Indicators of Quality in the pre-Analytical Phase for Determining Cardiac Markers in Acute Myocardial infarction—CK, CKMB and TnT

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Abstract: In order to provide consistent results for biomarkers of cardiac necrosis involved in the early diagnosis of acute myocardial infarction (AMI) and monitoring of patients with acute coronary syndrome (ACS), we carried out in our laboratory a prospective study tracking the influence of two types of preanalytical factors, known as indicators of quality (QIs): hemolysis and immunological status of the patient. We measured plasma levels of cardiac troponin T—TnT (hsst-ECLIA method) and serum levels for enzymatic biomarkers: CKMB (immunoturbidimetric method) associated with total CK and LDH (enzymatic method). The tests were performed after assessing the hemolysis degree through visual inspection of sera/plasma. We compared the results of the measurements performed in non-compliant samples with those obtained for compliant samples (repeated sampling request), and we validated the final analysis report based on available clinical data. The results analysis showed the occurrence of falsely elevated values for CKMB between 200%-700% higher for measurements performed on moderate hemolysed serum. For TnT, we had obtained very high values (even more than 10000 ng/mL), uncorrelated with CK and CKMB levels for patients with malignancy or autoimmune pathology. This preliminary data confirmed the importance of hemolysis degree as criteria for rejecting samples in preanalytical phase and provides premises for further study concerning the influence of immunological status on immunochemical methods for testing cardiac biomarkers.

Key words: Myocardial infarction, quality indicators, hemolysis, immunological status, troponin T, CKMB.

1. Background

In view of the increasing incidence of acute myocardial infarction (AMI), for early diagnosis and increasing patient safety by eliminating interference in pre-analytical phase is important the standardization of relevant quality indicators (QIs).

This study aims at the influence of two types of factors that interfere in pre-analytical phase in the process of quality assurance results related to the primary sample aspect (hemolysis), respectively those possible associated with the patient immunological status, in the latter case few information being available in the references data. We watched to assess the effect of these factors on the values from the panel

of biochemical markers with diagnostic and monitoring role in patients with acute coronary syndrome (ACS) according to ESC guidelines, which includes serum enzyme biomarkers (CKMB, LDH, CK) and the most relevant immunological biomarker of myocardial necrosis, troponin T (TnT).

2. Materials and Methods

According to ISO 15189:2012 [1], each accredited laboratory “shall establish quality indicators to monitor and evaluate performance throughout critical aspects of pre-examination, examination and post-examination processes” (Cap. 4.14.7.)

The prevalence of hemolytic specimens in medical laboratories can be around 3.3% of all of the routine samples, 5 times more than other causes for unsuitable

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samples (clotted, incorrect type, etc.) [2]. The occurrence of this kind of samples have a much higher incidence for specimens obtained in the Emergency Department (ED) then in the others phlebotomy services—in-patients or outpatients [3]. Hemolysis is a priority 1 quality indicator, being a potential rejection sample criteria test-specific for the unsuitable samples [4]. To evaluate the hemolysis degree, some labs have analyzers on which the hemolysis index (HI) is automatically calculated, while others labs are using a visual hemolysis scale, with different hemolysis degrees [5], in order to avoid false rejections of specimens.

We have studied the influence of the immune status of patients on plasma TnT levels, respectively the hemolysis degree influence on serum CKMB isoenzyme activity in specimens collected from patients with ACS or presumptive/confirmed AMI, mainly sourced from Cardiology and Cardiology ICU wards.

The prospective study was carried out for 6 months on 2 groups: in the first group were included 83 hospitalized patients with a presumptive/confirmed diagnosis of myocardial infarction (MI), with entry criteria TnT level at least 10 times over reference range upper limit, and in the second group were included 11 pairs of haemolysed/compliant serum samples collected from in-patients. In this first group were included also patients with outliers markers of cardiac necrosis, having TnT levels uncorrelated with the enzymatic activity levels according to the typical pattern of MI.

