G16 Improved Estimation of Postmortem Interval With Multiple Protein Markers and Improved Analytical Methods

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After attending this presentation, attendees will learn about the development and utility of cardiac Troponin I (cTnI) and Troponin T (cTnT) as time since death markers for PMI Estimation.

This presentation will impact the forensic community and/or humanity by improving the accuracy in postmortem interval estimates. PMI provides crucial information required in many criminal, civil, and forensic investigations.

Time since death markers have lagged behind advances in forensics technology. Knight (1994) explains," regrettably, the accuracy of estimating the postmortem interval (PMI) has by no means kept pace with the enormous strides made in technological sophistication." Early work on time since death focused on postmortem temperature measurements and algorithms to model postmortem cooling behavior. Current technology is still largely based on postmortem temperature methods similar to those described in the 1800's. Marshall summarized the temperature measurement method as follows, "It would seem that the timing of death by means of temperature can never be more than an approximation."

Biochemical markers for estimating time since death offer the possibility of increased accuracy and reliability in time since death estimates. Cardiac Troponin I (cTnI) and cardiac Troponin T (cTnT) are heart tissue proteins and selective markers of cardiac muscle damage. Investigation of these proteins as a marker for time since death shows great promise in mammalian heart tissue. cTnI and cTnT are found as intact protein in freshly sampled tissue at the onset of death, (T_0). These proteins are good substrates for several enzymes released in cardiac tissue upon death (necrosis). The proteolytic breakdown of these proteins by proteases in postmortem cardiac tissue is exploited to determine the postmortem interval. Both bovine and human heart tissue show similar banding patterns upon degradation. This technique takes a small sample of cardiac tissue that is homogenized and extracted with magnetic microparticles. The proteins are separated by SDS-PAGE electrophoresis and selectively visualized by Western blot. The Western blot is probed with mouse monoclonal antibodies against cardiac TnI and TnT. This step is followed by an anti-mouse conjugate labeled with alkaline phosphatase that is developed with a precipitating colored substrate. The degradation pattern of cTnI and cTnT is monitored using this bioanalytical protocol. The area of the bands within a lane is quantitated by scanning and digitizing the bands. Project methodology will be migrated to more automated system such as capillary electrophoresis. This technique exploits the use of separation of the complex fraction isolated followed by detection of the fragments.

The results show a linear relationship between percent protein degraded and the log of postmortem time. A fresh "reference" human heart tissue obtained at time T0 was incubated to obtain a temporal degradation profile. Comparison of human cardiac tissue samples with unknown time of death can be evaluated qualitatively against the "reference" human heart tissue. The time of death can be estimated by matching the "degradation fingerprint." Similarly, a calibration curve ($r > 0.95$) can be obtained with the percent cTnI degraded plotted against the log of the time postmortem using the reference human heart tissue. This curve can be used to estimate the time since death relative to the "reference" tissue based on the percent degradation. Data indicates that the degradation of cTnI in heart tissue shows very specific bands during a postmortem interval of a week. Troponin T is more stable in comparison to Troponin I so the degradation of cTnT should be longer. Data combined from cTnI and cTnT could be used for extended PMI estimate. Human cardiac tissue samples frozen at known time of death were analyzed by both semi-quantitative and qualitative techniques and both show similar agreement with the known time of death.

Overall, the data demonstrates that this technique represents a major advance in time of death determination providing a reliable semi- quantitative biochemical marker from a protected organ versus estimates based on direct temperature measurements. Tissue cardiac Troponin I and Troponin T shows excellent characteristics as time of death markers in the extended postmortem interval that is difficult to estimate with current methods.

TnI (Troponin I), TnT (TroponinT), Postmortem Interval (PMI)

 ** Presenting Author*