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Metalloproteinase Expression is Associated with Traumatic Wound Failure

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Background. **Matrix metalloproteinases (MMPs) are crucial in the inflammatory and remodeling phases of wound healing. We previously reported the correlation between pro-inflammatory cytokines and timing of successful combat-wound closure. We now extend our studies to investigate the correlation between wound-remodeling MMP expression and wound healing.**

Methods. **Thirty-eight wounds in 25 patients with traumatic extremity combat wounds were prospectively studied. Surgical debridement with vacuum-assisted closure (VAC) device application was repeated every 48 to 72h until surgical wound closure. Wound effluent and patient serum were collected at each wound debridement and analyzed for five matrix metalloproteinases using the Luminex multiplex system; Millipore Corp, Billerica, MA. The primary outcome was wound healing within 30 d of definitive wound closure. Impairment was defined as delayed wound closure ($>$ 21 d from injury) or wound dehiscence. MMP expression was compared between impaired and normal healing wounds.**

Results. **Elevated levels of serum MMP-2 and MMP-7 and reduced levels of effluent MMP3 were seen in impaired wounds ($n = 9$) compared with wounds that healed ($n = 29$; $P < 0.001$). Receiver operating characteristic (ROC) curve analysis yielded area-under-the-curve (AUC) of 0.744, 0.783, and 0.805, respectively.**

Conclusions. **Impaired wound healing is characterized by pro-inflammatory MMP-2 and MMP-7. Serum and effluent concentrations of MMP-2, MMP-3, and MMP-7 can effectively predict the outcome of traumatic war wounds and can potentially provide decision-supportive, objective evidence for the timing of wound closure.** Published by Elsevier Inc.

Key Words: **Luminex; MMP-2; MMP-3; MMP-7; effluent; multiplex; dehiscence; acute wound; VAC.**

INTRODUCTION

Advances in body armor have contributed to increased survival of some of our most severely injured combat casualties [1]. However, this survivability often comes at a devastating cost. Blasts from improvised explosive devices (IEDs) and other high-energy weaponry cause excessive extremity trauma that often renders limbs unsalvageable or requires extensive surgical care [1–4]. The resulting large wound beds are often difficult to manage due to location, extent of tissue damage, and the frequent additional complications of contaminating debris and recalcitrant bacteria [5–8].

Negative-pressure vacuum-assisted closure device (VAC) usage, in combination with serial debridements of devitalized tissue and high-pressure wound irrigation, has greatly reduced the morbidity of such traumatic extremity wounds [9, 10]. Although these methods have been accepted as the standard of care in many institutions, timing of surgical closure of a wound remains a subjective process in which the surgeon relies on criteria such as the patient's overall condition, wound location, wound bed gross appearance, and local perfusion. In spite of this quality care and success rate, some wounds still result in dehiscence. Conversely, unnecessary surgical debridements occur on wounds that could have been closed earlier than

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the surgeon had surmised, which results in additional patient risk and expense [8]. The study herein investigates the association of matrix metalloproteinases (MMPs) and the timing of successful surgical closure of acute traumatic wounds.

Acute wounds typically heal by a complex and interdependent sequence of events that can be divided into four phases: initiation (clotting), inflammation, proliferation, and maturation [11]. Progression through each phase from initial injury to wound healing and resolution is highly dependent on the molecular environment at the wound site [12]. MMPs play crucial roles in the inflammation, proliferation, and maturation phases by performing several functions related to inflammatory signaling and wound remodeling [13–15]. MMPs, which are proteins that incorporate a Zn^{2+} or Ca^{2+} ion in their enzymatic active sites [14], can be generally classified into five classes based on their primary substrate: collagenases, gelatinases, stromelysins, matrilysins, and membrane-type matrix metalloproteinases. However, recent research asserts that many MMPs have overlapping functions [16]. A thorough literature search has revealed that the major MMPs involved in wound remodeling are MMPs 1, 2, 3, 7, 8, 9, 10, 12, and 13, although, with ongoing research, it is likely that all MMPs play essential roles in wound healing. [13, 14, 16–20].

