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Integration between the Tele-Cardiology Unit and the central laboratory: methodological and clinical evaluation of point-of-care testing cardiac marker in the ambulance

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Abstract

The aim of this study was to identify patients with myocardial necrosis in pre-hospital phase during transport by ambulance, without ST-segment elevation (NSTE) on the ambulance ECG. The analytical performance of the i-STAT® troponin I (cTnI) method was assessed. A total of 53 NSTE ambulance ECG patients admitted to hospital were followed. The ambulance had experimental software able to receive data from the i-STAT device and transmit it to a protected address and server. cTnI mean values from 2.0 to 34 μg/L showed a total CV of 3.0–5.6%. The detection limit was 0.016 μg/L. A mean cTnI concentration of 0.09 μg/L was associated with a CV of 8.0% (decision limit). The i-STAT cTnI method was linear for concentrations from 0 to 35 μg/L. There was no effect ($p<0.05$) of interfering substances. For point-of-care testing (POCT), cTnI was >0.09 μg/L in 20 AMI patients (91%). The median ambulance turnaround time (TAT) was 12 min and median hospital TAT was 40 min, a difference of 28 min. The high sensitivity of the i-STAT cTnI method integrated with tele-medicine procedures could play an important role in the management of acute coronary syndrome patients related to the pre-hospital phase (early diagnosis and treatment in the ambulance). These approaches may allow improvements in patient outcomes and continuous monitoring of the POCT network in the central laboratory, thus meeting quality requirements.

Keywords: acute myocardial infarction; ambulance; Emergency Department; point-of-care testing (POCT); troponin I; turnaround time (TAT).

Introduction

In chest pain patients, the diagnosis of acute myocardial infarction (AMI) is established early in cases with ST elevation (STEMI) in the electrocardiogram (ECG), whereas in patients for whom no ST elevation (NSTE) is recorded in the ECG, confirmation of AMI is mainly dependent on serial testing of biochemical markers of myocardial necrosis (1). In particular, cardiac troponins have emerged as powerful tools for rule-in/rule-out AMI and risk assessment of recurrent cardiac events, including death and recurrent ischemia (2). Accurate early risk stratification of patients arriving at the Emergency Department (ED) with chest pain is important because it offers the advantage of early initiation of treatments aimed at improving the outcome in patients found to have acute coronary syndrome (ACS). To achieve quick assessments of chest pain in the ED, a short turnaround time (TAT) is necessary, for which clinical practice guidelines have been published. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (3) and the National Academy of Clinical Biochemistry (NACB) (4) recommended a 60-min TAT from the collection of blood to the reporting of results; the American Heart Association (AHA)/American College of Cardiology (ACC) (2) stated that a 30-min TAT was preferable. Point-of-care testing (POCT) was developed to reduce pre-analytical TAT (time for specimen transport) and, as patient outcomes are the goal for POCT, Total Quality Management principles must be incorporated into the management of POCT. In this setting we have implemented Stratus CS as POCT cardiac markers in the Emergency Cardiology Department (ECD) (5). Moreover, the management of patients with chest pain could be improved if therapeutic decision-making takes place before arrival at the hospital (6). In recent years, an increasing percentage of ambulance organizations use a 12-lead ECG. In our region, an average of 440 ambulance calls are recorded each month for patients with chest pain. The ECG performed en route to the hospital is transmitted to the Tele-Cardiology Unit; in the case of STEMI patients (18%), cardiologists are able to immediately diagnose AMI and give instructions on treatment (thrombolysis, aspirin, heparin) and the admission to the nearest Intensive Care Unit (ICU). In the case of NSTE ambulance ECG patients (82%), the decision is delayed until arrival at the ED and subsequent measurement of cardiac markers. In these cases, the measurement of cardiac troponin I (cTnI) in the pre-hospital phase might be of value.

The current study aimed to evaluate the possibility of performing cTnI POCT in the ambulance, in agreement with IFCC quality specifications. The analytical performance of the cTnI method was assessed in the central laboratory using both a lithium-heparin plas-

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ma pool and heparinized whole blood samples. Staff training and clinical evaluation of the POCT system were carried out in the ambulance. As information connectivity is a crucial component of a successfully planned and implemented POCT program, experimental software for remote control in the central laboratory related to troponin I results and the status of the device in ambulances was used. The software is able to receive data from the i-STAT device and transmit it via general packet radio system (GPRS) to a protected address and server; the central laboratory and the main Tele-Cardiology Unit both receive analytical data for troponin I online via an Internet connection. The central laboratory supervises the overall processes.

