Immunology Letters 200 (2018) 18–25

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

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

Lorena Bavia[1,2], Kárita Cláudia Freitas Lidani[1,2], Fabiana Antunes Andrade[2], Miguel Ibraim Abboud Hanna Sobrinho[2], Renato Mitsunori Nisihara[2], Iara Jose de Messias-Reason[*,2]

Laboratory of Molecular Immunopathology, Clinical Hospital, Federal University of Paraná, Curitiba, PR, Brazil

ARTICLE INFO

Keywords:
Complement activation
Acute myocardial infarction
Biomarker
C3d
sC5b9
Alpha-1-acid glycoprotein

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

* Corresponding author at: Setor de Ciências da Saúde, Hospital de Clínicas, Universidade Federal do Paraná, Rua General Carneiro 181, 80060-900, Curitiba, PR, Brazil.
E-mail address: iara.reason@hc.ufpr.br (I.J. de Messias-Reason).
[1] These authors contributed equally.
[2] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

https://doi.org/10.1016/j.imlet.2018.06.006
Received 15 February 2018; Received in revised form 19 May 2018; Accepted 13 June 2018

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

Table 1
Clinical parameters of AMI patients.

Parameters	AMI patients Total (N = 17)
ECG	Number (%)
Region	
Anterior heart wall	12 (70.6)
Inferior heart wall	03 (17.6)
Other	02 (11.8)
Description	
ST-segment	07 (41.2)
ST-segment and Q-wave	09 (52.9)
Other	01 (5.9)
Catheterization	Number (%)
Day of hospitalization[a]	18 (1,1/patient)
Not performed	03 (17.6)
1st	12 (70.6)
4th	01 (5.9)
5th	01 (5.9)
14th	01 (5.9)
Artery lesion[b]	14 (82.4)
Anterior descending	14 (82.4)
Right coronary	05 (29.4)
Left marginal	02 (11.8)
Left coronary trunk	03 (17.6)
Others arteries	02 (11.8)
Treatment	Number (%)
Thrombolytic	01 (5.9)
Angioplasty[c]	10 (58.8)
Anterior descending	09 (52.9)
Right coronary	02 (11.8)
Surgery	02 (11.8)
Evolution	Number (%)
Good	13 (76.5)
Death	04 (23.5)
Follow-up type	Number (%)
Angioplasty	08 (47.1)
Streptokinase	02 (11.8)
Clinical treatment	03 (17.6)
Death	04 (23.5)
Door-to-balloon time	Number (median, min-max)
Hours	17 (03, 02-05)
Discharge time/death (days)	Number (median, min-max)
Total	17 (08, 01-29)
Discharge	13 (09, 06-29)
Death	04 (01, 01-05)

Note:
[a] In one patient two catheterizations were performed, one on the first day and the other on the fourteenth day of hospitalization.
[b] More than one artery lesion per patient was observed.
[c] In one patient two angioplasties were performed: descending anterior and right coronary arteries.

the 17 patient with AMIs, twelve (70.6%) of them presented alterations in the anterior heart wall, and three (17.6%) in the inferior heart wall, besides two (11.8%) in other regions at admission to the hospital. In addition, nine (52.9%) patients presented STEMI and Q-wave, while seven (41.2%) presented only STEMI and one (5.9%) presented intraventricular conduction abnormality. The angiographic revealed that 14 patients (82.4%) presented lesion in the left anterior descending artery, five patients (29.4%) presented lesion in the right coronary artery, two patients (11.8%) presented lesion in the left marginal artery and two patients (11.8%) presented lesion in other arteries. In 8 patients (47%) were found lesion in more than one artery. Only one patient received thrombolytic treatment and two patients (11.8%) had undergone surgery intervention. The procedure of cineangiocoronariography was performed in 14 (82.4%) patients with AMI. In 12 patients (70.6%) this evaluation was performed on the first day of hospitalization and in 2 patients the cineangiocoronariography was performed on the fourth and fifth days of hospitalization. The AMI patients stayed at the hospital 12.8 +/− 8.1 days and 76.5% did not presented complications. Four patients (23.5%) died between the 1st and 5th days of hospitalization (Table 1).

Regarding the clinical alterations in cardiac catheterization (CC) group, the hemodynamic evaluation showed that two (11.8%) patients presented normal echocardiography, eight (47.1%) patients presented left ventricular hypertrophy, four (23.5%) patients presented diffuse hypocontractility, three patients (17.6%) presented localized akinesia and one patient presented mitral valve prolapse. One of the patients presented both diffuse hypocontractility and localized akinesia. No clinical alteration in asymptomatic group was recorded.

