Determining the Mineral Apposition Rate of Heterotopic Ossification in Military Healthcare System Patients after Total Joint Replacement: A Case Series

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ABSTRACT: Heterotopic ossification (HO) is frequently reported following total joint replacement (TJR) surgery. Symptomatic HO may limit the range of motion, cause pain, and require surgical excision. Deciding an appropriate time for ectopic bone excision is based on clinical judgment, and a more well-defined link between clinical predictors and histological analysis is needed to minimize recurrence. A case series was performed with military healthcare system patients undergoing TJR, who required removal of periarticular ectopic bone. Patients were prescribed oxytetracycline to assess the mineral apposition rate (MAR; ie, bone growth rate) of HO, and excised specimens were analyzed using scanning electron microscopy and light microscopy. Two males and one female were enrolled in this study, with height of 69.0 ± 7.8 inches, weight of 237.7 ± 28.3 pounds, and age of 61 ± 7 years at the time of HO removal. Ectopic bone occurred in two cases following total knee arthroplasty and one total hip arthroplasty. Data indicated that MAR rates were 1.7 times higher than that reported previously for nonpathological human bone (1.7 ± 0.7 µm/day; range: 1.3–2.6 µm/day), and microscopic imaging confirmed that the osseous tissues were still actively remodeling at the time of surgical intervention. Further characterization is needed to optimize HO excision timing and to better understand 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.1–3 These masses result from genetic abnormalities, neurologic injury, and/or musculoskeletal trauma and surgery.6 In the case of postoperative HO, ectopic bone has been commonly reported following total joint replacement (TJR).7–12 with postoperative rates varying between 2% and 90%,13,14 and severe cases occurring 3%–55% of the time.6 This wide range of HO incidence has been associated with patient demographics, surgical techniques, and the use of prophylactic treatments (radiation and nonsteroidal anti-inflammatory drugs).6 Ectopic bone may cause limited range of motion and/or pain6,15,16 and is clinically/radiographically detectable between 1 and 12 weeks postoperatively.17,18 HO remains a challenging comorbidity, and 3%–7% of total hip arthroplasty (THA) patients develop grade III/IV symptomatic HO19 (as scored by the Brooker scale)20 and require excision of their ectopic bone.

Most concerning is that the frequency of TJR-related HO is expected to rise in the coming years because of younger patients opting for this procedure,21 longer life expectancy,22 population growth,23 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 arthroplasty (TKA) and 332,000 THA procedures occur annually in the United States.24 Based on the abovementioned HO incidence data, tens or thousands of patients with symptomatic HO may require excision and advanced clinical management.

Although HO was first reported by El-Zahrawi (Albucasis) in 1000 C.E.,25 very little is known about the etiology of this pathological process. HO has been reported as a hybrid of cortical, cancellous, and woven bones, with...
varying degrees of mineralization and vascularity.\textsuperscript{1,26} However, only one previous study by our team has quantified the mineral apposition rate (MAR; ie, bone growth rate) of ectopic bone.\textsuperscript{26} This technique provides quantitative data on the rate of osteoid deposition, the unmineralized portion of bone, which is deposited by osteoblast prior to mature bone formation.\textsuperscript{1,26} In a study conducted by Isaacson et al, ectopic bone grew on average \( \pm 1.7 \times \) faster than the known standard for nonpathological tissue in combat-injured service members who experience blast-related trauma.\textsuperscript{26} To date, no study has investigated the MAR of ectopic bone that was resulted from TJR surgery. Similarly, only two studies, both from our group, have characterized HO bone architecture using scanning electron microscopy (SEM) and backscattered electron imaging (BSE).\textsuperscript{1,26} There is reason to believe that ectopic bone morphology, and growth rates may differ between patient populations given the induction mechanism and age of the patients—as not all HO processes are physiologically or histopathologically identical.\textsuperscript{1,12}

