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14. ABSTRACT
Our team completed the study and the PI published/submitted 8 manuscripts and 4 abstracts during the period of performance. Our final paper of 33 service members demonstrated that the MAR of trauma-induced HO was approximately 1.7 µm/day at the time of surgical intervention, a value I.7x higher than non-pathological human bone. The MAR and post-operative alkaline phosphatase (AP) values and AP pre-operative levels and the percent of osteoblastic activity were positively related and statistically significant (p0.509, p0.026, n J 9) and (p0.522, p 0.004, n29) respectively. When data was analyzed only within a two-year period from injury to excision (thereby removing outliers that were significantly longer than counterparts) and traumatic brain injury and non-steroidal anti inflammat01y drugs were controlled for in the statistical analysis (known correlates with HO development), MAR and recurrence severity were significantly related (p-0.572, p0.041, n J t). Data from this grant showed a link between bench top research and bedside care, and demonstrates that the MAR is elevated in HO and correlated with recurrence risk; however, a larger sample size and more clinical factors are needed to refine this model. A follow-up HO grant awarded using this data as a benchmark for developing a translatable animal model (CDMRP-MRMC).

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Recruitment, infrastructure, patient identification

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1. Introduction

Investigating ways to alleviate complications with heterotopic ossification (HO) has high clinical significance for wounded warriors. As noted in the original submission, approximately 63-65% of service members who sustain amputations as a result of improvised explosive device (IED) trauma, experience problems with ectopic bone formation (1). HO may require surgical revision and delay rehabilitation regimens for those seeking to return to active duty or reintegration to the community. Therefore, this grant focuses on using novel histological techniques to determine the rate of HO growth and markers such as osteoblast/osteoclast indices and mineral apposition rate (MAR) to improve surgical planning. The primary aim of this research initiative is to correlate HO growth with recurrence and thereby provide histological evidence to support clinical recommendations as to when the bony mass should be excised.

2. Body

2.1 Patient Recruitment

The investigators completed patient recruitment and collected samples from n=33 wounded warriors with symptomatic HO. All data has been reported and submitted to peer-reviewed manuscripts.

2.2 Peer-Reviewed Publications / Conference Abstracts

We submitted 2 manuscripts for publication (Appendix A) and presented findings at the Military Health System Research Symposium (Appendix B) during this final reporting period.

2.3 Literature Review

To ensure that no key information is omitted from future publications, the PI has focused a great deal of time reading a diverse collection of HO literature. An extensive list of articles has been collected and is being reviewed on a daily basis.

3. Key Research Accomplishments

* Demonstrated the ability to flurochrome label resected ectopic bone from wounded warriors
* Successfully created key histological techniques for quantifying HO bone growth/maturation
* Leveraged new knowledge for additional grant applications
* Eight manuscripts accepted/submitted for publication and 4 abstracts since grant funding initiated. The list includes the following:

Manuscripts

1. **Isaacson BM,** Potter BK, Bloebaum RD, Epperson RT, Kawaguchi BS, Swanson TM, Pasquina PF. A Link Between Clinical Predictors of Heterotopic Ossification and Histological Analysis for Improved Surgical Planning in Combat Injured Service Members. JBJS (Submitted).

2. **Isaacson BM,** Potter BK, Bloebaum RD, Epperson RT, Kawaguchi BS, Swanson TM, Pasquina PF. Determining the Mineral Apposition Rate of Heterotopic Ossification in Military Patients After Total Joint Replacement: A Case Series. BONE (Submitted).

3. Swanson, TM, **Isaacson, BM**, Cyborski, CM, French LM, Tsao, JW, Pasquina, PF. A Review of Traumatic Brain Injury (TBI) in the United States Military Population. J Pub Health (Submitted).

4. **Isaacson BM,** Swanson TR, Potter BK, Pasquina PF. Tourniquet Use in Combat Injured Service Members: A Link with Heterotopic Ossification? JORR 2014 Apr 6: 27-31.

5. **Isaacson BM,** Jeyapalina S. Direct Skeletal Fixation: A Review of Osseointegration Technology. JORR 2014 Apr 6: 55-65.

6. **Isaacson BM**, Williams DL The 5 Hallmarks of Biomaterials Success: An Emphasis on Orthopaedics. Adv Biosci Biotechnol. 2014 Mar 5(4): 283-293.

7. **Isaacson BM**, Weeks SR, Potter BK, Pasquina PF, Bloebaum RD. Relationship Between Heterotopic Ossification Volume and Clinical Screening Tools in Combat-Injured Transfemoral Amputees. JPO. 2012 July; 24(3): 138-143.

8. **Isaacson BM,** Swanson TR, Pasquina PF. The Use of A Computer Assisted Rehabilitation Environment (CAREN) for Enhancing Wounded Warrior Rehabilitation Regimens. J Spin Cord Med. 2013 July 36(4): 296-299.

Abstracts

1. **Isaacson BM**, Swanson TM, Potter BK, Epperson RT, Bloebaum RD, Pasquina PF. Clinical and Histological Predictors of Heterotopic Ossification Recurrence in Warfighters from OIF and OEF. *Military Health System Research Symposium, Ft. Lauderdale, Florida, August 17-19,2015.*

2. **Isaacson BM**, Swanson TM, Potter BK, Epperson RT, Bloebaum RD, Pasquina PF. Clinical and Histological Predictors of Heterotopic Ossification in Warfighters from OIF and OEF. *61st Annual Orthopaedic Research Society Conference, Las Vegas, Nevada, March 28-31 2015.*

3. **Isaacson BM**, Swanson TM, Potter BK, Epperson RT, Bloebaum RD, Pasquina PF. Predicting Heterotopic Ossification Formation: A Link between Bench top and Bedside? *60th Annual Orthopaedic Research Society Conference, New Orleans, Louisiana, March 15-18, 2014.*

4. **Isaacson BM**, Swanson TM, Potter BK, Epperson RT, Bloebaum RD, Pasquina PF. Establishing the Mineral Apposition Rate of Heterotopic Ossification for Prevention of Recurrence. *Military Health System Research Symposium, Ft. Lauderdale, Florida, August 12-15 2013.*

4. Reportable Outcomes and Conclusions

A full report of outcomes is listed in Appendix A.

5. References

(1) Potter BK et al. Heterotopic Ossification Following Traumatic and Combat-Related Amputations. The Journal of Bone and Joint Surgery. 89-A (3): 474-486, 2007.

Appendix A: Peer-Reviewed Manuscripts Submitted During this Reporting Period

Manuscript #1: A Link Between Clinical Predictors of Heterotopic Ossification and Histological Analysis for Improved Surgical Planning in Combat Injured Service Members

Authors: Isaacson BM[1-2], Potter BK[4-5], Bloebaum RD[6-7], Epperson RT[6], Kawaguci BS[6], Swanson TM[2], Pasquina PF[2-3]

Affiliation: [1]The Henry M. Jackson Foundation for the Advancement of Military Medicine; [2]The Center for Rehabilitation Sciences Research, Department of Physical Medicine & Rehabilitation, Uniformed Services University of Health Sciences; [3]Department of Rehabilitation, Walter Reed National Military Medical Center; [4]Department of Orthopaedics, Walter Reed National Military Medical Center; [5]Department of Surgery, Uniformed Services University of Health Sciences; [6]Bone and Joint Research Laboratory, Department of Veterans Affairs; [7]University of Utah, Departments of Bioengineering and Biology

Journal: Submitted to the Journal of Bone & Joint Surgery

Abstract: ***Background:*** Heterotopic ossification (HO) is a debilitating condition that occurs predominately following traumatic injury and may restrict range of motion and delay rehabilitation. Historical recommendations regarding the timing and efficacy of surgical resection have varied widely, and a gap exists between clinical predictors of HO recurrence and histological analysis. Peer-reviewed literature depicts HO as a metabolically active osseous tissue, but at present time, there is no quantifiable evidence to optimize surgical timing in order to minimize recurrence. ***Methods:*** Thirty-three service members at Walter Reed National Military Medical Center with symptomatic HO were enrolled in an institutional review board-approved prospective study. Participants were asked to take oxytetracycline on four scheduled days prior to HO resection to determine the mineral apposition rate (MAR; i.e., bone growth rate). ***Results:*** Detailed histological analyses included scanning electron microscopy with backscattered electron imaging and light microscopy. Data indicated that the MAR of trauma-induced HO was approximately 1.7 μm/day at the time of surgical intervention, a value 1.7x higher than non-pathological human bone. The MAR and post-operative alkaline phosphatase (AP) values and AP pre-operative levels and the percent of osteoblastic activity were demonstrated to be positively related and statistically significant (ρ=0.509, p=0.026, n=19) and (ρ=0.522, p=0.004, n=29) respectively. When data was analyzed only within a two-year period from injury to excision (thereby removing outliers that were significantly longer than counterparts) and traumatic brain injury and non-steroidal anti inflammatory drugs were controlled for in the statistical analysis (known correlates with HO development), MAR and recurrence severity were significantly related (ρ=-0.572, p=0.041, n=11). ***Conclusion:*** Data from this study provides a link between bench top research and bedside care, and demonstrates that the MAR is elevated in HO and correlated with recurrence risk; however, a larger sample size and more clinical factors are needed to refine this model. ***Clinical Relevance:*** Enhanced HO understanding may be achieved through further clinical study and/or the development of a physiologic translatable animal model that carefully isolates each predictor variable.

Manuscript #2: Determining the Mineral Apposition Rate of Heterotopic Ossification in Military Patients After Total Joint Replacement: A Case Series

Authors: Isaacson BM[1-2], Potter BK[4-5], Bloebaum RD[6-7], Epperson RT[6], Kawaguci BS[6], Swanson TM[2], Pasquina PF[2-3]

Affiliation: [1]The Henry M. Jackson Foundation for the Advancement of Military Medicine; [2]The Center for Rehabilitation Sciences Research, Department of Physical Medicine & Rehabilitation, Uniformed Services University of Health Sciences; [3]Department of Rehabilitation, Walter Reed National Military Medical Center; [4]Department of Orthopaedics, Walter Reed National Military Medical Center; [5]Department of Surgery, Uniformed Services University of Health Sciences; [6]Bone and Joint Research Laboratory, Department of Veterans Affairs; [7]University of Utah, Departments of Bioengineering and Biology

Journal: BONE

Abstract: ***Background:*** Heterotopic ossification (HO) is frequently reported following total joint replacement (TJR) surgery and symptomatic cases may limit range of motion, cause pain and require surgical excision. Deciding an appropriate time to remove HO is subjective and closing the gap between clinical predictors and histological analysis may minimize the likelihood for recurrence. ***Methods:*** A case series was performed with military healthcare system (MHS) patients undergoing TJR who required removal of periarticular ectopic bone. Patients were prescribed tetracycline to assess the mineral apposition rate (MAR, i.e. bone growth rate) of HO and surgical specimens were analyzed using scanning electron microscopy (SEM) and light microscopy. ***Results:*** Two males and one female qualified for the study and were 69.0±7.8 inches, 237.7±28.3 pounds and 61±7 years of age at the time of HO excision. Ectopic bone occurred in two cases following total knee arthroplasty and one total hip arthroplasty. Data indicated that MAR levels were 1.7 times higher than previously reported non-pathological human bone at the time the HO was excised (1.7±0.7 μm/day, range: 1.3-2.6 μm/day). SEM and light microscopic images showed that HO to be in a quiescent state and consisted of only cancellous bone. ***Discussion:*** HO bone architecture observed from veterans undergoing TJR was vastly different than the previously characterized specimens investigated by our team from wounded warriors. This variation may be attributed to differences in the induction mechanism (controlled operative procedure vs. blast injury) and patient age differences. ***Conclusion:*** HO is a metabolically active tissue that may reduce quality of life. Further characterization is needed to optimize symptomatic HO excision timing and further understand the etiology of this pathological bone disorder.

Appendix B: Abstract Presented at MHSRS during this Reporting Period

Title: Clinical and Histological Predictors of Heterotopic Ossification Recurrence in Warfighters from OIF and OEF

Authors: Isaacson BM[1-2], Swanson TM[1-4], Potter BK[4-5], Epperson RT[6], Bloebaum RD[6-7], Pasquina PF[2-3]

Affiliations: [1]The Henry M. Jackson Foundation for the Advancement of Military Medicine; [2]The Center for Rehabilitation Sciences Research, Department of Physical Medicine & Rehabilitation, Uniformed Services University of Health Sciences (USUHS); [3]Department of Rehabilitation, Walter Reed National Military Medical Center (WRNMMC); [4]Department of Orthopaedics, WRNMMC; [5]Department of Surgery, USUHS; [6]Bone and Joint Research Laboratory (BJRL), Department of Veterans Affairs; [7]University of Utah, Departments of Bioengineering and Biology

Background: Over 2200 major limb amputations have occurred as a result of Operations Iraqi Freedom (OIF) and Operation Enduring Freedom (OIF). Approximately 63-65% of these service members have developed heterotopic ossification (HO) in the musculature and peri-articular regions, with 20-40% requiring surgical excision. While results from early resection have been promising, premature removal may result in ectopic bone regrowth, which may require additional surgery and delays rehabilitation regimens. This study sought to determine if histological measures could predict HO growth/development in order to determine an optimal timing of surgery in order to reduce the likeliness of recurrence.

Methods: Service members at Walter Reed National Military Medical Center (WRNMMC) requiring surgery for removal of symptomatic HO were enrolled. Prior to ectopic bone excision, HO maturation was determined based on alkaline phosphatase (AP) levels, nuclear scintigraphy and orthogonal radiographs. Participants were given oxytetracycline (250mg/tid) on four separate dates before their scheduled surgery for fluorochrome double labeling. Following resection, HO samples were analyzed using scanning electron microscopy (SEM) and light microscopy/bone stains to calculate the mineral apposition rate (MAR). The percent of osteoblastic (%OBA), osteoclastic (%OCA) and resting bone (%RB) were evaluated. Quantitative data was analyzed using Pearson's correlation coefficients in SPSS.

Results: Twenty-eight service members who required removal of symptomatic HO following combat related trauma are reported. Twenty-seven of the subjects were male and improvised explosive devices were the primary injury mechanism. Subjects were 26.3 +/- 5.6 years of age at the time of injury and ectopic bone resection occurred on average 12.2 +/- 8.0 months from the date of their injury. Pre-operative radiographs were scored as 13 cases of mild HO, 7 moderate and 8 severe. Traumatic brain injury (TBI) occurred in 64% of the subjects (16 mild, 1 moderate, and 1 severe).

HO masses were actively modeling and remodeling at the time of surgical intervention as evident by elevated MAR levels (1.6 um/day), SEM images and the %OBA, %OCA and %RB values which were 28.9+/-13.7%, 8.8+/- 5.8% and 62.4 +/- 18.5% respectively. Bivariate correlations indicated that the %OBA and pre-surgical AP levels were significant and directly related ($p=0.018$, Pearson coefficient=0.469), thereby demonstrating a link between a clinical predictor and the amount of bone growing *in situ*. MAR and HO anatomical location were significant ($p=0.050$) with bone growth rates being highest for upper extremity injuries (2.01 um/day) followed by above knee (1.64 um/day), pelvis (1.64 um/day) and below knee (1.36 um/day).

Partial correlations controlling for non-steroidal anti-inflammatory drug (NSAID) usage, TBI and anatomical location indicated a significant relationship between MAR and recurrence ($p=0.024$). Linear regressions were able to predict within an 8% error the expected MAR rate of a warfighter with post-combat HO based on the following equation: MAR = 0.006 (AP post-op) – 0.009 (weight lb.) +0.187 (TBI severity) + 2.745).

Conclusion: A direct relationship between clinical predictors and histological markers was observed. Further studies may help optimize surgical schedules to minimize the likelihood of HO recurrence.

Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army, the Department of Defense, or the United States government.

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Establishing the Mineral Apposition Rate of Heterotopic Ossification for Prevention of Recurrence
BAA W81XWH-12-2-0017

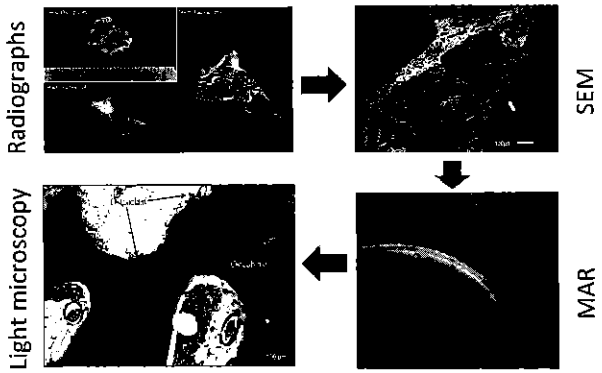
PI: Brad M. Isaacson, PhD, MBA, MSF **Org:** Henry M. Jackson Foundation **Award Amount** $680,226

Study Purpose / Deliverables

The objectives of this study are to improve clinical management for injured servicemen and women with heterotopic ossification (HO) and reduce ectopic bone recurrence. Developing new methods for assessing HO maturity remains of utmost importance since florid bone growth may result from premature HO resection and cause additional surgical procedures for injured service members.

Study Aims

- Aim 1: Determine the mineral apposition rate (MAR) of resected HO collected from service members.
- Aim 2: Improve HO bone characterization using scanning electron microscopy (SEM), light microscopy and measuring osteoclastic index (OCI) and osteoblastic index (OBI).



Accomplishments: Patient recruitment completed; successfully demonstrated flurochrome labeling of resected ectopic bone and established key histological techniques for quantifying growth/maturation; 4 abstracts accepted to major conferences and 8 manuscripts accepted/submitted for publication, follow-up HO grant awarded using this data as a benchmark for developing a translatable animal model (CDMRP-MRMC).

Timeline and Cost

Activities	CY 13	14
Obtain WRNMMC IRB approval		
Collect ectopic bone samples		
Analyze HO with advanced histology		
Determine the MAR of HO		
Publish data in peer-reviewed journals		
Estimated Budget ($K)	$236	$444

Updated: 8 September 2015

Goals/Milestones

CY13 Goals

☑ Demonstrate the ability to flurochrome label HO
☑ 2 conference abstracts accepted based on this novel research
☑ Achieve 50% recruitment of wounded warriors

CY14 Goals

☑ Complete patient recruitment
☑ Investigate if a correlation exists between histological markers and clinical predictors of HO growth/development
☑ Perform parametric / non-parametric statistical evaluations
☑ Disseminate knowledge through the military treatment facilities and publish manuscript(s) detailing the findings

Budget Expenditure to Date: $672,969

Projected Expenditure: $676,919
(based on 2 month recent burn rate through 30 September 2015).

A Comprehensive Review of Traumatic Brain Injury (TBI): A Focus on the United States Military Population

Swanson, TM[1-3]; Isaacson, BM[1-2]; Cyborski, CM[6]; Tsao, JW[2, 4]; Pasquina, PF[2, 5]

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Abstract: The use of explosive armaments in Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) has contributed to 313,816 diagnosed cases of traumatic brain injury (TBI). Over 82.5% of these incidences were documented as mild, while 8.3% were moderate, and 1.0% severe. Rehabilitation from TBI may be challenging due to polytrauma and limited by standardized screening methods and diagnostic criteria. For example, high variation has been noted to occur due to patient self-report error and inaccurate meta-analyses findings. As a result, the Department of Defense (DoD) has issued guidelines to (1) classify TBI based on injury mechanism and severity, (2) categorize symptoms in somatic, psychological, and cognitive groupings and (3) standardize the care received by service members during the acute and chronic/rehabilitation stages of treatment. While this is a beneficial plan, the vast majority of cases in the DoD consist of mild TBI (mTBI), a condition in which traditional biomarkers are unavailable and diagnostic findings remain inconsistent. Therefore, a thorough review of the literature is pertinent given the closure of OIF and OEF and transition of these TBI patients into long-term care within the Department of Veterans Affairs. It remains critical that clinicians and investigators evaluate long-term mTBI care for resilience and readiness training.

Epidemiology

Traumatic brain injury (TBI) is defined as a traumatically induced structural injury and/or physiological disruption of normal brain function as a result of an external force.[1] This presents with a combination of signs and symptoms including a period of lost or decreased level of consciousness, amnesia, altered mental state, neurological deficits, and/or intracranial lesion.[1] An estimated 1.7 million people sustain a TBI of any severity (mild, moderate, severe) in the United States each year,[2] of which approximately 1.4 million present to emergency and other acute medical settings, 275,000 require hospitalization, and 52,000 cases are fatal.[2]

In the civilian setting, TBI results mainly from falls, motor vehicle accidents, and other incidences of blunt trauma to the skull.[3] However, this is vastly different than the military community in which TBI often results from explosive armaments. During the period of 2000 to Q4 2014, there was 313,816 diagnoses cases of TBI during Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF);[4] representing 19%-23% of all returning service members.[5, 6] The vast majority of these incidents were mild (82.5%) followed by moderate (8.3%) and severe (1.0%).[5, 6] Given its high frequency, TBI has been dubbed the "signature wound" of service members returning from OIF/OEF.[6]

Military databases have reported significantly fewer traumatic thoracic injuries and gunshot wounds for OIF/OEF service members than in previous wars (Vietnam and World War II), but significantly more head and neck injuries due to improvised explosive devices (IED) and rocket-propelled grenades (RPG). These weapons have accounted for 88% of combat-related TBIs reported.[7] Improvements in body armor, tactical training, and overall safety precautions has

resulted in a 92% survival rate[8, 9] and contributes to these diagnostic trends.[10] As such, long-term research efforts for TBI patients will continue to be important for the military healthcare system.[2, 4, 7]

Mechanism

In the Department of Defense (DoD), penetrating and non-penetrating closed head injuries account for 1.5% and 91.7% of cases respectively with the remaining 6.8% typically being complex polytrauma cases with ambiguous diagnosis.[4] However, TBI is difficult to diagnose because it requires an investigation of patient history before, at the time of, and immediately following injury. Thus, implementing a service-wide standardized diagnostic protocol for screening TBI has been met with challenges and the accuracy of incidence statistics is frequently disputed. Estimations have been calculated through patient self-reports or meta-analysis of total traumatic injuries. These outputs vastly differ based on the dataset being utilized and limits the understanding of TBI frequency in the military population.[11] For example, previous studies have calculated TBI based on broad regional injury casualty reports and excluded subjects that returned to duty within 72 hours of injury.[12] As such, cases of mild TBI (mTBI) which were not accompanied by visible wounds were likely to have been underreported.[12] A later estimation by a nonprofit public policy organization utilized patient self-reports of signature symptoms without clinician confirmation in service members returning from OIF/OEF (n=1,965) and concluded that 320,000 instances of TBI had occurred.[5] What is most striking is that data collected the same year from the Armed Forces Health Surveillance Center stated only 130,000 cases of TBI were clinically confirmed.[11]

Self-reporting may also be responsible for overestimations in prevalence statistics. For instance, in a study examining the relationship between neuropsychological test performance and patient self-report of cognitive complaints (n=109), self-reported cognitive complaints were significantly correlated with psychological distress (i.e. co-occurrence of chronic depression) but generally not with overall neurocognitive functioning.[13] Researchers concluded from this study that there was a low rate of agreement between neurocognitive test scores and self-reported cognitive complaints.[13] Therefore, incidence discrepancies may be due to both inaccurate statistical extrapolations and errors in patient self-report as a result of heightened TBI awareness in the military community.[11]

Classification

TBI injuries may be broadly categorized based on neurological damage (diffuse, focal, or a combination of both),[3] penetrating (open) vs. non-penetrating (closed), and classification (mild, moderate, or severe). Mild TBI (mTBI) is the most frequent form of brain injury and is commonly known as a concussion[3, 4] often caused by IEDs and RPGs. In fact, due to its high prevalence in military healthcare, blast injury is subcategorized on a mechanism-specific schema of primary, secondary, tertiary, and quaternary injury (Fig. 1).[14] Additionally, tertiary blast injury is the principal mechanism of head, neck, and subsequent TBI in injured service members.[15]

Classification	Mode of Injury	Result
Primary	Over-pressurization of internal organs	Lung, ear, gastrointestinal insults
Secondary	Objects propelled via shockwave	Fragmentation, penetrating injuries
Tertiary	Air displacement throws victim	Fractures, coup-contrecoup injuries
Quaternary	Miscellaneous	Psychiatric, other injuries

Figure 1. Modes of injury and resulting symptoms following blast exposure.[14]

Per DoD definition, the extent of TBI injury is based on the status of the patient at the time of the incident. A mTBI is defined as having normal structural imaging, loss of consciousness (LOC) from 0-30 min, alteration of consciousness (AOC) from a moment up to 24 hours, and post traumatic amnesia (PTA) for 0-1 day (Fig. 2). Interestingly, the severity of injury does not predict functional or rehabilitative outcome of the patient, which adds to the complexity of treating this patient population.

