Assessment of Severe Extremity Wound Bioburden at the Time of Definitive Wound Closure or Coverage: Correlation With Subsequent Postclosure Deep Wound Infection (Bioburden Study)

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Summary: Infection remains the most common and significant complication after high-energy fractures. The Bioburden Study is a multicenter, prospective, observational cohort study of wound bacterial bioburden and antibiotic care in severe open lower extremity fractures. The aims of this study are to (1) characterize the contemporary extremity wound "bioburden" at the time of definitive wound closure; (2) determine the concordance between polymerase chain reaction results and hospital microbiology; (3) determine, among those who develop deep infections, the concordance between the pathogens at wound closure and at deep infection; and (4) compare the probability of deep infection between those who did and did not receive an appropriate course of antibiotics based on bioburden at the time of wound closure. To address these aims, sites collected tissue samples from severe lower extremity injuries at the time of wound closure and at first surgery for treatment of a deep infection, nonunion, flap failure, amputation, or other complications (because these surgeries may be due to undetected infection). Otherwise, if no further surgical treatment occurred, participants were followed for 12 months. The study was conducted at 38 US trauma centers and has enrolled 655 participants aged 18–64 years. This is the first large multi-institutional study evaluating the wound bioburden of severe open tibia fractures and correlating this bioburden with the risk of wound complications after definitive soft tissue closure.

Key Words: infection, antibiotic therapy, open fracture wound colonization, biofilm, hospital microbiology techniques, genomic sequencing, open tibia fractures

(*J Orthop Trauma* 2017;31:S3–S9)

BACKGROUND AND RATIONALE

Infection remains the most common and significant complication after high-energy fractures, with rates ranging from 15% to 40%.[1–4] The large traumatic wounds associated with these fractures are inoculated with environmental bacteria at the time of injury and then exposed to host and hospital flora colonization during the initial course of treatment.[5] To counter these bacteria, patients are typically exposed to a short burst of antibiotics in the emergency department or as close to injury as possible as well as at the time of definitive wound coverage. At present, antibiotics are delivered in an empiric fashion, as surgeons often do not know the bacterial profile of the open fracture wound at the time of closure. In addition, this lack of data at final closure makes it difficult to know if the wound bioburden at this time is associated with a subsequent deep infection. Better data are required to refine our current treatment strategies.

The Lower Extremity Assessment Project study, reported that the rehospitalization rate for complications after severe open fractures is as high as 57% and one of the major drivers of hospital readmission is deep infection.[2,6] Furthermore, deep infections after open fracture have been found to develop in up to 15% of recent combat casualties.[3] Despite significant knowledge gained about the consequences of infection after major trauma, prevention efforts have lagged behind. In contrast to significant advances made in the reconstruction of the fracture and the soft tissue injury, the strategies used to prevent deep infection after severe open fracture wounds have remained constant for many years. Wounds are debrided on the day of injury and as needed thereafter, until a stable wound bed is obtained. All necrotic tissue and

Accepted for publication January 13, 2017.

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Supported by DOD OETRP W81XWH-09-20108.

The authors report no conflict of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jorthotrauma.com).

ClinicalTrials.gov: NCT01496014.

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DOI: 10.1097/BOT.0000000000000805

organic and inorganic contaminants are removed. Systemic antibiotics are administered during the perioperative period. The antibiotic type, dosing, and duration of treatment varies with surgeon, hospital, and region. The wounds are closed or covered when deemed "ready" by the surgeon. At the time of wound closure, antibiotics are typically readministered for 1–14 days based on surgeon or institution preferences. Because of the unreliable results from the routine microbiology at predicting subsequent failure due to infection, few surgeons sample the wound bioburden at the time of closure.[3,7,8]

