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Molecular Diagnostics

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Abstract

Orthopaedic infections are complex conditions that require immediate diagnosis and accurate identification of the causative organisms to facilitate appropriate management. Conventional methodologies for diagnosis of these infections sometimes lack accuracy or sufficient rapidity. Current molecular diagnostics are an emerging area of bench-to-bedside research in orthopaedic infections. Examples of promising molecular diagnostics include measurement of a specific biomarker in the synovial fluid, polymerase chain reaction–based detection of bacterial genes, and metabolomic determination of responses to orthopaedic infection.

Surgical site and postoperative infections are among the most common and severe complications that affect orthopaedic patients. Universal diagnostics for these infections are still lacking. Although blood-based tests (eg, C-reactive protein [CRP] level, cell count) can suggest the presence of infection, they are not able to determine the species or antibiotic sensitivities of infecting organisms. Culture of tissue or fluid remains the current standard of care for diagnosing infections, but this method is not sensitive and can be time-consuming. In some cases, cultures produce false-negative results because of the use of empiric antibiotics or because low-virulence bacteria require specific nutrients to be grown in cultures. Accurate and rapid diagnosis of an infection is still sometimes the most difficult aspect of managing orthopaedic infections. Here, we present the current applications of molecular diagnostic tests as well as their advantages, limitations, and future directions for the diagnosis and personalized treatment of orthopaedic infections.

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Current Applications of Molecular Techniques

Recent advances in molecular diagnostics are beginning to shift from basic research to clinical reality. Some of the most popular and cost-effective diagnostic tests in medicine are based on quantification of a specific protein and are used frequently in hospitals across the world. For example, detection of β-human chorionic gonadotropin in blood or urine is used to diagnose pregnancy, and the detection of cardiac troponin is used to diagnose myocardial infarction. Currently, the CRP test is one of the most universally used blood biomarker tests for clinical infections.[1,2] CRP is also an archetypal blood biomarker for periprosthetic joint infections (PJIs). This test has been available for years and is commonly used by surgeons. It is sometimes regarded as nonspecific for diagnosis of infections because the CRP level may be increased by other inflammatory processes. However, studies have shown that a threshold blood CRP level of 10 mg/L provides a sensitivity and specificity of approximately 70% to 90% for detection of chronic PJI.[1,2]

The term *proteomics* describes a contemporary approach of analyzing proteins to identify diagnostic biomarkers for a disease. For the past decade, proteomics research has been active in the field of orthopaedics, with researchers attempting to identify biomarkers for PJI in blood and synovial fluid. Because infection-related biomarker levels in synovial fluid should be much greater than those in blood, it makes sense to specifically target the biomarkers in synovial fluid.[3] Several studies have systematically examined the synovial fluid proteome in relationship to PJI and have identified two protein families that provide a good diagnostic value for PJI: antimicrobial peptides and cytokines.[3–6] Described biomarkers include α-defensin, interleukin-1, interleukin-6, and neutrophil elastase, among others.[7] These studies have demonstrated the detection of specific synovial proteins as diagnostic biomarkers for PJI.

Detection of causative organisms, which is directly relevant to antibacterial treatment, remains an important challenge in the management of orthopaedic infections. However, treatment currently relies mainly on microbiological cultures. With strong demand for more appropriate and rapid detection of organisms, new technology is redefining how we diagnose infections and expanding our knowledge of the organisms involved in colonizing and infecting wounds and prostheses. In 1999, Tunney et al[8] used molecular detection methods to diagnose prosthetic hip infections and found evidence of bacterial colonization in >60% of retrieved arthroplasty samples from 120 patients. Standard microbiologic tests diagnosed infection in <25% of these patients. In this study, sonication of the components and the release of bacteria in biofilm were major technological advances. Biofilm detection and the observation of nonculturable bacteria continue to be emerging areas of research in orthopaedic surgery.

