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# Use of ischemia modified albumin for the diagnosis of myocardial infarction

## *Uso da albumina modificada isquêmica no diagnóstico de infarto do miocárdio*

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### ABSTRACT

**Introduction:** Literature reports addressing ischemia modified albumin (IMA) as a good marker for the early diagnosis of myocardial ischemia through albumin cobalt binding (ACB) test, that is before myocardial infarction (MI) occurs. **Objective:** To evaluate the IMA plasmatic levels in infarcted patients, in order to verify its potential as an early marker for early diagnosis of MI, investigate its correlation with existing cardiac biomarkers such as total creatine kinase (CK) and creatine kinase-MB fraction (CK-MB), as well as to assess the correlation between IMA and oxidative stress. **Methods:** The sample was divided into two groups according to serum troponin I (TnI) results; one group of infarcted patients (with MI) (TnI levels higher than 0.05 ng/ml), and the other group of non-infarcted patients (without MI) (TnI levels lower than 0.05 ng/ml). The results of total CK, CK-MB, thiobarbituric acid reactive substances (TBARS), and IMA were analyzed in both groups. **Results:** Regarding the existing cardiac markers, there was a significant increase of total CK and CK-MB levels in With MI group. In relation to the oxidative stress parameter, a significant increase was observed in with MI group compared to without MI group. However, IMA showed no significant difference between the groups; and also there was no significant correlation between IMA and the cardiac markers. There was no correlation between IMA and TBARS. **Conclusion:** Our results suggest that IMA cannot be used alone for the diagnosis of MI.

**Key words:** serum albumin; myocardial ischemia; infarction.

### INTRODUCTION

Albumin is the most abundant protein in human blood plasma and its level may vary according to age. In neonates, its level is approximately 3.9 g/dl. It decreases to 2.8 g/dl at 9 months of age and increases slowly (3.5 g/dl to 5.5 g/dl) until the adult age<sup>(1)</sup>. For albumin perform its main functions – to maintain the colloid osmotic pressure of intravascular fluid and to bind several substances in blood plasma such as bilirubin, fatty acid, calcium ion, magnesium ion and various drugs<sup>(2)</sup> – these levels must be maintained. Albumin contains 585 amino acids and, under normal conditions, the N-terminal region of this protein forms the N-Asp-Ala-His-Lys sequence. The first three amino acids show greater metal-binding capacity and specificity. Although this region contains an inherent affinity site for cobalt (Co), it also binds tightly to copper (Cu) and nickel (Ni)<sup>(1-4)</sup>. However, when exposed to ischemia, hypoxia and/or free radical damage, the

N-terminal region of albumin is more susceptible to degradation when its ability to bind to metals is reduced, forming ischemia-modified albumin (IMA)<sup>(2,3)</sup>.

The reduction of albumin affinity by Co, Ni and Cu, caused by the change that occurs at the N-terminal region, increases the concentration of these free metals in the blood<sup>(3)</sup>. Such change can occur within minutes after an ischemic event<sup>(5)</sup> and quickly elevates the IMA levels in the blood<sup>(1)</sup>. Therefore, some studies proposed the use of IMA as a useful rule-out marker for the diagnosis of acute coronary syndrome<sup>(1,5,6)</sup>. The term acute coronary syndrome (ACS) refers to any group of clinical symptoms compatible with acute myocardial ischemia, including angina and myocardial infarction (MI). This ischemic process is a result of insufficient blood flow in cells and inadequate oxygen and nutrient supplies to the site affected. According to the World Health Organization (WHO), the diagnosis of ACS may be based on three criteria: clinical symptoms, alterations in the

electrocardiogram (ECG), and biochemical markers. However, these criteria have low specificity and sensitivity, indicating that the clinical symptoms are not specific enough, although their report is necessary; ECG shows 50% sensitivity; and, finally, the biochemical markers frequently used present late results, after tissue injury<sup>(1)</sup>.

