Rev Bras Cardiol Invasiva.
2014;22(2):131-6

Original Article

# Immunohistochemical Characteristics of Coronary Thrombi in Patients with ST-Elevation Myocardial Infarction and Diabetes Mellitus: Pilot Study

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**ABSTRACT**

**Background**: Diabetes mellitus is associated with increased mortality rates in the setting of acute myocardial infarction. The aim of this study was to evaluate whether there are peculiarities in the atherothrombotic process that might be implicated in increased risk for this outcome in patients with diabetes. **Methods**: Pilot study in a cohort of patients with ST-elevation acute myocardial infarction undergoing primary percutaneous coronary intervention and aspiration thrombectomy. Clinical and laboratory variables were evaluated in all of the cases. Thrombi were analyzed for histopathological features as well as immunohistochemical expression of CD34, CD61 and factor VIII. **Results**: Our sample included the first ten diabetic patients with material available for analysis, who were matched according to age, gender and time elapsed since myocardial infarction with ten patients without diabetes. There was no significant association between the immunohistochemical expression of CD34, CD61 and factor VIII with other histopathological, clinical and laboratory variables, including the presence of diabetes mellitus. **Conclusions**: In this preliminary analysis, it was not possible to demonstrate any significant difference in the expression of endothelial cell activity, platelet function and activation of the coagulation cascade between thrombi of patients with and without diabetes undergoing primary coronary intervention.

**DESCRIPTORS**: Myocardial infarction. Diabetes mellitus. Coronary thrombosis. Immunohistochemistry. Percutaneous coronary intervention.

**RESUMO**

**Características Imuno-Histoquímicas de Trombos Coronarianos de Pacientes com Infarto do Miocárdio com Elevação de ST e *Diabetes Mellitus*: Estudo Piloto**

**Introdução**: No contexto do infarto agudo do miocárdio, o *diabetes mellitus* está associado à maior mortalidade. O objetivo deste estudo foi avaliar se existem, entre os diabéticos, peculiaridades no processo de aterotrombose que poderiam estar implicadas em maior risco para tal desfecho. **Métodos**: Estudo piloto, proveniente de coorte de pacientes com diagnóstico de infarto agudo do miocárdio com elevação do segmento ST submetidos à intervenção coronária percutânea primária e à tromboaspiração. Foram estudadas variáveis clínico-laboratoriais de cada caso. Os trombos foram analisados quanto às características histopatológicas e às expressões imuno-histoquímicas de CD34, CD61 e fator VIII. **Resultados**: Foram incluídos os primeiros dez pacientes portadores de *diabetes mellitus* com material disponível para análise, pareados por idade, sexo e tempo de evolução do infarto com dez pacientes sem *diabetes mellitus*. Não houve associação significativa entre as expressões imuno-histoquímicas de CD34, CD61 e fator VIII com relação às variáveis histopatológicas, laboratoriais e clínicas estudadas, inclusive com relação à presença de *diabetes mellitus*. **Conclusões**: Em análise preliminar, não foi possível demonstrar diferença significativa quanto à expressão da atividade de células endoteliais, da função plaquetária e da ativação da cascata de coagulação entre trombos de pacientes com e sem o diagnóstico de *diabetes mellitus* submetidos à intervenção coronariana primária.

**DESCRITORES:** Infarto do miocárdio. *Diabetes mellitus*. Trombose coronária. Imuno-histoquímica. Intervenção coronária percutânea.

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Received: 03/10/2014 • Accepted: 05/18/2014

Rev Bras Cardiol Invasiva.
2014;22(2):131-6

Coronary artery disease (CAD) is the leading cause of death worldwide, with most adverse events being associated with the occurrence of acute myocardial infarction (AMI).[1] With the optimization of antithrombotic therapies and reperfusion, a decrease in mortality from acute myocardial infarction with ST-segment elevation (STEMI) has been observed.[2] However, efforts are required to improve the management of this condition, as high rates of mortality have still been reported.[3] In most cases, STEMI is caused by the rupture of vulnerable atherosclerotic plaques associated with inflammatory activity and a dysfunctional endothelium. The ruptured plaque is the trigger for platelet activation and aggregation, and thrombin formation, resulting in total occlusion of the coronary artery by the thrombus.[4]