Furthermore, when symptomatic HO occurs following TJR, determining a period to excise the symptomatic mass remains a critically unresolved issue. The general consensus is that ectopic bone should not be removed until the mass has fully matured, as confirmed by radiographic evidence,\textsuperscript{18,27-29} and/or until the patients have demonstrated normalized serum alkaline phosphatase levels.\textsuperscript{30,31} While early resection has generally been promising, there is still uncertainty regarding surgical timing and recurrence,\textsuperscript{32} with some reports indicating that premature removal can result in as high as 100% recurrence in some cases.\textsuperscript{33} 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 military healthcare system patients treated for ectopic bone following THA or TKA. The goals of this study were (1) to use advanced histological techniques to quantify the rate of ectopic bone growth and (2) to compare HO bone morphology with a previous study by our team that investigated soldiers injured in combat\textsuperscript{26} to understand the differences of bone architecture and provide general clinical management recommendations for the military and general population.

### Methods and Materials

Patients treated between the periods of June 2012 and March 2015 with symptomatic HO following TJR were included in this institutional review board-approved study. The research was conducted in accordance with the principles of the Declaration of Helsinki. Patient enrollment, recruitment, and treatment were adhered to Isaacson et al’s protocol developed for wounded warfighters.\textsuperscript{26} In short, participants were identified by physician referrals, and once the determination was made that symptomatic HO required excision, subjects gave their written, informed consent, and were given oxytetracycline (250 mg/tid) on four separate dates prior to their scheduled surgery to determine their MAR. Tetracycline was used as the fluorochrome-labeling agent since it is a commonly prescribed antibiotic and has been extensively used in bone research.\textsuperscript{26,34,35} 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. HO excision was determined based on the surgeon’s clinical judgment and was not influenced by oxytetracycline dosing schedules. Patient’s radiographic data were blinded and reviewed by an attending orthopedic surgeon and the coauthor (BKP) to assess the HO severity prior to resection as 0%, none; <25%, mild; 25%–50%, moderate; and >50%, severe.\textsuperscript{36}

Following HO excision, samples were deidentified, photographed, radiographed, fixed in formalin, dehydrated in ascending grades of ethanol, and embedded in polymethyl methacrylate according to the standard laboratory procedures.\textsuperscript{37,38} Samples were analyzed for MAR, SEM, and light microscopy, as previously described by Isaacson et al.\textsuperscript{26} Demographic information was captured for each participant using the local electronic medical record systems and included gender, age, height, weight, date of initial and excision surgeries, and HO anatomical location.

### Results

Three patients were enrolled in this research study and experienced symptomatic HO following TJR (two cases of TKA and one of THA). Two subjects were male and one was female. The average and standard deviation of basic demographic information included height of 69.0 \( \pm 7.8 \) inches, weight of 237.7 \( \pm 28.3 \) pounds, and age of 61 \( \pm 7 \) years at the time of HO excision. Ectopic bone was graded as mild in all the cases, but required removal due to limited range of motion and pain. HO appeared to be mature and in a quiescent state based on radiographic images at the time of surgical intervention, and there was no sign of recurrence three months postoperatively.

Histological data indicated that MAR levels were 1.7 times higher than nonpathological human bone at the time of surgical intervention (1.7 \( \pm 0.7 \) \( \mu \)m/day; range: 1.3–2.6 \( \mu \)m/day) compared to the known 1.0 \( \mu \)m/day of nonpathological human bone.\textsuperscript{39} The amount of single and double labels calculated during fluorochrome labeling was 2.0 \( \pm 2.0 \) and 1.3 \( \pm 0.6 \), respectively; these values were substantially lower than ectopic bone observed in our previous study of soldiers who experienced traumatic combat-related injuries.\textsuperscript{26} Scanning electron microscopic and light microscopic images showed that HO had a trabecular structure with bone chips and varying degrees of mineralization (Figs. 1–4). Although the HO samples appeared radiographically mature at the time of surgery and the masses had ceased growing, several of the excised samples demonstrated areas of osteoclastic resorption at the outer boundaries, indicating that active remodeling was still occurring.
Discussion