Myocardial damage was evaluated by total serum CK, CK-MB, troponin-I and myoglobin (Fig. 1A–D). CK concentration in AMI patients were significantly higher in the samples collected at 6 and 12 h (Median: 620.5 and 363U/L, respectively) than at admission (73U/L; $p < 0.001$ and $p < 0.05$, respectively), discharge (27U/L; $p < 0.0001$ to both 6 h and 12 h), CC (30U/L; $p < 0.0001$ to both 6 h and 12 h) and asymptomatic controls (35U/L; $p = 0.0003$ and $p = 0.0001$). Besides that, CK at admission (73U/L) was significantly higher when compared with CC (30U/L; $p = 0.0038$) and no difference in CK concentrations between CC and asymptomatic groups (30U/L *vs.* 35U/L; $p = 0.3996$) was observed (Fig. 1A). In the same way, CK-MB concentration was significantly increased at 6 and 12 h (51.5 and 27U/L) when compared with admission (4U/L; $p < 0.0001$ and $p < 0.05$, respectively), discharge (2.5U/L; $p < 0.0001$ to both 6 h and 12 h), CC (4U/L; $p < 0.0001$ to both 6 h and 12 h) and asymptomatic controls (6.5U/L; $p < 0.0001$ and $p = 0.0005$). Additionally, CK-MB concentration was significantly higher in CC than asymptomatic ($p = 0.0237$). However, there was no difference on CK or CK-MB concentrations at 6 h *vs.* 12 h (Fig. 1B).

Troponin-I, a gold standard biomarker for AMI, concentration was significantly increased at 6 h and 12 h (Median: 69.7 ng/mL and 50.3 ng/mL) when compared with admission (3.51 ng/mL; $p < 0.05$ and $p < 0.001$, respectively), discharge (2.5U/L; $p < 0.05$ and $p < 0.001$, respectively), CC (0.78 ng/mL; $p = 0.0012$ and $p = 0.0003$, respectively) and asymptomatic controls (0.49 ng/mL; $p < 0.0001$ for both 6 h and 12 h). Additionally, troponin-I levels at admission, and CC were significantly higher than in asymptomatic ($p = 0.0002$and $p = 0.0053$, respectively) (Fig. 1C). Myoglobin serum level was significantly increased at admission, 6 h and 12 h (Median: 125 µg/L, 418 µg/L and 97 µg/L) in AMI patients than CC (25 µg/L; $p = 0.0004$, $p < 0.0001$ for both 6 h and 12 h) and asymptomatic controls (25 µg/L; $p = 0.0027$, $p < 0.0001$, and $p = 0.0003$). Additionally, higher levels of myoglobin were also observed at discharge than CC control (36.5 µg/L *vs.* 25 µg/L; $p = 0.0118$) (Fig. 1D).

3.2. Evaluation of early inflammation in AMI patients and controls

Serum AGP concentration (Fig. 1E) was increased at admission (Median: 134 mg/dL) *vs* 6 h (103 mg/dL; $p < 0.05$), CC (101 mg/dL; $p = 0.0002$) and asymptomatic (83 mg/dL; $p = 0.0003$). On the other hand, serum AGP concentration was lower at 6 h *vs* discharge (158 mg/dL; $p < 0.001$), and higher concentration than asymptomatic ($p = 0.0289$). Besides that, AGP levels were higher at 12 h (133 mg/dL) and discharge when compared with CC ($p = 0.0005$ and $p = 0.0002$) and asymptomatic ($p = 0.0009$ and $p = 0.0017$) controls.

Serum IL-6 concentration (Fig. 1F) was increased at 12 h (Median: 14.18 pg/mL) than admission (0 pg/mL) and discharge (0.05 pg/mL), $p < 0.0001$ for both groups. In addition, serum IL-6 was higher at admission, 6 h (5.96 pg/mL) and 12 h post admission, and discharge groups than CC [0 pg/mL ($p = 0.01$; $p < 0.0001$; $p < 0.0001$; $p = 0.0064$)] and asymptomatic controls [0 pg/mL ($p = 0.03$; $p < 0.0001$; $p < 0.0001$; $p = 0.0179$)] groups. Serum TNF-alpha concentration was detected at admission only in two patients (37 pg/mL and 1.32 pg/mL). The patient presenting higher levels of TNF-alpha had

