G77 Determination of Time Since Death— Cardiac Troponin I

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This presentation will describe the development and utility of cardiac Troponin I (cTnI) as a time since death marker. Estimates of time since death in the early postmortem interval 0–7 days are currently performed using different temperature based methods and other physical parameters that lack the desired forensic reliability. The qualitative and semi-quantitative analysis of the degradation of cTnI as a time since death marker will be discussed using a bovine tissue model followed by postmortem human cardiac tissue samples.

The importance of determining the time since death is crucial to criminal, civil and forensic cases. Time since death markers have lagged behind the advances in technology of the past century. Knight 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 documented works on time since death focused on temperature measurements postmortem and possible algorithms to model the behavior of postmortem cooling of the body. Current technology is based on postmortem temperature methods similar to those described back in the 1800s. Marshall, an expert in this area, best summarizes the general issues with temperature measurements as follows, 'It would seem that the timing of death by means of temperature can never be more than an approximation.' Biochemical markers investigated to estimate time since death include protein fractions, urea, creatinine, glucose, iron, potassium, calcium, enzymes, immunohistochemical detection of insulin in pancreatic β-cells, myo-albumin fraction and Strontium-90 calcium analogue levels. Postmortem muscle proteolysis has been researched to explain the muscle relaxation following rigor mortis. Temperature, as a time since death marker, remains a leading marker after many years of investigations and limitations.

Cardiac Troponin I emerged as the leading serum marker for myocardial infarction (heart attack) in both the U.S. and Europe in the mid 1990s. It has become the gold standard serum marker for cardiac damage. This research is focused on a technique exploiting the postmortem tissue degradation of cardiac Troponin I to determine the time since death.

The technique consists of isolating and separating troponin I and its proteolytic fragments from cardiac muscle tissue (myocardium). This is accomplished by using magnetic microparticles that capture this protein from a 1.0 g cardiac tissue homogenate extracted with a buffer that inhibits proteolytic activity. The capture microparticles are incubated for 1 hour and washed several times with extraction buffer. The proteins bound are eluted from the microparticles using a low pH buffer. The proteins are separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to paper using a semi-dry Western blot protocol. The proteins transferred are probed with monoclonal antibodies specific for cardiac Troponin I. The blot is then incubated with goat anti-mouse antibody labeled with alkaline phosphatase (GAM-ALP). The blot is developed after incubating with a precipitating colored substrate. The bands of cTnI and most of its proteolytic degradation products that retain the antibody-binding region (epitope) are visualized by this technique. Digitization of the Western blot is performed using a scanner and software that can integrate the area of the peaks for qualitative and semi-quantitative analysis.

Cardiac troponin I exists as an intact protein when sampled from fresh human cardiac tissue. The experiments focused on a model of bovine cardiac tissue followed by human cardiac tissue with known time since death. The samples were frozen until the analysis was performed to avoid proteolysis during storage. The results indicate a consistent cTnI banding pattern amongst different human cadavers and a pseudolinear relationship between percent cTnI degraded and the log of the time since death with a coefficient of correlation, $r > 0.95$. The unknown time since death degradation pattern can be qualitatively compared to a "reference heart" incubated under controlled conditions. The analysis matches the cTnI degradation pattern of the cadaver in question to the "reference heart" degradation pattern incubated at different time points. Thus, the extent of cTnI degradation serves to estimate time since death. Overall, this technique offers advantages over current methods such as wider postmortem interval, measurable degradation pattern and a temporal semi-quantitative relationship. In addition, at lower temperatures the postmortem prediction interval can be extended to provide a wider range. The degradation pattern of tissue cTnI is useful in the determination of the early postmortem interval (0 to 7 days), which is difficult to estimate with current technology.

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