Detection of bacterial genes with a polymerase chain reaction (PCR)–based technique has been used clinically to improve the diagnostic accuracy and determination of the causative organisms involved in orthopaedic infections.[9–11] PCR is a molecular biology technique used to amplify a single copy of a piece of DNA to generate thousands to millions of copies of a particular DNA sequence, thus enabling ready detection.[12] PCR-based techniques are typically real-time PCR assays, with the amplified DNA detected as the reaction progresses

in real time. This is accomplished by the use of nonspecific fluorescent dyes that intercalate with any double-stranded DNA and/or sequence-specific DNA probes that consist of oligonucleotides that are labeled with a fluorescent reporter detected as a function of hybridization of the probe with its complementary sequence.[13] PCR could determine drug resistance by detecting encoding genes of multidrug resistance (eg, *mecA* gene).[10,11,14] PCR also substantially reduces the time required to identify the causative organism,[14] as represented in clinical detection of tuberculosis.[15,16] Molecular detection has also led to an increased understanding of the nature and biology of orthopaedic infections. In a study of 11 patients with infected shoulder arthroplasties, *Proprionibacterium acnes* was isolated in more than a third of patients.[17] This organism can take up to 2 weeks to grow in culture and is thus particularly suitable for molecular detection because it has a substantially higher sensitivity than that of traditional techniques. The recognition of atypical, difficult-to-culture bacteria species as infecting organisms has led researchers to suggest long-term (2 weeks) cultures of specimens as standard practice. This approach facilitates detection of additional infecting organisms that may be missed with traditional cultures of shorter duration.

PCR-based molecular diagnostics have also been used in the form of reverse transcription-PCR (RT-PCR) to quantify the levels of messenger or ribosomal RNA (mRNA or rRNA, respectively), which relates to the level of protein synthetic activities. In two studies, bacterial RNA isolated from synovial fluid was measured after reverse transcription to DNA to detect active PJI and determine the causative bacterial species.[18,19] Because RNA rapidly degrades upon cell death, RT-PCR can detect only living bacteria and is thus able to estimate the viable bacterial load. Interestingly, a recent study has suggested that mRNA levels of Toll-like receptors 1 and 6 in periprosthetic tissue correlate well with PJI, although this pilot study included small numbers of patients, and their samples were limited to intraoperative specimens.[20]

Molecular diagnostics are a battery of widely applied, powerful, and sensitive techniques used to identify biologic markers in a genome and proteome by detecting bacterial genes (with PCR-based techniques) and measuring expressed bacterial infection–specific proteins (with enzyme-linked immunosorbent assay [ELISA] and proteomics). It is noteworthy that, metabolomics, a systematic study of the end-products of cellular processes (metabolites), has recently become a useful tool for understanding the body's response to various diseases and is being used to develop screening and diagnostic tools for cancer and other diseases.[21,22] With its highly sensitive response system, metabolomics may also become a valuable tool for analysis of orthopaedic infections.

Advantages, Limitations, and Future Applications of Molecular Diagnostics

PCR

PCR has been used clinically to detect infection and identify causative organisms. In contrast to conventional culture-based methods, PCR techniques target and rely on fragments of bacteria instead of viable culturable cells to make a diagnosis. The first PCR-based studies centered on the amplification of bacterial genetic material (DNA), specifically the 16S rRNA gene.[23] Despite its high sensitivity, the validity of this technique is limited by the number of false-positive results caused by both the high magnification power of DNA

amplification and the persistence of bacterial DNA long after bacterial death.[24] Detection of a target whose status better reflects the viability of bacteria is needed. Recently, propidium monoazide and ethidium bromide monoazide have been used to mitigate these false-positive results.[25] In theory, these chemicals do not penetrate intact cytoplasmic membranes in living bacterial but inhibit amplification of DNA in dead or membrane-compromised bacteria. However, the current methodologies may not inhibit all dead bacterial DNA; therefore, these techniques are not yet applicable for clinical use.[26]

mRNA has also been used as a marker of infection in simulated infections.[18] As transient carrier molecules of the genetic material in bacterial cells, mRNAs quickly degrade after cell death and are present only in active infections. However, the low number of copies of mRNAs and the lack of a universal target sequence for all bacteria somewhat limit its use as a sufficiently sensitive marker of infection.

