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# Analytical assessment and performance of the 0/3h algorithm with novel high sensitivity cardiac troponin I

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## ABSTRACT

**Background:** Recently, Ortho Clinical Diagnostics have launched a high sensitivity cardiac troponin I assay adapted on Vitros3600 / 5600. We aimed at evaluating the analytical and clinical performances using the 0/3-hour algorithm, of the Ortho hs-cTnI assay on the Vitros 3600® (Ortho Clinical Diagnostics, Illkirch, France) analyzer with comparison to Roche hs-cTnT assay on the Cobas8000/e801® module (Roche diagnostics, Meylan, France) and the Abbott STAT hs-cTnI assay on the Alinity i® (Abbott, Rungis, France) analyzer.

**Methods:** Imprecision studies, linearity, limit of detection, and the interference to hemolysis were performed for Ortho hs-cTnI assay. The concordance study was based on results of troponin obtained from 160 patients with chest pain to the emergency department. Testing was performed simultaneously measuring the Roche hs-cTnT and the Ortho hs-cTnI, then on remaining sample the concentration of Abbott hs-cTnI (n = 150). We applied the 0/3h algorithm to rule out/rule in, in acute myocardial infarction.

**Results:** Analytical performances were acceptable and in accordance with manufacturer's data. For late presenters 100, 92.8 and 88.8% patients could be rule-out with Roche, Ortho and Abbott assay, respectively with one NSTEMI missed with both hs-TnI assays. Applying the hs-cTn cut-offs from 0/3-hour algorithm, 107 (66.8%) could be rule out with Roche hs-cTnT with no AMI missed (sensitivity 100% [95%CI : 90.5 to 100] and NPV 100%); 89 with Ortho hs-cTnI (sensitivity 97.3% [95%CI : 85.8–99.9] and NPV 98.8% [95%CI :92.7–99.8]); and 61 with Abbott hs-cTnI (sensitivity 97% [95%CI : 84.2–99.9] and NPV 98.4% [95%CI : 89.8–99.7]). For rule-in, 23.7% (n = 37) from the total population would be designated in this group, with a specificity of 86.9% (95%CI: 79.7–92.4) and PPV of 69.8% (95%CI: 59.4–78.5) vs specificity of 72.3% (95%CI:63.5–80.0) and PPV of 51.4% (95%CI: 44.1–58.2) with Roche hs-cTnT vs Ortho hs-cTnI respectively; and with Abbott, 33 patients had constituted this group with a specificity of 52.1% (95%CI:42.7–61.4) and PPV of 36.4% (95%CI: 32.0–41.0).

**Conclusion:** In conclusion, according to ESC guidelines, our results indicate that the Ortho hs-cTnI assay for the Vitros 3600 instrument is a method that may be used in the clinical practice using 0/3-hour algorithm.

## 1. Introduction

Cardiac troponins I or T (cTnI or cTnT) are the most sensitive and specific biomarkers of myocardial injury and remain the gold standard biomarker for diagnosing acute myocardial injury (AMI). The implementation of high-sensitivity cardiac troponin I and T (hs-cTnI and hs-cTnT) assays allowed the rapid rule-out / rule-in cut-off and facilitated earlier decision-making in the management of patients with suspected

AMI especially in the emergency department (ED), compared with less sensitive contemporary assays [1]. Effectively, the higher sensitivity of cTn assay allows a shortened time interval to the second cardiac troponin assessment. L'European Society of Cardiology (ESC) implemented the most efficient algorithms for rapid triage of patients suspected of non-ST elevation myocardial infarction (NSTEMI). Until now, the 0/3-hour hs-cTn algorithm is the most commonly used scheme to diagnose acute myocardial infarction [2]. More recently as an

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alternative, 0 h/1-hour or 0/2-hour assessments are possible consisting of an absolute changes of the cTn levels within 1 h. However, in order to be able to be used, the algorithm for each hs-cTn assay should be validated. Currently, most manufacturers have their own hs-cTn assays on their immunoassay instrument for which the cut-off levels in ng/L within the 0 h/1-hour and 0/2-hour algorithms are available [3]. Recently, Ortho Clinical Diagnostics have launched the Ortho hs-cTnI assay adapted on Vitros3600 / 5600. The 0/1-hour algorithm using Ortho hs-cTnI assay were validated [4] and in the ESC guidelines specific cut-off levels in ng/L within the 0/1-hour and 0/2-hour algorithms were reported [3]. Alone one publication validated the use of a 0/3-hour algorithm of Vitros hs-cTnI assay in comparison to Ortho's contemporary cTnI assay. Although the different algorithms of Ortho hs-cTnI have been validated [4–6], no study comparison with other high sensitive cTn has been performed, especially with algorithm 0/3-hour which is the most used currently.

The aim of our study was to determine the analytical and clinical performances using the 0/3-hour algorithm, of the Ortho hs-cTnI assay on the Vitros 3600® (Ortho Clinical Diagnostics, Illkirch, France) analyzer with comparison to Roche hs-cTnT assay on the Cobas8000/e801® module (Roche diagnostics, Meylan, France) and the Abbott STAT hs-cTnI assay on the Alinity i® (Abbott, Rungis, France) analyzer.

## 2. Materials and methods

### 2.1. Study design

Patients admitted to the ED presenting chest pain or symptoms suggesting of acute coronary syndrome (ACS) were included. Blood sampling were performed on a lithium heparin tube at presentation and after 3 h according to the ESC recommendations [2] (Figure A-supplemental appendix). However, patients were re-classified a posteriori for symptom onset ≤ or > 6 h. Hs-cTnT was performed on a Roche hs-cTnT assay on the Cobas8000/e801® module using a fifth-generation assay and was used for the clinical diagnosis. In addition, hs-cTnI was tested using Ortho hs-cTnI assay on the Vitros 3600®, after evaluation of analytical performances, as well as the Abbott STAT hs-cTnI assay on the Alinity i® instruments. Only patients with both Roche hs-cTnT and Ortho hs-cTnI results obtained at 0 h and 3 h later have been taken as eligible. All investigations were performed in accordance with the principles of the Declaration of Helsinki and approved by the local ethics committee, institutional review Board of Montpellier university hospital center.

