Variability of high sensitivity troponin T concentrations in emergency settings: impact for the diagnosis of myocardial infarction

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Abstract

Objectives: Assess biological variation of troponin T in emergency settings and establish limits for the interpretation of serial results.

Methods: We studied 6,557 consecutive patients with troponin measurements. A stable reference subset was selected to estimate biological variation and threshold limits.

Results: The first troponin was elevated in 32% of patients. 2,490 had a second troponin with a prevalence of 16.2% of MI. In the stable reference group with at least one abnormal value, the 99th percentile of the absolute delta between the first two samples was 16ng/L. For MI diagnosis, the area under the ROC curve was 0.85 (CI:0.83-0.87) for the first troponin and 0.94 (CI:0.93-0.95) for the absolute delta.

Conclusions: An absolute delta of 16ng/L has good specificity in the emergency setting. This threshold is valid for any sex, age and sampling interval between 3 and 24 hours and is higher than published limits found in healthy outpatients.

1 Introduction

Many individuals without acute conditions have basal high-sensitivity troponin T (hs-TnT) values repeatedly above 14 ng/L, the recommended 99th percentile threshold for diagnosis of myocardial infarction (MI). Factors associated with increased values include older age, male sex, chronic heart disease, peripheral atherosclerotic vascular disease, hypertension, diabetes and chronic kidney disease (1-4). For patients with chronic elevations of troponin T, the challenge is to differentiate ''stable'' patients from patients with an acute condition that increases troponin, most importantly a myocardial infarction. Some authors have suggested to use age and sex-adjusted upper limits to improve discrimination (2, 5, 6). However, dynamic changes are still required in most cases to identify MI with certainty (7). Moreover, patients presenting to the emergency room in a clinical context are more likely to have comorbidities and have a chronic elevation of hs-TnT than typically studied reference populations with young and healthy individuals. Our objectives were to assess biological variability of hs-TnT in unselected patients presenting to the emergency room and define a 99th percentile limit for the delta in sequential troponin results in stable patients without acute injury. This limit could then be applied as a rule-in criterion to identify acute cardiac injury. Secondary objectives were to evaluate the diagnostic accuracy of the criterion to recognize MI and to identify factors associated with increased biological variability.

2 Methods

We performed a retrospective study of consecutive patients presenting to the emergency department of a university hospital (IUCPQ, Québec, Canada). 6,557 consecutive patients with at least one hs-TnT measurement were included. Only the first encounter was studied for each patient. Hs-TnT was measured in heparinized plasma on a stat basis with Modular analysers (Roche Diagnostics) according to the manufacturer's instructions. Imprecision is below 10% for values above 10 ng/L. All results are expressed as whole number without decimal. We predefined the following cut-offs: **(1)** 3 ng/L as the limit of detection, any value below 3 was set at 3 ng/L for statistical analyses; **(2)** 14 ng/L as the upper limit of normals as suggested by the manufacturer; **(3)** 50 ng/L as equivalent to the upper reference limit used with the previous

troponin T assay to identify a high-risk group. Variation of troponins was analyzed in patients with repeated troponin measurements within a limit of 24 hours (n = 2,490). Our strategy was first to select a reference cohort from the whole cohort with very low probability of acute events, apply the principle of symmetry as described below, determine a 99th percentile limit of variation and secondly evaluate its performance in the whole cohort. We focused our analysis on patients with normal or slightly abnormal (below 50 ng/L) since this is the critical zone where sequential analysis is most needed. Patients with MI or other acute events typically show increases in troponin. In contrast, patients without acute events may show either increasing or decreasing sequential values in a random manner. Patients who have no change (identical results) in troponin or decreasing values with an average below 50 ng/L are at very low risk of MI. McMullin et al. found that a decreasing troponin T in an emergency setting was not associated with increased odds of acute coronary syndrome in a cohort of over 1,800 patients (8). Among patients with a mean hs-TnT < 50 ng/L (n = 1,861), we thus defined a reference cohort of stable patients with equal or decreasing results. For stable patients, it is expected that hs-TnT values vary randomly around a mean such that for two sequential results, there are equal chances to have $R_1 > R_2$ or $R_2 > R_1$ with half going up and half going down. Thus, the absolute variation can be used as a surrogate of variability in stable low risk patients (principle of symmetry). To avoid statistical bias, we included only half of the patients with identical values selected randomly and excluded extreme outliers defined as a change exceeding three times the interquartile range (a standard criterion for extreme outliers). Figure 1 describes the selection process for the reference cohort.