The HO bone specimens analyzed in our patients following TJR consisted of trabecular bone with a mean MAR of 1.7 µm/day. This bone growth rate was the same as the warfighters previously studied by our team; however, one striking difference was the type of bone observed. More specifically, the HO samples investigated following blast-related injuries in combatants consisted of cortical, cancellous, and woven bones, with an average of 270 ± 280 single and 365 ± 371 double labels. The veterans observed in this study only demonstrated trabecular bone, and single and double labels were substantially lower at 2.0 ± 2.0 and 1.3 ± 0.6, respectively. These differences may be attributed to several factors that include the subject’s age (young vs. elder), traumatic insult (trauma vs. postoperative complication), and prophylactic treatments (used in younger soldiers, but not in elderly TJR patients). However, we believe that the most likely driver for the differences in bone types may be due to the intensity of the traumatic injury. Improvised explosive devices (IEDs) used in theater cause extensive polytrauma, which has the potential to displace microscopic 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 include hypoxia inducible factor alpha, vascular endothelial growth factor, transforming growth factor beta, and fibroblast growth factor. 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 combatants (1.7 µm/day) also represents a unique and previously unreported finding. Because data herein are only a case study, it may be possible that the mean HO growth may normalize around 1.7 µm/day across all the disease states, may be attributed to the limited sample size, or this similarity may be due to differences in vasculature between cancellous and cortical bones. As mentioned earlier, wounded soldiers demonstrated a hybrid of cortical, cancellous, and woven bones, whereas the veterans in this study only demonstrated trabecular bone. Trabecular bone porosity is reported to be between 50% and 95%, whereas haversian canals that provide perfusion to cortical bone comprise only 5%–10% of the cross-sectional area. Given the differences in bone types, volume, and anatomical location where HO developed (in the warfighters, it was mainly located in the musculature adjacent to long bones as compared to the periarticular region for veterans), samples tended to be larger for those with combat injuries.

Figure 1. (A) BSE micrograph from Patient 1’s resected HO. The dark gray areas (yellow arrows) of bone indicate the recent remodeling activity. (B) BSE micrograph showing a cluster of phosphorus (white) particles in the outer region of the specimen (red arrow), which likely occurred during the removal as no foreign body responses were noted during light microscopy.

Note: Gray represents bone and black represents pore space and soft tissue.

Figure 2. BSE micrograph from Patient 2’s HO. The bone resorption (yellow arrows) at the outer boundaries and bone fragment (red arrow) within the ectopic bone are to be noted.
Limitations
Although this study provides unique insights regarding the MAR of ectopic bone following TJR, the principle limitation is the small sample size that 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.

Conclusions
TJR is a valuable surgical alternative for persons with limited range of motion or pain due to degenerative, inflammatory, or post-traumatic arthritis. However, ectopic bone has been noted to occur following this procedure, and there are limited associations between the histology and clinical predictors to decide when surgery is most appropriate. Data from this study demonstrated that HO grew ~1.7× faster than nonpathological human bone and the type of HO bone may vary based on the patient population and injury mechanism. After further investigation, the rate of bone ectopic bone growth (ie, MAR) may be a predictor when deciding when to excise HO.

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.

Author Contributions
Conceived and designed the experiments: BI. Analyzed the data: BI, BP, TS, PP, RB, RE, BK. Wrote the first draft of the manuscript: BI. Contributed to the writing of the manuscript: BI, BP, RB, PP, RE, BK, TS. Agree with manuscript results and conclusions: BI, BP, RB, PP, RE, BK, TS. Jointly developed the structure and arguments for the paper: BI, BP, RB, PP, RE, BK, TS. Made critical revisions and

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

Figure 4. Light microscopic image from Patient 3 demonstrating that HO was (A) in a quiescent state (yellow arrow) and (B) bone chips were observed (red arrows).
Note: Pink represents bone, blue represents tissue/cells, and white represents pore space.
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REFERENCES