Evidence supports the initial administration of systemic antibiotics as effective in reducing the infection rate in open fracture care.[1,9,10] Tissues in the zone of injury are often hypoperfused, and the antibiotic concentration in the critically injured tissues is often less than the desired therapeutic level.[11] The wound bacteria can attach to soft tissues, bone, or metal implants and initiate biofilm production.[12] Biofilm colonies are increasingly recognized as the likely source of chronic and implant-related infections.[12] Traditional antibiotics are unable to penetrate the biofilm and thus multicomponent therapies that prevent biofilm development are needed.[13]

To address the challenges noted above, many surgeons use various local adjunctive antibiotic therapies in an effort to "sterilize" the wound. Antibiotics are combined with polymethyl methacrylate cement or bioabsorbable ceramics and placed into the wound area as beads, which can effectively deliver high concentrations of antibiotics to the local tissues with no appreciable systemic antibiotic exposure.[1,9,11]

At present, surgeons administer open fracture antibiotics based on national guidelines[14] or the historic deep infection profile at the treating center, without knowing the actual pathogen profile in the individual patient's wound. The surgeon rarely samples the severe wound at the time of initial closure for pathogen identification and less often delivers a therapeutic course of antibiotics that are specifically targeted to the dominant bacteria during the index hospitalization. If a deep infection develops (which can occur as often as 15%–40% of the time), deep tissue cultures are obtained and directed systemic therapy is provided based on the pathogen's susceptibility profile.

Standard microbiological techniques for open wound samples are considered unreliable by most surgeons and, therefore, often do not influence patient care decisions.[8] Wound surface samples obtained during the course of antibiotic therapy may falsely report "no growth" in cases where the pathogens were suppressed, but not eliminated by the treatment, or report the presence of readily culturable bacteria that is the principal agent of the later infection.

Molecular diagnostic methods [including polymerase chain reaction (PCR) techniques] offer an advantage over standard microbiology cultures in that they have been shown to identify the presence of bacteria that might not be detected by traditional culture techniques. Standardizing extremity wound sampling techniques along with the addition of enhanced molecular diagnostic methods to the wound bioburden analysis could enhance our ability to define the magnitude of the wound bioburden and the relationship of subsequent infections to the initial bioburden screen at the time of wound closure.[15,16]

METHODS: TRIAL DESIGN, PARTICIPANTS, AND INTERVENTION

The Bioburden Study is a multicenter, prospective, observational cohort study of wound bacterial bioburden and antibiotic care in severe open lower extremity fractures. The aims of this study are to (1) characterize the contemporary extremity wound "bioburden" at the time of definitive wound closure; (2) determine the concordance between PCR results with those obtained from hospital microbiology; (3) determine, among those who develop deep infections, the concordance between the pathogens at wound closure and at deep infection; and (4) compare the probability of deep infection between those who did and did not receive an appropriate course of antibiotics based on bioburden at the time of wound closure. To address these aims, sites collected tissue samples from lower extremity injuries at the time of wound closure and at first follow-up requiring operative treatment. A central laboratory used PCR and standard tissue culture microbiology techniques to assess wound bacterial bioburden. Enrolled patients were prospectively followed until the first surgical procedure performed after definitive wound closure or for 12 months, if no further surgical treatment was documented.

The study was conducted at 38 US trauma centers participating in the Major Extremity Trauma Research Consortium (METRC).[17] The list of participating centers can be found in Appendix 1. The study protocol, including the written informed consent form, was approved by the Johns Hopkins Bloomberg School of Public Health (location of the METRC Coordinating Center), the Department of Defense Human Research Protection Office (DoD HRPO, study sponsor), and the local institutional review board at each participating center. Furthermore, each site was required to obtain DoD HRPO approval of local institutional review board documents and certification by the Coordinating Center to ensure proper training on study procedures and data collection before the initiation of the study.

Participants

The study population consisted of patients aged 18–64 years with a Gustilo type III open tibia fracture or a traumatic amputation of the tibia that required repeat debridement before a definitive wound closure. Detailed inclusion and exclusion criteria are summarized in Table 1. Patients were not excluded based on having other fractures, risk factors for infection, other infections, or traumatic brain injury. Furthermore, initial treatment at an outside facility was not a basis for exclusion nor was the fixation method or treatment with a fasciotomy. The study injury for patients with multiple qualifying injuries was defined as the injury with the most severe soft tissue damage in the opinion of the treating surgeon.

