G4 Improved Estimation of Time Since Death With Multiple Protein Markers and Automated Analytical Methods

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After attending this presentation the attendee will learn how degradation of cardiac Troponin I (cTnI) and Troponin T (cTnT) analysis can help forensic scientists narrow their estimate of postmortem interval.

This presentation will impact the forensic community by improving the precision and rate of postmortem interval (PMI) estimates using an automated analytical method to better assist law enforcement in many criminal, civil, and forensic investigations.

Knowledge of the time since death (PMI) has enormous legal, crimi- nological, and psychological impact; but currently suffers from uncertain- ties on the order of days to weeks without mathematically defined confidence information and a lack of technological advances. The main principle of the determination of the time since death is the calculation of a measurable date along a time-dependent curve back to the start point. Characteristics of the curve slope and the start point are influenced by internal and external, ante- mortem and postmortem conditions which need to be taken into considera- tion. Current methods utilize temperature-based algorithms intended to model the cooling of the body after death in order to estimate the postmortem interval which introduces considerable inaccuracy due to influencing fac- tors. Livor mortis, rigor mortis, and to a lesser degree, algor mortis also have been used to estimate the postmortem interval. Forensic pathologists agree that these characteristics only provide "postmortem windows." Quantita- tion of the vitreous fluid potassium level has been of some value in evaluat- ing the early postmortem interval, but the accuracy of this method is dependent on external conditions, the availability of vitreous fluid and the purity of the sample. For practical purposes, a simple, relatively inexpensive assay performed on readily available cardiac tissues, less dependent upon external factors, and providing data that could be plotted on a reproducible control curve would be of value in determining the postmortem interval ac- curately.

Cardiac Troponin I (cTnI) and cardiac Troponin T (cTnT) are proteins found in heart tissue as selective markers of cardiac muscle damage, and investigation of these proteins for determining time since death shows great promise in mammalian heart tissue. These proteins are good substrates for several enzymes released in cardiac tissue upon death (necrosis); the proteolytic breakdown of theses proteins in postmortem cardiac tissue can be exploited to determine the PMI. This technique takes a small sample of cardiac tissue that is homogenized and the proteins are then extracted with magnetic microparticles, separated by SDS-PAGE and visualized by Western blot, which is probed with mouse monoclonal antibodies against cardiac TnI and TnT. This step is followed by labeling and precipitation with a colored substrate to monitor degradation patterns. The area of the bands within a lane is quantitated by scanning and digitizing the image using commonly available scanners. This methodology is also migrated to more automated capillary electrophoresis.

The results show a linear relationship between the percent protein degraded and the log of the postmortem time. A fresh "reference" human heart tissue obtained at time T_0 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 of death relative to the "reference" tissue based on the percent degradation. The data indicate that the degradation of cTnI in heart tissue shows very specific bands during a postmortem interval of a week. The Troponin T is a more stable protein in comparison to Troponin I, so the degradation of cTnT takes longer. Combining the data obtained from the cTnI and cTnT can then be used for extended PMI estimates. Frozen human cardiac tissue samples at known times of death were analyzed by both the semi-quantitative and the 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 fast and reliable semi-quantitative biochemical marker from a protected organ versus other measurements. Tissue cardiac Troponin I and Troponin T shows excellent characteristics as a time of death marker in the extended postmortem interval which is difficult to estimate with current methods.

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

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