Journal of Clinical Orthopaedics and Trauma 10 (2019) 560–565

Contents lists available at ScienceDirect

Journal of Clinical Orthopaedics and Trauma

journal homepage: www.elsevier.com/locate/jcot

Laboratory diagnosis of Prosthetic Joint Infections: Current concepts and present status

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ARTICLE INFO

Article history:
Received 30 June 2018
Received in revised form
28 September 2018
Accepted 15 October 2018
Available online 16 October 2018

Keywords:
Arthroplasty
Joint replacement
Prosthetic joint infection
Microbes
Laboratory diagnosis

ABSTRACT

Prosthetic joint infection (PJI) is a challenging complication of total joint arthroplasty for both a microbiologist and an Arthroplasty surgeon with respect to diagnosis and management. The various investigations used for diagnosis of PJI based on different reference criteria have their own fallacies. Amongst the newer biomarkers evaluated to strengthen the diagnosis of PJI, Alpha defensins has been found to be a useful synovial fluid biomarker. In this review, we have discussed the various laboratory investigations, their benefits and shortcomings for the diagnosis of PJI. Other newer diagnostic methods like molecular methods and modified culture techniques to increase the performance characteristics for the diagnosis of PJI have also been presented.

1. Introduction

Total joint arthroplasty is a highly successful and widely accepted standard of care in the therapeutic management of the severe degenerative joint disease.[1] However, infection around these implants (Prosthetic joint infections or PJI) is a nightmare for an orthopedic surgeon. Diagnosis of Prosthetic Joint Infections (PJI) is a significant challenge for both an Arthroplasty surgeon and a Microbiologist. The rate of PJI depends upon the type of arthroplasty. In primary joint replacement, the infection rate varies in different joints viz. hip and shoulder (<1%), knee (<2%), and elbow (<9%), in the first two years.[2] Since the clinical presentation of the post-prosthetic aseptic failure and infections are similar, but management is different, so optimum management depends upon timely and accurate diagnosis.

A positive synovial fluid culture can diagnose PJI, but a negative synovial fluid culture does not rule out PJI. Moreover, the isolation of the microorganisms which constitute the normal skin commensals like Coagulase-negative *Staphylococcus*, *Propionibacterium acnes,* etc also confuse diagnosing PJI until the same species is repeatedly isolated on multiple cultures along with certain criteria incorporated to support the diagnosis of PJI strongly. Besides culture, the various investigations which are used for the diagnosis of PJI in these criteria include Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), Synovial fluid White Blood Cell (WBC) count, Synovial Polymorphonuclear (PNL) percentage, Leukocyte esterase in synovial fluid, histological analysis of tissue. However, each investigation has its fallacy (Table 1). For reporting the synovial fluid culture as negative, a minimum incubation period of 14 days is recommended and which further hinders timely diagnosis and treatment of PJI. A negative synovial fluid culture can also be obtained when the patient is already on antibiotics before obtaining sample for culture. There are various guidelines for the diagnosis of PJI, e.g., Musculoskeletal Infection Society (MSIS), American Academy of Orthopedic Surgeons (AAOS), Infectious Diseases Society of America (IDSA), Zimmerli's criteria, International Consensus Group on PJI, further posing a challenge in diagnostically interpreting and treating PJI. Hence, the need for robust diagnostic criteria based modality for defining PJI timely and accurately.

In this review, we will discuss the development of PJI, the various investigations which are currently being used for the diagnosis of PJI as per the current standard guidelines and a new test Alpha-defensins which is coming up for the timely diagnosis of PJI.

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https://doi.org/10.1016/j.jcot.2018.10.006
0976-5662/

Table 1
Pros and Cons of various laboratory tests used for the diagnosis of Prosthetic Joint Infection.

