve analysis revealed an area under the curve value of 0.67 (95% CI 0.58–0.76). This indicated that cTnI levels could not discriminate between those with CAD and those without. For the purpose of this study the authors state that significant CAD is defined as either; (1) CAD with a stenosis greater than 70% on coronary angiography (2) A greater than 50% stenosis of coronary angiography in conjunction with flow fractional reserve (FFR) confirmed haemodynamic compromise and (3) 50-70% left main stem coronary artery stenosis on intravascular ultrasound (IVUS) assessment.

A recent retrospective study from 5 large centres in the UK (85) investigated the association of AF and cTn rises. The study consisting of 3121 patients with a primary diagnosis of AF and who had a cTn level measured during the admission. The patients included were admitted to these centres over a 7 year period (2010-2017). With a median follow-up 1462 days (interquartile range, 925-1975) a total of 586 (18.8%) deaths were recorded. 59.6% of patients were found to have cTn level above the ULN. A total of 216 (6.9%) patients underwent coronary angiography, with 36.1% of those who underwent coronary angiography requiring revascularization in the form of PCI (93.6%), CABG (2.6%) or both (3.8%). After adjustment for key demographic and baseline clinical factors the hazard ratio for mortality with cTn level above the ULN was 1.2 (95% CI, 1.01-1.43; $p<0.05$). In patients who underwent coronary angiography there was no relationship between cTn level and mortality. However, a higher mortality was seen in patients who did not undergo coronary angiography despite a cTn level above the ULN, with a worse short term survival seen on the Kaplan-Meier survival analysis ($p=0.02$). After adjusting for demographic and clinical factors (including cTn level), using a multivariate Cox regression analysis coronary angiography was shown to be associated with a 39% reduction in mortality (hazard ratio, 0.61; 95% CI, 0.42-0.89, $p=0.01$). A potential explanation for this is that the cohort of patients chosen to undergo coronary angiography may in fact be a relatively low risk group, as opposed to the patients who were treated with non-invasive medical therapy. In the 36.1% of patients who underwent coronary revascularization a statistically significant improvement in mortality was not seen (hazard ratio, 0.36; 95% CI, 0.12–1.10; $P=0.07$).

Currently, the data would not support the predictive value of cTn elevations in patients presenting with tachyarrhythmia for CAD. Furthermore, the role of coronary revascularisation in this heterogeneous cohort is also unclear. As such, the evidence would suggest that the checking of cTn levels in patients presenting with tachyarrhythmias should only be undertaken

where the clinical history and other investigations are indicative of myocardial ischaemia. There is however, a clear signal that raised cTn levels in tachyarrhythmia patients is associated with a poorer outcome.

1.7 Challenges: what is the "correct" 99th centile?

The Universal Definition of MI advocates the use of the 99th percentile (ULN) of a hs-cTn assay as the correct cut off level to diagnose MI (2, 48). This level is unique for each Hs-cTn assay that is used in clinical practice. In general, the 99th centile is defined by manufacturer's data derived as part of the internal validation for the assay: subsequently this level is quoted and usually used as a clinical "upper limit of normal".

There are many variables that can affect an individual's troponin level (discussed in detail below). This has a significant effect on the definition of the ULN for any assay. *This is particularly important as each individual manufacturer will have a different inclusion and exclusion criteria when defining the reference population used to quantify the 99th centile*. For example, it has been shown that the younger the reference population is, and the stricter the criteria that are used to define cardiac health are, the lower the 99th centile will be.(86) Furthermore, studies have shown different ULN have been defined for different sample populations when using the same assay.(87) Koerbin et al(87) have shown that the ULN for hs-cTn assays can be refined if individuals are excluded from the reference population when clinical factors such as eGFR, NT-proBNP, clinical criteria, clinical history, examination and echocardiogram, are used to highlight sub clinical disease. When individuals with possible subclinical disease are excluded from the reference population the 99th centile for an assay in men aged less than 75 the value drops from 22.9 ng/L to 10.3 ng/L. This reduction is followed in both sex and all ages, to lesser but still considerable extent. The important question that inevitably arises from this observation, that the 99th centile is dependent upon the general health of a population, is "which level should clinicians use as the upper limit of normal in routine practice"? Intuitively, one would assume the higher level is more

representative of the population as a whole and particularly relevant to the population that is admitted to hospital. *However, guidelines currently used in developing the ULN highlight that the ULN should be derived from a reference population that is healthy and free from cardiovascular disease* (42). This raises some important, and challenging, questions. These are the focus for this thesis.

Firstly, given that Hs-cTn assays are more sensitive, is it appropriate that the assay is still used as a 'rule in' / 'rule out' tool for the diagnosis of MI using a simple binary cut off? Secondly, and more importantly, what is an "abnormal" Hs-cTn level and exactly which population should be used to define that level? This question has important implications for the use of Hs-cTn in clinical practice. The hospital population includes individuals with a very wide spectrum of comorbidities: from outpatients with autoimmune disease to patients in intensive care. *How likely is it that a single troponin level can be used appropriately as a binary cut off for the ULN in such a heterogeneous population?* Furthermore, should we be using the ULN derived from a healthy young population to determine the management of the hospital population?

1.8 Underlying reasons for variability in Hs-cTn levels

1.8.1 Troponin and age

The variation in Hs-cTn levels in individuals is dependent on many factors. One outstanding and significant factor is age. This is highly pertinent to the discussion above: most studies used to define the 99th centile (and therefore, the ULN) of a Hs-cTn assay have used reference populations with a younger average age than the average age of patients who present with MI.(88) This clearly presents a potential logic gap: the application of the 99th centile for a younger healthy population as a putative ULN in clinical practice that is dealing with a much older and comorbid population of patients, who are therefore likely to have a higher troponin level.

The concern that the population of individuals who present with MI are not represented in these reference populations is given credence in work by Eggers et al(89). The authors investigated the influence of cardiovascular disease, sex and age on the 99th percentile. The study has shown that the 70 year olds who were free from cardiovascular disease had considerably higher 99th percentile than previous studies describing younger reference populations. Hammarsten et al(90) showed that in patients under the age of 65 the 99th centile was 12 ng/L with little age dependence whereas in those over 65 years the 99th centile was 82 ng/L and highly age dependent.

1.8.2 Troponin and sex

Prior to the use of cTn assays in clinical practice, creatinine kinase assays were used by clinicians to detect AMI. It was shown that creatinine kinase levels in healthy men was higher than in women (91, 92). However, with the early cTn assays concentrations sex-specific cut offs could not be established due to the fact that cTn concentrations could only be detected in 5% of healthy individuals (4, 93). The development of the Hs-cTn assays now allows for the detection of cTn concentrations in 80% of healthy individuals(93), with significantly higher concentrations detected in men(92). For example, the analysis of 19 cTn assays in 524 healthy individuals has shown that the 99th percentile was 1.2-2.4 times higher in men when compared to women(93). Further, Gore et al have shown that men aged between the age of 50-64 had a 99th percentile of 28ng/L using a hs-cTnT, compared with 14ng/L in contrast women aged 50-64 (94). There is therefore some theoretical concern that the lack of sex distinction on cTn cut-off levels could lead to a failure to correctly diagnose AMI in females (59). Despite this, Trambas et al(95) has shown that changing from a cTnI assay to hs-cTnI assay resulted in an significantly increased number of female patients with a cTnI concentration above the 99th percentile. The work by Eggers et al(89) also reported that men were found to have 24-46% higher median concentrations compared to women and, consequently, higher ULN. This is in keeping with other studies.(93, 96)

1.8.3 Troponin and heart failure

Hs-cTn has been shown to be raised both in patients with heart failure (HF) and in patients who go on to develop HF. Using the early cTn assays, 6% of patients presenting with acute decompensated HF have a raised cTn concentration(97). By contrast, the RELAX-AHF study (n=1,076) has shown that 90.1% (n=969) of patients admitted with acute decompensated HF have an elevated hs-cTnT concentration (98). Furthermore, hs-cTn is also a potent predictor of mortality in HF patients.(99) This includes both cardiac- and non-cardiac related mortality.(100) In addition, reductions in Hs-cTn levels have been found to closely correlate with improvements in the clinical status of HF patients(101). Unsurprisingly, it has also been shown that patients presenting with decompensated HF with severe coronary artery disease (CAD) have a higher Hs-cTn on admission when compared to patients without severe CAD. The explanation put forward for this is that patients with severe coronary stenosis are more liable to myocardial stress produced by an episode of acute decompensated HF.(102) Ergstrup et al have shown in a prospective study of 416 outpatients with chronic systolic heart failure and left ventricular (LV) systolic impairment of 45% or less, 57% of these individuals had a hs-cTnT level above the 99th percentile (103).

1.8.4 Troponin and hypertension

Raised troponin levels have traditionally been thought to represent myocardial necrosis. However, it is increasingly apparent that elevated levels using the modern Hs-cTn assays can be indicative of cardiomyocyte injury, as opposed to necrosis. This is evident by the fact that higher Hs-cTn levels have been detected in patients with hypertension compared to the normotensive population. In addition to this, rising Hs-cTn levels have been associated with cardiac remodeling from normal LV geometry to eccentric hypertrophy in hypertensive patients(104). This is independent of age, sex, diabetes mellitus and renal function

1.8.5 Troponin and renal function

The association of chronic kidney disease (CKD) and hs-cTn elevation has been well established (106, 107). cTn concentrations are often elevated in patients with CKD: this can be explained in two ways. Firstly, patients with CKD have an increased prevalence of cardiovascular disease, and, secondly, in CKD there is a reduction in troponin renal excretion. Thus, in the CRIC study, 81% patients with reduced renal function had detectable hs-cTnT concentrations (108). Furthermore, hs-cTnT concentrations have been shown to be a powerful marker of all-cause mortality in patients receiving haemodialysis(109). Twerenbold and colleagues have looked at the application of more sensitive cTn assays (7 in total) in patients presenting with suspected AMI in a multicentre prospective trial. In particular they were able to assess the diagnostic accuracy of these assays in patients with impaired renal function, and also looked to see if they could establish optimal cut offs to improve the specificity and sensitivity of the assays. A total of 2813 patients were included in the final study population, with renal impairment defined as an estimating glomerular filtration rate (eGFR) of less than 60 ml/min/1.73m^2. A total of 447 (16%) of patients recruited had impaired

renal function. Thirty-six percent of patients with impaired renal function had a final diagnosis AMI compared to 18% of individuals with normal renal function ($p<0.001$). Furthermore, T2MI was diagnosed in 23% patients with renal impairment compared to 10% of individuals with normal renal function ($p<0.001$). In patients whose final diagnosis was not AMI, the patients with renal impairment had higher baseline cTn levels than patients with normal renal function ($p<0.001$). Optimal cut offs were calculated for each assay, with the premise of optimising both the sensitivity and specificity of the assay. The authors found the optimal receiver operating characteristics (ROCs)-derived cut off levels were 1.9-3.4 times higher in patients with impaired renal function compared to individuals with normal renal function. The authors conclude that if assay specific optimal cut offs, which are higher in patients with renal impairment, are used, the diagnostic accuracy of cTn assays can be enhanced (110).

1.8.6 Troponin and cerebrovascular disease

The incidence of cardiovascular disease co-existing with cerebrovascular disease has been well described, in fact cardiac events following a cerebrovascular events are frequent(111). A meta-analysis of 15 studies has shown that 18.1% of patients who had suffered a stroke also had a cTn elevation, although these studies did not use the hs-cTn assays(112). However, in studies using the hs-cTn assay the prevalence of hs-cTn elevation is as high as 60% (113, 114). Anders et al(115) has shown that these troponin elevations were stable in 60% cases with no dynamic change in serial hs-cTn measurements, suggestive that a large proportion of these elevated levels were due to a chronic, as opposed to an acute, myocardial injury. Causes of the chronic injury would include age and comorbidities (such as renal failure, CAD and heart failure). In those patients with an acute myocardial injury it has been suggested that in addition to concomitant T1MI, an acute rise in hs-cTn could be due to an excessive release of

catecholamines in response to changes in the autonomic control of the heart(116). Weight is given to this theory by the fact that the TRELAS study (117) has shown that patients who have suffered an ischaemic stroke with a concomitant T1MI were less likely to have a culprit coronary lesion than age- and sex-matched patients who had suffered solely a T1MI, with 48.2% of the ischaemic stroke group showing no evidence of obstructive CAD on coronary angiography.

1.8.7 Troponin in critical illnesses

Patients who are critically ill also have a different distribution of troponin to a general population. One prospective observational study, for example, has shown 121 (84%) out of 144 critically ill patients had elevated Hs-cTn levels, but only 40% of these patients had study-identified MI.(118) Another study has shown Hs-cTn levels are raised within 12 hours of admission to an Intensive Care Unit in 75% of patients (n = 451). Furthermore, a clear link was demonstrated between raised Hs-cTn levels and morbidity and mortality. Specifically, patients with Hs-cTn level of $<$ 3ng/L had a risk of in hospital death of 0% whereas those with a level ≥ 50ng/L had a risk of death pre discharge of 31% ($p<0.001$). None of these patients were diagnosed as having an "ACS".(119) In patients labelled as "sepsis" Hs-cTn levels have been shown to be raised above the ULN in the majority of cases (80%) and the level is associated with disease severity(120). Interestingly, the elevated Hs-cTn levels were not associated with mortality in this population. Our group has recently published a comprehensive review about the relationship between hs-cTn and critical care (121)

In patients with rheumatoid arthritis Hs-cTn levels have been shown to be raised in comparison to healthy controls:(122) in the rheumatoid arthritis group 8% of patients had a

Hs-cTn level above 14ng/L versus 1% of the healthy controls ($p<0.007$). This was independent of cardiovascular risk factors.

Other conditions that can affect an individual's Hs-cTn level include pulmonary emboli(123), chronic obstructive pulmonary disease(124), cerebrovascular disease(125) and radiation(126).

1.9 So what is the ULN?

The concept of using a single value for the 99th centile as a cutoff to diagnose ACS/NSTEMI/STEMI appears to be flawed. This has been highlighted in several studies. Petrie et al(127) prospectively looked at Hs-cTn levels of 564 patients admitted to an acute medical unit in a district general hospital in the UK. 50% of these patients had their Hs-cTn level measured, and, of those measured, 80% (n = 224) had a raised Hs-cTn (≥14 ng/l). However, only 44 (20%) had a final diagnosis of acute MI. The authors proposed the low actual MI rate is likely to reflect the increased sensitivity, but reduced specificity, of the assay. These data are consistent with Saad et al(128). This study looked at 204 consecutive patients admitted to ED with symptoms suggestive of an ACS. When using a Hs-cTn assay 96 out of the 204 patients had Hs-cTn levels above the 99th centile cutoff but only 26 of these patients had an ACS diagnosis as ultimately defined by electrocardiogram changes and angiography. Stein et al(129) reported on 5,696 hospitalised patients, 61.6% of whom had a Hs-cTn level above the 99th centile. Serial measurements were taken 3,062 patients, a hs-cTnT delta change of 50% or more was seen in 24% of the patients. However, despite these relatively high numbers a confirmed ACS diagnosis accounted for only 6.1% of the total outcomes.

