Impact of the implementation of highly sensitive cardiac troponin T assay in a university hospital setting*

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ABSTRACT

Troponin is the gold standard for diagnosing myocardial necrosis. Roche Diagnostics has recently replaced the fourth-generation troponin T (TnT) assay with a new highly sensitive method (hsTnT). In our 600-beds university hospital, we replaced TnT (cut-off 0.03 µg/L) with hsTnT (cut-off 15 ng/L). After three months, we audited the impact of hsTnT by comparing data with the same period one year before. After hsTnT implementation, a 5.4% increase of troponin tests was recorded. A positive result was found in 31.7% of TnT and 58.7% of hsTnT (+85%), corresponding to 22.2% and 47.0% positive patients, respectively (P <0.0001). 64% of hsTnT positive results fell in the 16-65 ng/L range, determined as negative with TnT. Considering troponin curves, 39.1% for TnT and 69.0% for hsTnT had at least one result positive (P <0.0001). When the positive curves were classified as typical/atypical according to the reference change value, the difference in percentage of positive curves displaying a typical marker release became not significant (17.2% for TnT vs. 20.5% for hsTnT, P = 0.32). Using hsTnT there was an increase (+85%) in patients with positive troponin who were hospitalized, but also the rate of discharged positive patients increased (from 8.5% to 26.6%). The introduction of hsTnT markedly increased the number of positive tests. However, in interpreting positive results, the evaluation of marker release may keep specificity at the same level of TnT. The triage of hsTnT positive patients in Emergency Department showed that biomarker results are only one of factors considered for clinical decisions.

INTRODUCTION

The document issued in 2007 by the Task Force for the Redefinition of Myocardial Infarction (MI) and the complementary Laboratory Medicine Practice Guidelines of the National Academy of Clinical Biochemistry (NACB) have strengthened the role of cardiac troponin (I or T) increase as the key criterion for acute MI diagnosis, when an appropriate clinical and/or instrumental situation is present (1, 2). Both documents agree on defining myocardial necrosis as an elevation of troponin values exceeding the upper reference limit (URL) of the cardio-healthy population, set at the 99th percentile of the distribution (99th URL) to minimize false positive results. Given the cardio-specificity of troponins, any marker concentration above this limit theoretically mirrors a cardiac injury, worsening the patient outcome with increased risk of new adverse cardiac events. Until recently, most commercial assays for cardiac troponin determination have, however, shown suboptimal analytical sensitivity to consistently measure cardiac troponins in the blood of apparently healthy individuals and accurately derive 99th URL (3, 4).

As accurate discrimination between "minor" myocardial injuries vs. analytical noise requires assays that have lower limit of detection and display higher precision in measuring low troponin concentrations in blood, the new generation of assays enhances the analytical performance at this level in order to permit the clinical application of international recommendations (5). However, if from one hand this results in markedly improved assay sensitivity for detecting troponin elevations, on the other hand this also increases the number of positive troponin results not related to acute coronary syndrome (ACS) (6). Several papers have recently described this scenario (7-12).

A new highly sensitive troponin T assay (hsTnT) manufactured by Roche Diagnostics has recently become available and it has been evaluated in analytical and clinical pilot studies (10-14). We also had the

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opportunity to comprehensively validate the performance characteristics of the hsTnT in a multicenter international evaluation carried out before its release on the market (15). As in our experience hsTnT clearly exhibited improved analytical performance over the fourth-generation troponin T assay (TnT), a decision was taken in our institution during 2009 for replacing TnT with the new test once it was made commercially available. After three months from the hsTnT introduction in clinical practice, we performed a comprehensive audit on the impact of this implementation on test volumes, troponin positive rates, and interpretation of results. Here we present and discuss in detail results of this audit.