Measure/Severity	Mild	Moderate	Severe
Neuroimaging	Normal	Normal or abnormal	Normal or abnormal
LOC	<30 min	>30 min and <1 day	>1 day
AOC	<1 day	>1 day	>1 day
PTA	<1 day	>1 and <7 days	> 7 days

Figure 2. DoD diagnostic criteria for determination of injury severity.[1]

The most regularly referenced TBI grading system, the Glasgow Coma Scale (GCS), determines injury severity based on varying levels of consciousness following resuscitation.[1, 2, 16] The patient's verbal, motor, and eye-opening are used to score the patient on a scale of 3-15 to categorize the level of consciousness and severity of TBI (mild ≥13, moderate 9-12, and severe ≤8).[3] GCS, while valuable in early assessment, is affected by other factors including acute intoxication[17] and has limited prognostic ability as it does not account for the underlying pathological factors that occur during initial injury, acute treatment, and long-term rehabilitation stages that may strongly impact patient recovery.[18] The highest GCS score obtained within the first 24 hours following injury is generally utilized as the benchmark for later rehabilitation and testing.[1, 16]

Prognostication

When determine the prognosis of TBI, a health care provided may use PTA, LOC, and coma (or time to follow commands) to help provide a framework for rehabilitation (Fig. 2). PTA is a state of disorientation and confusion that may occur immediately following initial injury.[19] PTA may manifest in retrograde, memories prior to the injury, or anterograde, memories from the time of the injury on, amnesia with 33% of patients suffering from fragmented memory across all moderate to severe TBI cases.[20] An extended duration of LOC immediately following injury has also been negatively correlated with long term rehabilitative potential.[21, 22] An accurate depiction of LOC must be witnessed, as the patient would not know how long he/she was unconscious for given the possible PTA. Additionally, in war situations, the circumstances surrounding TBI also may make it difficult for witnesses to report LOC given the high kinetic activity. Despite its challenges, LOC is well-documented to occur 35.2% of returning OIF/OEF service members experiencing TBI.[23] Although more a means of description rather than prognosis, AOC manifesting in acute cognitive-behavioral changes such as disorientation, confusion, and delayed thinking is frequently reported alongside PTA, coma, and LOC as well.[24, 25]

Measure/Prognosis	Chronic disability	Complete recovery
Coma duration	>1 month	<2 weeks
PTA duration	>3 months	<2 months
Age	>65 years	<65 years
Injury	Bilateral, brainstem, or penetrating	No structural abnormality

Figure 3. DoD criteria of evidence-based guidelines for prognostication after TBI.[1]

Due to the prognostic shortcomings of GCS, PTA, LOC, and AOC taken individually, the DoD and Veteran Affairs (VA) have implemented an aggregate prognostic system that utilizes a combination of the various grading measures.[4] The Military Acute Concussion Evaluation

(MACE),[26] uses a combination of patient self-reports and neurological examination to provide an improved diagnosis. This methodology was implemented by the DoD in and mandated for rapid use in battlefield concussion screening in 2010. However, one limitation of the MACE is that it partially relies on patient self-report of symptoms and has been found to lose sensitivity when administered greater than 6 hours post-injury.[27]

Symptomatology

Non-penetrating mTBIs sustained in OIF/OEF may be largely uniform in modality but injury severity and promptness of treatment greatly influence whether symptoms resolve or persist long-term.[28-30] In general, symptoms may classified as being somatic, psychological, or cognitive.[1] Somatic symptoms manifest in headache, fatigue, hypersensitivity to light/noise, insomnia and sleep disturbance, drowsiness, dizziness, nausea, vestibular difficulties, and vomiting. In more severe cases, somatic changes of seizures and transient neurological abnormalities may also occur. Psychological changes associated with TBI may manifest in emotional distress, irritability, anxiety, and depression. Potential cognitive problems arising include challenges with memory, concentration, various cognitive disorders and subsequent functional status limitations. More critical and persistent symptoms associated with moderate and severe TBI may include asymmetrical pupil dilation, slurred speech patterns, dysarthria, aphasia, peripheral neuropathy, restlessness, and agitation.[29, 31, 32] In a general population evaluated 10 years post-injury, moderate and severe TBI patients had a significantly higher risk of developing and sustaining abnormal social behaviors, difficulties in maintaining attention, abnormal executive functioning, and alexithymia.[29, 31-33]

Neuroimaging

Radiographic evidence of neurological insult is most apparent in cases of severe, open head, injury. In these instances, Computed Topography (CT) and Positron Emission Topography (PET) provide a gross anatomical overview of brain function and physiology where injury is conspicuously apparent. However, these widely established detection modalities lack the necessary resolution to detect subtle changes in neurocircuitry and are therefore limited in their ability to explain post-injury behavioral deficits. In moderate to severe TBI patients, higher resolution neuroimaging studies have demonstrated extensive changes in white matter organization during both the acute and chronic rehabilitation stages.[34, 35] In an investigation of patients (n=13) with severe TBI undergoing serial diffuse tensor imaging (DTI) tested acutely (<6 weeks DOI), 2 years, and 5 years post-injury, fractional anisotropy (FA) was significantly lower in the genu and body of the corpus callosum and bilateral corona radiata regions, whereas radial diffusivity (RD) was significantly higher demonstrating persistent neurological damage and reorganization.[34] Significant differences in FA and RD further increased in severity after 2-year-post-injury reassessment when compared to controls and were significantly associated with neurocognitive sequelae of aphasia, dyspraxia, and amnesia after 5 years. Declarative memory impairment, a well-documented effect of severe and, to a lesser extent moderate, TBI, may be mediated and accompanied by decreased hippocampal white matter FA.[35] In another study, DTI was utilized to examine cortical thickness, white matter connectivity, and regional volume differences in severe TBI patients (n=26), revealing widespread atrophy in the prefrontal, parietal, and precuneus regions.[36] Atrophy of these regions was significantly correlated to poorer declarative memory scores.[36] Although hippocampal volume was significantly decreased in TBI patients when compared to controls, only decreases in FA correlated with declarative memory

capability.[36] These findings are particularly relevant to military healthcare as many injured service members enter the long-term care window where said behavioral problems may arise. Additionally, establishing causative injury-to-symptom pathophysiology may allow clinicians to develop improved treatment intervention strategies (i.e. pharmaceutical or neurosurgical).

In a meta-analytic review of 28 DTI studies of mTBI patients, random effect model demonstrated significant FA reductions (n=280) and RD increases (n=154) pertaining to the splenium, midbody, but not genu, subregions of the corpus callosum.[37] Advanced neuroimaging techniques such as DTI hold promise in identifying biomarkers for TBI. However, DTI is limited in practicality due the associated high expense, time commitment required for performing serial imaging sessions, and differences in basal neurophysiology that prevent cross-comparison between patients.[38-40] These results demonstrate long-term neurophysiological adaptations following TBI and may elucidate a potential pathophysiology for persistent psychological, somatic, and cognitive symptoms.

Biomarkers and Brain-Specific Proteins

Broad symptomatology and multiple diagnostic grading scales make confirming TBI severity, particularly challenging for clinicians. Biomarkers are thus being investigated as an objective scale which can quickly and accurately determining the presence and severity of TBI.[11, 27] Neuroimaging and brain-specific protein blood tests aim to provide a measurable indicator to physiologically assess a patient's condition. Clinical trials of brain-specific protein biomarkers such as S100B, glial fibrillary acidic protein (GFAP), Ubiquitin C-terminal hydrolase L1 (UCH-L1), Tau, Neuron Specific Enolase (NSE), and spectrin breakdown products (SBDP) have been

shown to be elevated following severe TBI.[11] GFAP (n=34) and UCH-L1 (n=96), in particular, has been corroborated in preliminary clinical trials as maintaining a high level of diagnostic sensitivity that can be accurately paired with acutely derived GCS scores.[41-43] GFAP and UCH-L1 tests accurately paired for clinician diagnoses of moderate and severe, but not mild TBI injuries, as consistency in test results was lost on the latter.[41-43] Although brain-specific protein blood tests hold promise for acute care improvements, there are conflicting study findings requiring further research and validation.

Treatment

Acute

Treatment for TBI is categorized into acute, transitory subacute, and rehabilitation stages. Following injury, the focus of acute treatment is stabilization of homeostatic neurophysiological function so as to stabilize the primary injury and to prevent secondary injury.[44] In the acute treatment of moderate to severe TBI, principal concerns consist of controlling for primary and secondary injury symptoms by maintaining appropriate cerebral blood flow and intracranial pressure (ICP).[30] Elevated ICP may be detected by neuroimaging and actively monitored through intraventricular catheterization to drain cerebrospinal fluid as necessary.[45, 46] Additionally, ICP may be controlled by use of analgesics, paralytic agents, sedatives, diuretics, controlled hyperventilation, or simple manipulation of the patient's skull.[47-49] Endotracheal intubation and mechanical ventilation is frequently used to ensure proper brain oxygenation while blood pressure is strictly controlled through use of intravenous fluid and medications.[30] Additionally, body temperature is tightly regulated to ensure the brain's metabolic resources remain constant so as to limit potential long-term damage.[50] In cases of moderate, severe, or

open-head TBI, urgent neurological surgery may be performed to remove penetrating matter, contusions, or hematomas causing significant intracranial structure shifting.[30, 50] In cases where ICP cannot be controlled by intraventricular cranial catheter or in the presence of critical hematomas, pharmacological intervention and/or decompressive craniectomy may be utilized whereby a portion of the skull is removed.[51] However, due to the practical limitations of in-theater acute care, intraventricular catheterization monitors are less frequently available whereby decompressive craniectomy is the approach more often utilized.[52] As it pertains to the majority of in-theater injuries, blast-related insults have been associated with a higher incidence of vasospasm and pseudoaneurysm formation.[52] Of particular concern, vasospasm has been identified as a common occurrence within 48 hours of injury and may manifest up to 14 days afterwards, thereby highlighting the critical importance of comprehensive acute care for injured service members.[53]

Clinical recommendations for acute treatment of concussion include rest and limited physical and cognitive exertion followed by a gradual, clinician-supervised, reintroduction to normal activities following a period of up to 3 days following injury.[54] However, service members having experienced 3 or more lifetime concussions have been demonstrated to show significantly impaired neurocognitive performance on simple tasks and increased distress symptoms and, as such, are pulled out of theater.[1, 55]

Rehabilitation

Rehabilitation of TBI patients is implemented following the stabilization of the patient, with the principal aim being to improve independent functioning, social integration, and disability

adaptation.[56] Depending on the complexity of their injury, TBI patients may require a comprehensive rehabilitation regimen from occupational therapists, cognitive-behavioral psychologists, physical therapists, speech-language pathologists, audiologists, neuro-optometrists, assistive technologists, and integrative medicine providers.[56, 57] Frequently encountered neuropsychiatric comorbidities such as chronic depression, post-traumatic stress disorder (PTSD), and generalized anxiety disorder may require treatment on both an acute and long-term basis to decrease social impairment.[58, 59] Patients who remain incapable of independent living despite rehabilitation efforts may require continued monitoring in supported living facilities, group homes, or respite care facilities.[56] Pharmacological therapy may be used as a conjunctive measure to help control lingering post-TBI deficits, such as epilepsy, depression, and PTSD.[58-60] Psychiatric services are most frequently utilized in combat/violence-related TBI cases.[59, 61]

Prognosis/Outcomes

Research suggests TBI may be a significant barrier to optimal rehabilitation during both acute and rehabilitation phases due to psychological and neurological impediments.[62] The added burden of TBI is of particular concern for a military population where injury frequently accompanies cases of polytrauma requiring extensive physical rehabilitation, high levels of self-motivation, positive patient morale, and a strong psychological foundation to optimize treatment success. However, comprehensive treatment approaches to moderate and severe TBI in military populations show encouraging results. In service members averaging 5 years post-injury with a previous diagnosis of mild to moderate TBI (n=96), community-integrated rehabilitation programs demonstrated marked improvement in psychosocial impairments with a mean

treatment time of 190 days.[63] Additionally, neuropsychological functioning has shown marked improvements from the time of injury following 4 months of intensive neurocognitive rehabilitation therapy in a group of service members (n=56) averaging 34 months after mild to moderate TBI diagnosis.[64]

At the time of this writing, there is no consensus as to persistent sequelae following mTBI nor is there agreement upon what time duration qualifies a symptom as being considered persistent.[1] Recovery for concussed patients is typically rapid and resolve of symptoms occurs in hours or days following time of injury. Although controversial and inconsistent in its findings, there is limited research suggesting the potential for long-term residual consequences following mTBI.[6, 28] A study of returning OIF/OEF service members at the Palo Alto VA (n=138) sustaining mTBI reported the large majority of subjects held significant self-reported cognitive and emotional barriers impairing successful community reintegration at initial and 2-year follow-ups – 90% and 77%, respectively.[65] mTBI incidence has also been correlated with higher rates of unemployment, which may lead to the development of secondary symptoms.[66] In one study investigating Quality of Life (QoL) and neurocognitive outcome assessments following mTBI in the general population (n=86) at admission for acute treatment, 1 year post-injury, and 10 years post-injury, incrementally higher scores were observed over time (higher scores indicate negative clinical outcome and decreased life satisfaction) with only 62.8% being within "normal" range at 10 years post-injury.[67] Further, initial assessment for patients with radiographically documented intracranial injury demonstrated QoLs score twice as high as those without injury and 9.3% of study subjects reported loss of employment due to persistent mTBI complaints such as fatigue, insomnia, and exhaustion, at 10-year assessment.[67] In a retrospective study of Vietnam service

members (n=3,214) who sustained combat-related injuries with and without concurrent mTBI diagnosis, the mTBI group demonstrated increased risk of chronic depression, psychosocial deficits, and subtle neurologic and neuropsychological conditions as compared to their non-TBI counterparts.[68, 69] Somewhat conflictingly, another study by Vanderploeg et al. examined preliminary long-term neuropsychological outcomes in service members a mean of 8 years duration post-mTBI diagnosis (n=254) and found no significant differences on a standard 15-measure test battery between controls.[69] As demonstrated by later research, this discrepancy is likely explained by a lack of congruency between neuropsychological testing scores and reported, self-perceived, cognitive symptoms (i.e. diagnostic errors caused by self-report of symptoms).[13] However, in focused tests of complex working memory and attention, mTBI subjects had significant, yet subtle, deficits associated with left-sided visual neglect and impaired tandem gait scores.[69]

PTSD and chronic depression frequently present with mTBI cases in the military.[25] Thus, research correlating mTBI incidence with persistent symptoms must account for the confounding influence of multiple psychiatric comorbidities in its analysis. PTSD, in which 118,829 cases were reported in post-deployment service members between September 2001 and Q1 2014,[70] shows considerable overlap in symptoms and in co-occurrence with TBI. [25] A survey of Army infantry returning from Iraq (n=2,525) indicated 43.9% of service members with LOC-confirmed mTBI received in combat operations met Diagnostic Statistical Manual IV (DSM-IV) criteria for PTSD.[25] Furthermore, those with mTBI were more likely to report poor general health, absentee work days, higher numbers of somatic symptoms, and more frequent medical visits.[25] Encouragingly, when analysis was adjusted to control for symptoms of PTSD and chronic

depression, the only persistent symptom following concussion was headache.[25] Likewise, chronic depression confounds mTBI diagnosis in its ability to influence self-report of symptoms.[13] One study found that, while depression is most likely to occur within one year following injury, mTBI patients remain at a significantly elevated risk of developing this condition up to 10 years following time of injury in one study.[59] However, as with PSTD, the link between depression and TBI remains poorly elucidated with meta-analyses finding concurrent incidence rates ranging from 10%-77%.[2, 59, 71] Thus, PTSD and chronic depression are suspected to mediate persistent symptoms reported by mTBI patients.[13, 25, 72] The relationship between mTBI and persistent sequelae are further complicated by lingering inconsistencies in diagnostic standards and heightened public awareness regarding military TBI and its symptoms. Additional research into persistent mTBI symptoms is necessitated before a consensus on long-term outcomes can be reached. Nonetheless, patients should be made aware that the majority of mTBI cases make full recoveries within hours or days following injury.[1, 28]

Conclusion

TBI presents a significant challenge and concern for military healthcare system. Accurately estimating TBI incidences is critical for developing immediate and long-term rehabilitation strategies. Although significant progress has been made in standard of care treatment, long-term sequelae and pathophysiological mechanisms underlying TBI, and particularly mTBI, remain poorly understood and require additional investigation. Clinical experience from OEF/OIF suggests an intensive multidisciplinary approach holds the most promise for improving the quality of care in the treatment of affected service members.

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Journal of Bone and Joint Surgery

A Link Between Clinical Predictors of Heterotopic Ossification and Histological Analysis for Improved Surgical Planning in Combat Injured Service Members

--Manuscript Draft--

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Question	**Response**
Is this a clinical trial? This is defined as any clinical research where patients are being randomized into two treatment groups OR where patients are being followed prospectively comparing two different treatments.	No
Was this study NIH funded?	No
Have any of the illustrations or tables used in this article been published previously (i.e. does another party now own the copyright to any illustration or table)?	No
How will this work influence the practice of Orthopaedics?	This study utilizes advanced histological measurements and provides direct quantitative evidence of ectopic bone growth. Further assessment may lead to improved surgical planning to reduce recurrence and help explain the etiology of this

	debilitating bone disease.
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Abstract:	Background: Heterotopic ossification (HO) is a debilitating condition that occurs following traumatic injury and may restrict range of motion and delay rehabilitation. The timing and efficacy of surgical resection have varied widely, and a gap exists between clinical predictors of HO recurrence and histological analysis. Peer-reviewed literature depicts HO as a metabolically active osseous tissue, but there is no quantifiable evidence to optimize surgical timing and reduce recurrence. Methods: Thirty-three service members at ***Blinded by JBJS*** with symptomatic HO were enrolled in an institutional review board-approved study. Participants took oxytetracycline on four scheduled days prior to HO resection to determine the mineral apposition rate (MAR; i.e., bone growth rate). Results: Detailed histological analyses included scanning electron microscopy with backscattered electron imaging and light microscopy. Data indicated that the MAR of trauma-induced HO was approximately 1.7 μm/day at the time of surgical intervention, a value 1.7x higher than non-pathological human bone. The MAR and post-operative alkaline phosphatase (AP) values and AP pre-operative levels and the percent of osteoblastic activity were demonstrated to be positively related and statistically significant (ρ=0.509, p=0.026, n=19) and (ρ=0.522, p=0.004, n=29) respectively. When data was analyzed only within a two-year period from injury to excision (thereby removing outliers that were significantly longer than counterparts) and traumatic brain injury and non-steroidal anti inflammatory drugs were controlled for in the statistical analysis (known correlates with HO development), MAR and recurrence severity were significantly related (ρ=-0.572, p=0.041, n=11). Conclusion: Data from this study provides a link between bench top research and bedside care, and demonstrates that the MAR is elevated in HO and correlated with recurrence risk; however, a larger sample size and more clinical factors are needed to refine this model. Enhanced HO understanding may be achieved through further clinical study and/or the development of a physiologic translatable animal model. Level of Evidence for Primary Research: Level II, Development of diagnostic criteria (consecutive patients with consistently applied reference standard and blinding)

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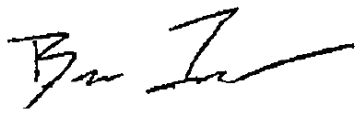
Dear Journal of Bone & Joint Surgery Editorial Team:

Enclosed is our manuscript entitled, "A Link Between Clinical Predictors of Heterotopic Ossification and Histological Analysis for Improved Surgical Planning in Combat Injured Service Members" that we submit for publication. Heterotopic ossification (HO) is a debilitating condition that occurs predominately following traumatic injury and may restrict range of motion and delay rehabilitation. Historical recommendations regarding the timing and efficacy of surgical resection have varied widely, and a gap exists between clinical predictors of HO recurrence and histological analysis. Therefore, thirty-three service members with symptomatic ectopic bone were enrolled in an institutional review board-approved prospective study. Data from this study provided a link between bench top research and bedside care, and demonstrates that the mineral apposition rate (ie bone growth rate) was elevated in HO and correlated with recurrence risk. Further clinical study and/or the development of a physiologic translatable animal model that carefully isolates each predictor variable may significantly advance the standard of care.

All listed co-authors are free from any conflicting interests and each has contributed significantly to this document. This manuscript has not been published previously nor is it being considered for publication elsewhere.

We look forward to your review of our manuscript and thank you for your time.

Best wishes,



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A Link Between Clinical Predictors of Heterotopic Ossification and Histological Analysis for Improved Surgical Planning in Combat Injured Service Members

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ABSTRACT

Background: Heterotopic ossification (HO) is a debilitating condition that occurs following traumatic injury and may restrict range of motion and delay rehabilitation. The timing and efficacy of surgical resection have varied widely, and a gap exists between clinical predictors of HO recurrence and histological analysis. Peer-reviewed literature depicts HO as a metabolically active osseous tissue, but there is no quantifiable evidence to optimize surgical timing and reduce recurrence.

Methods: Thirty-three service members at *****Blinded by JBJS***** with symptomatic HO were enrolled in an institutional review board-approved study. Participants took oxytetracycline on four scheduled days prior to HO resection to determine the mineral apposition rate (MAR; i.e., bone growth rate).

Results: Detailed histological analyses included scanning electron microscopy with backscattered electron imaging and light microscopy. Data indicated that the MAR of trauma-induced HO was approximately 1.7 μm/day at the time of surgical intervention, a value 1.7x higher than non-pathological human bone. The MAR and post-operative alkaline phosphatase (AP) values and AP pre-operative levels and the percent of osteoblastic activity were demonstrated to be positively related and statistically significant ($\rho=0.509$, $p=0.026$, $n=19$) and ($\rho=0.522$, $p=0.004$, $n=29$) respectively. When data was analyzed only within a two-year period from injury to excision (thereby removing outliers that were significantly longer than counterparts) and traumatic brain injury and non-steroidal anti inflammatory drugs were controlled for in the statistical analysis (known correlates with HO development), MAR and recurrence severity were significantly related ($\rho=-0.572$, $p=0.041$, $n=11$).

Conclusion: Data from this study provides a link between bench top research and bedside care, and demonstrates that the MAR is elevated in HO and correlated with recurrence risk; however, a larger sample size and more clinical factors are needed to refine this model. Enhanced HO understanding may be achieved through further clinical study and/or the development of a physiologic translatable animal model.

Level of Evidence for Primary Research: Level II, Development of diagnostic criteria (consecutive patients with consistently applied reference standard and blinding)

INTRODUCTION

Heterotopic ossification (HO) is abnormal osseous tissue that occurs in the musculature and periarticular regions following tissue injury and inflammation.[1] Although ectopic bone may develop from rare genetic disorders,[2-4] it is most frequently observed following orthopedic trauma,[2, 5-12] burns,[13, 14] arthroplasty,[15-20] spinal cord injury (SCI),[21-24] and traumatic brain injury (TBI).[23, 25-27] HO has been reported to occur in 20-30% of SCI, 10-20% of closed head injuries and following 16-53% of total hip arthroplasty procedures.[28, 29]

In most general population cases, HO following traumatic insult is usually radiographically minimal, clinically asymptomatic, and does not necessitate surgical intervention. However, for military service members injured by blasts sustained in the conflicts in Afghanistan and Iraq, the prognosis is quite different. These armaments generate extensive polytrauma, and hallmark injury profiles during overseas combat have included limb loss, TBI/SCI and HO.[1, 30-32] In fact, 1,573 wounded warriors have sustained one or more major limb amputations in the Operation Iraqi Freedom (OIF), Operation Enduring Fredom (OIF) and Operation New Dawn (OND) missions between the periods of 2000 and 2014;[33] approximately 63%-65% of these individuals experience post-traumatic HO[2, 6, 10] and 20%-40% require surgical excision.[6, 34-36] Symptomatic HO is problematic for service members since it delays rehabilitation regimens, causes pain, limits range of motion, and requires modifications to prosthetic limb componentry and socket size.[8, 34]

Wounded warriors represent a unique patient population given their relative youth, high fitness level prior to injury, and desire to aggressively rehabilitate in order to return to active duty or civilian recreational sports and activities.[37] Therefore, if/when ectopic bone becomes evident

(typically between one to twelve weeks after injury)[38, 39] patients may request that their mass be removed immediately. However, deciding when to excise HO requires careful consideration since delayed limb and patient immobilization may lead to fibro-fatty proliferation, muscle contracture, disuse osteoporosis, cartilage erosion and bone and fibrous ankylosis,[23, 39] whereas premature resection may lead to aggressive HO recurrence. While results from early resection have remained promising, premature surgical procedures often result in more florid ectopic bone regrowth; Genêt et al. noted that HO recurrence was present with radiographic and clinical investigation between 82%-100% and 17%-58% of the cases respectively.[40]

Early attempts to correlate HO recurrence with clinical predictors focused primarily on timing of the excision surgery and the severity of the neurologic insult; however, these attempts were unsuccessful.[2, 40] Genêt et al. provided the general recommendation that "HO should be carried out when it begins to be troublesome, as soon as comorbid factors are under control and the HO is sufficiently constituted for excision."[40] While this is logical, this recommendation lacks an objective measurement tool as to what exactly defines "sufficiently constituted" (i.e., mature), and does not address the absence of histological markers for optimizing resection schedules.

In an effort to bridge the clinical and histological gap, our team planned a prospective research study of combat injured service members who required removal of ectopic bone. The goals of this study were to use advanced imagining tools to (a) provide direct quantitative evidence of ectopic bone growth via the mineral apposition rate (MAR), percent osteoblast (%OBA), osteoclast (%OCA) and resting bone (%RB), (b) further understand HO's architecture through scanning electron microscopy (SEM), and (c) to assess relationships between ectopic bone

severity/recurrence with demographic information and previously attributed predictors (neurological injury, gender, etc.) in order to optimize surgical planning. Quantifying the metabolic rate of HO may validate conventionally used measures to determine ectopic bone development (pre-operative AP, nuclear scintigraphic (i.e., "bone scan") activity, and radiographic evidence of HO maturity). It was hypothesized that the presence of the elevated HO MAR, greater than traditional human bone remodeling (1 um/day)[41] would be a predictor of HO recurrence.

METHODS

Service members treated between the periods of June 2012 and March 2015 with symptomatic combat-related HO were included in this institutional review board-approved study. Participants were excluded if they were less than 18 years of age, allergic to tetracycline, or current/past usage of tetracycline within 3 months of enrollment. Patients were recruited through orthopedic surgeon referral after counseling and confirming that surgical resection was necessary and planned. All participants signed an informed consent document and were treated per the standard of care, other than that each participant was asked to take oxytetracycline (250mg/tid) on 4 separate dates prior to their scheduled surgery to determine their MAR (i.e. bone growth rate). Dosing was slightly variable per the participant's clinical schedule, but typically consisted of a 2 day dosing period, minimal of a 3 day hiatus, 2 day dosing period, 2 day washout, and then surgical excision. Lastly, the timing of surgical intervention was based on the clinical standard for assessing HO maturation (AP levels, cortication and stability on serial orthogonal radiographs, and consideration for TBI) and was at the surgeon's discretion.

Clinical

Demographic information and injury data was captured for each participant using the local electronic medical record systems. Specific recorded information included gender, age, date of injury, height and weight prior to injury, injury-to-excision latency, the injury mechanism which caused the limb loss, HO pre-operative and post-operative serum AP levels, history of non-steroidal anti-inflammatory (NSAID) use, TBI diagnosis, and HO anatomical location. TBI was coded as ordinal data, with 0=none, 1=mild, 2=moderate and 3=severe. Each patient's radiographic data was also blinded and reviewed by an attending orthopedic surgeon to assess HO severity prior to resection and recurrence 3-6 months post-operatively. Ectopic bone severity and recurrence were based on the Walter Reed method which uses anteroposterior and lateral radiographs to segment individuals based on the amount of ectopic bone within their residual limb (0%, none; < 25%, mild; 25%-50%, moderate; > 50%, severe).[6]

Histology

Following surgical intervention, HO samples were deidentified, and processed to perform post-operative analysis. All specimens were photographed, radiographed, fixed in formalin, dehydrated in ascending grades of ethanol and embedded in polymethylmethacrylate according to standard laboratory procedures (Figure 1).[42, 43] Two millimeter slices were sectioned using a high-speed, slow-feed cut-off saw with a diamond-impregnated rotary blade. HO sections were ground, polished and sputter-coated with carbon to increase conductivity for SEM analysis. Three specimens from each patient's HO were analyzed with backscattered electron imaging (BSE) at 10-2000x magnifications, working distance of 15mm, and 20kV accelerated voltage (Figures 2-5). Following SEM examination, the HO cross-sections were ground to approximately

75μm, polished, and prepared for MAR analysis. To calculate the bone growth rate, 3 bone sections were randomly selected which had double labeling and 63 total data points were measured at an average of 200x magnification using a mercury lamp microscope. The width of the newly mineralized bone layer was calculated in units of microns per day (μm/day), and was determined by measuring the distance between the midline of two parallel fluorescent labels (Equation 1). The numbers of double and single labels were also counted to assess the metabolic activity of the resected HO bone (Figure 7).

$$\text{MAR}(\mu\text{m/day}) = \frac{\Sigma x(e)(\pi/4)}{nt}$$

Σ_x = sum of all the measurements between double labels
$\pi/4$ = is the obliquity correction factor
n = total number of measurements
t = time interval expressed (days)

Equation 1: Formula for calculating MAR

Each bone slide was stained using Sanderson's bone stain and 30 light microscopic images were analyzed with light microscopy to compute the %OBA, %OCA and %RB by determining the proportion of quiescent and metabolically active bone (Figure 8).

RESULTS

Demographic Information

Forty-six service members were initially enrolled in this research study and met the inclusion criteria. However, 13 subjects were excluded since they did not adhere to the tetracycline dosing schedule (which would have prevented MAR assessment) or because of an infection or comorbid injury required immediate surgical intervention. Thus, 33 service members who experienced

symptomatic HO following combat-related trauma are reported herein. Thirty-two (97%) of the subjects were male and the average height and weight prior to injury was 69+/-3 inches and 186+/-23 pounds respectively. IEDs accounted for 76% of the injuries (25/33) followed by 15% "other"(5/33) which consisted of gun shot wounds, suicide bomber attacks and training injuries, 6% motor vehicle accident (2/33), and 3% RPG (1/33). Subjects were 27+/-6 years of age at the time of injury and ectopic bone resection occurred 13+/-9 months from the date of their traumatic insult. Pre-operative AP levels (recorded 1-3 months prior to surgery) were measured at 111+/-44 ui/L and post-operative AP levels (measured 3-6 months after surgical intervention) were 85+/-26 ui/L. HO occurred in 20 patients with transfemoral amputations (61%), six with transtibial amputations (18%), four upper extremity amputations (12%) and three hip disarticulations (9%). TBI was reported in 67% of the subjects (20 mild, 1 moderate, and 1 severe). Pre-operative radiographs using the Walter Reed HO severity scoring methodology resulted in 15 cases of mild HO (46%), 10 moderate (30%) and eight severe (24%). Post-operative review greater than 3 months after initial surgery showed no signs of recurrence in 25 subjects (76%), a minimal non-clinically relevant amount of ectopic bone in 4 subjects (12%), mild amount in 4 subjects which may require further observation (12%).