It is clear that the percentage of patients with "elevated" Hs-cTn levels is dependent (a) on the population that is being studied and (b) the derivation of the reference point. It is also clear that the normal population used to define the 99th centile cutoff for manufacturer's Hs-cTn assay is likely to be different to the population that is admitted to hospital. It is this difference between the normal population and the population admitted to hospital that could account for reduced specificity of the Hs-cTn assays. Furthermore, there is currently no policy for manufacturers on how to choose their reference population to determine the 99th centile for their specific Hs-cTn assay. It has indeed been shown that the selection strategy for the

reference population significantly influenced the 99th percentile reference values.(88) As described above, there are multiple factors that can influence any individual's Hs-cTn level including age, renal function, sepsis, heart failure, diabetes mellitus, and chronic inflammatory conditions such as arthritis. One would expect these factors to be more prominent in the population admitted to hospital than the reference population used to determine the 99th centile. Thus, we should expect that the distribution of hs-cTn in the hospital population would be shifted to the right when compared to a reference population of relatively healthy individuals, even of most of them do not actually have heart attack or even heart problems. The potential implication of this is twofold. Firstly, it calls into question the validity of using the 99th centile for the healthy population as an ULN for the hospital population. Secondly, it raises the question as to how to use the assay in the diagnosis of potential ACS/MI, when we would expect a (as yet unresolved) proportion of people attending hospitals to have an hs-cTn above the 99th centile.

Currently, there is a significant, and consistent, body of evidence to support the use of Hs-cTn levels of a patient as a 'rule out' test for ACS. In particular, the recent cohort study by Shah et al (130) has demonstrated that, in patients suspected of ACS, the Hs-cTn assays are able to identify two thirds of patients who are at very low risk of cardiac events and can therefore be discharged from hospital. This is a significant finding and, if implemented by clinicians, could result in dramatic reductions in hospital admissions. As a 'rule in' test, however, the evidence is less conclusive. Part of the reason for this is that the 99th centile for the normal reference population is likely lower than the true 99th centile for the hospital population. We would therefore propose that by trying to define the "true" 99th centile for the hospital population we may facilitate an improved understanding of the value of finding an elevated hs-cTn level in a hospital patient, particularly if they do not have a classical clinical presentation of ACS.

1.10 Aims and objectives

The development of Hs-cTn assays has allowed clinicians to rule out a diagnosis of MI in a timelier manner, thus allowing for a more rapid discharge from hospital. However, the utilisation of hs-cTn as a 'rule in' test for MI has important flaws and uncertainties that carry with them the frequent potential for misdiagnosis and inappropriate treatment. In this thesis the utilization of the hs-cTn assay in hospital practice will be explored.

The overall objectives are as follows:

Experiment 1

-To describe the relationship between tachyarrhythmias at hospital admission and hs-cTn elevations.

-To assess the proportion of patients presenting with tachyarrhythmia who were also found to have a rise in their hs-cTn concentration.

-To describe the outcome of patients presenting with a tachyarrhythmia and hs-cTn concentration rise and the comparison to patients diagnosed with NSTEMI.

Experiment 2

-To define the true 99th centile of the Beckman Coulter Access AccuTnI+3 assay for the hospital population.

-To define the distribution of hs-cTnI in subgroups of the hospital population in order to identify populations with elevated levels, as part of an ongoing project to assess the association with risk of acute cardiac events.

1.11 Proposal

To achieve the aims described above the following chapters will be presented.

High sensitivity troponin in the clinical practice and management of tachyarrhythmias (The STRIPE-MI Study)

Hypothesis:

1. Hs-cTn elevations in association with tachyarrhythmias at presentation is associated with short and mid-term outcomes.

In Chapter 3, the prevalence of patients presenting with a tachyarrhythmia and associated hs-cTn rise will be assessed. The management of these patients will be assessed. The short and mid-term outcome in these patients will also be described.

Is the current threshold for diagnosis of "abnormality", including non ST elevation myocardial infarction, using raised highly sensitive troponin appropriate for a hospital population? (The CHARIOT Study)

Hypothesis:

1. That the 99th centile for the hs-cTn level of a hospital population is significantly different to the current manufacturer's recommended 99th centile, derived from a 'healthy' population.

2. That describing the distribution of hs-cTn in a hospital population and its subgroups will sponsor further investigation regarding the utility of this assay as a biomarker for prediction of medium and long term cardiovascular risk.

In Chapter 4, the 99th percentile of high sensitivity cardiac troponin I concentration for an entire hospital population will be defined.

2.0 Methods

2.1 Setting

All the experiments described in this thesis were undertaken at the University Hospitals Southampton (UHS). UHS is a large tertiary centre in the UK, providing services for 1.9 million people living in Southampton and south Hampshire, specialist services such as neurosciences, cardiac services, and children's intensive care to more than 3.7 million people in central southern England and the Channel Islands. . With regards to cardiac services the centre provides a tertiary level service for both elective and emergency care in both cardiology and cardiothoracic care, including procedures such as CABG and transcatheter aortic valve intervention. The Trust in partnership with the University of Southampton is a major centre for research partners include Medical Research Council and the Wellcome Trust. Within the research directorate is the coronary research group (CRG). The CRG, led by Professor Nick Curzen, coordinates all the research related to coronary artery disease within the Trust. This includes a combination of original and investigator led research. For the purpose of the experiments described in this thesis the statistical analysis was undertaken by myself with the Cardiovascular Research Group at Keele University.

2.2 Cardiac troponin assay

The Beckman Coulter Access AccuTnI+3 assay (Beckman Coulter, Brea, CA, USA) is employed in routine clinical practice at our Trust and was used to measure hs-cTnI concentrations in the study populations in both experiments. The supplied 99th percentile (ULN) is 40 ng/L, which is the level used in routine clinical practice at our institution. The ULN for this assay was determined by analysing serum samples 330 healthy blood donors (260 men, 70 women age

range 18-70, median age 36years) in Italy (131). 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. 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. This involves the checking of the imprecision and bias using internal quality control materials at three levels, this was undertaken by an analyst. The internal quality control imprecision and bias were reviewed by senior biochemistry lab staff on a monthly basis.

2.3 Statistical analysis

The data for both experiments were stored on password encrypted computers and external hard drives owned by the CRG. The statistical packages used for the experiments were IBM SPSS V.22.0 (SPSS, IBM Corporation, Armonk, New York, USA) and Stata 14.0 (College Station, USA). For the purposes of the CHARIOT study a decision was made to collaborate with Keele Cardiovascular Research Group to assist with the statistical analysis. This decision was made given the proven track record of the Keele Cardiovascular Research Group with regards to original research involving the handling and analysis of large datasets.

2.4 Ethical approval

All the experiments described in this thesis were sponsored by the University Hospitals Southampton Foundation Trust Research and Development department. All the experiments were undertaken according to the principles of Good Clinical Practice and the Declaration of Helsinki. Ethical approval was sought for both experiments.

For the STRIPE-MI study, ethical approval was obtained via the South East Scotland Research Ethics Committee 01 and the Confidentiality Advisory Group (CAG), a subsidiary of the Health Research Authority (HRA).

The CHARIOT study also required ethical approval, however the process for the CHARIOT study was more protracted due the nature of the study. 20,000 consecutive patients would need to be recruited without written consent. Furthermore, the hs-cTnI levels measured on these patients would not be available to their clinical team irrespective of the level. The results would only be available to the clinical team if requested as part of the patient's clinical care. Initially, a favourable recommendation was given by the South Central - Hampshire B Research Ethics Committee. However, the study would also need approval from the CAG. To assist with this approval from the CAG, the protocol was reviewed by the British Cardiac Patients Association (BCPA). Following a review by the BCPA, a letter of recommendation was given by the Chair of the BCPA, Mr. Keith Jackson (see figure 5). Following this recommendation and a final review of the study by the CAG, the study was given ethical approval.

THE BRITISH CARDIAC PATIENTS ASSOCIATION

President: Professor John Wallwork CBE. DL. ~~EMed Sci~~
Vice-President: Mr A ~~Bowobet~~ DMS FFA.
Founder: Mr. Fred Roach MBE

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Re: Is the Current Threshold for Diagnosis of "Abnormality", including Non ST Elevation Myocardial Infarction, using Raised Highly Sensitive Troponin Appropriate for a Hospital Population? The CHARIOT study

Dear Nick

25th April 2017

Thank you for approaching the British Cardiac Patients Association to comment upon the CHARIOT Study.

I can confirm that, having seen the protocol and discussed the study with you over the phone, we the Association Trustees, feel that it is, indeed, appropriate that (a) the patients are not consented in this study and (b) the result of their troponin test will never be revealed to them or their supervising clinician unless troponin level was requested specifically for clinical reasons.

We feel that this is an important study that could help improve the care of a large number of patients, and that its conduct will be in favour of "the common good".

I am happy for this email to be shared with the Confidentiality Advisory Group and similar organisations

Best wishes

Yours sincerely

Keith Jackson

Keith Jackson
Chairman, British Cardiac Patients Association

Figure 5: Letter of approval from the chair of the BCPA, Mr Keith Jackson

3.0 Results : High sensitivity troponin in the clinical practice and management of tachyarrhythmias (The STRIPE-MI Study)

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Clinical

High sensitivity troponin in the management of tachyarrhythmias ☆, ☆☆, ★

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Abstract

Background

The introduction of the highly sensitive troponin (hs-trop) assays into clinical practice has allowed for the more rapid diagnosis or exclusion of type 1 myocardial infarctions (T1MI) by clinicians, in addition type 2 myocardial infarctions (T2MI) are now more frequently detected. Tachyarrhythmias are one of the common causes of T2MI, the medium and long term outcome for this cohort of T2MI is yet to be clarified.

Methods

Retrospective review of consecutive patients admitted with a diagnosis of either (a) non ST-elevation myocardial infarction (NSTEMI) or (b) tachyarrhythmia was performed. Data were collected on patient demographics and investigations. Patient mortality status was recorded through the Personal Demographics Service (PDS) via NHS Digital.

Results

A total of 704 patients were eligible for inclusion to the study. 264 patients were included in the study with a final discharge diagnosis of NSTEMI and 440 patients with a final discharge diagnosis of tachyarrhythmia. There was a significantly higher peak troponin in NSTEMI patients compared to the tachyarrhythmia troponin positive group (4552 ng/L vs 571 ng/L, $p < 0.001$). Mortality was significantly higher in the troponin positive tachyarrhythmia patients than the troponin negative patients (54 vs 34, 26.2% vs 14.5%, log rank $p = 0.003$), furthermore, the mortality of NSTEMI and troponin positive tachyarrhythmia patients was similar (55 vs 54, 20.8% vs 26.2%, log rank $p = 0.416$). Only one patient (0.14%) was given a formal diagnosis of T2MI.

Conclusions

These data suggest that troponin positive tachyarrhythmia is not a benign diagnosis, and has a mortality rate similar to NSTEMI. Formal labeling as T2MI is rare in real life practice. More investigation into the detection and management of T2MI and troponin positive arrhythmia patients is now warranted.

Abstract

Background

The introduction of the highly sensitive troponin (hs-cTn) assays in clinical practice have allowed for the more rapid diagnosis or exclusion of type 1 myocardial infarctions (T1MI) by clinicians. Conditions that cause myocardial ischaemia due to imbalance in oxygen supply and demand are known as type 2 myocardial infarctions (T2MI). The incidence of T2MI has increased with the advent of the hs-cTn assays. Arrhythmias are one of the common conditions associated with T2MI. The optimal management strategy for this cohort of patients is currently unknown.

Methods

Retrospective review of consecutive emergency admissions to our institution with a primary diagnosis of either a non ST-elevated myocardial infarction (NSTEMI) or arrhythmia was performed. Patients who were 18 years or older and had a hs-cTn level measured during their admission were included in the study. Patients were classified as having either NSTEMI or arrhythmia as their primary discharge diagnosis at the discretion of discharging clinician. Data were collected on patient demographics, presenting symptoms, traditional risk factors for coronary artery disease, heart rate on admission, highest recorded Hs-cTn, left ventricular systolic function, investigations and procedures carried out, and discharge medication. The mortality status of patients in this study was collected via NHS Digital through the Personal Demographics Service (PDS), which is the master demographic source for the NHS which records patient mortality status including date of death where applicable.

Results

A total of 2404 consecutive patients were discharged from our institution with a primary diagnosis of NSTEMI or arrhythmia between April 2014 and December 2015. Of these 2404 patients, a total of 704 patients were deemed eligible for inclusion to the study. The commonest reason for exclusion to the study were patients who were either electively admitted for procedures such as DC Cardioversion and Electrophysiology ablation procedures or no hs-cTn level measured during their admission. The patients included in the study comprised of 264 patients with a final discharge diagnosis of NSTEMI and 440 patients with a final discharge diagnosis of arrhythmia. 206 (46.8%) of arrhythmia patients had a hs-cTn level above the 99th percentile (>40 ng/L). There is a significantly higher troponin seen in NSTEMI patients compared to the arrhythmia troponin positive group (4552ng/L vs 571ng/L, p<0.001). Significant differences are observed in the presence of chest pain (238 vs 94, 90% vs 45.6%, p<0.001). Additionally, there is a significant difference between the number of patients who were referred for non-invasive ischaemia testing (NIIT) (29 vs 10, 11% vs 5%, p=0.018), coronary angiography (158 vs 35, 60% vs 17%, p <0.001), presence of coronary artery disease (127 vs 3, 80% vs 9%, p<0.001) and subsequent PCI (90 vs 0, 34% vs 0%, p<0.001) when comparing the NSTEMI and arrhythmia troponin positive groups respectively. A significant difference in mortality was observed between the troponin positive arrhythmia patients to the troponin negative arrhythmia patients (54 vs 34, 26.2% vs 14.5%, log rank p=0.003). Patients with an NSTEMI had similar mortality to arrhythmia patients with a raised hs-cTn level (55 vs 54, 20.8% vs 26.2%, log rank p=0.416). Of interest, only one patient (0.14%) was given a formal diagnosis of T2MI.

Conclusions

These data suggest that a raised hs-cTn level in tachyarrhythmia patients is not a benign diagnosis, and has a mortality rate similar to NSTEMI. Formal labeling as T2MI is rare in real life practice. More investigation into the management of troponin positive tachyarrhythmia patients is now warranted.

