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QUESTION 4: What is the role of quantitative evaluation (e.g., density of bacteria, cuti (propi) score) of positive cultures from the shoulder?

RECOMMENDATION: Semi-quantitative and quantitative reporting of bacterial culture results may have clinical utility for the diagnosis of shoulder periprosthetic joint infection (PJI) and may be used to interpret the relevance of positive cultures.

LEVEL OF EVIDENCE: Limited

DELEGATE VOTE: Agree: 96%, Disagree: 0%, Abstain: 4% (Unanimous, Strongest Consensus)

RATIONALE

Introduction

Approaches to quantifying the bacterial load at the time of revision shoulder arthroplasty.

Infection is an especially problematic and potentially devastating complication of elective major joint arthroplasty. There is extensive recent interest in PJI of knee and hip arthroplasty leading to protocols for prevention, evaluation and management of PJI. Investigation of PJI of the shoulder has lagged in part due to the limited numbers of primary shoulder arthroplasty cases, the relatively infrequent recognition of PJI, and the difficulty in applying the traditional criteria for hip and knee PJI to the shoulder due to the issue of "stealth" presentation of *Propionibacterium,* frequently occurring at times long after the index procedure.

The diagnosis and management of a prosthetic joint infection is dependent upon identifying the pathogen. Prior to the recognition of *Cutibacterium* as a definite pathogen, it was not uncommon for cases of shoulder PJI to be unrecognized. More recent studies have attempted to determine the optimal approach to evaluation of potential shoulder PJI. This includes specific approaches to specimen harvest, culturing method and culture observation appropriate for identifying *Cutibacterium.*

While the results of a specimen culture are often reported as being "positive" or "negative," it is now apparent that the degree of positivity – that is the number of bacteria in the specimen – can vary widely. Quantitative cultures have been used by clinicians to estimate the threshold above which the bacterial burden will likely be of clinical significance [1]. Low levels of bacterial growth from a specimen may be of less clinical significance than high levels. In determining the clinical importance of any level of bacterial growth, it is also important to know the degree to which control specimens (i.e., a sterile sponge opened in the operating room (OR) without contact with the patient's tissue) demonstrate bacterial growth [2,3].

Quantitative culture results have been used to evaluate wound infection, urinary tract infections and bronchial brushings. In the case of urine the actual colony count of a urine specimen is necessary (one colony equals one colony-forming unit or CFU) and a positive culture with 100,000 CFU is considered to be indicative of a urinary tract infection [4]. A number of studies have investigated the relevance of bacterial count to wound healing. Bacterial counts above 10,000 to 100,000 are thought to be indicative of infection and delayed healing [5]. More recent work supports this concept but suggests that there is little to no benefit of quantitative biopsy analyses or quantitative wound surface cultures, with several studies finding a low correlation of culture to infection. The problem with any threshold, such as 100,000 CFU is that there can be no clinically significant difference between a count of 100,010 and 99,990.

Most standard bacterial cultures are evaluated using a semi-quantitative technique in which cultures are inoculated onto medium using a sterile loop that sequentially dilutes the specimen from the first area or quadrant of the medium to the last area or quadrant. Results are often reported as 1+, 2+, 3+ or 4+ (or as text, using such terms as "trace," "few," "moderate" or "abundant"), depending on which areas or quadrants demonstrate bacterial growth [1,6,7].

Bacterial load, the virulence of the organism, variations in host response and wound environment all may contribute to determining the effect of the bacteria in the wound. Despite this, the literature on shoulder PJI suggests wide variability in culture practice and rarely considers semi-quantitative or quantitative culture results [8]. The purpose of this systematic review was to identify information regarding quantitative evaluation of bacterial cultures and to relate this to the evaluation and management of shoulder PJI.

Methods

A Scopus search was performed with the query "(shoulder OR "upper extremity") AND (arthroplasty OR replacement OR revision) AND (culture OR microbiologic OR microbiology)." The resulting titles, abstracts and full text (127) from this query were reviewed for relevance to the question of number of samples for culture, specimen type and anatomic locations. All pertinent articles were then fully reviewed and any other pertinent citations in these gathered articles were obtained and reviewed. Based upon the findings of this review and review of the manuscript reference lists, an additional

search was performed on PubMed using the term "quantitative culture."

Results

The initial search identified 127 articles. After review of these articles, 11 were included in the final summary. Due to the nature of the available data, it was not possible to perform a meta-analysis. Thus, this is a narrative report of the findings.