Histology

Histological data indicated that MAR levels from traumatic injury were approximately 1.7 times higher than non-pathological human bone at the time of surgical intervention (1.7+/-0.5 μm/day, range: 1.1-3.7 μm/day) (Figure 9). SEM images showed HO in varying stages of remodeling with %OBA, %OCA and %RB values at 28.1±14.9%, 8.3±5.8% and 63.6±19.8% respectively.

BSE demonstrated that the pathological masses were a composite structure of cortical (Figure 2b) and cancellous bone (Figure 6b), with bone chips and newly formed woven bone (Figures 5c). A wide range of low mineral to highly mineralized regions was noted within the HO structure. The low mineralized bone suggested recent appositional bone formation. Eroded resorption fronts along the periphery of bone fragments, as well as other regions of the newly formed tissue, complemented the ongoing remodeling (Figure 3a). Osteon type structures along with woven bone formation demonstrated the complexity of remodeling and bone formation that had occurred.

Bivariate Pearson correlations coefficients (ρ) indicated that MAR and HO anatomical location were significantly associated (ρ=0.353, p=0.047, n=32) with rates highest for upper extremities (2.6±1.1 μm/day, range: 1.5-3.7 μm/day), followed by pelvis/hip (1.8±0.3 μm/day, range: 1.6-2.1 μm/day), transfemoral (1.6±0.2μm/day, range: 1.1-2.0 μm/day) and transtibial (1.4±0.3 μm/day, range: 1.1-2.0 μm/day).

A direct relationship between histological markers and clinical predictors was established between the MAR and post-operative AP values (ρ=0.509, p=0.026, n=19), and AP pre-operative levels and the %OBA (ρ=0.522, p=0.004, n=29). The number of double labels counted during MAR analysis was significantly related with pre-operative AP levels (ρ=0.430, p=0.032, n=25). Furthermore, the MAR and the time from injury to excision were significantly and directly related (ρ=0.399, p=0.024, n=32).

When MAR was compared to HO recurrence, there was no significant relationship (ρ=-0.285,

p=0.120, n=31) identified with the numbers available. However, when data was analyzed only within a two-year period from injury to excision (thereby removing outliers that were significantly longer period than counterparts) and TBI and NSAID use were controlled for in the statistical analysis (known correlates with HO development), MAR and recurrence severity were significantly related (ρ=-0.572, p=0.041, n=11).

Linear regression analysis indicated that a significant relationship existed between MAR and several predictors. Data indicated that there was only an average 2.4% error between the predicted and actual MAR rates when using the following equation: MAR = 2.362 + 0.007*AP levels at the time of surgery (pre-op) – 0.008*weight of the patient prior to injury + 0.177*TBI classification (0-3 scoring system noted above) (Figure 10).

DISCUSSION

While the peer-review literature is replete with text suggesting that HO is more metabolically active than non-pathological bone,[28, 44-46] to date, there has not been a study to directly quantify this activity. MAR has been used to evaluate the grow rate of human and animal cortical and cancellous bone,[47] but this technique has not been utilized to examine ectopic bone growth. The current study addresses this limitation and suggests that ectopic bone growth is approximately 1.7 μm/day in traumatically injured patients, a rate 1.7x higher than non-pathological human bone. Furthermore, detailed BSE imaging showed HO as a cortical and cancellous hybrid with varying decrees of vascularity and mineralization. This finding may impact clinical practice since one of the hallmarks for deciding when to remove ectopic bone requires waiting until a well-defined neocortex to form which suggests HO maturity; however, data from this study

indicates that in some cases, this may lead to a protracted time period prior to excision if only or predominantly trabecular bone forms.

The most significant findings from this study include the new knowledge that there is a direct link between clinical predictors of HO and post-operative histological analysis. When data was analyzed on an aggregate level, MAR directly correlated with post-operative AP counts. This association is physiologically sound, since as osteoblasts actively deposit unmineralized bone (as indicated by the elevated MAR rates), these cells also release AP, which assist in the calcification process. This tightly coupled bone remodeling process clearly exists for HO as well as non-pathological bone, as highlighted in this study and confirmed through single and double flurochrome labeling.

In our samples, ectopic bone manifested most rapidly in the upper extremity, followed by pelvic region, above the knee, and lastly below the knee in wounded warriors following limb loss. As noted in detail by Isaacson et al.,[30] the use of IEDs and battle field tourniquets may in part explain the increased amount of ectopic bone noted in wounded warriors during the recent conflicts. A blast-injury drastically changes the micro-environment in the residual limb (pH, oxygenation, perfusion, etc.) and may trigger a cascade of chemotactic agents and upregulation of vascular endothelial growth factor, transforming growth factor beta, fibroblast growth factor and glucose transporters.[48] However, previous reports have indicated that increased HO volume most notably occurs in areas with increased muscle mass (and higher resident mesenchymal progenitor cell counts),[22] whereas this study suggests that if HO develops, its progression may be

linked with the anatomical location and the upper extremity could be impacted more rapidly than the lower limb.

The MAR was determined to be positively correlated and statistically significant. This would indicate that as the time between injury to surgical resection lengthened, the bone growth rate (MAR) increased as well. This relationship seems counterintuitive since one would expect that a more protracted period would lead to bone becoming quiescent or "mature." This phenomenon is likely attributed to: (1) patients with extensive polytrauma/comorbid injuries required a longer period to have their HO excised and this included a unique subset of self-selected patients and (2) these individuals may have had concurrent fractures or neurological insult which may have confounded AP level analysis. To test this principle, our team restricted the time to excision to only within 1 year from injury (to isolate the most acute patients) and the relationship between MAR and surgical timing was nearly significant an inversely related (ρ=-0.426, p=0.061, n=20). The latter association is the most logical and reaffirms that further analysis is needed with an increased sample size and more medical information from each patient.

When MAR and HO recurrence was analyzed on an aggregate level, there was no statistical relationship. However, when MAR and HO recurrence was analyzed only within a 2 year time period and data was controlled for known HO correlates (TBI and NSAID usage), the relationship was significant, but inversely related. This would seem to indicate that as MAR increased, the likelihood for recurrence would decrease. As noted above, there are likely exogenous factors in this model that necessitate further investigation since one would expect less recurrence with lower metabolically active bone.

The MAR equation developed in this study (MAR = 2.362 + 0.007*AP levels at the time of surgery – 0.008*weight of the patient at time of injury + 0.177*TBI classification), provides additional insight for surgeons in order to plan their surgical procedures. However, until the questions posed above can be answered, clinical discretion continues to be required. In the future, a carefully designed, physiologic and translatable large animal model may be developed which accurately reflects the clinical condition (and includes tourniquet and wound vacuum usage/duration, positive infection signals, controlled blasts, etc.) and may further our understanding of the etiology and pathophysiology of HO.

CONCLUSION

HO remains a frequent and troublesome clinical complication following both military and civilian trauma. While some patients will not develop HO following traumatic injury or present asymptomatically, others may experience florid symptomatic growth within a residual limb or periarticular space. Therefore, developing a sound link between clinical predictors and histological analysis holds tremendous value and may help surgeons improve their planning of HO excision, as well as refine patient counseling regarding recurrence risk. Data from this study highlights some direct relationships between bench top and bedside; however, additional factors must be further investigated to directly correlate MAR findings with development and recurrence. In this study, MAR and post-operative AP values were demonstrated to be positively related and statistically significant.

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FIGURE LEGENDS

Figure 1: Photographs of the amount of bone resected from Patient #28's residual limb. This service member was a male, 32 years of age at injury and experienced HO in above knee following an IED blast and limb loss.

Figure 2: SEM BSE micrographs of Subject 1. Grey=Bone and Black=Pore Space and Soft Tissue. (A) BSE micrograph showing newly formed bone (arrow). (B) BSE micrograph displaying osteon formation (arrow) that is common with cortical bone. (C) BSE micrograph showing bone resorption (arrow) on a trabecular type structure of bone. The images suggest that bone fragments were displace from the blast.

Figure 3: SEM BSE micrographs of Subject 4. Grey=Bone and Black=Pore Space and Soft Tissue. (A) BSE micrograph showing bone resorption (arrow) at hypermineralized region of bone, likely from endochondral bone formation. (B) Low power view presenting the structure of the HO bone. (C) High power view of image B (red box) exhibiting osteon formation (arrows) that is typically found in cortical bone. Note note the presence of bone fragments from the blast injury. The bright white regions in all specimens in this series suggest endochondral bone formation similar to that seen in fracture healing.

Figure 4: SEM BSE micrographs from Subject 7. Grey=Bone and Black=Pore Space and Soft Tissue. (A) BSE micrograph showing mature host cortical bone with new bone growth (darker grey areas). This micrograph shows a likely fragment of cortical bone that had been dislodged by

the blast injury and/or surgical procedure. (B) BSE micrograph showing an area with hypermineralized bone (arrows). (C) BSE micrograph displaying a bone chip (arrow) being incorporated by new bone growth.

Figure 5: SEM BSE micrographs from Subject 8. Grey=Bone and Black=Pore Space and Soft Tissue. (A) BSE micrograph showing mature host bone with new bone growth (darker grey areas). This micrograph shows secondary osteonal remodeling in the cortical bone fragment from the HO tissue. Cancellous bone structure with endochondral type (bright white) bone. It is interesting to see the cement lines in the cortical bone fragment. (B) BSE microscope showing bone resorption (arrow). (C) BSE micrograph showing trabecular type bone structure.

Figure 6: SEM BSE micrographs of Subject 2. (A) BSE micrograph showing bone chips (arrows) being incorporated by new bone growth. (B) BSE micrograph showing a mature trabecular structure. (C) High power view of image B (red box) showing mature bone.

Figure 7: The result of the MAR analysis for Patient 1 demonstrated that the HO bone was remodeling at 1.8±0.6µm per day.

Figure 8: Histological analysis used to calculate the %OBA, %OCA and %RB. Note that a histology sample was prepared with Sanderson Rapid Bone Stair and then was capture (A). In this image, pink=bone, blue=tissue/cells and white=pore space. The image as next traced using a

software program to determine active and quiescent areas of remodeling (B) and then color-coded for easy recognition (C).

Figure 9: MAR values calculated in this study. Note, that all values exceeded the peer-reviewed literature values for of 1.0 μm/day.

Figure 10: Comparison between the Actual MAR's calculated and the ones predicted using the linear regression developed in this study. Note there is a 2.4% an average error.

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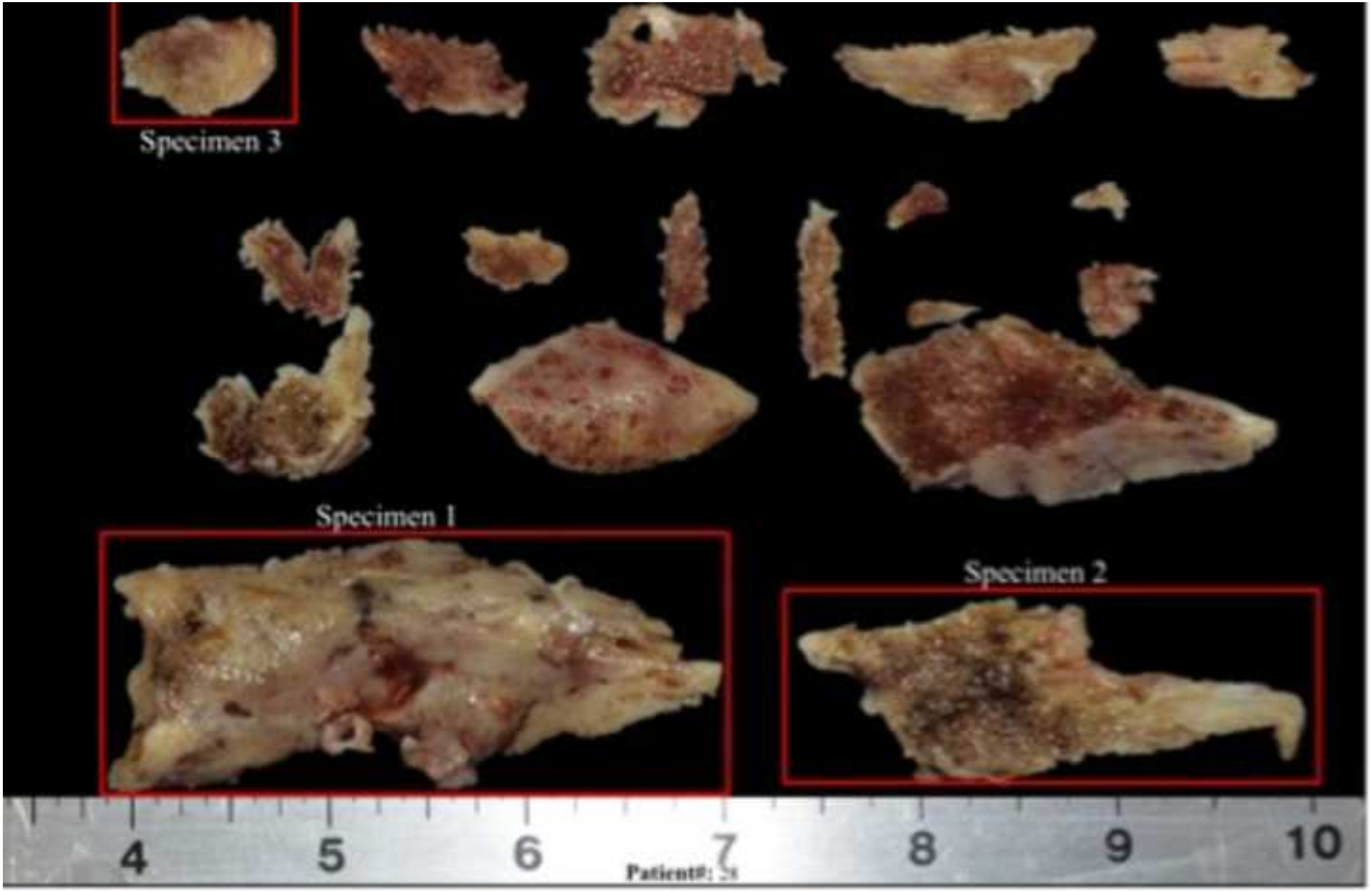

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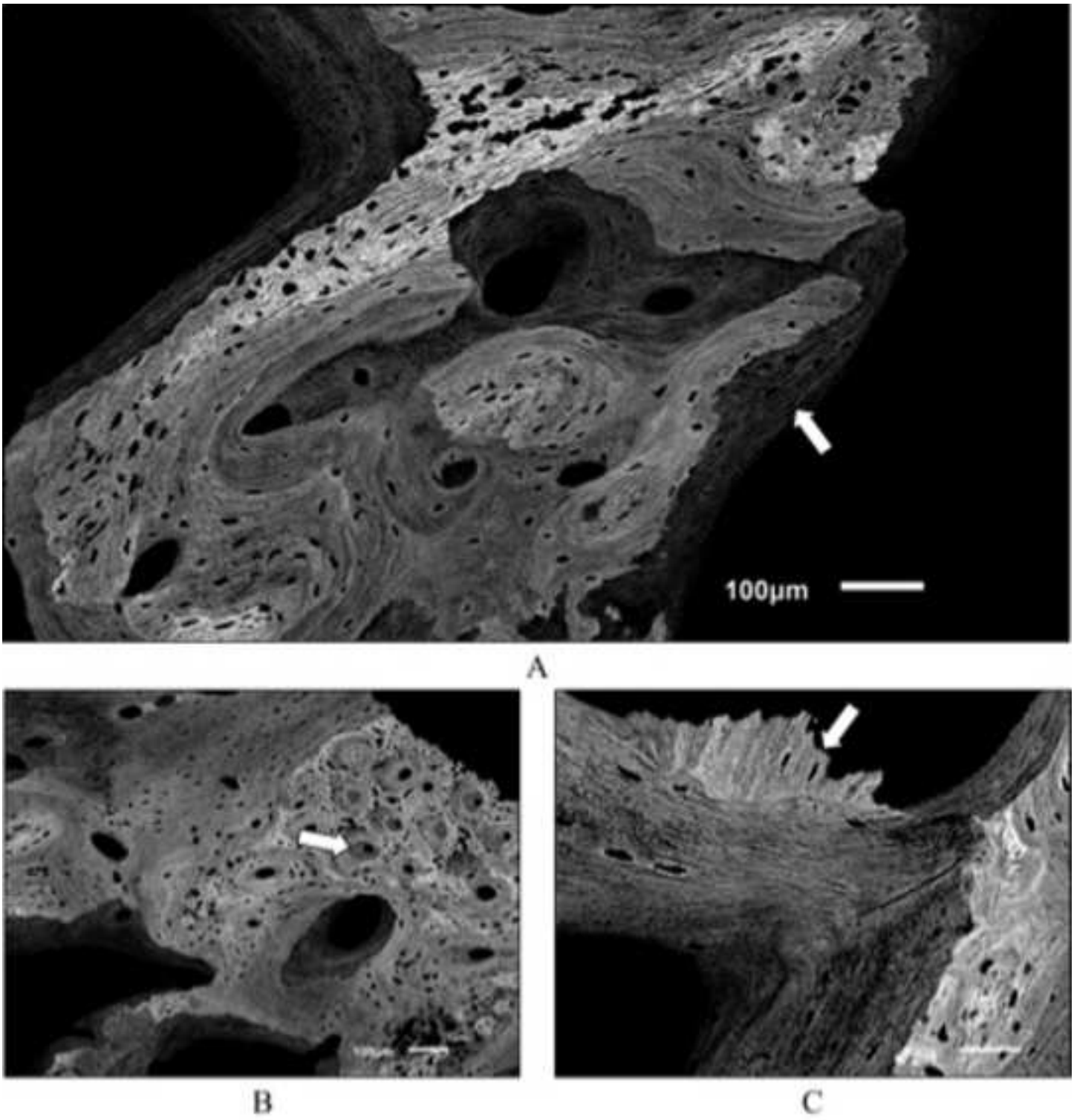

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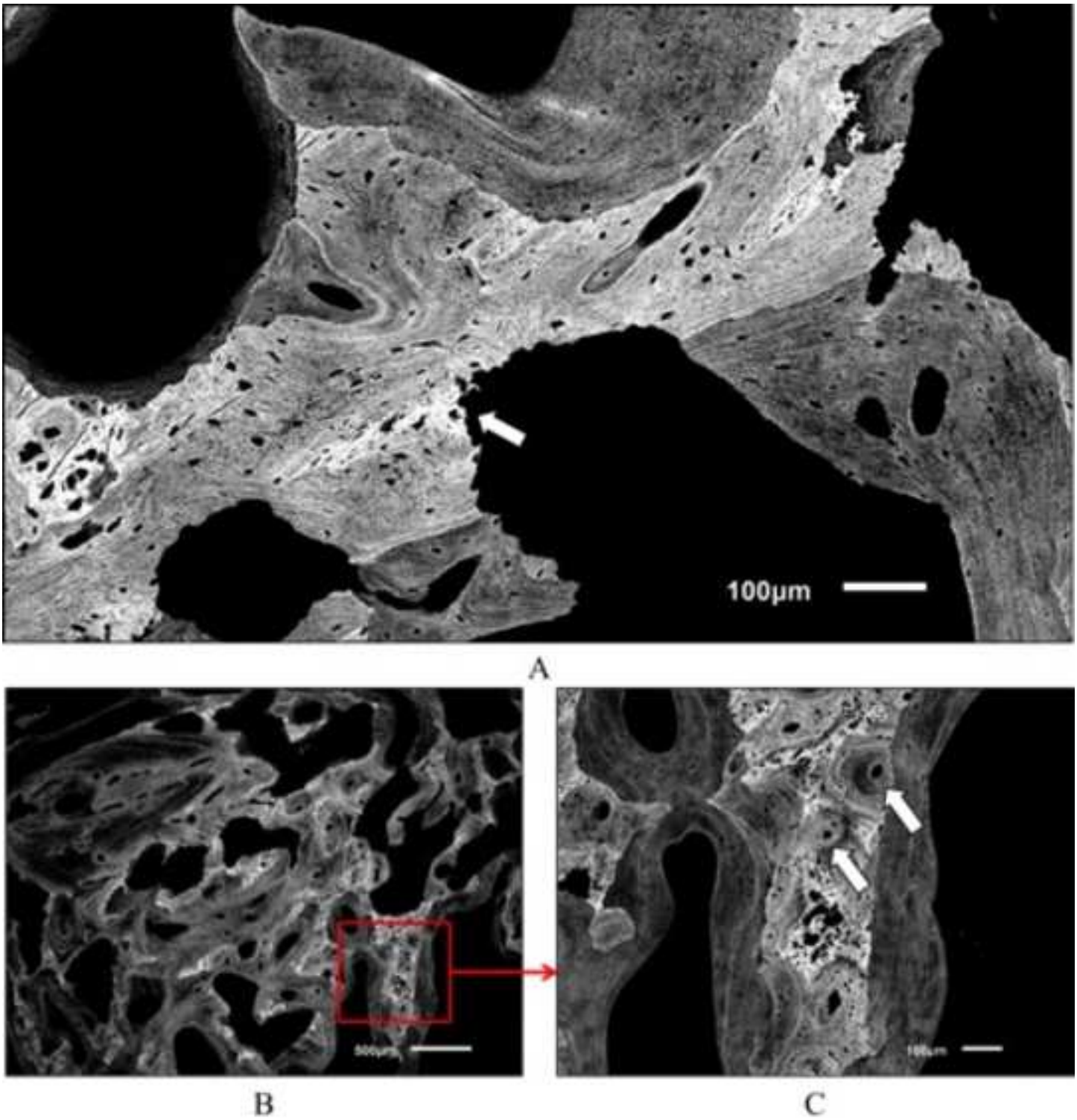

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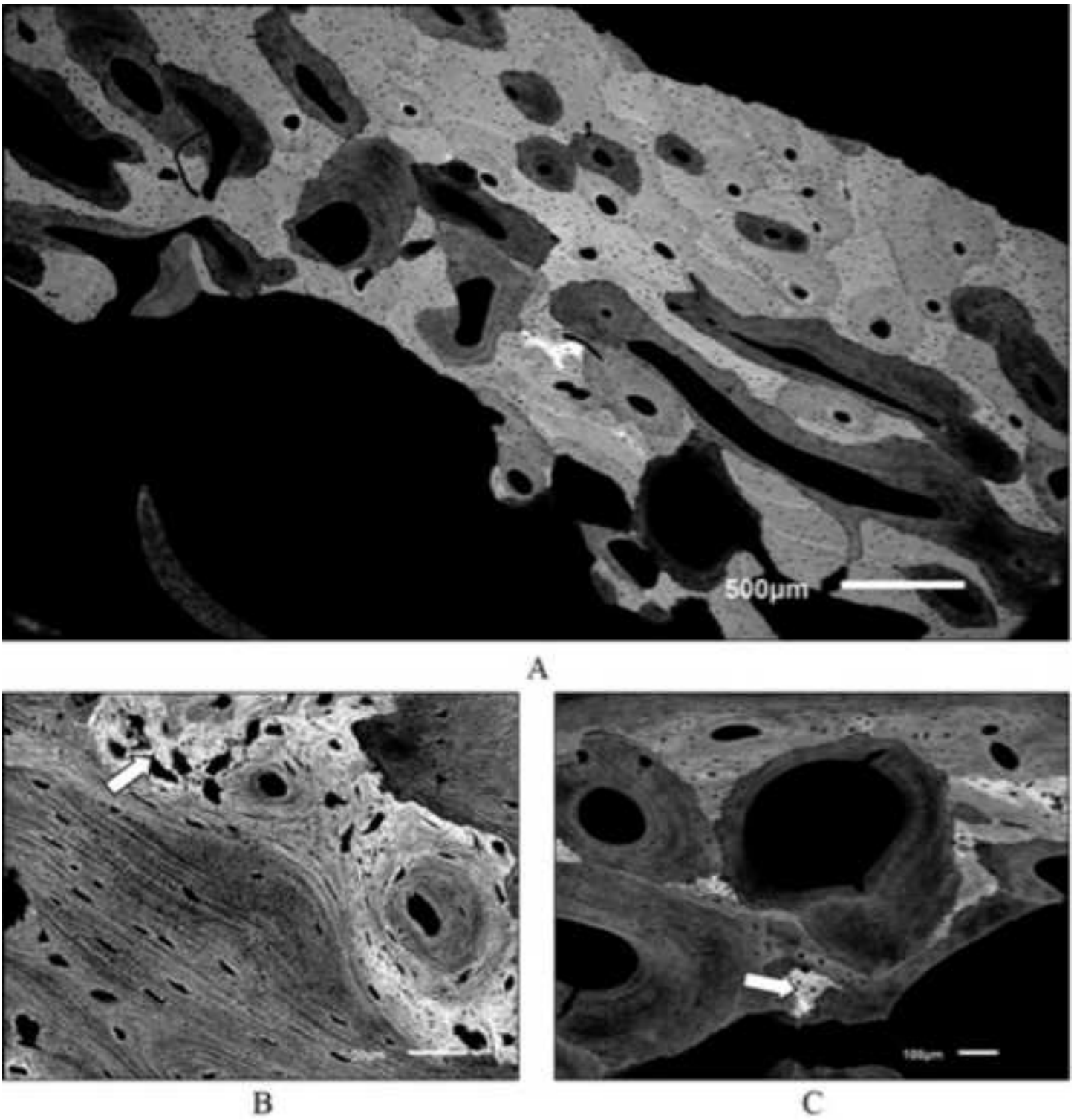