More recently, rRNA has been explored as a marker of infection.[19] rRNA is a component of ribosomes, which are abundant, integral structural subunits inside the bacterial cell involved in protein synthesis. Because of its abundance, the use of rRNA as a detection target offers sensitivity similar to that of DNA, but the abundance of rRNA declines rapidly with cell death. Although having universal sequences common to nearly all bacterial species allows the use of common primers for amplification, rRNA also has nucleotide sequences that are unique to specific bacteria and can be used in PCR-based identification. In clinical samples, as a diagnostic test, rRNA detection for PJI assessment had accuracy similar to that of cell count with differential and was more accurate than intraoperative cultures.[19] Importantly, the rRNA-based test was positive for infection in cases where cultures were negative, and the test had the potential to identify bacteria based on DNA sequencing. Several research groups are currently exploring molecular methods to detect periprosthetic infection and have had success in experimental and clinical settings.[8,9,10,18,20,27]

PCR-based amplification technology may be combined with other molecular techniques for more efficient diagnosis of bacterial infection, including quantification of bacterial load in patients with open fractures. The combination of this technology and mass spectrometry analysis may allow for direct determination of the types and quantity of bacterial colonization.[27] This approach exploits the ability of mass spectrometry to determine the sequence identity and the quantity of the amplified DNA fragments, thereby permitting bacterial speciation without a lengthy DNA sequencing step.

The detection and identification of bacterial orthopaedic infections is an ongoing biomedical challenge. Culture-positive cases may represent only a small percentage of infecting organisms and knowing the proper bacterial species and antibiotic sensitivity is crucial to effective treatment. Molecular techniques, including PCR- and microarray-based methods,[28] have shown early promise but need to be proven useful and cost-effective compared with other methods of infection detection before widespread adoption. In the future, information derived from these modalities may be prospectively compared to treatment outcomes to evaluate how molecular diagnosis may be applied and to assess antibiotic regimen and débridement courses for these orthopaedic wounds.

ELISA and Proteomics

Quantification of a specific protein with an ELISA is now a popular, cost-effective diagnostic test in medicine. Given the readily available platforms to conduct protein immunoassays in hospitals, it is no surprise that protein targets are highly desired for the diagnosis of orthopaedic infections. For the past decade, proteomics research in the field of orthopaedics has attempted to identify biomarkers for PJI in blood and synovial fluid samples. Diagnosis of PJI is currently dependent on the interpretation of a multitude of diagnostic results, including blood and synovial fluid tests. However, the advent of proteomics and the identification of biomarkers specific to PJI have the potential to make the diagnosis of orthopaedic infections simpler, more consistent, and more cost-effective. In the future, a biomarker test in conjunction with a molecular test to identify the pathogen may be the best combination to diagnose orthopaedic infections.

Blood Tests—Detection of a biomarker in the blood is likely the most accessible and convenient method of testing. The availability of phlebotomists combined with the general tolerability of a blood draw makes a blood test for PJI a desirable goal. A blood CRP level >10 mg/L is highly accurate for diagnosis of chronic PJI.[1,2] The use of a blood biomarker (eg, interleukin-6) for detection of infection has been explored in the literature.[29] However, among surgeons, this test has not become the standard of care and requires further research to be used appropriately[29] because of the substantial drawbacks of a blood test. One drawback is that blood tests reflect the systemic state of disease and can be confounded by other diseases and metachronous infections. Another drawback is that systemic treatments may affect blood tests in a way that does not accurately reflect the local state of disease. Therefore, although blood testing for orthopaedic infections is an ultimate goal, there are inherent limitations to the development of accurate blood tests.

