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Original Research

Performance of high sensitive cardiac troponin T assay to detect ischemia at PET-CT in low-risk patients with acute coronary syndrome

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ABSTRACT

Background

Highly sensitive troponin T (hs-TnT) assay has improved clinical decision-making for patients admitted with chest pain. However, this assay's performance in detecting myocardial ischemia in a low risk population has been poorly documented.

Purpose

To assess Hs-TnT assay's performance to detect myocardial ischemia at PET-CT in low-risk patient admitted with chest pain.

Methods

Patients admitted for chest pain with a non-conclusive electrocardiogram and negative standard cardiac troponin I results at admission and after 6 hours were prospectively enrolled. Their hs-TnT samples were at T0, T2 and T6. Physicians were blinded to hs-TnT results. All patients underwent a PET-CT at rest and during adenosine-induced stress. All patients with a positive PET-CT result underwent a coronary angiography.

Results

Forty-eight patients were included. Six had ischemia at PET-CT. All of them had ≥ 1 significant stenosis at coronary angiography. Areas under the curve [95% CI] for predicting significant ischemia at PET-CT using hs-TnT were 0.764[0.515; 1.000] at T0, 0.812[0.616; 1.000] at T2, and 0.813[0.638; 0.989] at T6. The receiver operating characteristic-based optimal cut-off value for hs-TnT at T0,T2, and T6 needed to exclude significant ischemia at PET-CT was <4 ng/L. Using this value, sensitivity, specificity, positive and negative predictive values of hs-TnT to predict significant ischemia were 83%/38%/16%/94% at T0, 100%/40%/19%/100% at T2, and 100%/43%/20%/100% at T6.

Conclusions

Our findings suggest that in low-risk patients, using the hs-TnT assay with a cut-off value of 4 ng/L demonstrates excellent negative predictive value to exclude myocardial ischemia detection at PET-CT, at the expense of weak specificity and positive predictive value.

Key Words

troponin ; acute coronary syndrome ; positron emission tomography ; ischemia

Strengths and limitations of this study

- In this study we showed an additive diagnostic value of high-sensitive troponin T over standard troponin in detection of myocardial ischemia as assessed by the gold standard imaging modality – positron emission tomography/computed tomography (PET/CT) in a clinically difficult population of patients with chest pain, non-conclusive ECG and negative standard cardiac troponin and low-risk of an ACS.
- Even within a normal range of high-sensitive troponin T concentration a cut-off level may be identified to further improve diagnostic accuracy and reduce false negative and false positive results indicative for myocardial ischemia as assessed by PET/CT.
- We provide data, supported by the PET/CT study, on non-inferiority of the shortening of troponin protocols for ruling out an ACS in the emergency department from 6 to 2 hours interval.
- The study sample and single center character of the study warrants careful interpretation of outcomes and further research on larger population, preferably multicentric.
- Time intervals of blood sampling were adopted from European guidelines so the implementation of outcomes is limited in respect to ongoing research on different strategies maximally shortening diagnostic protocols.

Introduction

Patients presenting with symptoms suggestive of acute coronary syndrome (ACS) represent a large population of those admitted to emergency departments[1]. Recent available high-sensitive troponin T (hs-TnT) assay has improved the detection of patients with acute myocardial infarction (AMI) in terms of its speed and sensitivity over standard cardiac troponin (cTn) assays[2]. The hs-TnT assay was recently incorporated into the clinical decision algorithm of the latest European Society of Cardiology (ESC) guidelines[3]. However, despite its better sensitivity, the hs-TnT assay has a lower specificity because positive values are driven by several non-coronary cardiac and non-cardiac clinical conditions. The introduction of the hs-TnT assay has therefore lead to more false-positive results and subsequent unnecessary hospitalizations. When cardiac troponin levels are negative at different time points, current recommendations propose using a stress imaging test to identify patients at risk of cardiac events. The added value of the hs-TnT assay over the standard cTn assay in this population of patients is poorly documented, however. The aim of this study was therefore to assess the performance of the Hs-TnT assay to detect myocardial ischemia at PET-CT in a population of patients admitted for chest pain with negative standard cTn.

Material and methods

Trial Oversight

The study was conducted after regulatory and ethical approval was obtained from the local ethical commission (protocol 18/11) and it was duly registered on clinicaltrial.gov (ClinicalTrials.gov Identifier: NCT01374607).

Sample population

Patients admitted to the emergency department for chest pain were prospectively enrolled in the study. Inclusion criteria were: acute chest pain lasting ≥ 5 min within the last 24 h and negative standard cTn results at admission (T0) and after 6 h (T6). Exclusion criteria were: ST-segment elevation myocardial infarction (STEMI); major organ dysfunction, infection or major medical conditions (uncontrolled asthma, severe chronic obstructive pulmonary disease, AV-block of the II- or III-degree without a pacemaker) that would compromise the patient's ability to undergo a hyperemic, adenosine-induced stress test; cancer with expected survival < 6 months; pregnancy; or age < 18 years old. At admission, a clinical examination was performed and an electrocardiogram (ECG) was used to screen for acute myocardial ischemia according to international standards[4]. Information on cardiovascular risk factors, any past medical history of cardiovascular diseases and current medical treatment were collected. All patients were stratified according to their thrombolysis in myocardial infarction (TIMI) risk score and the score of 0 or 1 was indicative for low risk of cardiovascular adverse events[5].

Troponin measurements

The standard cTn assay, routinely used in the institution throughout the enrollment process, was cardiac troponin I measured with AccuTnI assay (Beckman Coulter, Fullerton, CA, USA) with a 99th percentile level of 0.04 µg/L and a 20% coefficient of variation at 0.03 µg/L) and considered as positive if above the 99th percentile. The hs-TnT value was measured in parallel to the standard cTnI assay, using the same plasma samples at T0, T2 and T6. Concentrations of hs-TnT in plasma were measured using a Cobas e602 immunoanalyzer (Roche, Basel, Switzerland) based

on electrochemiluminescence technology (detection range of 3–10000 ng/L, with a 99th percentile level in a normal population of 14 ng/L and a 10% coefficient of variation level of 13 ng/L). The two hour diagnostic protocol using cTn has been described previously to carry a good diagnostic accuracy[6].

PET-CT and coronary angiography

All patients underwent a rubidium-82 (Rb-82) rest–stress cardiac PET-CT acquisition (Discovery 690, GE Healthcare, Milwaukee, WI, USA) according to a previously described method[7]. Patients were instructed to fast for 6 h and the absence of any caffeine intake in the previous 24 h was checked. Dynamic rest acquisition started after the beginning of an i.v. infusion of 10 MBq/kg of Rb-82 (Jubilant Draximage, Kirkland, Canada)[8]. Ten minutes later, a hyperemic stress test was performed using a slow intravenous infusion of adenosine (140 µg/kg/min) over 6 min. A second PET-CT acquisition was started 2 min after the beginning of adenosine infusion. PET-CT images were analyzed semi-quantitatively by two independent nuclear medicine specialists using the 17-segment AHA polar map[9] to reveal the extent and severity of perfusion defects at rest (summed rest score, SRS) and during stress (summed stress score, SSS), as well as inducible ischemia as defined by the summed difference score (SDS = SSS - SRS). Absolute quantitation of myocardial blood flow (MBF) at rest and during stress, as well as the myocardial flow reserve (MFR = Stress MBF / Rest MBF), were computed using FlowQuant (Ottawa Heart Institute, Ottawa, Canada). Both, SDS and MFR have been documented as having a prognostic value in patients investigated for ischemia[7, 10]. A PET-CT is likely to be positive for myocardial ischemia when SDS > 2 or MFR < 1.8. These two thresholds have been shown to be strong predictors of major cardiovascular events[7, 11].

Patients in the present study who were positive for myocardial ischemia on PET-CT images were scheduled for a coronary angiography.

The results of coronary angiography were assessed visually by two independent investigators, with an assessment by a third interventional cardiologist in cases of borderline stenosis. Stenosis of an epicardial coronary artery was defined as *significant* if the diameter of the stenosis was > 50% of the lumen diameter in an artery with a diameter > 2 mm.

Major Adverse Cardiac Events

Patients were followed-up with phone calls 30 days after discharge and evaluated for any major adverse cardiovascular event (MACE), defined as rehospitalization for a cardiovascular reason, repeated revascularization, non-fatal AMI, or death of a cardiovascular origin.

Statistics

Statistical analysis was performed using SPSS software (version 19, SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, California, USA). Variables are presented as a mean ± standard deviation (SD) or as a median and its interquartile range (IQR). Comparisons between groups were performed using the Mann-Whitney U test for continuous variables. Comparisons of categorical data were performed using the Fischer exact test or the chi-square test, as appropriate. A bilateral P value < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed by plotting each patient's values of hs-TNT at T0, T2, and T6 against the presence of ischemia at PET-CT. Best cut-offs were calculated using Youden's index.

Results

Clinical characteristics of the sample population

Of 50 eligible patients, 2 were excluded due to technical problems during the PET-CT quantitation of flow reserve. The remaining 48 patients participating in the study had a median (P25; P75) duration of chest pain of 2 h (1; 4); mean age was 58 ± 13 years; 33 (68 %) were male; 7 (15%) were diabetic; 3 (6%) had a history of myocardial infarction; 15 (31%) had prior percutaneous coronary intervention and 1 (2%) had prior coronary artery bypass graft The median TIMI risk score was 1 (0; 2) (**Table 1**).

Table 1. Patients' clinical characteristics.

Characteristic	Total population (n=48)
Past medical history	
Myocardial Infarction	3 (6%)
Percutaneous Coronary Intervention	15 (31%)
Coronary Artery Bypass Grafting	1 (2%)
Peripheral Artery Disease	1 (2%)
Stroke	2 (4%)
Renal insufficiency	1 (2%)
Cardiovascular risk factors	
Positive familial history	15 (31%)
Arterial hypertension	28 (58%)
Dyslipidemia	26 (54%)
Diabetes	7 (15%)
Atrial fibrillation	2 (4%)
Current/former smoking	20 (42%)
TIMI risk score	1 (0;2)
Clinical presentation	
Systolic Blood Pressure [mmHg]	135 (119;155)
Diastolic Blood Pressure [mmHg]	75 (66;82)
Heart Rate [beats/min]	72 (60;88)
Body Mass Index (BMI) [kg/m^2]	27.8 (25.3;30.4)
Medication	
ASA	22 (46%)
Clopidogrel	10 (21%)
Prasugrel	2 (4%)
RAA inhibitor	23 (48%)
Beta blocker	13 (27%)
Statine	16 (33%)
Nitrate	5 (10%)

Data are presented as n (%) or median (25th; 75th percentile).

Levels of hs-TnT in the sample population

As per the study design, standard cTn levels were < 99th percentile (< 0.03 mg/L) in all patients at T0 and T6. First blood sample for hs-TnT measurement was taken after a median of 4h8min after first chest pain and after a median of 1h28min after the last episode. Median hs-TnT levels for the sample population were 6.0 (3.0; 9.0) ng/L at T0; 5.5 (3.0; 9.0) ng/L at T2, and 5.0 (3.0; 9.0) ng/L at T6. These hs-TnT values

remained stable over time, with a mean absolute change of 0.29 ng/L within the first 2 h after admission, and of 0.021 ng/L in the following 4 h (**Figure 1**).

Identification of inducible ischemia with PET-CT

A PET-CT was performed with a median delay of 32 h (17; 65) from symptom onset. Among the 48 patients, 6 (12.5%) had a positive PET-CT for myocardial ischemia **(Figure 2).** In all patients diagnosed positive for myocardial ischemia, a coronary angiography confirmed at least one significant epicardial coronary artery stenosis.