Currently, the biochemical diagnosis of ACS is accomplished by the myocardial necrosis biomarkers most commonly used: cardiac troponins, creatine kinase-MB fraction (CK-MB) and total creatine kinase (total CK). However, these biomarkers increase after tissue injury, approximately 4 to 6 hours after the cardiac event<sup>(5)</sup> and detect only the consequences of prolonged ischemia. CK (EC-2.7.3.2) has several functions in cellular energy metabolism. It catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to adenosine diphosphate (ADP), to regenerate adenosine triphosphate (ATP)<sup>(7)</sup>. The major CK isoenzymes, whose names are given as a reference to the tissues in which they were historically isolated, creatine kinase BB fraction (CK-BB), and creatine kinase MM fraction (CK-MM) are found in the cytosol. Both isoenzymes exist as homodimers under specific physiological conditions and may be present as a heterodimer CK-MB in the heart. CK-MM and CK-MB isoforms can be easily detected in human serum. CK-MM is the main isoenzyme found in striated muscle (approximately 97% of the total CK). CK-MB is mainly found in cardiac muscle, where it comprises 15% to 40% of the total CK activity. However, trace amounts of CK-MB are found in skeletal muscle, therefore, patients with skeletal muscle injury will have increases in the absolute concentrations of total CK and CK-MB, but not associated with myocardial injuries. For this reason, it is used in combination with total CK and CK-MB measurements, and with cardiac troponins for the diagnosis of ACS. The regulatory troponin complex plays an important role in the regulation of striated muscle contraction. It consists of three different subunits – troponin C (TnC), troponin I (TnI) and troponin T (TnT). TnT and intracellular TnT are mostly bound to myofibrils in the cardiac myocyte, although a small amount of TnT (6%-8%) and TnI (3% to 4%) is found in the cytoplasm of myocardial cells. The elevation of both troponins in plasma is due to the continuous loss of myofibrils caused by ischemia. The great amount of troponins in myocytes suggests higher sensitivity and specificity of this test when compared to other markers<sup>(8, 9)</sup>. Data and clinical studies have shown that TnI is an early marker of myocardial injury<sup>(10)</sup>. However, it is important to recognize that troponin elevations can also be detected in conditions other than ACS, for example, in pulmonary embolism, stroke and severe renal insufficiency<sup>(8, 9)</sup>.

Although the biomarkers most widely used for the diagnosis of ACS are cardiac troponins, we must consider that improving the diagnosis of myocardial ischemia is still required, since it occurs before tissue necrosis, i.e. before MI. Despite the fact that ECG (along with the stress testing) is the most commonly used, it is not considered the gold standard for diagnosing heart diseases<sup>(11)</sup>.

IMA has been recently licensed by the US Food and Drug Administration (FDA) for the diagnosis of suspected myocardial ischemia. It is considered a very sensitive marker of myocardial ischemia. Although it has a high negative predictive value, IMA detection can corroborate the early diagnosis of cardiac ischemia and other existing conventional biomarkers<sup>(12)</sup>. However, high levels of IMA are found in many inflammatory diseases and also in diseases associated with oxidative stress, but little is known about the IMA levels in patients with ACS.

Considering that the N-terminal region of albumin is modified when exposed to ischemia, hypoxia, acidosis and free radical damage, and that its presence in the serum is an indicator of abnormalities, the main objective of the present study was to evaluate the plasmatic levels of IMA in patients with MI to verify its potential as a marker for the early diagnosis of MI, and to investigate its correlation with existing cardiac biomarkers, as well as to assess the association between IMA and oxidative stress.

## METHODS

The study used aliquots from the Hospital Getúlio Vargas, in the city of Sapucaia do Sul (RS), Brazil without interfering in the exams required for the patient in question. The levels of serum IMA were measured at the Laboratório de Pesquisa em Biofísica Celular e Inflamação of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) after obtaining approval by the Research Ethics Committee of the institution (protocol 254965).

## Samples

During the month of April, 2014, samples of individuals of both sexes and different age groups were analyzed. They were divided into two groups: with MI and with no MI. Only individuals admitted in the hospital with chest pain were included in the study. The without MI group ( $n = 32$ ) comprised patients with TnI levels lower than 0.05 ng/ml. The with MI group ( $n = 24$ ) comprised patients with TnI levels higher than 0.05 ng/ml. In both groups, the presence of hemolysis and a small sample size were used as exclusion criteria.

## Sample collection and processing

For sample collection, tubes without anticoagulant and with gel separator were used. Sample processing occurred after a maximum of 4 hours after the patient was admitted to the hospital emergency department. Upon arrival in the laboratory, the samples were centrifuged at 1,000 g for 10 minutes. For the albumin cobalt binding (ACB) test and the measurement of thiobarbituric acid reactive substances (TBARS) concentration, 450 µl of patient's serum was extracted, stored at -20°C until both tests were conducted. The aliquots were separated after completion and release of CK-MB, total CK and TnI tests, requested by the patient's physician. The samples remained frozen for approximately 4 weeks.