3.1 Introduction

The early cTn assays were introduced into clinical practice from 1995 onwards and aided clinicians in the diagnosis of acute MI. The value of the early assays was limited by their lack of sensitivity, meaning that a reliable rule in or rule out could only be given 10-12 hours after the onset of symptoms. Consequently, there has been a drive to develop more sensitive troponin assays that could facilitate early exclusion of MI (41, 42, 132-134). The new highly sensitive assays are able to detect troponin at much lower concentrations than the previous generation(4). The universal definition of MI recommends that a troponin assay used to diagnose MI should have a coefficient of variation of ≤ 10% at the threshold concentration representing the 99th percentile upper limit of a normal reference population (ULN) (135). However, these hs-cTn assays detect troponin in more than 50% of the general population with some assays able to detect troponin in everyone(44). The interpretation of assays results in patients is therefore more challenging.

According to the fourth universal definition (48, 135) there are different forms of MI. The classical form or Type 1 MI (T1MI) is defined as a troponin elevation related to an acute coronary plaque rupture (49, 50). Type 2 MI (T2MI) is defined as myocardial ischaemia resulting from increased oxygen demand or reduced oxygen supply. Clinical scenarios giving rise to T2MI include sepsis, anaemia, arrhythmia, hypertension, hypotension, coronary artery spasm and embolism (49). The evidence for antiplatelet therapy (136, 137) and early revascularisation (67, 68) in the setting of a T1MI is robust and compelling. By contrast further non-invasive or invasive investigations have failed to demonstrate a clinical outcome benefit in T2MI(52). Furthermore, patients with T2MI have been shown to have a 2-fold higher mortality rate than those with T1MI(138).

A common dilemma for clinicians is the optimal management of patients presenting with primary arrhythmia and troponin elevation. A simplistic view would be to define troponin rises associated with a primary diagnosis of arrhythmia as evidence of ACS and therefore manage these patients as such. However, many of these patients have not suffered a T1MI and may therefore undergo unnecessary invasive assessment and inappropriate revascularization and pharmacotherapy. The advent of the contemporary hs-cTn assay makes this more prevalent (139-141).

It is well described that troponin levels are often elevated in association with arrhythmias such as atrial fibrillation (AF), supraventricular tachycardia (SVT) and ventricular tachycardia (VT) and, furthermore, that many of these patients have unobstructed coronary arteries(142-151). However, studies have previously shown in patients with AF and elevated troponin levels, AF is a risk marker for cardiovascular morbidity and mortality (152-154).

The selective contribution of the arrhythmia itself, and concomitant coronary disease, in such patients is discrepant and unclear according to existing evidence. The aim of this study was to assess the tracked mortality outcome of a consecutive series of patients presenting with a primary diagnosis of tachyarrhythmia according to whether they were troponin positive or negative and using a cohort with a primary diagnosis of NSTEMI as a reference.

3.2 Methods

3.2.1 Patient selection

We conducted a retrospective search of patients who were admitted to the University Hospitals Southampton, Southampton, UK between April 2014 and December 2015 with a primary diagnosis of AF, SVT, VT or non ST-elevated myocardial infarction (NSTEMI). This was achieved by searching the trust database for patients who had been discharged with a primary diagnosis with the following International Classification of Diseases, version 10 (ICD-10) codes I48(AF), I47.1(SVT), I47.2(VT) and I21.4(NSTEMI) for the study period. This hospital is an 1100-bed teaching hospital.

Patients were deemed eligible for inclusion into the study if they were ≥ 18 years of age, UK residents, had a hs-cTnI level measured (Beckman Coulter Access AccuTnI+3 Hs-cTnI assay), and had a final primary diagnosis of either AF, SVT, VT and NSTEMI. Patients in the AF, SVT or VT group were excluded if their heart rate on admission was less than 100 beats per minute. Patients presenting with a STEMI were also excluded, as the vast majority of STEMI cases clinical decisions are made prior to the availability of hs-cTn level.

The population of patients included in this study was identified through the use of the coding system which is primarily used for hospital reimbursement. A total of 2404 patients were identified as having a final primary diagnosis of either AF, SVT, VT or NSTEMI, this final primary diagnosis was made by the discharging responsible clinician for the patient's hospital stay. From this group a total 705 patients were identified as eligible for inclusion to the study after a review of their clinical records. A further patient was excluded due to miscoding of their final

diagnosis. Initially the remaining 704 patients were broadly stratified into four primary diagnoses; NSTEMI (n=264), AF (n=344), SVT (n=40), VT (n=56) (figure 2). No re-interpretation of diagnosis was made by the researchers.

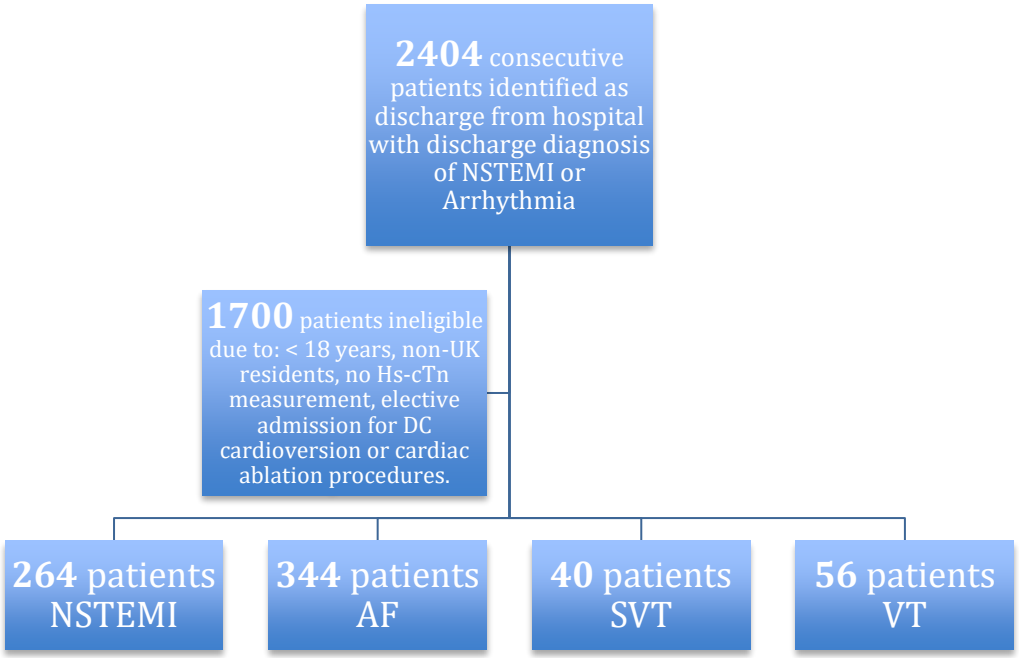


Figure 6: Flow diagram of patients screened and recruited to the STRIPE-MI study.

3.2.2 Data collection

Data on the patients included in the study were collected through a review of both electronic and paper patient records using a standardized data collection template, designed by the researchers in Microsoft Excel (version 15.26). Patient demographics, presenting symptoms, traditional risk factors for CAD, heart rate on admission, highest recorded hs-cTn reading, left ventricular systolic function, investigations and procedures carried out, and discharge medication.

In order to obtain robust outcome data, the mortality status of each patient was obtained via NHS Digital through the Personal Demographics Service (PDS) following ethical and Confidentiality Advisory Group (CAG) approval. The PDS is the master demographic source for the NHS, which records patient mortality status including date of death where applicable.

3.2.3 Troponin assay

The Beckman Coulter Access AccuTnI+3 Hs-cTn assay (Beckman Coulter, Brea, CA, USA) was used to determine the hs-cTn level of patients recruited to this study. The manufacturer's 99^{th} percentile cut off for the assay is 40 ng/L.

3.2.4 Statistical analysis

Continuous variables were presented as mean (+/- SD) or as medians and interquartile ranges (IQR). Differences in the continuous variables were evaluated using independent sample t tests. Categorical variables were compared by using χ^2 tests.

Cumulative survival curves were constructed using the Kaplan-Meier method with the log-rank test used to compare the survival curves.

For all analyses, two-sided pa values <0.05 were defined as significant. Statistical analyses were performed using IBM SPSS V.22.0 (SPSS, IBM Corporation, Armonk, New York, USA). Comparisons for baseline characteristics and mortality were made for the following groups:

1) NSTEMI versus tachyarrhythmia
2) Troponin positive tachyarrhythmia versus troponin negative tachyarrhythmia

3.2.5 Ethical approval

The study was sponsored by University Hospital Southampton NHS Foundation Trust Research and Development department, approved by the South East Scotland Research Ethics Committee 01 (REC reference: 16/SS/0194, IRAS project ID:206061) and the CAG (CAG reference: 16/CAG/0146).

3.3 Results

3.3.1 Observed data

Patients included in the study ranged from age 21 to 100 years with a median of 75 years. Three hundred and eighty-eight (55%) patients were male. The patients were divided into one of four primary diagnoses: NSTEMI (n=264), AF (n=344), VT (n=56) or SVT (n=40). Of interest, T2MI was listed as a diagnosis in only 1 patient in the whole study population.

Table 5 shows a comparison of the baseline demographics of patients in the NSTEMI and arrhythmia group. Significant differences are observed in hypertension, hypercholesterolaemia, and diabetes mellitus. Of the arrhythmia group (n=440), 206 (47%) were troponin positive (ie elevated troponin above the 99th percentile cut off 40ng/L) and 234 (53%) were troponin negative. Table 6 and7 compare the baseline demographics between NSTEMI and arrhythmia troponin positive and negative patients.

Table 5: Comparison of baseline demographics (SD= Standard deviation, IHD= Ischaemic heart disease)

	NSTEMI (n=264)	Total Arrhythmia (n=440)	p value
Age (years/SD)	73.0/14.33	72.9/13.56	0.891
Sex (male)	161(61%)	227(52%)	**0.015**
Sex (female)	103(39%)	213(48%)	**0.015**
Hypertension	189(72%)	255(58%)	**<0.001**
Hypercholesterolaemia	108(41%)	102(23%)	**<0.001**
Diabetes Mellitus	80(30%)	70(16%)	**<0.001**
IHD	76(28%)	92(21%)	0.261
Heart failure	61(23%)	121(27%)	0.208
AF	70(27%)	372(84%)	**<0.001**
SVT	7(3%)	52(12%)	**<0.001**
VT	12(4%)	53(12%)	**<0.001**

Table 6: Patient baseline demographics in NSTEMI and arrhythmia troponin positive patients

	NSTEMI (n=264)	Arrhythmia troponin positive (n=206)	p value
Age (years/SD)	73.0/14.33	74.9/13.37	0.152
Sex (male)	161(61%)	116(56%)	0.308
Sex (female)	103(39%)	91(44%)	0.308
Hypertension	189(72%)	128(62%)	**0.025**
Hypercholesterolaemia	108(41%)	54(26%)	**0.001**
Diabetes Mellitus	80(30%)	35(17%)	**0.001**
IHD	76(28%)	48(23%)	0.167
Heart failure	61(23%)	66(32%)	0.054
AF	70(27%)	168(81%)	**<0.001**
SVT	7(3%)	25(12%)	**<0.001**
VT	12(4%)	31(15%)	**<0.001**

Table 7: Patient baseline demographics in NSTEMI and arrhythmia troponin negative.

	NSTEMI (n=264)	Arrhythmia troponin negative (n=234)	p value
Age (years/SD)	73.0/14.33	71.1/13.53	0.130
Sex (male)	161(61%)	110(47%)	**0.002**
Sex (female)	103(39%)	124(53%)	**0.002**
Hypertension	189(72%)	129(55%)	**<0.001**
Hypercholesterolaemia	108(41%)	49(21%)	**<0.001**
Diabetes Mellitus	80(30%)	35(15%)	**<0.001**
IHD	76(28%)	44(19%)	**0.016**
Heart failure	61(23%)	56(24%)	0.996
AF	70(27%)	206(88%)	**<0.001**
SVT	7(3%)	28(12%)	**<0.001**
VT	12(4%)	21(9%)	**0.036**

Table 8 shows a comparison between the baseline demographics of arrhythmia troponin positive and negative patients. Troponin positive patients are older (74.9 Vs 71.1, p=0.004) and have fewer previous AF diagnoses (168(81%) Vs 206(88%), p=0.046).

Table 8: Patient baseline demographics in arrhythmia troponin positive and arrhythmia troponin negative

	Arrhythmia trop positive (n=206)	**Arrhythmia trop negative (n=234)**	**p value**
Age (years/SD)	74.9/13.37	71.1/13.53	**0.004**
Sex (male)	116(56%)	110(47%)	0.063
Sex (female)	91(44%)	124(53%)	0.063
Hypertension	128(62%)	129(55%)	0.115
Hypercholesterolaemia	54(26%)	49(21%)	0.160
Diabetes Mellitus	35(17%)	35(15%)	0.562
IHD	48(23%)	44(19%)	0.357
Heart failure	66(32%)	56(24%)	0.060
AF	168(81%)	206(88%)	**0.046**
SVT	25(12%)	28(12%)	0.847
VT	31(15%)	21(9%)	0.073

Table 9 displays differences in the clinical presentations, investigations and management of NSTEMI patients compared to those with a primary diagnosis of arrhythmia. There are significant differences in the occurrence of chest pain and heart rate between the two groups. In addition, there are significant differences in non-invasive ischaemia testing, coronary angiogram and subsequent PCI. A significant difference in prescribed dual antiplatelet therapy (DAPT) was also seen between the two groups.

Table 9: Clinical presentation, investigations and management in NSTEMI and arrhythmia patients. (eGFR= Glomerular Filtration Rate, PCI= Percutaneous Coronary Intervention, NIIT= Non-Invasive Ischaemia Tests, DAPT= Dual Antiplatelet Therapy)

	NSTEMI (n=264)	**Arrhythmia (n=440)**	**p value**
Chest pain	238(90%)	172(39%)	**<0.001**
Heart rate (SD)	84(22.9)	147(33.7)	**<0.001**
eGFR	65.10	64.65	0.781
LVSD	109(41%)	163(37%)	0.300
Troponin (ng/L)	4552	276	**<0.001**
Coronary angiography	158(60%)	40(9%)	**<0.001**
Lesion	127(48%)	3(0.7%)	**<0.001**
PCI	90(34.1%)	0(0%)	**<0.001**
NIIT	29(11%)	14(3%)	**<0.001**
DAPT	190(72%)	26(6%)	**<0.001**

Tables 10 and 11 describe the comparison between NSTEMI and arrhythmia troponin positive and negative patients.

Table 10: Clinical presentation, investigations and management in NSTEMI and arrhythmia troponin positive patients.