### 2.2. Assays and instruments

The main characteristics claimed by the manufacturers of each assay have been described in Table 1.

**Roche hs-cTnT assay**  
The quantitative determination of Elecsys hs-cTnT on plasma was based on ECLIA-Electrochemiluminescence immunoassay.  
The Elecsys hs-cTnT assay employs two monoclonal antibodies specifically directed against human cardiac troponin T [7]. The antibodies recognize two epitopes (amino acid position 125–131 and 136–147) located in the central part of the cardiac troponin T protein, which consists of 288 amino acids. The lowest limit value and the upper reference limit (99th percentile) for troponin T was determined at 3 and 14 ng/L, respectively.  
**Ortho hs-cTnI assay**  
The concentration of Ortho hs-cTnI is based on enhanced chemiluminescence detection. Cardiac troponin I present in the sample reacts simultaneously with streptavidin-conjugated antibody (mouse monoclonal anti-cTnI), bound by biotin-BSA on the wells, and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-cTnI). The antigen–antibody complex is captured by the antibody on the wells. The bound HRP conjugate is measured by a luminescent

**Table 1**  
Comparative analytical characteristics of hs-cTnT from Roche, and both hs-cTnI from Ortho and Abbott according to manufacturer's data.

	Elecsys hs-cTnT	Vitros hs-cTnI	Alinity STAT hs-cTnI
LoB, ng/L	3	0.14–0.51	1.0
LoD, ng/L	5	0.39–0.86	1.6
Concentration at 20% CV, ng/L	6	1.23	1.3
LoQ, ng/L at 10%	11	1.99	4.7
99th percentile [CI95%], ng/L	14 [12.7–24.9]	F = 9 [4.8–19] M = 13 [9.7–22.9] Total = 11 [8.4–14.8]	F = 15.6 [13.8–17.5] M = 34.2 [28.9–39.2] Total = 26.2 [23.2–29.7]
HI, g/L	1	6	5
Measuring range, ng/L	3–10 000	1.5–30 000	10–50 000
Time for first result, minutes	18	15	18
Sample size, µL	30	60	210

F. female, M: male, LoB: limit of blank, LoD: limit of detection, LoQ: limit of quantification, HI: hemolysis index.

reaction. The amount of HRP conjugate bound is directly proportional to the concentration of cardiac troponin I present. The assay consists of two capture antibodies recognizing the amino acids at position 24–40 and 41–49, and one of detection for amino acids at position 87–91 [7]. The lowest limit value and the 99th percentile for this assay troponin I was determined at 1.5 and 11 ng/L, respectively.

**Alinity hs-cTnI assay**  
The Alinity i STAT hs-cTnI assay is a chemiluminescent microparticle immunoassay used for the quantitative determination of troponin I in plasma and serum samples. One detection antibody and one capture antibody directed against the amino acids at position 24–40 and 41–49, respectively, allow determination of troponin concentrations in samples [7]. This assay has a recommended 99th percentile cut-off of 26.2 ng/L and a lowest limit value at 10 ng/L.

Analytical performances of the Ortho hs-cTnI assay  
Imprecision studies was based on the CLSI EP15 protocol (duplicate measurements twice per day on 4 levels for 5 days) [8] from plasma pool and quality controls. A plasma pool with Ortho hs-cTnI concentration of 48.6 ng/L was used to test the linearity. The pool was diluted at the following final concentrations: 24, 12, 6, 3, 1.5, 0.75 and 0.35 ng/L. Each concentration was measured in duplicate. The limit of detection (LoD) was determined according to the current CLSI standard [9] from plasma samples. The interference to hemolysis was performed according the Delgado's approach [10]. We selected 2 lithium heparin tubes for each concentration of troponins desired from donors who had a troponin value around 12, 19 and 117 ng/L. One of the 2 tubes was directly centrifuged (10 min, 2000g), while the other was subjected to 3 freeze–thaw cycles to induce hemolysis and subsequently centrifuged. Both plasma samples (for the same concentration) were mixed in different proportions to create a hemolysis gradient. To determine the allowable HI we used the the total change limit (TCL) as significant limit of bias. The TCL, as described by Oddoze et al., was calculated using Eq. (2) [11]:

$$[(2.77 \times CVa)^2]^{\frac{1}{2}} + [(0.5 \times CVw)^2]^{\frac{1}{2}},$$

where CVa is analytical imprecision and CVw is within-subject biological variation.  
When the bias (in percentage) was higher than TCL values, the variation was considered as significant.

### 2.3. Method comparison

The concordance study was based on results of troponin obtained



from patients with chest pain suggestive of ACS admitted to the emergency department of Lapeyronie (Montpellier, France). Baseline and 3 h samples were available in 160 patients. Testing was performed simultaneously measuring the hs-cTnT on the Cobas8000/e801® module and the cTnI on the Vitros 3600® instrument, then on remaining sample the concentration of hs-cTnI on the Alinity i® was determined (n = 150).

The clinical records of all patients were evaluated to confirm the diagnosis following the enrollment. The final diagnoses were adjudicated using the hs-cTnT from Roche by a senior emergency physician and by a cardiologist. Patients were divided into 2 diagnostic groups at discharge, AMI or non AMI.

Diagnosis of AMI was established according to the universal definition of myocardial infarction [3]: patients with symptoms of cardiac ischemia with simultaneously ECG changes and/or increased cardiac markers such as hs-cTn T or I, with at least one value above the 99th percentile of the upper reference limit, development of pathological Q waves on ECG, imaging evidence of loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology, intracoronary thrombus detected on angiography or autopsy.