We evaluated the diagnostic performance of the threshold found in the reference cohort study for the identification of MI. In the absence of an independent gold standard, we used the final diagnosis from the electronic medical records as established by the treating physicians without any patient chart reviews. Complementary investigations were at the discretion of the clinicians and all troponin results were available to physicians for their final judgement. The hospital research ethics review board approved the study. The validation cohort included patients with at least two hs-TnT measurements within a 24-hour period (Figure 1, n = 2,490). Because of the uncertainty about troponin evolution, patients with a diagnosis of cardiomyositis,

pericardial disease or circulatory shock were excluded (n = 52). All others (n = 2,438) were included in the validation cohort and were divided into positive or negative for myocardial infarction. Patients with cardiac arrest were included in the MI group (figure 1).

2.1 Statistical analyses

We made comparisons between groups with the standard t-test, the chi-square test and the non-parametric Kruskal-Wallis (KW) test or Mann-Whitney U test where appropriate. Outliers and extreme outliers were defined as, respectively, any value exceeding 1.5 and 3 times the interquartile range from the median. In the reference group, we defined the threshold to identify a significant change in hs-TnT based on the 99th percentile of the delta distribution between repeated measures. Variables associated with the absolute delta between two consecutive troponin results were analyzed by univariate and multivariate linear regression statistics in the reference group. To evaluate the performance of each testing strategy, we calculated sensitivity, specificity and area under the receiver operating characteristic curve (ROC). Logarithmic scale was used to better visualize results in the low range.

3 Results

We identified 6,557 consecutive patients with a hs-TnT measurement in the emergency department. Median age was 68 years and 55% were men. There were 435 MI cases, representing 6.6% of patients. The first hs-TnT was normal (≤ 14 ng/L) in 68%, slightly elevated (between 15 and 50 ng/L) in 23% and above 50 ng/L in 9%. In those three groups, MI was diagnosed respectively in 1.4%, 6.5% and 46.6%.

Of the whole cohort, 38% had a second sample within 24 hours (n = 2,490 with 16.2% MI). Of those, 1,861 had an average troponin below 50 ng/L among which 445 had decreasing and 751 had identical values. Figure 1 and table 1 describe the population and the subgroups studied. To select the reference stable group, we kept the patients with decreasing values (n = 445) and added half of the 751 patients with equal

values selected randomly (n = 376), thus generating a set of 810 patients after exclusion of 11 extreme outliers (delta above 20 ng/L). Basic characteristics of these groups are displayed in table 1. Differences between Group B and the reference group are statistically significant for age, sex, and troponin distributions ($p<0.01$), mainly du to the large sample size. However, we consider the reference group quite representative of the population served (Table 1).

The absolute delta between the 2 measurements increased progressively ($r = 0.56$, $p < 0.001$) with increasing hs-TnT (Figure 2a). However, for patients with values between 15 and 50 ng/L, the upper limit of dispersion was relatively stable, suggesting that a unique cut-off for absolute delta could be used. Relative change showed a progressive decrease in dispersion with increasing hs-TnT values (Figure 2b). For the reference group of 810 subjects, the 99th percentile of the absolute delta between samples 1 and 2 was 14 ng/L. However, for subjects with at least one value above 14 ng/L (a condition required for the diagnosis of MI), the calculated 95th and 99th percentile limits of absolute delta were 12 and 16 ng/L respectively. We used a conservative cut-off of 16 ng/L as the limit to define a clinically significant increase.

We evaluated factors associated with the observed variation in the stable group (Table 2). By univariate analysis, age ($p < 0.001$), male sex ($p = 0.029$), troponin concentration ($p < 0.001$) and sampling interval ($p < 0.001$) were all associated positively with absolute variability. However, only troponin concentration ($p < 0.001$) and sampling interval ($p < 0.001$) showed significant association with variability in multivariate analysis. In patients with at least one hs-TnT > 14 ng/L, the variability was quite stable when measurements where made 3 hours or more apart (Figure 3). The group with a sampling interval below 3 hours showed a lower variability (KW test, $p=0.002$), although the number was small (n=74).

In the validation group (n = 2,438) including 396 cases of MI, the area under the ROC curve for the absolute delta was significantly higher than for the first troponin alone (0.94 vs 0.85, $p < 0.0001$; Table 3). An absolute delta of 16 ng/L had a specificity of 94.2 % and a sensitivity of 83.2 % (Table 3 and figure 4).

Figure 5 illustrates the distribution of patients with a first troponin below 100 ng/L that would be identified using this criterion (absolute delta over 16 ng/L).

4 Discussion

In a large retrospective study in patients presenting to the emergency room, we derived a threshold for the absolute delta in two serial measurements of hs-TnT to identify patients with meaningful change suggestive of MI or other acute events. Our cohort is the largest to consider biological variation in the critical zone. For patients with values in between 14 and 50 ng/L, a delta of 16 ng/L was highly predictive of MI and was not influenced by age and sex. Globally, we observed a high prevalence (32%) of abnormal hs-TnT for the first measurement, consistent with previous reports. In ambulatory elderly patients without acute conditions (age over 75 years), the prevalence of hs-TnT above 14 ng/L has been reported between 40 and 50% (9-11). Thus, the cut-off of 14 ng/L provides limited specificity. Uncertainty in the low abnormal range can best be resolved by considering sequential samples.