	NSTEMI (n=264)	Arrhythmia trop positive (n=206)	p value
Chest pain	238(90%)	94(45.6%)	**<0.001**
Heart rate (SD)	84(22.9)	148(33.4)	**<0.001**
eGFR	65.10	62.12	0.122
LVSD	109(41%)	80(41%)	0.944
Troponin (ng/L)	4552	571	**<0.001**
Coronary angiography	158(60%)	35(17%)	**<0.001**
Lesion	127(80%)	3(9%)	**<0.001**
PCI	90(57%)	0(0%)	**<0.001**
NIIT	29(11%)	10(5%)	**0.018**
DAPT	190(72%)	19(9%)	**<0.001**

Table 11: Clinical presentation, investigations and management in NSTEMI and arrhythmia troponin negative patients.

	NSTEMI (n=264)	Arrhythmia trop negative (n=234)	p value
Chest pain	238(90%)	73(31%)	**<0.001**
Heart rate (SD)	84(22.9)	145(33.9)	**<0.001**
eGFR	65.10	66.89	0.344
LVSD	109(41%)	71(33%)	0.073
Troponin (ng/L)	4552	16	**<0.001**
Coronary angiography	158(60%)	3(1%)	**<0.001**
Lesion	127(80%)	0(0%)	**<0.001**
PCI	90(57%)	0(0%)	**<0.001**
NIIT	29(11%)	4(2%)	**<0.001**
DAPT	190(72%)	7(3%)	**<0.001**

Table 12 compares the clinical presentations, investigations and management of arrhythmia troponin positive and arrhythmia troponin negative patients. The troponin positive patients had significantly more cases of chest pain on admission, more coronary angiography, presence of coronary artery disease and significantly worse eGFR.

Table 12: Clinical presentations, investigations and management of Arrhythmia patients.

	Arrhythmia trop positive (n=206)	**Arrhythmia trop negative (n=234)**	**p value**
Chest pain	101(49%)	73(31%)	**<0.001**
Heart rate (SD)	148(33.4)	145(33.9)	0.337
eGFR	62.12	66.89	**0.013**
LVSD	80(41%)	71(33%)	0.083
Troponin (ng/L)	571	16	**<0.001**
Coronary angiography	35(17%)	3(1%)	**<0.001**
Lesion	3(9%)	0(0%)	**<0.001**
NIIT	10(5%)	4(2%)	**0.031**
DAPT	19(9%)	7(3%)	0.069

The distribution of troponin for each primary diagnosis is displayed as a box plot in figure 7. The boxplot only includes the data of troponin positive patients (n=470). Outliers are present in the AF group, presented as dots outside of the interquartile range (IQR) bar. The clinical records of the outliers were further screened to ensure that they were coded correctly.

Table 13 displays the mean, median, standard deviation (SD) and IQR of the Hs-cTn readings for each primary diagnosis group.

Figure 7: Hs-cTn boxplots for all patients with a troponin above 40 ng/L.

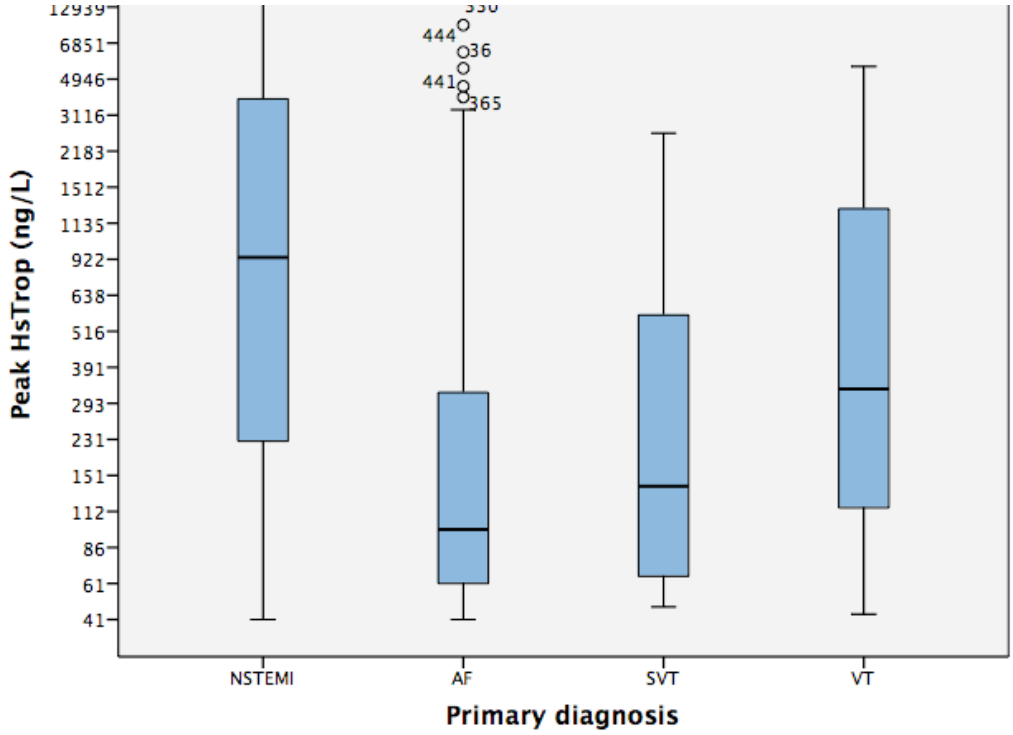


Table 13: Hs-cTn values for each primary diagnosis (troponin positive patients only)

Troponin (ng/L)	NSTEMI (264)	AF (149)	SVT (22)	VT (35)
Mean	4551.6	489.5	451.3	997.2
Median	924.5	99.0	136.5	322.0
SD	10494.8	1140.6	632.5	1481.0
IQR	3759.0	263.0	506.0	1312.0

3.3.2 Clinical outcomes

The mortality data was obtained via NHS Digital through the PDS. Follow-up was a median of 747 days, with a range of 1-1223 days. No significant differences were seen between the NSTEMI and tachyarrhythmia group in terms of survival rates at 3 months (251 vs 416, 95% vs 94%, p=0.662), at 12 months (223 vs 384, 84% vs 87%, p=0.307), and finally at 18 months (219 vs 373, 83% vs 85%, 0.529).

A Kaplan-Meier survival curve was constructed to compare the mortality in between both NSTEMI and tachyarrhythmia group, demonstrating no significant difference in mortality between the two groups (p=0.401). This is shown in figure 8.

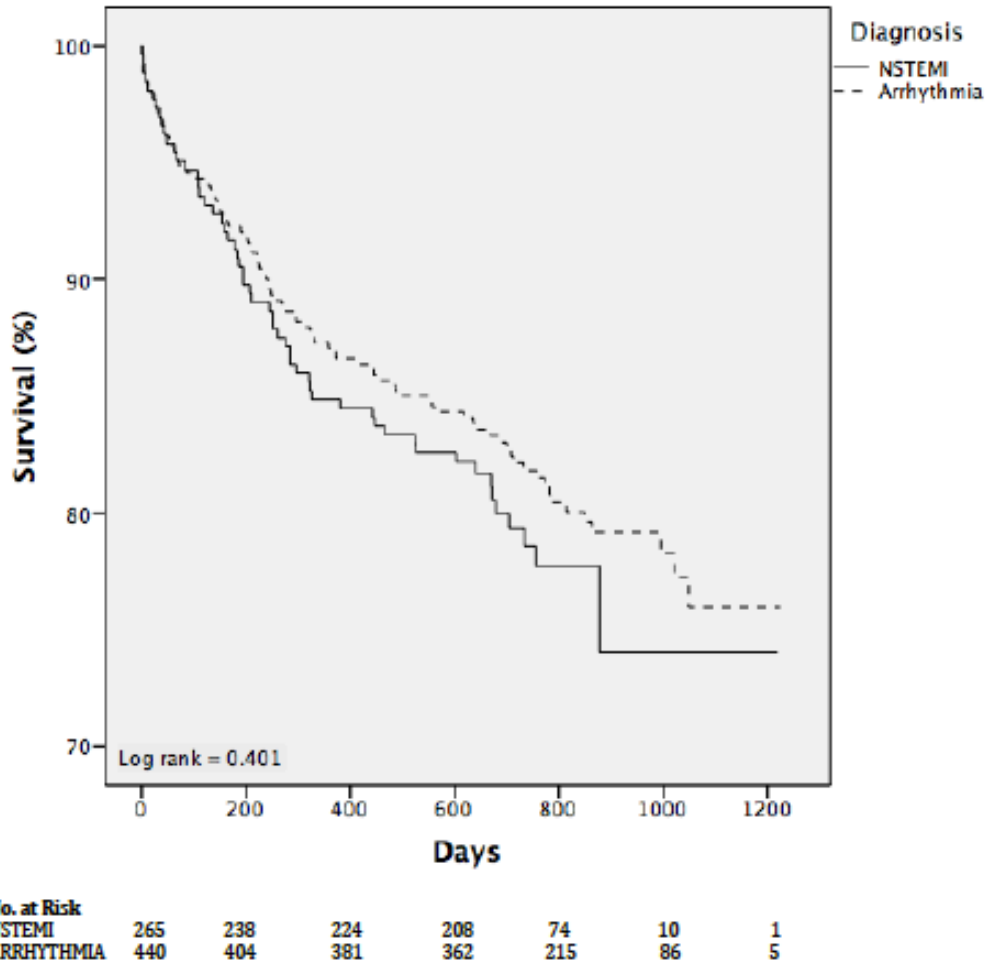


Figure 8: Kaplan-Meier survival curve comparing NSTEMI and tachyarrhythmia patients.

Tachyarrhythmia patients were stratified according to whether they had a raised Hs-cTn level above the ULN (+ve) or below the ULN (-ve). Kaplan-Meier survival analysis (figure 9) showed a significant difference in mortality between the two groups for the whole follow-up period (54 vs 34, 26.2% Vs 14.5%, p = 0.003).

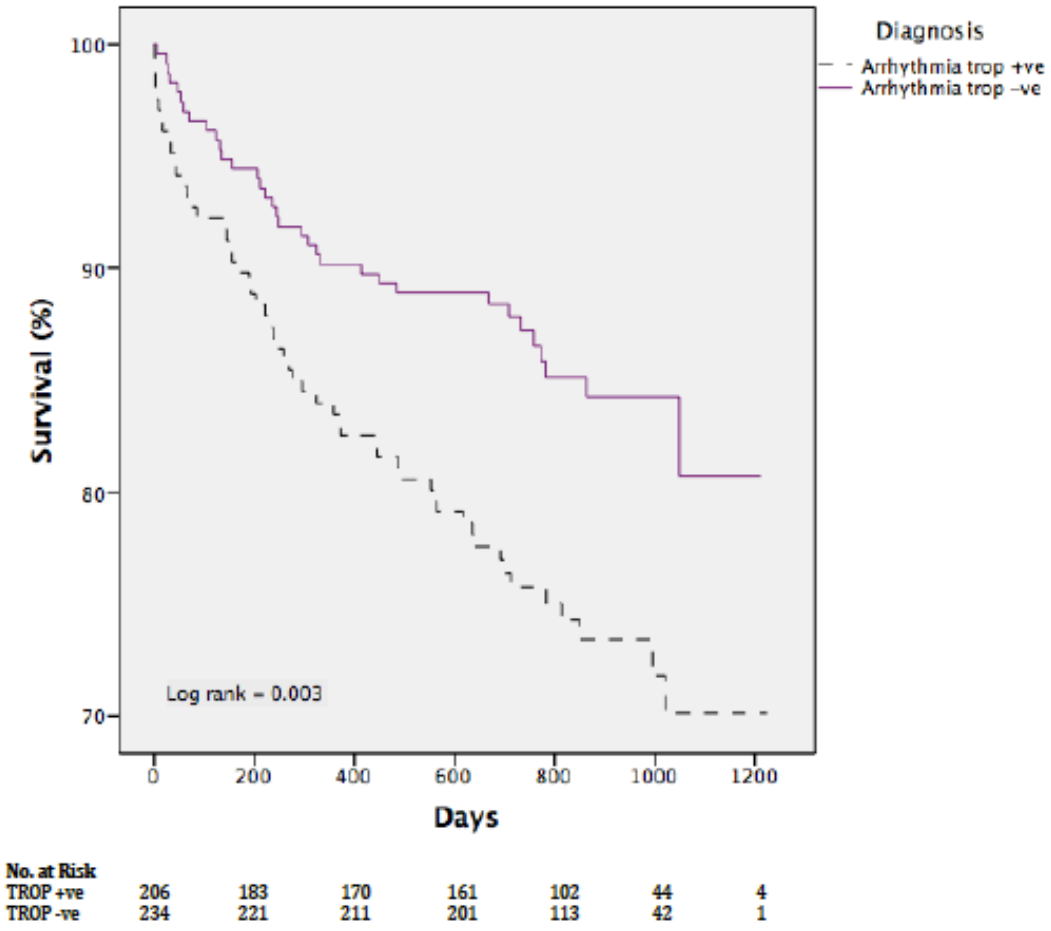
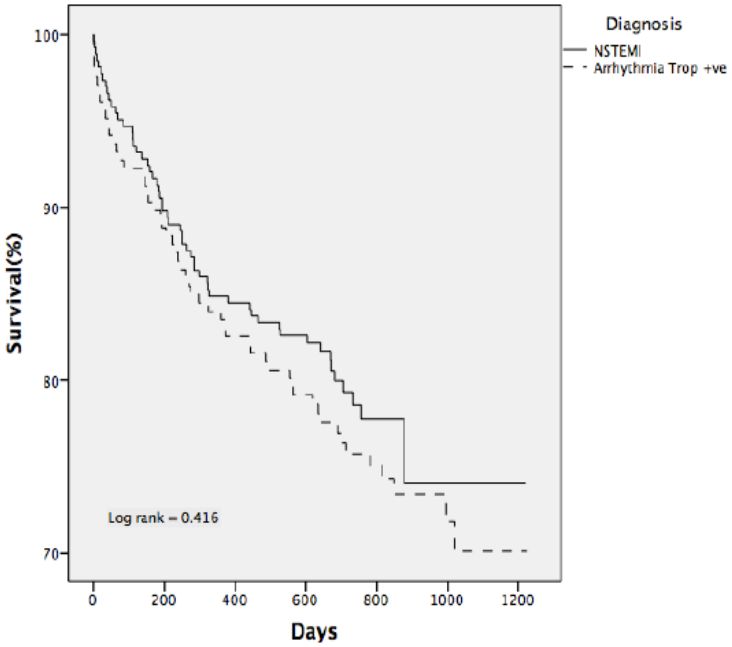


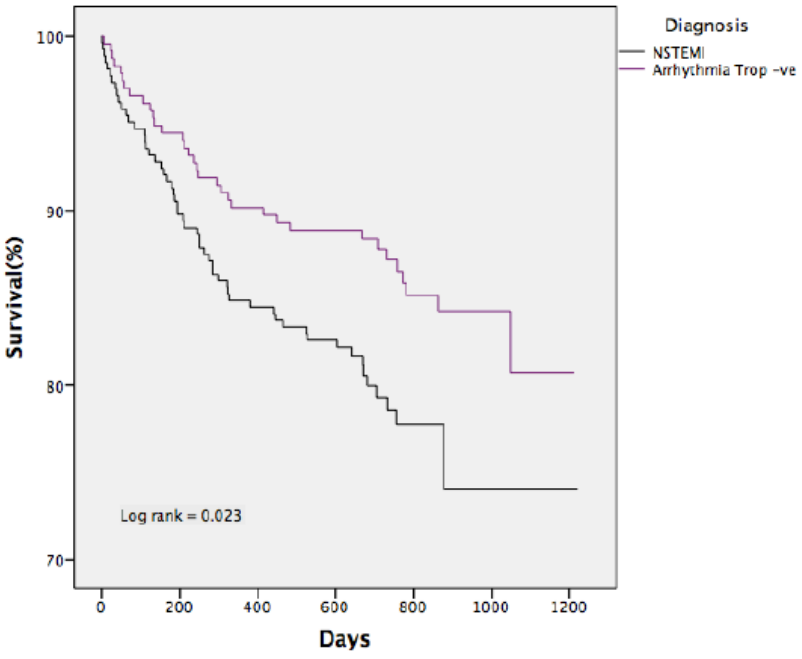
Figure 9: Kaplan-Meier survival curve for tachyarrhythmia patients.