MATERIALS AND METHODS

Methods

Effective July 2009, hsTnT was introduced in our emergency laboratory serving a 600-beds university metropolitan hospital in Milano. The new assay was implemented on the Cobas e 411 platform (Roche Diagnostics) and fully replaced TnT performed on the Elecsys 2010 analyzer (also from Roche). Both systems are fully automated immunoassay analyzers using electrochemiluminescent technique for signal detection. hsTnT utilizes the same antibodies used for TnT, the detection antibody being, however, genetically reengineered to reduce the interference by human anti-mouse IgG antibodies (5). Analytical sensitivity of hsTnT was improved by increasing the sample volume, increasing the ruthenium concentration of the detection antibody, and lowering the background signal by buffer optimization, giving a detection limit of 5 ng/L (14, 15). During the pre-market evaluation of hsTnT (15), we determined the 99th URL in our reference population at 15 ng/L and this concentration was then selected as decision limit once the assay was implemented. The previously used cut-off for TnT was 0.03 µg/L (30 ng/L) (16, 17). Although comparison between TnT and hsTnT over the whole measuring range was good, a limited comparability was observed at troponin T concentrations <100 ng/L (<0.10 µg/L) (14, 15). Particularly, in the data set obtained in our laboratory during the multicenter study (15), we established that on average a 0.03 µg/L (30 ng/L) TnT concentration was measured ~65 ng/L with the hsTnT. Lacking the comparability between the two assays in the clinically relevant concentration range, we decided to express results for hsTnT in nanogram per liter, instead of the traditional units, i.e. µg/L, previously used for TnT, to discourage direct comparisons between old and new assay values. Imprecision of both TnT and hsTnT was monitored during the study periods by the IQC performed daily on fresh-frozen serum pools (16): results showed an average CV of 9.1% for TnT (cumulative mean, 39 ng/L) and an average CV of 8.5% for hsTnT (cumulative mean, 17 ng/L), respectively.

Patient data

Using the laboratory information system, we retrieved all hsTnT results for the first 3-month period following hsTnT implementation (July 16th, 2009 to October 15th, 2009) and all TnT results from the same 3-month period one year before. The number of patients who had cardiac troponin testing during their hospital examination was also estimated. We considered as part of the same examination troponin test ordered within ≤24 h interval between consecutive samples. Accordingly, some patients underwent more than one examination, even during the same hospitalization. All data were split into different hospital wards; when patients were first admitted to the Emergency Department (ED) and then moved to other clinical wards, the troponin orders were split according to the requiring ward and the examination was assigned to the ED. To evaluate the impact of hsTnT on the ED triage of patients, we retrieved data regarding hospitalization/discharge of troponin positive patients examined in the two evaluated periods and compared them.

Criteria for interpretation of troponin test

As said before, the decision limits for myocardial damage were 15 ng/L for hsTnT and 0.03 µg/L for TnT, respectively. All results were dichotomized as positive or negative with respect to established cut-offs. Furthermore, among troponin positive patients with at least two results during their examination, we divided marker release curves on the basis of typical or atypical kinetic. We defined as 'typical' a rising and/or falling pattern showing a troponin variation between two consecutive samples exceeding +46% for increasing and -32% for decreasing troponin results. Otherwise the troponin pattern was considered as 'atypical'. Although Vasile et al. (18) have recently tried to assess biological variability of troponin T to derive its reference change value (RCV), their data were implausible as the majority of hsTnT results in selected individuals used for calculation were lower than the assay detection limit. So that, for definition of the above reported RCV we referred to data published by Wu et al. (19) for the troponin I short-term monitoring, considering that the analytical CV obtained from our IQC program (see above) was quite similar to that used in the original paper for RCV derivation. Finally, among typical positive rising patterns with at least 3 results obtained during the first 24 h after admission, we checked the ability of hsTnT to become positive earlier when compared to TnT, by evaluating how many curves became positive on the first, second and third sample using the two assays.

Statistical analysis

All statistical evaluations were performed using the SigmaStat program, version 1.0 (Jandel Scientific). The one-way ANOVA was utilized to compare population distributions and the chi-square test was used to compare observed frequencies in different populations. A P value <0.05 was considered statistically significant.