Diagnostic method	Reference value (as per MSIS guidelines)	Advantage	Disadvantage	Comments
C-reactive protein	>10 mg/l	• Can be done from blood • Helps in pre-operative diagnosis • Rapid and cost-effective	• CRP levels are also elevated post-operatively for 3–8 weeks and in other inflammatory conditions • CRP levels may be normal in low-grade PJI • The threshold value may be different in the early-postoperative and late-chronic PJI • In PJI settings, CRP levels may be increased due to underlying co-morbid conditions	Different studies have proposed different threshold values. CRP levels in synovial fluid may be more accurate but in that case, synovial fluid aspiration is required, and the threshold value is to be defined
Erythrocyte Sedimentation Rate (ESR)	>30 mm/h	• An only blood sample is required • Rapid and cost-effective	• ESR can remain elevated post-operatively for few weeks • Threshold values have a wide range	Technical methods for measuring ESR may also affect ESR measurement[3]
Synovial WBC count	>3000/μL	• The cut-off value of synovial WBC at 11,200 cells/μL has shown highest sensitivity (100%) and specificity (98.9%)[4] • Fast turn around time • Cost-effective	• Synovial fluid aspiration is required • Time of collection of sample and operative site may affect the result[5] • A wide range of the threshold value • Inflammatory arthropathy and use of antibiotics may affect the result	Cost effective and satisfactory diagnostic value is if correlated clinically
Synovial fluid Polymorphonuclear count	>65%	• The pooled sensitivity and specificity have been found to be 90% and 88% respectively[6] • Rapid and cost-effective	• Synovial fluid aspiration is required • A wide range of the threshold value • Inflammatory arthropathy and use of antibiotics may affect the result	Cost-effective and satisfactory diagnostic value is given if correlated clinically eliminating the confounding factors
Leucocyte Esterase test	Included as one of the minor criteria for the diagnosis of PJI as per International Consensus Group	• Rapid and cost-effective • Strongly correlate with Polymorphonuclear neutrophils[7]	• Synovial fluid aspiration is required • Blood can interfere with the results • Result interpretation is subjective	Can be used in place of synovial fluid white cell count
Microbiological culture	Isolation of a pathogen from at least two separate tissue or fluid samples obtained from the affected prosthetic joint (Major criteria) Isolation of a microorganism in one culture of periprosthetic tissue or fluid (Minor criteria)	• Definitive diagnostic method • Preoperatively, Positive Gram stain microscopical examination and the culture growth of the similar morphotype as seen in the microscopic examination together is diagnostic	• Low sensitivity • Use of antibiotics may also decrease the sensitivity • The growth of low pathogenic organism at a single instance may also lead to a diagnostic dilemma • Fastidious organisms and anaerobic bacteria and/or fungi requires special culture media and incubation conditions	The positive microbiological culture of the synovial fluid specimen taken pre-operatively is the definitive and diagnostic test
Histopathological analysis of tissue	Not Applicable (N.A.)	• Histological examination of periprosthetic tissue with at least five polymorphonuclear leukocytes per high power field can predict infection	• The surgical tissue sample is required • Diagnosis would be post-operative	
Molecular methods	Not included in PJI diagnostic criteria	• Detection of the pathogen at a lower concentration	• Expensive • Needs expertise to perform and interpret • Contamination of sample may lead to false positive results	Can be used as an adjunctive test for diagnosis of PJI

2. Prosthetic joint infection (PJI)

A PJI is mainly caused by Gram-positive cocci (65%) followed by aerobic Gram-negative bacilli (6%) and anaerobes (4%). The different routes of infections are perioperative, i.e., during surgery, the hematogenous, i.e., the spread of infection from other body sites (like as a result of severe urinary tract infection or respiratory tract infections, etc.) and contiguous, i.e., from an adjacent site.[8] Early onset PJI occurs within three months of surgery and delayed onset PJI occurs between 3 and 24 months postoperatively. Both of these are mainly due to contiguous spread from the cutaneous site while Late-onset PJI occurs after 24 months of surgery and the route is usually hematogenous.[9] These infections are difficult to diagnose and treat because the microorganisms grow in biofilm's which are formed on the surface of implants. Both neutrophilic defects due to implants and biofilm formation protect the microorganisms from host defense mechanisms, thereby increasing the susceptibility to infection. The various risk factors for PJI include male gender, co-morbidities like diabetes, malnutrition, obesity, previous surgery and immunocompromised or immunodeficient states.[10]

