H38 Early Ischemic Heart Injury: An Immunohistochemical Study of a Paradigmatic Case

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The goal of this presentation is to provide the results of an investigation concerning immunohistochemical methods that may allow for an accurate postmortem diagnosis of early ischemic lesions of the heart muscle.

This presentation will impact the forensic science community by providing the results of a postmortem immunohistochemical study of the myocardium in a case of sudden cardiac ischemic death, compared to a control matched by age and sex, resulting from a fall from a height.

Introduction: Ischemic heart disease is the major cause of sudden cardiac death. In forensic pathology, The need for new methods is particularly crucial in cases in which ischemic lesion occurs shortly before death: often, in very early ischemia (less than three hours), the damage can't be detected by light microscopy. On the basis of a previous immunohistochemical study, this study attempted to discover if a number of immunohistochemical markers could be useful in order to differentiate ischemic areas from well-perfused ones.

The goal of this presentation is to provide the results of a postmortem study of the myocardium in a case of sudden cardiac ischemic death compared to a matched control who died from a fall from a height.

Materials and Methods: A forensic autopsy was performed 72 hours after death. The whole heart was fixed and specimens for microscopic examinations were collected from each part of the heart. Each section was stained with Hematoxylin-Eosin (H&E) and with the following primary antibodies: antifibronectin, anti C5b9, anti Cx43, antiNpCx43, and antiZO1. Immunopositives of each marker in the myocardium were semi-quantitatively graded.

Results: A 39-year-old woman went to the hospital complaining of chest pain. The objective examination, blood exams, and Electrocardiogram (ECG) didn't point out any pathological findings. A few hours later, she was discharged. The next day cardiologists performed an ECG and an echocardiography (referenced as negative); the pain was diagnosed as angina pectoris and a stress test was scheduled. The morning after, the woman suddenly died at home. At the autopsy, the heart showed an increase of the left ventricle wall thickness and the subtotal stenosis of the interventricular anterior branch of the left coronary. At H&E staining, the myocardium showed intimal thickening of the walls of small arteries, contraction bands and fibers disarrangement, without necrosis. Immunostaining: Fibronectin (FN) — neither the scoring nor the distribution pattern seem to show any relationship with the site of ischemia. Among the case samples, different patterns of distribution could be noticed, but not related to the ipoperfused area. C5b9 was negative in the cytoplasm, in both the case and the control specimens. CX43, npCx43, ZO1 — in the specimens from the diseased area, a less marked and disarranged Cx43 staining was observed, whereas NpCX43 in these specimens showed a more marked positivity and disomogeneous distribution. ZO1 showed a ill-scattered pattern in the specimens collected from the ischemic area; it was weak in the control specimens.

Discussion: On the basis of a previous immunohistochemical study, attempts were made to ascertain if these markers could be useful in order to differentiate ischemic areas from the well-perfused areas.[1] By the analysis of many specimens from one heart (obtained from a patient whose clinical data, diseased coronary artery, and ipoxic-ischemic area were known), the study is free of any influence by the postmortem interval or by the time of fixation. The most useful marker seems to be the combination of Cx43 and its de-posphorilated form (npCx43). In the normal heart, Cx43 is phosphorylated and localized at the Intercalated Discs (ID); stimuli such as ischemia and hypoxia induce non-phosphorylation and redistribution to the cytoplasm and/or lateral cell border of cardiomyocytes.[2-4] In animal models, decreased Cx43 and increased npCx43 at ID were detected 15min after the beginning of ischemia.2 The less-defined pattern of npCx43 might indicate its redistribution from the cell junction to the cytoplasm. As the ZO1 binds and regulates the Cx43, it can be another marker of this junction disruption. In this case, a clear correlation between ZO1 pattern and ischemia was not observed.[5] All of these markers, despite the interesting findings about their distribution, have the disadvantage of being in any case positive and the scoring could be influenced by many factors, such as technical or operator-related issues.

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Reference(s):

1. Kawamoto O. et al. Immunohistochemistry of connexin43 and zonula occludens-1 in the myocardium as markers of early ischemia in autopsy material. *Histol e Histopathol* (2014) 29: 767-775.
2. Beardslee M.A. et al. Dephosphorylation and Intracellular Redistribution of Ventricular Connexin43 During Electrical Uncoupling Induced by Ischemia, *Circulation Research*, October 13, 2000: 655-662.
3. Barker R.J. et al. Increased association of ZO-1 with connexin43 during remodeling of cardiac gap junctions. *Circ Res*. 2002 Feb 22;90(3):317-24.
4. Hesketh G.G. et al. Ultrastructure and regulation of lateralized connexin43 in the failing heart. *Circ Res*. 2010 Apr 2;106(6):1153-63.
5. Palatinus J.A. et al. ZO-1 determines adherens and gap junction localization at intercalated disks *Am J Physiol Heart Circ Physiol* 300: H583–H594, 2011.

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