ROC curves

Areas under the ROC curves were calculated for hs-TnT at T0, T2, and T6, both with and without absolute delta changes. The areas under the curves [95% CI] obtained were: 0.764 [0.515; 1.000] (T0); 0.812 [0.616; 1.000] (T2); 0.806 [0.601; 1.000] (T0, T2, and delta change); 0.813 [0.638; 0.989] (T6); and 0.829 [0.634; 1.000] (T0, T2, T6, and delta changes) (**Figure 3**). Additional analyses with different absolute values for hs-TnT and incorportation of the absolute delta changes improved the area under the curves, but these differences were not statistically significant.

Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy

The ROC-based, optimal cut-off value for hs-TnT value at T0, T2, and T6 necessary to exclude a diagnosis of significant ischemia at a PET-CT was < 4 ng/L. Such concentration was met by 17 (35%) patients at T0 and T2 and 18 (38%) patients at T6. Using this value, the sensitivity, specificity, and positive and negative predictive values of the hs-TnT assay to predict significant ischemia at PET-CT were 83%/38%/16%/94% at T0, 100%/43%/20%/100% at T2, and 100%/40%/19%/100%

at T6. (**Table 2**). Using the recommended cut-off value of the 99th percentile at 14 ng/L, the sensitivity, specificity, positive and negative predictive value of the hs-TnT assay were 83%/38%/16%/94% at T0, 100%/40%/19%/100% at T2 and 100%/43%/20%/100% at T6.

Table 2. The hs-TnT assay's performance in predicting the detection of ischemia at PET-CT.

Hs-TnT >= 4ng/l at :	T0	T2	T6
Sensitivity	83,3%	100,0%	100,0%
Specificity	38,1%	40,5%	42,9%
Positive predictive value	16,1%	19,4%	20,0%
Negative predictive value	94,1%	100,0%	100,0%
Area under the curve	0.764 [0.515;1.000]	0.812 [0.616;1.000]	0.813 [0.638;0.989]

The performance accuracy of different hs-TnT cut-off values was assessed at T0, T2, and T6 (**Figure 4**). The highest prediction values at T0, T2, and T6 were observed with cut-off values of 19 ng/L, 18 ng/L, and 21 ng/L, respectively, with a performance accuracy of 91.6 % for each time point.

Follow-up

During the 30-day follow-up period, no adverse cardiovascular events were reported in any of the 48 patients.

Discussion

The recently introduced hs-TnT assays are the most sensitive markers of myocardial necrosis. Their recommendation in European guidelines for ACS management[3] have been validated by several clinical trials that showed the assay's high diagnostic

accuracy[2, 12] and greater prognostic accuracy than standard cTn assay[3]. However, the hs-TnT assay's diagnostic performance for the detection of myocardial ischemia in a low-risk profile population had been poorly documented. We chose myocardial blood flow quantitation, using a Rb-82 PET-CT, as a non-invasive stress test to detect myocardial ischemia.

The present study showed that hs-TnT assay measurements in a population of low-risk ACS profile patients at T0, T2 and T6 provided low specificity and low positive predictive values but an excellent sensitivity and negative predictive values (94%, 100%, and 100%, respectively) for predicting the detection of ischemia as assessed using a PET-CT at a cut-off of 4 ng/L. Moreover, measurements at T2 provided higher negative predictive values than at T0 and equal to values at T6. In other words, T2 might be considered as potentially valuable time point at which to exclude ischemia in this specific population but this finding warrants further studies on larger cohorts.

Current European guidelines on the management of ACS in patients presenting without persistent ST-segment elevation have introduced algorithms for ruling-in and ruling-out acute myocardial infarction. In patients with hs-TnT levels below the 99th percentile, without ischemic changes on ECGs and free of chest pain for several hours, these guidelines propose a stress imaging test at the time of admission or shortly after discharge. Nevertheless, the exact timing of these investigations remains unclear and the treatment regimen during a potential discharge remains a typical daily dilemma for clinicians. Indeed, a recent prospective study based on data from 1,400 patients with unstable angina suggested that adherence to the ESC guidelines was inadequate in nearly two thirds of patients in terms of over-treatment or under-treatment[13]. Furthermore, there have been only a few studies evaluating the safety

of discharge before stress testing based solely on negative hs-TnT values. In a retrospective study based on 344 patients with chest pain, negative serial ECG, and negative cardiac enzyme discharged before stress testing, 2 patients had a fatal out-of-hospital cardiac event, and 24 were readmitted to the emergency department prior to carrying out stress testing[14]. In addition, data based on 966 patients with unstable angina who were mistakenly discharged were analyzed as part of a multicenter trial. Their risk-adjusted mortality ratio was 1.7 times higher than those who were hospitalized (95% CI: 0.2 to 17.0)[15]. The present study identified a cut-off of 4 ng/L hs-TnT at T2 as being sufficient for an effective decision-making process in a low-risk ACS profile population. Indeed, no patient with T2 and T6 hs-TnT values below this cut-off had ischemia at PET-CT. The proportion of patients in the present study with hs-TnT < 4 ng/L at T2 was 35% (17 patients) and at T6 was 38% (18 patients). Accordingly, using an algorithm to allow the discharge of patients with a low-risk of ACS, based on a cut-off of 4 ng/L at T2 without any stress imaging, would have allowed these patients to have been discharged with a very small probability of an occurrence of any MACEs at the 30-day follow-up.

Regarding the hs-TnT assay's performance in the detection of myocardial ischemia, it showed a low positive predictive value for detecting myocardial ischemia at PET-CT in this specific population with a low-risk profile. Indeed, the lowest diagnostic accuracies in our study (44%, 48%, and 50%) were observed at the 4 ng/L cut-off at T0, T2, and T6, respectively. On the other hand, the highest diagnostic accuracies were found with cut-off values of 19 ng/L, 18 ng/L, and 21 ng/L, at T0, T2, and T6, respectively, but with lower negative predictive values (93%, 91%, and 91%). In other words, hs-TnT seems to be an excellent biomarker for excluding the diagnosis of myocardial ischemia at PET-CT, rather than for detecting it in this specific population.

Nevertheless, an algorithm based on hs-TnT measurements, coupled with a PET-CT stress test, seems to diagnose functionally significant coronary artery stenosis correctly at angiogram if hs-TnT ≥ 4 ng/L.This assumption should be verified by using a coronary angiogram for all patients.

Limitation

One major limitation of the present study is its small number of participants, particularly those with a positive PET-CT. Another limitation is a verification bias, as coronary angiograms were not performed on patients with negative PET-CT. Indeed, the study investigated a low-risk ACS population, in which an invasive diagnostic strategy is not indicated. However, it could also be argued that all these patients had a negative PET-CT and no MACE during 30 days of follow-up.

Conclusion

In conclusion, we found that a cut-off < 4 ng/L at T2 provided excellent negative predictive values (100%) for the exclusion of myocardial ischemia as measured by PET-CT in a low-risk profile ACS population. Furthermore, in patients with hs-TnT ≥ 4 ng/L, a strategy based on a PET-CT ischemia detection appears to be appropriate. These results should be confirmed in the study of a larger sample population.

Compliance with Ethical Standards

All authors declare that they do not have conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki

declaration and its later amendments or comparable ethical standards. Informed consent: Informed consent was obtained from all individual participants included in the study.

Founding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Authors' contributions

BM and SF contributed to the conception of the study, to aquisition and analysis of the data, performed analysis and prepared the draft of the manuscript, MT gave the design of the study, gathered and managed the data, JOP, PM and VD gathered the data, gave critical view to the design and the draft of the manuscript, NL and FR participated in al analysis and gathering of the data, CT, JFI, and DK gathered the data, performed analysis and participated in preparation ofthe manuscript, OB, DB and SL performed laboratory analyses and data management, EE, OH, and OM participated in the design of the study, approved all analyses and gave critical overview to the manuscript.

Data sharing

The dataset is not available.

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Figure Legends

Figure 1. Plot of hs-TnT concentrations at admission (T0) and at 2 h (T2) and 6 h (T6) afterwards.

Figure 2. Study chart.

Figure 3. ROC curves for the detection of myocardial ischemia.

Figure 4. Diagnostic accuracy.

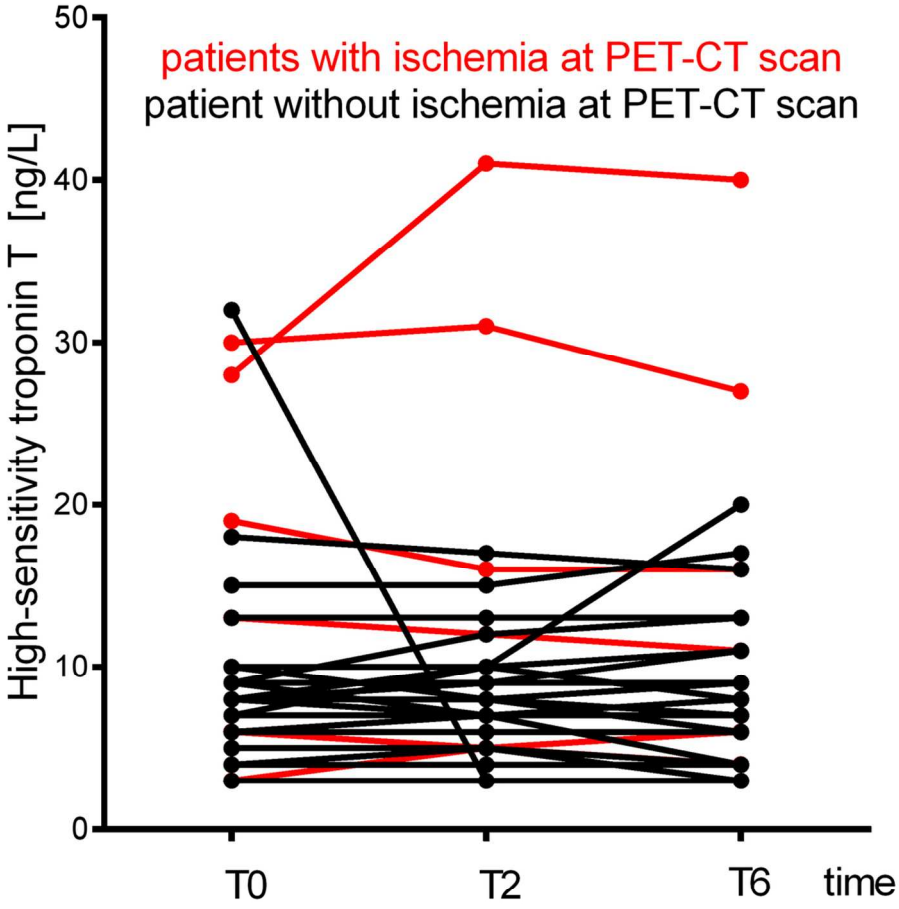

Figure 1. Plot of hs-TnT concentrations at admission (T0) and at 2 h (T2) and 6 h (T6) afterwards.