Patients categorized as having AMI were further subcategorized into ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) [3]. STEMI was defined by the persistent elevation of the segment ST of  $\geq 1$  mm in 2 contiguous electrocardiographic leads or by the presence of a new left bundle branch block with positive cardiac enzyme results. NSTEMI was defined as the occurrence of acute myocardial infarction in the setting of positive cardiac marker results with or without accompanying electrocardiographic changes other than ST segment elevation.

Unstable angina (UA) was diagnosed if the ECG was uncertain and with negative

cTn levels. Upon admission, patients with UA or NSTEMI may not have been distinguished and the diagnosis of NSTEMI was established according to cardiac biomarkers. All patients with excluded AMI (non AMI) were categorized to a number of clinical conditions cardiac, non cardiac or unknown origin (UO). Cardiac causes included heart failure, UA, arrhythmia, and TakoTsubo. Non-cardiac causes are composed of pulmonary disease as pulmonary embolism and acute respiratory distress syndrome, syncope, digestive disease, infection, stroke, renal disease, and alcohol related disorder.

2.4. Diagnostic performance of hs-cTn concentrations according to the ESC 0/3-hour algorithm

The application of the ESC 0/3h algorithm published since 2015 is possible by the availability of “highly sensitive” troponin assays. AMI may be ruled-out if the baseline troponin value is below the 99th percentile of the general population and the symptom onset is more than 6 h ago. For patients with symptom onset  $\leq 6$  h a second troponin measurement 3 h after presentation needs to be considered. If both troponin measurements are below the 99th percentile AMI may be ruled-out. To rule-in AMI, one of the two troponin measurement (0 or 3 h, respectively) needs to exceed the 99th percentile in combination with a change of 5-fold the upper limit of normal (ULN). All patients that are not included in group rule-in or rule-out by this assessment need further workup differential diagnoses.

2.5. Statistical analysis

The LoD, and 99th percentile cut point for hs-cTn assay from Roche, Ortho and Abbott were used (Table 1). Receiver operator characteristic (ROC) with 95% CI around the Area Under Curve (AUC) curves were calculated to assess diagnostic performance at admission and 3 h later taking into account the delta change. We applied the 0/3-hour algorithm with Roche hs-cTnT, Ortho hs-cTnI and Abbott hs-cTnI in our population and compared the diagnostic performance parameters such as the

negative predictive value (NPV) and sensitivity as well as positive predictive value (PPV) and specificity which were calculated. NPV and PPV were compared by the McNemar test. Clinical concordance between the methods with disease state samples was then assessed using Cohen’s K-test with values  $< 0$  as indicating no agreement, 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial and 0.81–1 as almost perfect agreement [12]. Statistical analyses were performed using XLSTAT® software, version 2016.06.35661 (NY, USA). A p value  $< 0.05$  was considered as statistically significant.

3. Results

3.1. Analytical performances of Ortho hs-cTnI assay

All results were presented in Table 2. The results of the imprecision study showed that the within-run and total imprecision (CV) was lower than 4.54% in accordance with manufacturer’s data. The LoD in our condition was 0.54 ng/L in agreement with the manufacturer. The HI tested on different concentrations of troponin exhibited two schemes. Effectively (Figure B-supplemental appendix), we observed a linear relationship between the increases in analyte value and HI with a positive bias for low values (11.9 and 29.04 ng/L), and a negative bias for high value (117.4 ng/L). However, with a TCL calculated at 7.8%, allowable HI was found at 4.5 g/L whatever the concentration of troponin. The Passing-Bablok regression of linearity showed a gradual decrease in recovery from 112.0% on the highest sample measured with Ortho assay (assigned value 48.6  $\mu$ g/L) to 67% on the low sample (assigned value 1.52  $\mu$ g/L). The CUSUM test did not show significant deviation from linearity.

3.2. Patient characteristics

Of admitted patients, a large proportion were excluded from follow-up due to no hs-cTnT at 3 h or insufficient samples. In the remaining 160 patients, a kinetic analysis was performed within 3 h after admission.

The final adjudicated clinical diagnoses were categorized to AMI (n = 37) including STEMI (n = 9), NSTEMI (n = 28), and non-AMI (n = 123). Subcategories were cardiac causes (n = 57) including heart failure (n = 23), UA (n = 9), arrhythmia (n = 7), TakoTsubo (n = 1), and NSCP (n = 17). Non-cardiac causes (n = 66) are composed of pulmonary disease as pulmonary embolism and acute respiratory distress syndrome (n = 11), syncope (n = 22), digestive disease (n = 8), infection (n = 8), stroke (n = 7), renal disease (n = 5), and alcohol related disorder (n = 3).

Table 2  
Analytical performances of Ortho hs-cTnI assay determined in our study.

	Plasma pool		Control level 1		Control level 2	
	Mean, ng/L	CV, %	Mean, ng/L	CV, %	Mean, ng/L	CV, %
Repeatability imprecision	8.7	2.6				
Intermediate precision	11.4	4.4	10.5	2.8	33.3	3.6
LoD, ng/L	0.54					
HI, g/L	4.5					
Linearity	Theoretical values, ng/L		Mean of observed values, ng/L (% of mean recovery)			
	48,60		54,64 (112)			
	24,30		26,68 (109)			
	12,15		12,73 (104)			
	6,07		5,76 (94)			
	3,04		2,77 (91)			
	1,52		1,02 (67)			
	0,76		0,63 (82)			
	0,38		0,27 (71)			

LoD: limit of detection, HI: hemolysis index.



For 2 STEMI and 2 NSTEMI patients, as well as for 3 cardiac and non cardiac causes, the plasma volume was insufficient to determine the concentration of Abbott hs-cTnI.