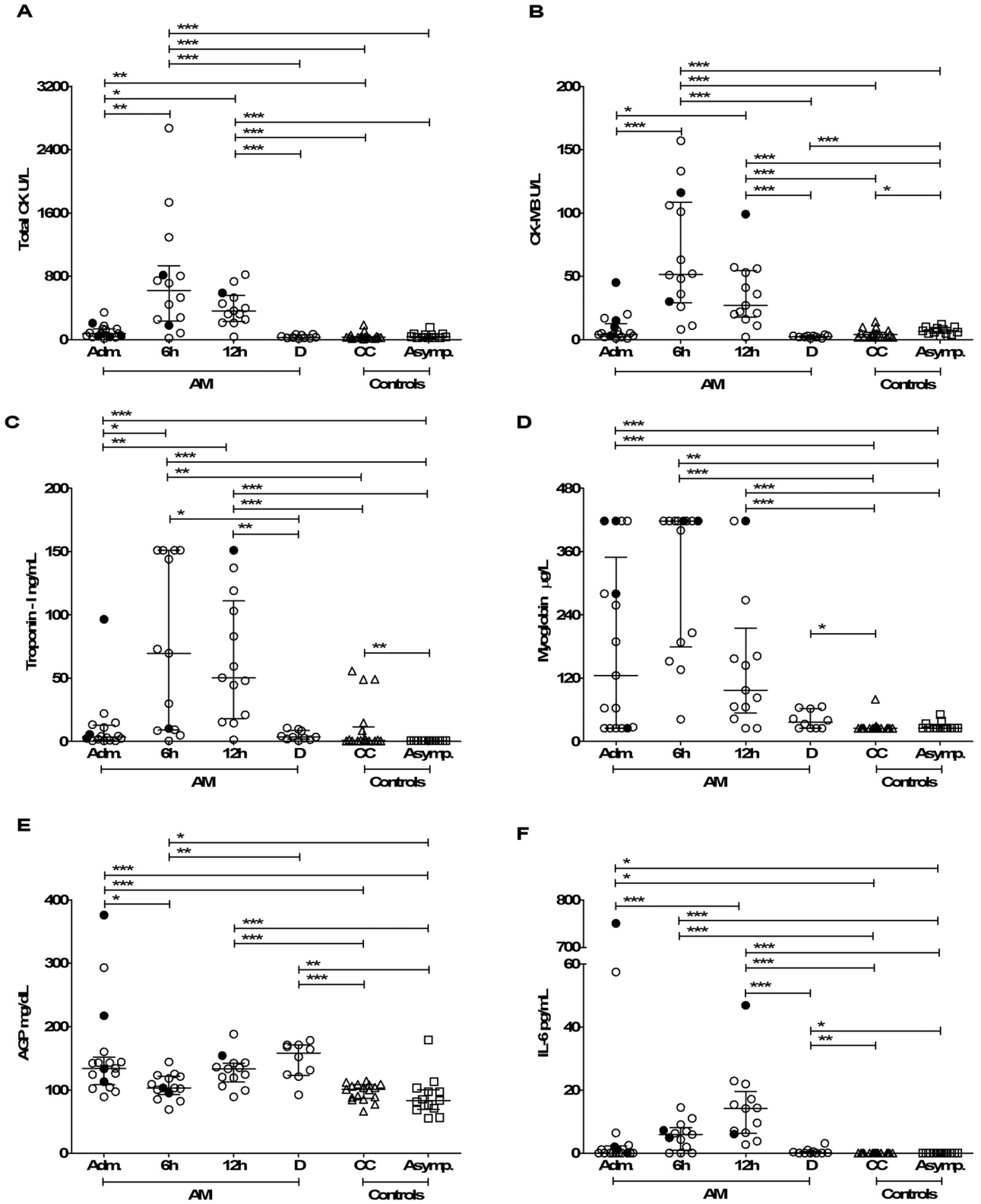

Fig. 1. Serum concentrations of CK, CK-MB, **troponin-I, myoglobin, AGP and IL-6 in patients with acute myocardial infarction.** Serum CK (**A**), CK-MB (**B**), troponin-I (**C**), myoglobin (**D**), AGP (**E**) and IL-6 (**F**) levels in AMI group were compared to each other's and with controls. Bars indicate median and interquartile values and significant differences are represented as * when $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$. Patients who died were identified with black balls. Time of admission (Adm.), 6 h post admission (6 h), 12 h post admission (12 h), discharge (D), cardiac catheterization (CC), asymptomatic (Asymp.).

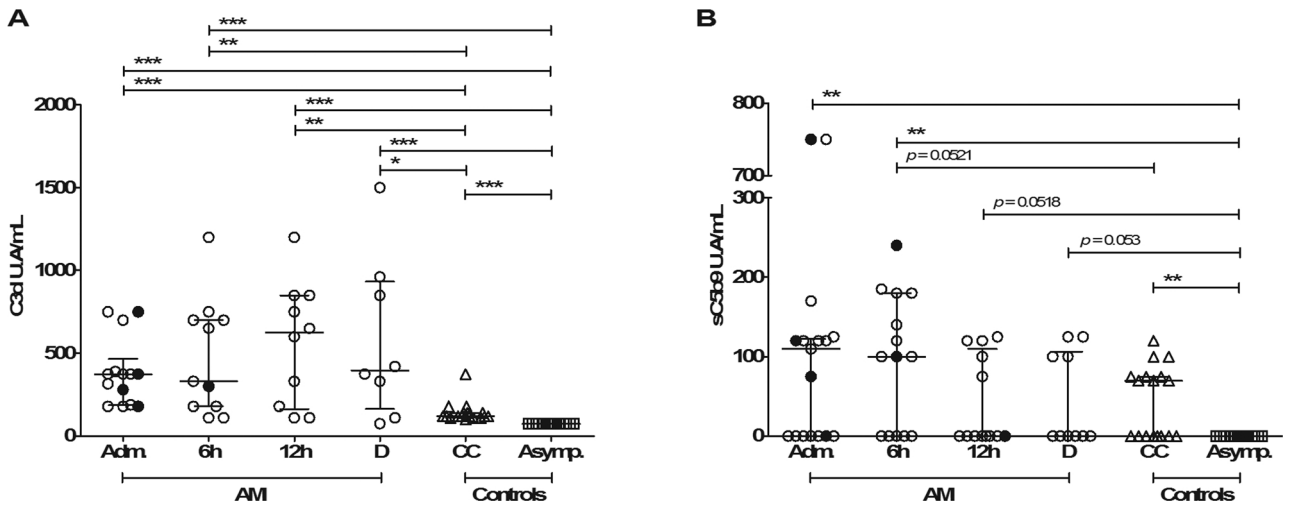

Fig. 2. Plasma levels of C3d and sC5b9 in patients with acute myocardial infarction. C3d (**A**) and sC5b9 (**B**) levels in AMI group were compared to each other's and with controls. Bars indicate median and interquartile values and significant differences are represented as * when $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$. Patients who died were identified with black balls. Time of admission (Adm.), 6 h post admission (6 h), 12 h post admission (12 h), discharge (D), cardiac catheterization (CC), asymptomatic (Asymp.).

also higher levels of IL-6 (750 pg/mL) and C3d (750U.A/mL), this patient died between admission and 6 h. There was no significant correlation between age and inflammatory markers considering each group.