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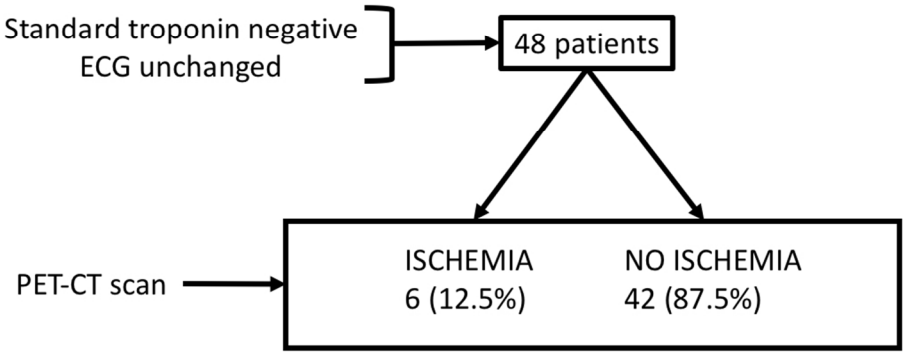

Figure 2. Study chart.

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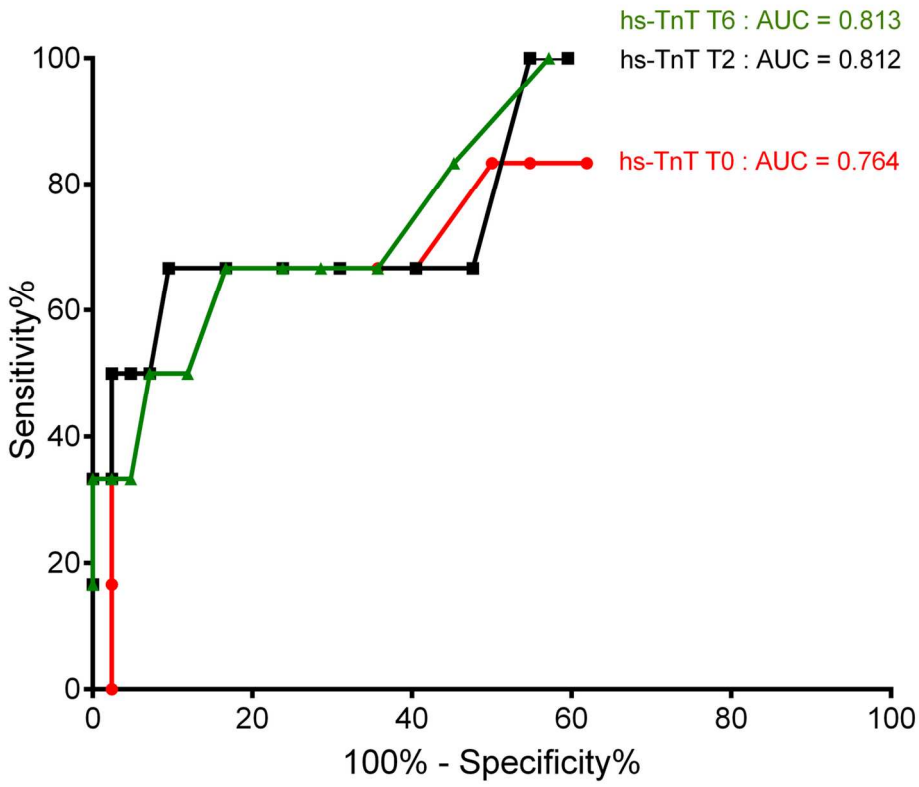

Figure 3. ROC curves for the detection of myocardial ischemia.

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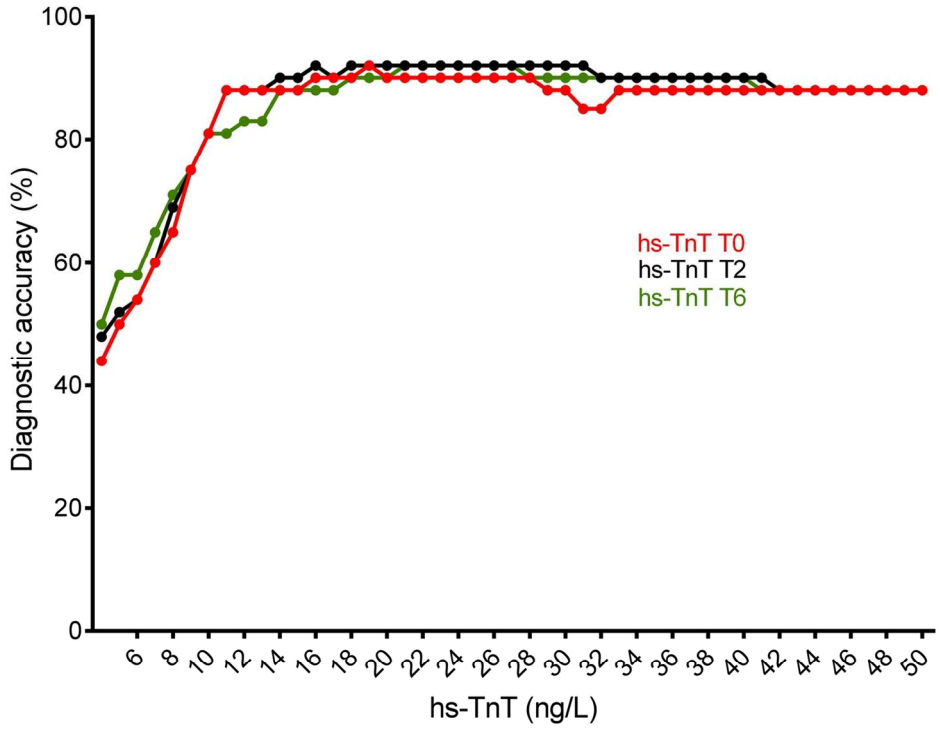

Figure 4. Diagnostic accuracy.

138x112mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	n/a
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	Fig 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	2
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) Summarise follow-up time (eg, average and total amount)	10-11
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11-12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

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Performance of high sensitive cardiac troponin T assay to detect ischemia at PET-CT in low-risk patients with acute coronary syndrome

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Keywords:	troponin, acute coronary syndrome, positron emission tomography, ischemia

Original Research

Performance of high sensitive cardiac troponin T assay to detect ischemia at PET-CT in low-risk patients with acute coronary syndrome

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ABSTRACT

Background

Highly sensitive troponin T (hs-TnT) assay has improved clinical decision-making for patients admitted with chest pain. However, this assay's performance in detecting myocardial ischemia in a low risk population has been poorly documented.

Purpose

To assess Hs-TnT assay's performance to detect myocardial ischemia at PET-CT in low-risk patient admitted with chest pain.

Methods

Patients admitted for chest pain with a non-conclusive electrocardiogram and negative standard cardiac troponin T results at admission and after 6 hours were prospectively enrolled. Their hs-TnT samples were at T0, T2 and T6. Physicians were blinded to hs-TnT results. All patients underwent a PET-CT at rest and during adenosine-induced stress. All patients with a positive PET-CT result underwent a coronary angiography.

Results

Forty-eight patients were included. Six had ischemia at PET-CT. All of them had ≥ 1 significant stenosis at coronary angiography. Areas under the curve [95% CI] for predicting significant ischemia at PET-CT using hs-TnT were 0.764[0.515; 1.000] at T0, 0.812[0.616; 1.000] at T2, and 0.813[0.638; 0.989] at T6. The receiver operating characteristic-based optimal cut-off value for hs-TnT at T0,T2, and T6 needed to exclude significant ischemia at PET-CT was <4 ng/L. Using this value, sensitivity, specificity, positive and negative predictive values of hs-TnT to predict significant ischemia were 83%/38%/16%/94% at T0, 100%/40%/19%/100% at T2, and 100%/43%/20%/100% at T6.

Conclusions

Our findings suggest that in low-risk patients, using the hs-TnT assay with a cut-off value of 4 ng/L demonstrates excellent negative predictive value to exclude myocardial ischemia detection at PET-CT, at the expense of weak specificity and positive predictive value.

Key Words

troponin ; acute coronary syndrome ; positron emission tomography ; ischemia

Strengths and limitations of this study

- In this study we showed an additive diagnostic value of high-sensitive troponin T over standard troponin in detection of myocardial ischemia as assessed by the gold standard imaging modality – positron emission tomography/computed tomography (PET-CT) in a clinically difficult population of patients with chest pain, non-conclusive ECG and negative standard cardiac troponin and low-risk of an ACS.
- Even within a normal range of high-sensitive troponin T concentration a cut-off level may be identified to further improve diagnostic accuracy and reduce false negative and false positive results indicative for myocardial ischemia as assessed by PET-CT.
- We provide data, supported by the PET-CT study, on non-inferiority of the shortening of troponin protocols for ruling out an ACS in the emergency department from 6 to 2 hours interval.
- The study sample and single center character of the study warrants careful interpretation of outcomes and further research on larger population, preferably multicentric.
- Time intervals of blood sampling were adopted from European guidelines so the implementation of outcomes is limited in respect to ongoing research on different strategies maximally shortening diagnostic protocols.

Introduction

Patients presenting with symptoms suggestive of acute coronary syndrome (ACS) represent a large population of those admitted to emergency departments[1]. Recent available high-sensitive troponin T (hs-TnT) assay has improved the detection of patients with acute myocardial infarction (AMI) in terms of its speed and sensitivity over standard cardiac troponin (cTn) assays[2]. The hs-TnT assay was recently incorporated into the clinical decision algorithm of the latest European Society of Cardiology (ESC) guidelines[3]. However, despite its better sensitivity, the hs-TnT assay has a lower specificity because positive values are driven by several non-coronary cardiac and non-cardiac clinical conditions. The introduction of the hs-TnT assay has therefore lead to more false-positive results and subsequent unnecessary hospitalizations. When cardiac troponin levels are negative at different time points, current recommendations propose using a stress imaging test to identify patients at risk of cardiac events. The added value of the hs-TnT assay over the standard cTn assay in this population of patients is poorly documented, however. The aim of this study was therefore to assess the performance of the Hs-TnT assay to detect myocardial ischemia at PET-CT in a population of patients admitted for chest pain with negative standard cTn.

Material and methods

Trial Oversight

The study was conducted after regulatory and ethical approval was obtained from the local ethical commission (protocol 18/11) and it was duly registered on clinicaltrial.gov (ClinicalTrials.gov Identifier: NCT01374607).

Sample population

Patients admitted to the emergency department for chest pain were prospectively enrolled in the study. Inclusion criteria were: acute chest pain lasting ≥ 5 min within the last 24 h and negative standard cTn results at admission (T0) and after 6 h (T6). Exclusion criteria were: ST-segment elevation myocardial infarction (STEMI); major organ dysfunction, infection or major medical conditions (uncontrolled asthma, severe chronic obstructive pulmonary disease, AV-block of the II- or III-degree without a pacemaker) that would compromise the patient's ability to undergo a hyperemic, adenosine-induced stress test; cancer with expected survival < 6 months; pregnancy; or age < 18 years old. At admission, a clinical examination was performed and an electrocardiogram (ECG) was used to screen for acute myocardial ischemia according to international standards[4]. Information on cardiovascular risk factors, any past medical history of cardiovascular diseases and current medical treatment were collected. All patients were stratified according to their thrombolysis in myocardial infarction (TIMI) risk score and the score of 0 or 1 was indicative for low risk of cardiovascular adverse events[5].

Troponin measurements

The standard cTn assay, routinely used in the institution throughout the enrollment process, was cardiac troponin I measured with AccuTnI assay (Beckman Coulter, Fullerton, CA, USA) with a 99th percentile level of 0.04 µg/L and a 20% coefficient of variation at 0.03 µg/L) and considered as positive if above the 99th percentile. The hs-TnT value was measured in parallel to the standard cTnI assay, using the same plasma samples at T0, T2 and T6. Concentrations of hs-TnT in plasma were measured using a Cobas e602 immunoanalyzer (Roche, Basel, Switzerland) based

on electrochemiluminescence technology (detection range of 3–10000 ng/L, with a 99th percentile level in a normal population of 14 ng/L and a 10% coefficient of variation level of 13 ng/L). The two hour diagnostic protocol using cTn has been described previously to carry a good diagnostic accuracy[6].