3. Pathogenesis of PJI

Microorganisms adhere to the surface of the prosthetic implant. The formation of biofilm occurs when organisms attach to the implant before their functional and structural integration with bone. The microorganisms aggregate and communicate with each other via quorum sensing and get surrounded by polysaccharide matrix and form biofilm. These biofilm's act as a virulence factor because most of the antimicrobial agents cannot penetrate the biofilm and also there is shedding of bacteria from the mature biofilm, and thus microorganisms migrate and disseminate to other sites. Immune cells are recruited at the site of inflammation occurring anywhere in the body. Inflammatory biomarkers are secreted by the cells of the immune system which are recruited at the site of inflammation or infection to contain the infection. These immune cells get activated by the microbial products and biofilm proteins and secrete biomarkers like cytokines, alpha-defensins, leucocyte esterase and other types of biological factors. Interleukin-1β is produced by local macrophages, Interleukin 6 is mainly produced by inflammatory cells, and to a lesser extent by synovium and chondrocytes.[11] Interleukin 1β and Interleukin-6 also stimulate the production of acute phase reactants like C-reactive protein (CRP) by the hepatocytes. CRP activates complement and helps in immune defense mechanism by inducing phagocytosis of the microbes. Interleukin 6 also acts as a chemoattractant and is an essential element for both innate and acquired immunity. The other biological factors like Alpha defensins, Tumor necrosis factor-α (TNF-α), lactoferrin, neutrophil gelatinase-associated lipocalin are also involved in enhancing the immune response. These biological factors are involved in enhancing host defense mechanisms by various mechanisms like chemoattraction of immune cells, bactericidal action, enhancing bacterial cell wall permeability etc.[11,12] Alpha defensin is produced by the cells of the innate immunity mainly neutrophils and has antimicrobial properties.

4. Laboratory diagnostic tests for PJI

Several direct and indirect tests have been used to suspect and diagnose a PJI. These include:

I) Biological markers:
   A) Serum biomarkers
   B) Synovial fluid biomarkers
II) Microbiological culture
III) Molecular methods

4.1. Biological markers for diagnosis of PJI

Biological markers for the diagnosis of PJI include synovial and serum biomarkers. These biomarkers are involved in the pathogenesis of PJI and get elevated in patients with PJI. So, these can be utilized for the diagnosis of PJI.

4.1.1. Serum biomarkers of PJI

The various serum biomarkers include CRP, Erythrocyte Sedimentation Rate (ESR), Interleukin-6, Procalcitonin, D-Dimer, Tumor necrosis factor-α, Intercellular adhesion molecule-1 (ICAM-1), Lipopolysaccharide binding protein (LBP).