Materials and methods

Assay method

We identified the i-STAT® 1 portable clinical analyzer (i-STAT Corporation, Windsor, NJ, USA) as being suitable for cTnI POCT in the ambulance. Each i-STAT cartridge is provided with sensors to detect cTnI, as well as all the reagents necessary to perform the test on whole blood samples with lithium heparin or plasma. The i-STAT cTnI method utilizes basic ELISA principles, involving monoclonal capture, antibody/antigen bound to a silicon chip surface, and polyclonal label antibody/alkaline phosphate, both directed to the central 30–100 amino acids of cTnI (7). Calibration coefficients are established at the manufacturer's site for each lot; the cTnI values assigned to the control materials for calibration of the i-STAT system are traceable and conform to the i-STAT calibration solution prepared using the full troponin complex (ITC) (Hy Test Ltd., Turku, Finland). As stated by the manufacturer, the total coefficient of variation (CV) ranges from 7.6% to 8.5% for cTnI mean concentrations from 0.53 to 31.8 μg/L; the linear range for the master curve is 0–35 μg/L.

Analytical performance of i-STAT cTnI method

The i-STAT cTnI method was evaluated according to IFCC guidelines (8).

Limit of detection (LOD) and precision studies The detection limit or analytical sensitivity of the i-STAT cTnI method was calculated as a signal of 3×SD added to the mean response for 20 measurements of a lithium-heparin plasma pool from samples of apparently healthy subjects who were free of troponin I when tested with the Stratus CS assay (Dade Behring, Glasgow, DE, USA). The LOD for the Stratus CS cTnI assay is 0.015 μg/L (5). The precision study was carried out based on NCCLS guideline EP5-A (9). Two commercial control samples produced by the manufacturer (i-STAT levels 2 and 3, cTnI mean concentrations 2.0 and 34 μg/L) and a lithium-heparin plasma pool (cTnI mean concentration 9.8 μg/L), obtained from routine samples, were tested at a frequency of two runs/day on 20 consecutive days. To define the precision of the i-STAT method in the low cTnI range, a lithium-heparin plasma pool with a cTnI concentration of 0.3 μg/L was diluted manually with a sample free of cTnI when tested by the Stratus CS method, until an expected concentration of cTnI equal to zero was achieved. Ten plasma pools were obtained, which were aliquoted, frozen at −20°C and analyzed (after defrosting and centrifugation) in duplicate on 20 consecutive days (10 consecutive working days per lot). For precision studies, two different reagent lots were used.

Linearity of dilution The linearity of the test was determined in both lithium-heparin plasma and heparinized whole blood pools with a cTnI concentration close to the upper linearity limit (35 μg/L according to the manufacturer). Pools were manually diluted at a ratio of 3:1, 2:2 and 1:3 using samples without cTnI by Stratus CS assay: five plasma pools and five whole blood pools with different cTn I concentrations were tested in quadruplicate in one single run. The linearity of the assay in different sample types was assessed in compliance with NCCLS guideline EP6-P (10).

Effect of interfering substances on the accuracy of cTnI results According to NCCLS EP7-P (11), the in vitro interfering effect of bilirubinemia and lipemia on the accuracy of i-STAT cTnI results was investigated based on two spiked lithium-heparin samples with a cTnI concentration of 1.5 and 8.6 μg/l, using the same approach described previously (5). The analytical interference of unconjugated bilirubin (20 mg/dL, 342 mmol/L; bilirubin mixed isomer, Sigma-Aldrich, St. Louis, MO, USA) and triglycerides (3000 mg/dL, 33.87 mmol/L; Intralipid 20% emulsion, Sigma-Aldrich) on the troponin I results was calculated using the paired-difference approach.