### 3.3. Method comparison

The baseline characteristics at admission of the 160 patients included were reported in Table 3. The mean age ( $\pm$ SD) was 73.3 (17), and close to 60% were male. The baseline levels with the three assays were significantly higher in patients with AMI ( $p < 0.001$ ) compared to values obtained in the patients without AMI. In AMI group, at presentation all patients (100%) had Roche hs-cTnT above the 99th percentile, and only 94% of patients had Ortho and Abbott hs cTnI results above 11 and 26 ng/L. For the three assays, the percentage of the distribution between AMI vs. non-AMI was statistically different ( $p < 0.03$ ). The mean hs-cTn concentrations at admission were higher with Abbott (750.7 ng/L  $\pm$  1662) than with Ortho (469.2 ng/L  $\pm$  154.6) without significant difference. We found a good correlation in all patients between the Roche hs-cTnT vs Ortho hs-cTnI values ( $r=0.77$ ), with a less good correlation in the group of AMI patients ( $r = 0.74$ ). The hs-cTnI values from Ortho and Abbott exhibited an excellent correlation in AMI patients ( $=0.94$ ), in all patients the correlation was lower ( $r = 0.85$ ).

Direct comparison of hs-cTn groups showed a weak concordance

**Table 3**  
Characteristics at admission of patients.

Number (%)	All patients*	AMI patients <sup>a</sup>	Cardiac <sup>b</sup>	Others <sup>c</sup>
Sex, n	65F, 95 M	9F, 28 M	24F, 33 M	33F, 33 M
Age, mean ( $\pm$ SD)	73.3 ( $\pm$ 17)	73.2 ( $\pm$ 17)	72.8 ( $\pm$ 16.4)	75.3 ( $\pm$ 18.3)
	Mean ( $\pm$ SD), ng/L	Mean ( $\pm$ SD), ng/L	Mean ( $\pm$ SD), ng/L	Mean ( $\pm$ SD), ng/L
Roche hs-cTnT	110.3 ( $\pm$ 286)	261.5 ( $\pm$ 538.8)	65.7 ( $\pm$ 132.8)	59.2 ( $\pm$ 62.0)
Ortho hs-cTnI	469.2 ( $\pm$ 154.6)	1205.6 ( $\pm$ 2697)	208.8 ( $\pm$ 759.0)	166.3 ( $\pm$ 457.1)
Abbott hs-cTnI	750.7 ( $\pm$ 1662.0)	1446 ( $\pm$ 2566)	554.4 ( $\pm$ 1250.3)	442.5 ( $\pm$ 1054.7)
Low linearity value, ng/L	%	%	%	%
Roche hs-cTnT < 3	1.8	0	5.3	0
Ortho hs-cTnI < 1.5	5.6	0	10.5	4.5
Abbott hs-cTnI < 10	8.0	3	14.8	4.8
99 <sup>o</sup> percentile, ng/L				
Roche hs-cTnT $\leq$ 14	11.8	0	24.6	7.6
Ortho hs-cTnI $\leq$ 11	28.7	5.4	40.3	31.8
Abbott $\leq$ 26	18.0	6.0	20.4	22.2
Roche hs-cTnT > 14	88.1	100	75.4	92.4
Ortho hs-cTnI > 11	71.2	94.6	57.9	69.7
Abbott hs-cTnI > 26	82	93.9	79.6	77.7

\*n = 160 results obtained with Roche hs-cTnT and Ortho hs-cTnI, n = 150 results obtained with Abbott hs-cTnI.

<sup>a</sup>n = 37 results obtained with Roche hs-cTnT and Ortho hs-cTnI including 28 NSTEMI and 9 STEMI, n = 33 results obtained with Abbott hs-cTnI including 26 NSTEMI and 7 STEMI.

<sup>b</sup>n = 57 results obtained with Roche hs-cTnT and Ortho hs-cTnI, n = 54 results obtained with Abbott hs-cTnI.

<sup>c</sup>n = 66 results obtained with Roche hs-cTnT and Ortho hs-cTnI, n = 63 results obtained with Abbott hs-cTnI.

F: female, M: male, NSTEMI: non-ST elevation myocardial infarction, STEMI: ST-segment elevation myocardial infarction, SD: standard deviation.

where only 65% and 72.6% of patients had simultaneously hs-cTn results higher their 99th percentile considering Roche vs Ortho and Roche vs Abbott, respectively ( $p > 0.5$ , NS).

The concordance was established using the lowest range value claimed by the manufacturer and the 99th percentile of Roche hs-cTnT, Ortho hs-cTnI, and Abbott hs-cTnI assays i.e 3, 1.5 and 10 ng/L and 14, 11 and 26 ng/L, respectively. Cohen's kappa agreements were poor at 0.43 [95%CI- 0.310 to 0.552] between Roche hs-cTnT vs Ortho hs-cTnI and a very slight agreement was found between Roche hs-cTnT vs Abbott hs-cTnI ( $<0.1$  [95%CI -0.016 to 0.202]). The concordance between both hs-cTnI was weak (Cohen's kappa 0.307; 95%CI 0.197–0.417).

### 3.4. Rule-in / rule-out at baseline (Fig. 1)

All patients had measurements of cTn at admission to ED and 3 h later. Among 160 patients eligible for analysis with hs-cTn, AMI was the final adjudicated diagnosis for 37 patients (23.1%) including 9 patients STEMI and 28 NSTEMI with Roche and Ortho, only 33 patients (22%) including 26 NSTEMI and 7 STEMI with Abbott. In our study, 69 patients (43%) presented > 6 h after onset of chest pain, and could be managed with a single blood draw selon the ESC 0/3-hour algorithm. For late presenters with hs-cTn below their respective URL, 100, 92.8 and 88.8% patients could be rule-out with Roche, Ortho and Abbott assay, respectively (Fig. 1). According to the initial value, one NSTEMI was missed with both hs-TnI assays, leading a NPV 100%, 93% and 88% for Roche hs-cTnT, Ortho hs-cTnI and Abbott hs-cTnI assay, respectively. However, the second troponin value 3 h later clearly identified the NSTEMI.

