G59 Early Markers of Myocardial Ischemia Relevant to the Forensic Pathology: An Immunohistochemical and Gene-Expression Study

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After attending this presentation, attendees will get new biological and methodological insights in the field of early myocardial ischemia and will get a panel of highly promising markers to apply to the routine activity in cases of sudden cardiac death.

This presentation will impact the forensic science community by providing solid diagnostic tools for early myocardial ischemia and will also impact human health, as the presented markers can be used to retrospectively analyze some clinical cases, to adjust the existing clinical strategies to prevent sudden cardiac death, and for the estimation of the cardiovascular risk for relatives of the deceased person.

Postmortem diagnosis of acute myocardial ischemia represents a current challenge for forensic pathologists, especially when death occurs within a short period of time (minutes to a few hours) after the onset of the ischemic injury.

Recent works have investigated, at the immunohistochemical level, some markers that accumulate in, or leak from, the human cardiomyocyte after the ischemic event. Nevertheless, these markers are not detectable in the very early phase of myocardial ischemia. Besides, the role of cardiomyocyte apoptosis as a diagnostic tool in cases of early myocardial injury has been investigated by Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assay, which has shown good sensitivity but controversial specificity.

This investigation has tested, under experimental conditions, the diagnostic potential of some immunohistochemical markers, as well as of the TUNEL assay, for the detection of early myocardial ischemia. Among the immunohistochemical markers investigated are: Troponin I and T, myoglobin, fibronectin (total and tissular), C5b-9, connexin 43, Jun B, and tenascin C. The same and additional markers (as HIF-1 alpha, caspases 3, 8, and 9) have been studied at gene-expression level as well, using the NanoString nCounter® gene-expression system.

A rat model of myocardial ischemia (ligation of Left Anterior Descending (LAD) coronary artery) was used. The immunohistochemical and gene-expression investigations were performed on the ischemic myocardium at different time points after LAD ligation, ranging from 5 minutes to 2 weeks. As comparison, hearts from control- and sham-operated groups were investigated by the same methods. The NanoString nCounter® is a novel gene-expression system which allows direct measurements of mRNA expression levels without enzymatic reactions or bias, with a sensitivity coupled with high multiplex capability, and a digital readout.

The earliest expressions following myocardial ischemia were observed for JunB and dephosphorylated connexin 43 (15 minutes) as well as for apoptosis and hypoxia markers (15-30 minutes), followed by total fibronectin (≤1 hour), C5b-9 (≤1 hour), myoglobin (≤1 hour), and troponins I and T (≤1 hour). The latest markers, expressed only in the healing phase of myocardial infarction, were tissular fibronectin and tenascin C.

This study identified, by immunohistochemical and gene-expression investigations performed on a pure experimental model of myocardial ischemia, early markers of ischemic injury as JunB, dephosphorylated connexin 43, and apoptosis effectors, expressed as early as 15 minutes after coronary artery ligation in rats. Moreover, this research confirmed the early expression of total fibronectin, C5b-9, myoglobin, and troponins (≤1 hour). This study has, therefore, identified a panel of markers to further apply to the routine forensic practice to improve the diagnosis in challenging cases of sudden cardiac death.

Sudden Cardiac Death, Immunohistochemistry, Gene-Expression

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