Approach for Molecular Diagnosis of Periprosthetic Joint Infections

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Periprosthetic joint infections (PJIs) represent some of the most challenging complications whose incidence will consequently rise in proportion to surgeries. The manifestation and evaluation of physical findings such as acute local inflammation, fever, and wound drainage, may correlate to the presence of PJIs; indeed, these clinical manifestation are of great value in raising the suspicion of PJIs.

prosthetic joint infection NGS PCR

1. Periprosthetic Joint Infections Classification

The period of symptom manifestation can vary and depends on microbial virulence [1][2]. Infections that have an outbreak within the first month after implantation are called early infections: high-virulent causative agents as *Staphylococcus aureus*, *Streptococci* spp., and *Enterococci* spp. elicit an evident local and systemic inflammation. On the contrary, low-virulent organisms such as *Cutibacterium* spp. and coagulase-negative *Staphylococci* (CoNS) might be the starting agents for infections manifesting within three months and one year after the implantation of the prostheses. These types of infection are named as delayed, and they show mainly attenuated symptoms such as joint pain or early loosening. Late infections present more than 24 months after surgery [2][3]. Not only may the bacterial colonization of the periprosthetic tissue represent a beginning factor for an inflammatory response, but also the high vascularity of those areas predisposes the patient to the risk of hematogenous infections within the first year after surgery [1][2]. Hence, the detection and elimination of the originating seeding focus is mandatory to prevent a relapse. The most common infectious bacterial species for hematogenous spreading are:

- *Staphylococcus aureus*, from skin and soft tissues infections that possibly leads to bacteraemia up to 34%;
- *Streptococcus pneumoniae*, spreading from respiratory tract;
- *Salmonella*, *Bacteroides*, *Streptococcus gallolyticus* from gastrointestinal infections;
- *Escherichia coli*, *Klebsiella*, *Enterobacter* spp. Affecting the urinary tract.

Other low-virulent bacteria such as *Staphylococcus epidermidis* may be responsible for a systematic infection, likewise the viridans group, *Streptococci*, can spread in the general blood circulation starting from dental procedure infections [2].

Notably, patients who underwent second-stage reimplantation and a subsequent doxycycline treatment can be colonized by *S. epidermidis* resistant to the molecule. This event appears be critical because doxycycline is a well-tolerated long-term use antibiotic [4].

Infections related to shoulder prostheses may relate to *Cutibacterium acnes* (formerly *Propionibacterium acnes*) [5] [6], a low-virulent anaerobic Gram-positive bacterium, and a commensal skin microbe that exists in different subtyping populations [7][8]. An abnormal distribution could lead to a failure and reversion surgery [8].

Staphylococcus lugdunensis is CoNS that generally colonizes hips and perineum and mutates under stress conditions as a limitation of nutrients or sub-lethal concentrations of antibiotics, inducing the formation of genetically modified small-colony variants (SCVs). Regarding pathogenicity, *S. lugdunensis* resembles *S. aureus* and might be misidentified in slide coagulase tests rather than tube tests because of the production of yellow pigments and DNase [9].

Infections caused by a direct contact are called *per continuitatem* and subdivide into two classes: a direct contact between the prostheses and the external world or a spread from a nearby infectious focus (e.g., osteomyelitis) [2].

Additionally, fungal PJIs are under-reported consequences in 1–3% of a failed joint arthroplasty and the symptomatologic profile is complicated in the presence of comorbidities and susceptibility to antifungal drugs. Vast majority of diagnoses due to a single infectious agent are attributed to *Candida* spp. and in particular to *C. albicans*. *Candida* species adapt to rapidly changing environments and express an adhesion capability to bind native tissues or implanted devices. Analogously to *C. albicans*, *Aspergillus* spp. present a dimorphic form between the growing hyphal structure and unicellular yeast or spores state, that is more difficult to eradicate [10].

Despite clinical evidence as purulence, sinus tract or histological acute inflammation, some hypotheses of infection, 7% to 15%, may result in culture-negative response whose main cause is a prior antimicrobial therapy of the patient [7].

2. Periprosthetic Joint Infections Diagnosis

Since the annual increase in primary and reversion arthroplasties, the need for a rapid and accurate diagnosis is ever more crucial for the management of PJI and an early bacteria detection, particularly within the first 2 weeks, allows a timely treatment of the biofilm [11].

The diagnosis is based on a combination of clinical findings and laboratory results obtained from multiple tests conducted through different techniques as peripheral blood, histological tissues, and microbiological cultures

evaluation and intraoperative findings evaluating numerous markers as D-dimer, C-reactive protein, synovial leukocyte esterase, and synovial alpha-defensin [12].