Kaplan-Meier survival curves also show no significant difference in mortality over the follow up period (p=0.416) between NSTEMI and troponin positive tachyarrhythmia (figure 10) but a significantly worse mortality outcome (p=0.023) in the NSTEMI group compared to the troponin negative tachyarrhythmia group (figure 11).



No. at Risk							
NSTEMI	265	238	224	208	74	10	1
TROP +ve	206	183	170	161	102	44	4

Figure 10: Kaplan-Meier survival curve NSTEMI and tachyarrhythmia troponin +ve.



No. at Risk							
NSTEMI	265	238	224	208	74	10	1
TROP -ve	234	221	211	201	113	42	1

Figure 11: Kaplan-Meier Survival Curve NSTEMI and tachyarrhythmia Troponin – ve.

Further survival analysis has shown that in the NSTEMI group the absence of chest pain confers a higher mortality risk (13 vs 42, 43.3% vs 17.9%, p=0.01) (see figure 12). This observation is also seen in the tachyarrhythmia patients with a raised hs-cTn level (42 vs 12, 37.2% vs 12.8%, p<0.001) (see figure 13). This is not seen in the tachyarrhythmia patients with a normal Hs-cTn level (22 vs 12, 13.7% vs 16.4%, p=0.603).

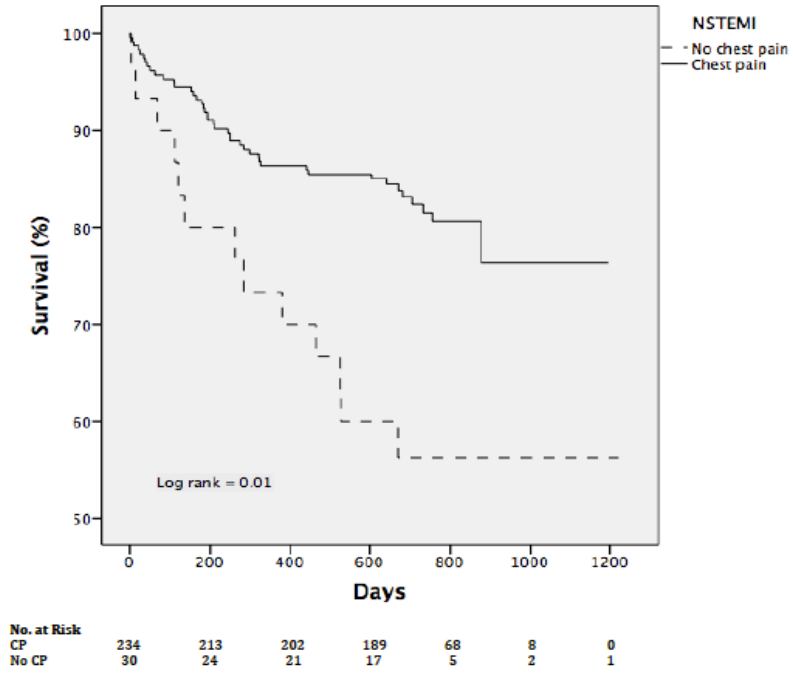


Figure 12: Kaplan-Meier curve NSTEMI with and without chest pain.

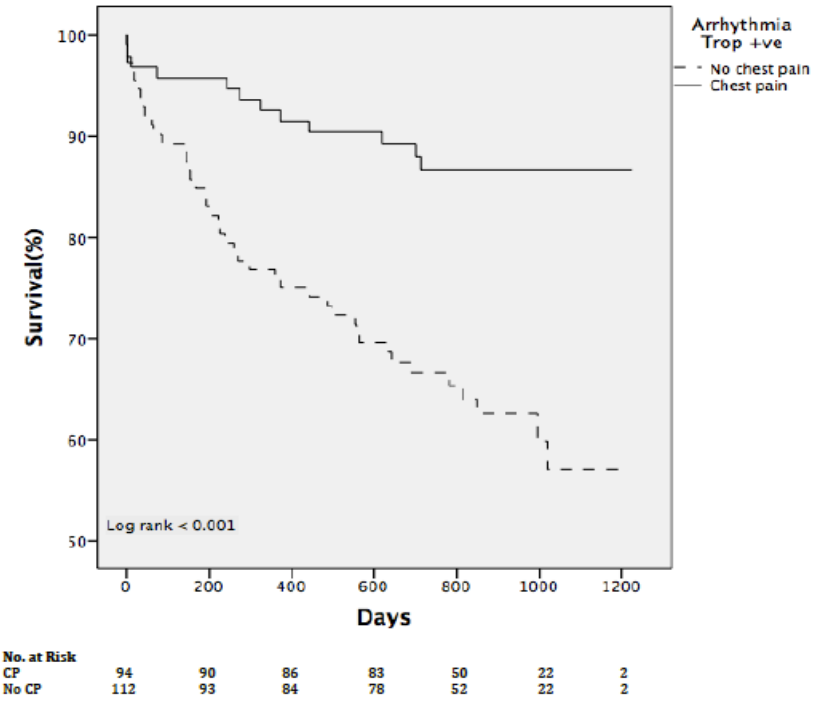


Figure 13: Kaplan-Meier survival curve tachyarrhythmia troponin +ve with and without chest pain.

3.4 Discussion

This study has described the distribution, management and associated mortality of a consecutive group of patients presenting with a primary diagnosis of tachyarrhythmia, and the impact of troponin results on these. A group of NSTEMI patients admitted during the study period is used for reference comparison.

Our main findings were as follows. Firstly, that 47% of patients presenting with a tachyarrhythmia are troponin positive but do not receive a formal diagnosis of either NSTEMI (T1MI) or T2MI. Secondly, that tachyarrhythmia patients with a raised hs-cTn level have a low rate of angiography and PCI compared to NSTEMI patients. Third, that the mortality of tachyarrhythmia troponin positive patients is similar to NSTEMI patients, and that this is higher than troponin negative tachyarrhythmia patients.

Our findings highlight the challenge that front line staff are presented with in the interpretation of troponin elevation in patients presenting with a tachyarrhythmia. Previous studies have shown that patients presenting with AF to the emergency department (n=2898) have shown an increased risk of mortality when they have a Hs-cTn level above the ULN compared to a normal Hs-cTn level(155). More recently, Thelin et al(156) investigated troponin elevations patients who presented with AF with a fast ventricular response who were previously not known to have coronary artery disease (CAD) (n=521). The authors hypothesized that significant CAD in this cohort of patients is the driver behind the troponin rise. They aimed to prove that there was an increased risk of cardiac events in AF patients with a troponin rise. The primary outcome of the study was ACS, revascularization or death due to ischaemic heart disease, patients were followed up for 30 months in total. The study found that 9.5% (n=49) had troponin levels above the ULN. The investigators went onto show in this cohort of patients, the age-adjusted hazard ratio for all-cause mortality was 3.8(95% CI: 1.7 to

8.5; p=0.001), however, they could not show a major increased risk of coronary events or death due to ischaemic heart disease. Thus suggesting that CAD may not play a significant role in the increased mortality risk seen in patients with AF and troponin rise.

In my study a total of 470 (66.8%) are diagnosed as having suffered an MI as per the fourth universal definition (50, 157), of this cohort of patients only one was formally diagnosed with a T2MI. This patient was admitted with AF and chest pain and a raised Hs-cTn level of 372ng/L. The patient was referred on for coronary angiography and this revealed only minor coronary artery disease. The patient was discharged on a combination of apixaban and aspirin, and at the point of follow up is alive. The low rate with which the diagnosis of Type 2 MI is actually used in clinical practice is highlighted by these findings.

This is in contrast to a study from Shah et al(52), which utilised Hs-cTn, has shown that 48% of patients with a raised hs-cTn concentration were diagnosed with either a T2MI or myocardial injury. Furthermore, a recent study from Cediel et al (160) reported that in the MI population the incidence of T2MI was 19.2%, while the incidence of non-ischaemic myocardial injury (NIMI) was documented at 43.6%. Interestingly, both T2MI and NIMI cohorts had similar levels of mortality (39.7% Vs 40.0%), that were, in fact, higher than the level of mortality for the troponin positive tachyarrhythmia group in this study (26.2%). This can be explained by the fact that my study focuses solely on the troponin positive tachyarrhythmia group of patients, who despite having a comparable mortality to risk to patients diagnosed with NSTEMI may in fact have a lower mortality risk than some of the groups comprising the T2MI population such as those with sepsis, heart failure and anaemia. It is also of note that 49% of troponin positive tachyarrhythmia patients presented with chest pain as their primary symptom, therefore it is certainly plausible that a proportion of these tachyarrhythmias were secondary to cardiac ischaemia and the cause of the troponin elevation. When the tachyarrhythmia troponin positive group is further analysed with regards to whether the

patients presented with chest pain or not; no significant difference was seen between the two groups in terms of sex, hs-cTn level, age, incidence of hypertension, diabetes mellitus and hypercholesterolaemia. This pattern is also seen when comparing the whole tachyarrhythmia troponin positive group with the tachyarrhythmia troponin negative group, no significant difference is seen in sex, incidence of hypertension, diabetes mellitus and hypercholesterolaemia. A significant difference is seen, however, in age (74.9 vs 71.1, p=0.004). Importantly, this data has shown that in the troponin positive tachyarrhythmia patients presenting without chest pain had a poorer outcome than those who presented with chest pain (log rank, p<0.001), this is also seen in the NSTEMI group but not in the troponin negative tachyarrhythmia group.

A greater incidence of hypertension, hypercholesterolaemia and diabetes mellitus is seen in the NSTEMI group when compared to the tachyarrhythmia troponin positive group. Despite these differences the short and mid-term outcomes in both the NSTEMI and tachyarrhythmia troponin positive are similar (log rank p=0.46). In contrast, despite similar demographics the tachyarrhythmia troponin positive group have worse short and mid-term outcomes when compared to the tachyarrhythmia troponin negative group (log rank p=0.003). Perhaps suggesting that in this a cohort a raised troponin is a superior marker for risk than the traditional risk factors of hypertension, diabetes mellitus and hypercholesterolaemia.

The measurement of troponin and the finding that the level is elevated does not appear to have a predictable impact on management in tachyarrhythmia patients according to our study. This is reflected in the relatively low rate of coronary angiography in this cohort. Whilst we cannot tell accurately why some patients with troponin positive tachyarrhythmia had coronary investigations and treatment, and some did not, it is clear that a formal diagnosis of T2MI is very rare in clinical practice. This is important, because previous studies have shown that T2MI is not appropriately managed using angiography/revascularization(52). Other

studies have demonstrated that the prognosis for T2MI is not benign (160), and indeed the cohort we observed with troponin positive tachyarrhythmia, had a relatively poor prognosis.

It is unclear from our analysis of these patients exactly how troponin positivity or negativity contributed to the patients' management. Further investigation is warranted into the interpretation of troponin level in patients presenting with tachyarrhythmia and, in particular, into how such patients should be optimally managed. It would certainly appear that the impaired prognosis associated with tachyarrhythmia-related troponin elevation warrants further investigation into potential disease-modifying therapy.

3.5 Study limitations

There are several limitations to this study. Firstly, it is observational. However, this has the advantage that we can show what actually happened to a consecutive, real life population. Secondly, we have used the diagnoses formulated by supervising physicians, rather than by independent assessment, for our analyses. It is clear that in some cases careful reinterpretation of the data may have led to a different categorization, but our aim was to describe what had actually happened. Thirdly, these are retrospectively derived data. Perhaps, however, choosing tracked mortality as the primary endpoint allows for more robust interpretation of patient outcome. Although quality control of the assay was undertaken on a daily basis by the biochemistry laboratory at our institution including validation of the CV. This involves the checking of the imprecision and bias using internal quality control materials at three levels, this was undertaken by an analyst. The internal quality control imprecision and bias were reviewed by senior biochemistry lab staff on a monthly basis. However, no data is provided on the CV, intra and inter assay CV during the study period.

3.6 Conclusions

In hospitalised patients with a tachyarrhythmia, 46.8% have been observed to have hs-cTn level above the 99th percentile. This is higher than previously reported for AF with cardiac troponin I assay. Overall, there is no difference in mortality in these tachyarrhythmia patients compared to reference patients presenting with an NSTEMI. This confirms that troponin-positive tachyarrhythmia is not benign, and certainly more research is required to assess potential specific therapies to alter this outcome in this group.

At present the value of detecting a raised hs-cTn level in the arrhythmia population is unclear. Clinicians can identify arrhythmia patients at a higher risk of mortality but at present no effective intervention is available to modify this risk. Finally, despite increasing research on T2MI by the scientific community, T2MI as a diagnosis is rarely used in the real life management of patients admitted to hospital. More investigations are warranted in this field to optimise patient diagnosis and outcome.