Method comparison with Stratus CS and Dimension RxL The agreement between methods was assessed in compliance with NCCLS guidelines (12). For comparison of the i-STAT and Stratus CS cTnI methods, 70 blood samples were collected in PET tubes with 68 IU of lithium-heparin for 4 mL of blood. For comparison of the i-STAT and Dimension RxL cTnI methods, since serum should be the matrix of choice for the RxL, agreement was assessed for cTnI concentrations between 35 paired randomized blood samples of lithium-heparin (collected as described above) and serum (in PET tubes with a silicone gel barrier; Becton Dickinson, Franklin Lakes, NJ, USA). All samples were centrifuged for 10 min at 4°C (lithiumheparin samples at 2500 rpm, 1250×*g*; serum samples at 3000 rpm, 1800×*g*) and analyzed in duplicate on both analyzers.

Preliminary clinical evaluation of the POCT: reference subjects, patients, TAT

To define the cTnI upper reference limit, 100 lithium-heparin plasma samples were collected from healthy Caucasian subjects: 63 men (aged 20–75; average 52±14 years) and 37 women (aged 24–65; average 56±11 years). The subjects' good state of health was determined using a personal interview as a report; patients with a history of cardiac diseases or with alterations of hematochemical parameters (S-creatinine, S-glucose, S-urea, alanine transferase, aspartate aminotransferase, C-reactive protein, CK-MB isoenzyme, leukocyte blood count, hemoglobin) were excluded. To evaluate the accuracy of the i-STAT cTnI method for early differential diagnosis of AMI in the pre-hospital phase, 53 NSTE ambulance ECG patients (34 men, 64±13 years; 19 women, 74±19 years) admitted to hospital were followed. The mean duration of symptoms was 2.0±1.1 h. Blood samples from chest pain patients were taken at the time of admission to the ambulance and the appropriateness of the i-STAT cTnI results was assessed in relation to the final diagnosis of AMI, according to European Society of Cardiology (ESC)/ACC diagnostic criteria.

Statistical analysis

The total imprecision (CV%) for the cTnI method was determined using the analysis of variance (ANOVA) method. Total CV values determined for each pool were used to construct the imprecision profile and the lowest cTnI concentrations corresponding to 10% CV were identified from the intercept of the total CV (y-axis). Linearity on dilution was determined by least-squares linear regression analysis with the lack-of-fit test (10). A paired t-test was used to assess statistical significance in the interference studies. Comparison between the i-STAT and Stratus CS and Dimension RxL cTnI methods was evaluated by Deming regression; Bland-Altman analysis was used to assess the agreement. Statistical analysis was carried out using Analyse-it Software for Microsoft EXCEL version 1.71 (Analyse-it Software Ltd., Leeds, UK).

Results

LOD and precision studies

The mean (SD) cTnI concentration of 20 pool measurements without troponin I by the Stratus CS assay was 0.0015 (0.005) μg/L and the i-STAT method LOD, calculated as 3×SD added to the mean response of the 20 measurements, was 0.016 μg/L. Data from the precision study were as follows: commercial control sample level 2: mean, 2.0 μg/L; within-run CV, 2.6%; total CV, 3.7%; commercial control 3: mean, 34 μg/L; within run CV, 2.2%; total CV, 3.0%; plasma pool: mean, 9.8 μg/L; within-run CV, 3.6%; total CV, 5.6%. The total troponin imprecision at different low concentrations was determined by ANOVA method: a mean cTnI concentration of 0.09 μg/L (95% CI, 0.085 to 0.099) was associated with a CV of 8.0% (decision limit). Figure 1 shows the imprecision profile at low cTnI concentrations.

Linearity of dilution The method was linear for concentrations from 0 to 35 μg/L. Regression analysis (least-squares linear regression) and the lack-of-fit test showed the following results:

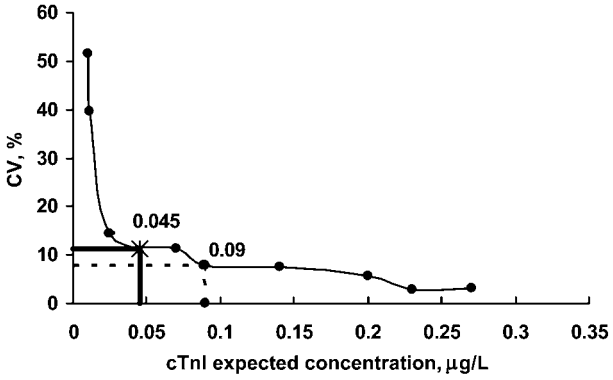

Figure 1 Precision profile of the i-STAT cTnI method at low concentrations. A plot of cTnI values (x-axis) vs. total imprecision as coefficient of variation (CV, %; y-axis) is shown. The dashed lines show the value of cTnI concentration (0.09 μg/L) with total CV of 8.0%; the thick lines show the value of cTnI concentration for the 99 percentile reference (0.045 μg/L) with a total CV of 11%.