Subsequent to clinical inspections, the following step is the culture of synovial fluid, and the evaluation of chemical and biochemical parameters through Gram coloration, especially for Gram-positive *Staphylococcus aureus*, *Streptococcus* spp., and Gram-negative diplococci [13][14]. Regardless many methodologies as sonication [15][16], or detection of serum inflammatory markers (C-reactive protein, erythrocyte sedimentation rate synovial fluid cell count) [17]. In regard to the analysis of sonication fluid cultures, 16S r RNA beacon-based fluorescent in situ hybridization (bbFISH) represents a novel molecular approach for the identification of bacterial pathogen in PJI [18].

Typically, the gold standard for the diagnosis of PJI remains a tissue culture examination [14]. The definition of a PJI diagnosis is made on the positivity of two or more periprosthetic tissue cultures for the same microorganism. However, conventional cultures may be error-prone because of an appropriate medium, short incubation time, loss of microbial load due to conservation conditions, or a prior antimicrobial therapy [19].

Imaging techniques are also used into the diagnose of PJI, although their low sensitivity and specificity. Computed tomography (CT) or magnetic resonance imaging (MRI) that provide a good resolution for soft tissues [20].

One of the major challenges that remains is the culture-negative PJI caused by numerous reasons as antibiotics treatment, low-virulent bacterial infections, or biofilm. In patients who are not capable to undergo surgery, a proper antibiotic treatment has a success in 23–83% of cases; however, the lack of standardization and the vast surgical and antibiotics options make the resolution difficult to achieve [21][22].

In the last years, the implementation of molecular biology techniques has reduced the turn-around time to few hours and completely automated the workflow [23]: specific real-time, broad range 16S-polymerase chain reaction (PCR) [19], or next-generation sequencing that could be a robust support for PJI diagnosis [11]. The application of an interdisciplinary approach of surgery, infectiology, and microbiology will reduce the incidence of PJI and prolong the infection-free survival time of patients [24].

3. Periprosthetic Joint Infections Molecular Diagnosis

The PCR represents a robust diagnostic tool for PJI that can be applied to different specimens: tissue samples, synovial fluid, or sonicated prosthetic fluid [25].

The sensitivity and the velocity of the PCR overtakes the ones of tissue cultures; therefore, it is applied into the characterization of the bacterial colonies [26].

The PCR target can be amplified for a specific single organism or for multiple bacteria, acquiring the name of multiplex-PCR in this instance [27].

The nature of a PCR technology can be a house-made methodology or a commercial kit; the establishment of a robust protocol remains a challenging perspective because of the standardization: the reproducibility often requires well-trained technicians. For that purpose, many pharma companies have commercialized multiplex-PCR protocols: examples are SeptiFast by Roche, Genotype by Hain, Xpert by Cephaid, Filmarray by Biofire [28]. Culture-negative infections can be detected and diagnosed through multiplex-PCR, also defining the gene profile of microorganisms through the comparison to databases [29].

On the other hand, a broad range PCR targeting the 16S gene has a sensitivity from 50% to 92% and a specificity from 65% to 94%. Broad range PCR remains susceptible to error; hence, the lack of sensitivity may not correctly identify the microorganism leading to a false-positive result; on the contrary, all bacteria can be identified in a polymicrobial infection reducing the turn-around time [27][29][30].

Lastly, the detection of *Kingella kingae*, an important paediatric osteoarticular infectious agent, requires a pathogen-specific PCR on 16S gene [27].

The false-positive yield of broad-range PCR remains an issue of concern: in order to reduce the misdiagnosis and contamination, some studies tried to develop genus-specific PCR targeting a subgroup of Gram-positive cocci and excluding *E.coli* [31].

In case of mixed cultures, the broad-range PCR can detect the dominant bacterial strain present in the infection site; hence, the definition of the proper causative microorganism is appointed to NGS. Through PCR, non-viable microorganisms can be identified in a small volume and within few hours [24], also in cultures of patients receiving an antibiotic treatment. Particularly, PCR can implement the culture-independent diagnosis [27].

A limitation of the PCR technique could be contamination: a proper quality control process could prevent a false-positive result [31][32].

The incapacity to distinguish between living or dead bacteria and DNA contamination represent the main limitations of PCR-based diagnosis yielding to a false positive result [33].

In 2021, Bourbon et al. have associated the high-resolution melt analysis (HRMA) to broad-range PCR positive specimens. Equally to PCR, HMRA targets 16S gene and relates to the denaturation of the bacterial DNA and the melting temperature depending on the G-C content of the sequence and its length. The detachment of the double strand DNA decreases the fluorescent emission intensity that represents a rapid and cost-effective method for bacterial detection [34].

A broad-range PCR is followed by Sanger or next-generation sequencing (NGS) and exploits the high conservation of the 16S region that varies in short subunits depending on the bacteria species [27].