Synovial Fluid Tests—Detection of a biomarker in synovial fluid has the main advantage of reflecting the local state of disease. Although synovial fluid is more difficult to obtain than blood, the advantages and potential increase in testing accuracy may make synovial fluid testing the best method for diagnosis of PJI. Importantly, the expression levels of biomarkers in synovial fluid are greater than those in blood[3] and may be less susceptible to perturbation by variation in the systemic levels of biomarkers. Recent studies have found that the measurement of antimicrobial peptides and cytokines in synovial fluid provides a sensitivity and specificity of >95% for diagnosis of PJI.[4–7]

The α-defensin biomarker test is highly accurate for diagnosis of PJI.[4,6] This antimicrobial peptide is the natural local tissue response to infection. In the setting of PJI, the level of α-defensin in the synovial fluid increases substantially, achieving levels that can be detected easily by immunoassay. The results of the α-defensin test mirror the Musculoskeletal Infection Society criteria for infection, which is currently considered the standard of care for diagnosis of PJI.[4] One study demonstrated that the α-defensin test for PJI outperformed the leukocyte esterase test strip.[4] Additionally, elevated α-defensin levels appear to be a general indicator of infection, responding to the wide variety of organisms that have been found to cause PJI.

Metabolomics

Metabolomics is the scientific study of chemical processes involving metabolites.[30,31] Metabolites are the end products of many cellular processes, and their levels can be regarded as the ultimate response of biologic systems to genotype, phenotype, and environmental conditions.[32] There are two basic methods for using metabolomics for analysis: chemometric (profiling) and quantitative (targeted) methods.[33] The chemometric method is an all-inclusive systematic review that uses principal component analysis to identify potential biomarkers from the pool of all potential metabolites. Once these potential markers are identified, they can be further analyzed using the targeted method. The quantitative (targeted) method can be used to determine the baseline level of expression of a specific biomarker in the standard population and establish standard deviations within healthy patients compared with biomarker levels in patients with PJI. This step is required when developing a diagnostic tool because biomarkers used for diagnosis need to have a predictable response to orthopaedic infections.

Metabolomics could be a powerful tool for diagnosis of orthopaedic infections or to help guide the optimal time for prosthethic reimplantation. Candidate metabolites may be conveniently identified by analysis of blood, urine, and synovial fluid. Drawbacks of metabolomics-based diagnosis include the time needed to analyze the samples and the current cost. There is a paucity of research on metabolomics and orthopaedic infections in the published literature, and this exciting field is wide open for potential breakthroughs in diagnosis and treatment.[33]

Personalized Medicine via DNA Profile

Diagnosis of orthopaedic infections is often inaccurate, with high numbers of false-negative culture results; personalized medicine and molecular diagnostics may play an important role in diagnosis of these infections. The cause of false-negative culture results has been debated, and classic microbiological tests have been expanded to include many different media and culture conditions to try to increase sensitivity and accuracy. Classic culture methods may not be effective for a subset of organisms because the conditions are not identical to those of an in vivo environment. In addition, infection can exist with low levels of pathogens; therefore, molecular techniques have become more popular for diagnosis of infection.

Levine et al[34] and others[35,36] optimized RNA extraction from synovial joints and used PCR-based technology to detect specific bacterial contaminants. These early tests were limited by potential false-positive results and insufficient information on the gene sequences of different bacterial species. Recent approaches based on RT-PCR have allowed more accurate assessment of the viable bacterial load.[18,19] The PCR-based method has also been used for detection of bacteria in the setting of chronic osteomyelitis.[37] New PCR technology and sequence information on the 16S rRNA genes of hundreds of different bacterial species and strains have facilitated analysis of whole genomes of a subset of bacteria; it is now possible to undertake identification of all bacterial species in a particular infection.[38,39] In orthopaedics, this technology has been used to retrospectively identify contaminating organisms in arthroplasty cases.[40] The sequence information has revealed the presence of bacteria common to deep infection (eg, *Staphylococcus aureus* and coagulase-negative

staphylococci) as well as *Streptococcus*, *Enterococcus*, and *Acinetobacter*. To date, application of these molecular techniques to tailor clinical therapy in orthopaedics has not yet been attempted and potentially involves a strategy that uses preoperative and intraoperative sampling and perioperative treatment.