Lithium-heparin plasma pool: $y=0.8286\ (\pm 0.003)\ x-0.0394\ (\pm 0.082)$; $r^2=1$; $F=0.19$; $p=0.9$.
Whole blood: $y=0.8709\ (\pm 0.027)x+0.3376\ (\pm 0.58)$; $r^2=0.98$; $F=1.83$; $p=0.18$.

Effect of interfering substances on accuracy of cTnI results No significant interference ($p>0.05$) was observed for cTnI concentrations in icteric (up to 20 mg/dL, 342 mmol/L unconjugated bilirubin) or lipemic (up to 3000 mg/dL, 33.87 mmol/L triglycerides) samples. The absolute and percentage mean differences in cTnI results between samples with and without added interfering substances were as follows:

Icteric samples:

1. cTnI concentration 1.5 μg/L: absolute mean difference, −0.025 μg/L (95% CI −0.053 to 0.003); percentage mean difference, −1.7% (95% CI −3.6 to 0.14)
2. cTnI concentration 8.6 μg/L: absolute mean difference, −0.2 μg/L (95% CI −0.7 to 0.2); percentage mean difference, −0.6% (95% CI −8.9 to 2.9)

Lipemic samples:

1. cTnI concentration 1.5 μg/L: absolute mean difference, 0.03 μg/L (95% CI −0.004 to 0.06); percentage mean difference, 1.96% (95% CI −0.2 to 4.1)
2. cTnI concentration 8.6 μg/L: absolute mean difference, 0.035 μg/L (95% CI −1.0 to 1.0); percentage mean difference, 0.6% (95% CI −12.3 to 13.5)

Method comparison between Stratus CS and Dimension RxL Agreement between the Stratus CS and the Dimension RxL cTnI methods was evaluated by Deming regression (Figure 2A) and Bland-Altman analysis (Figure 2B).

1. i-STAT vs. Stratus CS method (n=70 paired lithium-heparin samples; cTnI concentrations from 0.02 to 38.5 μg/L): i-STAT=1.045 (±0.051) Stratus CS–0.7553 (±0.45); Sy/x=2.0; absolute bias −0.5 (95% CI −0.2 to 0.1); percentage bias −10 (95% CI −22.7 to 1.8).
2. i-STAT vs. Dimension RxL (n=35 paired lithium-heparin and serum samples; cTnI concentrations from 0.02 to 8.0 μg/L): i-STAT=0.78 (±0.011) Dimension RxL–0.098 (±0.03); Sy/x=0.11; absolute bias −0.3 (95% CI −0.5 to −0.1); percentage bias −34 (95% CI −62.2 to −6.5).

Preliminary clinical evaluation of the POCT: reference subjects, patients, TAT

The 99th percentile for 100 reference subjects was 0.045 μg/L; 10% of samples showed measurable cTnI concentrations (i.e., >0.016 μg/L, assay LOD). This reference cTnI concentration was associated with a total CV of 11% (Figure 1). A total of 53 NSTE ambulance ECG patients admitted to hospital were followed. In all patients, cTnI was measured by POCT in ambulance. The blood samples collected upon arrival in the ED and after admission to hospital for more