Our results fully support that absolute deltas in troponin as seen in figure 4 should be preferred for diagnostic purposes (17-20). Strangely, some authors have focused solely on the analytical variation to recommend low relative cut-offs, such as 20% to identify a significant increase. This would require no variation due to sampling and no oscillation in plasma. Other authors have suggested limits between 20% and 112% from limited numbers of subjects (12-15). Our data clearly shows that it is not possible to define a single cut-off for relative change in percentage as the limit decreases progressively with increasing concentrations of hs-TnT (fig 2). We suggest that changes in hs-TnT should only be expressed in percentage in the range above 80 ng/L.

Since the diagnosis of MI requires at least one hs-TnT over 14 ng/L, we were especially interested in the stable patients with one value over 14 ng/L. Our observed limit is larger than those found in studies with

normal volunteers. For example, one study reported an upper 95th percentile limit of 8.3 ng/L for the maximum delta in observed troponins in 67 patients (20). However, 53/67 of the cases had all values ≤ 14 ng/L. Thus, this limit underestimates the variation for patients with troponin in the critical zone over 14 ng/L. In our opinion, studying variability in normal individuals with very low troponin levels has little relevance to older patients with chronically slightly abnormal levels. Although the absolute variation increases with average concentrations, an upper uniform limit of 16 ng/L is a reasonable option for concentrations between 15 and 80 ng/L. A delta of hs-TnT exceeding 16 ng/L (increasing or decreasing) is therefore an unusual occurrence in stable patients and is compatible with an acute event (MI or other diagnosis).

It is interesting to note that sex and age are not significant contributors of normal short-term troponin variability in multivariate analysis. This suggests that, when two results are available, interpretation of the delta should not be influenced by sex or age. This is a welcome conclusion as specific criteria are not required. In other words, the same limits of absolute delta can be used regardless of sex and age for the interpretation of sequential results.

The threshold for absolute delta was stable across different sampling intervals between 3 and 24 hours, suggesting that the periodicity of biological oscillation in stable patients is below a few hours. Similar observations were reported for hs-TnI in a recent study of biological variation in patients presenting to the emergency department (22). Data for sampling intervals below 3 hours was limited, but it is very likely that smaller cut-offs should be used for optimal strategic management. Recent studies have proposed different algorithms for rule out and rule in of myocardial infarction using sampling intervals as low as one hour (15,23). Recommendations to use a delta below 7 ng/L as a rule-out criterion to identify a low risk group is a reasonable strategy (17,18,22). Here, we suggest that a delta over 16 ng/L in the low range is a proper rule-in criterion with a high rate of acute events. Patients falling between 7 and 16 are in a grey zone of uncertainly and should be further investigated with additional testing.

A limitation of our study is that some patients with undiagnosed MI and decreasing hs-TnT values could have been included in the reference group and introduced some bias in the observed cut-offs. Furthermore, we relied on the acting physicians for the final diagnosis of MI, which was most likely influenced by the hs-TnT results, although nearly all patients with MI subsequently underwent imaging modalities as the hospital is a cardiac referral center. We could not assess precisely the timing of the MI in respect to the first two samples and this might have reduced the observed sensitivity and specificity. We could not assess the diagnostic performance for NSTEMI and STEMI specifically as our validation data set does not include that information. Furthermore, another limitation is that we did not try to evaluate delta as prognostic factor for mortality or other major coronary events at one month or one year. Globally, our results are consistent with other reports and allow comparisons between potential strategies. Since we used a large pool of real patients' data, we report robust evaluations that take into account the biological and analytical variations in a real clinical setting.

5 Conclusion

The absolute delta in hs-TnT is a dynamic reflection of the evolution of a potential acute event. Based on the variation observed in a clinical setting, we propose that an absolute cut-off of 16 ng/L as a specific limit (rule-in criterion) for the delta in serial measurements with sampling interval >= 3 hours to identify acute myocardial events. This criterion is proposed as a practical and easy way to interpret results in an emergency setting to identify significant alterations in hs-TnT in patients with moderately elevated values, independently of age and sex. Further studies are needed for fast protocols that include resampling before 3 hours since biological variability is expected to be lower within such a short time frame.

References

1. de Lemos JA, Drazner MH, Omland T, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. Jama. 2010;304(22):2503-12.

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

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

4. Biener M, Giannitsis E, Lamerz J, et al. Prognostic value of elevated high-sensitivity cardiac troponin T levels in a low risk outpatient population with cardiovascular disease. Eur Heart J Acute Cardiovasc Care. 2016 Oct;5(6):409-418

5. Franzini M, Lorenzoni V, Masotti S, et al. The calculation of the cardiac troponin T 99th percentile of the reference population is affected by age, gender, and population selection: A multicenter study in Italy. Clin Chim Acta. 2014;438C:376-81.