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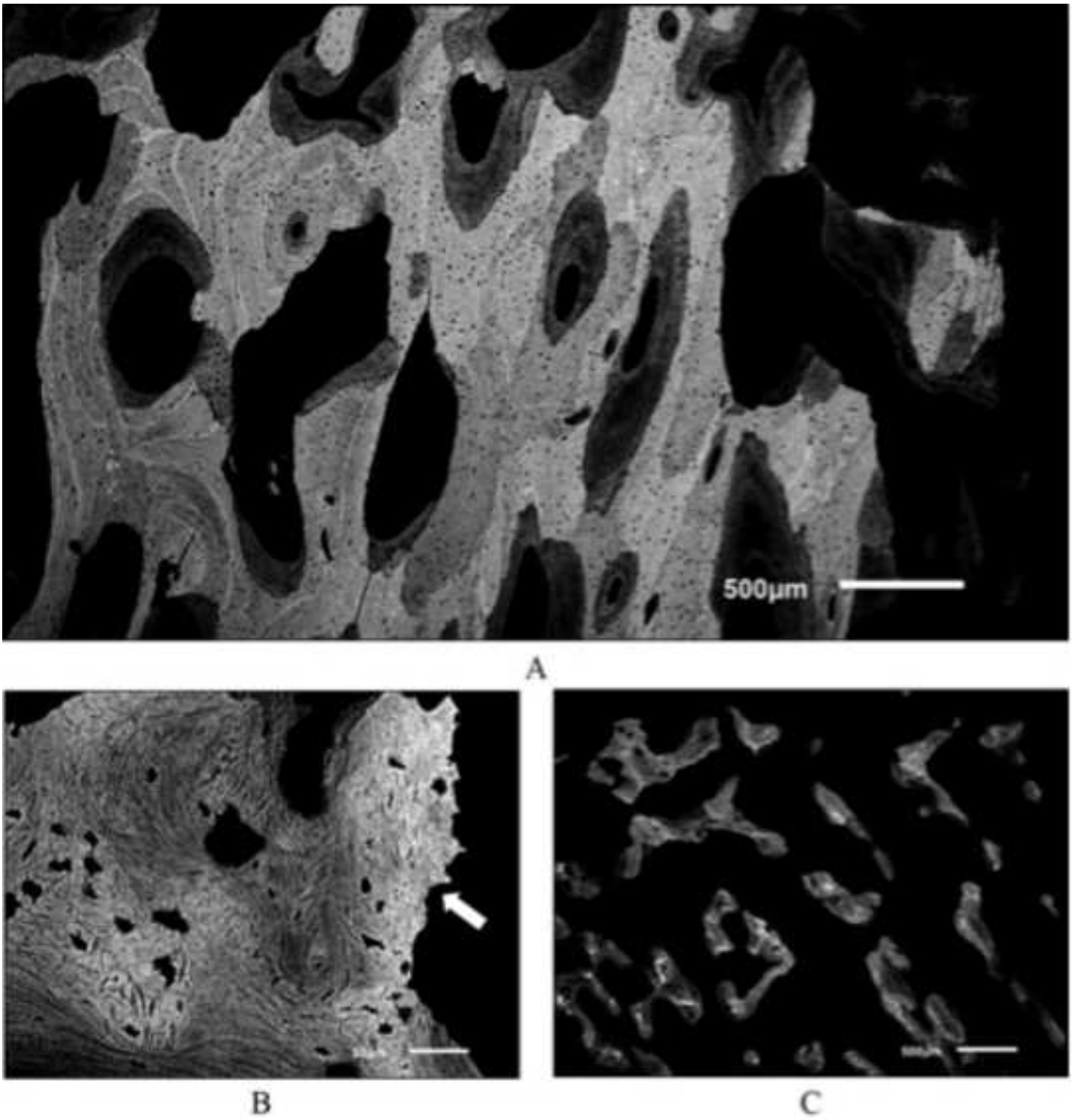

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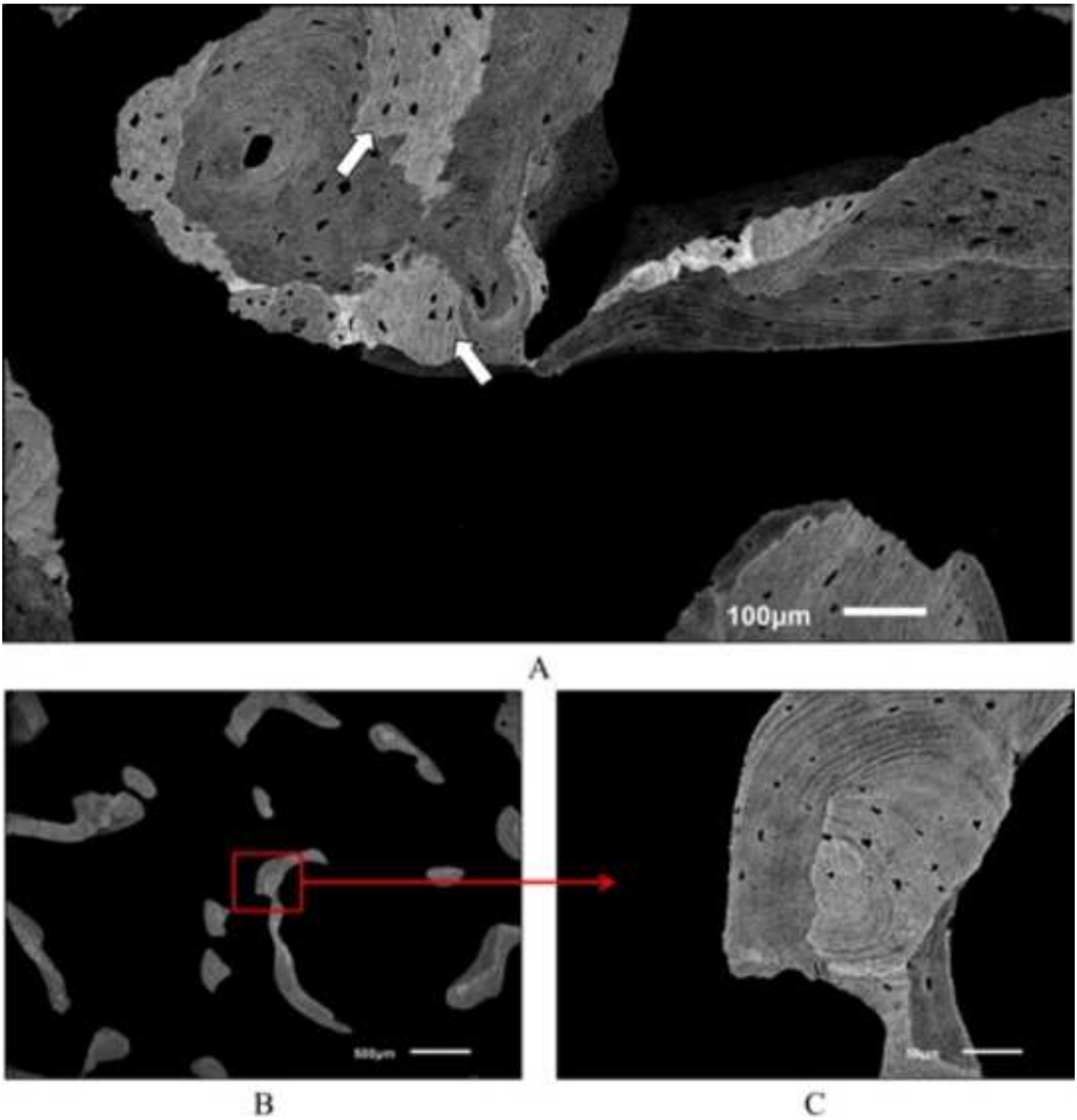

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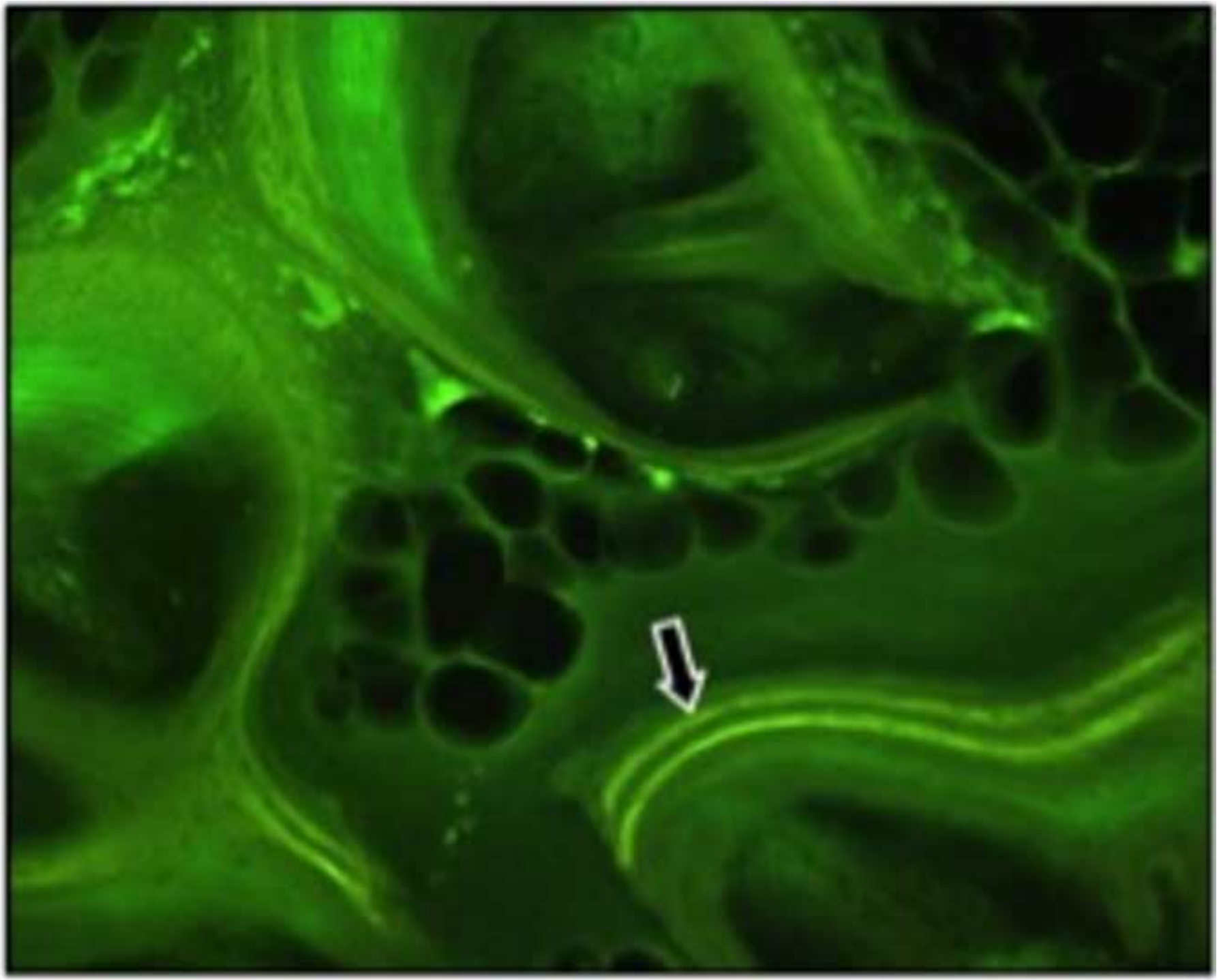

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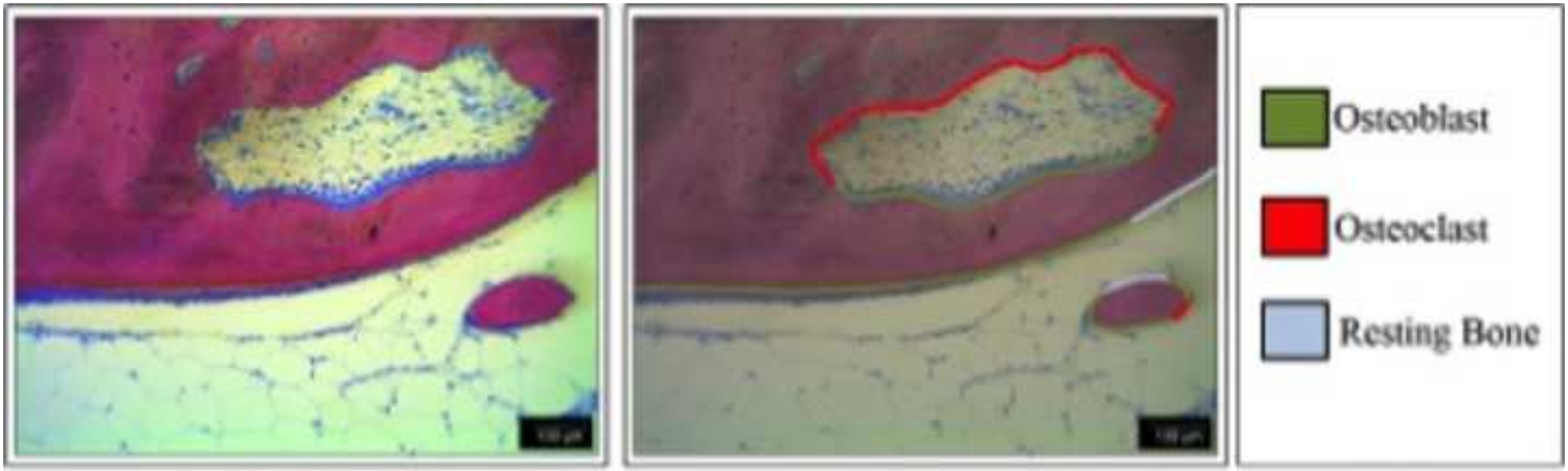

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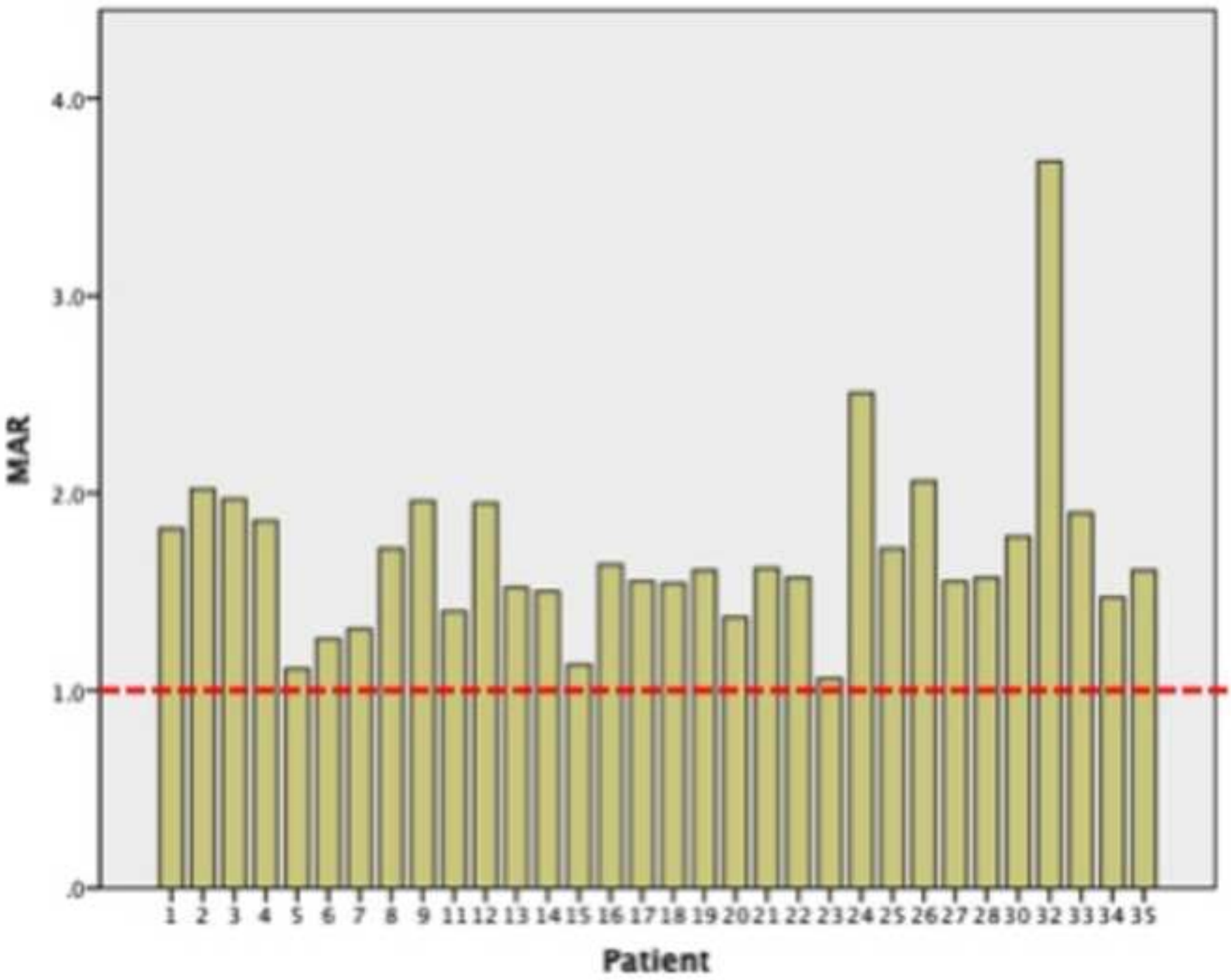

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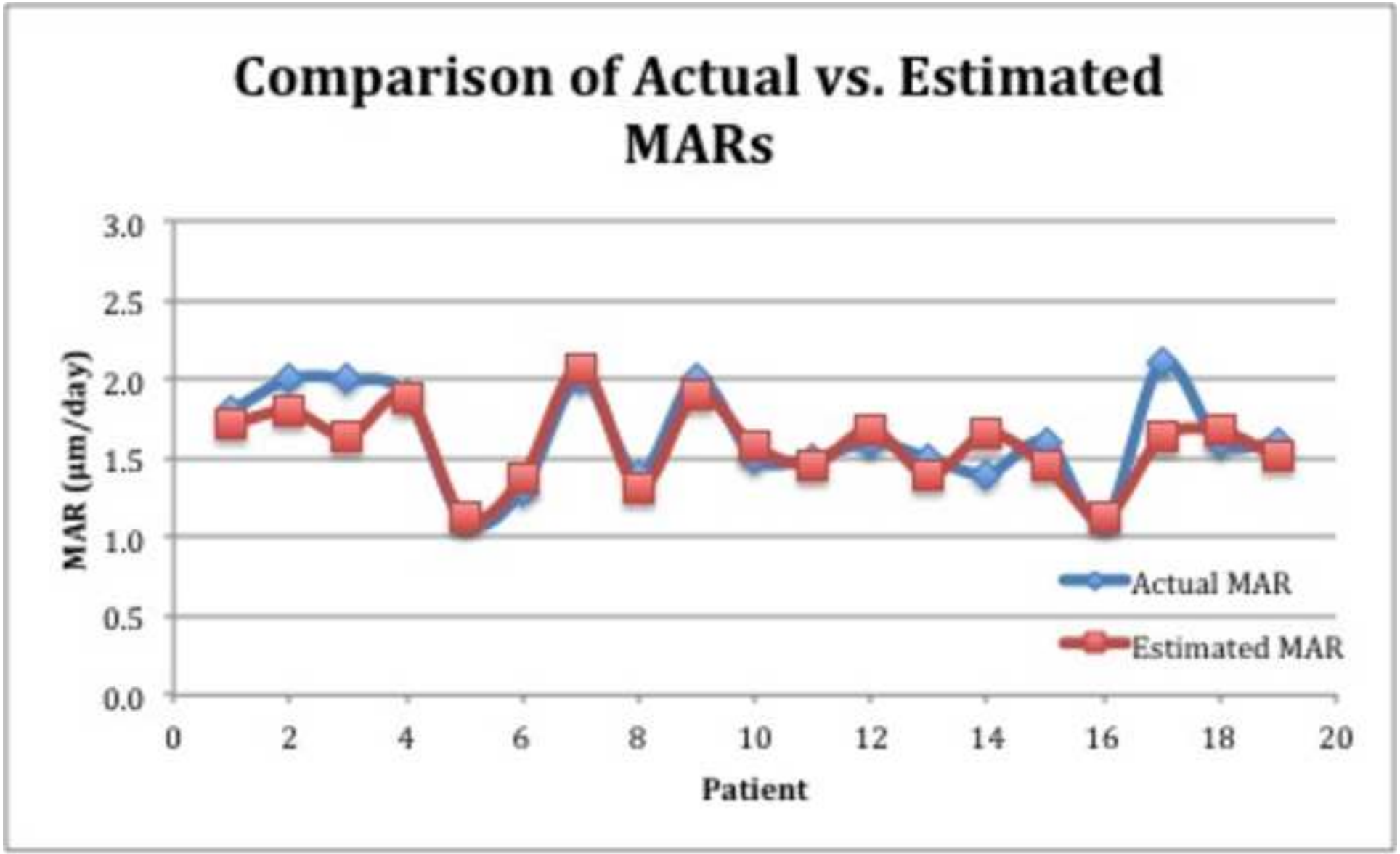

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February 4, 2015

MEMORANDUM

FROM: WRNMMC IRB
TO: Paul Pasquina

SUBJECT: WRNMMC IRB REVIEW OF Revision- Continuing Review Report/Amendment

PROJECT TITLE:	Establishing the Mineral Apposition Rate of Heterotopic Ossification for Prevention of Recurrence
REFERENCE #:	359978-28
ACTION:	APPROVED
APPROVAL DATE:	February 4, 2015
EXPIRATION DATE:	February 19, 2016
REVIEW TYPE:	Administrative Review
RISK LEVEL:	More than Minimal Risk

1. The WRNMMC IRB reviewed your continuing review report and amendment at their meeting on January 22, 2015 and the modifications required by the IRB were approved on February 4, 2015. Your protocol continues to meet the requirements under 32 CFR 219.111 and 21 CFR 56.111. This study continues to meet the requirements under 21 CFR 312.2(b)(1). This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All future research must be conducted in accordance with this approved submission.

2. The following documents have been updated with this submission:

- Protocol- Version 13, December 23, 2014
- HIPAA Consent/Authorization - Version 8, February 3, 2015

3. The IRB approved, stamped consent/HIPAA authorization form enclosed is to be duplicated and used to enroll subjects. Keep the signed, original consent forms in your project file; give each subject a signed copy of the consent form. **Please note: The Georgian Consent Form will not be reissued at this time at the request of the investigator, as they are no longer working with the NATO patient exchange program.**

4. You are reminded to provide all amendments, deviations, related serious adverse events, unanticipated problems involving risks to subjects or others, and any other pertinent information regarding this research protocol through IRBNet for reporting to the IRB.

5. You are reminded that all presentations and publications related to this work must be cleared through the publications clearance process.

6. If you have any questions, the POC is Debarati Dasgupta at 301 400-0692 or debarati.dasgupta2.civ@mail.mil. Please include your project title and reference number in all correspondence with this committee.

This document has been electronically signed in accordance with all applicable regulations, and a copy is retained within our records.

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Manuscript Number: BONE-D-15-00852

Title: Determining the Mineral Apposition Rate of Heterotopic Ossification in Military Patients After Total Joint Replacement: A Case Series

Article Type: Case Report

Keywords: heterotopic ossification; ectopic bone; veterans; total joint replacement; mineral apposition rate

Corresponding Author: Dr. Brad Isaacson, PhD, MBA, MSF

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First Author: Brad Isaacson, PhD, MBA, MSF

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Abstract: Background: Heterotopic ossification (HO) is frequently reported following total joint replacement (TJR) surgery and symptomatic cases may limit range of motion, cause pain and require surgical excision. Deciding an appropriate time to remove HO is subjective and closing the gap between clinical predictors and histological analysis may minimize the likelihood for recurrence. Methods: A case series was performed with military healthcare system (MHS) patients undergoing TJR who required removal of periarticular ectopic bone. Patients were prescribed tetracycline to assess the mineral apposition rate (MAR, i.e. bone growth rate) of HO and surgical specimens were analyzed using scanning electron microscopy (SEM) and light microscopy. Results: Two males and one female qualified for the study and were 69.0±7.8 inches, 237.7±28.3 pounds and 61±7 years of age at the time of HO excision. Ectopic bone occurred in two cases following total knee arthroplasty and one total hip arthroplasty. Data indicated that MAR levels were 1.7 times higher than previously reported non-pathological human bone at the time the HO was excised (1.7±0.7 µm/day, range: 1.3-2.6 µm/day). SEM and light microscopic images showed that HO to be in a quiescent state and consisted of only cancellous bone. Discussion: HO bone architecture observed from veterans undergoing TJR was vastly different than the previously characterized specimens investigated by our team from wounded warriors. This variation may be attributed to differences in the induction mechanism (controlled operative procedure vs. blast injury) and patient age differences. Conclusion: HO is a metabolically active tissue that may reduce quality of life. Further characterization is needed to optimize symptomatic HO excision timing and further understand the etiology of this pathological bone disorder.

Suggested Reviewers: Dustin Williams
dustin.williams@hsc.utah.edu
Dr. Williams has 8 years of bone biology experience.

Jonathan Forsberg

jonathan.a.forsberg.mil@mail.mil
Dr. Forsberg is one of the world's foremost experts on HO growth/development.

Jason Wilken
jason.m.wilken.civ@mail.mil
Dr. Wilken is the Director of the Center for the Intrepid and is very aware of military HO.

Opposed Reviewers:

3 September 2015

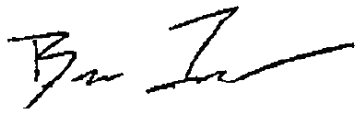
Dear Dr. Khosla:

Enclosed is our manuscript entitled, "Determining the Mineral Apposition Rate of Heterotopic Ossification in Military Patients After Total Joint Replacement: A Case Series" that we submit for publication in BONE. Heterotopic ossification (HO) is frequently reported following total joint replacement (TJR) surgery and symptomatic cases may limit range of motion, cause pain and require surgical excision. Deciding an appropriate time to remove HO is subjective and closing the gap between clinical predictors and histological analysis may minimize the likelihood for recurrence. Therefore, a case series was performed with military healthcare system (MHS) patients undergoing TJR who required removal of peri-articular ectopic bone. Patients were prescribed tetracycline to assess the mineral apposition rate (MAR, i.e. bone growth rate) of HO and surgical specimens were analyzed using scanning electron microscopy (SEM) and light microscopy. Data from this study demonstrated HO bone architecture observed from veterans undergoing TJR was vastly different than the previously characterized specimens investigated by our team from wounded warriors. This may be attributed to differences in the induction mechanism (controlled operative procedure vs. blast injury) and patient age differences.

All listed co-authors are free from any conflicting interests and each has contributed significantly to this document. This manuscript has not been published previously nor is it being considered for publication elsewhere.

We look forward to your review of our manuscript and thank you for your time.

Best wishes,



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Determining the Mineral Apposition Rate of Heterotopic Ossification inMilitary Patients After Total Joint Replacement: A Case Series

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ABSTRACT

Background:Heterotopic ossification (HO) is frequently reported following total joint replacement (TJR) surgery and symptomatic cases may limit range of motion, cause painand require surgical excision. Deciding an appropriate time to remove HO is subjective and closing the gap betweenclinical predictors and histological analysis mayminimize the likelihood for recurrence. ***Methods:*** A case series was performed with military healthcare system (MHS) patients undergoing TJR who required removal of periarticular ectopic bone. Patients were prescribed tetracycline to assess the mineral apposition rate (MAR, i.e. bone growth rate)of HO and surgical specimens were analyzed using scanning electron microscopy (SEM) and light microscopy. ***Results:***Two males and one femalequalified for the study and were 69.0±7.8 inches, 237.7±28.3 pounds and61±7 years of age at the time of HO excision. Ectopic bone occurred in two cases following total knee arthroplasty and one total hip arthroplasty. Data indicated that MAR levels were 1.7 times higher than previously reported non-pathological human bone at the time the HO was excised (1.7±0.7 μm/day, range: 1.3-2.6 μm/day). SEM and light microscopic images showed that HO to be in a quiescent state and consisted of only cancellous bone. ***Discussion:***HO bone architecture observed from veterans undergoing TJR was vastly different than the previously characterized specimens investigated by our team from wounded warriors. This variation may beattributed to differences in the induction mechanism (controlled operative procedure vs. blast injury) and patient age differences. ***Conclusion:***HO is a metabolically active tissue that may reduce quality of life. Further characterization is needed to optimize symptomatic HO excision timing and further understand the etiology of this pathological bone disorder.