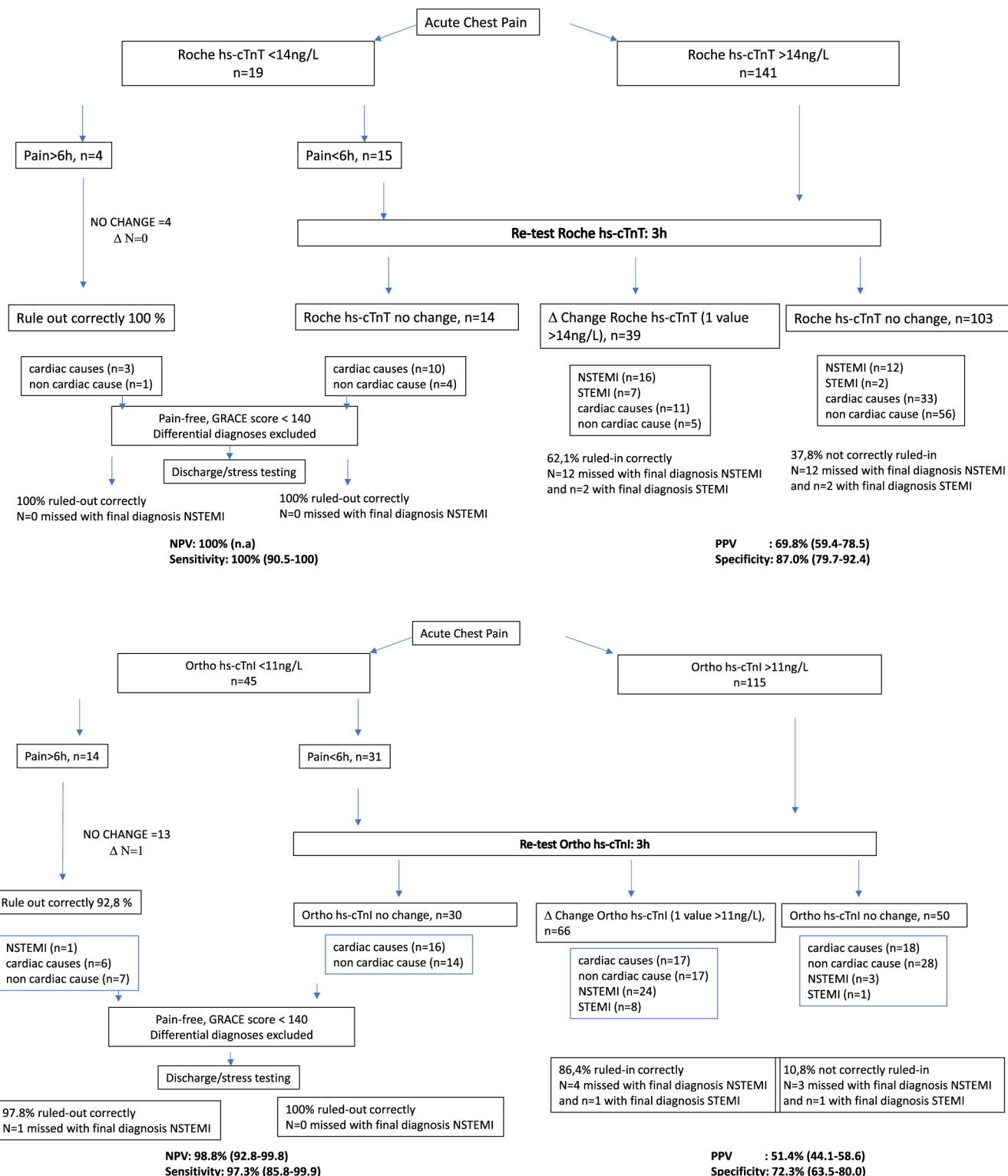
### 3.5. Diagnostic performance of hs-cTn concentrations according to the ESC 0/3-hour algorithm

Applying the hs-cTn cut-offs from 0/3-hour algorithm to the 160 patients with serial blood drawn, 107 (66.8%) could be rule out with Roche hs-cTnT with no AMI missed (sensitivity 100% [95%CI : 90.5 to 100] and NPV 100%); 89 with Ortho hs-cTnI (sensitivity 97.3% [95%CI : 85.8–99.9] and NPV 98.8% [95%CI :92.7–99.8]); and 61 with Abbott hs-cTnI (sensitivity 97% [95%CI : 84.2–99.9] and NPV 98.4% [95%CI : 89.8–99.7]). For rule-in, 23.7% (n = 37) from the total population would be designated in this group, with a specificity of 86.9% (95%CI: 79.7–92.4) and PPV of 69.8% (95%CI: 59.4–78.5) vs specificity of 72.3% (95%CI:63.5–80.0) and PPV of 51.4% (95%CI: 44.1–58.2) with Roche hs-cTnT vs Ortho hs-cTnI respectively; and with Abbott, 33 patients had constituted this group with a specificity of 52.1% (95% CI:42.7–61.4) and PPV of 36.4% (95%CI: 32.0–41.0). Diagnostic accuracy of 0–3-hour deltas values for myocardial infarction, as quantified by AUCs, for Roche hs-cTnT was 0.81 [95%CI: 0.72–0.90], for Ortho hs-cTnI was 0.84 [95%CI: 0.75–0.93], and for Abbott hs-cTnI 0.77 [95%CI 0.65–0.88].