4.0 Results : Is the current threshold for diagnosis of “abnormality”, including Non-ST elevation myocardial infarction, using raised highly sensitive troponin appropriate for a hospital population? (The CHARIOT Study)

True 99th centile of high sensitivity cardiac troponin for hospital patients: prospective, observational cohort study

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ABSTRACT

OBJECTIVE

To determine the distribution, and specifically the true 99th centile, of high sensitivity cardiac troponin I (hs-cTnI) for a whole hospital population by applying the hs-cTnI assay currently used routinely at a large teaching hospital.

DESIGN

Prospective, observational cohort study.

SETTING

University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom, between 29 June 2017 and 24 August 2017.

PARTICIPANTS

20 000 consecutive inpatients and outpatients undergoing blood tests for any clinical reason. Hs-cTnI concentrations were measured in all study participants and nested for analysis except when the supervising doctor had requested hs-cTnI for clinical reasons.

MAIN OUTCOME MEASURES

Distribution of hs-cTnI concentrations of all study participants and specifically the 99th centile.

RESULTS

The 99th centile of hs-cTnI for the whole population was 296 ng/L compared with the manufacturer's quoted level of 40 ng/L (currently used clinically as the upper limit of normal; ULN). Hs-cTnI concentrations were greater than 40 ng/L in one in 20 (5.4%, n=1080) of the total population. After excluding participants diagnosed as having acute myocardial infarction (n=122) and those in whom hs-cTnI was requested for clinical reasons (n=1707), the 99th centile was 189 ng/L for the remainder (n=18 171). The 99th centile was 563 ng/L for inpatients (n=4759) and 65 ng/L for outpatients (n=9280). Patients from the emergency department (n=3706) had a 99th centile of 215 ng/L, with 6.07% (n=225) greater than the recommended ULN. 39.02% (n=48) of all patients from the critical care units (n=123) and 14.16% (n=67) of all medical inpatients had an hs-cTnI concentration greater than the recommended ULN.

CONCLUSIONS

Of 20 000 consecutive patients undergoing a blood test for any clinical reason at our hospital, one in 20 had an hs-cTnI greater than the recommended ULN. These data highlight the need for clinical staff to interpret hs-cTnI concentrations carefully, particularly when applying the recommended ULN to diagnose acute myocardial infarction, in order to avoid misdiagnosis in the absence of an appropriate clinical presentation.

TRIAL REGISTRATION

Clinicaltrials.gov NCT03047785.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Current guidelines recommend the use of troponin assays to help exclude or diagnose acute myocardial infarction

Manufacturers of troponin assays provide a recommended 99th centile that is based on a few hundred healthy individuals; this level is often used as the upper limit of normal when applied to the hospital population

A variety of clinical factors affect the troponin level, such as age, sex, and renal function, but little is known about the true distribution of the troponin level across the whole hospital population

WHAT THIS STUDY ADDS

In a hospital population of 20 000 consecutive patients, one in 20 of all patients had a high sensitivity troponin I concentration greater than the manufacturer's recommended 99th centile; in most of these patients there was no clinical suspicion of acute myocardial infarction

It is important to interpret the troponin result in hospital patients according to individual patients, their clinical presentation, and the guideline recommendations for correct diagnosis of type 1 and type 2 myocardial infarction

These results could help to avoid misdiagnosis and inappropriate treatment

Introduction

The use of increasingly sensitive troponin assays for excluding or diagnosing acute myocardial infarction has become universal. A diagnosis of acute myocardial infarction is defined, in the context of an appropriate clinical presentation, by a rise or fall in cardiac troponin concentration, now the gold standard biomarker,[1 2] with at least one value greater than the 99th centile derived from a reference population of healthy individuals.[3-5]

Under most circumstances, the troponin assay is requested by frontline clinical staff to determine whether a patient is having a type 1 myocardial infarction caused by coronary plaque rupture or erosion. Robust evidence has shown symptomatic and prognostic benefit from applying early pharmacological and interventional treatment strategies in these patients. However, particularly with the advent of newer assays, this strategy has two potential challenges.

Firstly, raised cardiac troponin concentrations, particularly in patients not presenting with a typical history of cardiac pain, are often caused by myocardial injury or type 2 myocardial infarction.[6 7] These

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.

Trial registration

The study is registered with Clinicaltrials.gov, number NCT03047785.

4.1 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, 4, 48).

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)(64, 160), which is secondary to ischaemia due to either increased oxygen demand or decreased supply rather than a plaque erosion event (56, 59, 161). 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(52), with some exceptions including spontaneous coronary dissection, coronary embolism and coronary spasm (161, 162). 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 (76, 77, 163, 164), 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 (4). Consequently, new highly sensitive cardiac troponin (hs-cTn) assays (41, 42, 132-134) 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(44). 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(165). Given the number of factors that are well known to affect an individual's troponin (165), including age(89), sex(166), glomerular filtration rate(167), left ventricular function(103), and the presence of significant inflammatory conditions (122), 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 (94, 96, 168).

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.

4.2 Methods

4.2.1 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. Figure 14 is a flow diagram of how the final study population was derived.

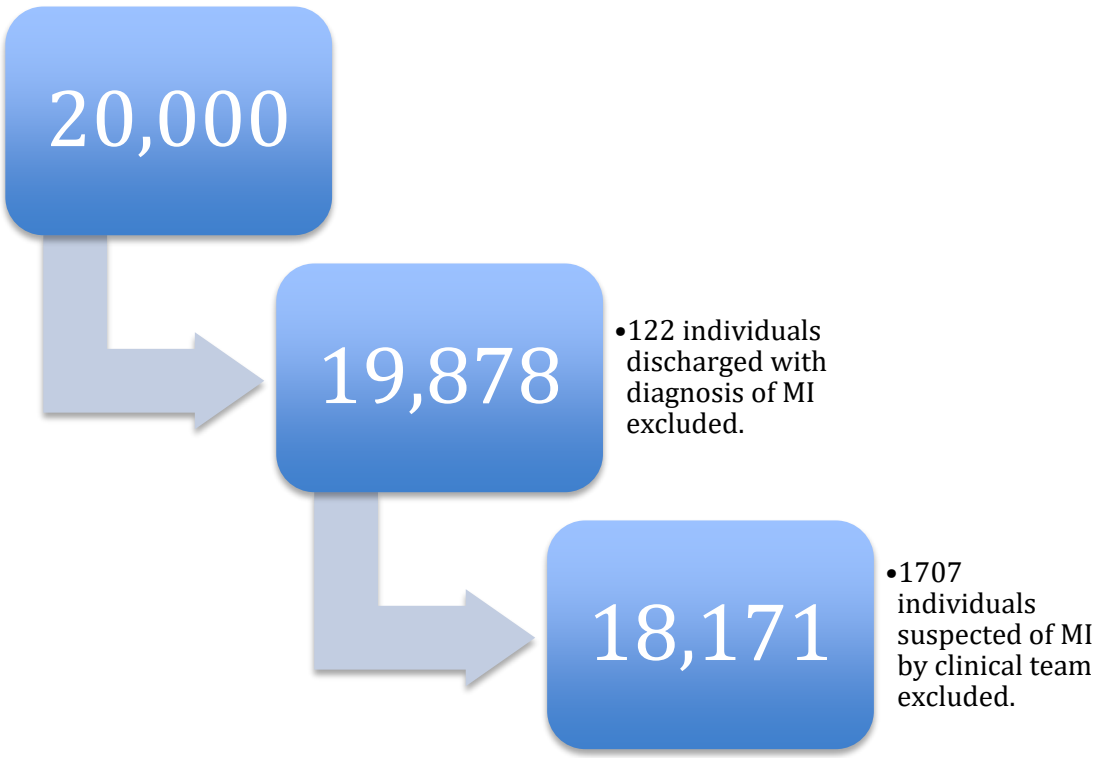


Figure 14: Flow diagram of how final study population was derived.

4.2.2 Ethics and 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.

4.2.3 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 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 ULN for this assay was determined by analysing serum samples 330 healthy blood donors (260 men, 70 women age range 18-70, median age 36years) in Italy (131). 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. This involves the checking of the imprecision and bias using internal quality control materials at three levels, this was undertaken by an analyst. The internal quality control imprecision and bias were reviewed by senior biochemistry lab staff on a monthly basis.

4.2.4 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.

4.2.5 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.

4.2.6 Statistical analysis

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, estimated glomerular filtration rate and location.

4.3 Results

A total of 20,000 consecutive patients were included in CHARIOT between 29/06/2017 to 24/08/2017. Once all the patients who were either diagnosed with MI or had been suspected of suffering an MI had been excluded this left a final study population of 18,171 patients. The median age was 61 (standard deviation 19.89 years) and 53.5% were female, (n = 9729). Baseline characteristics are shown in Table 14.

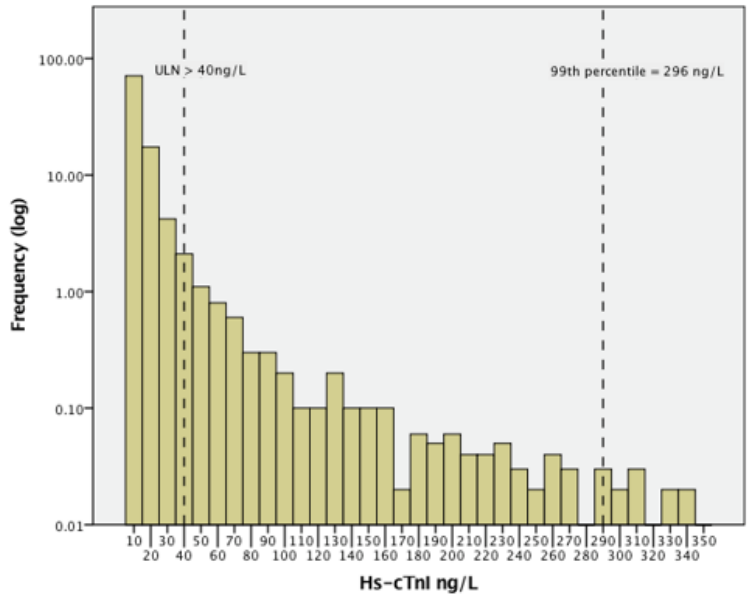
Table 14: Baseline characteristics stratified by hs-cTnI levels (ng/L) below or above the ULN (Upper limit of normal = 40ng/L)

	Hs-cTnI below ULN (n=17329)	Hs-cTnI above ULN (n=842)	P value
Age (years)	57.7	74.6	<0.001
Male Sex (%)	46	56	0.005
eGFR	79.1	58.7	<0.001
Inpatients(%)	25.0	41	<0.001
Outpatients(%)	52	22	<0.001

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) (Figure 15a). In the final study population (n=18,171) the 99th percentile was 189 ng/L, with 4.6% (n=836) above 40 ng/L (Figure 15b).

Of the 1707 patients in whom hs-cTnI concentrations were requested by the clinical team, 48% (n=821) had presented with chest pain, with arrhythmia (n=52) and suspected blackouts (n=63) the next most common reason for the test.

15a



15b

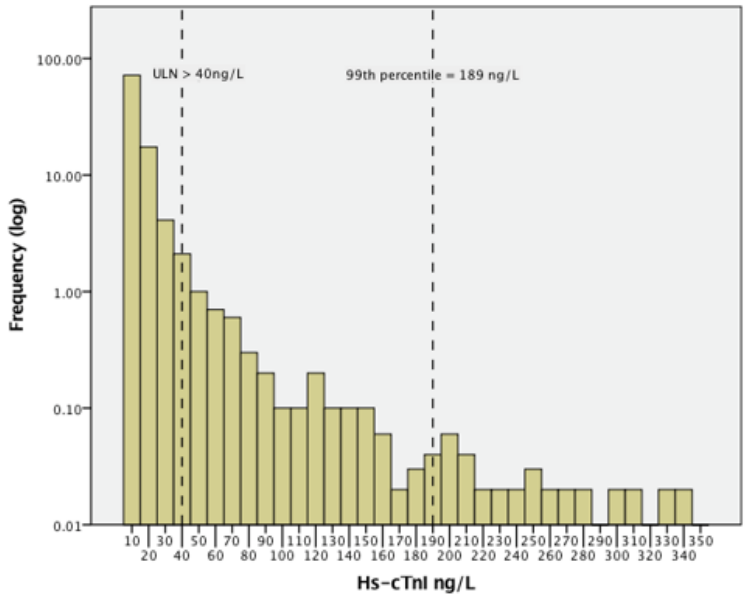


Figure 15: A log distribution of high sensitivity cardiac troponin (Hs-cTnI) concentration (15a) in the whole population (n=20,00) and (15b) in the final study population (n=18,171). (ULN = Manufacturer's recommended upper limit of normal for Hs-cTnI concentration (>40ng/L).

4.3.1 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.

A total of 821 patients included in this study had blood sampling undertaken due to chest pain. The vast majority of these patients were from the ED (93.7%, n= 769) with 8.9% of this ED cohort (n=69) being discharged from hospital with a formal diagnosis of MI. The mean hs-cTnI level for this group was 93.87 ng/L, median of 7 ng/L, and a 99th centile of 2279 ng/L. Table 15 shows the distribution of hs-cTnI levels in patients presenting with chest pain from the different locations in the hospital.

Table 15: Distribution of hs-cTnI in patients presenting with chest pain

Location	Median (ng/L)	Interquartile Range (ng/L)	Range (ng/L)	MI Diagnosed (n, %)
Whole (n=821)	**7**	**9**	**27155**	**75, 9.1%**
Emergency Department (n=769)	**7**	**9**	**27155**	**69, 8.9%**
Resuscitation Room (n=43)	**22**	**55**	**2221**	**15, 34.9%**
Cardiac (n=10)	**26**	**127**	**1321**	**3, 30%**
Acute Surgical Unit (n=1)	**17**	**-**	**-**	**0, 0%**
Medical Wards (n=7)	**11**	**27**	**4826**	**1, 14.3%**
Acute Medical Unit (n=2)	**2423**	**-**	**4822**	**1, 50%**
Outpatients (n=15)	**8**	**9**	**11**	**0, 0%**

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. Figure 16 shows the distribution Hs-cTnI in the different locations, with figure 17 and table 16 demonstrating the percentage of patients from each location with a Hs-cTnI concentration above the ULN.