PET-CT and coronary angiography

All patients underwent a rubidium-82 (Rb-82) rest–stress cardiac PET-CT acquisition (Discovery 690, GE Healthcare, Milwaukee, WI, USA) according to a previously described method[7]. Patients were instructed to fast for 6 h and the absence of any caffeine intake in the previous 24 h was checked. Dynamic rest acquisition started after the beginning of an i.v. infusion of 10 MBq/kg of Rb-82 (Jubilant Draximage, Kirkland, Canada)[8]. Ten minutes later, a hyperemic stress test was performed using a slow intravenous infusion of adenosine (140 µg/kg/min) over 6 min. A second PET-CT acquisition was started 2 min after the beginning of adenosine infusion. PET-CT images were analyzed semi-quantitatively by two independent nuclear medicine specialists using the 17-segment AHA polar map[9] to reveal the extent and severity of perfusion defects at rest (summed rest score, SRS) and during stress (summed stress score, SSS), as well as inducible ischemia as defined by the summed difference score (SDS = SSS - SRS). Absolute quantitation of myocardial blood flow (MBF) at rest and during stress, as well as the myocardial flow reserve (MFR = Stress MBF / Rest MBF), were computed using FlowQuant (Ottawa Heart Institute, Ottawa, Canada). Both, SDS and MFR have been documented as having a prognostic value in patients investigated for ischemia[7, 10]. A PET-CT is likely to be positive for myocardial ischemia when SDS > 2 or MFR < 1.8. These two thresholds have been shown to be strong predictors of major cardiovascular events[7, 11].

Patients in the present study who were positive for myocardial ischemia on PET-CT images were scheduled for a coronary angiography.

The results of coronary angiography were assessed visually by two independent investigators, with an assessment by a third interventional cardiologist in cases of borderline stenosis. Stenosis of an epicardial coronary artery was defined as *significant* if the diameter of the stenosis was > 50% of the lumen diameter in an artery with a diameter > 2 mm.

Major Adverse Cardiac Events

Patients were followed-up with phone calls 30 days after discharge and evaluated for any major adverse cardiovascular event (MACE), defined as rehospitalization for a cardiovascular reason, repeated revascularization, non-fatal AMI, or death of a cardiovascular origin.

Statistics

Statistical analysis was performed using SPSS software (version 19, SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, California, USA). Variables are presented as a mean ± standard deviation (SD) or as a median and its interquartile range (IQR). Comparisons between groups were performed using the Mann-Whitney U test for continuous variables. Comparisons of categorical data were performed using the Fischer exact test or the chi-square test, as appropriate. A bilateral P value < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed by plotting each patient's values of hs-TNT at T0, T2, and T6 against the presence of ischemia at PET-CT. Best cut-offs were calculated using Youden's index.

Results

Clinical characteristics of the sample population

Of 50 eligible patients, 2 were excluded due to technical problems during the PET-CT quantitation of flow reserve. The remaining 48 patients participating in the study had a median (P25; P75) duration of chest pain of 2 h (1; 4); mean age was 58 ± 13 years; 33 (68 %) were male; 7 (15%) were diabetic; 3 (6%) had a history of myocardial infarction; 15 (31%) had prior percutaneous coronary intervention and 1 (2%) had prior coronary artery bypass graft The median TIMI risk score was 1 (0; 2) (**Table 1**).

Table 1. Patients' clinical characteristics.

Characteristic	Total population (n=48)	Without ischemia (n=42)	With ischemia (n=6)
Past medical history			
Myocardial Infarction	3 (6%)	1 (2%)	2 (33%)
Percutaneous Coronary Intervention	15 (31%)	12 (29%)	3 (50%)
Coronary Artery Bypass Grafting	1 (2%)	1 (2%)	0 (0%)
Peripheral Artery Disease	1 (2%)	0 (0%)	1 (17%)
Stroke	2 (4%)	1 (2%)	1 (17%)
Renal insufficiency	1 (2%)	1 (2%)	0 (0%)
Cardiovascular risk factors			
Arterial hypertension	28 (58%)	22 (52%)	6 (100%)
Dyslipidemia	26 (54%)	20 (48%)	6 (100%)
Diabetes	7 (15%)	6 (14%)	1 (17%)
Familial history	15 (31%)	14 (33%)	1 (17%)
Current/former smoking	20 (42%)	18 (43%)	2 (33%)
TIMI risk score	1 (0;2)	0 (0;0)	2 (2;3)
Clinical presentation			
Systolic Blood Pressure	135 (119;155)	135 (122;152)	142 (129;154)
Diastolic Blood Pressure	75 (66;82)	75 (69;82)	64 (62;78)
Heart Rate	72 (60;88)	73 (62;88)	65 (53;75)
Body Mass Index (BMI)	27.8 (25.3;30.4)	27.3 (25.3 ; 29.8)	28.8 (26.5 ; 30.7)

Data are presented as n (%) or median (25^{th}; 75^{th} percentile).

Levels of hs-TnT in the sample population

As per the study design, standard cTn levels were < 99th percentile (< 0.03 mg/L) in all patients at T0 and T6. First blood sample for hs-TnT measurement was taken after a median of 4h8min after first chest pain and after a median of 1h28min after the last episode. Median hs-TnT levels for the sample population were 6.0 (3.0; 9.0) ng/L at T0; 5.5 (3.0; 9.0) ng/L at T2, and 5.0 (3.0; 9.0) ng/L at T6. These hs-TnT values remained stable over time, with a mean absolute change of 0.29 ng/L within the first 2 h after admission, and of 0.021 ng/L in the following 4 h (**Figure 1**).

Identification of inducible ischemia with PET-CT

A PET-CT was performed with a median delay of 32 h (17; 65) from symptom onset. Among the 48 patients, 6 (12.5%) had a positive PET-CT for myocardial ischemia **(Figure 2).** In all patients diagnosed positive for myocardial ischemia, a coronary angiography confirmed at least one significant epicardial coronary artery stenosis.

ROC curves

Areas under the ROC curves were calculated for hs-TnT at T0, T2, and T6, both with and without absolute delta changes. The areas under the curves [95% CI] obtained were: 0.764 [0.515; 1.000] (T0); 0.812 [0.616; 1.000] (T2); 0.806 [0.601; 1.000] (T0, T2, and delta change); 0.813 [0.638; 0.989] (T6); and 0.829 [0.634; 1.000] (T0, T2, T6, and delta changes) (**Figure 3**). Additional analyses with different absolute values for hs-TnT and incorportation of the absolute delta changes improved the area under the curves, but these differences were not statistically significant.

Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy

The ROC-based, optimal cut-off value for hs-TnT value at T0, T2, and T6 necessary to exclude a diagnosis of significant ischemia at a PET-CT was < 4 ng/L. Such concentration was met by 17 (35%) patients at T0 and T2 and 18 (38%) patients at T6. Using this value, the sensitivity, specificity, and positive and negative predictive values of the hs-TnT assay to predict significant ischemia at PET-CT were 83[36;99]%, 38[24;54]%, 16[6;34]%, 94[69;100]% at T0, 100[52;100]%, 40[26;57]%, 19[8;38]%, 100[77;100]% at T2, and 100[52;100]%, 43[28;59]%, 20[8;39]%, 100[78;100]% at T6. (**Table 2**). Using the recommended cut-off value of the 99th percentile at 14 ng/L, the sensitivity, specificity, positive and negative predictive value of the hs-TnT assay were 50[14;86]%, 93[79;98]%, 50[14;86]%, 93[79;98]% at T0, 50[14;86]%, 95[83;99]%, 60[17;93]%, 93[79;98]% at T2, and 50[14;86]%, 93[79;98]%, 50[14;86]%, 93[79;98]% at T6.

Table 2. The hs-TnT assay's performance in predicting the detection of ischemia at PET-CT.

Hs-TnT >= 4ng/l at :	T0	T2	T6
Sensitivity	83.33 % [36.48;99.12]	100% [51.68;100.00]	100 % [51.68;100.00]
Specificity	38.10 % [23.99;54.35]	40.48 % [26.02;56.65]	42.85% [28.08;58.93]
Positive predictive value	16.13 % [6.09;34.47]	19.35 % [8.12;38.06]	20% [8.40;39.13]
Negative predictive value	94.12 % [69.24;99.69]	100 % [77.08;100.00]	100% [78.12;100.00]
Area under the curve	0.76 [0.52;1.00]	0.81 [0.62;1.00]	0.81 [0.64;0.99]

The performance accuracy of different hs-TnT cut-off values was assessed at T0, T2, and T6 (**Figure 4**). The highest prediction values at T0, T2, and T6 were observed with cut-off values of 19 ng/L, 18 ng/L, and 21 ng/L, respectively, with a performance accuracy of 91.6% for each time point.

Follow-up

During the 30-day follow-up period, no adverse cardiovascular events were reported in any of the 48 patients.

Discussion

The recently introduced hs-TnT assays are the most sensitive markers of myocardial necrosis. Their recommendation in European guidelines for ACS management[3] have been validated by several clinical trials that showed the assay's high diagnostic accuracy[2, 12] and greater prognostic accuracy than standard cTn assay[3]. However, the hs-TnT assay's diagnostic performance for the detection of myocardial ischemia in a low-risk profile population had been poorly documented. We chose myocardial blood flow quantitation, using a Rb-82 PET-CT, as a non-invasive stress test to detect myocardial ischemia.

The present study showed that hs-TnT assay measurements in a population of low-risk ACS profile patients at T0, T2 and T6 provided low specificity and low positive predictive values but an excellent sensitivity and negative predictive values (94%, 100%, and 100%, respectively) for predicting the detection of ischemia as assessed using a PET-CT at a cut-off of 4 ng/L. Moreover, measurements at T2 provided higher negative predictive values than at T0 and equal to values at T6. In other words, T2 might be considered as potentially valuable time point at which to exclude ischemia in this specific population but this finding warrants further studies on larger cohorts.