***Keywords*:** heterotopic ossification; ectopic bone; veterans; total joint replacement; mineral apposition rate

INTRODUCTION

Heterotopic ossification (HO) is classified as abnormal osseous tissue that occurs in the musculature and periarticular regions.[1-5] These masses result fromgenetic abnormalities, neurologic injury, and/or musculoskeletal trauma and surgery.[6] In the case of post-operative HO, ectopic bone has been commonly reported following total joint replacement (TJR),[7-12] with post-operative rates varying between 2-90%,[6,13-16]and severe cases occurring3-55% of the time.[6]This wide range of HO incidence has been associated withpatient demographics, surgical technique and the use of prophylactic treatments (radiation and non-steroidal anti-inflammatory drugs (NSAIDs)).[6]Ectopic bone maycause limited range of motion and/orpain,[6,15,16]and is clinically/radiographically detectablebetween 1 to 12 weeks post-operatively.[17,18]HO remains a challenging comorbidity and 3-7% of THA patients develop grade III/IV symptomatic HO[20] (as scored by the Brooker scale)[19] andrequire excision of their ectopic bone.

Most concerning is that the frequency of TJR-related HO is expected to rise in the coming years due to increased life expectancy,[21] availability of advanced medical care globally, and a demand for a high quality of life. As noted by the 2010 Center for Disease Control and Prevention inpatient surgery census, 719,000 total knee replacement (TKA) and 332,000 total hip arthroplasty (THA) procedures occur annually in the United States.[23] Based on HO incidence data noted above, tens of thousands of patients with symptomatic HOmay require excision and advanced clinical management.

Although HO was first reported by El Zahrawi (Albucasis) in 1000 C.E.,[24] very little is known about the etiology of this pathological process. HO has been reported as a hybrid of cortical, cancellous and woven bone, with varying degrees of mineralization and vascularity.[1,25] However, only one previous study by our team has quantified the mineral apposition rate (MAR; i.e. bone growth rate) of ectopic bone. In a study conducted by Isaacson et al., ectopic bone grew approximately 1.7x faster than the known standard for non-pathological tissue in combat-injured services members who experience blast-related trauma.[25]To date, no study has investigated the MAR of ectopic bone that resulted from TJR procedures. Likewise, only two studies, both from our group, have characterized HO bone architecture using scanning electron microscopy (SEM)and backscattered electron imaging (BSE).[1,25]There is reason to believe that ectopic bone morphology and growth rates may differbetween patient populations given the differences in the induction mechanismand age of the patients, as not all HO processes are physiologically or histopathologically identical.[12,26,27]

Furthermore, when symptomatic HO occurs following TJR, determining a period to excise the symptomatic mass remains a critically unresolved issue. The general consensus is thatectopic bone should not be removed until the mass has fully matured as confirmed by radiographic evidence[18,28-30] and/or until patients have demonstrated normalized serum alkaline phosphatase (AP) levels.[27,26] While early resection has been generally promising, there is still uncertainty regarding surgical timing and recurrence,[31] with some reports indicating that premature removal results in nearly 100% recurrence rate.[32] To date, there remains a paucity of histological evidence to support clinical predictors for assessing HO.

In an effort to bridge the clinical and histological gap, our team planned a prospective case series of MHS patientsat Walter Reed National Military Medical Center treated for ectopic bone following total hip arthroplasty (THA) or total knee arthroplasty (TKA). The goals of this study were(1) to use advanced histological techniques to quantify the rate of ectopic bone growth (MAR) and (2) to compare HO bone morphology with a previous study by our team which investigatedwounded warriors injured in combat[25] to understand bone architecture differences and provide general clinical management recommendations for the military and general population.

METHODS

Patientstreated between the periods of June 2012 and March 2015 with symptomatic HOfollowing TJR were included in this institutional review board-approved study. Patient enrollment, recruitment and treatment adhered to Isaacson et al.'s protocol developed for wounded warriors.[25] In short, participants were identified by physician referral and once the determination was made that symptomatic HO required excision, subjectswere given oxytetracycline (250mg/tid) on four separate dates prior to their scheduled surgery to determine their MAR (i.e. bone growth rate). Dosing was slightly variable per the participant's clinical schedule, but typically consisted of a two day dosing period, six day hiatus, two day dosing period, and two day washout, followed by surgical excision. Surgery date was determined per the referring surgeon's clinical judgment and was not influenced by oxytetracycline dosing schedules. Patient radiographic data was blinded and reviewed by an attending orthopedic surgeon (BKP) to

assess HO severity prior to resection as 0%, none; < 25%, mild; 25%-50%, moderate; > 50%, severe.[33]

Following HO excision, samples were deidentified, photographed, radiographed, fixed in formalin, dehydrated in ascending grades of ethanol and embedded in polymethylmethacrylate according to standard laboratory procedures.[34,35] Samples were analyzed for MAR, SEM and light microscopy as previously described by Isaacson et al.[25]Demographic information was captured for each participant using the local electronic medical record systems and included gender, age, date of initial and excision surgeries, height, weight and HO anatomical location.

RESULTS

Threepatientswere enrolled in this research study and met the inclusion criteria and experiencedsymptomatic HO following TJR (two cases of TKA and one of THA).Two subjects were male and one was female, 69.0±7.8 inches, 237.7±28.3 pounds and61±7 years of age at the time of HO excision. HO was graded as mild in all cases, but required removal due to limited range of motion and pain. HO was mature and in a quiescent state at the time of surgical resection based on SEM and light microscopy.Clinical and radiographic evidence demonstrated no sign of recurrence three months post-operatively in all patients.

Histological data indicated that MAR levels were 1.7 times higher than non-pathological human bone at the time of surgical intervention (1.7±0.7 μm/day, range: 1.3-2.6 μm/day) compared to the known 1.0μm/day.[36] The amount of single and double labeled

calculated during flurochrome labeling were 2.0±2.0 and 1.3±0.6 respectively; these values were determined to be significantly lower than ectopic bone observed in our previous study of wounded warriors (as confirmed by an independent samples t-test after verifying homogeneity of variance ($p<0.0001$)). SEM and light microscopic images showed HO to have a trabecular structure with bone chips (Figures 1-4).

DISCUSSION

The HO bone specimens analyzed in our patients following TJR consisted of mature trabecular bone with a meanMAR of 1.7μm/day. This bone growth rate was the same as the wounded warrior segment previously studied by our team; however, one striking difference was thetype of bone observed.More specifically, the HO samples investigated following blast-related injuries in wounded warriors consisted of cortical, cancellous and woven bone with an average of 270±280single and 365±371double labels.[25]The veterans observed in this study only demonstrated trabecular bone and single and double labels were significantly lower at 2.0±2.0 and 1.3±0.6 respectively ($p<0.0001$). These differences may beattributed to severalfactors that include the differences in subject's age (young vs. elderly), traumatic insult (trauma vs. post-operative complication) and prophylactic treatments (used in younger wounded warriors but not in elderly TJR patients). However, we believe that the most likely driver for the differences in bone types is the trauma mechanism. Improvised explosive devices (IEDs)used in theater causeextensive polytrauma, which has the potential todisplacemicroscopic bone in the localized area in addition to instigating a dysplastic progenitor cell healing response. Although the limb is debrided prior to surgical intervention, extensive bone injury

following an IED, disrupts the tightly coupled process between osteogenesis and angiogenesis, and sets off a cascade of potent factors which includes, hypoxia inducible factor alpha (HIF), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGFβ), and fibroblast growth factor (FGF).[3]Remaining microscopic bone fragments may be a catalyst for developing HO when the localized microenvironment is alkaline and conducive for osseous growth.

The fact that the MAR levels of the TJR patients were the same as the wounded warriors (1.7μm/day)also representsa unique and previously unreported finding. Because data herein is only a case study it may be possible that the mean HO growth is approximately 1.7 μm/day across all diseases states, or this similarity may be due to differences in vasculature between cancellous and cortical bone. As noted above, wounded warriors demonstrated a hybrid of cortical, cancellous and woven bone,[25] whereas the veterans in this study only demonstrated trabecular bone. Trabecular bone porosity is reported to be between 50-95%,[37] whereas Haversian canals which provide perfusion to cortical bone comprise only 5-10% of the cross sectional area.[37]Given the differences in bone types, volume and anatomical location where HO developed (in the wounded warriors it was mainly located in the musculature adjacent to long bonesas compared to the periarticular region for veterans), samples tended to be larger for those with combat injuries.

Limitations

Although this study provides unique insights regarding the MAR of ectopic bone following TJR, the principle limitation is that the small sample size, which does not allow

for robust statistical analysis. In order to fully understand ectopic bone architecture and MAR, a larger, more highly powered study is required.

CONCLUSION

TJR is valuable surgical alternative for persons with limited range of motion or pain due to degenerative, inflammatory, or post-traumatic arthrosis. This problem is further compounded by the fact that there is limited association between histology and clinical predictors for deciding when surgical intervention is appropriate. Data from this study demonstrates that HO grew approximately 1.7x faster than non-pathological human bone, and the type of HO bone may vary based on the patient population and injury mechanism. Additional work is necessary to further elucidate HO pathophysiology and the relationships between clinical criteria, histology/MAR, and HO recurrence.

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FIGURES LEGENDS

Figure 1: (A) BSE micrograph from Patient 1's resectedHO. Note the dark grey areas (yellow arrows) of bone that indicate recently remodeling activity. (B) BSE micrograph showing a cluster of phosphorus (white) particles in the outer region of the specimen, which likely occurred during the removal since no foreign body responses were noted during light microscopy.Note:Grey=Bone and Black=Pore Space and Soft Tissue.

Figure 2: BSE micrograph from Patient 2 HO. Note the bone resorption (yellow arrows) at the outer boundaries and bone fragment (red arrow) within the ectopic bone.

Figure 3: BSE micrograph from Patient 3 showing a trabecular like bone structure of HO. Note the unincorporated bone chip (yellow arrows) and (b) bone chip (red arrow) that has been incorporated by new bone growth.

Figure 4: Light microscopy image from Patient 3 demonstrating that HO was (A) in a quiescent state (yellow arrows) and (B) bone chips that were observed (red arrow). Note: Pink=Bone, Blue=Tissue/Cells & White=Pore Space.

Figure 1

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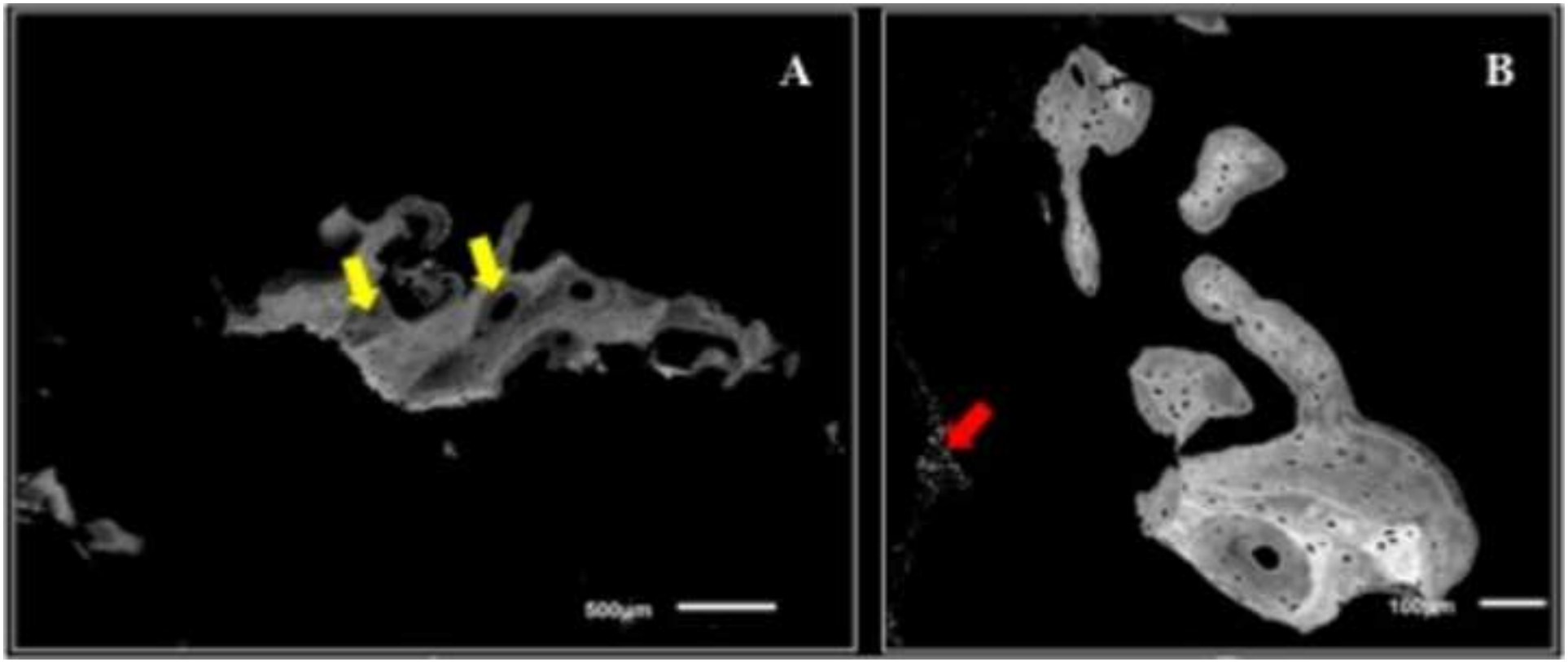

Figure 2

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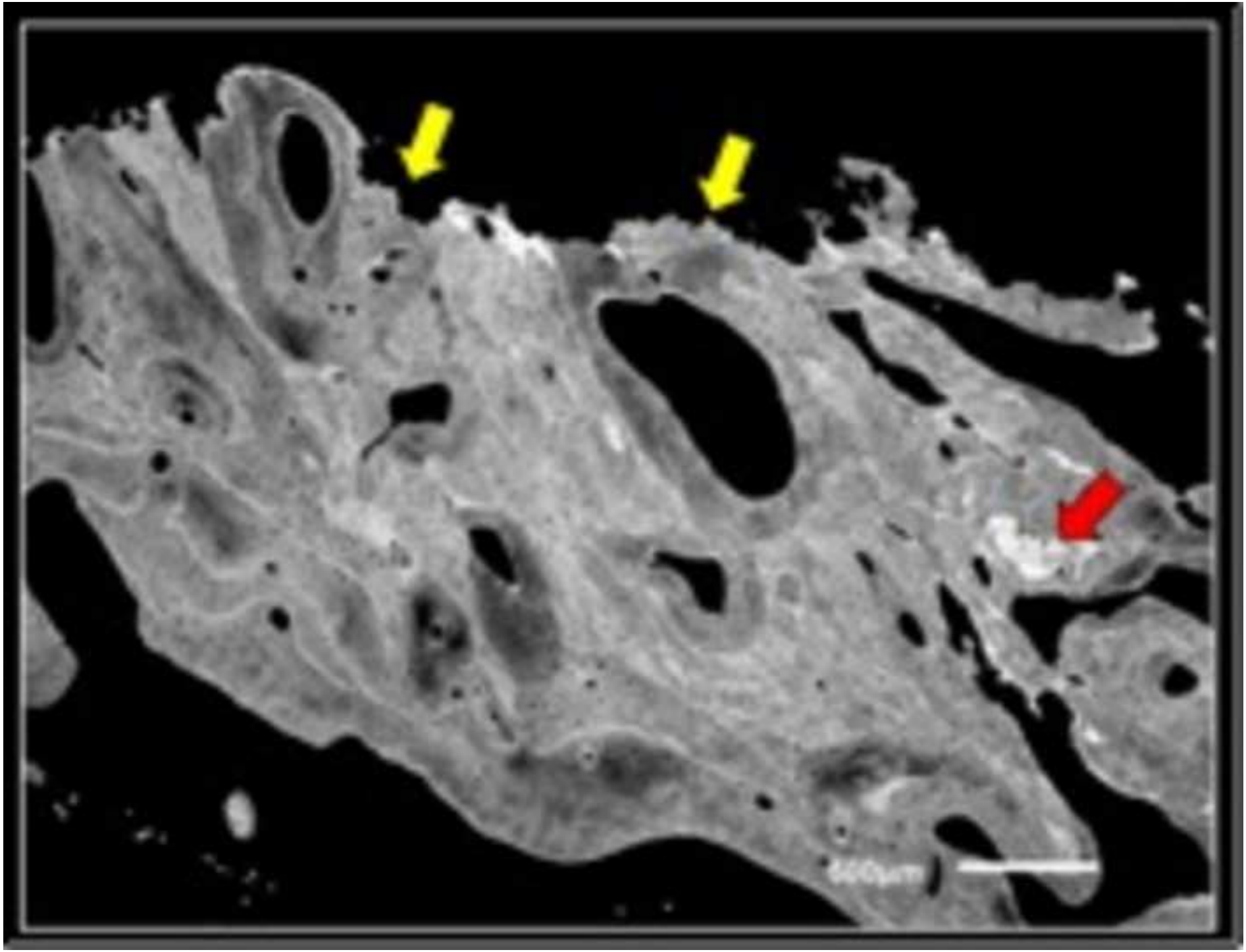

Figure 3

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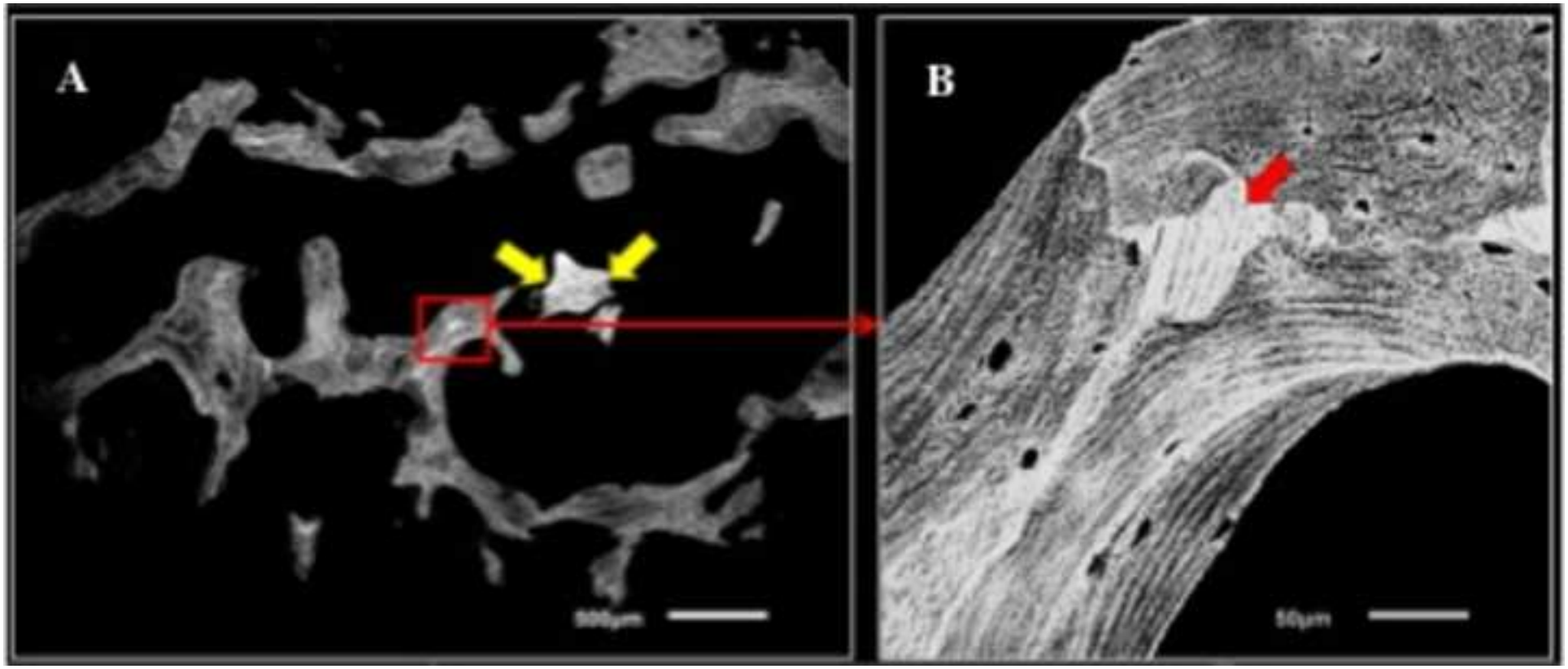

Figure 4

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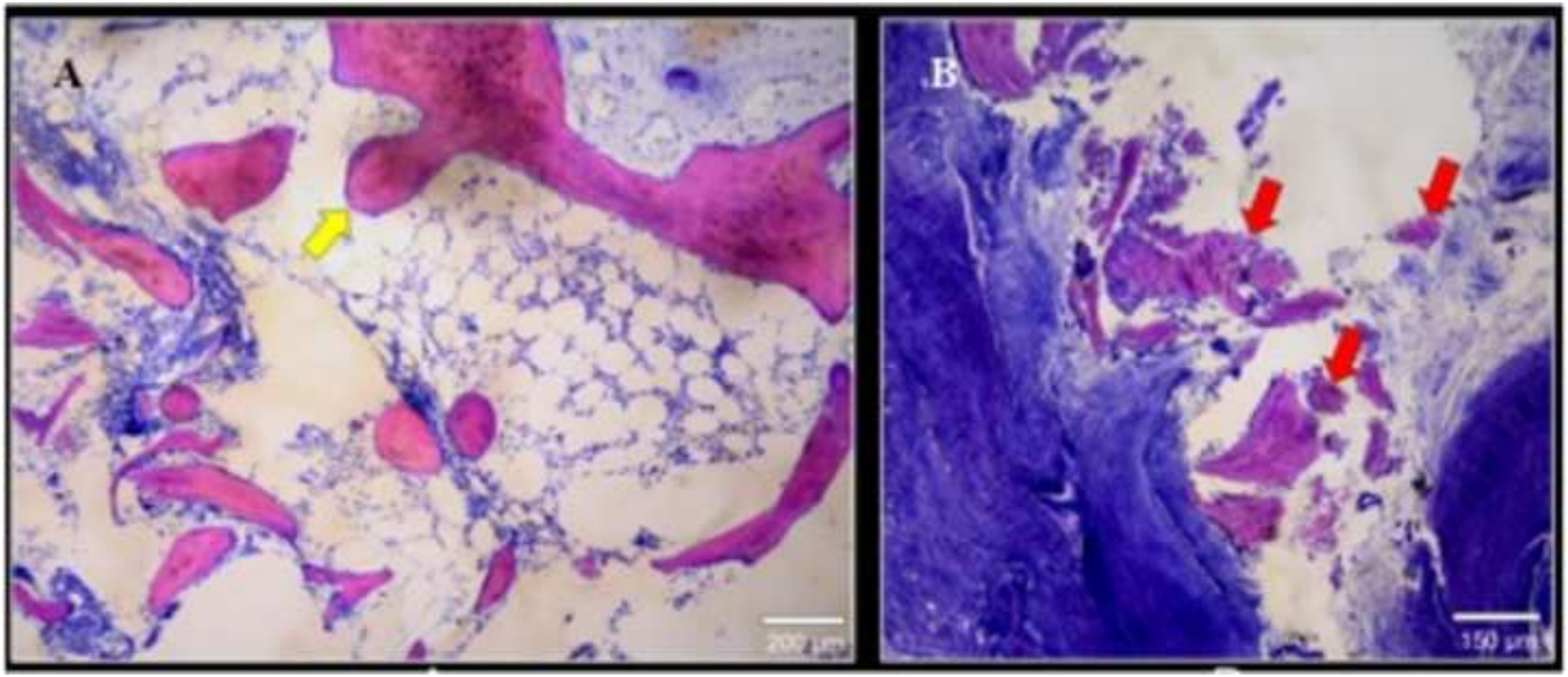

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PERSPECTIVES

Tourniquet use in combat-injured service members: a link with heterotopic ossification?

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Abstract: Tourniquet use during Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) has contributed to the high survival rate of combat-injured service members. While preservation of a life – even at the potential expense of a limb – should always take precedence, delayed perfusion in traumatized residual limbs may alter the proliferation, differentiation, and function of endothelial and osteoprogenitor cells. Given the synergistic relationship between angiogenesis and osteogenesis, and the influence of environmental conditions on bone formation, hypoxic conditions from tourniquets may in part explain the higher frequency of heterotopic ossification (HO) present during OIF/OEF. Determining a correlation between tourniquet usage/duration on subsequent HO formation remains challenging. Long-term retrospective investigations have been limited, since the United States Army's Institute of Surgical Research did not standardized tourniquet issuance until July 2004. Thus, associating tourniquet-induced HO in previous military conflicts is not feasible, since poor medical documentation and inadequate application of these medical devices prevent large-scale meta-analyses. Therefore, this article focuses on the basics of bone biology and how tourniquet usage following combat trauma may impact osteogenesis, and subsequently, ectopic bone formation.

Keywords: heterotopic ossification, osteogenesis, combat, trauma, ectopic bone, osteoprogenitor cells, Operation Iraqi Freedom, Operation Enduring Freedom

Introduction

Medical advancements in Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) have led to a 92% survival rate of combat-injured service members, a higher proportion than previous military conflicts.[1] Congressional databases have indicated that, as of December 2013, returning US military combatants have sustained 1,558 major limb amputations (762 from OEF and 796 from OIF/Operation New Dawn [OND]).[2] Utilizing tourniquets for controlling hemorrhaging on the battlefield has contributed to this progression, as 70% of injuries sustained in OIF and OEF have been to the musculoskeletal system,[3] with uncontrolled bleeding accounting for 50% of all combat fatalities.[4] While preservation of a life – even at the potential expense of a limb – has always taken and should always take precedence,[5] concerns about gangrene and neuromuscular damage from prolonged vascular occlusion prevented widespread acceptance of tourniquets during World War I, World War II, and the Vietnam War.[5,6] However, retrospective reviews from these military conflicts have noted that approximately 7%–9% of battlefield deaths may have been prevented with tourniquet usage.[4,5] This statistic coupled with the rapid evacuation strategies in OIF/OEF and data from the Israeli Defense Forces

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Medical Corps have demonstrated the effectiveness of tourniquets for preventing exsanguinations in the military pre-hospital setting.[7] As such, each US service member is now equipped with a tourniquet.

When a tourniquet is appropriately applied and inflated to 300–400 mmHg in theater, all arterial bleeding within the extremity ceases[8] and hypoxemic conditions remain until surgical intervention is possible. In some instances, postoperative wound hypoxia may persist upon tourniquet release, due to vasoconstriction and distal microvasculature blockage from cellular debris.[8] Delayed perfusion in traumatized residual limbs may exacerbate damage to the underlying endothelial cells and initiate a cascade of potent mitogenic factors known to control the proliferation, differentiation, and function of osteoprogenitor cells.[9] Given the synergistic relationship between angiogenesis and osteogenesis,[10] and the influence of environmental conditions on bone formation,[9] hypoxic conditions from tourniquets may in part explain the high frequency of heterotopic ossification (HO) seen during OIF/OEF.

HO is a pathological process characterized by ectopic bone growth in musculature and/or periarticular regions and frequently manifests following tissue trauma, traumatic limb amputation, and brain/spinal-cord injury[11–16] (Figure 1). The occurrence of HO following combat-related injury has been reported in the US military medical literature since the Civil War[17] and World War I;[18] however, OEF and OIF have been unique. The peer-reviewed literature has indicated that approximately 63%–65% of wounded service members with traumatic combat-related limb loss have experienced HO during OIF/OEF,[11,19,20] a much higher frequency than in other military conflicts. Ectopic bone growth has been significantly correlated with the trauma mechanism, zone of injury, and postoperative negative-pressure treatment,[21] but, to the author's knowledge, to date, no studies have examined the potential link between tourniquet use/duration and the potential manifestation of ectopic bone formation in combat-injured service members.