4.1.1.1. C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR). CRP and ESR together constitute one of the minor MSIS criteria for the diagnosis of PJI. The CRP is an acute phase serum protein which is synthesized in the liver as a result of inflammation of the body. Generally, the level of CRP is elevated in PJI, but CRP levels may also be high as a result of post-operative inflammation and other conditions. Also, CRP levels may be decreased as a result of prior antibiotic administration. The sensitivity and specificity of CRP for the diagnosis of PJI have been found to be variable in different studies, and it depends upon the cut-off value taken for the diagnosis of disease. For Acute PJI, Kim et al. found 100% sensitivity and 90.3% specificity with CRP level >34.9 mg/l.[4] In a meta-analysis, the pooled sensitivity, and specificity for CRP in serum was found to be 97% (95% CI, 93%–99%) and 91% (95% CI, 87%–94%), respectively.[13] It is also studied that the sensitivity and specificity of CRP in synovial fluid sample gets increased up to 96%–97% and 90%–93% respectively.[14] ESR is also a nonspecific marker of PJI. The red cell aggregation is increased due to an upsurge in fibrinogen and other normal plasma proteins and production of abnormal circulating proteins from necrotic tissue. It increases the settling of erythrocytes, thus increasing measured ESR. An ESR is estimated along with CRP to increase the sensitivity and specificity for the diagnosis of PJI. Sigmund IK et al. found that the sensitivity of CRP and ESR using modified MSIS criteria is 70.6% and 40.6% respectively and the sensitivity is increased to 75% when these two parameters are used in combination.[15] To determine the normal temporal trends, Park KK et al. evaluated levels of CRP and ESR before surgery and on the first, second, fifth, seventh, fourteenth, forty- second, and ninetieth postoperative days in 320 uncomplicated primary TKAs. They found temporal changes in CRP to be faster in comparison to ESR. CRP levels increases rapidly on second postoperative day and decreases in a biphasic manner. In the first phase, it decreases rapidly from the peak value in two weeks and in the second phase there is gradual decrease and it decreases to less than the normal reference level on the forty-second day but returns to preoperative levels on the ninetieth day. On the other hand, there is slight decrease in levels of ESR on the first postoperative day and then it increases to peak on the fifth day. There is gradual decrease in ESR levels and it remains elevated above the normal reference level (20 mm/h) on the forty-second postoperative day. It returns close to the preoperative levels only on the ninetieth postoperative day.[16]

4.1.1.2. Interleukin-6 and Procalcitonin. Interleukin-6 is a cytokine which is produced by monocytes and macrophages in case of inflammation and it induces the release of CRP by liver cells. Procalcitonin is a precursor of calcitonin which is primarily synthesized by C cells of the thyroid gland but in case of inflammation or infection lipopolysaccharide, microbial toxins and other

inflammatory mediators induces the production of Procalcitonin from various other cells of the body like white blood cells, spleen, kidney, liver etc.[17] Both Interleukin-6 and Procalcitonin levels have been evaluated for the diagnosis of PJI in both serum and synovial fluids. For Interleukin-6, it has been found that the diagnostic performance is good with synovial fluid IL-6 with accuracy more than 0.9 and high sensitivity. However, serum Procalcitonin was earlier found to be more relevant in comparison to synovial fluid Procalcitonin to differentiate between septic and aseptic arthritis.But, it was later found that PCT cannot be utilized as a diagnostic marker for localized infections and should be used only for systemic bacterial infections.[18]

The diagnostic utility of other serum biomarkers like TNF-α, ICAM-1, LBP has been found to be uncertain.[12]

4.1.2. Synovial fluid biomarkers of PJI

The various synovial fluid biomarkers which have been evaluated for the diagnosis of PJI include inflammatory proteins, cytokines, and antimicrobial peptides. Out of these antimicrobial peptides have shown the promising results. Antimicrobial peptides include Alpha-defensins, Human neutrophil elastase 2, bactericidal/permeability protein, neutrophil gelatinase-associated lipocalin,and Lactoferrin. Out of these the best synovial fluid biomarker for the development of immunodiagnostic test has been found to be Alpha defensin protein. The cut-off concentration of Alpha-defensins has been found to be 5.2 mg/L and mean concentration is 65 mg/L which is far above the Alpha-defensins level in patients with osteoarthritis and rheumatoid arthritis and aseptic arthroplasty failure.[19] Synovial fluid cytokines like Interleukin 1β and Interleukin 6 may be detected in the synovial fluid and may help in the diagnosis of PJI.

4.1.2.1. Alpha-defensins. Alpha-defensins are the cationic peptides which are released by the neutrophils as a defense mechanism against various types of microbes. Their molecular weight varies from 3 to 4 kDa and is present within azurophilic granules of neutrophils. These are released on the surface of microbes when azurophilic granules fuse with the phagosomes, and these not only act as powerful antimicrobial peptides but anti-inflammatory also.[20] Increased levels of Alpha-defensins in blood and body fluids may be associated with various disease conditions and are being evaluated for diagnostic and prognostic purpose.[6]

Alpha-defensins are now being evaluated as a synovial fluid biomarker for the diagnosis of PJI, and it has been found that their values are not affected by prior antibiotic administration, other inflammatory conditions in the body and the type of microorganism. The sensitivity and specificity of Alpha-defensins in synovial fluid for the diagnosis of PJI has been found to 100% taking MSIS criteria as the gold standard (Table 2). Thus, it has been found to be a useful tool for deciding on revision surgery.