Current European guidelines on the management of ACS in patients presenting without persistent ST-segment elevation have introduced algorithms for ruling-in and ruling-out acute myocardial infarction. In patients with hs-TnT levels below the

99th percentile, without ischemic changes on ECGs and free of chest pain for several hours, these guidelines propose a stress imaging test at the time of admission or shortly after discharge. Nevertheless, the exact timing of these investigations remains unclear and the treatment regimen during a potential discharge remains a typical daily dilemma for clinicians. Indeed, a recent prospective study based on data from 1,400 patients with unstable angina suggested that adherence to the ESC guidelines was inadequate in nearly two thirds of patients in terms of over-treatment or under-treatment[13].In a retrospective study based on 344 patients with chest pain, negative serial ECG, and negative cardiac enzyme discharged before stress testing, 2 patients had a fatal out-of-hospital cardiac event, and 24 were readmitted to the emergency department prior to carrying out stress testing[14]. In addition, data based on 966 patients with unstable angina who were mistakenly discharged were analyzed as part of a multicenter trial. Their risk-adjusted mortality ratio was 1.7 times higher than those who were hospitalized (95% CI: 0.2 to 17.0) [15]. Different studies evaluating the safety of discharge before stress testing based solely on negative hs-TnT values have been recently published. In a general population of patients suspected for ACS, it was documented that single normal troponin measurement at admission (below the 99th percentile) was not a reliable method to safely rule-out patients suspected for ACS [16]. The safety of ruling-out an ACS was also examined in regard to undetectable levels of hs-Tn at admission, showing high NPV for this strategy [17, 18]. The present study identified a cut-off of 4 ng/L for hs-TnT at T2, and not admission, as being sufficient for exclusion of ischemia at PET-CT and effective decision-making process in a low-risk ACS profile population. Indeed, no patient with hs-TnT values at T2 and T6 below this cut-off had ischemia at PET-CT and none of them experienced MACE. The proportion of patients in the present study with hs-

TnT < 4 ng/L at T2 was 35% (17 patients) and at T6 was 38% (18 patients). Accordingly, using an algorithm to allow the discharge of patients with a low-risk of ACS, based on a cut-off of 4 ng/L at T2 without any stress imaging, would have allowed these patients to have been discharged with a marginal probability of an occurrence of any MACEs at the 30-day follow-up. In a prospective cohort study of patients with suspected acute coronary syndrome, Shah et al concluded that low plasma troponin concentrations identify two-thirds of patients at very low risk of cardiac events who could be discharged from the hospital [19]. However, in this study, the outcomes were index myocardial infarction, subsequent myocardial infarction or cardiac death at 30 days. In our study, we further support the safety of proposed hs-TnT algorithm, by showing no ischemia in PET-CT for patients in the rule-out zone in a preselected low-risk population. However, even if the strategy of ruling-out AMI with single value of hs-TnT ranging from 3 to 5ng/L appears safe (as reported in a recent meta-analysis [20]), safety concern remains regarding patients who present less than three hours after symptom onset.

Regarding the hs-TnT assay's performance in the detection of myocardial ischemia, it showed a low positive predictive value for detecting myocardial ischemia at PET-CT in this specific population with a low-risk profile. Indeed, the lowest diagnostic accuracies in our study (44%, 48%, and 50%) were observed at the 4 ng/L cut-off at T0, T2, and T6, respectively. On the other hand, the highest diagnostic accuracies were found with cut-off values of 19 ng/L, 18 ng/L, and 21 ng/L, at T0, T2, and T6, respectively, but with lower negative predictive values (93%, 91%, and 91%). In other words, hs-TnT seems to be an excellent biomarker for excluding the diagnosis of myocardial ischemia at PET-CT, rather than for detecting it in this specific population. Considering the combined accuracy for ruling-in and ruling-out, the algorithm of

2-hour change in hs-Tn level seems to outperform a single measure, what was shown and validated for hs-TnI in a general cohort of patients with chest pain [21]. Nevertheless, an algorithm based on hs-TnT measurements, coupled with a PET-CT stress test, seems to diagnose functionally significant coronary artery stenosis correctly at angiogram if hs-TnT $\geq$ 4 ng/L. This assumption should be verified by using a coronary angiogram for all patients.

Limitation

One major limitation of the present study is its small number of participants, particularly those with a positive PET-CT. Another limitation is a verification bias, as coronary angiograms were not performed on patients with negative PET-CT. Indeed, the study investigated a low-risk ACS population, in which an invasive diagnostic strategy is not indicated. However, it could also be argued that all these patients had a negative PET-CT and no MACE during 30 days of follow-up.

Conclusion

In conclusion, we found that a cut-off < 4 ng/L at T2 provided excellent negative predictive values (100%) for the exclusion of myocardial ischemia as measured by PET-CT in a low-risk profile ACS population. Furthermore, in patients with hs-TnT $\geq$ 4 ng/L, a strategy based on a PET-CT ischemia detection appears to be appropriate. These results should be confirmed in the study of a larger sample population.

Compliance with Ethical Standards

All authors declare that they do not have conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent: Informed consent was obtained from all individual participants included in the study.

Declaration of competing interests and founding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Authors' contributions

BM and SF contributed to the conception of the study, to aquisition and analysis of the data, performed analysis and prepared the draft of the manuscript, MT gave the design of the study, gathered and managed the data, JOP, PM and VD gathered the data, gave critical view to the design and the draft of the manuscript, NL and FR participated in al analysis and gathering of the data, CT, JFI, and DK gathered the data, performed analysis and participated in preparation ofthe manuscript, OB, DB and SL performed laboratory analyses and data management, EE, OH, and OM

participated in the design of the study, approved all analyses and gave critical overview to the manuscript.

Data sharing

The dataset is not available.

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Figure Legends

Figure 1. Plot of hs-TnT concentrations at admission (T0) and at 2 h (T2) and 6 h (T6) afterwards.

Figure 2. Study chart.

Figure 3. ROC curves for the detection of myocardial ischemia.

Figure 4. Diagnostic accuracy.

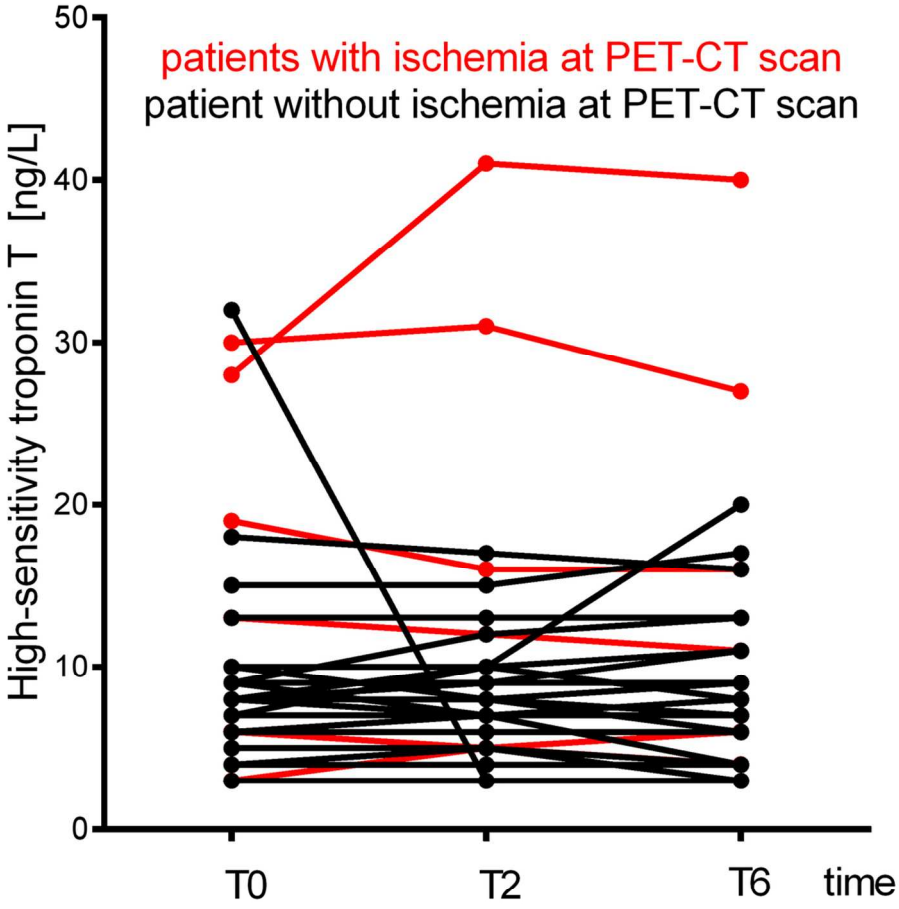

Figure 1. Plot of hs-TnT concentrations at admission (T0) and at 2 h (T2) and 6 h (T6) afterwards.

111x119mm (300 x 300 DPI)

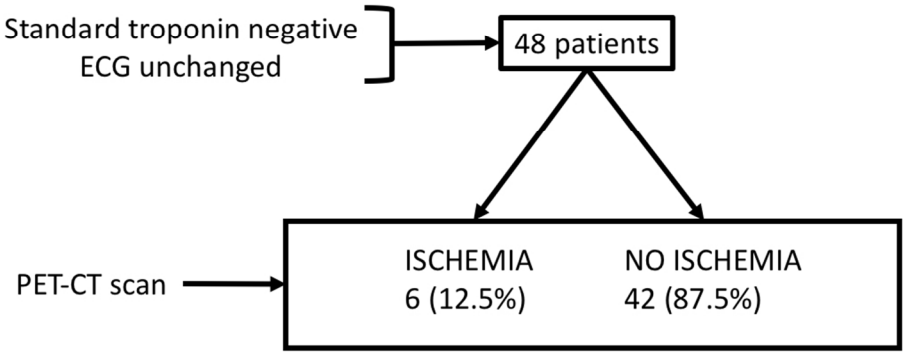

Figure 2. Study chart.

299x141mm (96 x 96 DPI)

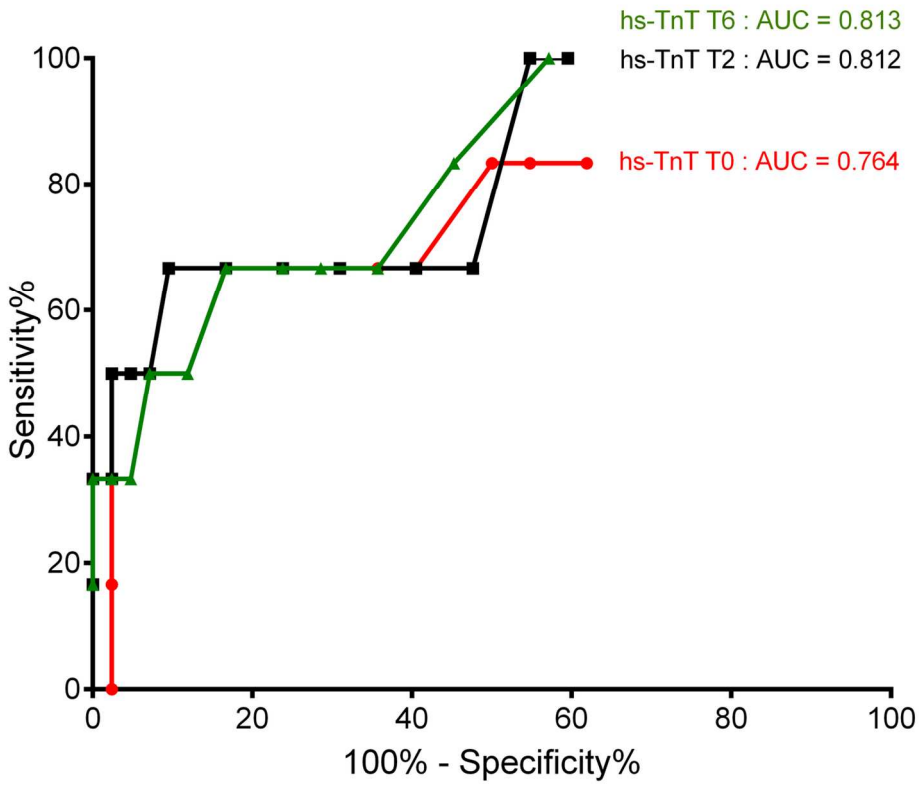

Figure 3. ROC curves for the detection of myocardial ischemia.