Assessing hypoxia-induced HO from tourniquet usage remains challenging, as it was not until July 2004 that the United States Army's Institute of Surgical Research issued a recommendation that every soldier carry a modern tourniquet.[22] Additionally, correlating tourniquet-induced HO in previous military conflicts is not feasible, since poor medical documentation and inadequate application of these medical devices prevent large-scale meta-analyses.[5] Therefore, this article focuses on the basics of bone biology and how tourniquet usage following combat trauma may impact osteogenesis, and subsequently, ectopic bone formation.

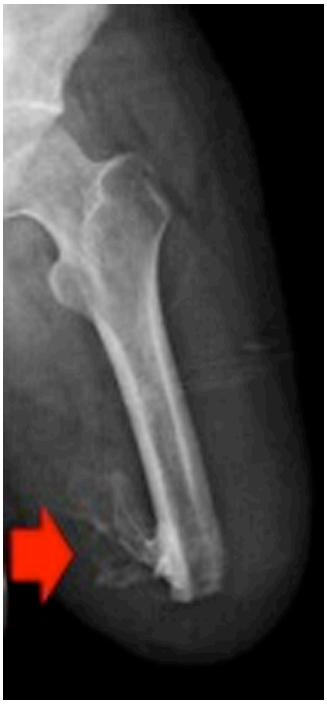

Figure 1 Radiographic image demonstrating heterotopic ossification within the residual limb of a combat-injured service member.

Narrative

Research has indicated that endothelial cells, which play a key role in angiogenesis, are of critical importance for bone formation, repair, and HO development.[23] Impairment to the underlying endothelial cells from an oxygen-deprived microenvironment (as is the case with tourniquet usage) sets off a cascade of events once hypoxia-inducible factor-1 alpha (HIF-1α), an oxygen-sensitive proteolytic mechanism, becomes stabilized.[10] HIF-1α translocation from the cytoplasm to nucleus may initiate chemotactic agents, including upregulation of vascular endothelial growth factor (VEGF), transforming growth factor beta, fibroblast growth factor, and glucose transporters.[24] In fact, overexpression of HIF-1α through the selective deletion of the *Von Hippel–Lindau* gene in a small-animal model has demonstrated a significant increase in VEGF, which resulted in extremely dense, well-vascularized bones.[24]

Elevations in HIF-1α and VEGF have been noted to directly influence blood-vessel invasion into ossification centers and are key to chondrocyte survival.[25] For instance, HIF-1α levels impact chondrocyte proliferation at the epiphyseal plate,[10] an anatomical location renowned for having an alkaline pH and decreased oxygen content[26] during skeletal

growth and development. Decreased oxygen content and hypoxic conditions have also been linked with increases in alkaline phosphatase (AP) levels[27] and HIF-2α, which may regulate RunX2, a master osteoblast factor necessary for differentiation.[10] These observations may in part explain the rise in tourniquet-induced hypoxic HO formation, as Isaacson et al previously noted that ectopic bone formation occurred from endochondral ossification (Figure 2).[13]

The hypoxic conditions that transpire following tourniquet usage may also influence mesenchymal stem cell (MSC) function. In vitro experimentation with bone-marrow stromal cells has shown that MSC differentiation accelerated threefold in hypoxic versus normoxic conditions;[27] while a separate study also indicated that a deoxygenated environment increases bone morphogenetic protein 2 messenger RNA expression 2.5-fold after only 2 hours.[9] The importance of MSC function and its link with HO occurrence has previously been identified in war-traumatized muscle tissue.[28] In a study by Nesti et al, debrided muscle from soldiers sustaining traumatic open extremity injuries were harvested, enriched, expanded in culture, and exposed to induction media for osteogenesis, chondrogenesis, and adipogenesis.[28] Genetic markers in the traumatized tissue demonstrated the potential of these cells to differentiate into multiple mesenchymal lineages.[28] War-traumatized muscle tissue also exhibited a significant increase in AP activity, production of a mineralized bone matrix, and upregulation of osteoblast-associated gene *CBFA1*,[29] all of which are correlative factors for HO. Therefore, disruption of homeostatic MSC processes following tourniquet use may adversely mediate MSC differentiation through a similar mechanism as seen in war-traumatized soft tissues, with the mutual malefactor being extended peripheral hypoxia as a result of inadequate blood circulation.

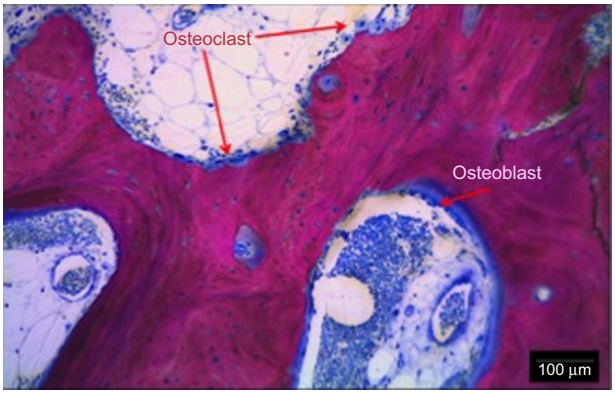

Figure 2 Heterotopic ossification bone sectioned, stained, and analyzed using light microscopy. Note the distinct regions of bone formation and resorption due to osteoblasts and osteoclasts.

Hypoxic environments and endothelial cell damage have been previously linked with ossification in two murine HO models; however, there remains a knowledge gap and translational concern between small-animal models and the clinical condition. In one study by Lounev et al, researchers isolated vascular endothelial and smooth-muscle cells to determine their respective contribution to stages of ectopic bone development.[23] Cells expressing the vascular endothelial tissue-specific Tie2/Tek marker at some point in their development contributed heavily to the osteogenic stages of HO, while the role of smooth-muscle cell lineages was negligible.[23] The identification of endothelial-specific precursors suggests the tissue plays a critical role in the disease's progression and may be a cause for HO resulting from hypoxia-induced cellular damage.

Understanding the link between tourniquet use, persistent tissue hypoxia, and HO formation may improve the standard of care for wounded service members suffering combat-related amputations. Persistent tissue hypoxia remains a frequent problem in the management of open wounds, as low oxygenation has been demonstrated to have deleterious effects on wound closure rates, latency to resumption of an unperturbed blood flow, and may delay the final stages of healing.[30] Although there are several diagnostic measures available to determine the rate of hypoxia in localized tissue, none of the methods is without its faults and requires strict adherence to protocols to collect reliable data. Direct measurement, albeit the most accurate method, is generally avoided due to its invasiveness.[31] Several imaging-derived techniques (positron emission tomography and magnetic resonance imaging scans) are primarily applicable to research rather than clinical applications due to their costliness and lengthy procedures; while other techniques such as duplex ultrasonography and arteriography are most helpful for mapping the revascularization of under-perfused tissue.[31,32] New techniques like near-infrared spectroscopy and blood oxygen level-dependent magnetic resonance imaging may provide noninvasive, precise, and time-effective means to determining tissue hypoxia, but their outputs still require fine-tuning by developers before they can become clinically relevant diagnostic tools.[31,33]

Future directions

While this narrative provides plausible rationales as to why hypoxic environments, such as is the case with tourniquets, may be associated with the higher incidences of HO formation in our nation's service members during OIF/OEF/OND, further empirical evidence is required to fully understand the

etiology of HO. The next steps for assessing the link between tourniquet duration, tissue hypoxia, and HO formation may include conducting simulated blast-related ovine models (rather than small murine studies), since the mineral apposition rate of bone in these animals more closely matches that of human bone.[34] Improved diagnostic techniques are also required to better understand hypoxic tissue pathology independent of tourniquet use.[31,33] By further developing these preclinical models, researchers may begin to bridge the current knowledge gap in HO research.

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REVIEW

Osseointegration: a review of the fundamentals for assuring cementless skeletal fixation

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Abstract: Direct skeletal fixation, termed osseointegration, has expanded in the last century and includes use in total joint replacements, the edentulous mandible and maxilla, and percutaneous osseointegrated prosthetics. Although it is well known that titanium and bone have the ability to form a durable bone–implant interface, new applications have emerged in the field of orthopedics, which requires a more thorough assessment of the literature. This review aims to introduce the basic biological principles for attaining osseointegration and discusses the major factors for assuring successful cementless fixation.

Keywords: osseointegration, bone, skeletal attachment, total joint replacements, dental implants, percutaneous

Introduction to osseointegration

Surgical implantation of metals and ceramics has been used to restore function for individuals with diseased and compromised tissue for the past 200 years.[1] However, the success of direct skeletal attachment with metal substrates remained limited until Per-Ingvar Brånemark discovered the integration potential between titanium and bone.[2] Brånemark and his coworkers coined the term "osseointegration" (OI) to describe the ability of titanium to form a mechanical and functional interconnection with osseous tissue without the formation of interpositioned connective tissue.[3] The definition of OI has continued to evolve over the years given the advancement in imaging and microscopic tools available for assessing the bone–implant interface (Table 1). Current descriptions of OI include the need of the periprosthetic bone to resist shear and tensile forces[4] and to be within 50 μm distance from the implant surface to host bone to prevent fibrous tissue attachment.[5]

Since the initial scientific discovery by Brånemark and his colleagues, fixation of metallic and nonmetallic implants to bone has increased exponentially in the fields of dentistry and orthopedics. OI has been used as a means to fix dental implants, bone-anchored hearing aids, spinal fusion implants, and endo-exo prostheses. Clinical follow up of oral, craniofacial, and cementless total joint replacements (TJR) has reported long-term clinical success rates with high implant survivorship.[6–17] The principle factors for achieving direct skeletal fixation have been reported to include: the implant surface properties; quality of the host bone; surgical site preparation; loading conditions; implant design; and preventing initial and chronic infections. These factors are reported within this review, with the goal of improving the current understanding of OI and spurring future innovation in this field.

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Table 1 Advantages and disadvantages of various testing modalities

Testing modality	Advantage	Disadvantage	References
Light microscopy	Inexpensive technique	Does not provide sufficient detail at the interfacial zone because the resolution capacity is only 0.1 mm	143
Microcomputed tomography (μ-CT)	Provides three-dimensional images of the bone–implant construct	Image artifacts occur due to the opaque nature of the titanium-based implant	144–148
Resonance frequency	A nondestructive technique shown to correlate with mechanical removal forces and bone ingrowth or ongrowth	Implant stability quotient values do not provide sufficient detail of host bone–implant integration	149–154
Backscatter electron imaging	High resolution	Expensive technique	155,156

Bone biology and osseointegration

The implant surface

Various metals, ceramics, and biostable polymers have been used to achieve OI. The major metal types have included: cobalt chromium,[18–20] tantalum,[21] stainless steel,[19,20,22] zirconium,[23,24] and commercial pure titanium and its alloys.[19,20,22] However, titanium has been widely advocated as the most biocompatible material for promoting OI, due to its excellent mechanical properties,[25] resistance to corrosion,[25,26] and its ability to develop an oxide layer on the surface (comprised of a dioxide chemical structure, TiO_2).[27,28] Most interestingly, this oxide layer thickness has been noted to be dynamic, ranging between 1,000–2,000 Å at 7 years postoperative follow up – much higher than the initial measurement of 60–100 Å reported at the time of implantation.[4,21] The ability for bone to both mechanically and chemically bind to the surface of titanium has been known to facilitate durable OI and long-term implant survivorship (Figure 1).

Roughness, porosity, topography, and surface energy all contribute to the host response to a titanium implant placed in apposition with cortical and/or cancellous bone.[29,30] While a complete review of each of these topics is not within the scope of this paper, some brief generalizations regarding the material surface are worth noting. It is well observed that the implant surface morphology directly influences osteoblast and osteoclast attachment and metabolism.[31] Skeletal fixation is most effective when using porous implants (50–400 μm)[32] with roughened surfaces, where ingrowth and interdigitation of the newly formed bone into the porous structure stabilizes the interface (Figure 2). As stated by Boyan et al, implant surfaces should have a 4–7 μm layer of roughness to ensure proper osteoblast cuboid morphology,[33] an essential characteristic for assuring OI. Osteoblasts seated on roughened surfaces have demonstrated increased proliferation, and previous in vivo animal models have reported that the textured surfaces required higher removal torques compared with smooth controls.[29]

The implant surface is a key factor in direct skeletal fixation, with implant survivorship dependent upon the specific device design and anatomical location for OI. Given the differences in mechanical loading conditions, vascular integrity, host bone quality (bone mineral density [BMD] and bone mineral content [BMC]), and bone type (cortical vs cancellous), surface properties may in future be tailor-made for each unique application (Figure 3). While in general, smooth implants do not have a microtexture conducive for osteoconduction, Balshe et al noted, when comparing 2,182 smooth-surface dental implants and 2,425 roughened implants postoperatively, that survival rates were 94.0% and

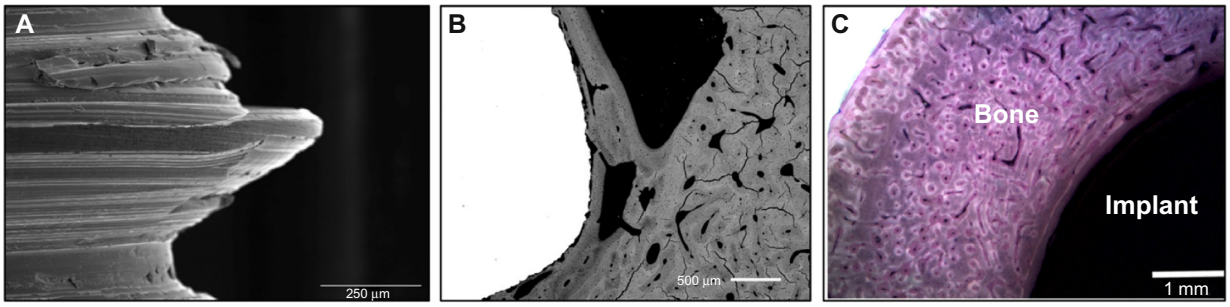

Figure 1 (**A**) Representative scanning electron microscope image demonstrating high resolution along the screw threads of an implant used for osseointegration. (**B**) BSE micrograph of bone–implant cross section, clearly depicting the bone on-growth (gray) onto the implant (white) within 50 μm. (**C**) Bone–implant cross-section stained with Sanderson's Rapid BoneStain™ and counter stained with acid fuchsin, showing bone and implant interconnection.
Note: Sanderson's Rapid Bone Stain™ (Surgipath Medical Industries, Richmond, IL, USA).
Abbreviation: BSE, back-scattering electron.

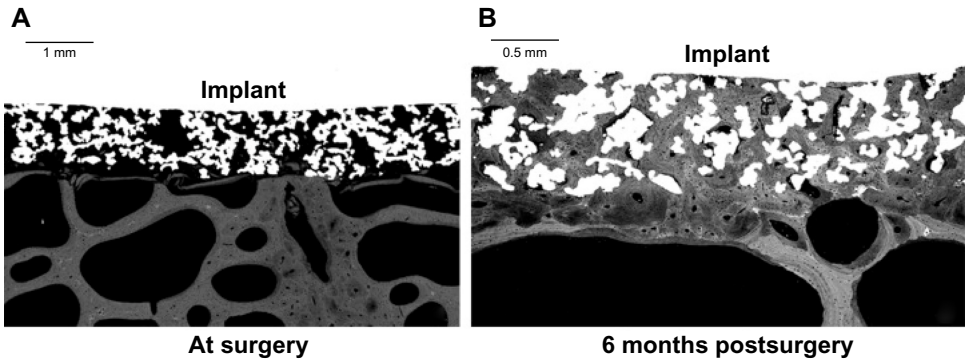

Figure 2 A representative set of BSE micrographs showing the ingrowth and interdigitation of new bone tissue into the porous, coated region at 6 months postsurgery (**B**) compared with time 0 (at surgery), when the implant was placed in close apposition with the host bone (**A**). The image shows porous coating (white), bone (gray), and marrow cellular components (black).
Abbreviation: BSE, back-scattering electron.

94.5%, respectively.[34] However, Balshe et al reported that the implant length and anatomic location were significant predictors for smooth implant failure[34] and that surface properties may be overridden when implants were placed at a sufficient depth within the osseous tissue. Pak et al[35] supported the potential for smooth implant attachment and noted, in their histomorphometric studies of dental implants with three separate surface treatments (commercially pure titanium, tricalcium phosphate, and anodic corrosion), that there were no differences in bone–implant contact or localized bone volume density at 3 and 6 weeks, respectively, thereby signifying the importance of proper implant "fit and fill."

Quality of the host bone

Biological fixation between a titanium implant and host bone depends upon the quality and architecture of the supporting bone used in the OI procedure.[36] The human skeleton is comprised of approximately 80% cortical bone and 20% cancellous bone; however, the ratio between these bone types varies greatly between anatomical locations. For instance, the cortical to cancellous bone ratio of the vertebra is 25:75, compared with 50:50 in the femoral head and 95:5 in the radial diaphysis.[37] Given that cortical bone is typically less metabolically active than trabecular bone,[37] the placement of an orthopedic implant is critical for long-term success. Also, bone formation at the periprosthetic interface has shown to be a slow but a dynamic and tightly coupled process[38] coordinated between cells,[39] hormones,[40] and enzymes.[38] Modeling and remodeling of bone tissue around an OI implant results from complex chemical interactions and mechanical stimuli.

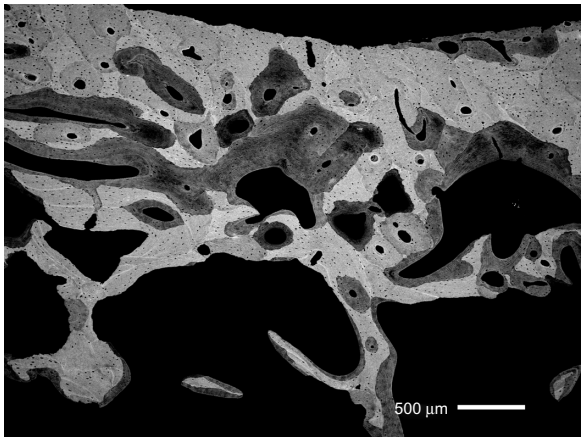

Figure 3 BSE micrograph demonstrating the wide range in bone mineralization levels during remodeling.
Abbreviation: BSE, back-scattering electron.

It has been largely accepted that bone adapts to mechanical loads in accordance with Wolff's law.[41] The functional adaption of bone, most studied in the proximal femur, demonstrates the unique ability of bone to alter its trabecular orientation as a result of loading conditions.[42] Bone biologist, Harold Frost also described the transformation of bone as a strain-driven event.[43] Frost hypothesized that a "minimal effective strain" was required to maintain bone architecture[43] and that physiologic bone strains rarely exceeded 3% in vivo.[44] In the absence of the minimum effective strain, bone volume will be reduced (as was the case with early astronauts who went into space). Moreover, loss of crestal bone may also result from highly localized stresses that induce microfractures. Thus, in order to maintain a healthy host bone volume and to preserve bone tissue, dental and orthopedic implants should permit adequate mechanical stimulation to the surrounding skeletal tissue.

A complete review of bone biology and the mechanical effects on bone formation has been reported in the literature previously.[45,46] However, it should be noted that both BMC and BMD significantly impact the durability of OI by altering cell proliferation and protein synthesis.[47] Minor increases in

bone mineralization exponentially increase the modulus of elasticity of bone[48,49] and subsequently, the durability of the bone–implant construct. However, there is a known inverse relationship between bone stiffness and fracture toughness, so minor decreases in BMC may allow the host bone to absorb higher energy prior to deformation.[49] This balance in BMC may affect the longevity of OI implant survivorship, as highly mineralized bones may fracture due to their inability to absorb the kinetic energy[49] – which may occur from an abrupt fall, to a patient with an OI implant.

In the case of OI within long bones, cortical bone porosity ranges between 5%–10% in skeletally mature individuals, while the porosity of cancellous bone varies between 50%–95%.[39] The increased pore space of cancellous bone results in an approximate three- to eightfold reduced bone density compared with cortical bone[44] and explains the 30-fold reduction in strength and stiffness between the two bone types.[44] Aside from the biomechanical advantage of cortical bone, Charnley also noted that cancellous bone does not have a periosteum along the surface of the trabeculae,[50] thus contributing to one of the known metabolic differences between cortical and cancellous bone remodeling.[5,51,52] Moreover, cancellous bone heals in an appositional manner, with very little callus formation (<1%), which significantly differs from the healing patterns/cascades of fractured cortical bone; this would affect bone remodeling if accidental trauma occurred to the site where an osseointegrated implant was placed.

Surgical site preparation/implant stability

While proper instrumentation and operative techniques help to minimize disturbance to the localized vascular network during OI procedures, uncontrolled thermal or mechanical factors (reaming, rasping, or drilling) used to ensure proper implant "fit and fill" or fixation may damage the host bone's ability to remodel.[53,54] Insertion of an orthopedic implant into the host bone results in a localized region of necrotic tissue.[55] While it has been generally agreed upon that this amount of necrotic bone should be reduced during the initial implantation, Albrektsson et al have speculated that a minor region of dead bone may act as an early implant stabilizer during the preliminary phase of bone remodeling[21] and may even be beneficial for anchoring osseointegrated implants in situ. In order to prevent premature implant failure, primary implant stability must occur immediately[56] to eliminate micromotion at the bone–implant site[57] and to also prevent fibrous tissue formation.[58] Gaps in excess of 50–150 μm between the implant surface texture and host bone may lead to fibrous tissue without skeletal attachment.[5,59,60]

To improve the likelihood for dental implant survivorship, novel techniques have been developed that use computed tomography scans from the patient's mouth, and computer-aided design.[61] Advanced implant planning in a virtual environment may improve the accuracy of dental implant fabrication and provide patient-specific replicas for surgery. In fact, a study performed by Valente et al, using computer-aided oral surgery in a series of 25 patients resulted in a 96% implant survivorship, with mean deviations being less than 2 mm in any direction[62] – thereby demonstrating the usefulness of this technique for positioning and for selecting an appropriate implant size.

Trauma to the host bone tissue during surgery may also accelerate local bone turnover.[63–65] This has been termed the "regional acceleratory phenomenon" (RAP), which was first defined by Frost, using noxious stimuli, and then by Bloebaum et al.[64,66,67] The RAP may occur for two reasons: the first being that placement of an intramedullary OI implant alters the dynamic strains to the host bone tissue. Depending on the "fit and fill," the implant may result in high concentrations of localized stress or "stress shielding;"[66,68,69] second, the surgical procedure itself disrupts the blood supply to the endosteal wall (which results in a local tissue response to reestablish bone vascularity) – thus causing an increase in cortical bone porosity.[70,71] This increased vascular network is optimal for bone remodeling but will impact overall strength. Knowledge of the RAP is vital for the success of OI implants. In dentistry, increasing the severity of the RAP has been reported to accelerate the rate of orthodontic tooth movement.[72]

Loading conditions

One challenge with cementless fixation has been preventing micromotion during the early phases of healing and allowing the bone to form a strong skeletal interlock;[21,73] if this is not achieved, a fibrous tissue interface (Figure 4) may form and prevent OI.[74–77] As noted above, limiting the initial forces on an OI implant has been based on the principle that stress must be exerted gradually to promote firm skeletal attachment since under- or overloading may compromise the integrity of the host bone. To prevent mechanical loosening at the bone–implant construct, OI procedures for dental applications initially have required periods of restricted load-bearing, to avert overloading.[78–87] However, the dental and TJR literature now indicates that immediate implant loading may not compromise the integrity of the bone–implant interface or prevent OI if micromotion is controlled with properly designed implants.[73,80–82,86–90] However, key design elements must be

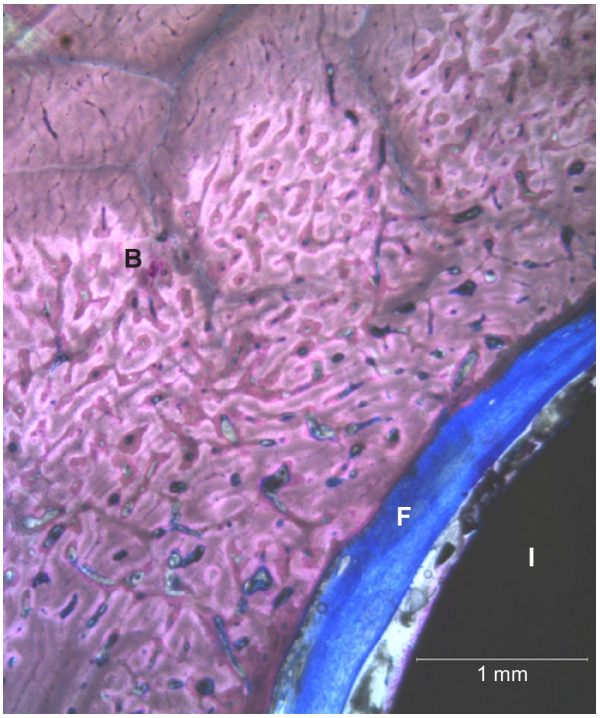

Figure 4 A representative bone–implant cross section that was stained with Sanderson's Rapid BoneStain™ showing the interpositioned fibrous capsule (**F**) between the implant (**I**) and the host bone tissue (**B**).
Note: Sanderson's Rapid Bone Stain™ (Surgipath Medical Industries, Richmond, IL, USA).

considered and include the implant neck design, screw shape, abutment design, etc during the oral implant design.

Most importantly, a delayed weight-bearing protocol deviates from the TJR paradigm, in which patients with total knee arthroplasty (TKA) or total hip arthroplasty (THA) bear loads within hours of the procedure. Literature further indicates that immediate load-bearing may occur without compromising skeletal attachment.[91,92] Implant-retrieval studies have further demonstrated that early load-bearing may be permitted if careful operative protocols and implant designs with optimal porous coatings are used.[52,76,77]

Since the time when delayed loading for dental and orthopedic implants was first introduced, several authors have evaluated immediate loading and found high success rates that are comparable with or better than short-term protocols that require a "nondisturbed healing period."[93–97] Degidi and Piattelli studied the clinical prognosis of 646 immediately loaded dental implants placed in 152 patients and found only six failure cases within the first 6-month period.[98] Additionally, recent studies by Jeyapalina et al confirmed that when an immediate-loading protocol was used with percutaneous OI implants placed within the intramedullary canal, there were no signs of implant loosening postoperatively for up to 1 year.[63,99–101] The appositional bone index, calculated at predetermined time points, demonstrated progressive bone interconnection and further validated the importance of "fit and fill" (Figure 5). These findings provide further evidence for an immediate implant loading once primary implant stability has been achieved.