Immunoturbidimetric method has been used to quantify CK-MB serum levels, associated with total CK and LDH (IFCC method), respectively ECLIA TnT hs STAT (high sensitivity Short Turn Around Time) for the determination of plasmatic troponin T (TnT) levels. In order to interpret the data, we used declared reference ranges for our laboratory: 0-24 U/L for CK-MB, 26-174 U/L for CK, 135-225 U/L for LDH and respectively 0-30 pg/mL for TnT values.

The tests were performed on automated analyzers without preinstalled software to calculate the hemolysis index (HI), so that the assesment of non-compliance degree was made through visual inspection of biological samples after primary processing of specimens. We have used a coloured scale to evaluate the hemolysis degree (slightly/minor, moderate/medium, severe). Following quantifying of hemolysis degree, hemolytic samples management involved a second specimen request for some cardiac markers, such as CK-MB, LDH [6] and sometimes for CK too. The reliability of visual assessment of the degree of hemolysis is influenced by serum bilirubin concentration [7].

3. Results

We have compared the results of the measurements performed on inconsistent specimens with those obtained for compliant specimens received after repeated request for biological sampling. To validate the results and drafting the final analysis report we took into consideration the biological status of the patient, according to clinical data available in EHR.

The study of the pathologies types distribution associated with markedly elevated TnT levels showed that 34% from patients had impaired immunological status in the selected group, from which 16% with malignancies (solid tumors or haematological malignancies such as leukemia, monoclonal gammopathies) and 27% with altered immune status, majority in the age group over 60 years, because of age-related immunodefficiency (Fig. 1).

Data analysis concerning distribution of types of pathologies related to impaired immune status (endocrine diseases, infections of various etiologies, HCV) showed too an increased incidence age-related (90% from this group of patients had over 60 years old, only 2 patients being in the under 60 years study group) for infectious and endocrine pathologies, considering their possible immune suppressed status (Fig. 2).

Results obtained for TnT plasmatic levels in patients

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Fig. 1 Malignant and immunologic pathology distribution by age in the study group.

Fig. 2 Distribution of types of pathologies associated with altered immune status.

with immune altered status (in oncologic, endocrine and infectious pathology) and ACS symptoms (Fig. 3) showed that 34% of them had very high TnT values (range values between 1500-over 10000 pg/mL), uncorrelated with CK and CKMB dynamics. The highest TnT value (over 10000 pg/mL) was found in a breast cancer female, aged 60, with associated pulmonary pathology; the TnT marked increase level in this last case was described previously [8].

It is also possible that an endocrine disease

associated with a malignant hematological pathology may produce a false marked increase of TnT value (clinical case—4600 pg/mL in a 70 years old man with myeloma and hypothyroidism). In patients with hematological malignant/ possible malignanat pathology, miocardic enzymes levels showed an ordinary pattern, but for plasmatic TnT was obtained high/very high levels (even 62 times higher than reference range upper limit) against those reported for enzymatic activities (Table 1).

Fig. 3 TnT outliers in patients with immune altered status.

Table 1 Myocardial necrosis markers values in patients with malignant/ possible malignant haematological diseases.

Pathology	CK (U/L)	CK-MB (U/L)	LDH (U/L)	TnT (pg/mL)
Myeloma and hypothyroidism	1175	51.6	1139.3	4600
Modified FL, free Plt, hypergammaglobulinemia	123	16.0	349	1857
Leukemia	23	11.0	136	65.67
Severe anemia—To be investigated	545	56.0	-	1526

CKMB out of range values analysis, based on the upper limit of reference range (Fig. 4), showed that the higher values were registered in ACS patients with endocrine diseases - including an increase of 4.83 times, respective 2.15 fold in two patients with typical dynamics of enzymes in MI. However in this cases the TnT values were very high—80.43 respectively 153.3 times higher than the upper limit of the reference range, possibly due to the test method interferences with endocrine diseases specific antibodies.

Another patient, an 82 years old female, with oncologic pathology and severe hypothyroidism, had a 3.51 fold increase of CKMB and a 40.6 fold increase of TnT level, which may also be associated with malignant and endocrine diseases specific antibodies in this particular case (Table 2).