The use of nucleic acid analysis has been most successful in the setting of treatment of chronic wounds that affect elderly and diabetic patients.[41] Deep sequencing or multiplex analysis has allowed identification of multiple pathogens present in the wound. This nucleic acid-based strategy has been used by Rhoads et al[41] at the Southwest Regional Wound Care Center for a personalized approach to treatment of chronic wound infections. Importantly, there is ready access to the biofilm-contaminated bacteria in a chronic wound, ensuring accurate identification of pathogens. Based on nucleic acid sequencing analysis, combination antibiotic therapy was devised for the wound bed, resulting in a marked decrease in wound size and depth. Another study found that, when treated with either an improved selection of antibiotics or customized therapeutics based on the results of molecular tests, the time to complete wound closure decreased by 26% and 45.9%, respectively.[42] It is important to note that, at the present level of infection control, identification of biofilm pathogens does not ensure that the therapeutic intervention (however well-targeted to the individual pathogens) will be able to eradicate the biofilm bacteria. The use of the appropriate antibiotics at the required therapeutic levels will be more efficacious and prevent bacterial resistance.

Additional challenges are also presented in the context of total joint surgery, specifically with regard to tissue sources for sampling. Accuracy in identifying the bacterial pathogen can be increased by effective sampling of the biofilm itself, not just the wound fluid. Sampling of the synovium is also required and, in patients undergoing revision surgery, a sample from the implant itself must be obtained, as well. Therefore, optimization of sampling method and timing will impact the success of this nucleic acid based strategy for diagnosis, detection, and perioperative eradication of pathogens in patients with infected total joint arthroplasty. The use of deep sequencing to provide important information about the state of joint infection is still in its early stages but can aid the physician in tailoring a personalized antimicrobial regimen.

Summary

Molecular diagnostics have contributed to improved diagnosis of orthopaedic infections. PCR-based techniques are capable of identifying bacterial DNA or RNA, which can aid determination of pathogenic organisms and drug resistance, even in orthopaedic infections that are not culturable or have a low virulence. These techniques can be used to assess viable bacterial load. Measurement of specific host proteins in synovial fluid, such as cytokines and antimicrobial peptides, also represents an attractive strategy for effective detection of orthopaedic infections. Recent advances in metabolomics provide another means to understand the biological basis of orthopaedic infections and new parameters for infection diagnosis. Finally, DNA profiling with deep sequencing technology may be used to tailor personalized antimicrobial regimens for patients with orthopaedic infections.

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References

1. Ghanem E, Antoci V Jr, Pulido L, Joshi A, Hozack W, Parvizi J. The use of receiver operating characteristics analysis in determining erythrocyte sedimentation rate and C-reactive protein levels in diagnosing periprosthetic infection prior to revision total hip arthroplasty. Int J Infect Dis. 2009; 13(6):e444–e449. [PubMed: 19473865]
2. Piper KE, Fernandez-Sampedro M, Steckelberg KE, et al. C-reactive protein, erythrocyte sedimentation rate and orthopedic implant infection. PLoS One. 2010; 5(2):e9358. [PubMed: 20179760]
3. Deirmengian C, Hallab N, Tarabishy A, et al. Synovial fluid biomarkers for periprosthetic infection. Clin Orthop Relat Res. 2010; 468(8):2017–2023. [PubMed: 20300901]
4. Deirmengian C, Kardos K, Kilmartin P, et al. The alpha-defensin test for periprosthetic joint infection outperforms the leukocyte esterase test strip. Clin Orthop Relat Res. 2014
5. Jacovides CL, Parvizi J, Adeli B, Jung KA. Molecular markers for diagnosis of periprosthetic joint infection. J Arthroplasty. 2011; 26(6 suppl):99–103. [PubMed: 21570803]
6. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J. Combined measurement of synovial fluid α-Defensin and C-reactive protein levels: Highly accurate for diagnosing periprosthetic joint infection. J Bone Joint Surg Am. 2014; 96(17):1439–1445. [PubMed: 25187582]
7. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J. Diagnosing periprosthetic joint infection: Has the era of the biomarker arrived? Clin Orthop Relat Res. 2014; 472(11):3254–3262. [PubMed: 24590839]
8. Tunney MM, Patrick S, Curran MD, et al. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol. 1999; 37(10):3281–3290. [PubMed: 10488193]
9. Kobayashi N, Procop GW, Krebs V, Kobayashi H, Bauer TW. Molecular identification of bacteria from aseptically loose implants. Clin Orthop Relat Res. 2008; 466(7):1716–1725. [PubMed: 18438724]
10. Choe H, Aota Y, Kobayashi N, et al. Rapid sensitive molecular diagnosis of pyogenic spinal infections using methicillin-resistant Staphylococcus-specific polymerase chain reaction and 16S ribosomal RNA gene-based universal polymerase chain reaction. Spine J. 2014; 14(2):255–262. [PubMed: 24231777]
11. Choe H, Inaba Y, Kobayashi N, et al. Use of real-time polymerase chain reaction for the diagnosis of infection and differentiation between gram-positive and gram-negative septic arthritis in children. J Pediatr Orthop. 2013; 33(3):e28–e33. [PubMed: 23482277]
12. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1988; 239(4839):487–491. [PubMed: 2448875]
13. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008; 3(6):1101–1108. [PubMed: 18546601]
14. Kobayashi N, Inaba Y, Choe H, et al. Simultaneous intraoperative detection of methicillin-resistant Staphylococcus and pan-bacterial infection during revision surgery: Use of simple DNA release by ultrasonication and real-time polymerase chain reaction. J Bone Joint Surg Am. 2009; 91(12): 2896–2902. [PubMed: 19952253]
15. Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. FEMS Immunol Med Microbiol. 2012; 66(1):20–36. [PubMed: 22574812]