## 4. Discussion

Our study, confirms (1) the good analytical performances of Ortho hs-cTnI assay consistent with a recent study [13], (2) the good correlation with Roche hs-cTnT and Abbott hs-cTnI assays (3) the acceptable diagnostic performance in comparison with Roche hs-cTnT, with the use of the 0/3-hour algorithm to rule out/rule in patients with suspected ACS.

The LoD of Ortho hs-TnI determined in lithium heparin plasma was in accordance with manufacturer's data but lower than reported in Kavsak' study [13]. At concentration close to the 99th percentile, CVs were acceptable < 5% on plasma pool as well as on quality control. The concentrations with Ortho hs-cTnI assay in our population were systematically lower than values with Abbott hs-cTnI as previously observed [6], and higher than Roche hs-cTnT assay. Higher hs-cTnT and hs-cTnI concentrations were observed in group of patients with AMI in



**Fig. 1.** 0 h/3h rule out algorithm for AMI according to ESC recommendations; (A) Using Roche hs-cTnT assay, (B) using Ortho hs-cTnI assay, (C) using Abbott hs-cTnI assay. NPV: negative predictive value; PPV: positive predictive value; n.a.: not applicable.

comparison with the others groups of patients ( $p < 0.001$ ). Previously [14], a discordance was described according to the acute or non acute conditions suggesting different mechanisms of release for troponin I and T in the chronic setting. We did not find this difference in the correlation according to the status of patients between Roche hs-cTnT and Ortho hs-cTnI. Effectively, whether in patients with AMI or with other causes of

chest pain, the correlation was the same order ( $r = 0.80$ ). The lower correlation between both hs-cTnI assays in patients without AMI could be due to the difference of capture or detection antibodies. Effectively, Ortho assay consists of three antibodies, against two antibodies for Abbott. Although located between amino acid residues 30–110 of cTnI, which is the most stable part of the molecule, we could speculate that the

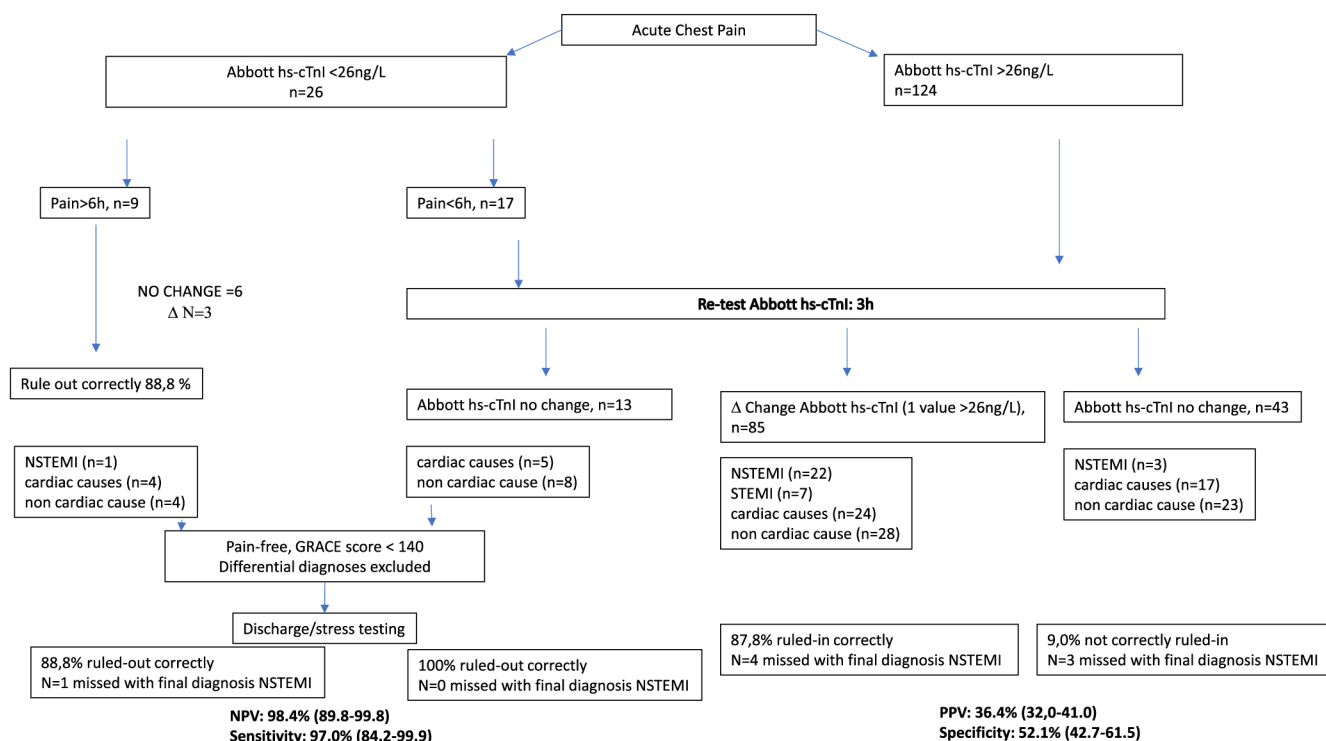


Fig. 1. (continued).

presence of number higher cTnI proteolytic fragments could explained according to the assay, these differences. Several authors have proposed a common cut-off of 5 ng/L for all the hs-cTn assays whatever the manufacturer to management of NSTEMI [13,15–16]. Based on our results, this does not seem reasonable to use the same cut-off for either hs-cTnI or hs-cTnT to rapid rule-out of patients at presentation in ED. The poor concordance demonstrates the heterogeneity in cTn assays and the difficulty of comparing data across studies. However, neither the analytical imprecision of the respective assays, nor the interference of heterophilic or other antibodies, could explain this poor agreement.

