HK36: What is the diagnostic relevance of molecular techniques for polymicrobial periprosthetic joint infections?

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Response/Recommendation:

Molecular diagnostics can enhance the diagnosis of polymicrobial periprosthetic joint infections (PJI). Multiplex PCR (mPCR) and Next Generation Sequencing (NGS) are the two most extensively studied molecular diagnostic technologies in recent years. The average sensitivity of molecular diagnostics for diagnosing polymicrobial PJI is higher than that of conventional microbial culture; however, the average specificity is lower compared to culture. A combination of culture and molecular diagnostics is recommended when polymicrobial PJI is suspected, as originally diagnosing and treating polymicrobial PJIs as monomicrobial is associated with worse outcomes.

Level of Evidence: Limited

Rationale:

To answer the question above (HK36), we conducted a comprehensive literature review across two databases (PubMed and Cochrane Library), utilizing the MeSH terms developed by our librarians. After screening the potentially eligible studies, we included 1,315 studies for full review and data extraction; ultimately, 75 were selected.

Periprosthetic joint infections (PJI) are considered polymicrobial when at least two different organisms are isolated from synovial fluid or intraoperative tissue cultures (1).

Polymicrobial PJIs account for up to 46% of all PJIs (2) (3). Historically, polymicrobial PJIs have demonstrated a lower success rate than monomicrobial PJIs due to the increased morbidity, costs, and complications usually associated with modern polymicrobial PJI treatments (4). Understanding the microbiological profile of the causative organisms remains central to the treatment strategy, as several species of pathogens are notoriously difficult to eradicate since these pathogens reside in a biofilm rather than in a more metabolically active planktonic form.

There is no consensus on the typical microorganisms responsible for polymicrobial PJIs: Yapar et al. (5) averaged 2.4 microorganisms in their series of polymicrobial PJIs; in the same study, those authors reported *Staphylococcus epidermidis* and *Cutibacterium acnes* (well-known low-virulence bacteria) as the leading pathogens in polymicrobial PJIs. In contrast, Wouthuyzen-Bakker et al. (6), Löwik et al. (7), and Li et al. (1) suggested that additional organisms with relatively low or variable virulence (e.g., *Enterococcus spp.,* anaerobes) or high-virulence organisms (e.g., *Staphylococcus aureus*, gram-negative bacilli) were more often responsible for polymicrobial PJIs.

To counteract the high culture negativity rate (up to 41% in some reports)(8)(9), molecular diagnostic technologies (DNA- and RNA-based) have been recently introduced to the market to quickly identify the infecting microorganisms and their antibiotic resistance (10). Among them, multiplex polymerase chain reaction (mPCR) (11), next-generation sequencing (NGS) (12), including metagenomic NGS (mNGS) (13) (14) (15), showed the best accuracy in identifying polymicrobial PJIs.

The utility of broad-range PCR with Sanger sequencing was lower for polymicrobial infections than that with standard culture or NGS techniques, as the presence of multiple organisms results in overlapping reads that were difficult to interpret (16). Multiplex PCR sensitivity in diagnosing monomicrobial PJI ranged between 33 and 85% (17) (18), while its specificity ranged between 91% and 100% (19) (20); only a few authors (18) (19) supported the diagnostic role of mPCR in polymicrobial PJIs. Lausmann et al. (18) were able to identify additional microorganisms in 12.5% of their intraoperative samples from infected joints analyzed with a new generation mPCR system; Gardete-Hartmann (19) recommended mPCR as an intraoperative test at the time of a 2-stage revision to rule out an incomplete initial diagnosis of complex polymicrobial infection that was not initially captured by culture.

Several authors have compared standard microbial cultures with NGS in systematic reviews and meta-analyses. Kato et al. (21) examined the detection rates of pathogens between NGS and microbial cultures using synovial fluid samples taken from replaced joints; the detection rate for NGS was significantly higher than that for culture (OR 4.52). Different findings emerged from Kildow et al. (22): those authors analyzed samples from 116 patients suspected of having PJI. They demonstrated that NGS did not yield superior sensitivity or specificity results compared to culture. Hantouly et al. (23), in a systematic review and meta-analysis, compared the sensitivity and accuracy of NGS and