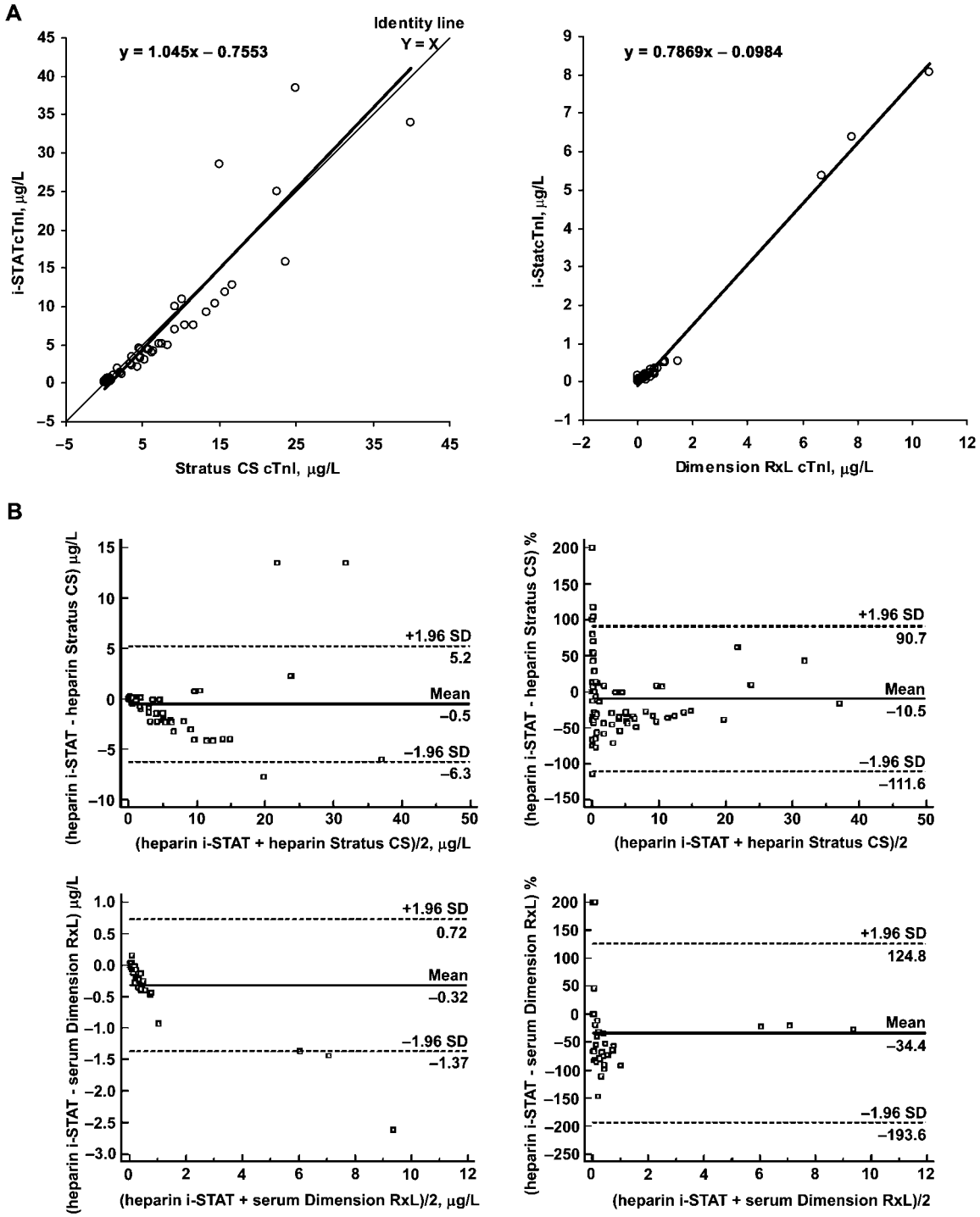

Figure 2 Method comparison (A) and Bland-Altman difference plot (B) for i-STAT vs. Stratus and Dimension RxL. (A) Deming method for comparison between i-STAT and Stratus CS (n = 100; left panel) and between i-STAT and Dimension RxL (serum samples) (n = 35; right panel). Values shown are the slope and intercept of the regression analysis, as well as the regression line (black line) and identity line (gray line). (B) Absolute results (left panels), percentage bias (right panels) and 95% limits of agreement of bias (dashed lines). Upper panels: bias plots between i-STAT and Stratus CS, n = 100. Lower panels: bias plots between i-STAT (lithium heparin whole blood samples) and Dimension RxL (serum samples), n = 35.