3.3. Complement activation in AMI patients and controls

In order to evaluate the complement activation the product of central component C3 activation, C3d, and the soluble component sC5b9 from terminal complement pathway were investigated in the plasma of AMI patients and controls (Fig. 2). Although there was median age difference among the groups, no correlation between age and C3d or sC5b9 plasma levels for both patients and controls were found. Plasma C3d was higher in all samples at admission (Median: 375UA/mL), 6 h (330 A/mL), 12 h (675UA/mL), and discharge (397UA/mL) than CC (120UA/mL; $p < 0.0001$, $p = 0.0061$, $p = 0.0081$, $p = 0.044$) and asymptomatic (75UA/mL; $p = 0.0001$ to admission, 6 h and 12 h, and $p = 0.0002$ for discharge group) controls. However, plasma levels of sC5b9 were significantly higher only at admission (Median: 110UA/mL) and 6 h (100UA/mL) than asymptomatic (0UA/mL; $p = 0.0031$ and $p = 0.0019$). Besides that, C3d and sC5b9 were higher in the CC (120 and 70UA/mL, respectively) group when compared with asymptomatic (75 and 0UA/mL, $p = 0.0001$ and $p = 0.007$, respectively). It is expected since the catheterism is an invasive procedure and can activate the complement.

3.4. Correlation between the parameters evaluated in AMI patients

A positive correlation was observed between troponin-I and CK (r = 0.6283; $p = 0.0091$) at admission time. In addition, a positive tendency was observed between troponin-I and CK-MB (r = 0.4496; $p = 0.0806$), CK and myoglobin (r = 0.4226; $p = 0.0911$), and IL-6 and AGP levels (r = 0.4599; $p = 0.0632$) at admission. Moreover, at 6 h post admission positive and negative correlation were observed between myoglobin and CK-MB (r = 0.5768; $p = 0.0308$) and myoglobin and AGP (r = -0.5921; $p = 0.0257$), respectively. As expected for classical parameters of cardiac damage positive correlations were found at 6 h and 12 h between CK and CK-MB (r = 0.9341; $p < 0.0001$ and r = 0.8352; $p = 0.0004$, respectively), CK and troponin-I (r = 0.8831; $p < 0.0001$ and r = 0.8516; $p = 0.0002$, respectively), CK and myoglobin (r = 0.5768; $p = 0.0308$ and r = 0.5840; $p = 0.0361$, respectively), CK-MB and troponin-I (r = 0.8497; $p = 0.0002$ and r = 0.6813; $p = 0.0103$, respectively), myoglobin and troponin-I (r = 0.6723; $p = 0.0118$ and r = 0.5758; $p = 0.0395$, respectively) levels. Interesting, at 12 h a tendency of positive correlation was observed between C3d and the pro-inflammatory parameters AGP (r = 0.6159; $p = 0.0580$) and IL-6 (r = 0.6051; $p = 0.0843$). Finally, at discharge a positive correlation was observed between AGP and IL-6 (r = 0.6809; $p = 0.032$). Otherwise, a negative tendency of correlation was observed between AGP and CK (r = -0.6201; $p = 0.0558$). No further correlation was observed.

3.5. Concomitant profile of complement activation products and cardiac parameters

A temporal representation of C3d and sC5b9 concentrations, inflammatory and cardiac parameters of necrosis highlights that complement activation occurred previously to the elevation of classical markers of myocardial damage total CK, CK-MB, troponin-I and myoglobin at 6 h post admission (Fig. 3A). We propose a schematic representation of longitudinal events since ischemia to discharge of AMI patients evaluated highlighting the importance of C3d and sC5b9 as biomarkers candidates of AMI (Fig. 3B).

4. Discussion

Evidence of the participation of complement in AMI has been documented by several authors. Since the 1950 s increase in serum complement has already been recognized as a serological marker for AMI [19]. In the following years, classical pathway activation and the presence of inactivation products of C4 (C4i) and C3 (C3c) were observed in the serum of patients with AMI [20]. Likewise, the consumption of classical complement components *in vitro* and in patients after AMI was observed [21]. Later, Reyes et al. (1984) [22] showed that products of C3 degradation were presented in the plasma of AMI patients and correlated with the severe evolution and bad prognostic of AMI. Plasma concentration of native C3 and C4 [23], as well as C3d and C4d, sC5b9 [12,23], C3a and C5a [24] were also shown to be increased in AMI patients.

