H73 The Alterations of HMGB and Troponin in Postmortem Interval (PMI): The First Experimental Study on Humans and a Review of Literature

Matteo A. Sacco, MD, Chair of Legal Medicine, University of Catanzaro, Catanzaro 88100, ITALY; Fabrizio Cordasco, MD, Università Magna Graecia CZ, Catanzaro, ITALY; Francesco Sicilia, MD, University Magna Graecia of Catanzaro, Catanzaro 88100, ITALY; Luigi De Aloe, MD, Institute of Legal Medicine, Catanzaro 88100, ITALY; Carmen Scalise, MD, University of Catanzaro, Catanzaro, ITALY; Natalia Malara, PhD, Viale Europa, Germaneto, Catanzaro 88100, ITALY; Ivan Presta, PhD, University of Catanzaro, Catanzaro, ITALY; Pietrantonio Ricci, PhD, University of Catanzaro, Catanzaro, ITALY; Roberto Raffaele, BE*, University Magna Graecia of Catanzaro, Crotone 88900, ITALY; Santo Gratteri, MD, Catanzaro 88100, ITALY; Isabella Aquila, MD, PhD*, Institute of Legal Medicine, Catanzaro, Italy 88100, ITALY*

Learning Overview: After attending this presentation, attendees will understand the role of High-Mobility Group Box-1 (HMGB) and troponin in PMI estimation.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing the quantitative alterations of these proteins in the PMI on humans.

Time of death represents a challenge in forensic research. Multiple extrinsic factors may make it challenging to estimate PMI. Several authors have suggested employing new scientific approaches, such as proteomics, to help improve estimation accuracy. In the forensic literature, numerous studies have emerged describing the changes proteins within tissues or biological fluids undergo with advancing PMI. However, most of these studies were carried out on animals or on corpses found after several hours, without knowing the exact hour of death. In fact, before the collection of the samples, the corpse has already been exposed to the action of extrinsic factors that are often unknown. This presentation proposes the results of an Enzyme-Linked Immuno-Sorbent Assay (ELISA) analysis carried out on human plasma taken at the exact time of death. The markers examined were HMGB and Troponin T (TnT). This is the first experimental study on human samples performed at "zero time."

The experimental model was created at the Institute of Legal Medicine of the University Magna Graecia of Catanzaro. The model is based on taking peripheral blood samples from patients who died at the intensive care unit. The study was approved by the Ethics Committee. The informed consent was signed by family members before the death of the patient. The corpses were exposed to constant room temperature. The samples were taken at seven predefined time intervals, starting from the exact moment of death up to the next two hours. The samples were immediately centrifuged and stored at -80°C. Subsequently, the samples were analyzed by ELISA 96-wells in order to examine the quantitative changes of the HMGB and TnT markers. The kits used were MyBioSource® with human target. The following procedure was conducted: (1) add 100µL standard or sample to each well. Incubate for 90min–2hrs at 37°C; (2) remove the liquid. Add 100µL Biotinylated Detection Ab. Incubate for one hour at 37°C; (3) aspirate and wash three times; (4) add 100µL Horseradish Peroxidase (HRP) conjugate. Incubate for 30min at 37°C; (5) aspirate and wash five times; (6) add 90µL substrate reagent. Incubate for 15–30min at 37°C; (7) add 50µL stop solution. Read at 450nm immediately; and (8) the results were compared with the data published in literature.

Twenty-four cases were collected and divided into two main groups by age (cut-off 69.6 years), sex, and cause of death. Of these, ten cases were selected in proportional numbers from each group in order to guarantee the homogeneity of the sample and reduce the role of intrinsic potential variables. The patients were both male and female, aged between 56 and 86 years, and died from cardiogenic shock (seven cases), respiratory failure (two cases), and septic shock (one case).

This study showed a variability of HMGB-1 over time, with increasing levels of the marker at 2h compared to "zero time" (seven cases/ten) regardless of the cause of death. In the other three cases, an oscillation of the marker was observed with substantial stability. These results appear in agreement with the published literature regarding the potential increase in HMGB-1 expression levels as a function of PMI.[1,2] TnT revealed levels of variable expression within a wider range. Troponin levels were higher at 2h in seven cases out of ten. The increase was progressive and linear in cases of myocardial ischemia. A reduction in expression at 2h was observed in three cases of cardiogenic shock with systemic failure, pulmonary fibrosis, and septic shock. These results, also in agreement with the literature data, suggest that the marker levels strongly depend on the cause of death.[3,4] Being a marker of muscle damage, its expression would therefore be expected to be greater in cases of cardiac death. Although the analyzed sample is small, the proposed model is the first to perform proteomic investigations on human biological samples from the exact moment of death without exposing the corpse to temperature variations or other extrinsic factors.

In summary, this operating model is intended to identify the possible role of peripheral blood protein biomarkers procured at the precise time of death in the estimation of PMI, verify and evaluate the variation of the proteomic profile of markers already published in the literature, and improve analysis of the so-called "early PMI" for forensic purposes.

Reference(s):

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*Presenting Author