G98 Age Estimation of Wounds Using the Proximity Ligand Assay

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After attending this presentation, attendees will learn how a novel immunohistochemical technique can improve the interpretation of histological samples from injuries.

This presentation will impact the forensic science community by showing how the proximity ligand assay has an advantage over other immunohistochemical methods by providing reactivity only when two marker proteins bind to each other, or are in close proximity, which may be an indication of a transient reaction after injury, not seen in uninjured tissue.

In the forensic pathology routine casework determining the time of infliction of wounds may be a critical issue. Conventional histological methods allow for a rough age estimation, but suffer from high imprecision. Platelet-selectin (P-sel) shows rapid dynamics in the early phase of an injury. Under normal conditions, it is stored in Weibel-Palade bodies in endothelial cells and in α-granules in platelets. After an injury, a degranulation occurs in these cells and P-Sel is transferred to the cell membrane; however, only for a short time, implying that membrane-bound P-Sel will disappear within hours after an injury. Deciding to take advantage of the possible co-localization of P-Sel and the Platelet Endothelial Cell adhesion molecule (PECAM-1), abundant in the cell membrane of both endothelial cells and platelets, by applying the proximity ligand assay (PLA) technique. This assay uniquely produces a reaction only when two secondary antibodies are physically very close. Further, von Willebrand factor (vWF) is also stored in the same granules as P-sel. This co-localization should therefore be expected to produce a PLA reaction under normal conditions. Degranulation due to vessel injury should reduce the positivity when these cells release P-Sel to the cell membrane and vWF to the circulation. Other combinations of antibodies, to factors involved in the coagulation and complement systems, as well as some early inflammatory markers were also investigated. The positivity of P-Sel - PECAM-1 showed a discrete time window. PSGL-1 is the natural ligand to P-Sel when exposed as a receptor in the membrane. P-Sel- PSGL-1 also showed a limited, and earlier, time window. A number of reactions in the coagulation cascade were futher examined. The reactivity of thrombin and fibrinogen showed a reactivity in the early phase of an injury, but the reactivity remained for a prolonged period. Antibodies against a number of other factors in the coagulation cascade were also examined, but the results were difficult to interpret. The methodology requires that each antibody shows a reasonable staining pattern in normal and injured tissue, and that their close co-localization occurs during a limited time period after an injury. Collagen III and Glycoprotein VI each showed a decent reactivity in normal tissue when applied separately, but still failed to produce a distinct reactivity in the early phase after injury when tested by the PLA technique. This may either be explained by a too large distance between the epitopes on each protein not allowing for the antibodies to produce a reaction. Alternatively, it might indicate that these factors do not interact, and that observation would then constitute a novel finding. The appearance of coagulation factor complexes of various kinds showed variable time patterns that need further studies. Having stated that, the reactivity of the different antibody combinations of the coagulation and complement systems consistently produced a negative reaction in uninjured samples, as well as in injuries of older age. It is believed that this methodology, using suitable combination of antibodies, will improve the age estimation of injuries, and conclude that the technique can be used by unexperienced users, since the all-or-none response that this method provides allows for an easy interpretation.

Wound Age Estimation, Immunohistochemistry, Postmortem

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