Comparison of the Ortho hs-cTnI with an established Roche hs-cTnT assay revealed similar diagnostic capacity according to the 0–3 h algorithm. In ROC analyses, both assays showed identical AUC values and both assays provided comparably high NPVs for rule out (100% for the hs-cTnT vs 98.8% for the Ortho hs-cTnI,  $p = \text{NS}$ ). The NPV with Abbott hs-cTnI was 98.4%, close to data reported in the literature [17–19]. For rule in of the relatively small number of NSTEMI ( $n = 28$ ), a higher PPV was observed for the hs-cTnT algorithm (69.8% for the hs-cTnT vs 51.4% for the hs-cTnI,  $p < 0.05$ ). This result is in total agreement with a previous report on Ortho hs-cTnI 0/1-hour highlighting a comparable diagnostic accuracy with Roche hs-cTnT and Abbott hs-cTnI [17]. However, one NSTEMI was missed using baseline value of both cTnI assays, strongly suggesting that the 0–3 h algorithm was a better option for hs-TnI.

Some limitations of our study merits consideration. First, the present study was performed in a single centre – the Montpellier University hospital – and a relatively small number of patients were included. However, in order to avoid potential bias in the interpretation of results we decided (1) to include – only patients with sufficient plasma volume to determine the concentration of troponins on the 3 instruments, (2) to perform measurement only on fresh plasma to avoid bias due to frozen / thawed samples [20], (3) to recruit only patients during the day to be able to carry out the 3 measurements on fresh samples, (4) to exclude patients who did not have available sample at 3 h and (5) to confirm the final diagnosis with an independent medical team including cardiologists. Because the prevalence of AMI (23%) in our study is substantially

higher than that reported by previous epidemiological investigations [4] our study may underestimate the NPV and overestimate the PPV. Lastly, as in similar studies, the GRACE score, included in the ESC recommendation was not forever taken into account and could have influenced the rule out/rule in procedure.

In conclusion, according to ESC guidelines [2], our results, together with those reported in other very recent studies [6,13], indicate that the Ortho hs-cTnI assay for the Vitros 3600 instrument is a method that may be used in the clinical practice using 0/3-hour algorithm.

#### Author contributions

(1) We declare that we participated following: - in the concept and design :Anne Marie Dupuy and Jean Paul Cristol, - in acquisition of subjects and/or data : Valentin Montagnon, Mustapha Sebbane, Olivier Beauflis , - in analysis and interpretation of data : AM Dupuy, François Roubille, Mustapha Sebbane, Olivier Beauflis, J P Cristol, - in preparation of manuscript : AM Dupuy, Stéphanie Badiou, J P Cristol and that we have seen and approved the final version. In addition all authors declare the absence of conflict of interest. (2) The Ortho hs-cTnI reagents used in this study were generously provided by Ortho Clinical Diagnostics France. (3) Sponsors not have any role in the design, methods, subject recruitment, data collections, analysis and preparation of paper.