RESULTS

In the 3-month period evaluated, a total of 2287 hsTnT tests during 1371 hospital examinations in 1137 patients were carried out. The corresponding data for TnT were 2170 tests during 1409 examinations in 1205 patients, respectively. Therefore, after hsTnT implementation a 5.4% increase of the hospital-wide volume of troponin tests was recorded, even if the number of admitted patients (-5.6%) and examinations (-2.7%) was slightly decreased. This gives a mean of 2.0 hsTnT tests vs. 1.8 TnT tests per patient, respectively. In the two evaluated periods, in the ED the same number of tests (1472 TnT vs. 1465 hsTnT) was spread on a slightly different number of patients (from 1019 to 958, -6.0%) and examinations (from 1089 to 1011, -7.2%). The second greatest amount of troponin orders among hospital wards was in charge of the Internal Medicine Department (184 TnT vs. 293 hsTnT), with a 59% increase when hsTnT was introduced. This largely accounts for the total increase in the hospital-wide test volume.

Over the three months audited, 145 (12.0%) patients before and 163 (14.3%) after hsTnT implementation had more than one examination, the difference being not significant (P = 0.12). As expected, this happened more frequently in clinical wards (13.9% for TnT and 20.7% for hsTnT, respectively) than in ED (11.7% for TnT and 13.1% for hsTnT, respectively). The number of troponin tests per examination averaged (±SD) 1.54 (±1.0) before and 1.67 (±1.1) after hsTnT implementation (P <0.0001), with a single test ordered in 951 (67.5%) and 825 (60.2%) examinations, respectively. In the remaining examinations, troponin was ordered at least twice. The frequency of the number of troponin tests ordered for each examination in the two evaluated periods is reported in Figure 1 that shows the greater tendency to repeat the test after hsTnT implementation.

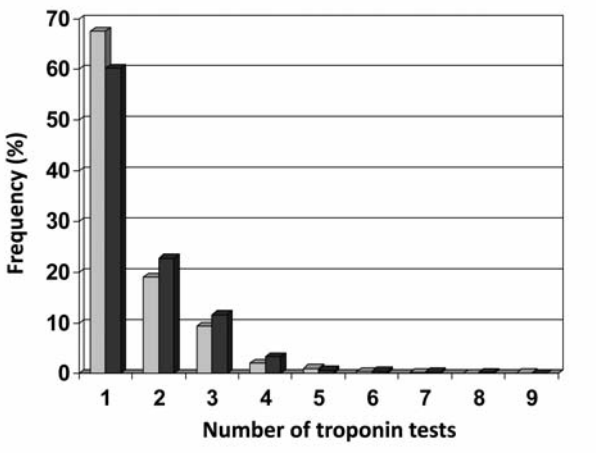

Figure 1
Number of troponin tests ordered per examination in the two evaluated periods. Pale and dark grey histograms indicate the number of fourth-generation troponin T (TnT) and highly sensitive troponin T (hsTnT) tests, respectively. The increasing trend in repeating the test after hsTnT implementation was statistically significant (P <0.05).

A positive result was found in 31.7% of TnT tests and 58.7% of hsTnT tests (relative difference, +85%), corresponding to 25.3% and 51.6% positive examinations, respectively (P <0.0001). Particularly, in ED 18.5% TnT and 45.3% hsTnT positive results (relative increase, +145%), corresponding to 196 (18.0%) and 434 (42.9%) examinations, were respectively audited (P <0.0001). Among positive examinations in ED, we were able to retrieve 187 (95.4%) and 410 (94.5%) clinical reports to evaluate the patient triage. After hsTnT implementation, the number of hospitalized patients with positive troponin increased from 158 to 292 (+85%), but the rate of admission in intensive and non intensive care departments was unchanged (P = 0.11). In the same periods, 16 (8.5%) TnT and 109 (26.6%) hsTnT positive patients were discharged. Among those, one TnT positive and 13 hsTnT positive patients were readmitted to the ED in the following two months (P = 0.80 between the two assays). Among subjects with elevated hsTnT who returned to the ED, 7 were discharged again, 4 were hospitalized in non intensive (Medicine) ward, and only one was admitted to the Intensive Care Unit for acute dispnea and suspected heart failure. The remaining patient refused hospitalization.

Among TnT negative results, 52.7% were <0.01 µg/L, i.e. the assay detection limit, and 15.6% were between the detection limit and the adopted cut-off (0.03 µg/L). Using hsTnT, the corresponding figures were 15.5% (results below the detection limit) and 25.8% (results between 5 and 15 ng/L). Among all hsTnT positive results (n=1342), 857 (64%) were in the range between 16 ng/L and 65 ng/L, and the remaining 485 (36%) were >65 ng/L, the hsTnT concentration that approximately corresponded to the TnT cut-off concentration (0.03 µg/L) (see Methods). Therefore, about 2/3 of hsTnT positive results fell in the 16-65 ng/L concentration range, previously determined as negative with TnT.

