H36 The Detection of Microscopic Markers of Haemorrhaging and Wound Age on Dry Bone: Beating the Barriers Between Forensic Anthropology and Forensic Pathology

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After attending this presentation, attendees will learn that it is worth trying to apply immunohistochemical techniques on dry bone in order to verify the antemortem nature of a fracture.

This presentation will impact the forensic community and/or humanity by demonstrating that the application of histopathology techniques for detection of survival may be useful also on dry bone and therefore in forensic anthropology.

An example of the barriers and conceptual differences between forensic anthropology and pathology can be seen in determining the vitality of a wound. Pathology can make use of skin color and microscopic techniques, anthropology needs different criteria. The diagnosis of the vitality of a wound (whether it is produced ante-mortem or postmortem) as well as determination of the time elapsed between the production of the wound and death is a crucial issue in forensic pathology. In fresh skin, the red-purplish coloration of a cut or bruise will reveal its vitality whereas the change in coloration, from a macroscopic perspective, will reveal the time of survival. In more difficult cases, microscopic analyses can be performed. A plethora of data from classical histological techniques and newer immunohistochemical techniques provides more accurate statements of survival time, although this depends on the stain used, time from death, etc. One however must keep in mind that unless there has been at least a four hour survival time, histological distinction between ante-mortem and postmortem skin wounds is not possible, regardless of sporadic studies which declare better results for short survival times with immunohistochemistry. Similar statements can be said concerning bone trauma, in particular fractures. Bone follows similar “laws” with regards to the progression of histological changes during healing. Even if the beginning of the healing process (periosteal bone production and callus formation) can be detected macroscopically and radiologically, these processes require a long time.

The scope of this pilot study was therefore to collect bone fractures from cadavers with a known time of survival, have them undergo a simulated putrefaction procedure until they became “dry bone” and perform macroscopic and microscopic analysis to verify the potential of histology in identifying “vital” processes in putrefied soft-tissue-free bone. This would allow one to verify if histopathological techniques can be useful in determining the antemortem nature of fractures in dry bone.

Six samples of fractured bone (cranium, rib, tibia) were taken from cadavers with known time of survival between trauma and death. Time intervals ranged from a few seconds after the bone fracture had been inflicted, to several hours, days, and weeks. A negative control was included (postmortem fracture). At the time of sampling, before starting the decalcifying process (using the following solution: distilled water 80%, HCl 10%, formic acid 10%), ink was painted onto the fracture surface to be studied. Fractured edges were observed macroscopically and then macerated in water in order to clear the bone of residual soft tissue and “simulate” dry bone. The bone was then decalcified and stained with H&E, PERLS (for the demonstration of hemosiderin deposits), and PAS, PTAH and Weigert (for the demonstration of fibrin). Immunohistochemistry was performed using a monoclonal antibody anti-human Glycophorin A. Two staining methods were used for antigen detection, the first with boiling of the sections and the second with incubation at room temperature.

Preliminary results indicate that not only do the PTAH and glycophorin tests show the presence of thrombi and red blood cells residues, respectively, on the fractured margin, but also within Haversian canals. This study, though certainly not conclusive, shows that it may be worth pursuing the study of bone fractures from a histopathological point of view even on “dry bone.”

Bone Fractures, Microscopy, Haemorrhaging

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