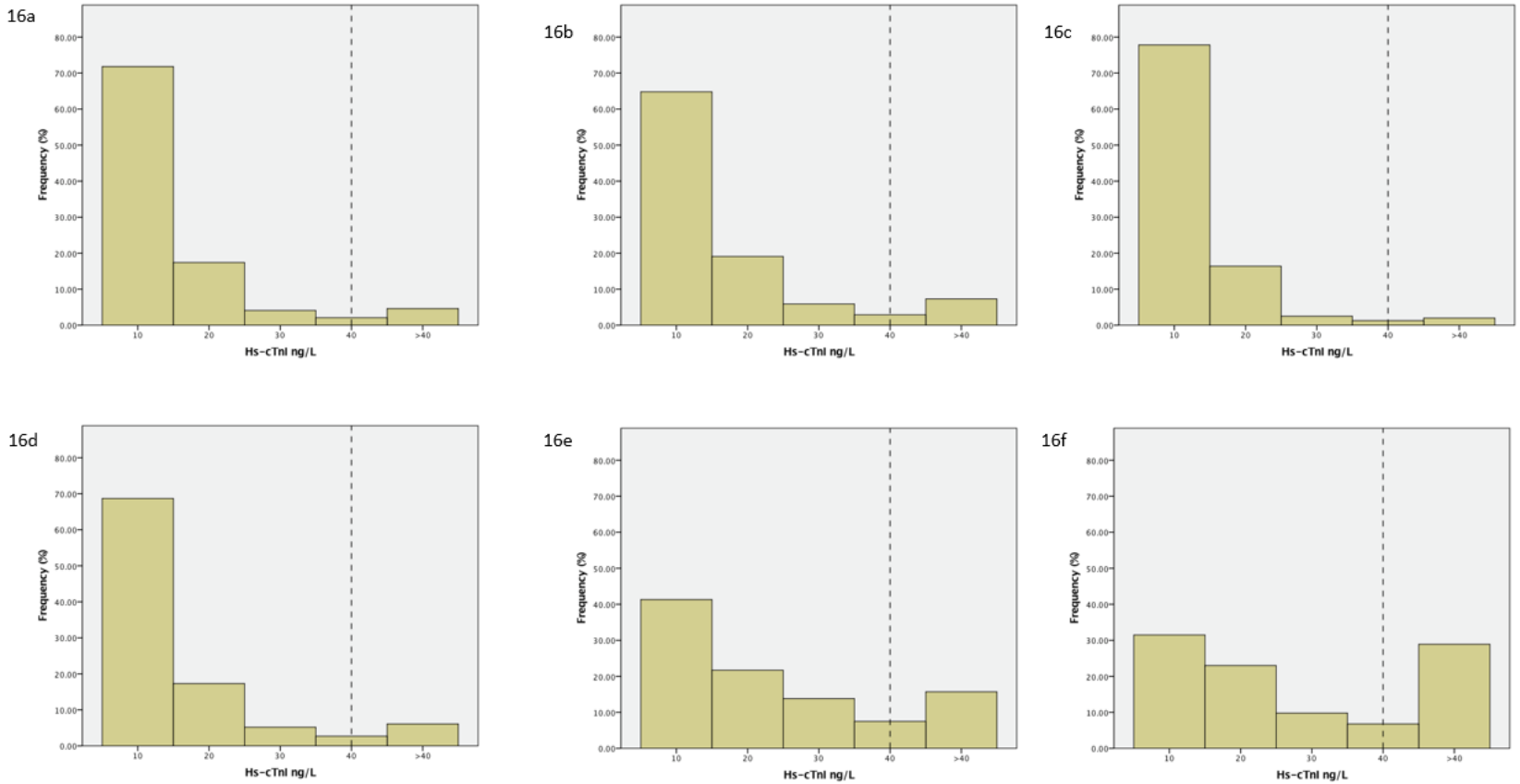


Figure 16: High-sensitivity cardiac troponin I (Hs-cTnI) concentrations in (16a) final study population, (16b) inpatients, (16c) outpatients, (16d) emergency department, (16e) medical inpatients, (16f) cardiac inpatients, (16g) resuscitation room, (16h) medicine for older people (16i) critical care units (16j) acute surgical unit and (16k) orthopaedic inpatients.

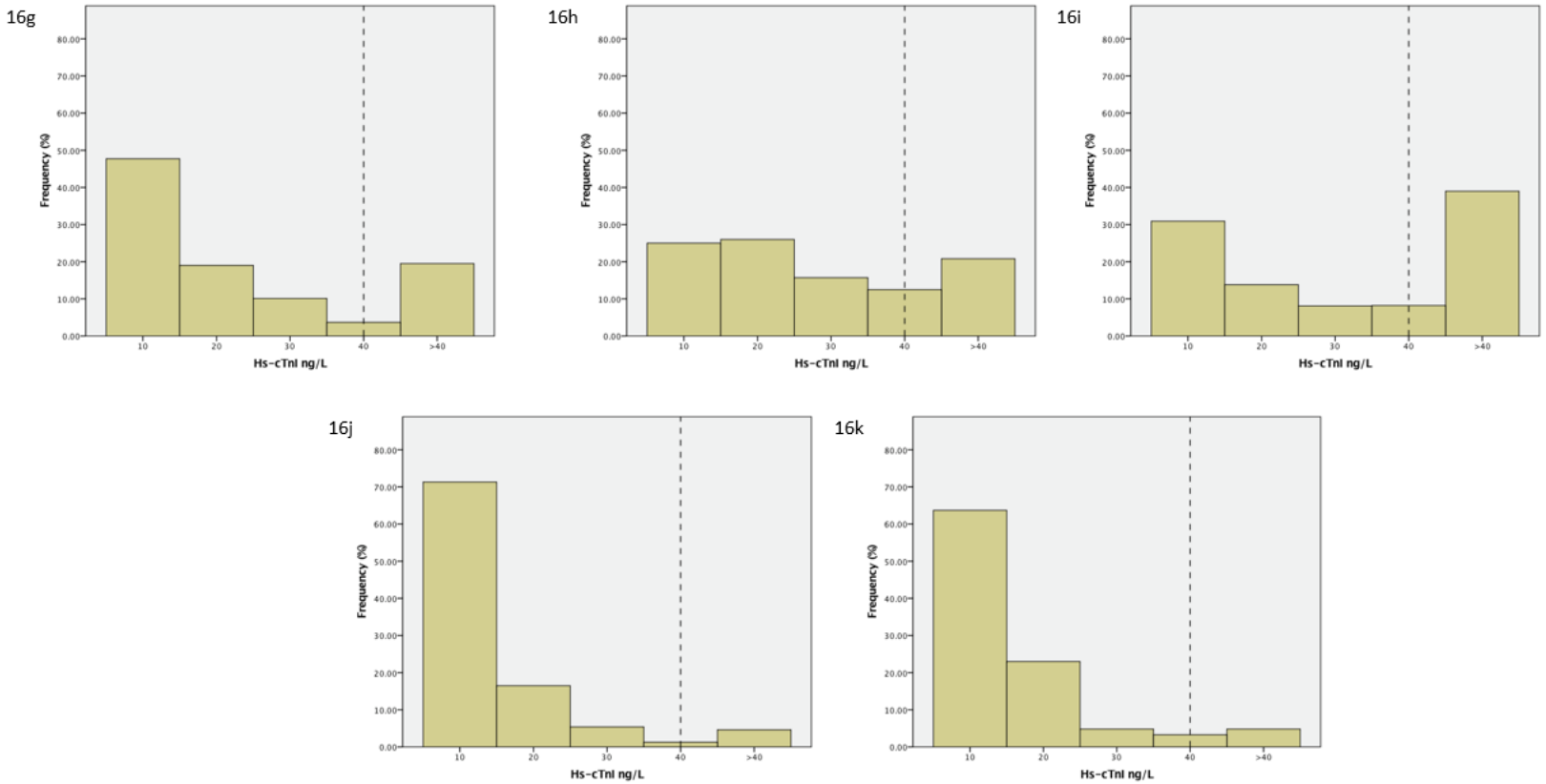


Figure 16: High-sensitivity cardiac troponin I (Hs-cTnI) concentrations in (16a) final study population, (16b) inpatients, (16c) outpatients, (16d) emergency department, (16e) medical inpatients, (16f) cardiac inpatients, (16g) resuscitation room, (16h) medicine for older people (16i) critical care units (16j) acute surgical unit and (16k) orthopaedic inpatients.

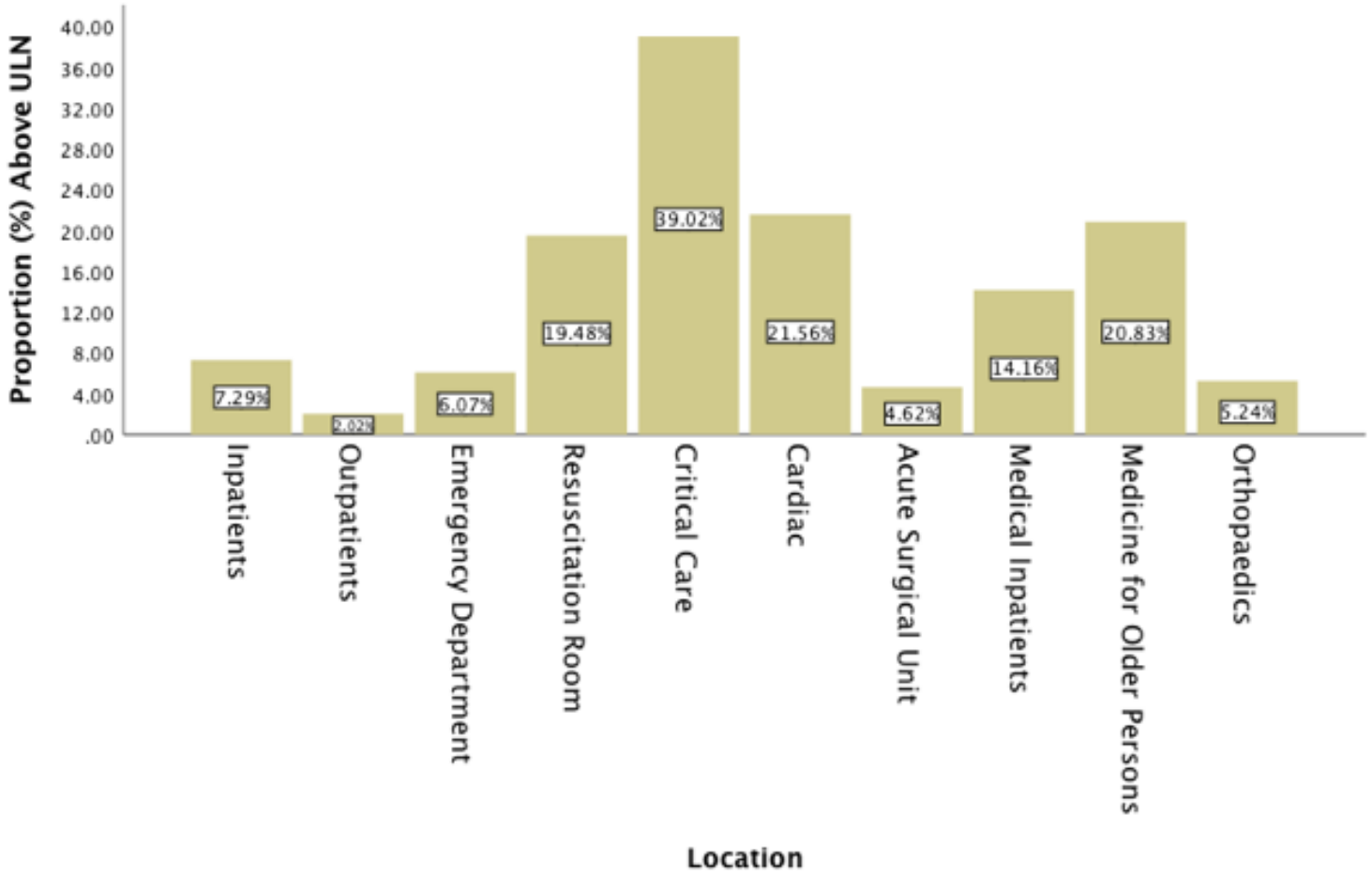


Figure 17: Proportion of patients with high sensitivity cardiac troponin I concentration above the upper limit of normal (ULN = 40ng/L) according to location.

Table 16: 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

4.3.2 Age

There was an association between increasing age and distribution of troponin concentration. Percentiles (25th, 50th, 75th, and 99th) is shown in tables 17 and 18. The proportion of patients with hs-cTnI above the ULN according to age is shown in figure 18.

Table 17: 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

Table 18: 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

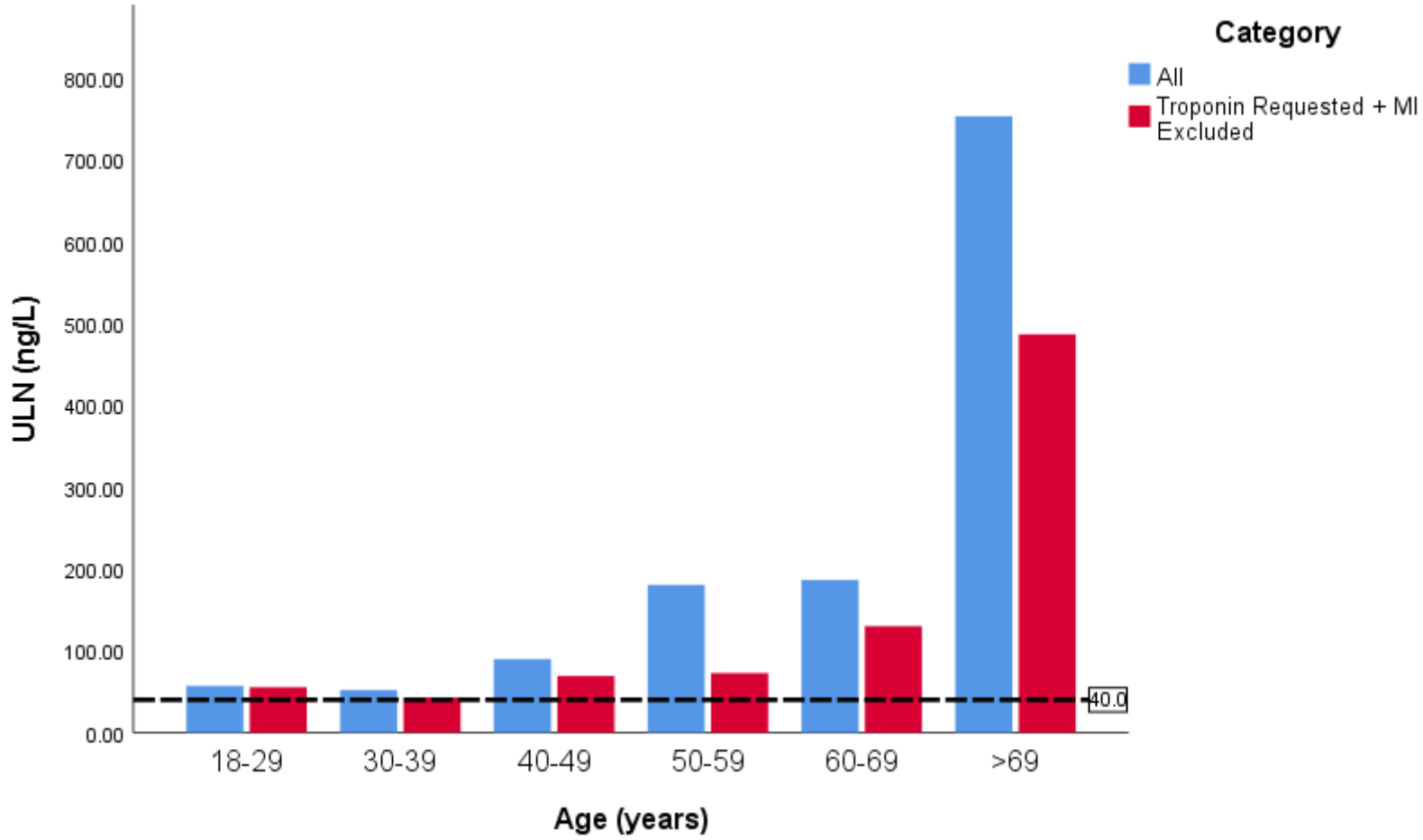


Figure 18: Upper limit of normal (ULN) high sensitivity cardiac troponin I concentration according to age

4.3.3 Sex

The 99th 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), the same pattern is seen once those diagnosed with MI had been excluded (46 vs 29 ng/L, p<0.001). Table 19 shows the distribution of Hs-cTnI concentrations in both males and females, table 20 reveals the distribution once those diagnosed with MI had been excluded.