Although several reports suggest that complement activation is crucial in the pathophysiology of cardiovascular events [25], few studies have investigated the association between serum levels of complement and AMI in a longitudinal follow-up from patient admission to discharge from the hospital. Thus, in order to identify an early biomarker of the disease process, we evaluated classical markers of

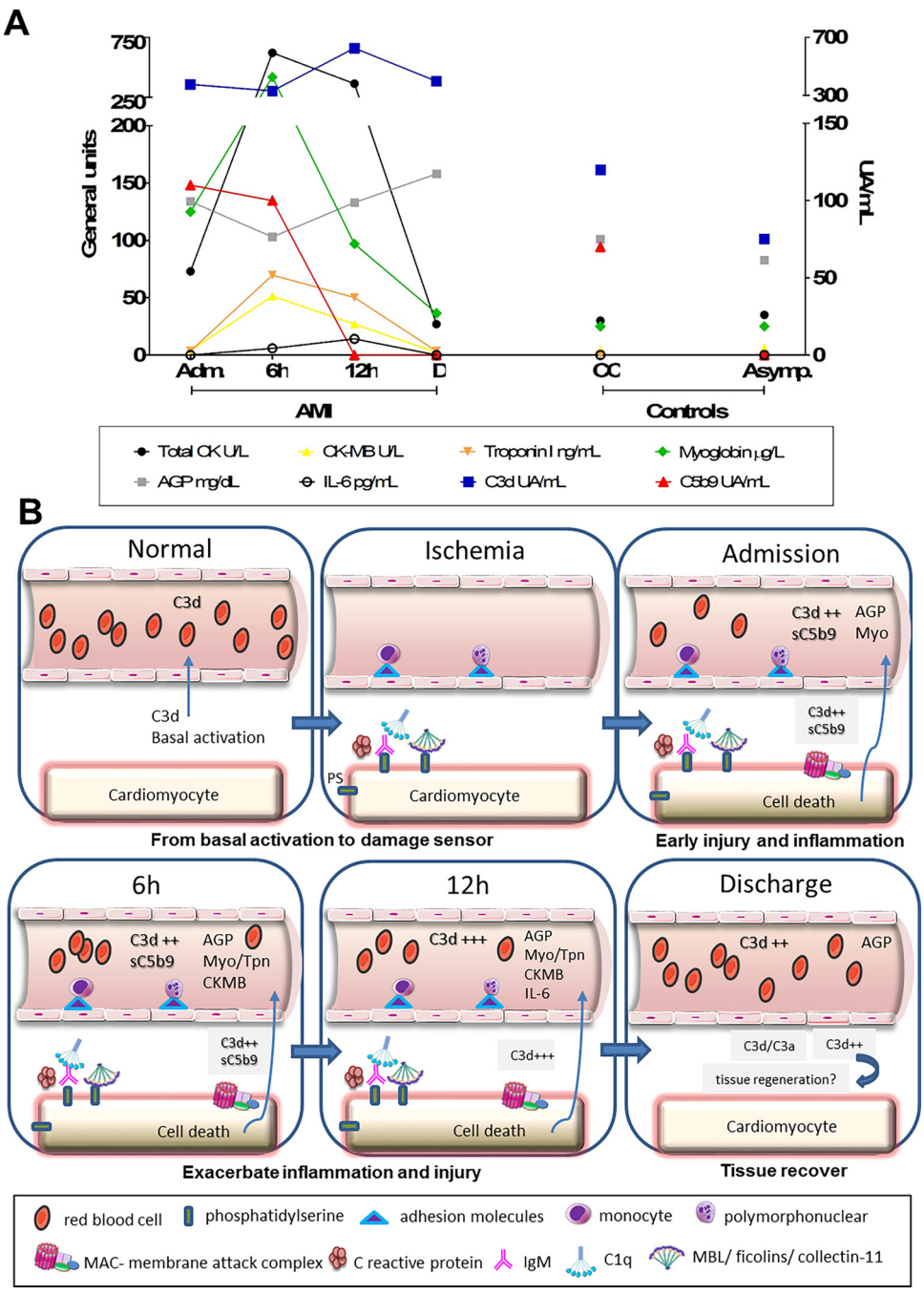

Fig. 3. A schematic representation of inflammatory/necrosis cardiac markers dynamic on AMI and its longitudinal events from ischemia to discharge. In (**A**) we present the median of the values obtained for each parameter measured to patients and controls. The error bar was omitted to make the information clear. In the right Y axis is presented the units complement activation products (UA/mL) and in the left Y axis is presented as general units (U/L, ng/mL, pg/mL, ug/L and mg/dL) from the clinical and inflammatory parameters evaluated. (**A**) High levels of complement breakdown products C3d and sC5b9 as well as AGP are concomitantly found in AMI patients at admission and 6 h post-admission. (**B**) **From basal activation of complement to damage sensor:** At physiological condition the C3d can be detected in plasma at low levels due to basal alternative pathway activation under strict regulation. During an ischemic event the inadequate blood supply on myocardial tissue leads to generation of damage-associated molecular patterns that activates endothelial cells and pattern recognition receptors including complement proteins such as, pentraxin 3 (PTX3), ficolins, and mannose binding lectin (MBL). (**B**) **Early injury and inflammation:** Blood flow is established and inflammatory cells are recruited to the damaged site. Likewise, more proteins can reach to cardiac tissue increasing the complement activation (represented as C3d + +, sC5b9 and membrane attack complex-MAC) which contribute to the severity of cardiomyocyte injury and inflammation (AGP). (**B**) **Exacerbate inflammation and injury:** As consequence, an exacerbated inflammatory process, represented by AGP, IL-6 and excessive complement activation (Cd3 + + +), is established promoting more cardiomyocyte injury and releasing damage cardiac muscle proteins as, myoglobin (Myo), creatine kinase-MB isoform (CKMB) and troponin-I (Tpn). (**B**) **Tissue recovery:** While inflammatory response regressed to homeostasis, the presence of C3d might suggest the importance of C3 (or C3a and its receptor) to myocardial regeneration.