## Measurement of cardiac markers

The total CK and CK-MB levels were measured using a CM200 clinical analyzer (Wiener Lab) by optimized ultraviolet (UV) method. The test results were expressed as U/l. The immunochromatographic assay (Bioassay) was used for the qualitative detection of cardiac TnI. The minimum detection level was 0.5 ng/ml. The results were expressed as ng/ml. The positive results (with test line) and the negative results (without test line) were higher or lower than 0.5 ng/ml, respectively.

## Measurement of oxidative stress marker

The measurement of TBARS was carried out by means of a commercial reagent kit (Cayman Chemical Company), which is used to measure the formation of malondialdehyde (MDA), a naturally occurring product of lipid peroxidation induced by reactive oxygen species (ROS) in membrane lipids<sup>(13)</sup>. The results were expressed as µM.

## Measurement of IMA

IMA was determined using the ACB test, a spectrophotometric assay that measures the binding of Co to albumin in serum samples. As described in some studies, during ischemia, the metal binding capacity of albumin for certain transition metals like Co is reduced<sup>(14-16)</sup>. Therefore, the concentration of IMA was determined by the addition of a known amount of exogenous Co (CoCl<sub>2</sub>) to a serum specimen and measurement of unbound cobalt using a colorimetric assay after adding a coloring substance – dithiothreitol (DTT) – which binds any excess (unbound) Co. This assay is based on the inverse relationship between the intensity and formation of the color<sup>(14, 16)</sup>.

The ACB test was performed according to the protocol previously described<sup>(17)</sup>. This test measures the intensity of the

color formed as a result of the reaction between DTT and unbound Co to albumin. For this technique, 96-well test plates were used and kept at room temperature. This procedure was prepared in duplicate wells. The ACB test was prepared by mixing 95 µl of the patient's serum, 5.0 µl of 1.0 g/l solution of Co chloride, 20 µl of barbital and homogenization, followed by 5 minutes incubation in water bath at 37°C. After incubation, 25 µl of DTT was added to the mixture. Again it was homogenized and then incubated for 2 minutes. The absorbance was measured at 492 nm (ASYS Expert Plus microplate reader, Biochrom). The blank of the essay was prepared similarly, after excluding DTT from the assay. All reagents, including cobalt chloride and DTT, were purchased from Sigma-Aldrich. The result was provided by the absorbance reading.

## Statistical analysis

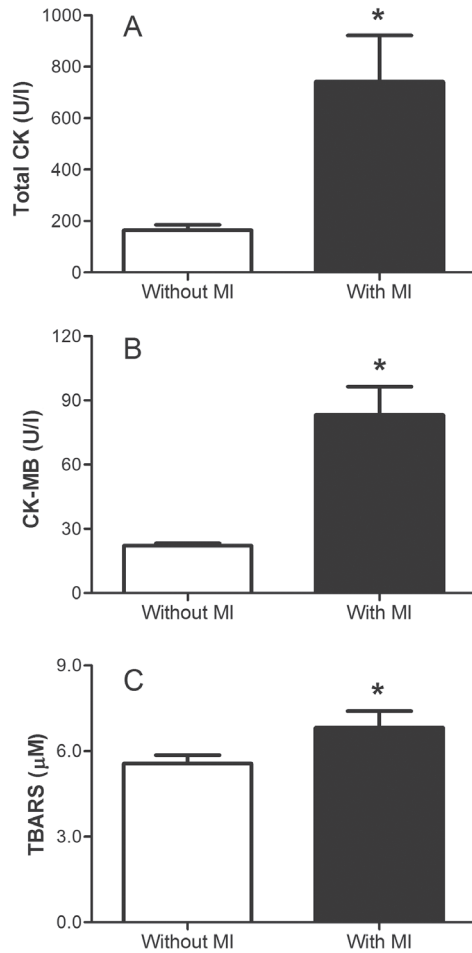
The results were expressed as means ± standard error of the mean (SEM). The Shapiro-Wilk test was applied to determine the normality of the data. Student's *t*-test and Pearson's correlation were used for statistical analysis. The analyses were performed using Statistical Package for the Social Sciences Version 18.0 (SPSS Inc.). The level of significance was set at 5%.