Implant design

Novel designs for orthopedic implants have recently been developed using finite element analysis as a prerequisite. Hansson[102,103] used computational modeling and finite element analysis of the femoral neck to reduce the peak

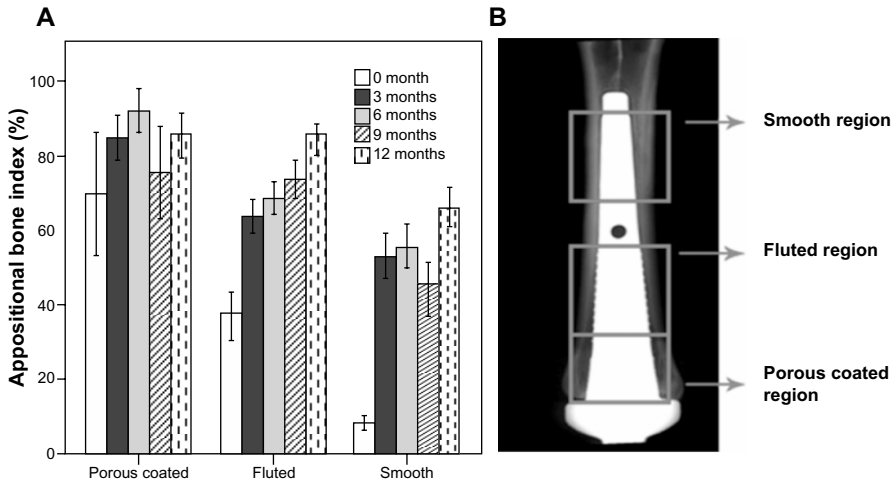

Figure 5 (**A**) ABI values at the time of the surgery (time 0) and at 3, 6, 9, and 12 months postsurgery. Data was obtained from a translational animal model, where sheep were implanted with a percutaneous OI implant in their fused right metacarpal III, IV bone. Statistically significant differences were found, between time 0 and all other time points, of fluted and smooth regions. (**B**) A radiographic image of the intramedullary implant, schematically showing the regions used for the ABI measurements.
Notes: ABI values are expressed as %. The error bars indicate 95% CI ($P<0.05$).
Abbreviations: ABI, average appositional bone index; CI, confidence interval; OI, osseointegration.

interfacial shear forces and promote axial load transfer over a greater area of peri-implant bone interfaces. Furthermore, tapered implants using this design approach and microtextured surface features, such as a porous coating, may provide more effective force dissipation over a greater bone volume – thus improving the likelihood of successful OI.[12] For instance, follow up studies of the Zweymüller® hip implant system (Zimmer Holdings, Inc., Warsaw, IN, USA) have demonstrated no stem revisions and exceptionally high implant survivorship using a tapered design.[104,105]

Preventing and treating initial and delayed infections

Although most of the OI procedures performed in controlled sterile clinical settings are successful, implant failures have been reported and may require revision surgery. The three primary reasons for OI implant revisions are due to 1) osteolysis and related aseptic implant loosening; 2) mechanical failures due to lack of OI; and/or 3) infection.[106,107] A discussion of infection is as follows.

Total joint replacement

Implant-related infection is one of the challenging obstacles to THA and TKA. It has been reported that 0.8%–1.9% of TKAs and 0.3%–1.7% of primary THAs fail due to infection, aseptic loosening, dislocation, or fracture.[108] In the case of infection, the most common conventional therapy is antibiotics. However, if antibiotic therapies are unsuccessful, then the implant is often removed and reimplanted in a revision surgery. However during the revision surgery, the risk of infection is increased and has been reported to be as high as 10%[109] (this is because the dermal barrier is broken once more, allowing bacteria to reach the surgical site). In some instances the pathogen may include methicillin-resistant *Staphylococcus aureus*, which has high patient morbidity and mortality. One study by Mortazavi et al noted that 57% of the staphylococcal organisms cultured following deep infections after revision TKA were methicillin-resistant.[110] Further compounding this problem, these bacteria may establish biofilms (sessile communities), which are difficult to eradicate with conventional antibiotic therapy.[111,112] Since most chronic infections are attributed to biofilms, reoccurring deep tissue infection that cannot be managed by antibiotic therapy may require removal of all infected, devitalized, and foreign materials including the arthroplasty components. Often, the biofilm-forming bacteria may readhere to the implant if they are still present within the surrounding tissue. Therefore, in order for OI between the implant and host tissue to be successful, the revised implant must be placed in a sterile environment. To ensure sterility of the site, a two-stage reconstruction surgery is often considered, with local and systemic antibiotic treatments used in between the surgeries for cementless fixation.[113–115]

Dental OI implants

Bacterial colonization on dental implants may not lead to ultimate implant failure; however, prolonged exposures may generate host tissue inflammatory reactions, which slow OI progression. There are two major types of dental implant infection: peri-implant mucositis and peri-implantitis.[116] While peri-implant mucositis is defined as a reversible inflammatory reaction in soft tissues surrounding an OI dental implant, peri-implantitis is considered to be an inflammatory reaction with the loss of supporting bone surrounding an implant.[116,117] Pontoriero et al studied the clinical and microbiological response to the development of experimental gingivitis and experimental peri-implant mucositis and concluded that there were no significant differences found between them.[118] The treatment option for peri-implant mucositis largely is based upon the management of plaque control, where surface debridement constitutes the basic element for treatment.

Peri-implantitis has an overall incidence rate of 12%–43%.[119] If the early stages of peri-implantitis persist, implant–bone integration may be compromised, and subsequently, the implant will be lost. Presently, no single pathogen has been closely associated with infection of any implant system;[120] however, the microbial floras of failing implants have been associated with the pathogens of periodontitis.[120] Several reports cited that these implants were colonized with putative periodontal pathogens, including *Peptostreptococcus micros*, *Fusobacterium* spp., enteric gram-negative rods, and yeast.[120–123] Moreover, the frequency of peri-implantitis in patients with a history of periodontitis has been reported to be four- to fivefold higher than that of individuals with no histology of periodontitis,[124] thereby indicating a closer tie between both types of infections. A review of the treatment used for peri-implantitis has revealed that surgical removal of the lesion followed by cleaning of the affected implant with hydrogen peroxide, chlorhexidine, citric acid, tetracycline, lasers, etc, and a systemic antibiotic therapy are effective methods.[120,122,125–128]

Craniofacial OI implants

Given the reduced number of craniofacial implants performed annually, less data is available for scrutinizing bacterial colonization on these implants. However, clinical studies on the skin penetrating abutments in the temporal region show that infections are rare. As reported by Albrektsson et al, 96% of the cases of craniofacial implant had minimal

to no skin irritation.[129] When infections have occurred, they have often been mitigated by proper implant site hygiene. Topical applications of antibiotics have been used to control superficial infection, if present.

Percutaneous OI implants

Although over 200 percutaneous OI prostheses have been fit to European patients with limb loss,[130–132] there have been limited published reports on infection outcomes.[130,133–136] When an infection signal is present, these have been frequently treated with topical/systemic antibiotic treatment and cleaning of the device abutment. However, with deep infections, device removal becomes almost necessary. The clinical resolution of deep infections for these OI prosthetic systems resembles that of the two-stage treatment protocol used in TJR surgeries, where, the first-stage is the removal of the infected endoprosthetic components and insertion of temporary spacer with antibiotic treatment, followed by a second-stage operation to insert a new implant system.[130]

Although Gunterberg et al reported 75% superficial and 37.5% deep infections in his earlier patient population of 16 individuals,[134] their infection rate decreased to 37% and 18%, respectively over a 3-year study period after a standardized treatment protocol was introduced in 1999.[133] The suspected pathogens in these cases were reported to be *S. aureus*, coagulase-negative *Staphylococcus* spp., *Enterococcus faecalis*, and *Escherichia coli*.[133] The reported rate was also in agreement with the UK experience of the Brånemark OI system, which had deep infection rate of approximately 18% and in some cases, required implant removal.[137] In spite of the significant improvements – such as surgical techniques, implant design, material selection, and implant exit site hygiene – infection still remains a concern with this implant system. Bragdon et al reported approximately one infection per 2 patient-years with the OPRA (Osseointegrated Prostheses for the Rehabilitation of Amputees) implant system (Integrum AB, Mölndal, Sweden).[138]

A publication by Juhnke et al from Lübeck, Germany appears promising.[136] After initially having a high frequency of stomal-associated infections and revision surgery (70%), this team reduced infections to 0% in their final design iteration.[136] The researchers reported that the best infection prevention strategy is daily cleansing of the skin/implant stoma with water and a mild soap and gentle debridement of the detritus and biofilm from the interface using a shaving brush. Finally, the data from an ongoing UK clinical trial led by Dr. Blunn indicated a successful skin-to-implant integration when HA coating is used.[131] A recent personal communication with this group revealed a great clinical success of this implant type in 15 transfemoral amputees. One of these amputees has already climbed mount Kilimanjaro with his percutaneous OI device.

Conclusion

Titanium and its alloys have been used in orthopedic and dental applications for the past 200 years and have significantly improved functionality for patients. While novel surface treatments continue to be developed, the basic bone healing principles still remain pertinent for OI and skeletal attachment. The initial attachment at the bone–implant construct is a vital prerequisite for successful OI. Durable biological fixation relies heavily on implant design and sizing in order to limit micromotion. The long-term implant survivorship varies based on the anatomical location and mechanical loading conditions.

In order to achieve durable implant–bone contact, adequate implant surface characteristics (roughness, porosity, depth of pores, etc)[32] must be carefully designed to achieve skeletal fixation. Excessive micromotion between the implant and host bone will not have the structural integrity needed to withstand the dynamic shear/tensile/compressive forces occurring with load-bearing during ambulation.[139] While initial implant fixation is required to prevent micromotion and fibrous encapsulation,[5,29,59,74,79,82,140–142] the long-term success of OI implants requires firm skeletal attachment, which may take up to 3 to 9 months postoperatively in human cancellous bone.[79] Immediate full load-bearing in the postoperative period has several benefits, including a shorter hospital stay, lower hospitalization cost, and an earlier return to daily living.

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Disclosure

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Scientific Research

The 5 Hallmarks of Biomaterials Success: An Emphasis on Orthopaedics

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Abstract

Over the past 200 years, there has been significant advancements in the fields of bioengineering and orthopaedics. Investigators, clinicians and manufactures are learning that the success of implant systems is not limited to a single factor, but a combination of variables that must work in unison to provide stability and high survivorship. Innovations continue to advance these fields and include: biomimetic alterations, three-dimensional, patient-specific reconstructions and novel coatings to mitigate aseptic loosening or other pathologies. However, implant systems continue to fail in clinical practice since they do not adhere to key fundamental principles. Therefore, this article is intended to highlight 5 hallmarks of biomaterials that should be considered during design, surgery, and post-operative rehabilitation.

Keywords

Biomaterials; Orthopaedics; Implant; Bone; Biofilm

1. Introduction

Much of what is known about biomaterials derived from non-biomedical engineering endeavors. For example, during World War II pilots who sustained windshield shrapnel in their eyes from combat experienced a minimal foreign body reaction. Thus Dr. Harold Ridley investigated polymethylmethacrylate (PMMA) as a biocompatible material for lens replacement [1]. Similarly, the large-scale production of titanium for jet aircrafts prompted researchers to investigate its use as a biomaterial (due to its excellent mechanical properties and resistance to

corrosion [2] [3]).

In the mix of the war, Bothe *et al*. published results suggesting for the first time that bone may fuse with titanium [4]. Eleven years later, Leventhal published further results demonstrating minimal soft tissue reaction to titanium and its potential use as a metal in orthopaedic applications [5]. Approximately the same time as Leventhal, Per-Ingvar Brånemark independently observed that bone attached to titanium chambers that were used to visualize blood cell formation in rabbit marrow. However, it is Brånemark's research with titanium and bone integration that is mostly recognized as contributing to the current understanding of "osseointegration" [6]. From this work, titanium has become commonplace in dental implants, total joint replacements, fracture fixation plates, intramedullary nails and external fixators [7] [8].

Prior to the use of titanium as an orthopaedic implant, various other metals—including stainless steel and Vitallium™—were used for fracture fixation plates [5] and dental implants [9] [10], but these metals were not investigated as a substitute for hip replacements until the 1940s [11]. Stemming from the work by Glück in 1891, who used ivory as a ball and socket joint to create a hip fixation implant, Moore was the first surgeon to implant a total hip fixation device made of Vitallium™ [12]. Sir John Charnley then revolutionized the design by developing a separate acetabular cup and femoral component made of Teflon™ and titanium respectively, which were cemented into place with PMMA. The use of Teflon™ by Charnley marked one of the early uses of a polymer in orthopaedic implants. However, because of premature wear [13]-[15], Teflon™ was found to not be a suitable material for acetabular components, and by 1962 was replaced by ultra-high-molecular-weight-polyethylene (UHMWPE) since it produced fewer wear particles during cyclic loading [13] [16]. This pioneering work by Charnley prompted joint arthroplasty, which once was a rare procedure, to become commonplace [17]. Total knee arthroplasty (TKA) in particular, is one of the most frequent elective surgical procedures and accounts for 450,000 cases in the United States annually [18].

The success of orthopaedic implants (similar to those designed by Brånemark, Glück, Charnley, etc.) cannot be attributed solely to good intuition or the material type alone. While titanium is a preferred biomaterial for many orthopaedic applications, additional factors significantly impact implant survivorship. Therefore, this article is intended to provide an overview of critical principles and will discuss what we define as the 5 hallmarks of orthopaedic biomaterials success: 1) biocompatibility, 2) physician technique, 3) design, 4) mechanical stability/initial fixation, and 5) infection prevention.

1.1. Biocompatibility

Over the past two decades, several definitions of "biocompatibility" have been proposed. Williams stated that "biocompatibility is the ability of a material to perform with an appropriate host response in a specific application" [19]. Mardis and Kroeger defined biocompatibility as being "the utopian state where a biomaterial presents an interface with a physiologic environment without the material adversely affecting the environment or the environment adversely affecting the material" [20]. These definitions have provided general guidelines for which researchers have compared the response of host tissue to biomaterials, and vise versa. However, as discoveries continue to be made, these definitions must be modified to conform to the ever-increasing understanding of biomaterial-host interactions.

Although no material known to man is completely biocompatible (*i.e.* no wound will heal in the same manner when a biomaterial is present than if it is not), it may be that our understanding of protein-surface interactions contributes most significantly to how we define biocompatibility. More specifically, shortly after implanting a biomaterial in the body, a conditioning film containing small molecules including water, electrolytes, cholesterol, complement, vitamins, lipids and proteins (such as albumin, IgG, fibronectin, fibrinogen, laminin, collagen and of interest to orthopaedics, osteopontin) forms on the surface of the implant long before cells are present and reach a state of equilibrium thereon [21]-[23]. This layer is dynamic and ever changing due to the differential diffusion and mass transport of these molecules/cells toward the implant surface. Competitive binding occurs on the surface due to the affinity of the molecules/cells towards the surface. Thus, it can be hypothesized that cells never "see" the entire surface of a biomaterial, but more correctly respond to and interact with the conditioning film that already developed *in situ*. The same would be true for bacteria that might be present near the implant surface (discussed in subsequent sections).

Attachment-dependent cells secure themselves to these proteins or protein matrices using integrin receptors, thus this conditioning film becomes very important in the reaction of cells to the surface of an orthopaedic bioma-

terial. This interface is what has prompted researchers to investigate protein-preconditioned surfaces of biomaterials [24]. Importantly, this conditioning film plays a significant role in biocompatibility because of the conformational changes that occur to adsorbed proteins, which often result in proinflammatory signals by the host immune system. As proteins adsorb to a biomaterial surface, they often change conformation and expose epitopes that are not typically identified as self-produced by the body's immune cells [23]. Immune cells then react as they detect what once were normal physiological proteins as foreign body materials. The result of this effect may be a cascade of blood coagulation and/or chronic inflammation, which can further lead to occlusion of nutrients, changes in oxygen tension, excessive fibrous capsule formation [25] and most importantly rejection of an implant system.

The extent of protein deformation and the assortment of proteins that adsorb onto an orthopaedic implant vary based on the material type [23] [26] [27]. For example, in an attempt to make metal surfaces more "passive," *i.e.* more resistant to corrosion, chemical treatments are often added during the manufacturing process. Passivation with nitric acid of stainless steel devices creates a less reactive oxide layer for enhanced biocompatibility. However, passivation also has one added benefit; it serves as a means for removing foreign material from the surface of metals such as machining oil and bacteria, (including bacteria that reside in a biofilm) [28]-[31]. However, our team recently grew biofilms of *Staphylococcus epidermidis* on the surface of titanium metal and found that despite being sonicated in detergent, passivated with nitric acid, rinsed with copious amounts of water and autoclaved, the surface of titanium still contained biofilm on the surface in over 30% of cases (unpublished data). Thus, if bacteria remain on the surface of an implant, dead or alive, their foreign materials and endotoxins may foster inflammation and lead to subsequent implant failure.

In the case of titanium, these specific metals naturally develop an oxide layer on the surface [32] which helps chemically bond the surface with the osseous cells during cementless skeletal fixation. While this is generally desired, in specific applications, long chain alcohol treatment of titanium may be used to make the surface more hydrophobic [33]; generally, hydrophilic surfaces have greater biocompatibility due to water retention at the surface. Therefore, a unique surface may be developed if only transient bone attachment is desired. Hydrophobic surfaces are also more apt to attract the adsorption of albumin, the most abundant protein in plasma that contains several hydrophobic residues. It is almost always undesirable to have albumin on the surface of an orthopaedic implant due to the inability of attachment-dependent cells to adhere to it. If albumin is the dominating protein at the surface, greater fibrous capsule formation occurs and there is problems maintaining a durable cementless skeletal fixation. An implant which does not remain fixed at the bone-implant construct may generate more wear particulate and this leads to bone loss and implant loosening [34] [35]. Taken together, any change in the treatment/production of orthopaedic implants should be noted and the success rate of the implants documented to determine the effect of treatments and modifications.

1.2. Physician Techniques

Perhaps the most difficult measure for predicting the success of an implanted device is the variation of physician technique. Multiple instruments have been designed to optimize the approach to total joint replacements (TJRs), however, the aspect of human variability will never be entirely removed. Importantly, it should be recognized that despite a surgeon's best efforts, the dissimilarity of each patient's bone quality, porosity, vasculature and lifestyle play a significant role in the success of an implant [36]-[39].

One option for reducing host rejection of orthopaedic implants requires careful surgical procedures and controlled drilling techniques. Attention must be paid to the temperature of the implantation site since excessive heat generated from frictional forces may lead to necrosis of the host bed—thus increasing the likelihood of scar tissue formation, which lacks the tensile strength of normal connective tissue and cannot sustain the loads exerted on an orthopedic implant [40]. Once a soft-tissue reaction has occurred, the healing process resembles pseudarthrosis, and repair is unlikely [41]. Determining the critical temperature of bone necrosis also compounds this problem. Literature indicates that that temperatures must be maintained below 56˚C since alkaline phosphatase (AP) is denatured at this temperature threshold [42]. AP is an enzyme produced during osteogenesis and may be an important phosphate transporter [43]. However, during conventional surgery, temperatures may exceed 65˚C [44] and have been recorded as high as 89˚C [41].

1.3. Design

An ideal implant design is one that models the anatomical geometry of living tissue and contains the same ratios

of physiological byproducts. However, tissue engineering models are still in their infancy and will require years of research prior to widespread usage during orthopaedic applications. Biocompatible metals and non-biodegradable polymers, on the other hand, are readily available and have been used for hundreds of years [45]. Despite the host of available bioactive agents which may be deposited to the exterior of a material surface, the fixation of all orthopedic implants depends on establishing a strong mechanical interlock with the bone, proper surgical technique, and the implant design altogether [46].

Because TJRs are subjected to high cyclic loads, clinical reports have indicated that approximately 25% of surgical implants fail from aseptic loosening and have been attributed to wear from articular bearings [47]. Altered loading patterns on newly implanted TJRs may compromise the material integrity since hip contact forces may exceed 409% the body weight with disturbed gait patterns [48]. Abnormally high non-physiological loads may not be supported and wear improperly given that hip and knee joints are cyclically loaded approximately 2×10^6 times annually [3].

To prevent implant loosening and ensure firm skeletal attachment, the orthopaedic industry has looked to porous coated surface treatments. Increasing implant roughness has improved the longevity of TJRs, but has also raised concerns with coating disassociation at the bone-implant interface. Metal particulate released from orthopaedic implants have been noted to appear in the urine, blood, and lungs remote from an implantation site [49]; and some of the metal alloys may be toxic and dissolve in the body fluids [50]. While there has not be a direct association with detached coatings and health problems due to underpowered studies [51], high aggregations of metal from orthopaedic implants may be linked with pathological diseases such as marrow fibrosis [52], cystic destruction of bone [52], granulomatosis [53], necrosis of the bone marrow [54], neoplasia [55]-[57], sarcoma [58]-[60], bone resorption [61], cardiomyopathy [62] and thyroid dysfunction [62].

1.4. Mechanical Stability/Initial Fixation

Attaining a strong skeletal interlock at the bone-implant interface is a prerequisite for long-term implant function and stability [63] [64]. While PMMA may be used for patients with inadequate bone stock, evidence of monomer leakage or exothermic curing reactions [41] [65] [66] are some reasons why some patients advocate for osseointegration procedures. Immediate weight bearing in joint arthroplasty is often advocated and does not compromise the integrity of the periprosthetic bone as long as micromotion is carefully controlled [64] [67]-[71].

Despite the signs of adequate implant "fit and fill", "it is evident that there must always be some movement between and artificial joint component and bone, even if its amplitude is minuscule and the precise site of its occurrence obscure [46]." However, the complete lack of integration between the host bone and implant leads to excessive micromotion and premature failure. This condition does not provide the skeletal attachment required for secondary implant stability (which results from bone remodeling that occurs over time [72]) and does not have the structural integrity to withstand the dynamic mechanical forces from during ambulation [73]. While initial implant fixation is required to prevent micromotion and fibrous encapsulation [7] [69] [74]-[80], the long-term success of orthopaedic implants requires firm skeletal attachment, which may require up to 9 months in human cancellous bone [74]. Therefore, the primary step in initial implant fixation is to minimize gaps greater than 50 μm since this has been noted to be unstable and prevents integration [7].

Roughness, porosity and surface topography may be specifically tailored based on the application and will impact the host response to an implant [76] [81] More specifically, the implant surface is vital in cementless skeletal fixation, as specific profiles influence osteoblast and osteoclast attachment and metabolism [82]. Boyan *et al*. previously noted that implant surfaces should be between 4 - 7 μm in roughness to ensure proper osteoblast cuboid morphology [83]; while others in the peer-reviewed literature note that skeletal fixation is most effective with porous implants in the 50 - 400 μm range [84] and with roughened surfaces. This hallmark has been demonstrated by observing that osteoblasts seated on roughened surfaces have increased proliferation and *in vivo* animal models revealed that textured surfaces required higher removal torques compared with smooth controls during bone-implant removal [76].

1.5. Infection Prevention

Orthopaedic implant-related infections are catastrophic to patients and physicians. These occurrences are often accompanied by extensive and expensive strategies of debridement, implant removal, antibiotic therapy and rehabilitation. The severity and concern of implant-related infections has been amplified in the past several dec-

ades with an increased understanding of bacterial biofilms that have the potential to form on the surface of implanted materials [85]-[93]. Biofilms are communities of bacteria that have the ability to communicate, transfer genetic material, protect themselves with secreted polymeric substances that encapsulate the community in a hydrated matrix and preferentially adhere to solid surfaces [86] [87] [90] [93]. Biofilms may serve as reservoirs of infection in patients who have indwelling devices [91]. More specifically, as antibiotics are administered, planktonic cells within the body may be killed and alleviate symptoms of infection short term. However, once antibiotic treatment has been discontinued, infection may recur. This cycle may continue for years until a biofilm-ridden device or tissue is removed [89] [91] [94] [95].

In light of the ever-present risk of biofilm implant-related infection, emphasis is placed on rigorous sterilization techniques for instrumentation and implants prior to surgery [86]. Yet despite these efforts, infection remains a significant problem. Adherence of bacteria to orthopaedic devices begins with contamination of the surgical site or implant. This contamination may come from multiple sources, the most likely of which is the patient, surgeon, or healthcare worker. The surrounding environment, such as air from filtration systems, may likewise contain bacteria. As noted by Williams and Costerton [96], skin preps have the ability to remove the top few layers of skin and kill approximately 99.9% of bacteria, *i.e.*, a 3 $\log_{10}$ reduction [96]. However, mature bacterial biofilms may reside up to 7 layers deep in human skin [97]. Thus, when an incision is made, contamination of tissues may follow despite extensive treatment with surgical prep packs. In addition, bacteria may be released from the nose, mouth or skin of surgeons, a patient or healthcare workers in an operating room. Ventilation systems may also transport bacteria from one room to another even in laminar flow surgical suites.

Once an incision site has been contaminated, microorganisms reproduce in high quantity. The adhesion process becomes almost irreversible as extracellular polymeric substances act as an adhesive between the biofilm and an implant surface [98]. Bacteria express membrane adhesins, which help to prevent phagoctyosis by neutrophils and adhere to host cells, thus increasing their virulence. One of the most difficult aspects of orthopaedic device-related infections is the diagnosis of biofilm-related infections. For example, Sir John Charnley, credited with the creation of the artificial hip, was unaware of biofilm formation in the early 1970s and noted "a rather high incidence of manifestations of infections, months or years after the implant was made… which might be blood-borne in origin or even the result of chemical reaction [99]." Failure of orthopedic implants is often misclassified as aseptic because due to the lack of clinical evidence [100]. However, new promising technologies involve 16s DNA sequencing to identify non-culturable bacteria, may improve diagnosis and increase implant success [101]-[103].

Once a medical device is placed *in vivo*, biofilms may cause damage by inducing a significant inflammatory response and colonizing the host tissue [86]. Biofilm-related infections may develop months or years after implantation and often require excision of necrotic bone or implant removal [86]. Once the onsite of infection has been determined, antibiotics may be administered, but have marginal efficacy since biofilms require exponentially higher antibiotic concentrations [104] [105]. It has been reported that biofilms are between 500 - 5000x more difficult to eradicate because they are in a non-planktonic form [86] [99] [106] [107].

In short, understanding the development of sessile, biofilm communities is a fundamental factor for determining how to prevent biofilm implant-related infections from occurring. In an attempt to prevent these infections, multiple technologies have been developed including: passive and active release antimicrobial coatings, antimicrobial loaded bone cements and beads, antimicrobial loaded sleeves for fracture fixation plates, and novel antimicrobial compounds that are specifically synthesized to be active against biofilms [108]-[117].

2. Conclusion

Orthopaedic devices are expected to be implanted at much higher rates in the upcoming decades since individuals are living longer and still demand the same quality of life. Successful procedures will continue to improve based on optimized surgical technique and advanced implant designs/coatings. However, to further increase the likelihood for long-term implant survivorship, the 5 hallmarks for biomaterials noted above should be considered. While this narrative is intended to provide an overview, the authors recognize that there is not a perfect roadmap to prevent early implant loosening, infection or other failures. Adherence to these principles may not guarantee success, however, ignoring these principles will likely yield future complications. In conclusion, future designs should emphasize infection prevention, early mechanical stability and geometry which ensure proper fit and fill.

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Relationship Between Volumetric Measurements of Heterotopic Ossification in Wounded Service Members and Clinically Available Screening Tools

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ABSTRACT

Heterotopic ossification (HO) often causes symptoms requiring surgical resection and may delay rehabilitation regimens for wounded service members. Clinical screening tools for assessing HO have included serum alkaline phosphatase (AP), nuclear scintigraphic activity, and patient pain scores. However, no studies to date have investigated the relationship of these clinical predictors with HO incidence and volume. Ten servicemen with transfemoral amputations were included in this retrospective study. Volumetric measurements of HO were calculated using thresholding software, and computed tomography scans were performed 12.6 ± 6.8 months after injury. Subject AP levels, white blood cell (WBC) counts, and pain scores were assessed to determine if these factors were predictors of ectopic bone volumes. The mean volume of HO was 44.73 ± 39.35 cm^3. Statistical analysis demonstrated that the volume of HO and serum AP levels were significantly correlated ($p = 0.002$). However, average pain scores were not a significant predictor of HO volume ($p = 0.212$). Infections developed in 9 of the 10 subjects, and WBC counts and HO volumes were significantly correlated ($p = 0.028$). The magnitude of serum AP levels and WBC counts may be effective factors for predicting the expected volume of ectopic bone in combat-injured service members with transfemoral amputations. (*J Prosthet Orthot*. 2012;24:138–143.)

