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QUESTION 4: Does the type of organism (i.e., fungi, *C. acnes*, *S. aureus*) influence the thresholds for serum and synovial markers in acute and chronic periprosthetic joint infection (PJI)?

RECOMMENDATION: Yes. Emerging data suggests that the type of organism influences the diagnostic thresholds for most serum and synovial biomarkers in the diagnosis of acute and chronic PJI.

LEVEL OF EVIDENCE: Moderate

DELEGATE VOTE: Agree: 94%, Disagree: 4%, Abstain: 2% (Super Majority, Strong Consensus)

RATIONALE

Diagnosis of PJI is currently a challenging process. There is no absolute diagnostic test and clinicians thus must rely on a combination of findings. The American Academy of Orthopaedic Surgeons (AAOS) [1,2] and the International Consensus Meeting (ICM) on PJI [3] currently recommend the serological markers of serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as the first line tests due to their reported high sensitivity in patients with suspected PJI. In addition, synovial white blood cell (WBC) counts, synovial polymorphonuclear percentage (PMN%) and leukocyte esterase (LE) will be frequently obtained, through aspiration, if there is high clinical suspicion for infection or if there is an elevation in the serological markers. Other serum and synovial biomarkers are used to make the diagnosis of PJI including serum interleukin-6 (IL-6), procalcitonin, D-dimer, tumor necrosis factor alpha (TNF-a), intercellular adhesion molecule-1 and lipopolysaccharide-binding protein. Synovial markers include WBC count, PMN%, CRP, IL-6, interleukin 8, LE and alpha-defensin, among others [4,5]. In general, synovial fluid biomarkers are considered to have superior accuracy when compared to serum biomarkers [6–9].

While each organism varies in virulence to elicit an inflammatory response, the aforementioned biomarkers are also dependent on the host's ability to mount a response [10] and recent studies have suggested that they may be influenced by a variety of factors, including the use of antibiotics [11].

While antibiotics can reduce the levels of these inflammatory markers, it is suspected that the infecting organism may influence the levels of these markers depending on the organism's ability to elicit an immune response in the host. Thus, low virulence organisms, such as *C. acnes* and coagulase-negative *Staphylococcus* (CNS) may demonstrate lower levels of inflammatory markers. If less-virulent organisms produce a less-robust inflammatory response, it is reasonable to expect that serum and synovial markers for inflammation may be lower as well and have a higher false negative rate when using traditional cutoffs for diagnosing PJI [12]. If this is the case, one would expect that differing thresholds are needed for diagnostic criteria. Two recently-published investigations highlight this issue. One study demonstrated that synovial CRP levels were dependent on the infecting organism and that false negative results were more likely for less virulent organisms such *as S. epidermidis* and yeast [13]. Another study reported that seronegative PJI was common with less-virulent infecting organism such as *Staphylococcus epidermidis, C. acnes, actinomyces, corynybacterium, candida* and *mycobacterium* [14].

Recent data from the Rothman Institute demonstrates that organism type does indeed influence serum and synovial biomarker levels [15]. The authors of the study performed a retrospective review of all PJI cases over a 15-year period to determine whether biomarker levels differ among organisms and to identify new cutoff values for biomarkers for each organism type. The results of the study found that more traditionally virulent organisms, such as resistant organisms or *S. aureus*, result in higher inflammatory markers while less virulent organisms and culture-negative cases demonstrated lower levels. The authors observed similar results for synovial markers, WBC and PMN%. Thus, the particular infecting organism influences the false negative rate and the levels of routine synovial and serum tests for diagnosing PJI. New cutoff values were determined for each biomarker predicting PJI and stratified by organism type. The values were variable and highly dependent on the organism. Thus, it is important to consider clinical suspicion for diagnosing PJI as the accuracy of serum and synovial inflammatory markers are dependent on the infecting organism. Of note, this is especially true for CNS and for culture-negative infections as serum ESR, CRP, synovial WBC and PMN% are generally much lower for these cases and thus have lower cutoff values. Given that the sensitivity is low for certain organisms, it is important for surgeons to be cognizant that there may be a higher rate of false negatives with certain organisms.

