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Complement activation in acute myocardial infarction: An early marker of inflammation and tissue injury?

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ABSTRACT

Background: Acute myocardial infarction (AMI) is a potentially fatal condition, being a major cause of death worldwide. Ischemia suffered during AMI causes tissue damage, leading to an inflammatory process. Moreover, myocardial injury can generate damage-associated molecular patterns that activate pattern recognition molecules including some complement proteins.

Methods: Here we investigated products of complement activation, C3d and soluble C5b9 (sC5b9), as potential biomarkers for myocardial injury and inflammation, as well as serum cytokines (IL-6 and TNF-alpha), alpha-1-acid glycoprotein (AGP), and classical markers of myocardial necrosis (creatine kinase, creatine kinase-MB isoform, myoglobin and troponin-I) in a longitudinal study of patients with AMI (from admission, 6 h and 12 h post admission, and at discharge from hospital). Individuals undergoing cardiac catheterization (CC) with normal coronary arteries and asymptomatics with no history of cardiovascular disease or invasive procedures were included as controls.

Results: Plasma C3d was higher in AMI at admission, 6 h, 12 h, and discharge *vs* CC ($p < 0.0001$; $p = 0.0061$; $p = 0.0081$; $p = 0.044$) and asymptomatic ($p = 0.0001$ for admission, 6 h and 12 h; $p = 0.0002$ for discharge). Moreover, sC5b9 was higher only at admission and 6 h *vs* asymptomatic ($p = 0.0031$ and $p = 0.0019$). Additionally, AGP levels were elevated at admission, 6 h, 12 h, and discharge *vs* asymptomatic ($p = 0.0003$; $p = 0.0289$; $p = 0.0009$, $p = 0.0017$). IL-6 concentration was low at admission and 6 h and reached a peak at 12 h ($p < 0.0001$ for all groups). All classical markers of myocardial necrosis presented higher concentration at 6 h.

Conclusions: Our results showed that complement activation is an early event in AMI occurring before the elevation of classical markers of myocardial necrosis such as creatine kinase, creatine kinase-MB isoform, myoglobin and troponin-I. These findings indicated C3d and sC5b9 as possible biomarkers for inflammation and tissue damage in AMI.

1. Introduction

Cardiovascular diseases (CVD) are the primary cause of death worldwide with 17.7 million deaths in 2015. Among these, an estimated 7.4 million were attributed to coronary heart disease [1]. Acute myocardial infarction (AMI) is an event of myocardial necrosis caused by an unstable ischemic syndrome, and is a potentially fatal condition if not promptly and correctly managed [2]. So, an early diagnosis of AMI is crucial for the timely institution of pharmacotherapy in order to prevent myocardial damage and preserve cardiac function. Ischemic insults during AMI cause myocardial tissue damage, leading to a potent inflammatory process [3] and releasing of heart muscle proteins [4], both useful as molecular diagnostic markers. Among cardiac enzymes used as biochemical markers of myocardial damage are total creatine kinase (CK), creatine kinase-MB isoform (CK-MB) and troponin [5].

During myocardial infarction event several innate immune pathways are activated. The necrotic myocardial injury can generate damage-associated molecular patterns that activate pattern recognition receptors including those of the complement system in the early steps of the inflammatory response following infarction [3]. The complement

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consists of more than 35 tightly regulated proteins that play an important role in host defense and inflammation. Complement proteins are widely distributed in the circulation and in tissues, being synthesized and secreted by a number of cells under various stimuli, including cytokines and hormones [6]. It can be activated by the classical, lectin and alternative pathways culminating in the formation of C3 convertases which cleaves the component C3 in C3a and C3b, small and large fragments respectively. The incorporation of C3b to C3 convertases results in the formation of the C5 convertases, which cleaves C5 into C5a and C5b, ultimately resulting in the formation of the multimeric MAC (C5b9) [7]. The fragment C3b plays an important role in the opsonization of pathogens, clearance of immune complexes, B cell activation and amplification of the three pathways. Due to the potential activation of complement cascade during the inflammatory response, C3b is strictly regulated and can be cleaved by Factor I in the presence of its cofactors such Factor H, complement receptor 1 (CR1) and membrane cofactor protein in the final products iC3b, C3dg and C3d [8,9].

C3 breakdown products and leukocyte infiltration have been demonstrated in infarcted myocardium of rats [10]. Moreover, complement inhibition consistently attenuated leukocyte recruitment following myocardial infarction highlighting the critical role of the complement cascade in triggering inflammation in the ischemic myocardium [11]. In addition, Yasuda et al. [12] investigated the role of complement as a mediator of myocardial inflammation by quantifying the products of complement activation, C3d, C4d, Bb, and sC5b-9, in patients with AMI, unstable angina pectoris, stable angina pectoris and normal volunteers. These authors found that plasma C4d, Bb, and sC5b-9 were increased only in patients with AMI, while C3d levels increased in both patients with AMI and with unstable angina pectoris. Thus, stable angina pectoris was not related to complement activation [12]. On the other hand, plasma levels of C3 and C4 were found elevated in acute coronary syndromes and stable angina. However, the systemic levels of inflammatory markers in patients with stable angina were lower than those found in the AMI [13]. More recently, serum elevation of C1r and C3 but low Factor B in the early phase of AMI was demonstrated with C1r levels being correlated with necrotic mass of the myocardium and troponin-T levels [14]. In addition, C3, C4 and C5b9 were found to be significantly elevated in the early phase of acute coronary syndrome patients one day after admission [15], reinforcing the involvement of complement activation in the pathophysiology of myocardial damage.