References

1. Corvec, S.; Portillo, M.E.; Pasticci, B.M.; Borens, O.; Trampuz, A. Epidemiology and new developments in the diagnosis of prosthetic joint infection. Int. J. Artif. Organs 2012, 35, 923–934.
2. Izakovicova, P.; Borens, O.; Trampuz, A. Periprosthetic joint infection: Current concepts and outlook. EFORT Open Rev. 2019, 4, 482–494.
3. Tande, A.J.; Patel, R. Prosthetic joint infection. Clin. Microbiol. Rev. 2014, 27, 302–345.
4. Kelly, M.P.; Gililland, J.M.; Blackburn, B.E.; Anderson, L.A.; Pelt, C.E.; Certain, L.K. Extended Oral Antibiotics Increase Bacterial Resistance in Patients Who Fail 2-Stage Exchange for Periprosthetic Joint Infection. J. Arthroplast. 2022, 37, S989–S996.
5. Scholz, C.F.P.; Kilian, M. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus Propionibacterium to the proposed novel genera Acidipropionibacterium gen. nov., Cutibacterium gen. nov. and Pseudopropionibacterium gen. nov. Int. J. Syst. Evol. Microbiol. 2016, 66, 4422–4432.
6. Dréno, B.; Pécastaings, S.; Corvec, S.; Veraldi, S.; Khammari, A.; Roques, C. Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: A brief look at the latest updates. J. Eur. Acad. Dermatol. Venereol. 2018, 32 (Suppl. S2), 5–14.
7. Nair, R.; Schweizer, M.L.; Singh, N. Septic Arthritis and Prosthetic Joint Infections in Older Adults. Infect. Dis. Clin. N. Am. 2017, 31, 715–729.
8. Hsu, J.E.; Matsen, F.A., 3rd; Whitson, A.J.; Bumgarner, R.E. Cutibacterium subtype distribution on the skin of primary and revision shoulder arthroplasty patients. J. Shoulder Elb. Surg. 2020, 29, 2051–2055.
9. Askar, M.; Bloch, B.; Bayston, R. Small-colony variant of Staphylococcus lugdunensis in prosthetic joint infection. Arthroplast. Today 2018, 4, 257–260.
10. Gross, C.E.; Della Valle, C.J.; Rex, J.C.; Traven, S.A.; Durante, E.C. Fungal Periprosthetic Joint Infection: A Review of Demographics and Management. J. Arthroplast. 2021, 36, 1758–1764.
11. Ahmad, S.S.; Shaker, A.; Saffarini, M.; Chen, A.F.; Hirschmann, M.T.; Kohl, S. Accuracy of diagnostic tests for prosthetic joint infection: A systematic review. Knee Surg. Sports Traumatol. Arthrosc. 2016, 24, 3064–3074.
12. Parvizi, J.; Tan, T.L.; Goswami, K.; Higuera, C.; Della Valle, C.; Chen, A.F.; Shohat, N. The 2018 Definition of Periprosthetic Hip and Knee Infection: An Evidence-Based and Validated Criteria. J. Arthroplast. 2018, 33, 1309–1314.e2.
13. Font-Vizcarra, L.; García, S.; Martínez-Pastor, J.C.; Sierra, J.M.; Soriano, A. Blood culture flasks for culturing synovial fluid in prosthetic joint infections. Clin. Orthop. Relat. Res. 2010, 468, 2238–2243.

