Biomarkers of Neutrophil Extracellular Trap Formation (NETosis) and Their Relationship to Wound Healing in Ankle Fracture Surgery

William Kent, MD; Mathew Whittaker, BS; Vitor Martins, BS; Simon Schenk, PhD; Paul Girard, MD; Alexandra Schwartz, MD

Purpose: A variety of patient factors have been shown to contribute to wound complications after open reduction and internal fixation (ORIF) of ankle fractures. However, there are few studies analyzing molecular biomarkers associated with wound complications in ankle fractures. NETosis is a process by which neutrophils release their nuclear contents to trap and kill pathogens. Originally recognized as a component of host immune defense, NETosis has been demonstrated to contribute to delayed wound healing. The purpose of this study was to investigate biomarkers of NETosis in ankle fracture patients undergoing ORIF and correlate these markers with postoperative wound healing.

Methods: 32 adipose samples were collected from the surgical site in patients (17 male, 15 female) undergoing ORIF of bimalleolar and trimalleolar ankle fractures from September 2016 to May 2017. Total RNA was isolated and used for semiquantitative real-time polymerase chain reaction to assess gene expression for markers of NETosis. Patients were followed at regular postoperative intervals to assess wound healing.

Results: The percentage of patients with detectable protein arginine deiminase 4 (PAD4) was significantly higher in patients with delayed wound healing compared to normal wound healing patients (P = 0.012). Gene expression of interleukin 8 (IL-8; P = 0.02), IL-6 (P = 0.002), and monocyte chemoattractant protein 1 (MCP-1; P <0.001), all neutrophil-related biomarkers, were significantly elevated in abnormal wound healing patients compared to normal wound healing patients.

Conclusion: PAD4 is an enzyme necessary for NETosis, a process shown to contribute to delayed wound healing. The significantly higher percentage of detectable PAD4 in patients who went on to develop delayed wound healing or infection suggests potential predictive value for this biomarker. Furthermore, the significant association between delayed wound healing and expression of cytokines important for neutrophil recruitment (IL-8 and MCP-1), and IL-6, a cytokine released from neutrophils, provide additional support for the role of neutrophils and NETosis in delayed wound healing and associated complications.

The FDA has stated that it is the responsibility of the physician to determine the FDA clearance status of each drug or medical device he or she wishes to use in clinical practice.