than 24 h were sent to the central laboratory for analysis by the cTnI Dimension RxL method. According to ESC/ACC criteria, 22 patients (41.5%) received a final AMI diagnosis. Of these AMI patients, the i-STAT cTnI concentration measured in the ambulance was >0.09 μg/L (decision limit for myocardial damage) in 20 patients (91%); the mean (SD) cTnI concentration was 1.23 (2.35) μg/L. For non-AMI patients (n = 31, 58%), the i-STAT cTnI concentration was <0.09 μg/L in 27 patients (87%). Data on comparison of the TAT (arm to report) are shown in Figure 3. From the ambulance, cTnI results were sent to the Tele-Cardiology Unit in 12 min (median; 95% CI, 11.0 to 13.0), while in the ED, Dimension RxL cTnI results were available from the central laboratory in 40 min (median; 95% CI, 40 to 45) after the arrival of the patient; the differ-

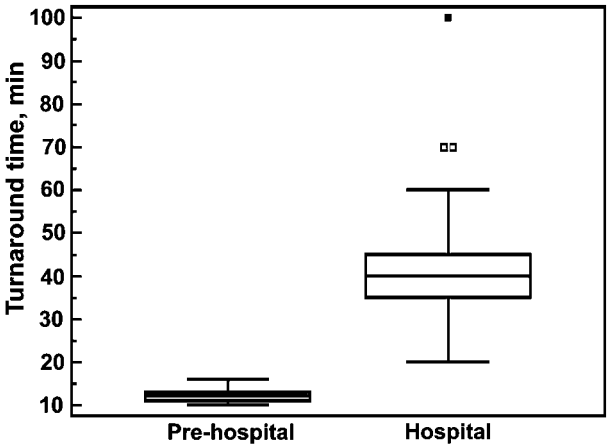

Figure 3 Turnaround time (TAT) for cTnI: pre-hospital vs. within-hospital phase. The box represents the values from the lower to the upper quartile (25 to 75th percentile); the middle line represents the median; the vertical lines extend from the minimum to the maximum value, excluding outlier values, which are displayed as separate empty squares, and an extreme outlier value, which is displayed as a separate black square.

ence between the TAT medians was statistically significant (28 min; 95% CI 26.0 to 31.5; $p < 0.0001$).

Discussion

A large percentage of patients who call for an ambulance have chest pain or other symptoms indicative of possible ACS. Patients with low or intermediate probability of AMI (non-diagnostic ECG at presentation) comprise the majority of acute chest-pain patients (in our area, 82%). An early rule-in/rule-out AMI decision in this group represents a huge challenge to the medical community. In a previous paper (5), we suggested that the introduction of a POCT system for cardiac markers in the ECD may be perfectly integrated with the clinical laboratory. Moreover, the use of a highly sensitive troponin method to recognize minimal cardiac damage could play an important role in the early identification of patients with AMI in a low- to intermediate-ACS-risk population admitted to the ECD. With the aim of implementing POCT for cardiac markers in the ambulance in compliance with quality requirements, we assessed the analytical performance of the i-STAT method for cTnI and conducted a preliminary clinical valuation in NSTE ECG ambulance patients with chest pain.

To validate the performance of the method, our study focused on analytical issues. The i-STAT cTnI method provides reproducible within- and between-run results; for the control levels and plasma pool tested, the total imprecision (CV) was <5.6%. The method showed linearity in the range from 0 to 35 μg/L. The analytical sensitivity was 0.016 μg/L and imprecision studies in the low range showed that mean cTnI concentrations of 0.09 μg/L were associated with CV <10%. The 99th percentile reference limit for whole blood was 0.045 μg/L and the imprecision (CV%) of the method at this cTnI reference concentration was 11%. The different reference limit (cTnI 0.08 μg/L) indicated by Apple et al. (7) could be correlated to the different characteristics of the population studied (race, age, sex, etc.), the different criteria used to select individuals, and the sample size. Interference studies confirmed that no significant interference on the accuracy of the results was correlated to presence of the unconjugated bilirubin and triglycerides in lithium-heparin whole blood samples. Nevertheless, the use of Intralipid does not mimic the effect of either triglycerides or lipoproteins, so further studies should be carried out to assess interference effects in samples from patients with hyperlipidemia. Comparison studies between the i-STAT and Stratus CS methods were carried out by testing cTnI in matched lithium-heparin whole blood samples; no significant bias (absolute or percentage) was found. Since serum is the matrix of choice for the Dimension RxL cTnI method, we evaluated agreement with the i-STAT method using the respective type of reference sample. Comparison of the two methods showed a significant negative bias (absolute and percentage), indicating that the two methods are not interchangeable. Our comparison data confirm heterogeneity due to the lack of standardization and suggest that different ''hybrid'' results between POCT and the central laboratory must be considered to guarantee appropriate interpretation of the monitoring results for patients with suspected ACS. In this first implementation phase of POCT in the ambulance, we assessed the capacity of the i-STAT cTnI method to correctly discriminate patients with myocardial damage requiring hospitalization in the ICU.