Patients were screened before definitive wound closure and those confirmed eligible were approached to provide informed consent to participate in the study. In addition, enrolled participants were approached for consent to allow a portion of tissue samples to be stored for future genomic studies. METRC has adopted a comprehensive informed consent process for all of its studies that involve the treating surgeon, the clinical site research coordinator, and material and resources for patients and family members to facilitate

TABLE 1. Inclusion and Exclusion Criteria for the Bioburden Study

Inclusion Criteria	Exclusion Criteria
Patients aged 18–64	Patient speaks neither English nor Spanish
Injury meeting at least one of the following criteria:	Patients likely to have severe problems maintaining follow-up, including:
Gustilo type III tibia fractures (plateau, shaft, and pilon) requiring a second procedure for wound closure after fixation	Diagnosed with a severe psychiatric condition
	Intellectually challenged without adequate family support
	Living outside the hospital's catchment area
Traumatic transtibial amputations requiring delayed primary closure, skin grafting, and/or flap coverage.	Planning to follow-up at another medical center
	Being a prisoner
	Being homeless

informed decision-making about participation. Details of this process are described in **Supplemental Digital Content 1** (see **Figure**, http://links.lww.com/BOT/A888). A legally authorized representative was permitted to consent on behalf of patients who were unable to do so before definitive wound closure. All screened and enrolled patients had inclusion and exclusion documented in REDCap,[18] the web-based, distributed data collection system used for all METRC studies.

Intervention

This is an observational study and as such, it did not have study treatments. All patients were treated per the participating center's standard of care for their injuries. The key study procedure involved the surgical collection of wound samples during the final soft tissue closure surgery and during the first follow-up surgical treatment of the study injury, if it occurred within the 12-month study period.

Protocol Changes

Two protocol changes were initiated after the first study enrollment. The first change increased the maximum number of enrolled patients because the study experienced lower than expected complication rates and greater heterogeneity in complications, with fewer coming from infections than originally expected. The second change replaced specific partners and technologies named in the protocol with generic equivalents to allow the use of state-of-the-art technologies unavailable at the beginning of the study. Neither amendment altered the patient risk profile or consent forms.

METHODS: DATA COLLECTION

Baseline and Follow-Up Data

Data collection for the Bioburden Study consisted of 3 primary activities: medical record review, patient interviews, and tissue sampling and evaluation (Fig. 1). All 3 of these tasks were done at the index hospitalization to record details regarding the patient's medical history, injury attributes, and treatment characteristics. Tissue sampling was performed during the surgical procedure for final soft tissue closure using a standardized collection technique. In addition to any standard of care microbiology cultures at the local institution, the study samples were shipped to a central laboratory according to a protocol for processing using both standard microbiology culturing and PCR techniques. Details regarding tissue sampling and shipping procedures can be found in **Supplemental Digital Contents 2 and 3** (see **Figure**, http://links.lww.com/BOT/A889, http://links.lww.com/BOT/A890, respectively).

Critical to the success of the study was the prospective identification of rehospitalizations to collect follow-up tissue samples at the time of infection or revision surgery at the fracture site. As such, the study did not have a standardized study follow-up schedule after discharge from the definitive wound closure

