Short communication

Creatine-kinase MB mass: Age and sex-associated reference limits in two different platforms that use the same method

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Background: Recent updated NACB guidelines suggest that troponins are the biomarker of choice for the detection of myocardial necrosis, but the CK-MB mass is still considered an effective and alternative indicator when troponin assays are not available. The aim of the present study was to compare the reliability of two different analytical platforms in establishing the gender-specific 99th percentile for the CK-MB mass.

Methods: Serum samples collected from healthy subjects were investigated in two different laboratories. LAB 1 (354 subjects: 222 men, 132 women; median age, 40 years, range 19–64 years) and LAB 2 (330 subjects: 224 men, 106 women; median age, 41 years, range 18–71 years), in order to determine the CK-MB mass (µg/L) using the Access® CKMB® method (Beckman Coulter), a two-site immunoenzymatic sandwich assay, on UniCel® DxI 800 (LAB 1) and Access® 2 (LAB 2) analyzers (Beckman Coulter). The related plasma samples (lithium-heparin) were also evaluated in LAB 2.

Results: Total imprecision (CV%), calculated in control materials, ranged from 6.00 to 9.05 (concentration range, 3.82–36.37) in LAB 1 and from 7.05 to 5.02 in LAB 2 (concentration range, 3.63–34.18). A statistically significant gender-related difference (p< 0.05) was found in the whole population studied, values in men being higher than those in women: median = 1.86 vs 1.22; 99th percentile = 7.64 vs 5.19. The median values in subjects aged 18–28 years (group 1) were lower than those in the other 4 groups (2–5); 1.12 vs 1.59, vs 1.78 vs 1.95 and vs 2.03. The same age-related trend was also observed for CK-MB plasma values, which were comparable to those observed in the matched-serum samples: median 1.12 vs 1.10 (group 1), 1.45 vs 1.50 (group 5).

Conclusions: The two different analytical platforms provide comparable results. The finding that CK-MB mass values are significantly higher in males than in females represents a relevant information, that will impact on patient classification when a myocardial necrosis has been suspected. Actually, however, numerous assays commercially available, lack of this information.

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and 59 females, aged 18–71 years), the related plasma samples (lithium heparin) were evaluated using the Access® 2 platform.

Serum CK-MB mass was measured by a two-site immunoenzymatic (sandwich) assay using murine monoclonal anti-human CK-MB antibody-alkaline phosphatase conjugate and paramagnetic particles coated with murine monoclonal anti-human CK-BB. The addition of a specific ALP chemiluminescent substrate (Lumi-Phos 530), allows the release of a light signal that is detected by a luminometer. The measurand (antigen) in CK-MB calibrators (5 levels ranging from 0 to 300 µg/L) is traceable to AACC recombinant human CK-MB antigen.

The principle for both assays and reagents (capture and tracer antibodies, enzyme substrate, calibrators) were identical for the methods applied to two different platforms considered in our study, both systems being automated analyzer that provides random, continuous and STAT access.

The main difference concerns the throughput because UniCel® DxI 800 uses four reagent pipettors enabling the instrument to perform dilutions and to add reagents to four samples simultaneously. As stated by the manufacturer, the analytical sensitivity was lower than 0.10 µg/L, the reportable range 0.10–300 µg/L and the stability of calibration curve 56 days. LAB 1 carried out the assay on healthy subjects using UniCel® DxI 800 and LAB 2 on Access® 2 instruments.

The statistical analysis was performed using Analyse-it® software program for Microsoft Excel (Method Evaluation Edition, version 2.07) (Pearson's r, Mann Whitney and Altman Bland test): A p-value less than 0.05 was considered indicative of a statistically significant difference.

During the evaluation protocol, the total imprecision (CV%) calculated from control materials (Seronorm, Levels 1 and 2) ranged from 6.00 to 9.05 (range of concentrations 3.82–36.37 µg/L) for UniCel® DxI 800 and from 7.05 to 5.02 on the same control material for Access® 2 system (range of concentrations 3.63–34.18 µg/L). The obtained performance is in agreement with the manufacturer's claims (total CV% from 2.66 to 3.54, range of concentration 6.6–172.3 µg/L).