## RESULTS

The Shapiro-Wilk test confirmed the normal correlation between the data and the corresponding normal scores. The results showed a significant increase of total CK levels in group with MI ( $741.8 \pm 180.6$  U/l) compared to without MI group ( $164.7 \pm 20.5$  U/l),  $p < 0.05$  (**Figure 1A**). There was also an increase in CK-MB levels (**Figure 1B**) in group with MI ( $83.2 \pm 13.1$  U/l) compared to group without MI ( $22.1 \pm 1.0$  U/l),  $p < 0.05$ . The presence of oxidative stress was confirmed by the increased levels of TBARS: the group with MI (**Figure 1C**) showed a significant increase ( $6.8 \pm 0.6$  µM) compared to the group without MI ( $5.5 \pm 0.3$  µM),  $p < 0.05$ . However, the IMA levels (**Figure 2**) showed no significant difference between group with MI ( $0.780 \pm 0.050$ ) and group without MI ( $0.770 \pm 0.042$ ). **Figures 3A, 3B and 3C** show no significant correlation of IMA levels with cardiac biomarkers total CK and CK-MB, neither with oxidative stress marker TBARS ( $r = 0.055$ ,  $r = 0.092$  and  $r = 0.093$ , respectively).

## DISCUSSION

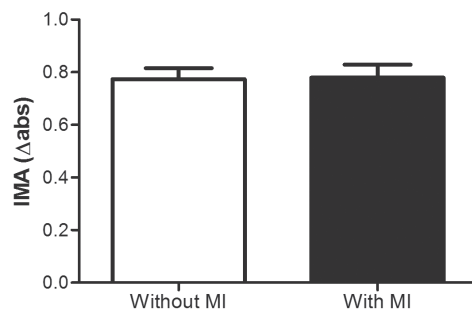
MI is now considered part of a spectrum referred to as acute coronary syndrome and is one of the main events caused by myocardial ischemia that can result in irreversible myocardial cell



**FIGURE 1** – Quantification of cardiac and oxidative stress makers in different experimental groups

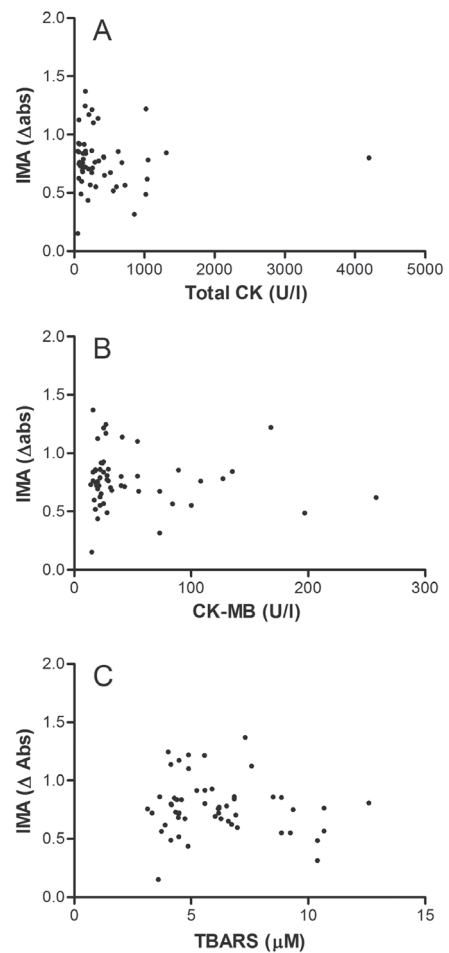
A) total CK; B) CK-MB; C) TBARS. Data are expressed as mean  $\pm$  SEM; \*  $p < 0.05$  when compared to group without MI.

CK: creatine kinase; CK-MB: creatine-kinase MB fraction; TBARS: thiobarbituric acid reactive substances; SEM: standard error of the mean; MI: myocardial infarction.



**FIGURE 2** – IMA in different experimental groups. Data are expressed as mean  $\pm$  SEM

IMA: ischemia modified albumin; SEM: standard error of the mean; MI: myocardial infarction.



**FIGURE 3** – Correlation between IMA and cardiac and oxidative stress markers in patients with MI and those without MI

A) correlation between IMA and total CK ( $r = 0.055$ ); B) correlation between IMA and CK-MB ( $r = 0.092$ ); C) correlation between IMA and TBARS ( $r = 0.093$ ).

IMA: ischemia modified albumin; MI: myocardial infarction; CK: creatine kinase; CK-MB: creatine kinase-MB fraction; TBARS: thiobarbituric acid reactive substances.

damage or death<sup>(16)</sup>. Some studies suggest that unlike injury and cellular necrosis markers, such as total CK, CK-MB and TnI, IMA can be used as a marker for the early prediction of myocardial ischemia<sup>(1, 15, 16)</sup>. According to Sinha<sup>(18)</sup>, IMA sensitivity for the diagnosis of acute ischemic chest pain is significantly higher than that of ECG and TnT. These results corroborate the findings of Christenson<sup>(5)</sup>, who also observed high sensitivity and high negative predictive values of IMA, demonstrating that the ACB test could be used to safely identify low-risk patients, and therefore, reduce the admission of patients in emergency hospitals. However the presence of IMA may not confirm myocardial ischemia but other medical conditions such as diabetes mellitus, peripheral vascular disease, glaucoma, skeletal muscle ischemia and systemic sclerosis<sup>(12)</sup>.