135x110mm (300 x 300 DPI)

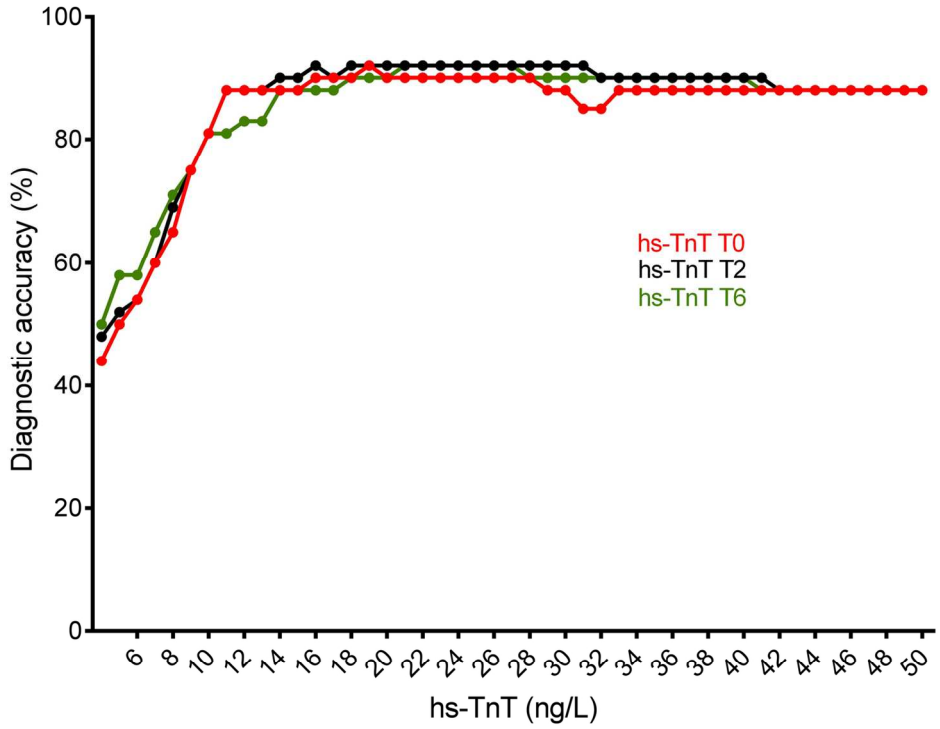

Figure 4. Diagnostic accuracy.

138x112mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	n/a
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	Fig 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	2
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) Summarise follow-up time (eg, average and total amount)	10-11
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11-12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

BMJ Open

Performance of high sensitive cardiac troponin T assay to detect ischemia at PET-CT in low-risk patients with acute coronary syndrome: a prospective observational study.

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Secondary Subject Heading:	Diagnostics
Keywords:	troponin, acute coronary syndrome, positron emission tomography, ischemia

Original Research

Performance of high sensitive cardiac troponin T assay to detect ischemia at PET-CT in low-risk patients with acute coronary syndrome: a prospective observational study.

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References: 21

Figures: 4

Tables: 2

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ABSTRACT

Background

Highly sensitive troponin T (hs-TnT) assay has improved clinical decision-making for patients admitted with chest pain. However, this assay's performance in detecting myocardial ischemia in a low risk population has been poorly documented.

Purpose

To assess Hs-TnT assay's performance to detect myocardial ischemia at PET-CT in low-risk patient admitted with chest pain.

Methods

Patients admitted for chest pain with a non-conclusive electrocardiogram and negative standard cardiac troponin T results at admission and after 6 hours were prospectively enrolled. Their hs-TnT samples were at T0, T2 and T6. Physicians were blinded to hs-TnT results. All patients underwent a PET-CT at rest and during adenosine-induced stress. All patients with a positive PET-CT result underwent a coronary angiography.

Results

Forty-eight patients were included. Six had ischemia at PET-CT. All of them had ≥ 1 significant stenosis at coronary angiography. Areas under the curve [95% CI] for predicting significant ischemia at PET-CT using hs-TnT were 0.764[0.515; 1.000] at T0, 0.812[0.616; 1.000] at T2, and 0.813[0.638; 0.989] at T6. The receiver operating characteristic-based optimal cut-off value for hs-TnT at T0,T2, and T6 needed to exclude significant ischemia at PET-CT was <4 ng/L. Using this value, sensitivity, specificity, positive and negative predictive values of hs-TnT to predict significant ischemia were 83%/38%/16%/94% at T0, 100%/40%/19%/100% at T2, and 100%/43%/20%/100% at T6.

Conclusions

Our findings suggest that in low-risk patients, using the hs-TnT assay with a cut-off value of 4 ng/L demonstrates excellent negative predictive value to exclude myocardial ischemia detection at PET-CT, at the expense of weak specificity and positive predictive value.

Key Words

troponin ; acute coronary syndrome ; positron emission tomography ; ischemia

Strengths and limitations of this study

- In this study we showed an additive diagnostic value of high-sensitive troponin T over standard troponin in detection of myocardial ischemia as assessed by the gold standard imaging modality – positron emission tomography/computed tomography (PET-CT) in a clinically difficult population of patients with chest pain, non-conclusive ECG and negative standard cardiac troponin and low-risk of an ACS.
- Even within a normal range of high-sensitive troponin T concentration a cut-off level may be identified to further improve diagnostic accuracy and reduce false negative and false positive results indicative for myocardial ischemia as assessed by PET-CT.
- We provide data, supported by the PET-CT study, on non-inferiority of the shortening of troponin protocols for ruling out an ACS in the emergency department from 6 to 2 hours interval.
- The study sample and single center character of the study warrants careful interpretation of outcomes and further research on larger population, preferably multicentric.
- Time intervals of blood sampling were adopted from European guidelines so the implementation of outcomes is limited in respect to ongoing research on different strategies maximally shortening diagnostic protocols.

Introduction

Patients presenting with symptoms suggestive of acute coronary syndrome (ACS) represent a large population of those admitted to emergency departments[1]. Recent available high-sensitive troponin T (hs-TnT) assay has improved the detection of patients with acute myocardial infarction (AMI) in terms of its speed and sensitivity over standard cardiac troponin (cTn) assays[2]. The hs-TnT assay was recently incorporated into the clinical decision algorithm of the latest European Society of Cardiology (ESC) guidelines[3]. However, despite its better sensitivity, the hs-TnT assay has a lower specificity because positive values are driven by several non-coronary cardiac and non-cardiac clinical conditions. The introduction of the hs-TnT assay has therefore lead to more false-positive results and subsequent unnecessary hospitalizations. When cardiac troponin levels are negative at different time points, current recommendations propose using a stress imaging test to identify patients at risk of cardiac events. The added value of the hs-TnT assay over the standard cTn assay in this population of patients is poorly documented, however. The aim of this study was therefore to assess the performance of the Hs-TnT assay to detect myocardial ischemia at PET-CT in a population of patients admitted for chest pain with negative standard cTn.

Material and methods

Trial Oversight

The study was conducted after regulatory and ethical approval was obtained from the local ethical commission (protocol 18/11) and it was duly registered on clinicaltrial.gov (ClinicalTrials.gov Identifier: NCT01374607).

Sample population

Patients admitted to the emergency department for chest pain were prospectively enrolled in the study. Inclusion criteria were: acute chest pain lasting ≥ 5 min within the last 24 h and negative standard cTn results at admission (T0) and after 6 h (T6). Exclusion criteria were: ST-segment elevation myocardial infarction (STEMI); major organ dysfunction, infection or major medical conditions (uncontrolled asthma, severe chronic obstructive pulmonary disease, AV-block of the II- or III-degree without a pacemaker) that would compromise the patient's ability to undergo a hyperemic, adenosine-induced stress test; cancer with expected survival < 6 months; pregnancy; or age < 18 years old. At admission, a clinical examination was performed and an electrocardiogram (ECG) was used to screen for acute myocardial ischemia according to international standards[4]. Information on cardiovascular risk factors, any past medical history of cardiovascular diseases and current medical treatment were collected. All patients were stratified according to their thrombolysis in myocardial infarction (TIMI) risk score and the score of 0 or 1 was indicative for low risk of cardiovascular adverse events[5].

Troponin measurements

The standard cTn assay, routinely used in the institution throughout the enrollment process, was cardiac troponin I measured with AccuTnI assay (Beckman Coulter, Fullerton, CA, USA) with a 99th percentile level of 0.04 µg/L and a 20% coefficient of variation at 0.03 µg/L) and considered as positive if above the 99th percentile. The hs-TnT value was measured in parallel to the standard cTnI assay, using the same plasma samples at T0, T2 and T6. Concentrations of hs-TnT in plasma were measured using a Cobas e602 immunoanalyzer (Roche, Basel, Switzerland) based

on electrochemiluminescence technology (detection range of 3–10000 ng/L, with a 99th percentile level in a normal population of 14 ng/L and a 10% coefficient of variation level of 13 ng/L). The two hour diagnostic protocol using cTn has been described previously to carry a good diagnostic accuracy[6].

PET-CT and coronary angiography

All patients underwent a rubidium-82 (Rb-82) rest–stress cardiac PET-CT acquisition (Discovery 690, GE Healthcare, Milwaukee, WI, USA) according to a previously described method[7]. Patients were instructed to fast for 6 h and the absence of any caffeine intake in the previous 24 h was checked. Dynamic rest acquisition started after the beginning of an i.v. infusion of 10 MBq/kg of Rb-82 (Jubilant Draximage, Kirkland, Canada)[8]. Ten minutes later, a hyperemic stress test was performed using a slow intravenous infusion of adenosine (140 µg/kg/min) over 6 min. A second PET-CT acquisition was started 2 min after the beginning of adenosine infusion. PET-CT images were analyzed semi-quantitatively by two independent nuclear medicine specialists using the 17-segment AHA polar map[9] to reveal the extent and severity of perfusion defects at rest (summed rest score, SRS) and during stress (summed stress score, SSS), as well as inducible ischemia as defined by the summed difference score (SDS = SSS - SRS). Absolute quantitation of myocardial blood flow (MBF) at rest and during stress, as well as the myocardial flow reserve (MFR = Stress MBF / Rest MBF), were computed using FlowQuant (Ottawa Heart Institute, Ottawa, Canada). Both, SDS and MFR have been documented as having a prognostic value in patients investigated for ischemia[7, 10]. A PET-CT is likely to be positive for myocardial ischemia when SDS > 2 or MFR < 1.8. These two thresholds have been shown to be strong predictors of major cardiovascular events[7, 11].

Patients in the present study who were positive for myocardial ischemia on PET-CT images were scheduled for a coronary angiography.

The results of coronary angiography were assessed visually by two independent investigators, with an assessment by a third interventional cardiologist in cases of borderline stenosis. Stenosis of an epicardial coronary artery was defined as *significant* if the diameter of the stenosis was > 50% of the lumen diameter in an artery with a diameter > 2 mm.

Major Adverse Cardiac Events

Patients were followed-up with phone calls 30 days after discharge and evaluated for any major adverse cardiovascular event (MACE), defined as rehospitalization for a cardiovascular reason, repeated revascularization, non-fatal AMI, or death of a cardiovascular origin.

Statistics

Statistical analysis was performed using SPSS software (version 19, SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, California, USA). Variables are presented as a mean ± standard deviation (SD) or as a median and its interquartile range (IQR). Comparisons between groups were performed using the Mann-Whitney U test for continuous variables. Comparisons of categorical data were performed using the Fischer exact test or the chi-square test, as appropriate. A bilateral P value < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed by plotting each patient's values of hs-TNT at T0, T2, and T6 against the presence of ischemia at PET-CT. Best cut-offs were calculated using Youden's index.