KEY INDEXING TERMS: heterotopic ossification, ectopic bone, alkaline phosphatase, bone growth, infection

Heterotopic ossification (HO) refers to ectopic bone formation, typically in residual limbs and/or periarticular regions, after trauma and injury.[1] This pathological process manifests outside the skeleton[2] and is composed of a hybrid of cortical and cancellous bone.[3] Heterotopic ossification was first reported by El Zahrawi (Albucasis) in 1000 CE, in which he noted that stony hard prominences occasionally developed during fracture healing and demanded urgent removal.[4] Although the etiology of HO has not been elucidated in the 1000 years since its initial observance,[5,6] there has been a general agreement in the orthopedic literature that HO is induced from damage to soft tissue and inflammation,[5,7]; and ectopic bone growth has been most frequently observed after combat-related trauma to service members with blast injuries.[8]

Reviews of orthopedic injuries from Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) have reported that approximately 70% of war wounds have involved the musculoskeletal system,[9] largely in part from the use of improvised explosive devices (IEDs) and rocket-propelled grenades (RPGs). Given the intense nature of blast injuries, which require rapid tourniquet use, debridement, and surgical intervention, HO has been reported to occur in approximately 63% to 65% of wounded service members.[10–12] Reports of recent OIF and OEF combat-related amputees with known HO have indicated that approximately 7% required surgery to excise their bony masses.[13] Symptomatic HO may delay rehabilitation regimens, as ectopic bone resection often requires modifications to prosthetic limb componentry and socket size.[13,14]

Current methods for assessing HO growth in periarticular regions have involved the collection of serum alkaline phosphatase (AP) during inpatient care, nuclear scintigraphic (i.e., "bone scan") activity, patient pain scores, observation of redness to the affected region, and radiographic/radiologic evidence of HO maturity based on the appearance of a clearly defined cortical rind. Most physicians note that the osseous overgrowth should not be removed until HO has fully matured[7,15–17] and/or until patients have demonstrated normalized AP levels.[11] However, other surgeons have cautioned

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against using this approach, as AP levels may not correlate with the severity of HO,[18] and in some instances, HO may manifest within normal AP levels.[19] To date, no clear experimental findings have indicated a mechanism for quelling or preventing metabolically active HO.[1] Correlative factors such as sex,[1,20] genetics,[7,19,21,22] bioelectric signals,[7] infection,[23] and age[20] have been associated with ectopic bone growth, but HO studies have often lacked histologic corroboration or advanced radiologic quantification.[24]

The gold standard for assessing periarticular HO severity after total hip arthroplasty (THA) was developed by Brooker et al.,[25] in which supine anteroposterior radiographs were used to classify ectopic bone on a I–IV grading scale. Although the criteria of Brooker et al.[25] are acceptable for ranking periartcular HO after total joint replacement, this method lacks reliable objectivity and is an insufficient tool for assessing HO in the residual limb. To offset this limitation, Potter et al.[12] developed a scale for assessing the magnitude of HO within the residual limb of injured service members using anteroposterior and lateral radiographs and by grouping individuals based on the cross-sectional area of ectopic bone within their residual limb (<25%, mild; 25% to 50%, moderate; >50%, severe). However, a more thorough method for calculating HO volumes has since been developed by Isaacson et al.[1] for quantifying the volume of ectopic bone formation within the residual limb or at other anatomic locations. We hypothesized that by using the method developed by Isaacson et al.,[1] there would be a direct correlation between volumetric HO calculations and clinical factors including serum AP levels, subjective pain scores, and white blood cell (WBC) counts.

MATERIALS AND METHODS

STUDY POPULATION

To determine if a relationship existed between the volume of HO in the residual limb of service members and currently used clinical assessment tools, previous computed tomography (CT) scans were collected in accordance with Walter Reed National Military Medical Center (WRNMMC) and University of Utah Institutional Review Board approvals.[1] Ten servicemen with prior CT scans of their transfemoral amputations were included in this study. Computed tomography was selected as the preferred imaging modality because this diagnostic tool provided clear distinction between tissue types and was necessary for determining the volume of HO. The small study population was necessitated by the frequent presence of metal fragments or fixation devices within the residual limbs of many amputees in the WRNMMC database at the time of radiologic review. Metal debris has been well known for generating image artifacts during three-dimensional reconstructions and thus would have created errors during volume calculations.[26] Subjects were on average 22.0 ± 5.2 years old at the time of injury and sustained limb loss because of combat-related injuries. In this study, IEDs accounted for the highest frequency of traumatic amputations, occurring in 9 of 10 subjects, whereas an RPG served as the other mechanism for limb loss (Table 1). Of the 10 patients included in this study, 3 did not have radiologic signs of HO and were included to establish baseline AP levels, WBC counts, and subject pain scores.

DATA COLLECTION

The 10 servicemen included in this retrospective study were monitored as inpatients for up to 3 months to access fluctuations in AP levels, pain score ratings, and WBC counts starting at the date of their arrival at WRNMMC. Because HO has been noted to occur within several weeks after combat-related trauma, a 3-month assessment period ensured that ectopic bone formation had adequate time to manifest within the residual limb. Heterotopic ossification formations were confirmed to be mature at the time of radiologic review, and CT scans were performed an average of 12.6 ± 6.2 months (range, 6–22 months) after injury.

Table 1. Demographic information and collected data from the 10 service members

Subject no.	Age, y	Injury mechanism	Volume of HO, cm^3	Pain score rating, mean ± SD	WBC count, mean ± SD, $\times 10^3/\mu L$	AP levels, mean ± SD, U/L	TBI
1	27	IED	47.88	1.93 ± 2.03	15.95 ± 7.43	212.12 ± 163.99	Yes
2	24	IED	74.25	1.65 ± 2.66	17.95 ± 6.26	243.01 ± 166.84	Yes
3	22	IED	115.96	3.64 ± 2.53	14.90 ± 7.22	461.22 ± 380.94	No
4	32	IED	00.00	5.01 ± 2.72	4.69 ± 1.40	–	No
5	30	IED	26.53	2.06 ± 2.32	9.20 ± 3.61	114.29 ± 32.80	No
6	39	RPG	00.00	3.40 ± 2.47	10.20 ± 4.24	126.89 ± 10.39	No
7	24	IED	12.75	5.33 ± 2.01	8.20 ± 0.97	88.00 ± 12.33	No
8	28	IED	47.78	3.56 ± 1.74	8.83 ± 3.30	135.08 ± 70.54	No
9	23	IED	77.43	3.73 ± 2.65	9.10 ± 5.17	170.46 ± 103.68	No
10	31	IED	00.00	5.12 ± 2.97	6.10 ± 1.16	45.50 ± 0.50	Yes

– indicates no data available for retrospective review.
HO, heterotopic ossification; WBC, white blood cell; AP, alkaline phosphatase; TBI, traumatic brain injury; IED, improvised explosive device; RPG, rocket-propelled grenade.

Subject chart reviews were used for reporting localized and systemic infections, and bacterial colonization was determined by using wound cultures, blood cultures, and in specific cases, peritoneal fluid (Table 2). Pain score ratings were documented twice daily using a Likert scale, and subjects were asked to rate their pain on a measure of 0 to 10, with 0 being absolutely no pain and 10 representing excruciating pain (Table 1). Alkaline phosphatase levels and WBC counts were recorded daily.

VOLUMETRIC MEASUREMENTS

Ectopic bone volume was computed using a model developed previously by Isaacson et al.[1] In short, software that multiplied voxel height and width by CT slice thickness was used to determine the volume of HO (Analyze 9.0, Mayo Clinic, OH, USA). Axial CT slices were manually inspected to determine HO connected to the periosteum and bony islands, which manifested within the soft tissue (Fig.1). All HO sections were identified, thresholded, and computed separately to determine ectopic bone volumes.

STATISTICAL EVALUATION

Serum AP levels, WBC counts, and subject pain scores were independently assessed to determine if these factors were significant predictors of HO volume. To accurately associate the predictor and outcome measures, without introducing over fitting or having confounding variables, each factor was correlated independently. All statistical evaluations were performed using a linear regression and were conducted with commercially available software at $\alpha \leq 0.05$ (SPSS, Inc, Chicago, IL, USA).

Table 2. Infection data from the 10 service members

Subject no.	Infection	Organism type	Culture location
1	Yes	*Acinetobacter baumannii*	Blood
		Pseudomonas aeruginosa	Wound site
2	Yes	*Stenotrophomonas maltophilia*	Wound site
		Aspergillosis	Tracheal aspirate
3	Yes	*Staphylococcus* *Acinetobacter baumannii*	Wound site
4	Yes	*Klebsiella Pneumonia* *Acinetobacter baumannii*	Wound site
5	Yes	*Acinetobacter baumannii*	Blood
		Peritoneal fluid	
6	Yes	*Acinetobacter baumannii*	Wound site
7	Yes	*Staphylococcus*	Wound site
8	Yes	*Enterococcus*	Blood
9	Yes	*Acinetobacter baumannii*	Tracheal aspirate
10	No	—	—

Of the 10 servicemen, 9 had an infection, with 4 of 10 experiencing infections from multiple strains of bacteria. – indicates no report of positive or negative cultures.

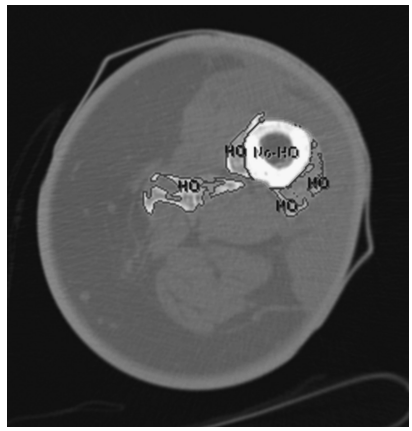

Figure 1. Axial cross-section demonstrating HO in the residual limb of an injured service member. Note that the ectopic bone connects to the periosteum and manifests as bony islands in the musculature. Each axial slice was combined in Analyze 9.0 to compute HO volumes. HO, heterotopic ossification.

RESULTS

Ten service members were included in this retrospective study to assess the relationship between HO volumes, AP levels, WBC counts, and patient pain scores. However, one subject (serviceman 4) did not have AP laboratory documentation and was omitted from the HO volume and AP level assessment. Data from 9 of the 10 servicemen indicated that the volume of HO (44.73 ± 39.35 cm^3) and average serum AP levels (177.40 ± 122.39 U/L) were significantly correlated ($p = 0.002$). An R^2 value of 0.782 indicated that a positive linear relationship existed, in which higher volumes of HO were associated with elevated AP levels. When average pain scores (3.5 ± 1.3) were compared with HO volumes to assess if ectopic bone formation increased subject pain, this association was not significantly correlated ($p = 0.212$). An R^2 value of 0.187 demonstrated no relationship between these two variables. However, there is reason to believe that disassociation between pain and HO volumes may have been influenced by subject comorbidities or neurological complications such as a traumatic brain injury (TBI). Traumatic brain injuries occurred in 3 of the 10 servicemen. In almost all cases, the service members in this patient series experienced concurrent bone fractures and soft tissue injuries aside from HO formation. This likely skewed patient pain scores as values were not solely dependent on just ectopic bone formation.

Infection and tissue culture data were reported for 9 of 10 servicemen. For the one subject without documented infection data (serviceman 10), medical records did not indicate positive or negative cultures, and therefore, this person was not included in this phase of analysis. Review of

the subjects' medical records indicated that bacterial colonization consisted of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Aspergillosis*, *Staphylococcus*, *Klebsiella pneumoniae*, and *Enterococcus* (Table 2). In fact, four of the nine of the subjects with positive infection signs also had multiple strains of cultured bacteria. When WBC count was compared with the volume of HO to determine if this was a predictor for ectopic bone growth, there was a significant correlation ($p = 0.028$). An R^2 value of 0.474 indicated a low to moderate association.

DISCUSSION

The prevalence of HO in combat-wounded servicemen and women has been reported to be approximately 63% to 65% for those returning from theater,[10–12] a significantly higher proportion than the documented HO rate within civilian trauma facilities. Because blast injuries sustained in OIF and OEF induce orthopedic trauma, neurovascular damage, and soft tissue injuries (key contributors to HO induction[1]), ectopic bone formation has and will remain a challenging orthopedic and rehabilitation issue. Therefore, the objective of this study was to use a novel HO volume measurement method to determine if a relationship existed between ectopic bone formation and serum AP levels, WBC counts, and patient pain scores. To the authors' knowledge, directly computing HO volume has not been evaluated by any other team to date.[27] Conventional methods for assessing ectopic bone development have included measuring the length of HO using anteroposterior or lateral radiographs[28] and by developing grading scales to group HO severity based on a percentage of occupied space around the affected region.[5] However, direct HO volumetric measurements serve as a more accurate mechanism for assessing ossification severity, preventing observer bias, and, of course, providing quantitative data of ectopic bone volume.

It is worth noting that ectopic bone formation is not unique to combat-related blast injuries, as HO has been reported after burns,[29] TBIs,[30] spinal cord injuries (SCIs),[16,20] rotator cuff surgery,[31] and THA.[5,32] However, the severity/magnitude of HO has been most pronounced in the residual limbs of individuals with combat wounds, potentially because of the greater volume of space for ectopic bone to manifest as well as the massive zones of polysystemic injury and associated inflammation. Ectopic bone percentages have been known to drastically differ based on the injury mechanism. In the case of THA, HO has been most noted to occur in approximately 10% to 30% of patients,[33] in 3.1% of burn victims,[15] and in 63% to 65% of the military population injured in theater.[10–12] Ectopic bone formation continues to be a problem for wounded service members with limb loss who wish to return to active duty or an energetic lifestyle,[34] as an improper interface between the residual limb and prosthetic socket may lead to skin breakdown[35] and significantly limit their mobility.[8,36]

Although an association between serum AP levels and HO formation seems conceptually clear (given that AP is an enzyme secreted by osteoblast and has long been associated with calcification),[37] the relationship between AP and HO development has been subject to frequent debate in the literature. Mollan[37] previously reported in his study of 131 THA patients that elevated serum AP levels resulted in an almost threefold increase in postoperative HO. Data from the present study agreed with the reported relationship between HO and elevated AP levels, as a direct positive correlation existed between AP and the volume of HO within the residual limb of injured service members.

The data from our study are also supported by Kjaersgaard-Andersen et al.,[38] who noted that an increase in AP levels of greater than 250 IU/L 12 weeks after surgery was associated with the development of severe heterotopic bone in 13 of 17 patients. In this study, the highest serum AP level occurred in subject 3 (461.22 ± 380.94 U/L), who also had the largest volume of ectopic bone formation within their residual limb (115.96 cm^3). Therefore, it may be postulated that AP levels monitored within 3 months of combat-injured service members may be an accurate predictor for developing HO and may directly correlate with ectopic bone volume.

Increased ectopic bone volume because of neurological impairment remains highly likely, as Forsberg et al.[10] noted that the presence and severity of a TBI were significantly associated with HO. Studies conducted on neurological-based HO by Furman et al.[16] noted that ectopic bone formation occurred in 47% (7/15) of his patient population with SCIs and that HO development was accompanied by elevations in serum AP. Hsu et al.[18] also reported that 100% (20/20) of their SCI subjects had periarticular HO around the hip and experienced increased AP levels as well. Data from this patient series demonstrated that TBIs occurred in 30% of the patient population, a higher rate than that reported in the literature and was likely attributed to the subject sample size. Symptomatic HO requiring surgical intervention has been noted to occur in approximately 11% of patients with a TBI and 20% of SCI patients.[39,40] Although a correlation between HO volumes and neurological-based HO was not possible in this study, it is worth noting that two of the three subjects with TBIs had the third and fourth highest volumes of HO present within their residual limb. Future studies assessing if a relationship exists between HO volumes and TBI and SCI subjects would provide valuable data as to the impact of nervous system damage and ectopic bone volume.

One prospective HO induction factor underreported in the orthopedic literature has been the potential of elevated WBC counts or infection for increasing the likelihood of HO development. Potter et al.[23] noted that although it has been well regarded that infections inhibit bone formation and fracture healing, six of six service members who had intraoperative cultures during surgical resection of HO all tested positive for bacterial contamination. Data from our study confirms the ability for HO to manifest concurrently with positive infection signals; however, it is important to note that the only 6 of the 10 servicemen in our study had a documented infection on their residual limb, whereas the

remaining positive cultures were determined using tracheal aspirate, blood, and peritoneal fluid. Therefore, before conclusions can be made on the association between combat-injured service members with infections and HO induction, a large retrospective study must be performed within the military healthcare facilities to ensure adequate statistical power.

Lastly, subjective patient pain scores demonstrated to not be an effective tool for assessing HO development, as this quantitative data did not correlate with HO volumes. Although HO development is often observed using the four cardinal signs of inflammation (redness, swelling, heat, and pain), the sole factor of pain alone was not a predictor for the servicemen included in this study. One explanation for this disassociation may have been the individualized pharmacologic regimens, extensive comorbidities and concurrent injuries, and variable personal pain tolerances. When subjects reported their pain scores, the Likert-based scale used in this study did not distinguish between overall pain and pain related to HO, and therefore, pain was grouped based as a personal whole.

CONCLUSION

Although ectopic bone formation has been previously categorized using bone scans and serum AP levels, no quantitative measurement method has existed for assessing HO volume. In a previous study by Isaacson et al.,[1] the coauthors used a thresholding tool to determine the volume of HO within the residual limb of service members but did not corroborate this model with clinical predictors of HO presence, maturation, or severity. Data from this study indicated that serum AP levels and WBC counts were significant predictors of HO volumes. However, patient pain scores were not a valid predictor. In the future, the magnitude of serum AP levels and WBC counts may better predict the expected volume of trauma-related HO, but requires large-scale studies with adequate power to confirm the findings of this small patient series. Improved ectopic bone diagnostic tools in both the military and general populations have high clinical relevance, as these may influence HO prognostication, operative resection timing, and treatment strategies and subsequently reduce recurrence rates.

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Special article

The use of a computer-assisted rehabilitation environment (CAREN) for enhancing wounded warrior rehabilitation regimens

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Purpose: This paper seeks to describe how novel technologies such as the computer-assisted rehabilitation environment (CAREN) may improve physical and cognitive rehabilitation for wounded warfighters.
Design/methodology/approach: The CAREN system is a dynamic platform which may assist service members who have sustained improvised explosive device injuries during Operation Enduring Freedom, Operation Iraqi Freedom, and Operation New Dawn. The complex nature of warfighter injuries present unique rehabilitation challenges that demand new tools for quick return to active duty or the civilian community.
Findings: Virtual reality-based gait training programs may directly influence physiological and biomechanical performance for those who have endured combat injuries. The CAREN system provides a safe, interactive environment for the user while capturing kinematic and kinetic data capture to improve rehabilitation regimens.
Conclusions: This paper provides an overview of the CAREN system and describes how this dynamic rehabilitation aid may be a translational tool for collecting biomechanical and physiological data during prosthetic training. The CAREN platform allows users to be fully immersed in a virtual environment while healthcare providers use these simulations to improve gait and stability, obstacle avoidance, or improved weight shifting. As such, rehabilitation regimens may be patient specific.

Keywords: Rehabilitation, CAREN, Service members, Wounded warriors

Narrative

The use of improvised explosive devices during Operation Iraqi Freedom (OIF), Operation New Dawn (OND), and Operation Enduring Freedom (OEF) often results in complex orthopedic/neurological trauma which may include limb loss, spinal cord injury, and traumatic brain injury[1]. While advancements in military medicine during OIF/OEF has resulted in 92% of wounded warriors surviving blast-related injuries,[2] musculoskeletal extremity trauma has been estimated to occur in 50% of all injuries in theatre[1] – with 2% of warfighters incurring limb loss.[3] Military databases have indicated that as of May 2012, returning US military combatants have sustained 1356 major limb amputations (775 from OIF/OND and 581 from OEF) and 243 minor amputations (213 from OIF/OND and 30 from OEF) (Fischer, 2012). The primary rehabilitation goal for these individuals is to provide them an expedited recovery and progressive reintroduction in the civilian or active duty populations.

The relative youth and high fitness level of injured service members with amputations make them an ideal population for new challenging rehabilitation methods which may require more physical/cardiac output than the civilian community.[4,5] Novel technologies such as the computer-assisted rehabilitation environment (CAREN) provide virtual simulations as a means to improve physical and cognitive skills for wounded warfighters while promoting resilience and recovery. The CAREN system, developed by MOTEK Medical (Amsterdam, Netherlands) consists of a motion capture system and a base driven by hydraulic and mechanical actuators. The base where the user stands is retrofit with force

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plates and a treadmill, with up to 6 degrees of freedom.[6] This allows the operator to generate visual and physical perturbations that require the user to make dynamic responses during their gait patterns. The CAREN system may also be equipped with varying degrees of virtual reality immersion ranging from a flat video, dual-channel audio, theater in its "base" model to a 360°, surround sound dome enclosure in its "high end" version. Real-time motion tracking technology enables the CAREN system to follow patient movements frame-by-frame for detailed kinematic and biomechanical analysis using up to 24 mounting locations. Numerous studies have also been conducted assessing over ground walking vs. virtual reality treadmill-based rehabilitation indicating that the CAREN system is an effective rehabilitation aid for patient assessment.[7] As demonstrated in Fig. 1, the CAREN system is equipped with a harness to ensure patient safety while simulations are being conducted.

The CAREN system is unique in that it allows a wounded warrior to be immersed in a realistic clinical environment, while therapist and physicians collect kinematic and kinetic data in order to plan future rehabilitation regimens. In everyday life, warfighters with lower extremity trauma may experience uneven terrain, cracks in pavements, slippery conditions, etc. – all potential scenarios that would may increase fall risk or injury.[8] However, when using the CAREN system, specific physical perturbations may simulate these environmental conditions in a more safe and controlled setting. New rehabilitation methods and gait/prosthetic limb training may be developed for these individuals to mitigate falling risks outside of the clinic.[8,9]

Figure 1 Photograph of a wounded warrior with a lower limb prosthetic using the CAREN system. (Image courtesy of Erik Wolf, Ph.D., Director of the Center for Performance and Clinical Research, WRNMMC).

Depending on the warfighters' rehabilitation goals, simulations in the CAREN system may challenge reactive balance, reaction time, and muscle activation in order to improve gait and stability, obstacle avoidance or improved weight shifting (Fig. 2).[10] The D-flow control software suite that the CAREN is equipped with allows for personalized monitoring modalities to be integrated during real-time during data collection. Owing to the modular and customizable nature of the software suite, D-flow can be easily programmed to accept a variety of complementary software packages that may run simultaneously using real-time feedback. Supplementing the CAREN system with complementary diagnostic tools enables clinicians and researchers to investigate a range of concurrent clinically relevant health markers during virtual simulations (Geijetbeek *et al.*, 2011).

Drawing on the customizable nature of the CAREN system, electro-myographic, and other physiological measures such as heart rate, VO_2, VCO_2, and ventilation data may be integrated directly into the feedback stream. Data gloves may be fitted to the patient during virtual reality immersion to track upper extremity limb, hand, and digit macro-movements when investigating grasping, reaching, and vestibular perturbation response (Subramanian *et al.*, 2007). In addition, in-shoe pressure measurement and haptic/resistance may also be integrated into the system software (Mert *et al.*, 2010). These supplementary systems significantly enhance the diagnostic power of the CAREN system and may enable a higher degree of patient-specific treatment and enhance rehabilitation regimens (Mert *et al.*, 2010).

As a result of the rehabilitation benefits of the CAREN system, many of the United States military treatment facilities have been equipped with these platforms. Researchers at Walter Reed National Military Medical Center (WRNMMC) and the Center for the Intrepid (CFI) at Brooke Army Medical Center have used the CAREN to better understand gait patterns of warfighters who have sustained lower extremity trauma. One specific study by Werner *et al.*[12] demonstrated that when eight transfemoral amputees were subjected to lateral perturbations on the CAREN platform, these wounded warriors avoided falling by using their uninjured limb and that there was little change in prosthetic ankle or knee kinematics on the affected side. This finding has important rehabilitation implications and may require improved prosthetic training – since overuse of an intact limb may lead to future complications (osteoarthritis and lower back pain),[11] factors which may be correctable, if discovered early in the therapy process.

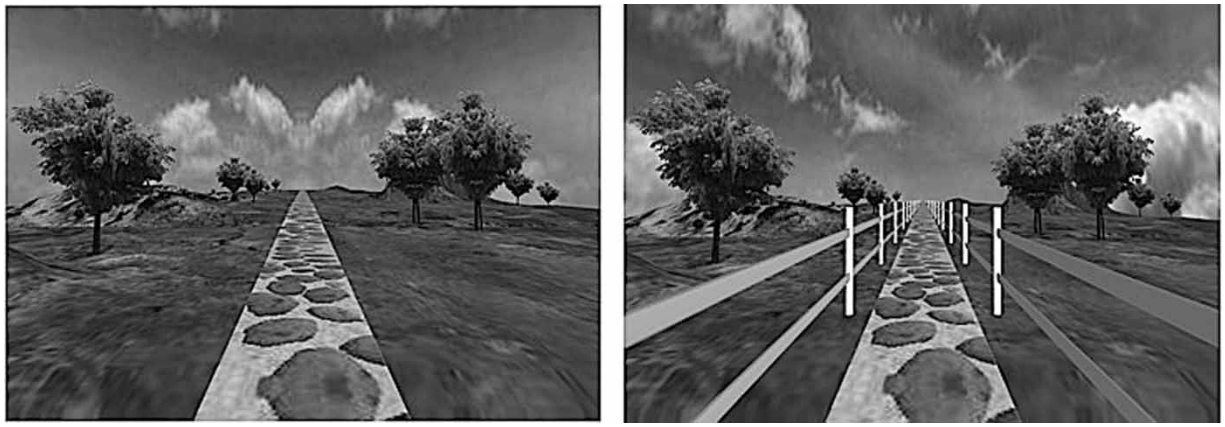

Figure 2 Side-by-side images of the two CAREN programs developed for wounded warrior rehabilitation. Note that the image on the left uses a basic path to encourage the individual to walk straight, while the image to the right has additional visual obstructions for those who are more experienced with their prosthetic device. (Image courtesy of Erik Wolf, Ph.D., Director of the Center for Performance and Clinical Research, WRNMMC).

Another distinct advantage of the CAREN system is that testing may be conducted in a controlled environment in order to regulate mechanical and visual cues. As such, rehabilitation outcomes may be specifically investigated without the risk of confounding variables. Once such example is from a study by Nottingham *et al.*[13] in which this team compared temporospatial parameters while using several prosthetic limb options: the conventional single axis hydraulic (SAH) knee, a microprocessor-controlled (MP) prosthetic knee and the X2 microprocessor knee (Otto Bock Healthcare, GmbH, Duderstadt, Germany) during slope ambulation. Twenty unilateral transfermoral amputees demonstrated that when descending a 10° slope that X2 users self-selected a faster walking speed, took longer steps than those using either the SAH or MP knee, and also did not require ambulatory aids; thereby demonstrating how MP knees may be beneficial for wounded warriors in slope descent. Data from this study may impact which prosthetic limb service members with lower limb amputations are fitted with, since the ultimate goal is to return these individuals to the active life they had prior to injury.

The applications for the CAREN system have continued to demonstrate how this rehabilitation tool is an important element in wounded warrior care. One distinct advantage of this system is that it provides physical and cognitive aid for those with multitrauma and traumatic brain injury. Gait training has, and will always remain, an important therapy element for individuals with lower extremity amputations.[14] While traditional therapy methods will always have a place in the clinic, virtual reality-based gait training programs have demonstrated the ability to directly influence physiological and biomechanical performance.[11,15] However, it is worth noting that while the CAREN system has numerous clinical advantages, the financial and spatial requirements for the platform may preclude this from being a rehabilitation aid for individuals in the general public.

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