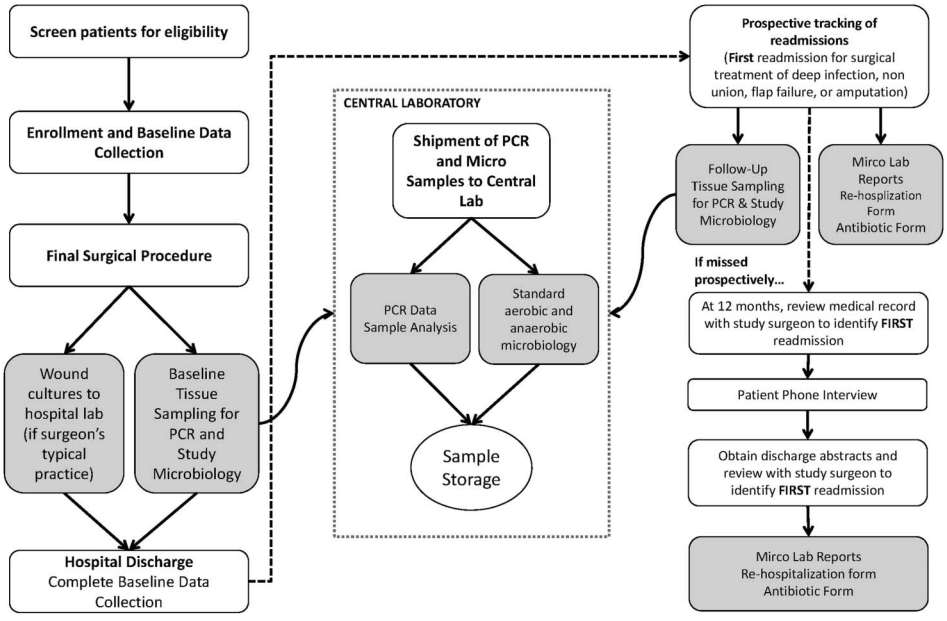

FIGURE 1. Bioburden study process flowchart.

hospitalization. Rather, patients were prospectively monitored for rehospitalization for deep infection, nonunion, flap failure, amputation, bone grafting, or any other complications that would necessitate opening the original study injury wound. In the event that a patient was rehospitalized in the 12 months after the injury, complications related to this event and treatment details were documented. If surgery was performed during this rehospitalization, a tissue sample (similar to those collected at baseline) was collected during the first surgical follow-up procedure.

To ensure all rehospitalizations due to complications were captured, medical records were reviewed, and participants for whom no rehospitalization was documented were contacted by phone to gather self-reported utilization in the year after the injury. Self-reported data were verified against medical record data to determine if any rehospitalizations were missing. The data collected at each timepoint are summarized in **Supplemental Digital Content 1** (see **Table**, http://links.lww.com/BOT/A893).

Primary Outcome: Deep Infection

The primary study outcome is the first surgery for treatment of a deep infection, which prompts the collection of a follow-up tissue sample. Also obtained were tissue samples from wounds at fracture sites that required surgical treatment of a nonunion, flap failure, amputation, or other complications because these surgeries may be due to undetected infection. The presence of a deep surgical site infection (SSI) was determined using the criteria of the Centers for Disease Control.[19] Deep SSI is defined as occurring within 30 days after the operation, if no implant is left in place, or within 1 year, if implant is in place and the infection appears to be related to the operation. In addition, the infection must involve deep soft tissues (eg, fascial and muscle layers) of the incision and at least one of the following: (1) purulent drainage from the deep incision; (2) a deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain, or tenderness; (3) an abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination; or (4) diagnosis of a deep SSI by a surgeon or attending physician.

Secondary Outcome: Appropriate Antibiotic Care

A secondary goal of this study is the classification of appropriate antibiotic care. As part of this study, information was collected on both antibiotic regimens and wound flora. An expert panel consisting of the study Principal Investigator, 2 additional orthopaedic trauma surgeons, and at least 3 infectious disease experts will be convened to develop a classification scheme for the most common and/or expected microbial species found in this study and the related antibiotic treatment regimens used in the initial care of these patients. Using the classification scheme, the expert panel will classify each patient as having received antibiotic care that was "appropriate" or "not appropriate." Appropriateness will be based on the selection of the antibiotic, the dosage, and the duration and assessed in the context of adherence to established clinical guidelines.[14]

Monitoring and Quality Assurance

Details of the METRC-wide standard operative procedures for monitoring can be found in **Supplemental Digital Content 4** (see **Figure**, http://links.lww.com/BOT/A891). The monitoring plan is designed to verify site compliance with the protocol, with study specific standard operative procedures on the data collection and procedures. The plan facilitates compliance with good clinical practice guidelines (5.18.1). An independent Data Safety Monitoring Board reviewed study progress, all reported complications, and serious adverse events during meetings held twice a year. The chair of the Data Safety Monitoring Board served as the medical monitor who reviewed each serious adverse event, as it was reported in real-time.