Results

Clinical characteristics of the sample population

Of 50 eligible patients, 2 were excluded due to technical problems during the PET-CT quantitation of flow reserve. The remaining 48 patients participating in the study had a median (P25; P75) duration of chest pain of 2 h (1; 4); mean age was 58 ± 13 years; 33 (68 %) were male; 7 (15%) were diabetic; 3 (6%) had a history of myocardial infarction; 15 (31%) had prior percutaneous coronary intervention and 1 (2%) had prior coronary artery bypass graft The median TIMI risk score was 1 (0; 2) (**Table 1**).

Table 1. Patients' clinical characteristics.

Characteristic	Total population (n=48)	Without ischemia (n=42)	With ischemia (n=6)
Past medical history			
Myocardial Infarction	3 (6%)	1 (2%)	2 (33%)
Percutaneous Coronary Intervention	15 (31%)	12 (29%)	3 (50%)
Coronary Artery Bypass Grafting	1 (2%)	1 (2%)	0 (0%)
Peripheral Artery Disease	1 (2%)	0 (0%)	1 (17%)
Stroke	2 (4%)	1 (2%)	1 (17%)
Renal insufficiency	1 (2%)	1 (2%)	0 (0%)
Cardiovascular risk factors			
Arterial hypertension	28 (58%)	22 (52%)	6 (100%)
Dyslipidemia	26 (54%)	20 (48%)	6 (100%)
Diabetes	7 (15%)	6 (14%)	1 (17%)
Familial history	15 (31%)	14 (33%)	1 (17%)
Current/former smoking	20 (42%)	18 (43%)	2 (33%)
TIMI risk score	1 (0;2)	0 (0;0)	2 (2;3)
Clinical presentation			
Systolic Blood Pressure	135 (119;155)	135 (122;152)	142 (129;154)
Diastolic Blood Pressure	75 (66;82)	75 (69;82)	64 (62;78)
Heart Rate	72 (60;88)	73 (62;88)	65 (53;75)
Body Mass Index (BMI)	27.8 (25.3;30.4)	27.3 (25.3 ; 29.8)	28.8 (26.5 ; 30.7)

Data are presented as n (%) or median (25^{th}; 75^{th} percentile).

Levels of hs-TnT in the sample population

As per the study design, standard cTn levels were < 99th percentile (< 0.03 mg/L) in all patients at T0 and T6. First blood sample for hs-TnT measurement was taken after a median of 4h8min after first chest pain and after a median of 1h28min after the last episode. Median hs-TnT levels for the sample population were 6.0 (3.0; 9.0) ng/L at T0; 5.5 (3.0; 9.0) ng/L at T2, and 5.0 (3.0; 9.0) ng/L at T6. These hs-TnT values remained stable over time, with a mean absolute change of 0.29 ng/L within the first 2 h after admission, and of 0.021 ng/L in the following 4 h (**Figure 1**).

Identification of inducible ischemia with PET-CT

A PET-CT was performed with a median delay of 32 h (17; 65) from symptom onset. Among the 48 patients, 6 (12.5%) had a positive PET-CT for myocardial ischemia **(Figure 2).** In all patients diagnosed positive for myocardial ischemia, a coronary angiography confirmed at least one significant epicardial coronary artery stenosis.

ROC curves

Areas under the ROC curves were calculated for hs-TnT at T0, T2, and T6, both with and without absolute delta changes. The areas under the curves [95% CI] obtained were: 0.764 [0.515; 1.000] (T0); 0.812 [0.616; 1.000] (T2); 0.806 [0.601; 1.000] (T0, T2, and delta change); 0.813 [0.638; 0.989] (T6); and 0.829 [0.634; 1.000] (T0, T2, T6, and delta changes) (**Figure 3**). Additional analyses with different absolute values for hs-TnT and incorportation of the absolute delta changes improved the area under the curves, but these differences were not statistically significant.

Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy

The ROC-based, optimal cut-off value for hs-TnT value at T0, T2, and T6 necessary to exclude a diagnosis of significant ischemia at a PET-CT was < 4 ng/L. Such concentration was met by 17 (35%) patients at T0 and T2 and 18 (38%) patients at T6. Using this value, the sensitivity, specificity, and positive and negative predictive values of the hs-TnT assay to predict significant ischemia at PET-CT were 83[36;99]%, 38[24;54]%, 16[6;34]%, 94[69;100]% at T0, 100[52;100]%, 40[26;57]%, 19[8;38]%, 100[77;100]% at T2, and 100[52;100]%, 43[28;59]%, 20[8;39]%, 100[78;100]% at T6. (**Table 2**). Using the recommended cut-off value of the 99th percentile at 14 ng/L, the sensitivity, specificity, positive and negative predictive value of the hs-TnT assay were 50[14;86]%, 93[79;98]%, 50[14;86]%, 93[79;98]% at T0, 50[14;86]%, 95[83;99]%, 60[17;93]%, 93[79;98]% at T2, and 50[14;86]%, 93[79;98]%, 50[14;86]%, 93[79;98]% at T6.

Table 2. The hs-TnT assay's performance in predicting the detection of ischemia at PET-CT.

Hs-TnT >= 4ng/l at :	T0	T2	T6
Sensitivity	83.33 % [36.48;99.12]	100% [51.68;100.00]	100 % [51.68;100.00]
Specificity	38.10 % [23.99;54.35]	40.48 % [26.02;56.65]	42.85% [28.08;58.93]
Positive predictive value	16.13 % [6.09;34.47]	19.35 % [8.12;38.06]	20% [8.40;39.13]
Negative predictive value	94.12 % [69.24;99.69]	100 % [77.08;100.00]	100% [78.12;100.00]
Area under the curve	0.76 [0.52;1.00]	0.81 [0.62;1.00]	0.81 [0.64;0.99]

The performance accuracy of different hs-TnT cut-off values was assessed at T0, T2, and T6 (**Figure 4**). The highest prediction values at T0, T2, and T6 were observed with cut-off values of 19 ng/L, 18 ng/L, and 21 ng/L, respectively, with a performance accuracy of 91.6% for each time point.

Follow-up

During the 30-day follow-up period, no adverse cardiovascular events were reported in any of the 48 patients.

Discussion

The recently introduced hs-TnT assays are the most sensitive markers of myocardial necrosis. Their recommendation in European guidelines for ACS management[3] have been validated by several clinical trials that showed the assay's high diagnostic accuracy[2, 12] and greater prognostic accuracy than standard cTn assay[3]. However, the hs-TnT assay's diagnostic performance for the detection of myocardial ischemia in a low-risk profile population had been poorly documented. We chose myocardial blood flow quantitation, using a Rb-82 PET-CT, as a non-invasive stress test to detect myocardial ischemia.

The present study showed that hs-TnT assay measurements in a population of low-risk ACS profile patients at T0, T2 and T6 provided low specificity and low positive predictive values but an excellent sensitivity and negative predictive values (94%, 100%, and 100%, respectively) for predicting the detection of ischemia as assessed using a PET-CT at a cut-off of 4 ng/L. Moreover, measurements at T2 provided higher negative predictive values than at T0 and equal to values at T6. In other words, T2 might be considered as potentially valuable time point at which to exclude ischemia in this specific population but this finding warrants further studies on larger cohorts.

Current European guidelines on the management of ACS in patients presenting without persistent ST-segment elevation have introduced algorithms for ruling-in and ruling-out acute myocardial infarction. In patients with hs-TnT levels below the

99th percentile, without ischemic changes on ECGs and free of chest pain for several hours, these guidelines propose a stress imaging test at the time of admission or shortly after discharge. Nevertheless, the exact timing of these investigations remains unclear and the treatment regimen during a potential discharge remains a typical daily dilemma for clinicians. Indeed, a recent prospective study based on data from 1,400 patients with unstable angina suggested that adherence to the ESC guidelines was inadequate in nearly two thirds of patients in terms of over-treatment or under-treatment[13].In a retrospective study based on 344 patients with chest pain, negative serial ECG, and negative cardiac enzyme discharged before stress testing, 2 patients had a fatal out-of-hospital cardiac event, and 24 were readmitted to the emergency department prior to carrying out stress testing[14]. In addition, data based on 966 patients with unstable angina who were mistakenly discharged were analyzed as part of a multicenter trial. Their risk-adjusted mortality ratio was 1.7 times higher than those who were hospitalized (95% CI: 0.2 to 17.0) [15]. Different studies evaluating the safety of discharge before stress testing based solely on negative hs-TnT values have been recently published. In a general population of patients suspected for ACS, it was documented that single normal troponin measurement at admission (below the 99th percentile) was not a reliable method to safely rule-out patients suspected for ACS [16]. The safety of ruling-out an ACS was also examined in regard to undetectable levels of hs-Tn at admission, showing high NPV for this strategy [17, 18]. The present study identified a cut-off of 4 ng/L for hs-TnT at T2, and not admission, as being sufficient for exclusion of ischemia at PET-CT and effective decision-making process in a low-risk ACS profile population. Indeed, no patient with hs-TnT values at T2 and T6 below this cut-off had ischemia at PET-CT and none of them experienced MACE. The proportion of patients in the present study with hs-

TnT < 4 ng/L at T2 was 35% (17 patients) and at T6 was 38% (18 patients). Accordingly, using an algorithm to allow the discharge of patients with a low-risk of ACS, based on a cut-off of 4 ng/L at T2 without any stress imaging, would have allowed these patients to have been discharged with a marginal probability of an occurrence of any MACEs at the 30-day follow-up. In a prospective cohort study of patients with suspected acute coronary syndrome, Shah et al concluded that low plasma troponin concentrations identify two-thirds of patients at very low risk of cardiac events who could be discharged from the hospital [19]. However, in this study, the outcomes were index myocardial infarction, subsequent myocardial infarction or cardiac death at 30 days. In our study, we further support the safety of proposed hs-TnT algorithm, by showing no ischemia in PET-CT for patients in the rule-out zone in a preselected low-risk population. However, even if the strategy of ruling-out AMI with single value of hs-TnT ranging from 3 to 5ng/L appears safe (as reported in a recent meta-analysis [20]), safety concern remains regarding patients who present less than three hours after symptom onset.

Regarding the hs-TnT assay's performance in the detection of myocardial ischemia, it showed a low positive predictive value for detecting myocardial ischemia at PET-CT in this specific population with a low-risk profile. Indeed, the lowest diagnostic accuracies in our study (44%, 48%, and 50%) were observed at the 4 ng/L cut-off at T0, T2, and T6, respectively. On the other hand, the highest diagnostic accuracies were found with cut-off values of 19 ng/L, 18 ng/L, and 21 ng/L, at T0, T2, and T6, respectively, but with lower negative predictive values (93%, 91%, and 91%). In other words, hs-TnT seems to be an excellent biomarker for excluding the diagnosis of myocardial ischemia at PET-CT, rather than for detecting it in this specific population. Considering the combined accuracy for ruling-in and ruling-out, the algorithm of

2-hour change in hs-Tn level seems to outperform a single measure, what was shown and validated for hs-TnI in a general cohort of patients with chest pain [21]. Nevertheless, an algorithm based on hs-TnT measurements, coupled with a PET-CT stress test, seems to diagnose functionally significant coronary artery stenosis correctly at angiogram if hs-TnT $\geq$ 4 ng/L. This assumption should be verified by using a coronary angiogram for all patients.

Limitation

One major limitation of the present study is its small number of participants, particularly those with a positive PET-CT. Another limitation is a verification bias, as coronary angiograms were not performed on patients with negative PET-CT. Indeed, the study investigated a low-risk ACS population, in which an invasive diagnostic strategy is not indicated. However, it could also be argued that all these patients had a negative PET-CT and no MACE during 30 days of follow-up.