6. Chenevier-Gobeaux C, Bailleul S, Mzabi A, et al. Upper reference limits of high-sensitivity cardiac troponin T in a general population: comparison with those of sensitive cardiac troponin I. Clin Lab. 2013;59(3-4):333-6.

7. Roffi M, Patrono C, Collet JP, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J. 2015.

8. McMullin N, Lindsell CJ, Lei L, et al. Outcomes associated with small changes in normal-range cardiac markers. Am J Emerg Med. 2011;29(2):162-7.

9. Menacer S, Claessens YE, Meune C, et al. Reference range values of troponin measured by sensitive assays in elderly patients without any cardiac signs/symptoms. Clin Chim Acta. 2013;417:45-7.

10. Mansour M, Clark L, Scott T, et al. Considerations for establishing a reference interval for elderly individuals in the emergency department with the high-sensitivity cardiac troponin T assay. Clin Chim Acta. 2013;421:85-6.

11. Sajeev JK, New G, Roberts L, Menon SK, et al. High sensitivity troponin: Does the 50% delta change alter clinical outcomes in chest pain presentations to the emergency room? Int J Cardiol. 2015;184:170-4.

12. Buccelletti F, Galiuto L, Marsiliani D, et al. Comparison of diagnostic accuracy between three different rules of interpreting high sensitivity troponin T results. Intern Emerg Med. 2012;7(4):365-70.

13. Frankenstein L, Wu AH, Hallermayer K, et al. Biological variation and reference change value of high-sensitivity troponin T in healthy individuals during short and intermediate follow-up periods. Clin Chem. 2011;57(7):1068-71.

14. Vasile VC, Saenger AK, Kroning JM, et al. Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. Clin Chem. 2010;56(7):1086-90.

15. Nordenskjold AM, Ahlstrom H, Eggers KM, et al. Short- and long-term individual variation in cardiac troponin in patients with stable coronary artery disease. Clin Chem. 2013;59(2):401-9.

16. Reichlin T, Schindler C, Drexler B, et al. One-hour rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. Arch Intern Med. 2012;172(16):1211-8.

17. Biener M, Mueller M, Vafaie M, et al. Comparison of a 3-hour versus a 6-hour sampling-protocol using high-sensitivity cardiac troponin T for rule-out and rule-in of non-STEMI in an unselected emergency department population. Int J Cardiol. 2013;167(4):1134-40.

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

19. Reichlin T, Irfan A, Twerenbold R, et al. Utility of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. Circulation. 2011;124(2):136-45.

20. Pretorius CJ, Wilgen U, Ungerer JP. Serial cardiac troponin differences measured on four contemporary analyzers: relative differences, actual differences and reference change values compared. Clin Chim Acta. 2012;413(21-22):1786-91.

21. Scharnhorst V, Krasznai K, van 't Veer M, et al. Variation of cardiac troponin I and T measured with sensitive assays in emergency department patients with noncardiac chest pain. Clin Chem. 2012;58(8):1208-14.

22 Simpson AJ, Potter JM, Koerbin G, et al. Use of observed within-person variation of cardiac troponin in emergency department patients for determination of biological variation and percentage and absolute reference change values. Clin Chem. 2014 Jun;60(6):848-54.

23 Reichlin T, Cullen L, Parsonage WA, et al. Two-hour algorithm for triage toward rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. Am J Med. 2015 Apr;128(4):369-79.

TABLES

Table 1. Cohort characteristics

Group*	Complete cohort	Validation cohort	Reference cohort
Number	6557	2438	810
Age (Median) *	68.3	70.4	68.8
Age (Mean ± SD) *	66 ± 16	69.4 ± 14	67.8 ± 14
Sex (Male)	55.4 %	58.9%	55.2 %
First troponin T (ng/L), median*	6	13	7
% 0 – 14 ng/L *	68%	52.5 %	65.8 %
% 15 – 50 ng/L *	22.6 %	27.2 %	32.6 %
% > 50 ng/L *	9.4 %	20.3 %	1.6 %
Second troponin T(ng/L) median *	-	16	5
Sampling interval (hr), median*	-	6.7	6.2

*note : comparison between cohorts are statistically significant p<0.01

Table 2. Regression analysis of variables associated with hs-TnT variability in the reference group.