Analyzing the out of range values for selected miocardial biomarkers, based on the upper limit of reference range (Figs. 4 and 5) showed that the higher values were registered in ACS patients with endocrine

diseases (Table 2). The very high increase for TnT values may be possibly caused by the test method interferences with endocrine diseases specific antibodies, specifying that for the case 1 the severe hypothyroidism is associated with oncological pathology.

On the other hand, in one particular case not included in the above graphs—an 57 years old male with advanced stage malignant pathology without MI paraclinical signs (confirmed by 8.47 pg/mL TnT value), for a 88 U/L CK value and a 1465 LDH U/L value (this one as malignancy marker), we had obtained a CK-MB value much higher than CK (178.6 U/L). Also in this situation, this CKMB value greather than CK can be case-related to altered immune status of the patient. We could not exclude/confirm the presence in patient's sera of autoantibodies generating macro CK type 1 [9], because we do not use in our laboratory CK MB mass method.

When asked for determining of cardiac enzymes

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Fig. 4 CKMB values variation toward reference range upper limit in patients with immune altered status.

Fig. 5 TnT values toward reference ranges upper limits in patients with immune altered status.

Table 2 Myocardial necrosis markers values in patients with endocrine diseases.

Miocardial biomarker increase toward the upper limit of the reference range	CK-MB	TnT
Case 1	3.51 fold	40.6 fold
Case 4	4.83 fold	80.43 fold
Case 5	2.15 fold	153.3 fold

(CK, CK-MB and LDH), on specimens with identified hemolysis (phlebotomy or improper handling of biological samples being the cause of *in vitro* hemolysis) in pre-analytical phase and on repeated sampling from the same patient (compliant samples), where performed enzyme activity measurements. The results included in Fig. 6 showed a minimum growth of these parameters for minor (light) hemolysated specimens toward compliant samples for CK values. Our results indicate an increase between 1.2 to 2 times in hemolysed specimens for CK-MB, respectively between 1.29 to 1.53 times in case of serum LDH activity. For this reason, it is recommended to ask for repetition of sampling, in order to avoid misdiagnosis caused by the results provided by the laboratory, when the levels of cardiac enzymatic markers are near the upper limit of the reference range. The same recommendation is applicable to moderate hemolysed samples, in whose case was found an increase between 1.4 to 3.0 times toward compliant samples for all

miocardial enzymes which are involved in SCA diagnosis.

For cases 2 and 3 (Fig. 6), higher values of CK-MB (increases by 6.23 respectively 7.63 times over the upper limit of reference) obtained for samples with moderate degree of hemolysis may be caused by interference problems with drugs or by the clinical status of patients from department of interventional cardiology. This may be one possible explanation because on consistent samples samples, taken after few hours, results were obtained within the reference range for both CK-MB and LDH.

In severe hemolysed specimens, we had obtained much higher values for CK-MB and LDH enzymatic activities compared to non-hemolysed sera, two sets of selected data not included in previous charts being presented in Table 3.

The severe hemolysis [10] is a pre-analytical phase criteria for rejection of blood specimens applicable in our laboratory for the miocardial biomarkers request,

Fig. 6 Haemolysis degree influence on enzymatic markers values in miocardial necrosis.

Table 3 Influence of severe hemolysis on cardiac enzyme biomarkers.

Biomarker	Hemolysed (1)	Non-hemolysed (1)	Hemolysed (2)	Non-hemolysed (2)
CK (U/L)	231	196	78	70
CK-MB (U/L)	64.6	33.7	90.6	13.8
LDH (U/L)	411.7	296.8	684	349

even in the case of a critical patient, taking into account the importance of hemolysis degree as a valuable tool for quality assurance and in order to ensure patient safety in health care processes [11]. The clinicians need compliant data to take the right medical decisions for a correct diagnostic and for therapeutically purposes; they also must be warned on the probability of in vivo hemolysis, if it's not a biological sampling accident.

4. Conclusions

The study results confirmed the importance of hemolysis as QI established samples rejecting criteria in pre-analytical phase and the need for an effective communication between clinicians and medical laboratory specialist in both pre-analytical and post-analytical TTP phases. Considering these first data obtained, we propose to further study for checking the effect of the immune status on biomarkers values for diagnostic and monitoring in patients with ACS.

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