We audited 458 TnT and 546 hsTnT curves (i.e. at least two marker results during patient examination), among which 179 (39.1%) and 377 (69.0%) had at least one positive result, respectively (P <0.0001). The positive curves were then classified as typical/atypical according to the marker release kinetic and the established RCV. Quite surprisingly, the difference in percentage of positive curves with the two assays displaying a marker release typical for an acute cardiac event became statistically not significant (17.2% of TnT curves and 20.5% of hsTnT curves, respectively; P = 0.32) (Figure 2). On the other hand, a higher absolute number of typical positive curves was shown after hsTnT implementation (from 79 to 112). This increased ability in detecting events involving acute marker release was a consequence of the increased sensitivity of hsTnT, being fully explained by the number of typically positive curves in which hsTnT never exceeded 65 ng/L (n=38).

Over 72 typical positive raising hsTnT patterns with at least 3 troponin results, 87.5% showed a marker elevation already on the first sample and the remaining

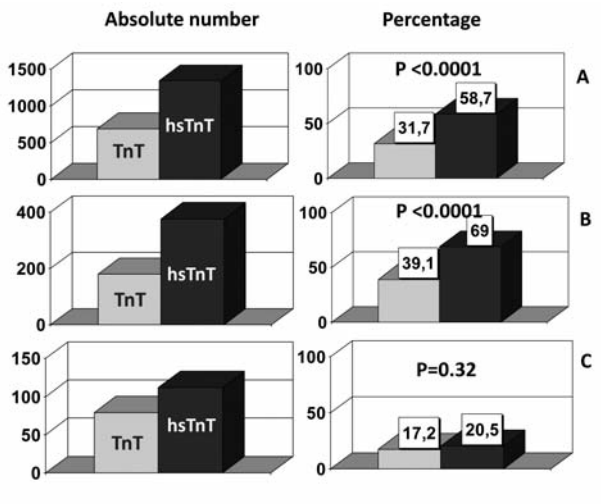

Figure 2
Absolute number and percentage of (A) positive individual troponin results, (B) positive troponin curves (i.e. at least one result positive during patient examination), and (C) positive troponin curves with typical marker release (>reference change value).
TnT, fourth-generation troponin T assay; hsTnT, highly sensitive troponin T assay.

12.5% became positive on the second sample in a time ranging from 3 to 10 h (median, 6 h) after collection of the first one. By assaying TnT in the comparative three-month period, only 32 (60.4%) out of 53 typical raising curves were positive on the first sample, 17 (32.1%) became positive on the second, and 4 (7.5%) on the third one. The difference in sensitivity between TnT and hsTnT at different sampling points was statistically significant (P <0.001).

DISCUSSION

In this study we performed a comprehensive audit on the impact of hsTnT use during the first three months after its implementation in our university hospital. This was done by comparing data with those of the same period of the year before when the previous generation of troponin T assay was used. As no important changes in the hospital organization occurred and, consequently, no variations in the belonging case-mix between the two periods were expected, we considered this approach the best way to obtain the desired information. The stability and, consequently, the comparability of the clinical setting in the two evaluated periods were indeed confirmed by the similar number of admitted patients and of corresponding examinations requiring troponin test.

In our experience the replacement of TnT with hsTnT markedly increased the rate of troponin positive test. This outcome was largely expected given the enhanced analytical sensitivity of hsTnT over TnT previously demonstrated in single-centre and multicenter evaluations (14, 15, 20). A similar outcome was also described in a study reporting the impact of implementation of a new generation cardiac troponin I assay (8). What is unique here is the information on the magnitude of the increase of positive results after hsTnT introduction, as this is, in our knowledge, the first experience implementing the new assay in a routine protocol. Practically, the number of positive examinations increased from approximately ¼ to more than half of total. The number of troponin tests per examination also increased reflecting, at least partly, the need to confirm by serial testing positive hsTnT results on the first sample.