	UNIVARIATE		MULTIVARIATE	
variable	Standard Coefficient	p-Value	Standard Coefficient	p-Value
Sex (male)	0.037	0.029	-0.017	0.57
Age (years)	0.22	<0.001	-0.047	0.15
Mean of troponins	0.56	<0.001	0.58	<0.001
Sampling interval (hours)	0.14	<0.001	0.12	<0.001

NB: multivariate model included all 4 variables

Table 3. Diagnostic performances of different strategies in the validation cohort n = 2,438

test	ROC surface	Confidence interval	Criterion (ng/L)	sensitivity	specificity
troponin 1	0.85*	0.830 - 0.864	> 14	85.1%	59.8%
			> 50	63.4%	88.1%
troponin 2	0.95	0.941 - 0.958	> 14	97.7%	57.5%
			> 50	91.9%	86.5%
Abs. delta 2-1	0.94	0.927 - 0.946	>16	82.3%	91.7%
Relative difference %	0.82*	0.805 – 0.842	50%	58.1%	88.1%

*** surface significantly different from T2 and Abs diff p<0.0001**
other comparisons: not significant

FIGURE TITLES AND LEGENDS

Figure 1: Cohort selection flowchart

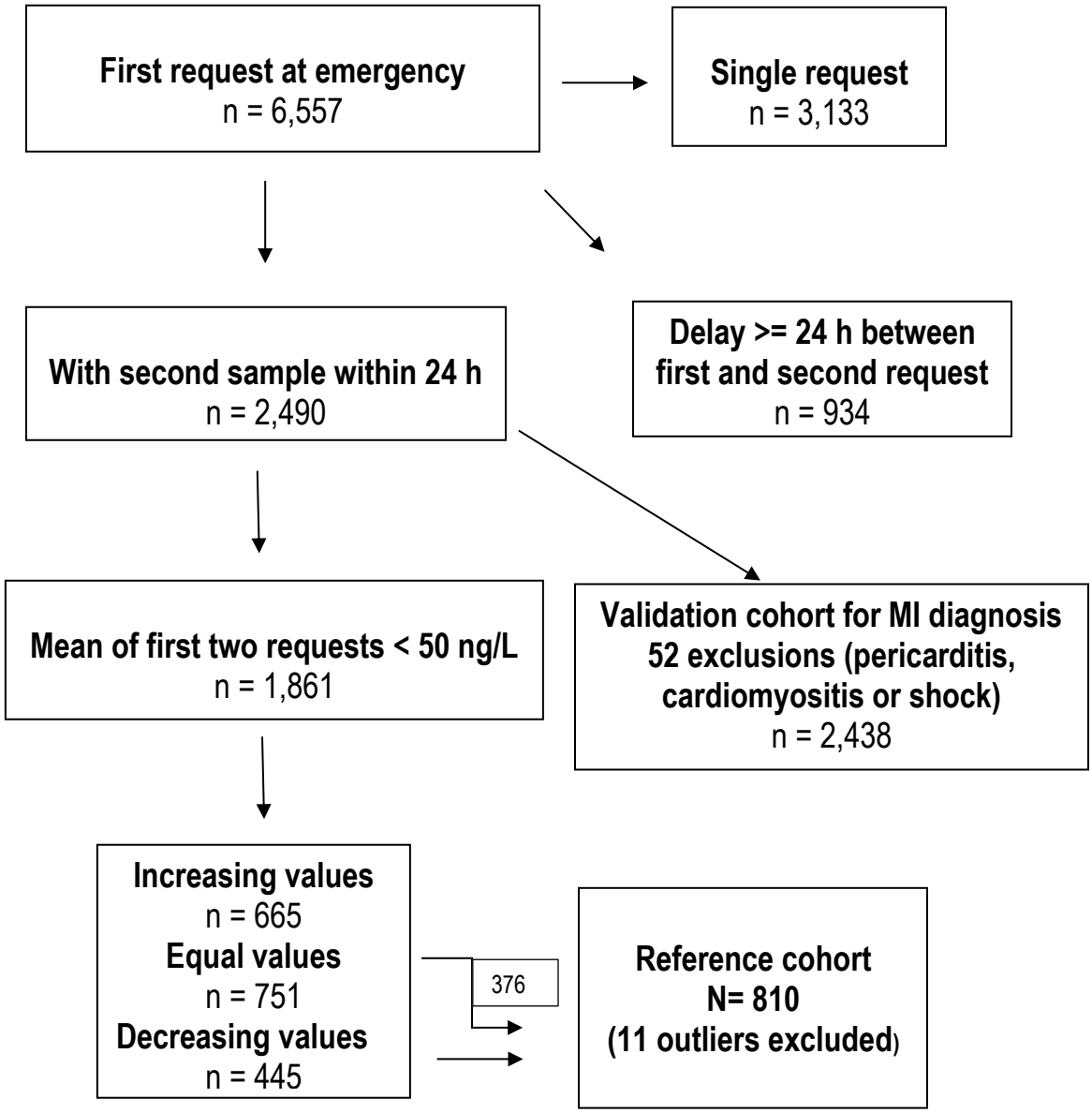
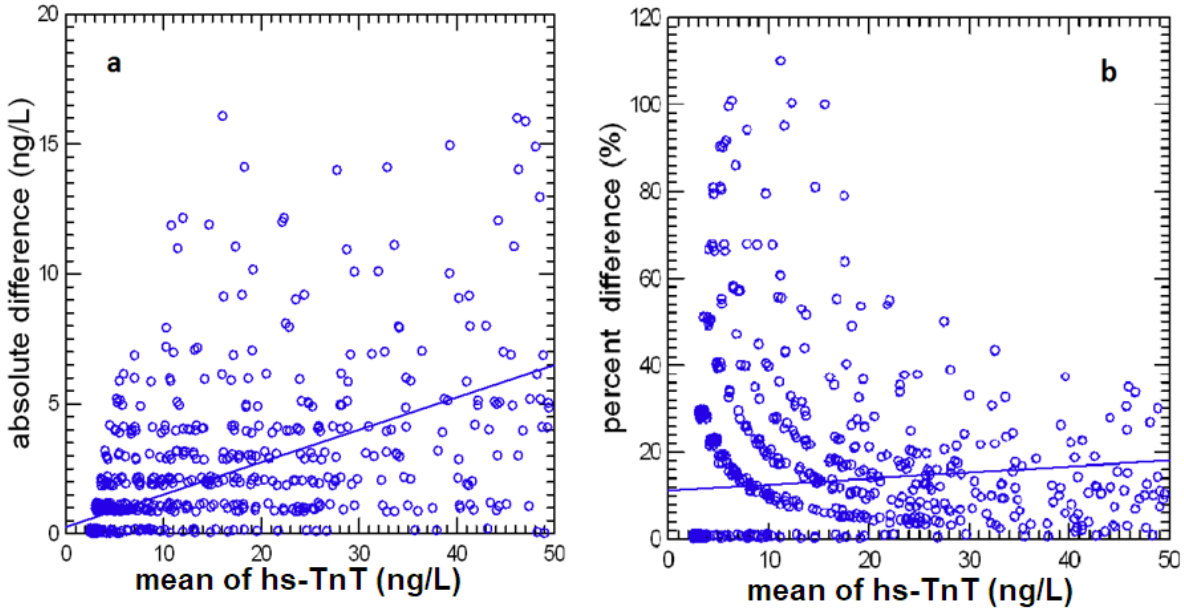
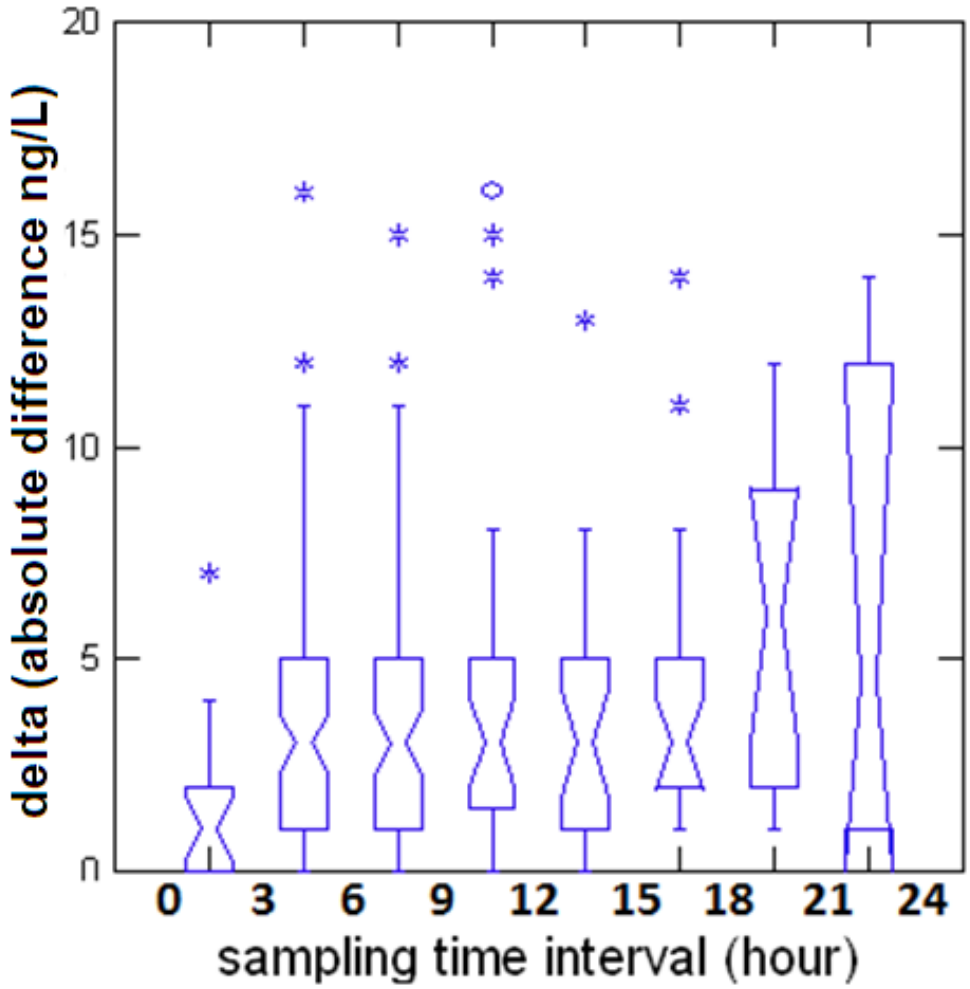


Figure 2: Absolute (delta) and relative differences in hs-TnT as a function of mean hs-TnT concentrations in the reference cohort.



