Transforming Growth Factor-ß2 Gene Expression Early May Be Predictive of the Severity of Future Development of Heterotopic Ossification

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Background/Purpose: Heterotopic ossification (HO) remains a significant problem for wounded warriors. As many as 60% of extremity injuries resulting from high energy blasts will result in HO formation. HO may result in skin breakdown, which can complicate prosthetic fitting and limit joint range of motion. Although there have been many studies into the etiology of HO formation, there is still no current way of determining which patients will develop significant HO and which patients will not. In this regard, not all patients that develop HO will have symptomatic HO that requires reoperation. The purpose of this study was to correlate severity of HO formation with gene expression levels within the traumatized tissue early after the sustained injury.

Methods: We retrospectively reviewed 175 patients who had tissue collected during surgical debridements following combat injury. Patients were identified who positively developed HO with adequate radiographic evidence between 2 and 9 months postinjury. Patients were excluded if tissue samples were not obtained within 2 weeks of injury. Additionally, patients were excluded if the tissue sample was not adequate to perform RNA isolation. The included patients had their HO graded and were stratified into mild, moderate, or severe HO. Real time quantitative polymerase chain reaction (qPCR) was used to identify significant changes in gene expression between the groups.

Results: We identified 13 patients who meet the criteria for inclusion. All patients were male with an average age of 28 years (range, 23-39). Tissue was collected on average 10 days from injury (range, 5-16 days). All patients were injured as a result of IED (improvised explosive device) blast. Three patients were identified as having transtibial amputations, three had open femur fractures, and seven patients had transfemoral amputations. Following stratification of HO severity, four patients were identified as severe HO, four as moderate, and five as mild HO. Several genes demonstrated upregulation from mild to severe HO. Patients classified as moderate or severe HO had significant upregulation of collagen 3A1 (COL3A1) and β2 microglobulin expression compared to those with mild HO. However, the difference in collagen 3α1 and β2 microglobulin expression was not statistically significant when comparing severe to moderate HO. Transforming growth factor beta-2 (TGFβ-2), however, demonstrated over a twentyfold upregulation in mild compared to moderate HO and over a sixtyfold upregulation in mild to severe, which correlated to a threefold upregulation from moderate to severe HO, which was a statistically significant difference in gene expression. TGFβ2 was the only gene expressed that demonstrated a linear increase between the various grades of HO severity, and was also the only gene that demonstrated a statistical significant difference when comparing the moderate to severe HO formers.

See pages 47 - 108 for financial disclosure information.

Conclusion: The ability to predict which patients will develop severe HO compared to those that will develop mild would allow clinicians to counsel patients about the potential need for future reoperation and provide them with closer follow-up. TGFβ-2 expression as a marker may have predictive value for severity of later HO development. This known mediator of fibrotic tissue development is increasingly upregulated in the patient developing mild, moderate, or severe HO. There were other genes expressed, such as COL3A1, which demonstrated a significant upregulation in the moderate and severe groups when compared to mild HO formers; however, none of these showed the linear progression from moderate to severe demonstrated by TGFβ-2. Furthermore, these markers may serve to identify patients in need of HO prophylaxis, as they may be at a higher risk of developing moderate to severe HO, which could necessitate additional operations to address symptomatic HO or other complications typically encountered with increased severity of HO.

Fig 1: Volcano plot demonstrating fold increase in TGFB2 expression in moderate and severe compared to mild HO group