Collagenases (MMP-1, MMP-8, and MMP-13) mainly cleave type I, II, and III fibrillar collagen present in the extracellular matrix (ECM). This allows for properly-oriented keratinocyte migration and ultimately sets the stage for later wound remodeling [13, 14, 21]. Similarly, gelatinases (MMP-2 and MMP-9) have been shown to participate in wound remodeling by cleaving type IV, V, VII, and X collagen, elastin, and other basement-membrane proteins [13–15]. More recently, MMP-2 and MMP-9 have been implicated in inflammatory cell recruitment as well as in the establishment of chemotactic gradients that direct immune cell migration [14]. Similar to the actions of gelatinases, stromelysins (MMP-3, MMP-10, and MMP-11) have been shown to degrade elastin and collagens IV, V, and X [13, 14]. MMP-3 has also been shown to possess regulatory functions in chemokine signaling as well as a critical role in both reepithelialization and wound contraction[14]. Matrilysin (MMP-7) shares many of the functions of MMP-3 such as processing elastin and wound bed reepithelialization, but also demonstrates the inflammatory functions of MMP-2 and MMP-9 by enhancing neutrophil migration across epithelial layers via chemokine processing [14]. Membrane-type matrix metalloproteinases (MMPs 14–17) are present on cell membranes and appear to aid in binding other MMPs to cell surfaces for local activation and wound reepithelialization [13].

These highly regulated and interdependent processes exist during “normal” wound healing, defined herein as wounds that are surgically closed within 21 d post-injury with no dehiscence within the following 30 d. We have observed that wounds exhibiting delayed healing (surgical closure>21 d post-injury) or subsequent dehiscence are the result of an inflammatory dysregulation of the wound-bed molecular environment [7]. This state of excessive or prolonged inflammation seems to ‘stall’ progression through the wound healing phases, thus resulting in impaired wound healing. Elevated levels of the proteases MMP-2 and MMP-3 and low levels of their respective inhibitors have been measured in wound effluent of chronic pressure ulcers treated with VAC [17]. Such elevated MMP expression and disproportionate expression of their inhibitors has been proposed as a cause of wound chronicity [15, 22–24].

By analyzing concentrations of MMP-2, MMP-3, MMP-7, MMP-9, and MMP-13 in traumatic extremity wound effluent throughout the treatment process, this study sought to parallel these studies in acute wounds. Additionally, our goal was to build a systemic picture by monitoring serum levels of these representative MMPs. We hypothesized that MMP expression, as an objective marker of healing, is indicative of timing for successful surgical wound closure and avoidance of dehiscence.

MATERIALS AND METHODS

Study Methodology

This serial, observational study with prospective data collection was conducted in accordance with the ethical standards of the committee on human experimentation as approved by the institutional review board of the National Naval Medical Center (NNMC) and the Naval Medical Research Center (NMRC). Study participants were recruited from wounded U.S. service members evacuated to the National Capital Area from Iraq and Afghanistan between January 5, 2007 and May 30, 2008, and treated at the National Naval Medical Center (Bethesda, MD). Informed consent was obtained for all participating patients. Inclusion criteria for this study were defined as all service men and women who sustained penetrating injuries to one or more extremities. Up to three wounds per patient were studied. Patients with confounding comorbid conditions, such as immune disorders, connective tissue disorders, or any conditions requiring immunosuppressive agents, were excluded. One patient was excluded from data analysis due to death prior to wound closure. One patient declined the study. Ten eligible patients were not entered as a third party could not be contacted for consent prior to the first surgical procedure at our facility. Although a third party was contacted by telephone, two eligible patients were not entered into the study as third-party consent was only accepted if the party was physically present. Recorded demographic variables included age, gender, date, body mass index, nicotine use, injury severity score (ISS), concomitant traumatic brain injury, location and mechanism of injury, wound size, associated major vascular injury to the affected limb, type of wound closure, number of wound debridements (Table 1), and Acute Physiology and Chronic Health Evaluation II (APACHE-II) scores (data not shown). Surgical debridement, pulse lavage, and VAC application were repeated every 48 to 72 h until surgical wound