Clinical Validation of a Novel ELISA Serum Assay Test for Detection of Staphylococcus aureus Biofilm Antibodies in Serum of Orthopedic Trauma Patients

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Purpose: Postoperative wound infections are common after high-energy orthopaedic trauma and cause additional surgeries, increased length of hospital stay, escalated cost of care, and are associated with increased morbidity and even mortality. The most common pathogens for these infections are known to be Staphylococcus aureus (methicillin-resistant S. aureus [MRSA] and methicillin-sensitive S. aureus [MSSA]); however there is typically a lag time of many weeks between the index surgery and the development of clinical symptoms. This delay allows for biofilm growth and spread of infection and makes treatment with surgery necessary in an attempt to clear the infection. We have developed a novel blood test to detect infection early before it become clinically apparent and have validated this test in animal sera and human joint fluid. Our hypothesis was that our novel test could differentiate between orthopaedic trauma patients who were positive for S. aureus surgical site infection and those who were not.

Methods: As part of a prospective trial to validate our novel serum test, patients (n = 72) were enrolled in a prospective study who had fractures treated operatively that were deemed to be at high risk of infection (open fractures, periprosthetic fractures, calcaneus, tibial plateau, and pilon fractures) or had known diagnosis of surgical site infection. 10-mL blood samples were collected from patients at 3 time points. From the larger sample of patients we selected a smaller subset of patients who were clinically determined to be infected and had blood samples that were drawn at or near the time of deep bone biopsies (<14 days). Biopsies were sent to Clinical Microbiology for culture and microbial identification. Sera samples were obtained from blood specimens by centrifugation (200g, 15 min) and then frozen at -20°C until use. Samples were blinded as to diagnosis of S. aureus infection (n = 7) versus controls that had no history of infection (n = 4) and tested. All samples were tested blindly and in duplicate to verify accuracy. The novel test utilizes ELISA (enzyme-linked immunosorbent assay) to detect host antibodies in clinical sera samples against biofilm-specific antigens produced by S. aureus during biofilm-mediated infection. SACOL0688, a biofilm in vivo-expressed antigen, was used to capture host antibodies via ELISA.

Results: Our novel ELISA based test was able to differentiate with accuracy between patients with and without S. aureus infections up to 30 days preclinical and 30 days postclinical positive culture and determination of infection. Patients that expressed antibodies above 1.0 OD (optical density) were determined to be S. aureus infected and patients below 1.0 were considered negative. All patients with S. aureus culture positive infection (7/7) were positive, while all patients that were culture negative for S. aureus (0/4) were negative (Fisher's exact, P <0.003).

Conclusion: The results of our clinical validation test are very encouraging and it appears that we have developed a novel serum-based test that can detect antigens to S. aureus biofilm in patients' serum at the time of infection. This test may allow detection of infection prior to the infection becoming clinically apparent, perhaps allowing infections to be treated earlier before infection spreads more and even perhaps avoid surgery if antibiotics can be started before biofilms become so established that they must be treated surgically. The SACOL0688 antigen is presently being used to capture host IgG (immunoglobulin G) in a simple lateral flow assay that is fast (<10 min), inexpensive (<$10), and, based on animal studies and our validation data, very accurate. This appears to hold great promise and may have profound effects on the treatment of orthopaedic trauma patients.

See pages 49 - 106 for financial disclosure information.