Considering the two study sites' populations, no differences in sex (Pearson's test, p=0.3350) and age (Mann Whitney test, p=0.8704) distributions were observed. Moreover CK-MB concentrations measured on the analytical platforms show a satisfactory correlation. In a previous study (data not published) CK-MB was measured in specimens collected from the laboratory routine analysis of hospitalized patients' samples showing concentrations from 0.59 to 107.36 µg/L (n=152, group 1, total population) being the CK-MB concentrations from 2 to 10 µg/L in 112 specimens (group 2). The linear regression analysis evidences the following results: group 1) CK-MB DxI 800=0.9638 CK-MB Access 2 −0.0681, R²=1.0 (Altman Bland test, bias, 95%CI=0.03, −0.09/+0.06); and group 2) CK-MB DxI 800=0.9626 CK-MB Access 2 +0.050, R²=0.99 (Altman Bland test, bias, 95% CI=−0.062, −0.093/+0.031) respectively. On the basis of these data, all the donors recruited in the two sites were grouped together and a population of 684 healthy subjects was used for the study.

The CK-MB concentrations, subdivided by gender, are reported in Table 1. A statistically significant gender-related difference was found (Mann–Whitney test, p<0.05), values in men being higher than those in women (median: 1.86 vs 1.22 µg/L); consequently, the 99th percentile was 1.47 fold higher in men (7.64 vs 5.19 µg/L). Fig. 1 shows the trend for CK-MB concentrations across the five age groups. An increase in values was found in relation to age, although an elderly adult population was not fully covered in the present study. Group 1 (18–28 years) had a median concentration of 1.12 µg/L (range: 0.0–8.89 µg/L) lower than those observed in all groups being 1.59 µg/L (range: 0.50–10.10 µg/L) and 1.78 µg/L (range: 0.40–8.43 µg/L) respectively. The median concentrations in groups 2 (29–38 years), 3 (39–48 years), 4 (49–58 years) and 5 (59–68 years) respectively. Even if the median values of groups increased with age, the analysis of covariance (ANCOVA) revealed no statistically significant effect of age (p=0.3350) on CK-MB concentrations. However, the median value of group 5 showed a 1.81 fold increase over that of group 1. The CK-MB values, observed using the Access® 2 platform in a subgroup of plasma samples, showed the same age-related trend, and the concentrations found were comparable to those observed in matched-serum samples being the median concentrations 1.10 µg/L (range: 0.30–2.70 µg/L); serum 1.1 µg/L; range: 0.0–2.80 µg/L in group 1 and 1.45 µg/L (range: 0.70–6.70 µg/L; serum 1.50 µg/L; range: 0.70–7.0 µg/L) in group 5, without any statistically significant differences being observed.

The results obtained in our study confirm data reported in the recent literature showing that CK-MB mass values were significantly higher in men than in women [3,4], men having a greater muscle mass and skeletal muscle turnover with higher measurable concentrations.

These relevant gender-related differences will impact on patient classifications and endpoints. To our knowledge, no clinical acute coronary syndrome, myocardial infarction or interventional trial or epidemiology study has taken gender-related differences into account when using CK-MB as discriminating biomarker [5,6]. Furthermore, in spite of the clinical relevance of this information to correctly classify patients with suspected acute myocardial infarction, a significant part of the assays commercially available lacking of these data. Moreover, our results provide the evidence-based appropriate and gender related serum cut-off concentrations for CK-MB assayed using an immuno nochemiluminescence method applied in two different platforms (Access® 2 and the UniCel® DxI 800 analyzers, Beckman Coulter) according to the recently published ESC/ACC [7] and the NACB documents [1], in which the gender-specific 99th percentile, has been proposed as the recommended myocardial infarction cut-off value. Our results are of value in order to appropriately use CK-MB measurements in the diagnosis of myocardial necrosis: in fact, even if cardiac troponins have been recognised as biochemical gold standard, the recently published guidelines [1,7] suggest CK-MB assays as the best alternative strategy. Noteworthy seem to be the findings showing that two different platforms, which used the same method but performed differently, provided comparable results. In fact, no significant difference has been found between their analytical performance (imprecision in particular),
and the absolute values provided for CK-MB mass. This approach allows, on the basis of the organizational needs, the simultaneous use of different platforms with different throughputs for the biochemical monitoring of patients with suspected cardiac diseases in the clinical and emergency settings.

References