The various technical methods which have been used for the determination of Alpha-defensins are Lateral Flow assay and enzyme-Linked Immunosorbent Assay. Lateral flow assay is a rapid method for the determination of Alpha-defensins in synovial fluid and has been evaluated by various authors both pre-operatively and intra-operatively to diagnose PJI. It is based upon the principle of Immunochromatography (IMCG). In this test, few drops of sample diluted in the buffer added to the test device containing a reagent strip. The solution then migrates to the buffering pad and reacts with the anti-defensin antibody labeled with the gold conjugate. If alpha-defensins is present in the sample above the cut-off value, then it forms a colored complex which is visible as a colored test line on the test device. There is a control line also to check the validity of the test (Fig. 1).

The accuracy of ELISA based detection of Alpha-defensins has been found to more than Lateral flow assay, but like other ELISA assays, it is more time consuming and not cost-effective concerning a single test run. It is also observed that unlike leucocyte esterase, blood in the synovial fluid does not affect the detection of Alpha-defensins in synovial fluid.[22]

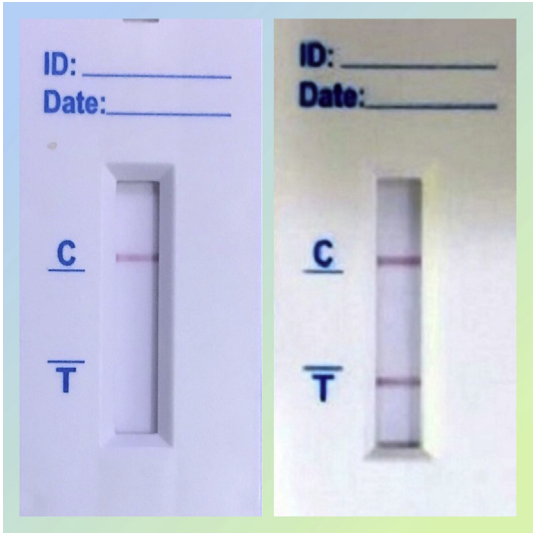

Fig. 1. Alpha Defensin test by Immunochromatography technique-showing negative and positive results.

Table 2
Studies on Alpha Defensin test for Prosthetic Joint Infection.

Reference	Gold standard	Sensitivity	Specificity	Alpha-defensin method	Comments
[21]Deirmengian C et al., 2014	MSIS criteria	97%	96%	ELISA	Specificity improved to 100% with concurrent interpretation with synovial C-reactive protein. Alpha-defensins levels were not affected by concurrent antibiotic treatment
[22]Deirmengian C et al., 2015	MSIS criteria	100%	100%	Immunochromatography (ICMG)	Alpha-defensin immunoassay was found to be superior and reliable than Leucocyte esterase test
[23]Bonanzinga T et al., 2017	International Consensus Group on PJI	97%	97%	ELISA	False positive was due to metallosis and polyethylene wear and false-negative presented with draining sinus, and intraoperative cultures were also negative
[24]Berger P et al., 2017	MSIS criteria	97.1% (95% confidence intervals)	96.6% (95% confidence intervals)	Lateral flow assay (ICMG)	Positive predictive value was 91.7% (95% CI 77.7% to 98.3), and the negative predictive value was 98.8% (95% CI 93.6 to 99.9) Results were found to be promising as per MSIS criteria
[15]Sigmund IK et al., 2017	Modified MSIS criteria	69%	94%	Lateral flow assay (ICMG)	Detection of Alpha-defensins by Lateral flow assay was found to be comparable to histology and bacteriology with one positive culture and can be used as an adjunct in the diagnosis of PJI