Table 19: The distribution of hs-cTnI concentrations in males and females

Sex	Mean (ng/L)	25th centile (ng/L)	50th centile (ng/L)	75th centile (ng/L)	99th centile (ng/L)
Male (n=9420)	**62**	**3**	**7**	**13**	**373**
Female (n=10580)	**31**	**2**	**5**	**10**	**236**

Table 20: The distribution of hs-cTnI concentrations in males and females with MI excluded.

Sex	Mean (ng/L)	25th centile (ng/L)	50th centile (ng/L)	75th centile (ng/L)	99th centile (ng/L)
Male (n=6919)	**46**	**4**	**8**	**13**	**302**
Female (n=7830)	**29**	**2**	**6**	**11**	**261**

4.3.4 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 to1.04), p<0.001), male sex (OR 1.33, (1.14 to1.54), p<0.001) and reducing estimated glomerular filtration (OR 0.98(0.97 to 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 to 3.43), p<0.001), resuscitation room(OR 9.91 (7.3 to 13.46), p<0.001), critical care units (OR 36.62(23.86 to 56.2), p<0.001), cardiac wards (OR 9.08, (6.44 to 12.81), p<0.001), acute surgical unit (OR 2.52(1.47 to4.33), p<0.001), medical wards (OR 4.74(3.45 to 6.50), p<0.001), MOP wards (OR 3.70 (2.16 to 6.34), p<0.001) and orthopaedic wards (OR 2.24 (1.23 to 4.05), p=0.008) were independent predictors for hs-cTnI concentration above the ULN (table 21). Independent predictors for the full cohort (n=20,000) are shown in the table 22. Figure 19 is a forest plot of the independent predictors.

Table 21: 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 sex	1.33 (1.14-1.54) (p<0.001)	0.90 (0.66-123) (p=0.513)
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)

Table 22: Independent predictors for the full cohort (n=20,000)

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 sex	1.38 (1.21-1.58) (p<0.001)	1.00 (0.75-1.34) (p=0.998)
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)

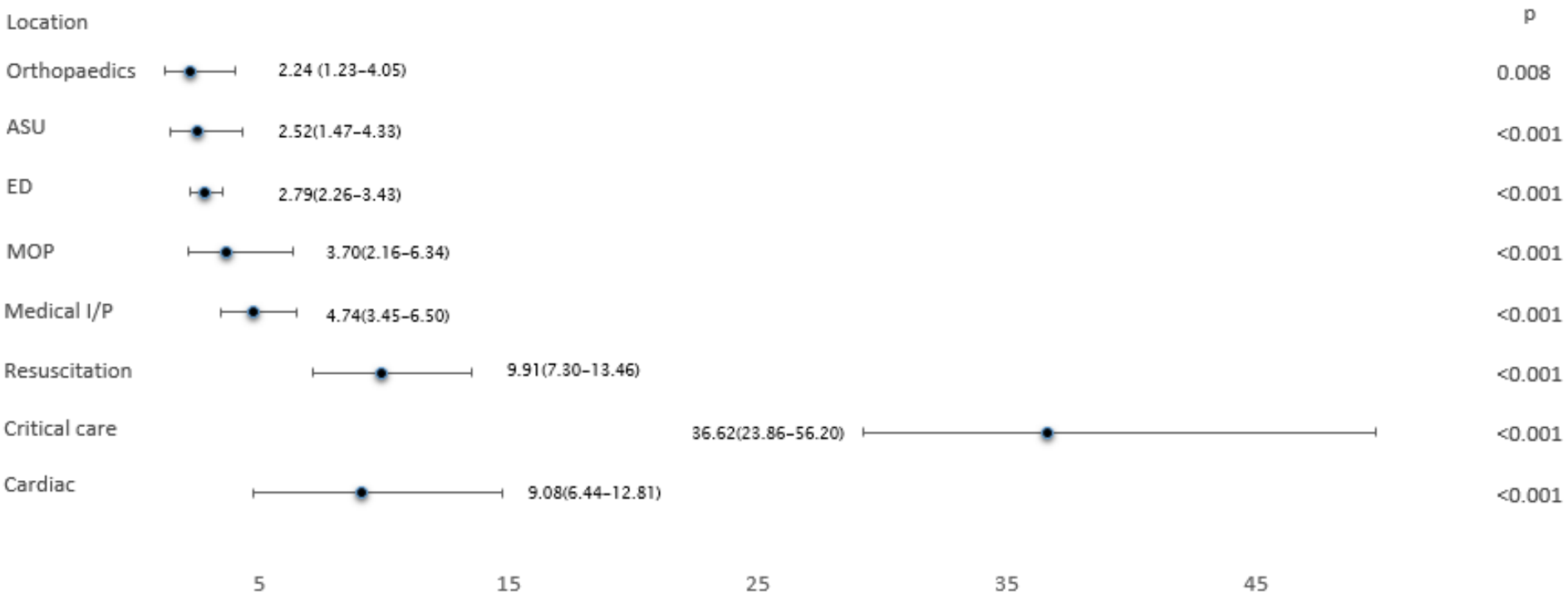


Figure 19: Forest plot of the independent predictors for hs-cTnI concentrations above the ULN.

4.4 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 sex, 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 (61, 77, 163, 164). The Fourth Universal Definition (48) 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 sex. 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 sex-specific quoted levels for ULN.

4.5 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 (61, 77, 163, 164). 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 to 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 (169-178). 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 (179, 180). It is conceivable that the “elevated” hs-cTn concentrations in a stable patient always indicates myocardial injury or unwellness (181).

4.6 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 (59, 87, 89, 90, 93, 96, 166, 182, 183). 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.

4.7 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. Although quality control of the assay was undertaken on a daily basis by the biochemistry laboratory at our institution including CV validation. This involves the checking of the imprecision and bias using internal quality control materials at three levels, this was undertaken by an analyst. The internal quality control imprecision and bias were reviewed by senior biochemistry lab staff on a monthly basis. However, no data is provided on the CV, intra and inter assay CV during the study period. 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.

4.8 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 sex 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.

5.0 : Discussion

5.1: Summary of findings

In chapter 3, the association between hs-cTn elevations and arrhythmias was explored. In this retrospective study it was shown that 46.8% of patients presenting with an tachyarrhythmia had a hs-cTnI concentration above the ULN (40 ng/L). Furthermore, it was shown that arrhythmia patients with a hs-cTnI concentration above the ULN had a higher mortality rate when compared to arrhythmia patients with a hs-cTnI below the ULN (26.2% vs 14.5%, log rank p=0.003). There was actually no difference in mortality rate when comparing arrhythmia patients with a hs-cTnI concentration above the ULN to patients diagnosed with an NSTEMI (26.2% vs 20.8%, log rank p=0.416). Importantly, only one patient (0.14%) in the whole cohort was diagnosed with a T2MI. The data from this study show that a raised hs-cTn level in arrhythmia patients is not a benign diagnosis, and has a mortality rate similar to NSTEMI. Formal labeling as T2MI is rare in real life practice.

Chapter 4 observed the distribution of hs-cTnI concentrations in an unselected hospital population of 20,000 individuals. It has been shown that one in twenty of this population have hs-cTnI concentrations above the ULN, irrespective of whether the patient had suffered an MI. Importantly, two percent of outpatients had hs-cTnI concentrations above the ULN. As expected, hs-cTnI concentrations were higher in males than females, and higher hs-cTnI were also associated with increasing age. Certain locations where the blood was sampled were associated with higher hs-cTnI such as the critical care areas, resuscitation room and cardiac wards. The results from this study highlight the importance of clinical judgement when using hs-cTnI concentrations to aid in the diagnosis of MI in patients.

5.2: Interpretation and relevance of study findings

The research described here highlight the many factors that can contribute to an individual's hs-cTnI concentrations. The body of work presented in my thesis brings into question how clinicians interpret hs-cTnI concentrations in all individuals. The advent of the more sensitive troponin assays has been driven by the need to make clinical decisions about a possible diagnosis of acute MI, and Type 1 MI in particular, on patients presenting to the acute services within hospitals in a timely manner. As a result, clinicians are now seeing more individuals with a raised troponin concentrations. However, not all these individuals have presented with a clinical history suggestive of an MI: this, in itself, of course, raises the question as to why the test was requested. Specifically, was the primary objective of the requesting clinician to identify Type 1 MI in such patients? If not, given that there is no specific treatment intervention for Type 2 MI or myocardial injury, which is in direct contrast to Type 1 MI, what was the objective when requesting the test? Whilst there is no question over the use of troponin as a rule out test for MI, the legitimacy of its use as the sole 'rule in' factor, however, is manifestly flawed. Several questions stimulated by my thesis remain:

a) *How should clinicians manage arrhythmia patients who have a raised hs-cTnI concentration?*

Chapter 3 has shown that nearly half of patients with an arrhythmia presenting to hospital have a raised hs-cTnI. Despite the prominence of the classification of MI from T1MI to T5MI in both the third (2) and fourth (48) universal definition of MI, only one patient (0.14%) in the cohort was labelled as having T2MI by their supervising clinicians. This suggests a disparity in the current guidelines and clinical practice. Furthermore, arrhythmia patients with raised hs-cTnI concentrations are shown to have a worse outcome than counterparts who do not have

raised hs-cTnI concentrations. This calls into question the notion that raised hs-cTnI concentrations in arrhythmia patients are inconsequential. In absolute contrast to this notion, the data from this study would suggest that this cohort of patients should be the focus of more research effort to find treatments that might modify their poor prognosis. It also plausible that a proportion of the arrhythmia patients with a raised hs-cTnI concentration may have in fact suffered a T1MI and been incorrectly managed with the troponin rise being attributed to the tacharrhythmia. This could have contributed to the mortality rate observed in these patients. Above all, the study highlights the fact that a raised troponin is a significant finding and is a marker of poorer outcomes in the tachyarrhythmia population. The optimal management of this cohort of patients is yet to be established and more work on this important clinical entity is required.

b) *How should clinicians use hs-cTn assays in the management of patients presenting to hospital?*

The data from chapter 4 brings into to question the use of ULN hs-cTnI concentrations derived from healthy individuals and the application of these cut-offs as the dominant arbiter of MI in patients in hospital. Specifically, the data from this study shows that the ULN quoted by manufacturers is *not* consistent with ULN of the hospital population, which is in fact much higher. There are many well described factors which can effect an individual's troponin concentrations such as age, sex and their clinical condition. Despite this, the ULN derived from healthy individuals is indeed apparently used by clinicians when adjudicating whether a patient has suffered a T1MI. This is of particular importance when the clinical history is not consistent with a diagnosis of T1MI. The potential risk of systematic misdiagnosis is highlighted by the observed 99th centile for hs-cTnI in our subpopulations of ED (215 g/dL) and acute medical admissions (1432 g/dL), and that close to 40% of patients in some clinical

settings have hs-cTnI levels above the manufacturers ULN. The misdiagnosis of patients with a T1MI is potentially harmful as it can expose patients to unnecessary pharmacology and invasive procedures. The fact that 2% of outpatients were shown to have hs-cTnI concentrations above the ULN, highlights the need to evaluate how the ULN are derived and the validity of their use in a distinct population, which is older with comorbidities.

The work also raises questions about the need for education of front line clinicians in the use of troponin test requesting and interpretation. For example, when a hs-cTn is requested in a hospital inpatients without a classical history of Type 1 MI, what was the specific objective in mind? Was it a misguided attempt to make a diagnosis of Type 1 MI? If it was to screen for Type 2 MI or myocardial injury, how exactly could a "positive" result then contribute to the management of that patient?

c) Raised hs-cTnI concentrations in non T1MI patients – should clinicians be concerned?

The short answer is yes. The data from this thesis show that a raised hs-cTnI concentration is not benign. There is data to support the notion that in patients with chronic conditions both cardiac and non-cardiac, raised troponin concentrations are associated with a higher risk of cardiovascular events (169-178). Despite this data, currently no studies have demonstrated how this risk can be reduced. Furthermore, evidence is accumulating that troponin can be used as a biomarker for mortality and risk in the non-cardiac population (184-186). The evidence base is apparently heading towards the notion that an elevated troponin level in the blood, allowing for the fact that the work presented here and elsewhere raise important questions about exactly what constitutes "elevated" never means nothing in terms of clinical outcome for that individual. This notion has been the subject of review by our group (121, 181)

5.3: Future work

The data from the CHARIOT study was observational and provided a snapshot into the hs-cTnI concentrations in the hospital population. The next step from this is to assess tracked 1 year mortality in this cohort to establish a possible association between the snapshot troponin result and subsequent prognosis. The results from this ongoing work are imminent. This will provide an insight on the use of troponin as a biomarker of risk in the non T1MI population.

Further work will also be undertaken looking at the application of hs-cTnI concentrations in patients managed in the intensive care setting. Given the prevalence of elevated troponin in this cohort, and the notion that troponin may be an independent biomarker for prognosis, our group is currently investigating the hypothesis that admission troponin and change in serial troponin levels in critical care patients may be associated with clinical progress and outcome, including both in-hospital and subsequent mortality. Such an association, if demonstrated, may provide the opportunity to use troponin testing as one factor in future risk stratification and prognostication in such patients: a glimpse into this potential application has been recently provided during the COVID 19 pandemic.

6.0 References

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