A68 Estimation of the Postmortem Interval (PMI) of Skeletonized Human Remains Using Nile Blue and Indophenol Colorimetrics on Femoral Cortical Thin Sections

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After attending this presentation, attendees will understand the potential of colorimetrics for the estimation of a PMI when applied to femoral bone sections.

This presentation will impact the forensic science community by testing the staining reaction of Nile Blue (NBL) and Indophenol (IND) on thin sections of the femoral cortex and by quantifying the correlation between the coloration and the PMI.

PMI is an essential piece of information for estimation, especially when dealing with skeletonized dry human remains, as it determines whether the case is forensically relevant or historical/archaeological. Although the prescription period varies depending on the country's criminal code, it generally spans between 10 and 30 years (with no limit in homicidal cases for some contexts). PMI estimation is complex after complete skeletonization, and even with a thorough analysis of the taphonomy and surrounding environmental factors, there is no straightforward method available to clearly quantify a PMI. Following Berg and Specht's observations in 1958, the colorants NBL and Indophenol IND tend to reveal differential affinities with cortical bone depending on the PMI.[1] The variations in coloration were visually assessed but not discussed. It was noted that NBL was more sensitive to ancient bones and IND more accurate for coloring recent bones. The protocol was tested later by Knight and Lauder, but judged too unreliable for routine use.[2] As part of the research within the French National Forensic Institute (IRCGN), the PMI estimation through colorimetrics of NBL and IND was further investigated.

A sample of 32 femoral sections from individuals with a known PMI was collected from a Swiss osteological reference collection, a number of French archaeological collections, and a number of recent forensic cases. The coloration protocol followed the procedures described by Berg.[3] Cortical thin sections were sliced at 1.2mm, immersed in 2% NBL or IND for 10 to 20 minutes, rinsed, and differentiated for 12 hours with acetic acid or 70% alcohol (respectively, for NBL and IND). For a more objective assessment of the resulting color, the dried sections were measured using a Konica Minolta CM-2600d/2500d spectrocolorimeter, which quantifies the color using the L=lightness, a=ambiance, b=brightness (Lab) system. The consensus-measured color is the average Lab for 30 measurements of the same section. Some sections were measured on both sides to evaluate potential systematic variations. Additionally, some steps of the protocol for the NBL were tested (i.e., different concentrations of both the NBL solution and acetic acid and different immersion times).

The dispersion of PMI within the tested sample was not homogeneous as there were too few recent individuals (<30 years of age) versus old (30-100 years of age) and archaeological individuals (>100 years of age). Consequently, correlations and regressions did not produce results coherent enough to build estimation models; however, computing the raw Lab data in a principal component analysis allowed for an objective visualization of the distribution of the recent, old, and archaeological individuals. Although this preliminary study did not take into account the taphonomy factors (environmental influence on cortical bone composition and the coloration process), NBL demonstrated a moderate potential to distinguish individuals with a PMI inferior or superior to 100 years, and IND demonstrated a stronger potential to distinguish individuals with a PMI inferior or superior to 30 years. A larger sample that is taphonomically controlled and has a homogenous PMI distribution is required before building a robust estimation model using IND colorimetrics, but this rapid and cost-efficient technique is promising.

Reference(s):

1. Berg S., Specht W. Untersuchungen zur bestimmung der liegezeit von skeletteilen. *Int J Legal Med.* 1958:47(2):209–241.
2. Knight B., Lauder I. Methods of dating skeletal remains. *Hum Biol.* 1963:41(3):322–341.
3. Berg S. The determination of bone age. In: Lundquist F., Curry A., editors. *Methods of Forensic Science, Volume 2*. New York: Interscience, 1963: 231–252.V

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