The marked increase of hsTnT positive rate was quite exclusively due to the results falling into the 16-65 ng/L range that could not be quantitatively determined with acceptable sensitivity and uncertainty by TnT. In other words, hsTnT test results in the 16-65 ng/L range likely represent the portion of low-positive troponin values previously missed with TnT, for which a higher conservative cut-off based on the actual assay performance (10% CV concentration) was employed (16, 17). Indeed, previous population studies using a lower TnT cut-off, i.e. the assay detection limit, already demonstrated a significant improvement in sensitivity for detecting adverse outcome in chest pain patients (21). However, the application of this lower cut-off for TnT in everyday practice was not recommended given the associated high variability of measurement.

It has been highlighted that the high sensitivity of hsTnT could sometimes appear as a confounding factor making the test less specific for the diagnosis of acute MI (22). However, we experimentally showed that there was no difference in percentage of curves with typical marker release when TnT and hsTnT were compared, showing that the use of an interpretative approach based on the demonstration of a pathophysiologically plausible acute release of troponin in blood can permit to keep the same test performance in terms of specificity when using different generation of troponin T assays. This means that implementation of hsTnT makes critical to interpret test results in the context of serial samples comparing percent concentration changes to the RCV in order to identify typical hsTnT curves (23). Estimate of biological variation of the analyte allows determination of RCV that includes the analytical imprecision and the intraindividual biological variability. Lacking robust data on the biological variability of troponin T, we applied data published by Wu et al. for troponin I (19). We are aware that the two cardiac troponins may have different biological release and clearance in blood, so that their biological variation may be different. However, our data show a RCV >+46% for increasing and >-32% for decreasing marker concentrations, when added to a hsTnT value >15 ng/L (99th URL), improves the specificity of hsTnT, keeping unchanged its higher sensitivity for detection of myocardial injury. We also showed that two measurements of hsTnT are probably enough, the first being at patient admission or, if hospitalized, at time of symptom onset and the second approximately 6 h later. The exact time sampling should, however, be defined in specifically designed studies (24, 25).

The major problem in the practical application of the RCV approach is, however, that the majority (in our hospital ~60%) of troponin tests are ordered just one time without any follow up of biomarker concentrations. This situation, also reported in previous audits (26, 27), clearly needs a strong educational effort to be improved (28, 29). It should be noted that, after hsTnT implementation, in our study over all examinations the percentage of cases with a single test ordered decreased by 10.8%. Hopefully, with greater experience in managing hsTnT results, more than one test will be requested to every patient before deriving any clinical hypothesis. On the other hand, also in the patients evaluated on the basis of consistent marker release curves (independent of the generation of the assay employed) one should be careful about the interpretation of results that can not always easily be reported to a univocal diagnostic criterion (acute MI/no acute MI). In our experience (data not reported), by comparing typical (rising and/or falling) and atypical troponin curves to the assigned final diagnoses, we found that serial changes >RCV could not be a perfect indicator of acute MI, both for the presence of MI also in some patients showing atypical curves and for the well-known possibility that other acute cardiac diseases (e.g., pulmonary embolism or myocarditis) display a typical marker release. In the majority of MI cases with atypical pattern, however, troponin concentrations greatly exceed the established cut-off and then become pathognomonic for MI per se. Using lower percentage changes in defining typical curves (for instance, expert opinion offered by the NACB Practice Guideline suggested a 20% change between two time points as indicative of a real change (2)) may give greater sensitivity, but more false positive may also occur. We are, however, convinced that the best scientific approach of defining significant troponin serial changes should rely on the widely accepted RCV criterion based on analytical and biological variation (30).

Comparing the ED triage of hsTnT positive patients with that of TnT positive patients admitted in the same period one year before, we found that, after hsTnT implementation, the absolute number of hospitalized patients with positive troponin significantly increased, even if the rate of admission in intensive and non intensive care wards was unchanged. On the other hand, about ¼ of hsTnT positive patients were discharged and, importantly, in the follow-up their rate of readmission did not increase when compared with the TnT period. The available data do not permit estimation of the final economic impact when balancing the cost of the increased number of hospitalised subjects with the improved ability to early detect acute cardiac events; recently published results however show that the implementation of a more sensitive troponin assay can be associated with major reductions in morbidity and mortality (31).

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