16. Drobniewski F, Nikolayevskyy V, Maxeiner H, et al. Rapid diagnostics of tuberculosis and drug resistance in the industrialized world: Clinical and public health benefits and barriers to implementation. BMC Med. 2013; 11:190. [PubMed: 23987891]
17. Dodson CC, Craig EV, Cordasco FA, et al. Propionibacterium acnes infection after shoulder arthroplasty: A diagnostic challenge. J Shoulder Elbow Surg. 2010; 19(2):303–307. [PubMed: 19884021]
18. Birmingham P, Helm JM, Manner PA, Tuan RS. Simulated joint infection assessment by rapid detection of live bacteria with real-time reverse transcription polymerase chain reaction. J Bone Joint Surg Am. 2008; 90(3):602–608. [PubMed: 18310711]
19. Bergin PF, Doppelt JD, Hamilton WG, et al. Detection of periprosthetic infections with use of ribosomal RNA-based polymerase chain reaction. J Bone Joint Surg Am. 2010; 92(3):654–663. [PubMed: 20194324]
20. Cipriano C, Maiti A, Hale G, Jiranek W. The host response: Toll-like receptor expression in periprosthetic tissues as a biomarker for deep joint infection. J Bone Joint Surg Am. 2014; 96(20): 1692–1698. [PubMed: 25320195]
21. Serkova NJ, Spratlin JL, Eckhardt SG. NMR-based metabolomics: Translational application and treatment of cancer. Curr Opin Mol Ther. 2007; 9(6):572–585. [PubMed: 18041668]
22. Bartella L, Thakur SB, Morris EA, et al. Enhancing nonmass lesions in the breast: Evaluation with proton (1H) MR spectroscopy. Radiology. 2007; 245(1):80–87. [PubMed: 17885182]
23. Mariani BD, Tuan RS. Advances in the diagnosis of infection in prosthetic joint implants. Mol Med Today. 1998; 4(5):207–213. [PubMed: 9612800]
24. Choe H, Inaba Y, Kobayashi N, et al. Evaluation of the time period for which real-time polymerase chain reaction detects dead bacteria. Pol J Microbiol. 2014; 63(4):393–398.
25. van Frankenhuyzen JK, Trevors JT, Lee H, Flemming CA, Habash MB. Molecular pathogen detection in biosolids with a focus on quantitative PCR using propidium monoazide for viable cell enumeration. J Microbiol Methods. 2011; 87(3):263–272. [PubMed: 21963489]
26. Taylor MJ, Bentham RH, Ross KE. Limitations of using propidium monoazide with qPCR to discriminate between live and dead *Legionella* in biofilm samples. Microbiol Insights. 2014; 7:15–24. [PubMed: 25288885]
27. Melendez DP, Uhl JR, Greenwood-Quaintance KE, Hanssen AD, Sampath R, Patel R. Detection of prosthetic joint infection by use of PCR-electrospray ionization mass spectrometry applied to synovial fluid. J Clin Microbiol. 2014; 52(6):2202–2205. [PubMed: 24719438]
28. Metso L, Mäki M, Tissari P, et al. Efficacy of a novel PCR- and microarray-based method in diagnosis of a prosthetic joint infection. Acta Orthop. 2014; 85(2):165–170. [PubMed: 24564748]
29. Berbari E, Mabry T, Tsaras G, et al. Inflammatory blood laboratory levels as markers of prosthetic joint infection: A systematic review and meta-analysis. J Bone Joint Surg Am. 2010; 92(11):2102–2109. [PubMed: 20810860]
30. Stitt M, Fernie AR. From measurements of metabolites to metabolomics: An ‘on the fly’ perspective illustrated by recent studies of carbon-nitrogen interactions. Curr Opin Biotechnol. 2003; 14(2):136–144. [PubMed: 12732314]
31. Fuhrer T, Zamboni N. High-throughput discovery metabolomics. Curr Opin Biotechnol. 2014; 31C:73–78. [PubMed: 25197792]
32. Adams SB Jr, Setton LA, Nettles DL. The role of metabolomics in osteoarthritis research. J Am Acad Orthop Surg. 2013; 21(1):63–64. [PubMed: 23281473]
33. Zhang A, Sun H, Wang P, Han Y, Wang X. Modern analytical techniques in metabolomics analysis. Analyst. 2012; 137(2):293–300. [PubMed: 22102985]
34. Levine MJ, Mariani BA, Tuan RS, Booth RE Jr. Molecular genetic diagnosis of infected total joint arthroplasty. J Arthroplasty. 1995; 10(1):93–94. [PubMed: 7730838]
35. Mariani BD, Levine MJ, Booth RE Jr, Tuan RS. Development of a novel, rapid processing protocol for polymerase chain reaction-based detection of bacterial infections in synovial fluids. Mol Biotechnol. 1995; 4(3):227–237. [PubMed: 8680929]
36. Mariani BD, Martin DS, Levine MJ, Booth RE Jr, Tuan RS. The Coventry Award: Polymerase chain reaction detection of bacterial infection in total knee arthroplasty. Clin Orthop Relat Res. 1996; 331:11–22. [PubMed: 8895614]