While the literature is marginal given the large sample size needed to stratify the accuracy of diagnostic laboratory values by organism, several studies have suggested that the sensitivity of diagnostic tests are dependent on the organism. Deirmengian et al. [13] demonstrated that the median synovial fluid CRP level was significantly lower for less-virulent organisms, when compared to those organisms classified as virulent (15.10 mg/L vs. 32.70 mg/L, $p < .0001$). Perez-Prieto et al. [16] also demonstrated that CRP and ESR may be falsely negative in up to 32% and 23% of PJIs, respectively. In this study, the clear majority of these patients' cultures grew low-virulence organisms, CNS, or *C. acnes*. Similarly, in our study [17] we found that inflammatory markers were lower in the serum in patients infected with less virulent organisms as well as in culture-negative cases.

Certain organisms may elicit a weak host response whereas others mount a much more robust response, which may help explain why the amount of gross purulence discovered intraoperatively may differ depending on the bacterial organism. A study by Alijanipour et al. [18] demonstrated that intraoperative purulence was more commonly found in PJI caused by *streptococcus* spp. (88%) and *S. aureus* (85%) compared with CNS (73%) and gram-negative bacteria (73%, $p = 0.04$). Although the orthopaedic literature does not have much discrete data on the effect of organism virulence on biomarker levels, we do see frequent implications of low virulence organisms, such as *C. acnes*, in shoulder arthroplasty infection. It has been shown that ESR and CRP have poor sensitivity to detect prosthetic shoulder infection when using previously-established cutoffs of 30 mm per hour or 10 mg/L, respectively [19]. This is presumably due to the low virulence of *C. acnes* and the need for optimized cutoff values for this particular organism implicated in prosthetic infections. Similarly, in our study we see that the biomarker sensitivities differ among organisms and thus optimal cutoff values vary based on the organism growing.

However, not all markers are affected by organism type. Neutrophils in the synovial fluid secrete specific proteins in response to infection. These proteins, such as alpha-defensin, have shown sensitivity and specificity above 96% for the diagnosis of PJI [6,20,21]. A large-scale study reviewed the results of 1,937 samples that simultaneously had a synovial fluid culture performed [8]. The organisms recovered from 244 alpha-

defensin positive, culture-positive fluids were recorded and grouped based on characteristics such as Gram stain, species, virulence, oral pathogenicity and source joint. Alpha-defensin negative samples served as uninfected controls. The alpha-defensin test for PJI was positive in the setting of a wide spectrum of organisms typically causing PJI. There was no difference in the magnitude of the alpha-defensin level regardless of Gram stain characteristics, specific organism, virulence, oral or non-oral pathogen or anatomic source. The test provides consistent results regardless of the organism type, Gram stain, species or virulence of the organism, and could be considered a standard diagnostic tool in the evaluation for PJI whenever synovial fluid is aspirated for a PJI work-up.

There is paucity of literature on fungal and acid-fast PJIs due to the rarity of such organisms. Fungal PJIs only represent 1% of PJIs [22]. Early knowledge of the microbe involved would aid in selecting appropriate antimicrobial therapy and would yield better treatment outcomes. The characteristics of systemic inflammatory markers in patients with fungal PJIs have not been fully assessed. In a single center review of 44 patients with culture-positive diagnosed fungal PJIs, the mean values for C-reactive protein and ESR were compared with 59 patients with bacterial PJI, including coagulase-negative *Staphylococcus* species, *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus species* [23]. The mean ESR for fungal and bacterial PJIs were 40 mm per hour (95% confidence interval (CI); 30, 50 mm per hour) and 41 mm per hour (95% CI 33, 49 mm per hr), respectively (p = 0.61). The mean CRP values for fungal and bacterial PJIs were 42 mg/l (95% CI 22, 62 mg/L) and 65 mg/L (95% CI 43, 88 mg/L), respectively (p = 0.42). Systemic inflammatory markers do not discriminate between bacterial and fungal infections. Due to the rare nature of fungal PJIs, multicenter collaborations are a possible research avenue to further study this question.

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