The type of microorganism does not influence the result of Alpha-defensins test in synovial fluid. It has been found that it provides consistent results with aerobic bacteria, anaerobic bacteria, and yeasts which implies that it is released by neutrophils in response to all type of microorganism.[25] The performance of Alpha-defensins test in synovial fluid for the diagnosis of PJI has been found to be useful in various studies. However, Scholten R et al., found the low sensitivity of Alpha-defensins lateral flow assay to exclude PJI in patients with suspected aseptic loosening.[26] So, it is essential to know whether this test can be alternatively and more reliably used in place of current existing tests like CRP and ESR in blood, Leucocyte esterase/Leucocyte count in synovial fluid,etc. Table 2 shows the performance characteristics of this test in various studies.

4.1.2.2. Leucocyte esterase test. Leucocyte Esterase (LE) strip test is also a rapid test based on immunochromatography (ICMG), and it detects Leucocyte esterase enzyme secreted by neutrophils, and it detects the concentration of neutrophils in synovial fluid which reflects PJI. The sensitivity and specificity of LE test was found to be similar to the histological analysis of synovial tissue by Li Rui et al.[27] In comparison to Leucocyte esterase strip test, Alpha-defensins has shown more promising and reliable results regarding sensitivity and specificity. Leucocyte esterase strip test results get affected with a bloody synovial fluid tap while Alpha-defensins results are not compromised due to the presence of blood in synovial fluid. Synovial fluid CRP may be used to exclude false positive Alpha-defensins results which occur as a result of metallosis.

4.2. Microbiological culture

It is the standard gold test for the diagnosis of PJI, but the limitation of culture is that it requires prolonged incubation and many-a-times the bacterial growth is not achieved because of previous antibiotics or biofilm formation in the prosthesis. Various methods are being used to improve the sensitivity of the culture. Some crucial steps to increase the bacterial yield from the specimens have been suggested.[28] These techniques include:

1. Synovial fluid collection in blood culture bottles.
2. Sonication of the prosthetic implant before putting culture.
3. Grinding of tissue, before inoculation on the culture media.
4. The use of optimum culture media both for transportation and inoculation.
5. The use of optimum incubation conditions like anaerobic conditions.
6. Prolonging the incubation period, for up to 14 days.

4.3. Molecular methods

The molecular methods like Polymerase Chain Reaction (PCR) can rapidly detect the microorganisms in low concentrations. PCR can also be used for the detection of microorganisms from synovial fluid or peri-prosthetic tissue in suspected cases of PJI, but the sensitivity of PCR has been found to be variable in different studies. Also, the performance characteristics of PCR also depend upon the type of specimen. The sensitivity and specificity of PCR have been found to be 76% and 93% respectively with peri-prosthetic tissue. However, with sonication fluid, the sensitivity and specificity were increased to 95% and 97% respectively.[29] The lower limit of detection also depends upon the amount of specimen used for DNA extraction and the presence of PCR inhibitors in the specimens. However, PCR may also be false positive due to contamination which may add to a diagnostic dilemma.

5. Conclusion

PJI is a catastrophic complication of an Arthroplasty, as not only increases the misery of the patients but its management increases the financial burden on the patients. However, the definitive diagnosis of PJI is very challenging because of the decreased sensitivity of the culture and variable sensitivity and specificity of various serum and synovial fluid inflammatory biological markers. Real-time PCR and other molecular studies including sequencing studies are more specific to define the etiological agents, but the sensitivity of tests requires more studies for accuracy and along with the availability and cost it could be a limiting factor in some countries. Although Alpha-defensins is a nonspecific marker for the diagnosis of infections as it is increased in other conditions also, the presence of Alpha-defensins in the synovial fluid can be used to diagnose or rule out PJI along with clinical correlation and MSIS/modified MSIS criteria. It is a promising and simple test to perform, which takes only 10 min to provide the result. However, more multi-centric and large prospective studies are required to confirm its validity and accuracy in different patient populations. Similarly, other newer diagnostic tests cannot be used alone and should be used judiciously with proper clinical correlation following standard guidelines/criteria.

Conflicts of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgement

None.

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