myocardial infarction (CK, CK-MB, troponin-I and myoglobin), inflammatory cytokines (IL-6 and TNF-alpha), an acute-phase protein (APG) and the products of complement activation C3d and sC5b9 at four different times (admission, 6 h, 12, discharge). We observed that complement activation products in plasma of AMI patients were present before the classical markers of myocardial necrosis, and they were significantly increased at all times in the AMI group when compared with controls. In fact, cardiac ischemia promotes the exposure of phosphatidylserine (PS) on cardiomyocyte membrane which allows the binding of serum molecules such as IgM, C reactive protein and complement proteins at reperfusion leading to complement activation, inflammation, and cell death. Subsequently, cardiac enzymes are released as result of cardiomyocyte death [25]. Corroborating with this hypothesis, Yasojima et al. [26] demonstrated that complement activation is directly involved in myocardial damage after ischemia, and that expression of complement mRNA molecules are strongly upregulated in cardiac tissue when compared to the liver, indicating that complement proteins are endogenously produced by the human heart. On the other hand, the murine model of chronic myocardial infarction showed that C3 contributes to myocardial preservation and regeneration stimulating the proliferation of cardiac stem/progenitor cells [27].

Moreover, Väkevä et al. [28] observed that the amount of CD59, a regulator of C5b9, correlated with the level of soluble terminal complement complexes sC5b9 in the plasma of AMI patients. Corroborating these findings, in a murine model of coronary occlusion, the deposition of terminal complement complex C5b9 was observed in the endothelial surface three hours after the procedure and human soluble CR1 administration reduced myocardial necrosis and inflammation [11]. Taken together, these studies indicate that complement activation contributes to tissue injury during early reperfusion. The dynamic change of complement C3, C4 and sC5b9 levels in patients with acute coronary syndrome was investigated by Shao et al. [15]. These authors

observed that serum complement components (C3, C4 and sC5b9) in infarcted patients with ST-segment elevation reached a peak on the third day after patient admission [15]. In our study, plasma levels of C3d and sC5b9 were detected from admission to discharge time (12.8 +/− 8.1 days) reinforcing the idea that the activation of complement is an earlier event, occurring previously to myocardial injury.

Acute-phase proteins such as C-reactive protein, serum amyloid A, and pentraxin 3 are important activators of complement through their binding to cellular remnants like nuclear fractions, neutralizing enzymes, scavenging free hemoglobin and radicals, and modulating the host's immune response [29]. These proteins, which are represented by AGP, complement components (including C2, C3, C4, C5, coagulation proteins and others are produced in acute-phase response to metabolism changes). AGP is an acute phase protein [29], whose levels raise several fold during an acute phase response reflecting a systemic answer to a local inflammatory stimulus [30]. Ours results showed that serum concentration of AGP in AMI patients were higher than in asymptomatic individuals. And its elevation occurred concomitantly with C3d and sC5b9 at admission time when compared with controls. It is important to mention that high AGP concentrations were observed in two patients who died post admission, affecting the AGP median in this group. In addition, these patients presented high levels of C3d and sC5b9. Interestingly, AGP was shown to have a relevant prognostic value in the follow-up of patients with AMI, with high levels being related to death in AMI [31]. Moreover, AGP has an immunomodulatory role and can inhibit both the alternative and the classical complement activation pathways [32].

In our study the elevation of IL-6 levels only in 6 h and 12 h post admission might be related to inflammatory process involved in myocardial ischemia and necrosis. IL-6 has been demonstrated as a proinflammatory cytokine involved in reperfusion injury, repair processes and scar tissue formation after myocardial infarction [3]. Morover, circulating IL-6 have been associated to the extent of myocardial necrosis [33,34] or ST-elevation [35], suggesting a role for IL-6 in the pathophysiology and severity of AMI.

Regarding the classical parameters markers for AMI, serum concentration of total CK generally reaches a peak 4–8 h post AMI and returns to baseline values at 2–3 days. On the other hand, CK-MB rises in serum 4–9 h after the onset of chest pain. And returns to baseline values at 48–72 h and myoglobin is released extremely early into the circulation, one hour after the onset of myocardial injury, peaks at 4–12 h and returns to baseline values immediately. Finally, troponin-I is released into the serum 6–8 h after myocardial injury, with peak at 12–24 h and remain elevated for 7–10 days [4,5]. In our study, serum concentrations of total CK, CK-MB, troponin-I and myoglobin in AMI patients peaked 6 h after admission and remained higher when compared to admission. Likewise, total CK presented a positive and significant correlation with CK-MB, troponin-I and myoglobin at 6 h and 12 h. These results are in agreement with previous reports, where plasma levels of total CK and CK-MB were increased between 4 and 8 h after AMI and tended to disappear in 2–3 days [36]. Moreover, it is well known that CK-MB is a more sensitive marker of myocardial injury than total CK activity [5] and considered the most accurate biomarker in determining infarct size [37].

Although the sample size of AMI patients is a limitation of this study which was due to the difficulty of following-up patients under death risk in a critical care unit, our results showed constant complement activation in AMI patients since admission to hospitalization discharge. This is the first study to report early complement activation in AMI and suggests complement activation products such as C3d and sC5b9 as useful precocious marker for early diagnosis and targets for immune directed therapies in AMI.