Primary percutaneous coronary intervention (PPCI) is considered the preferred method of reperfusion in AMI.[5] In recent years, the adjunct thrombo aspiration technique has been increasingly used, with improvement in angiographic results and clinical outcomes in some studies.[6] Additionally, thrombus removal has allowed for a new line of research regarding the analysis of the morphology, histology, and factors associated with different types of thrombi.[7-9] Variables, such as red color and thrombus chronology, have been associated with increased mortality,[10,11] but the physiopathological role of the different elements that constitute the thrombus has been seldom studied.

Immunohistochemical analysis of thrombus aspirates can be an important complementary tool in the search for these answers. The expression of the endothelial cell activity, of platelet function, of and coagulation cascade activation can be estimated by the immunohistochemical expression of the antigens CD34, CD61, and factor VIII, respectively.[12-15] In the scenario of STEMI, few studies have evaluated the immunohistochemical profile of intracoronary thrombus *in vivo* and its correlation with clinical variables.[13-16] In this context, the importance of diabetes mellitus (DM) is well-known, either by increasing the risk of CAD and thrombosis, or because it is associated with higher mortality in STEMI.[17,18] However, there are doubts as to whether this risk is mediated by differences in the physiopathological processes of atherothrombosis or by the increase in comorbidities associated with DM. Thus, this study aimed to evaluate the immunohistochemical expression of the antigens CD34, CD61, and factor VIII in coronary thrombi in patients with DM and STEMI.

## METHODS

### Patients

This was a pilot study of sample of patients consecutively admitted to this hospital with a diagnosis of STEMI, between December 2010 and November 2012. STEMI was defined as chest pain at rest lasting more than 30 minutes, associated to ST-segment elevation of at least 1 mm in two contiguous leads in the frontal plane, or at least 2 mm in the horizontal plane, or associated with new left bundle branch block. Patients with evolution > 12 hours, younger than 18 years, or those who refused to participate in the study were excluded.

All participants signed an informed consent. The study was approved by the Ethics Committee in Research of Instituto de Cardiologia, Fundação Universitária de Cardiologia, Porto Alegre (RS), Brazil.

### Clinical characteristics and follow-up

All patients were prospectively evaluated regarding their clinical and demographic characteristics by one of the researchers. The clinical, laboratory, and angiographic variables were recorded in a dedicated database. During hospitalization, the patients were followed daily by one of the investigators for verification of in-hospital events. After hospital discharge, patients were contacted by telephone or assisted at the outpatient clinic for outcome assessment.

### Percutaneous coronary intervention procedures

At admission, all patients received 300 mg of acetylsalicylic acid and 300 to 600 mg of clopidogrel. For the performance of the PPCI procedure, 60 to 100 U/kg of unfractionated heparin were administered. PPCI procedures were performed as recommended in the literature.[19,20] Decisions regarding technical aspects of the procedure, such as access route (radial or femoral), whether or not to perform thromboaspiration, type and number of stents, and use of glycoprotein IIb/IIIa inhibitors were left to the discretion of the surgeons. Regarding thromboaspiration, three different catheters were used: Export (Medtronic Vascular Inc, Santa Rosa, CA, United States), Diver (Invatec, Brescia, Italy), or Pronto (Vascular Solutions, Minneapolis, MN, United States). In all cases, the aspirations were performed prior to balloon inflation, passing more than once through the site of occlusion. The aspirated blood and intracoronary material were retained on a filter.

### Thrombus analysis

Immediately after thrombus removal from the coronary artery, the sample was embedded in 10% formalin and fixed in paraffin for 24 hours. The specimens were cut with a microtome and stained with the hematoxylin-eosin method for histopathological analyses, which were performed by a pathologist blinded to the clinical characteristics of patients. The following characteristics of the thrombi were studied: volume ($cm^3$), color (white or red), microscopic pattern (recent, with lysis, or in formation) and the percentage of leukocytes, fibrin, and erythrocytes, as well as the immunohistochemical expression of CD34, CD61, and factor VIII. Recent thrombi were characterized by the presence of fibrin,