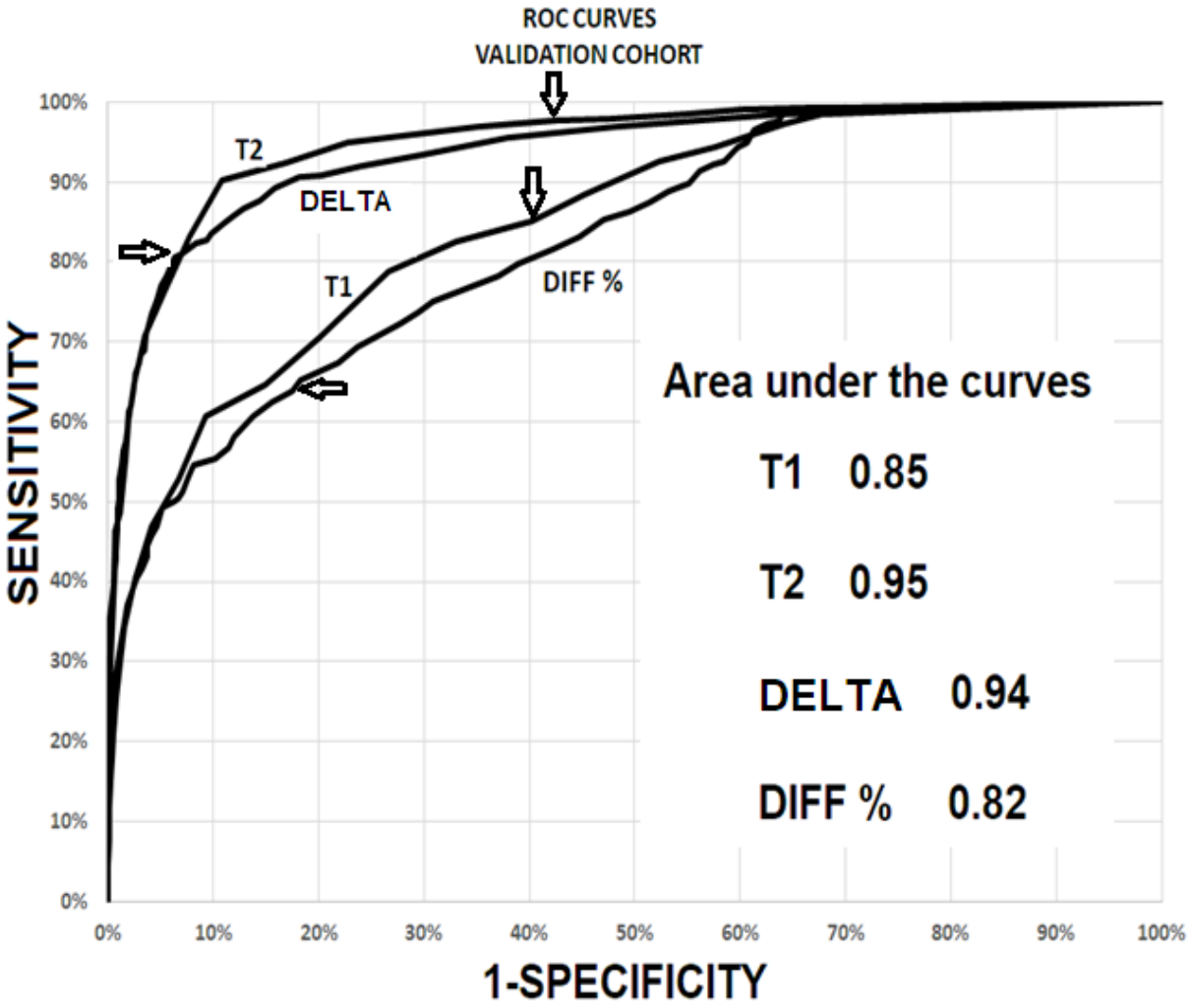
NB: The upper limit of the distribution is more stable across concentrations in the left panel in comparison with the right panel which shows progressive drop as concentration increases.
(N=810) Fig2a : y=0.125x+0.25 R=0.56 p<0.001 Fig2b: y=0.135x+11 R=0.088 p=0.013

Figure 3: Effect of sampling time interval on hs-TnT differences in the reference cohort by box plots.