5. Conclusion

The activation of complement seems to occur as an early event before the serum elevation of classical myocardial damage-related enzymes in AMI patients, concomitantly with the acute phase protein AGP. Since C3d is related to the inflammatory process it might be a promising tool as a non-invasive approach in the detection and estimation of the degree of myocardial injury.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

[1] World Health Organization (WHO), Cardiovascular Diseases (CVDs), (2017) (Accessed 13 September 2017. (n.d.)), http://www.who.int/mediacentre/factsheets/fs317/en/.
[2] J.L. Anderson, D.A. Morrow, Acute myocardial infarction, N. Engl. J. Med. 376 (2017) 2053–2064, http://dx.doi.org/10.1056/NEJMra1606915.
[3] N. Frangogiannis, Regulation of the inflammatory response in cardiac repair, Circ. Res. 110 (2012) 159–173, http://dx.doi.org/10.1161/CIRCRESAHA.111.243162.Regulation.
[4] S. Mythili, N. Malathi, Diagnostic markers of acute myocardial infarction, Biomed. Rep. 3 (6) (2015) 743–748, http://dx.doi.org/10.3892/br.2015.500.
[5] G.S. Bodor, Biochemical markers of myocardial damage, EJIFCC 27 (2016) 95–111 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975226/.
[6] M. Noris, G. Remuzzi, Overview of complement activation and regulation, Semin. Nephrol. 33 (2013) 479–492, http://dx.doi.org/10.1016/j.semnephrol.2013.08.001.
[7] J.V. Sarma, P. Ward, The complement system, NIH Publ. Access. Author Manuscr. 343 (2011) 227–235, http://dx.doi.org/10.1007/s00441-010-1034-0.
[8] D. Ricklin, G. Hajishengallis, K. Yang, J.D. Lambris, Complement: a key system for immune surveillance and homeostasis, Nat. Immunol. 11 (2010) 785–797, http://dx.doi.org/10.1038/ni.1923.
[9] N.S. Merle, S.E. Church, V. Fremeaux-Bacchi, L.T. Roumenina, Complement system part I – molecular mechanisms of activation and regulation, Front. Immunol. 6 (2015) 1–30, http://dx.doi.org/10.3389/fimmu.2015.00262.
[10] J.H. Hill, P. Ward, The phlogistic role of C3 leukotactic fragments in myocardial infarcts of rats, J. Exp. Med. 133 (1971) 885–900, http://dx.doi.org/10.1084/jem.133.4.885.
[11] H.F. Weisman, T. Bartow, M.K. Leppo, H.C. Marsh, G.R. Carson, M.F. Concino, M.P. Boyle, K.H. Roux, M.L. Weisfeldt, D.T. Fearon, Soluble human post-ischemic myocardial inflammaton and necrosis, Science 249 (1990) 146–151.
[12] M. Yasuda, K. Takeuchi, M. Hiruma, H. Iida, A. Tahara, H. Itagane, I. Toda, K. Akioka, M. Teragaki, H. Oku, The complement system in ischemic heart disease, Circulation 81 (1990) 156–163, http://dx.doi.org/10.1161/01.CIR.81.1.156.
[13] K. Iltumur, A. Karabulut, G. Toprak, N. Toprak, Complement activation in acute coronary syndromes, APMIS 113 (2005) 167–174, http://dx.doi.org/10.1111/j.1600-0463.2005.apm1130303.x.
[14] J. Cubedo, T. Padró, L. Badimon, Coordinated proteomic signature changes in immune response and complement proteins in acute myocardial infarction: the implication of serum amyloid P-component, Int. J. Cardiol. 168 (2013) 5196–5204, http://dx.doi.org/10.1016/j.ijcard.2013.07.181.
[15] A. Shao, X. Qi, Q. Li, W. Jia, L. Wei, W. Hou, Y. Qi, Y. Liu, Dynamic changes of complement level in patients with acute coronary syndrome and its relationships with myocardial injury, Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 29 (2017) 515–519.
[16] G.S. Huggins, P.T. O'Gara, Clinical presentation and diagnostic evaluation of ST-segment elevation myocardial infarction, in: E.J.T. Valentín Fuster, Russell Ross (Eds.), Atheroscler. Coron. Artery Dis. 2nd ed., Lippincott-Raven, Philadelphia, 1996, p. 1703.
[17] I. Brandslund, H.C. Siersted, S.-E. Svehag, B. Teisner, Double-decker rocket immunoelectrophoresis for direct quantitation of complement C3 split products with C3d specificities in plasma, J. Immunol. Methods 44 (1981) 63–71, http://dx.doi.org/10.1016/0022-1759(81)90107-1.
[18] T.E. Mollnes, T. Lea, S.S. Frøland, M. Harboe, Quantification of the terminal complement complex in human plasma by an enzyme- linked immunosorbent assay based on monoclonal antibodies against a neoantigen of the complex, complement, Scand. J. Immunol. 22 (1985) 197–202.
[19] A.J. Boltax, E.E. Fischel, Serologic tests for inflammation, Am. J. Med. 20 (1956) 418–427, http://dx.doi.org/10.1016/0002-9343(56)90127-9.
[20] J.M.B. Versey, R. Gabriel, Soluble-complex formation after myocardial infarction, Lancet 304 (1974) 493–494, http://dx.doi.org/10.1016/S0140-6736(74)92017-0.
[21] R.N. Pinckard, M.S. Olson, P.C. Giclas, R. Terry, J.T. Boyer, R.A. O'Rourke, Consumption of classical complement components by heart subcellular membranes

