



US DEPARTMENT OF DEFENSE

# BLAST INJURY RESEARCH PROGRAM COORDINATING OFFICE

## Diagnostics and Biomarkers

### Identification of Biomarkers of Heterotopic Ossification Following Extremity Blast Amputation: Animal Model Correlation with Human Disease

Heterotopic ossification (HO) is an ongoing issue for wounded Service Members, particularly after blast injury. Complications related to HO in blast amputation residual limbs include pain, overlying skin and muscle breakdown, poor fitting and functioning of prosthetic limbs, reoperation for amputation revision, and impaired limb function that delays rehabilitation. Current treatments are poor, and limited to mitigation rather than prevention. Furthermore, removal of heterotopic bone after it has formed can be difficult; this frequently requires resection of substantial amounts of soft tissue and risks injury to adjacent neurovascular structures that are often intimately associated with the ectopic bone. It is preferable to address the issue of HO before it begins. Developing a rat model to better characterize gene and protein level expression is critical to the identification and treatment of HO in wounded Service Members. To address this need, researchers at Uniformed Services University of the Health Sciences (USUHS) have developed a survival animal model for HO after extremity blast amputation in the Sprague Dawley rat subjected to a controlled explosion that closely resembles injuries observed in Service Members. The model has a high incidence (~90 percent) of ectopic bone formation in the amputation site with reliable animal survival following the blast.<sup>1,2</sup> Controlled study of this injury holds promise for development of effective interventions to prevent complications related to HO in the residual limb for survivors of blast type amputations. To date, all hind-limb blast amputation procedures on 75 animals have been completed, as well as related scheduled biopsies. The harvested specimens are currently undergoing ribonucleic acid (RNA) profiling using an Osteogenesis pathway specific RT2 Polymerase Chain Reaction array (SABiosciences) to determine the correlation of osteogenic marker expression between the treatment groups. Many of the genes determined from these analyses still need further molecular and cellular biology investigation to better understand their function.

Correlation of animal and human HO findings will allow identification of common biomarkers that are present early in the process and are predictive of HO formation in wounded Service Members at greatest risk. Biomarker identification of Service Members likely to develop HO, as well as potential prevention of HO is needed to offer amputation survivors the best possible quality of life (QOL). In the future high-risk individuals will ultimately be able to enroll in a clinical trial of therapeutic interventions known to effectively prevent HO in the civilian setting. Human tissue sample collection from wounded Service Members treated at Walter Reed National Military Medical Center (WRNMMC) will start as soon as Institutional Review Board (IRB) approval is received. This research is being conducted at USUHS and is funded by the Peer Reviewed Orthopedic Research Program (PRORP) of the Congressionally Directed Medical Research Programs (CDMRP).

- 1 Tannous, O., Griffith, C., O'Toole, R. V., & Pellegrini, V. D. J. (2011). Heterotopic ossification after extremity blast amputation in a Sprague-Dawley rat animal model. *Journal of Orthopaedic Trauma*, 25(8), 506–510. <https://doi.org/10.1097/BOT.0b013e31821f6265>
- 2 Jaffe, D. E., Yoo, D., Blevins, J., Gasbarro, G., Hughes, T., Paryavi, E., ... Pellegrini, V. D. J. (2015). Does Blast Medium Affect Heterotopic Ossification in a Blast- amputation Model? *Clinical Orthopaedics and Related Research*, 473(8), 2680–2687. <https://doi.org/10.1007/s11999-015-4320-z>