14. Dudareva, M.; Barrett, L.; Figtree, M.; Scarborough, M.; Watanabe, M.; Newnham, R.; Wallis, R.; Oakley, S.; Kendrick, B.; Stubbs, D.; et al. Sonication versus Tissue Sampling for Diagnosis of Prosthetic Joint and Other Orthopedic Device-Related Infections. J. Clin. Microbiol. 2018, 56, e00688-18.
15. Yehia, S.R.; Duncan, H. Synovial fluid analysis. Clin. Orthop. Relat. Res. 1975, 107, 11–24.
16. Sambri, A.; Cadossi, M.; Giannini, S.; Pignatti, G.; Marcacci, M.; Neri, M.P.; Maso, A.; Storni, E.; Gamberini, S.; Naldi, S.; et al. Is Treatment with Dithiothreitol More Effective than Sonication for the Diagnosis of Prosthetic Joint Infection? Clin. Orthop. Relat. Res. 2018, 476, 137–145.
17. Kim, S.G.; Kim, J.G.; Jang, K.M.; Han, S.B.; Lim, H.C.; Bae, J.H. Diagnostic Value of Synovial White Blood Cell Count and Serum C-Reactive Protein for Acute Periprosthetic Joint Infection After Knee Arthroplasty. J. Arthroplast. 2017, 32, 3724–3728.
18. Birlutiu, R.M.; Birlutiu, V.; Cismasiu, R.S.; Mihalache, M. bbFISH-ing in the sonication fluid. Medicine 2019, 98, e16501.
19. Stylianakis, A.; Schinas, G.; Thomaidis, P.C.; Papaparaskevas, J.; Ziogas, D.C.; Gamaletsou, M.N.; Daikos, G.L.; Pneumaticos, S.; Sipsas, N.V. Combination of conventional culture, vial culture, and broad-range PCR of sonication fluid for the diagnosis of prosthetic joint infection. Diagn. Microbiol. Infect. Dis. 2018, 92, 13–18.
20. Del Pozo, J.L.; Patel, R. Clinical practice. Infection associated with prosthetic joints. N. Engl. J. Med. 2009, 361, 787–794.
21. Watanabe, S.; Kobayashi, N.; Tomoyama, A.; Choe, H.; Yamazaki, E.; Inaba, Y. Clinical characteristics and risk factors for culture-negative periprosthetic joint infections. J. Orthop. Surg. Res. 2021, 16, 292.
22. Li, C.; Renz, N.; Trampuz, A.; Ojeda-Thies, C. Twenty common errors in the diagnosis and treatment of periprosthetic joint infection. Int. Orthop. 2019, 44, 3–14, correction in Int Orthop. 2019.
23. Arvieux, C.; Common, H. New diagnostic tools for prosthetic joint infection. Orthop. Traumatol. Surg. Res. 2019, 105, S23–S30.
24. Karczewski, D.; Winkler, T.; Renz, N.; Trampuz, A.; Lieb, E.; Perka, C.; Müller, M. A standardized interdisciplinary algorithm for the treatment of prosthetic joint infections. Bone Jt. J. 2019, 101, 132–139, correction in Bone Jt. J. 2019, 101, 1032.
25. Qu, X.; Zhai, Z.; Li, H.; Li, H.; Liu, X.; Zhu, Z.; Wang, Y.; Liu, G.; Dai, K. PCR-based diagnosis of prosthetic joint infection. J. Clin. Microbiol. 2013, 51, 2742–2746.
26. Morgenstern, C.; Cabric, S.; Perka, C.; Trampuz, A.; Renz, N. Synovial fluid multiplex PCR is superior to culture for detection of low-virulent pathogens causing periprosthetic joint infection.

Diagn. Microbiol. Infect. Dis. 2018, 90, 115–119.

27. Higgins, E.; Suh, G.A.; Tande, A.J. Enhancing Diagnostics in Orthopedic Infections. J. Clin. Microbiol. 2022, e0219621, online ahead of print.
28. Esteban, J.; Gómez-Barrena, E. An update about molecular biology techniques to detect orthopaedic implant-related infections. EFORT Open Rev. 2021, 6, 93–100.
29. Gomez, E.; Cazanave, C.; Cunningham, S.A.; Greenwood-Quaintance, K.E.; Steckelberg, J.M.; Uhl, J.R.; Hanssen, A.D.; Karau, M.J.; Schmidt, S.M.; Osmon, D.R.; et al. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. J. Clin. Microbiol. 2012, 50, 3501–3508.
30. Bémer, P.; Plouzeau, C.; Tande, D.; Léger, J.; Giraudeau, B.; Valentin, A.S.; Jolivet-Gougeon, A.; Vincent, P.; Corvec, S.; Gibaud, S.; et al. Evaluation of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: A prospective multicenter cross-sectional study. J. Clin. Microbiol. 2014, 52, 3583–3589.
31. Vandercam, B.; Jeumont, S.; Cornu, O.; Yombi, J.-C.; Lecouvet, F.; Lefèvre, P.; Irenge, L.M.; Gala, J.-L. Amplification-based DNA analysis in the diagnosis of prosthetic joint infection. J. Mol. Diagn. 2008, 10, 537–543.
32. Goh, G.S.; Parvizi, J. Diagnosis and Treatment of Culture-Negative Periprosthetic Joint Infection. J. Arthroplast. 2022. online ahead of print.
33. Šuster, K.; Podgornik, A.; Cör, A. An alternative molecular approach for rapid and specific detection of clinically relevant bacteria causing prosthetic joint infections with bacteriophage K. New Microbiol. 2020, 43, 107–114.
34. Bourbour, S.; Emaneini, M.; Jabalameli, M.; Mortazavi, S.M.J.; Tahmasebi, M.N.; Taghizadeh, A.; Sharafatvaziri, A.; Beigverdi, R.; Jabalameli, F. Efficacy of 16S rRNA variable regions high-resolution melt analysis for bacterial pathogens identification in periprosthetic joint infections. BMC Microbiol. 2021, 21, 112.