H143 The Histomorphology of Cranial Fracture Healing: Preliminary Observations

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Learning Overview: After attending this presentation, attendees will have an understanding of important histological features of cranial fracture healing and a proposed method for evaluating them in four differently stained slides.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by contributing to the field's knowledge of how cranial fractures heal and the cells and tissues involved in this process. In addition, this work is laying the foundation for a method to evaluate healing cranial fractures.

Understanding how cranial fractures heal and time-since-injury are important goals of forensic pathology and anthropology, especially as these biological responses relate to the context of accidental versus non-accidental injury. The scientific literature does not provide useful data or methods for estimating times since injury of cranial fractures. As such, this project has two primary research goals: (1) documenting the histomorphology of healing cranial fractures, and (2) establishing histomorphologic phases of cranial fracture healing.

Histologic sections from subadults and adults with known cranial fractures and/or surgical interventions of varying healing times have been collected and stained with: hematoxylin and eosin, Masson's trichrome, alcian blue hematoxylin/orange G, and Russell-Movat pentachrome. These stains were chosen for their ability to highlight different elements of bone healing. Specifically, evaluators are charged with identifying the following cells and tissues associated with bone healing: hematoma, pigment-laden macrophages, fibrin, inflammation, fracture edge morphology, mesenchyme/loose connective tissue, fibroblasts and/or fibrocytes, fibrous connective tissue/collagen, new capillaries, cartilage matrix, bone matrix, bone resorption, woven bone, lamellar bone, and reversal/cement lines.

To prepare samples for histology, the following methodology is employed: a gross sample of the fracture is removed (approximately 20–30mm); the sample is decalcified using either nitric acid, Ethylenediaminetetraacetic acid (EDTA), or Hydrochloric Acid (HCL); sections of the fracture are cut and the tissue is paraffin embedded; four thin sections are cut; and each section is stained and mounted on a glass slide. The slides are digitized and uploaded to a digital microscopy database. Glass slides are placed in four-slide kits and assessed by three randomly selected evaluators. As previous investigators have found variation in bone healing between the outer and inner tables, three different zones were identified for evaluation: the outer table, diploë, and inner table. For each slide, the evaluators assess the presence, absence, and quantity of the cells or tissues listed above. A separate evaluation form is used to assess each zone and stain; as such, each evaluator collects data on 12 different evaluation forms for each sample, resulting in 396 observations on a single sample (and 1,188 total observations by three evaluators per sample). When all slides in a set are assessed, evaluation forms are entered into a database for use in various statistical analyses. Analyses include evaluation of intra- and inter-observer variability; patterns in the presence, absence, and relative quantity of cells and tissues; and correlation of various cells and tissue.

Early findings suggest that HCL is an inferior method for decalcification, compared to EDTA and nitric acid. In addition, the cell and tissue types differ in juvenile versus adult individuals. Additionally, evaluators are noting considerable variability in the type of tissues present in the fracture gap among age-cohorts. Some of these differences include the presence of woven bone and cartilage matrix in juveniles, but these tissues appear to be absent in adult cranial injury healing. As more samples are collected, researchers will be able to provide evidence-based timelines for how different types of cranial bone injuries heal among various age-cohorts. Furthermore, the results of this research will provide a framework for forensic pathologists and anthropologists to sample, document, and classify fractures, as well as provide basic science data for future research goals of creating a method for interpretation of time-since-injury.

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Fracture, Histology, Time-Since-Injury

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