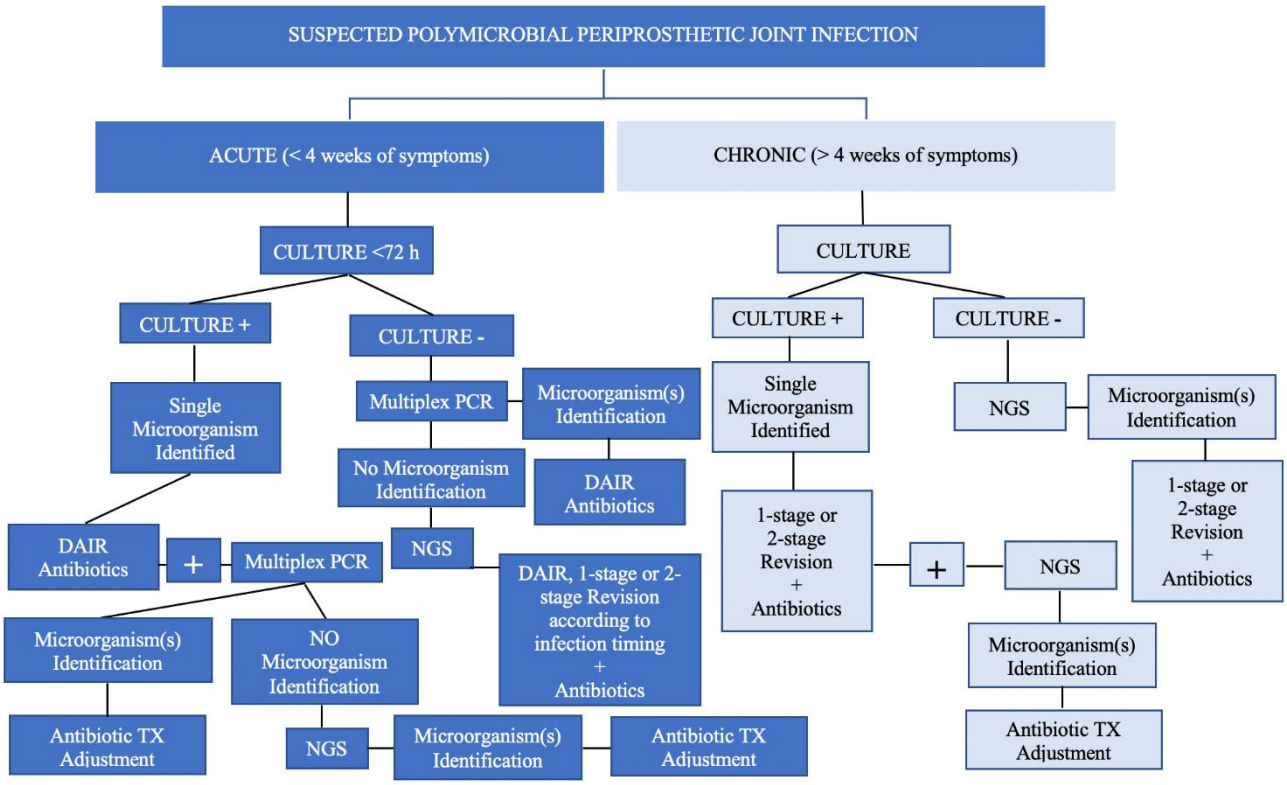
standard culture: data from 341 patients (seven studies) were reviewed, revealing an overall pooled sensitivity of NGS at 94%, while the pooled specificity was 89%; conversely, the pooled sensitivity of culture was 70%, and the specificity was 94%. In the same study (23), the accuracy of NGS and culture was reported as 91.9% and 80.5%, respectively. Unfortunately, most of these studies (18-20) did not assess the performance of molecular diagnostics in general or NGS specifically for diagnosing polymicrobial PJIs.

Mei et al. (15), in a mid-sized cohort study, were among the few to report that mNGS exhibited high sensitivity (85.7%), but moderate specificity (60%) and accuracy (65.2%) for the diagnosis of polymicrobial PJIs. Interestingly, in that study (15), the specificity, positive predictive value (PPV), and diagnostic accuracy of conventional microbial culture for diagnosing polymicrobial PJI were higher than those of mNGS; in contrast, the sensitivity and negative predictive value (NPV) of conventional microbial culture for diagnosing polymicrobial PJI were lower than those of mNGS. Goswami et al. (24) utilized NGS in a series of culture-negative PJIs: NGS was able to identify a pathogen in 65.9%; in the same study, NGS revealed a polymicrobial PJI in 91% of culture-negative PJIs.

In summary, it is recommended that different testing methods (standard culture and molecular diagnostics) be combined when a polymicrobial PJI is suspected. In clinical practice, the diagnosis of polymicrobial PJI made only by standard culture may be influenced by the concurrent use of antibiotics (25) and the competitive inhibition effects of multiple bacteria during culture (26).

Figure 1 proposes a diagnostic flow chart for the suspect of a polymicrobial PJI.

Figure 1: **Diagnostic flow chart for suspected polymicrobial PJI** when molecular techniques (multiplex PCR; NGS) are available at the treating Institution. The distinction between Acute and Chronic PJI has been made according to the 2018 ICM criteria (27). **Acute PJI:** Debridement, Antibiotics, and Retention of the Implant (DAIR) are recommended only if symptoms persist for less than four weeks with the possible identification of the microorganism; contraindications to DAIR also include the presence of a sinus tract, difficult-to-treat (DTT) infections, loosening of the implant, and antibiotic availability (28) (29). In culture-negative PJI with the presence of a sinus tract, the use of molecular diagnostics must be supported by future research. **Chronic PJI:** contraindications to a 1-stage revision also include the presence of sepsis, an infection caused by drug-resistant bacteria or DTT, the presence of a sinus tract, severe soft-tissue deficiency over the joint, and a history of multiple revisions (30) (31) (4).



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