Conclusion

In conclusion, we found that a cut-off < 4 ng/L at T2 provided excellent negative predictive values (100%) for the exclusion of myocardial ischemia as measured by PET-CT in a low-risk profile ACS population. Furthermore, in patients with hs-TnT $\geq$ 4 ng/L, a strategy based on a PET-CT ischemia detection appears to be appropriate. These results should be confirmed in the study of a larger sample population.

Compliance with Ethical Standards

All authors declare that they do not have conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent: Informed consent was obtained from all individual participants included in the study.

Declaration of competing interests and founding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Authors' contributions

BM and SF contributed to the conception of the study, to aquisition and analysis of the data, performed analysis and prepared the draft of the manuscript, MT gave the design of the study, gathered and managed the data, JOP, PM and VD gathered the data, gave critical view to the design and the draft of the manuscript, NL and FR participated in al analysis and gathering of the data, CT, JFI, and DK gathered the data, performed analysis and participated in preparation ofthe manuscript, OB, DB and SL performed laboratory analyses and data management, EE, OH, and OM

participated in the design of the study, approved all analyses and gave critical overview to the manuscript.

Data sharing

The dataset is not available.

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Figure Legends

Figure 1. Plot of hs-TnT concentrations at admission (T0) and at 2 h (T2) and 6 h (T6) afterwards.

Figure 2. Study chart.

Figure 3. ROC curves for the detection of myocardial ischemia.

Figure 4. Diagnostic accuracy.

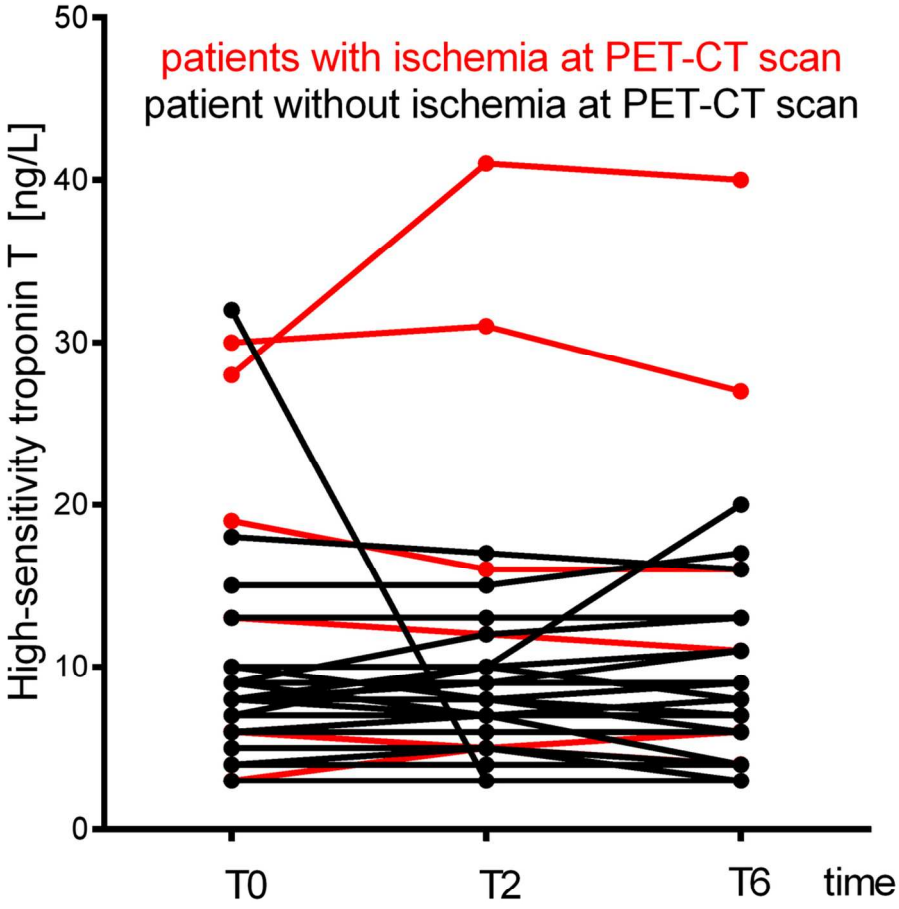

Figure 1. Plot of hs-TnT concentrations at admission (T0) and at 2 h (T2) and 6 h (T6) afterwards.

111x119mm (300 x 300 DPI)

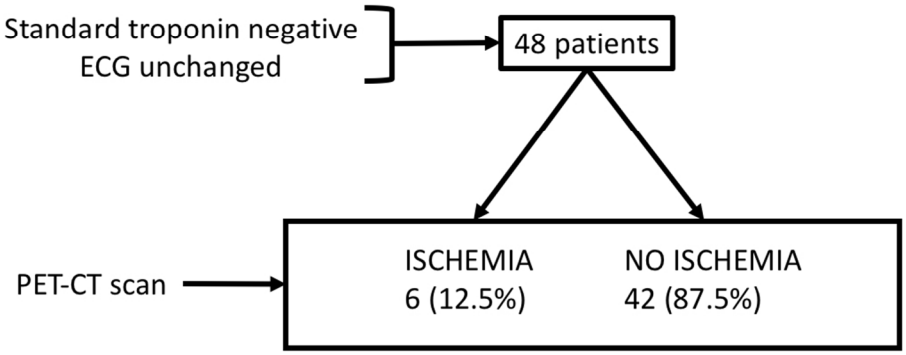

Figure 2. Study chart.

299x141mm (96 x 96 DPI)

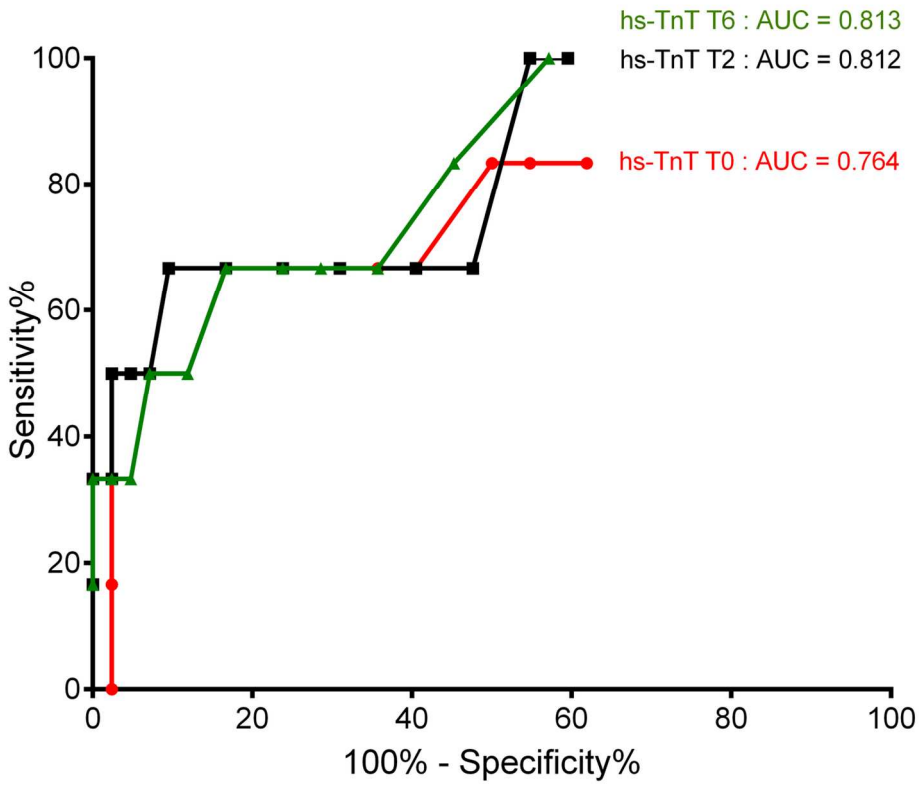

Figure 3. ROC curves for the detection of myocardial ischemia.

135x110mm (300 x 300 DPI)

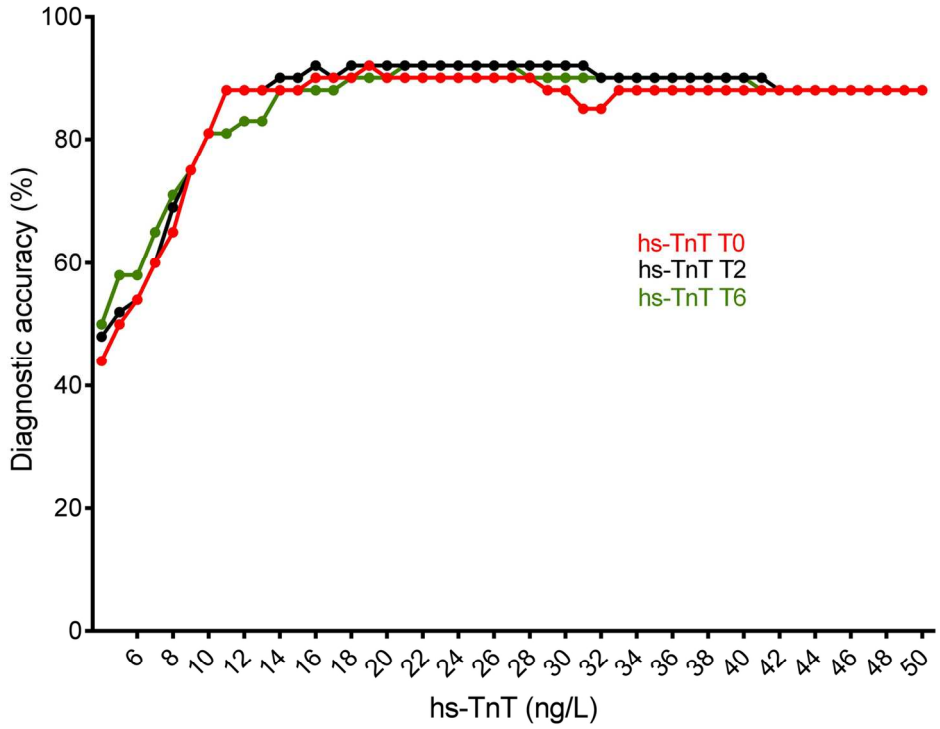

Figure 4. Diagnostic accuracy.

138x112mm (300 x 300 DPI)

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	4-5
Participants	**6**	Eligibility criteria	5
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	5-6
	10b	Reference standard, in sufficient detail to allow replication	6-7
	11	Rationale for choosing the reference standard (if alternatives exist)	7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	5-6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6-7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	-
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	-
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	-
	16	How missing data on the index test and reference standard were handled	-
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	-
	18	Intended sample size and how it was determined	-
RESULTS			
Participants	**19**	Flow of participants, using a diagram	9
	20	Baseline demographic and clinical characteristics of participants	8
	21a	Distribution of severity of disease in those with the target condition	8-9
	21b	Distribution of alternative diagnoses in those without the target condition	8-9
	22	Time interval and any clinical interventions between index test and reference standard	9
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	9-10
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10
	25	Any adverse events from performing the index test or the reference standard	11
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	14
	27	Implications for practice, including the intended use and clinical role of the index test	13-14
OTHER INFORMATION			
	28	Registration number and name of registry	4
	29	Where the full study protocol can be accessed	4
	30	Sources of funding and other support; role of funders	15

STARD 2015

AIM

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

EXPLANATION

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

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

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

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

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

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

DEVELOPMENT

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

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

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	n/a
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	Fig 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	2
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) Summarise follow-up time (eg, average and total amount)	10-11
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11-12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15