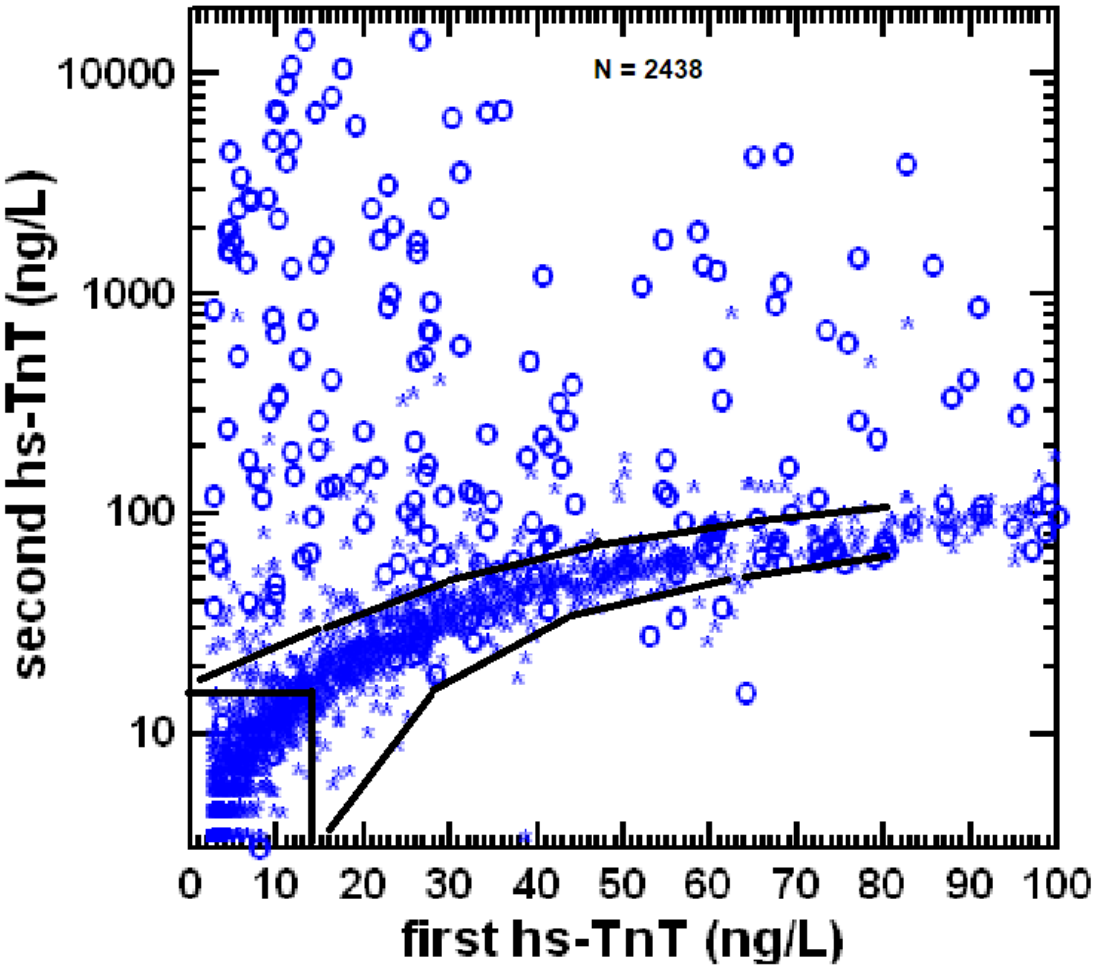
NB: Each box represents the 25th and 75th percentiles with the notches showing the confidence interval of the median. A significant difference between medians can be assumed when notches do not overlap such as between the first two columns. Outliers are represented as stars and extreme outliers as circles. Standard criteria to define these outliers are, respectively, any value extending more than 1.5 and 3 times the interquartile range from the median.

Figure 4: Receiver operating characteristic curve of 4 parameters for the diagnosis of MI in the validation cohort.



T1= first hs-TnT , T2=second hs-TnT, DELTA is the absolute difference in ng/L between T1 and T2, DIFF % is the absolute difference expressed in % of T1. Arrows indicate the coordinates position corresponding to the cut-off of 14 ng/L for T1 and T2 , 16 ng/L for DELTA and 30% for DIFF%.

Figure 5: Representation of the first hs-TnT and the second hs-TnT with reference range lines in the validation cohort.



NB The scale for hs-TnT 1 is linear and limited to the range below 100 ng/L where most of the diagnostic challenges are. The y axis is in logarithmic format to show all second hs-TnT results. Straight lines indicate the 99th upper limit of 14 ng/L while the curve lines represent the delta of +/- 16 ng/L between hs-TnT 1 and 2. Data points outside these lines are considered rule-in for acute heart injury. (MI diagnosis shown as circles)