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## References

- [1] P.A. Kavsak, L.C. Allen, F.S. Apple, R.A. Booth, P.C. Chan, E. Delvin, A. Fraser, L. Fu, D. Gornall, C. Collier, S. Hill, B. Hoffman, Y. Huang, J. Lavoie, A. Lou, A. Mattman, M. McQueen, Q.H. Meng, C. Oleschuk, M. Pudek, E. Randell, K. Y. Sohn, L. Thorlacius, P.M. Yip, N. Dahdah, P.J. Devereaux, S. Dhesy-Thind, S. J. Hotte, *Worst A Cardiac troponin testing in the acute care setting: ordering, reporting, and high sensitivity assays—an update from the Canadian society of clinical chemists (CSCC)*, *Clin. Biochem.* 16 (2011) 1273–1277.
- [2] Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP, Gencer B, Hasenfuss G, Kjeldsen K, Lancellotti P, Landmesser U, Mehilli J, Mukherjee D, Storey RF, Windecker S; ESC Scientific Document Group. 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.*, 37(3) (2016) 267–315.
- [3] Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, Dendale P, Dorobantu M, Edvardsen T, Folliguet T, Gale CP, Gilard M, Jobs A, Jüni P, Lambrinou E, Lewis BS, Mehilli J, Meliga E, Merkely B, Mueller C, Roffi M, Rutten FH, Sibbing D, Siontis GCM; ESC Scientific Document Group. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur. Heart. J.* 2020 Aug 29;ehaa575.
- [4] Boeddinghaus J, Twerenbold R, Nestelberger T, Badertscher P, Wildi K, Puelacher C, du Fay de Lavallaz J, Keser E, Rubini Giménez M, Wussler D, Kozhuharov N, Rentsch K, Miró Ó, Martín-Sánchez FJ, Morawiec B, Stefanelli S, Geigy N, Keller DI, Reichlin T, Mueller C; APACE Investigators. Clinical Validation of a Novel High-Sensitivity Cardiac Troponin I Assay for Early Diagnosis of Acute Myocardial Infarction. *Clin. Chem.* 64 (2018) 1347–1360.
- [5] Twerenbold R, Neumann JT, Sörensen NA, Ojeda F, Karakas M, Boeddinghaus J, Nestelberger T, Badertscher P, Rubini Giménez M, Puelacher C, Wildi K, Kozhuharov N, Breitenbuecher D, Biskup E, du Fay de Lavallaz J, Flores D, Wussler D, Miró Ó, Martín Sánchez FJ, Morawiec B, Parenica J, Geigy N, Keller DI, Zeller T, Reichlin T, Blankenberg S, Westermann D, Mueller C. Prospective Validation of the 0/1-h Algorithm for Early Diagnosis of Myocardial Infarction. *J. Am. Coll. Cardiol.* 72 (2018) 620–632.
- [6] P.A. Kavsak, S.E. Mondoux, J. Sherbino, J. Ma, N. Clayton, S.A. Hill, M. McQueen, S.R. Mehta, L.E. Griffith, P.J. Devereaux, A. Worster, *Clinical evaluation of Ortho Clinical Diagnostics high-sensitivity cardiac Troponin I assay in patients with symptoms suggestive of acute coronary syndrome*, *Clin. Biochem.* 80 (2020) 48–51.
- [7] <http://www.ifcc.org/media/276661/IFCC>, Version October 2014 and 2019. (Accessed december 2020). High sensitivity cardiac troponin I and T assay analytical characteristics designated by manufacturer v072020.
- [8] EP15-A3. User verification of precision and estimation of bias-approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute, 2014.
- [9] EP17-A2. Protocols for determination of limits of detection and limits of quantitation. Wayne, PA: Clinical and Laboratory Standards Institute, 2004.
- [10] J.A. Delgado, D. Morell-Garcia, J.M. Bauça, *Hemolysis Interference Studies: The Particular Case of Sodium Ion*, *EJIFCC* 30 (2019) 25–34.
- [11] Christiane Oddoze, Elise Lombard, Henri Portugal, *Stability study of 81 analytes in human whole blood, in serum and in plasma*, *Clin. Biochem.* 45 (6) (2012) 464–469.
- [12] Irene Guggenmoos-Holzmanna, *The meaning of kappa: probabilistic concepts of reliability and validity revisited*, *J. Clin. Epidemiol.* 49 (7) (1996) 775–782.
- [13] Peter A. Kavsak, Tara Edge, Chantele Roy, Paul Malinowski, Karen Bamford, Lorna Clark, Shana Lamers, Stephen Hill, Andrew Worster, *Analytical assessment of ortho clinical diagnostics high-sensitivity cardiac troponin I assay*, *Clin. Chem. Lab. Med.* 59 (4) (2021) 749–755.
- [14] D.M. Kimenai, R.J.H. Martens, J.P. Kooman, C.D.A. Stehouwer, F.E.S. Tan, N. C. Schaper, P.C. Dagnelie, M.T. Schram, C.J.H. van der Kallen, S.J.S. Sep, J.D. E. van Suijlen, A.A. Kroon, O. Bekers, M.P. van Dieijen-Visser, R.M.A. Henry, S.J. R. Meex, *Troponin I and T in relation to cardiac injury detected with electrocardiography in a population-based cohort - The Maastricht Study*, *Sci. Rep.* 26 (2017) 6610.
- [15] A.R. Chapman, T. Fujisawa, K.K. Lee, J.P. Andrews, A. Anand, D. Sandeman, A. V. Ferry, S. Stewart, L. Marshall, F.E. Strachan, A. Gray, D.E. Newby, A.S.V. Shah, N.L. Mills, *Novel high-sensitivity cardiac troponin I assay in patients with suspected acute coronary syndrome*, *Heart.* 105 (2019) 616–622.
- [16] K.M. Eggers, T. Jernberg, L. Ljung, B. Lindahl, *High-Sensitivity Cardiac Troponin-Based Strategies for the Assessment of Chest Pain Patients-A Review of Validation and Clinical Implementation Studies*, *Clin. Chem.* 64 (2018) 1572–1585.
- [17] J. Boeddinghaus, R. Twerenbold, T. Nestelberger, L. Koechlin, D. Wussler, M. Meier, V. Troester, T. Zimmermann, P. Badertscher, K. Wildi, M. Rubini Giménez, P. Lopez-Ayala, E. Potlukova, Ö. Miró, F.J. Martín-Sánchez, D. Kaweck, N. Geigy, D.I. Keller, T. Reichlin, C. Mueller, APACE investigators. *Clinical Use of a New High-Sensitivity Cardiac Troponin I Assay in Patients with Suspected Myocardial Infarction*, *Clin. Chem.* 65 (2019) 1426–1436.
- [18] N. van der Linden, K. Wildi, R. Twerenbold, J.W. Pickering, M. Than, L. Cullen, J. Greenslade, W. Parsonage, T. Nestelberger, J. Boeddinghaus, P. Badertscher, M. Rubini Giménez, L.J.J. Klinkenberg, O. Bekers, A. Schöni, D.I. Keller, Z. Sabti, C. Puelacher, J. Cupa, L. Schumacher, N. Kozhuharov, K. Grimm, S. Shrestha, D. Flores, M. Freese, C. Stelzig, I. Strebel, Ö. Miró, K. Rentsch, B. Morawiec, D. Kaweck, W. Kloos, J. Lohrmann, A.M. Richards, R. Troughton, C. Pemberton, S. Osswald, M.P. van Dieijen-Visser, A.M. Mingels, T. Reichlin, S.J.R. Meex, C. Mueller, *Combining High-Sensitivity Cardiac Troponin I and Cardiac Troponin T in the Early Diagnosis of Acute Myocardial Infarction*, *Circulation.* 138 (2018) 989–999.
- [19] K. Wildi, B. Nelles, R. Twerenbold, M. Rubini Giménez, T. Reichlin, H. Singeisen, S. Druey, P. Haaf, Z. Sabti, P. Hillinger, C. Jaeger, I. Campodarve, P. Kreutzinger, C. Puelacher, Z. Moreno Weidmann, M. Gugala, G. Pretre, S. Doerflinger, M. Wagener, F. Stallone, M. Freese, C. Stelzig, K. Rentsch, S. Bassetti, R. Bingisser, S. Osswald, C. Mueller, *Safety and efficacy of the 0 h/3 h protocol for rapid rule out of myocardial infarction*, *Am. Heart. J.* 181 (2016) 16–25.
- [20] P.A. Kavsak, C. Roy, P. Malinowski, L. Clark, S. Lamers, K. Bamford, S. Hill, A. Worster, A.S. Jaffe, *Sample matrix and high-sensitivity cardiac troponin I assays*, *Clin. Chem. Lab. Med.* 57 (2019) 745–751.