METHODS: DATA MANAGEMENT AND ANALYSIS

Data Management

Site research coordinators and clinical investigators collect data using paper case report forms designed specifically for this study and then entered REDCap. Details about data handling and data management can be found in **Supplemental Digital Content 5** (see **Figure**, http://links.lww.com/BOT/A892).

Data Analysis and Sample Size

Analyses will vary by aim. Exact and nonparametric hypothesis testing procedures will be used. Estimates and 95% confidence intervals will be reported. Multiple imputation will be used to handle missing baseline covariates but missing outcomes will not be imputed. In addition, sensitivity analyses will be conducted to evaluate the robustness of the study results to various untestable assumptions about the missing data mechanism.

For aim (1), both standard microbiology and PCR-based techniques will be used to characterize the contemporary extremity wound "bioburden" at the time of definitive wound closure. PCR-based analyses will focus on amplification and sequencing of 16s microbial rDNA, which has been widely used for phylogenetic analyses, as it is highly conserved across bacterial species.[20] Species level identification will be conducted based on genomic analyses of the hypervariable regions within this gene, using protocols developed as part of the Human Microbiome Project.[21]

For aim (2), the probability of concordance between PCR and standard microbiology techniques will be estimated, with concordance defined as agreement between the pathogens identified between the 2 technologies. A 95% exact lower confidence limit will be calculated for the probability of concordance using the Clopper–Pearson method. A simulation study indicates that if the true probability of concordance is 95%, then the proportion of lower confidence limits that are greater than 92.5% is greater than 80%.

Aim (3) focuses on patients who develop deep infections. Among these patients, the probability of concordance between the pathogens at wound closure and at deep infection will be

estimated. It is anticipated that 100 of the enrolled patients will develop a deep infection, based on grade III open tibia fracture rates from the previous studies.[2] With the anticipated sample size, a 95% confidence interval for the true probability of concordance whose width is no larger than 20% can be constructed.

Aim (4) is focused on comparing the probability of deep infection between 2 groups of patients: those who did and did not receive an appropriate course of antibiotics. Logistic regression will be used to estimate an adjusted (for baseline risk factors) odds ratio for deep infection between antibiotic subgroups. A panel of clinicians and infectious disease experts estimated 50% of enrolled patients would be treated by a course of antibiotics that is appropriate for the pathogens identified at the time of wound closure. For purposes of assessing the adequacy of the sample size, it was assumed that the antibiotic subgroups were comparable with respect to baseline risk factors. Under this assumption, the null hypothesis of no difference in the probability of deep infection between antibiotic subgroups would be tested using Fisher exact test. A simulation study indicates that the study has 80% power to detect an odds ratio for deep infection between these 2 groups of 1.55 or greater.

ENROLLMENT AND BASELINE PATIENT CHARACTERISTICS

The Bioburden Study enrolled patients between October 2011 and May 2015. A total of 1559 patients were screened for eligibility, and of these, 895 (57%) were eligible at the time of consent. Of those determined eligible, 699 (78%) were consented into the study and 655 (94%) had an initial tissue sample collected, confirmed eligible, and enrolled (Fig. 2). Forty-nine patients had no surgical treatment documented after wound closure and could not be contacted for their 12-month interviews.

Enrolled participants were aged 39 years on average and primarily male (79%), white (73%), and insured (75%). More than half (58%) of the cohort were current or former smokers, and the majority (84%) had none of the limited set of comorbidities most relevant to infection and other complications as study outcomes.