in vitro and in patients after acute myocardial infarction, J. Clin. Invest. 56 (1975) 740–750, http://dx.doi.org/10.1172/JCI108145.
[22] P.A. Reyes, G.F. de la Reguera, C. Enríquez, Patogenia del infarto agudo del miocardio: I. Activación del complemento in vivo y evolución del infarto/the pathogenesis of acute myocardial infarction: I. In vivo complement activation and the course of infarction, Arch. Inst. Cardiol. Mex. 54 (1984) 327–332.
[23] T.E. Mollnes, K.E. Tambs, Y. Myreng, L.F. Engebretsen, Acute phase reactants and complement activation in patients with acute myocardial infarction, Complement 5 (1988) 33–39.
[24] P.F. Langlois, M.S. Gawryl, Detection of the terminal complement complex in patient plasma following acute myocardial infarction, Atherosclerosis 70 (1988) 95–105, http://dx.doi.org/10.1016/0021-9150(88)90103-7.
[25] R.W. Emmens, D. Wouters, S. Zeerleder, S.M. van Ham, H.W.M. Niessen, P.A.J. Krijnen, On the value of therapeutic interventions targeting the complement system in acute myocardial infarction, Transl. Res. 182 (2016) 103–122, http://dx.doi.org/10.1016/j.trsl.2016.10.005.
[26] K. Yasojima, C. Schwab, E.G. McGeer, P.L. McGeer, Human heart generates complement proteins that are upregulated and activated after myocardial infarction, Circ. Res. 83 (1998) 860–869, http://dx.doi.org/10.1161/01.RES.83.8.860.
[27] M. Wysoczynski, M. Solanki, S. Borkowska, P. van Hoose, K.R. Brittian, S.D. Prabhu, M.Z. Ratajczak, G. Rokosh, Complement component 3 is necessary to preserve myocardium and myocardial function in chronic myocardial infarction, Stem Cells 32 (2014) 2502–2515, http://dx.doi.org/10.1002/stem.1743.
[28] A. Väkevä, T. Lehto, A. Takala, S. Meri, Detection of a soluble form of the complement membrane attack complex inhibitor CD59 in plasma after acute myocardial infarction, Scand. J. Immunol. 52 (2000) 411–414, http://dx.doi.org/10.1046/j.1365-3083.2000.00783.x.
[29] S. Jain, V. Gautam, S. Naseem, Acute-phase proteins: as diagnostic tool, J. Pharm. Bioallied Sci. 3 (2011) 118, http://dx.doi.org/10.4103/0975-7406.76489.
[30] T. Hochepied, F.G. Berger, H. Baumann, C. Libert, α1-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties, Cytokine Growth Factor Rev. 14 (2003) 25–34, http://dx.doi.org/10.1016/S1359-6101(02)00054-0.
[31] J.P. Chapelle, A. Albert, J.P. Smeets, C. Heusghem, H.E. Kulbertus, The prognostic significance of serum alpha 1-acid glycoprotein changes in acute myocardial infarction, Clin. Chim. Acta 115 (1981) 199–209.
[32] W. Van Dijk, S. Do Carmo, E. Rassart, B. Dahlbäck, J.M. Sodetz, The plasma lipocalins alpha1-acid glycoprotein, apolipoprotein D, apolipoprotein M and complement protein C8gamma, Lipocalins (2013) 1–27, http://dx.doi.org/10.4103/0975-7406.76489.
[33] Y. Miyao, H. Yasue, H. Ogawa, I. Misumi, T. Masuda, T. Sakamoto, E. Morita, Elevated plasma interleukin-6 levels in patients with acute myocardial infarction, Am. Heart J. 126 (1993) 1299–1304 http://www.ncbi.nlm.nih.gov/pubmed/8249785.
[34] U. Ikeda, F. Ohkawa, Y. Seino, K. Yamamoto, Y. Hidaka, T. Kasahara, T. Kawai, K. Shimada, Serum interleukin 6 levels become elevated in acute myocardial infarction, J. Mol. Cell. Cardiol. 24 (1992) 579–584, http://dx.doi.org/10.1016/0022-2828(92)91042-4.
[35] V.N. Ritschel, I. Seljeflot, H. Arnesen, S. Halvorsen, T. Weiss, J. Eritsland, G.T. Andersen, IL-6 signalling in patients with acute ST-elevation myocardial infarction, Results Immunol. 4 (2014) 8–13, http://dx.doi.org/10.1016/j.rinim.2013.11.002.
[36] J.E. Adams, D.R. Abendschein, A.S. Jaffe, Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990s? Circulation 88 (1993) 750–763, http://dx.doi.org/10.1161/01.CIR.88.2.750.
[37] D. Ternant, F. Ivanes, F. Prunier, N. Mewton, T. Bejan-Angoulvant, G. Paintaud, M. Ovize, D. Angoulvant, Revisiting myocardial necrosis biomarkers: assessment of the effect of conditioning therapies on infarct size by kinetic modelling, Sci. Rep. 7 (2017) 1–12, http://dx.doi.org/10.1038/s41598-017-11352-4.