Kallstrom, in a review article, discussed the role of quantitative cultures in determining if a nonhealing wound is infected [1]. Despite early work that emphasized the importance of quantitative wound tissue cultures, the current thought is that there is little to no benefit of quantitative biopsy analyses or quantitative wound surface cultures, with several studies finding a low correlation of culture to infection. Quantitative wound cultures of tissue is challenging, as the tissue must be accurately weighed, homogenized and serially diluted prior to inoculation of media for each dilution under aerobic and anaerobic conditions. Variations in biopsy collection processing and inoculation can often confuse the interpretation of quantitative wound culture results. The delay in reporting results from quantitative cultures makes clinical management difficult, so direct Gram staining has been used as a surrogate to determine bacterial loads in wounds. Early advocates of quantitative wound cultures were correct in realizing that clinical infection was influenced by an imbalance in the bacterial load, variations in the host response and wound type.

Ashan et al. studied a cohort of 137 patients who underwent revision shoulder arthroplasty and had at least one positive culture [6]. The subjects all had pain, stiffness or component loosening but did not have obvious clinical evidence of infection. The authors excluded subjects that did not have at least four culture specimens. The focus of the study was to use the semi-quantitative culture results to determine a measure of bacterial burden specific to *C. acnes*. They assigned numerical values (Specimen Propi Values) to the semi-quantitative Propionibacterium (now *Cutibacterium*) culture results: 0.1 (broth only), 0.1 (1 colony), 1, 2, 3, and 4 (1+, 2+, 3+, or 4+, respectively) and referred to this number as the "degree of positivity" for each specimen with the idea that this value "roughly" reflected the amount of bacterial growth [9]. They also calculated the sum for each type of specimen (humeral stem explant, humeral head explant, glenoid explant, collar membrane [between the modular head and stem], humeral membrane [between the humeral stem and humeral bone], other soft tissue, fluid, or "other") from each shoulder. The Specimen Propi Values for all of the specimens from a particular shoulder were summed to derive the Shoulder Propi Score for that shoulder. In order to account for the number of culture specimens in each case they calculated the Average Shoulder Propi Score, which they defined as the Shoulder Propi Score divided by the total number of specimens from that shoulder submitted for culture.

They reported that the average Specimen Propi Value for fluid (0.35 ± 0.89) was significantly lower than that for soft tissue (0.92 ± 1.50) and explant specimens (0.66 ± 0.90) ($p < 0.001$). Men had a significantly higher mean Shoulder Propi Score (3.56 ± 3.74) than women (1.22 ± 3.11) ($p < 0.001$), and men had a significantly higher Average Shoulder Propi Score (0.53 ± 0.51) than women (0.19 ± 0.43) ($p < 0.001$). Patient age did not have a significant effect on either score.

They further reported that, although the Shoulder Propi Score and Average Shoulder Propi Score varied among the shoulders that were culture-positive for Propionibacterium (now *Cutibacterium*), they could not identify a clear threshold above which they could be confident that a positive culture result represented a clinical infection, as opposed to contamination or commensal presence of an organism. The findings of this study clearly demonstrate that the identification of *C. acnes* is highly dependent upon the source of the culture specimen. The findings of this work have limitations, because the authors did not clearly determine what level of *C. acnes* burden constitutes a periprosthetic infection. Thus, true the value of semi-quantitative reporting of cultures is not clearly delineated. However, if one considers that the clinical manifestations of an infection are the result of an interaction between a host and a pathogen, then it is logical to consider that the amount of bacterial burden is important.

In a separate publication, Hsu and co-workers studied the results of epidermal, dermal and deep cultures obtained from subjects undergoing revision shoulder arthroplasty [7]. Based upon their data, they calculated that four different specimens would need to be cultured to have a 95% chance of detecting the organism and that, in order to achieve 95% of the positive cultures, the cultures need to be held for at least 14 days.

Carli et al. studied a mouse model of acute periprosthetic knee infection [10]. The experimental animals were inoculated with *S. aureus*. The infected animals demonstrated clinical signs of infection with impaired gait, implant loosening and elevated inflammatory markers. Viable *S. aureus* was quantified from the retrieved implant surfaces, and the infected animals had greater than 10^6 CFUs at 2 weeks and greater than 10^5 CFUs at 6 weeks.

Esteban et al. used quantitative culture analysis to study cases of PJI in which antibiotic loaded cement spacer was used during two-stage revision reconstruction [11]. Culture specimens were obtained from sonicated implants. Infection was defined by having one of the following criteria: (1) fistulae or wound dehiscence at the time of the second-stage surgery, (2) persistent pain around the joint associated with elevated C-reactive protein or (3) clinical appearance of infected tissue during surgery according to the surgeon. Thirteen of 50 specimens had positive sonicate cultures, 9 from infected cases and 4 from non-infected ones ($p = 0.001$, Fisher's exact test). The presence of high colony counts or a different isolate individually showed a strong statistical association with infection.