According to ESC/ACC criteria, a final diagnosis of AMI was made on the basis of serial cTnI monitoring in the central laboratory with the Dimension RxL method; the i-STAT method was able to detect cardiac damage in 20 (91%) of the 22 AMI patients in the pre-hospital phase. Using the same criterion, the i-STAT cTnI concentration was below the 0.09 μg/L decision limit in 27 (87%) of 31 non-AMI patients. Our experimental software was able to receive data from the i-STAT device and transmit them to central laboratory pathologists and Tele-Cardiology Unit cardiologists within 12 min of patient admission to the ambulance. Analysis of our preliminary clinical data suggests that, like the Stratus CS cTnI method, the high sensitivity of the i-STAT troponin method in recognizing minimum myocardial damage at the decision limit used could play an important role in the early identification of patients with AMI in a low- to intermediate-ACS-risk population in the diagnostic and therapeutic pre-hospital phase. Qualitative control of the remote diagnostic data allows different diagnostic tool synergies (cTnI and ECG) in situations of clinical emergency.

This integration rationale may provide an improvement in the managerial efficiency and efficacy of patient outcome.

References

1. Myocardial infarction redefined – a Consensus Document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction. J Am Coll Cardiol 2000;36:959–69; Eur Heart J 2000;21:1502–13.
2. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, et al. ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: executive summary and recommendations. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients with Unstable Angina). Circulation 2000;102:1193–209.
3. Panteghini M, Apple FS, Christenson RH, Dati F, Mair J, Wu AH. IFCC Scientific Division, Committee on Standardization of Markers of Cardiac Damage. Use of biochemical markers in acute coronary syndromes. Clin Chem Lab Med 1999;37:687–93.
4. Wu AH, Apple FS, Gibler WB, Jesse RL, Warshaw MM, Valdes RJ. National Academy of Clinical Biochemistry standards of laboratory practice: recommendations for the use of cardiac markers in coronary artery diseases. Clin Chem 1999;45:1104–21.
5. Di Serio F, Amodio G, Campaniello M, Coluccia P, Trerotoli P, Antonelli G, et al. Integration between pointof-care cardiac markers in an emergency/cardiology department and the central laboratory: methodological and preliminary clinical evaluation. Clin Chem Lab Med 2005;43: 202–9.
6. Goldman L, Cook EF, Johnson PA, Brand DA, Rouan GW, Lee TH. Prediction of the need for intensive care in patients who come to emergency departments with acute chest pain. N Engl J Med 1996;334:1498–504.
7. Apple FS, Murakami MM, Christenson RH, Campbell JL, Miller CJ, Hock KG, et al. Analytical performance of the i-STAT cardiac troponin I assay. Clin Chim Acta 2004; 345:123–7.
8. Panteghini M, Gerhardt W, Apple FS, Dati F, Ravkilde J, Wu AH. Quality specifications for cardiac troponin assays. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). IFCC Scientific Division Committee on Standardization of Markers of Cardiac Damage. Clin Chem Lab Med 2001;39:174–8.
9. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS guideline EP5-A. Wayne, PA: NCCLS, February 1999.
10. National Committee for Clinical Laboratory Standards. Evaluation of the linearity of quantitative measurement procedures: a statistical approach; proposed guideline. NCCLS guideline EP6-P. Wayne, PA: NCCLS, September 1986.
11. National Committee for Clinical Laboratory Standards. Interference testing in clinical chemistry: proposed guideline. NCCLS guideline EP7-P. Wayne, PA: NCCLS, 1986.
12. National Committee for Clinical Laboratory Standards. Method comparison and bias estimation using patient samples; approved guideline. NCCLS guideline EP9-A. Wayne, PA: NCCLS, December 1995.