Ertekin<sup>(19)</sup> reported that in early ischemia there is an increase in the IMA levels, which remain high. These findings corroborate those of other studies<sup>(1, 5, 12)</sup> that confirmed the efficiency of IMA as an early marker in the diagnosis of myocardial ischemia. In addition, Christenson<sup>(5)</sup> demonstrated that IMA can be an early predictor of TnI results after 6-24 hours in patients with ACS, suggesting an association between IMA and TnI. In contrast, our results did not show an association between IMA and the cardiac markers total CK and CK-MB analyzed in this study. However, our results confirmed those found in previous studies<sup>(15, 16)</sup> that showed that the ACB test does not differentiate ischemic from non-ischemic patients, although there is an increase in IMA levels before the TnI levels increase in patients with myocardial ischemia<sup>(1)</sup>.

The great disadvantage of IMA is related to the concentration of albumin in serum. This proposes the exact need to evaluate IMA values together with those of albumin to avoid possible false-positive or false-negative values in individuals with hypoalbuminemia or hyperalbuminemia<sup>(12)</sup>. Moreover, Michelis<sup>(20)</sup> suggested that oxidative modifications of serum albumin led to underestimation of albumin concentrations using conventional assays. When there is an imbalance between activities of ROS and their detoxification by cellular defense mechanisms, known as antioxidants, oxidative stress occurs<sup>(21)</sup>. According to Ellidag<sup>(12)</sup>, there is evidence that oxidative stress

plays an important role in cardiac pathologies. It reduces metal to albumin-binding capacity, including Co. However, measurement of ROS, which cause oxidative stress, becomes a challenge because most ROS are highly reactive and short lived<sup>(22)</sup>. Techniques to measure these reactive intermediates have been extensively reviewed. In the present study we have confirmed the damage caused by ROS in patients with MI and in patients with no MI using the TBARS technique. There was a significant increase of TBARS concentration among these patients, however, no relationship was observed between IMA and TBARS, suggesting that there is no positive correlation between oxidative damage and the presence of IMA. This finding is in agreement with that of Ellidag<sup>(12)</sup> who did not observe any correlation between IMA and oxidative stress markers.

## CONCLUSION

In conclusion, the levels of total CK, CK-MB and TBARS were increased in patients with MI, however, IMA levels showed no difference between patients with MI and those without MI. Likewise, there was a correlation of IMA with the cardiac markers and TBARS. Therefore, our results suggest that IMA cannot be used alone for the diagnosis of MI because the results may depend on the concentration of serum albumin detected in the patient.

## RESUMO

**Introdução:** Relatos na literatura abordam a albumina modificada isquêmica (AMI) como um bom marcador precoce para o diagnóstico de isquemia miocárdica por meio do albumin cobalt binding (ACB) test, ou seja, antes do infarto do miocárdico (IM). **Objetivo:** Avaliar os níveis plasmáticos de AMI em pacientes infartados a fim de verificar o seu potencial como marcador precoce para o diagnóstico antecipado do IM, investigar sua correlação com os biomarcadores cardíacos já existentes, como creatinaquinase (CK) total e creatinaquinase fração MB (CK-MB), além de avaliar a correlação de AMI com o estresse oxidativo. **Métodos:** Foram separados dois grupos de acordo com resultados séricos da troponina I (TnI), um com pacientes infartados (TnI superior a 0,05 ng/ml) e outro com pacientes não infartados (TnI inferior a 0,05 ng/ml). Foram analisados os resultados de CK total, CK-MB, substâncias reativas ao ácido tiobarbitúrico (TBARS) e AMI em ambos os grupos. **Resultados:** Em relação aos marcadores cardíacos existentes, houve aumento significativo de CK total e CK-MB no grupo dos infartados; já em relação ao parâmetro de estresse oxidativo, foi observado aumento significativo no grupo dos infartados quando comparado com o dos não infartados. Contudo, a AMI não apresentou diferença significativa entre os grupos; também não houve correlação relevante entre AMI e os marcadores cardíacos, bem como não foi observada correlação de AMI com TBARS. **Conclusão:** Nossos resultados sugerem que AMI não pode ser utilizada isoladamente como diagnóstico de IM.

**Unitermos:** albumina sérica; isquemia miocárdica; infarto.



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