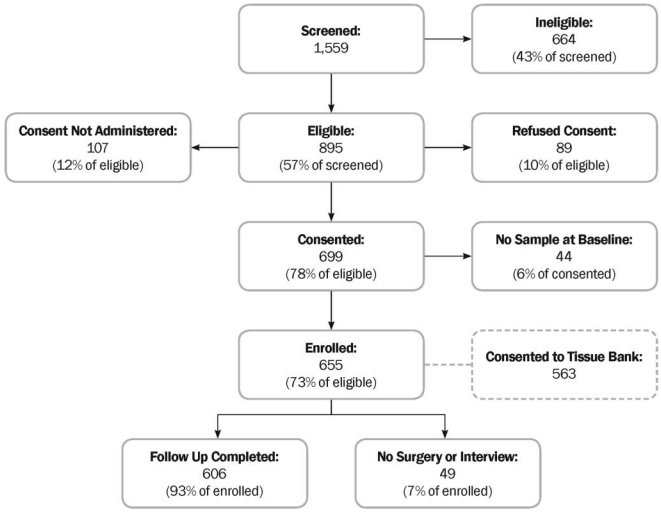

FIGURE 2. Bioburden enrollment summary.

DISCUSSION

This is the first large prospective multi-institutional study evaluating the wound bioburden of severe open tibia fractures and correlating this bioburden with the risk of wound complications after definitive soft tissue closure. A major strength of the study is the generalizability of results, given its inclusion of patients treated at 38 level 1 trauma centers from across the United States. An additional strength is the use of a centralized laboratory for both routine microbiology culture and PCR-based assays to evaluate wound bioburden, as standard microbiological techniques are not considered reliable by many clinicians. The use of a central laboratory ensures greater consistency in both standard culture and PCR techniques, regardless of the enrolling site of the patient.

There are several important limitations to the study design. First, the study is observational in which only data collection procedures were standardized. It did not prescribe treatment or postoperative care for the study injury; surgeons were allowed to deliver what they thought was the best possible care for the patient. This lack of standardization resulted in heterogeneity in the care patients received, and these differences could directly impact the study outcomes. A second limitation of the study is the lack of a fixed patient follow-up schedule to facilitate regular opportunities to check in with the patient after the definitive soft tissue closure procedure. While sites were instructed to carefully monitor hospital census logs and medical records to ensure that complications, which necessitated the collection of a tissue sample, were identified before any surgical treatment, some surgical procedures were inevitably missed. Regular follow-up visits may have eased the burden of prospective tracking and reduced the number of missed samples, but this would not fully eliminate the challenge in identifying complications in cases where treatment was sought at other non-METRC facilities. A final interview with patients for whom no rehospitalization was prospectively recorded identified the extent of missed infections and samples. Finally, tissue sampling techniques also are a potential limitation of this study. Although surgeons did receive instruction in when and how to collect tissue samples for the study, there may still have been natural variations in what organisms might be detected in microbiology cultures and PCR assays downstream. These variations could be due to the specific wound locations sampled, the relative abundance and distribution of organisms throughout the wound, the amount of tissue sampled, and subsequent shipping and handling procedures.

The Bioburden Study should provide important and clinically relevant information regarding the wound bioburden at the point of definitive soft tissue closure for severe open tibia fractures. Furthermore, it will document the breadth of potential pathogens that may be responsible for infection and other complications in this patient population after discharge and the extent to which infectious organisms correlate with what remains in the wound bed at soft tissue closure. Combined with extensive information regarding antibiotic

regimens used for prophylactic and continued treatment of wounds associated with these injuries, the Bioburden Study has the potential to provide clinicians important information that can guide choices in antibiotic selection for treating such injuries. This study will likely provide the foundation for future prospective randomized trials that would focus on patient-specific therapies to address the wound bioburden in open fracture care.

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