Grosso et al. studied implant sonication culture for the diagnosis of shoulder periprosthetic infection [12]. They defined infection according to their published guidelines that included four groups: definite infection, probable infection, probable contaminant or no evidence for infection. Their culture technique report quantified the number of CFUs for each specimen. Prior work by Trampuz et al. suggested that sonication fluid cultures of hip and knee arthroplasty implants had greater sensitivity than periprosthetic tissue cultures [13]. In contrast, Grosso et al. reported that there was no significant benefit to the shoulder implant sonication culture technique compared with standard intraoperative cultures. Using the cutoff value of > 20 CFU/mL to exclude contaminants, implant sonication culture had a low sensitivity (56%) but high specificity (93%). While without a cutoff value, implant sonication culture had a high sensitivity (96%) but low specificity (64%). Standard intraoperative cultures (tissue and fluid) had a better overall performance compared with the cutoff and non-cutoff sonication results.

Piper et al. also studied the role of sonication of shoulder implants and evaluated the relevance of quantitative reporting of the culture results [14]. In their previous work on hip and knee implants, they used a cutoff of 5 CFU per plate of sonicated fluid culture. In the study of shoulder implants they found that a cutoff of 20 CFU per plate with concentrated sonicate fluid resulted in a sensitivity and a specificity similar to those in their hip and knee work. In contrast to Grosso et al., they concluded that sonicate fluid culture is useful for diagnosing shoulder PJI.

Discussion

The clinical manifestations of an infection are the results of the interaction between a pathogen and the host. Kravitz wrote that "we

think of infection in terms of bioburden, which refers to the presence of bacteria in a wound and the number of microorganisms that contaminate an object" and subdivided bioburden into 4 categories: (1) contamination-bacteria within a wound without host reaction, (2) colonization-bacteria within the wound that multiply or initiate a host reaction, (3) critical colonization-bacteria that multiply to cause a delay in wound healing, often with increased pain but not with an acute host reaction and (4) infection-bacteria that multiply and cause a host reaction [15]. It seems logical that the presence of greater numbers of bacteria would correlate with the presence and severity of a periprosthetic shoulder infection. The results of this systematic review point out the paucity of available information, knowledge and understanding of the role of quantitative culture in the evaluation and management of shoulder PJI.

The limited data available suggests that standard fluid and tissue cultures are better than sonication cultures for diagnosis of shoulder PJI. However, there is insufficient experience and study of this technique to make definitive evidenced based recommendations. From a practical standpoint sonication is not readily available in all institutions. However, it seems that if sonication is used the quantitative culture results should reported.

New culture independent techniques and assays employed to identify the presence of bacteria including polymerase chain reaction, next generation sequencing and labeling techniques hold promise to aid both in the actual diagnosis of shoulder PJI as well as reduce the time to diagnosis. Nevertheless, the results of culture remain an important means to identify and characterize pathogenic microorganisms, to determine antibiotic susceptibility and to confirm the results of culture-independent methods. Previous experience demonstrates that the actual presence of bacteria does not always correlate with clinical manifestations of infection and that a number of pathogen and host factors must be considered in the diagnosis and management of shoulder PJI.

In summary, the results of prior studies in other specialties suggest that determining bacterial load with semi-quantitative and quantitative culture assessment in shoulder arthroplasty is of value in the evaluation and management of cases in which PJI is suspected. The application of these semi-quantitative and quantitative culture results to the evaluation of a failed shoulder arthroplasty requires (1) a standardized approach to harvesting specimens (source, number and technique), (2) using standardized culturing protocols designed to detect the presence of *Cutibacterium*, (3) standardized approach to reporting of the semi-quantitative or quantitative results and (4) documentation of the semi-quantitative or quantitative results of control specimens from the OR that have not been in contact with the patient.

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2.2. DIAGNOSIS: CULTURE TECHNIQUE

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QUESTION 1: What is the optimal culture technique (e.g., culture medium, days of incubation) in evaluating patients for shoulder periprosthetic joint infection (PJI)?

RECOMMENDATION: Current evidence suggests that culture of tissue samples for the diagnosis of shoulder PJI is best performed using both aerobic and anaerobic conditions. For solid culture media, diagnostic accuracy may be improved by using enrichment media. Fourteen days is the most common culture duration cited.

LEVEL OF EVIDENCE: Limited

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)