37. Mariani BD, Martin DS, Chen AF, Yagi H, Lin SS, Tuan RS. Polymerase Chain Reaction molecular diagnostic technology for monitoring chronic osteomyelitis. Journal of Experimental Orthopaedics. 2014; 1:9.

38. Ehrlich GD, Post JC. The time is now for gene- and genome-based bacterial diagnostics: "You say you want a revolution. JAMA Intern Med. 2013; 173(15):1405–1406. [PubMed: 23857368]

39. Stoodley P, Kathju S, Hu FZ, et al. Molecular and imaging techniques for bacterial biofilms in joint arthroplasty infections. Clin Orthop Relat Res. 2005; 437:31–40. [PubMed: 16056023]

40. Jacovides CL, Kreft R, Adeli B, Hozack B, Ehrlich GD, Parvizi J. Successful identification of pathogens by polymerase chain reaction (PCR)-based electron spray ionization time-of-flight mass spectrometry (ESI-TOF-MS) in culture-negative periprosthetic joint infection. J Bone Joint Surg Am. 2012; 94(24):2247–2254. [PubMed: 23318615]

41. Rhoads DD, Cox SB, Rees EJ, Sun Y, Wolcott RD. Clinical identification of bacteria in human chronic wound infections: Culturing vs. 16S ribosomal DNA sequencing. BMC Infect Dis. 2012; 12:321. [PubMed: 23176603]

42. Dowd SE, Wolcott RD, Kennedy J, Jones C, Cox SB. Molecular diagnostics and personalised medicine in wound care: Assessment of outcomes. J Wound Care. 2011; 20(5):232, 234–239. [PubMed: 21647068]