Although several studies have accessed complement activation and heart function enzymes in AMI, there is still necessity for more accurate biomarkers or signatures that could be helpful in early AMI diagnosis or prognosis. In this study we aimed to evaluate products of complement activation C3d and sC5b9 as biomarkers of early cardiomyocyte injury. For this, during AMI plasma concentration of C3d and sC5b9, inflammatory cytokines (IL-6 and TNF-alpha), acute phase protein (alpha-1-acid glycoprotein) and classical markers of cardiomyocyte injury (creatine kinase, creatine kinase-MB isoform, myoglobin and troponin-I) were measured and correlated with cardiac impairment since admission to hospital, following up to 6 h and 12 h post admission and at discharge of patients with AMI attended at an University Hospital, in Southern Brazil. This is the first study investigating products of complement activation C3d and sC5b9 at an early phase (admission, 6 h and 12 h post-admission) amongst AMI patients.

2. Material and methods

2.1. Patients and controls

A total of 17 patients with acute myocardial infarction (AMI) attended at the University Cajuru Hospital in Curitiba, Southern Brazil, were investigated [mean age 58.35 years; 2 (11.76%) female; 15 (88.24%) male]. AMI diagnosis was based on at least two of the following criteria [5,16]: 1. Angina symptoms with more than 30 min.; 2. Electrocardiographic changes such as: ST-segment elevation (STEMI) of 1 mm or more in at least 2 leads, with or without Q-wave association; 3. Increased cardiac enzymes in serum (CK, CK-MB, troponin-I and myoglobin) at least twice the upper reference limit. The patients reported the beginning of symptoms up to 5 h before their hospital admission. After patient consent in participating in the study, peripheral blood was collected in four times: at admission, 6 h post admission (6 h), 12 h post admission (12 h), and at discharge. All the patients were admitted at Chest Pain Unit (CPU) of the hospital, with a preferential management in order to reduce the delay of door-to-balloon time. Four patients (23.5%) with AMI died, three of them at 6 h and one at 12 h. These patients are presented with black color in the figures.

As controls a total of 17 patients undergoing cardiac catheterization (CC) with normal coronary arteries attended at the University Caruju Hospital were investigated [CC.: mean age 48.59 years; 9 (52.94%) female; 8 (47.06%) male]. And, 13 asymptomatic individuals with no history of cardiovascular disease or invasive procedures were investigated [(asymptomatic: mean age 39.92 years; 5 (38.46%) female; 8 (61.54%) male]. For CC and asymptomatic controls only one sample of peripheral blood was collected due to difficult in maintain these individuals hospitalized since they had no clinical complains. Formal written consent was obtained from each individual and the study was approved by the local medical ethics committee.

2.2. Clinical and laboratory findings

For evaluation of complement activation, blood samples were collected in tubes containing EDTA and kept in ice at all time. The samples were centrifuged at 4 °C, plasma were harvested, aliquot and kept at −80 °C until use. Complement plasma C3d and sC5b9 levels were assessed by double-decker rocket immunoelectrophoresis using the antibody anti-Human C3d Complement (Cat. nº A006302, Dako) [17] and Enzyme-linked immunosorbent assay (ELISA) using the antibody anti-Complement C5b9 (Cat. nº DIA 011-01, Bioporto) [18], respectively.

Serum CK and CK-MB measurement were performed with CK and CK-MB UV Test kits (Merck) exactly as recommended by the manufacturer. Alpha-1-acid glycoprotein (AGP) was measured by nephelometry (Boehringer Nephelometer). Troponin-I and myoglobin were measured by ELISA. Serum levels of IL-6 and TNF-alpha were measured by ELISA (R&D systems). Reference values: Total CK ♂ 35-232U/L and ♀21-215U/L, CK-MB until 24 U/L, Myoglobin ♂ 10–95 µg/L and ♀10-65 µg/L, Troponin-I 0-0.1 ng/mL, AGP ♂ 50–135 mg/dL and ♀40-120 mg/dL.

2.3. Statistical analysis

The normality distribution of each variable was assessed by Shapiro-Wilk test. Cardiac biochemical parameters, inflammatory proteins and complement activation products levels were assessed in AMI patients and compared among different times of the study (admission, 6 h, 12 h and discharge) using Kruskal-Wallis test (with Dunn's Multiple Comparison Test) or Mann Whitney test for AMI groups *vs* controls (CC or asymptomatic). Pairwise associations were done using Spearman's rank correlation test for nonparametric variables. Statistical analysis was undertaken using the STATA 12.0 (StataCorp, College Station, Texas, USA) and p-values < 0.05 were considered statistically significant. The GraphPad Prism program (version 6.0) (GraphPad Software, La Jolla, CA, USA) was used to generate graphics that show median and interquartile range.

3. Results

3.1. Clinical evaluation of AMI patients and controls

Clinical evaluation of AMI patients are presented in Table 1. Among