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Update on the role of pathology and laboratory medicine in diagnosing periprosthetic infection

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ABSTRACT

Technological and implant design advances have helped reduce the frequency of aseptic total joint arthroplasty failure, but periprosthetic joint infections (PJI) remain a clinical important problem with high patient morbidity. Misinterpreting PJI as aseptic mechanical loosening commonly leads to unsatisfactory revision arthroplasty, persistent infection, and poor long-term results. While there is no single "gold standard" diagnostic test for PJI, recent collaborative efforts by Orthopaedic and Infectious Disease Societies have developed algorithms for diagnosing PJI. However, the efficacy of individual tests as well as diagnostic thresholds are controversial. We review the recommended thresholds for commonly used screening tests as well as tissue histopathology and confirmatory tests to diagnose periprosthetic infection. We also update lesser-known laboratory tests, and we briefly summarize rapidly evolving molecular tests to diagnose periprosthetic infection. Pathologists hold a critical role in assisting with PJI diagnosis, maintaining laboratory test quality and interpreting test results. Collaboration between clinicians and pathologists is essential to provide optimal patient care and reduce the burden of PJI.

1. Introduction

The results of total joint arthroplasty are usually excellent, but occasional joint replacements become clinically unsatisfactory and need to be revised. Improved implant materials and designs have decreased the rate of aseptic loosening caused by mechanical factors or wear debris. Dislocation and adverse local tissue reactions persist, but one of the most common causes of arthroplasty failure is periprosthetic joint infection (PJI) [1,2]. The distinction between aseptic failure and infection is important, because in the absence of infection the implant is usually revised in a single operation, whereas infection requires more complex one-stage or two-stage operations as well as prolonged antibiotics. Misinterpreting a periprosthetic infection as aseptic loosening often leads to persistent pain and ultimately additional operations.

There is no single "gold standard" test for diagnosing PJI, so physicians often use a combination of tests. Previous working groups from the American Academy of Orthopaedic Surgeons [3,4], the Musculoskeletal Infection Society [5], the Infectious Diseases Society of America [6], as well as International Consensus Meetings [1,7–9] and other publications [10] have described testing algorithms to diagnose PJI, and comprehensive reviews have described the clinical features and risk factors for PJI [11–14]. In this review we will focus on the role the Pathologist and Pathology Lab can play in helping diagnose periprosthetic infection, with special focus on tests that have not been included in the recent consensus reviews. We will also discuss variability in instrumentation and procedures that may influence diagnostic thresholds and reference ranges.

1.1. Definition of periprosthetic joint infection

The sensitivity, specificity and predictive value of laboratory tests are best calculated with reference to a "gold standard" test. Unfortunately, no single test for periprosthetic infection is perfect, so several workshops and consensus meetings have attempted to define a combination of factors considered diagnostic of infection. Among the first of these was a multidisciplinary working group selected by the American Association of Orthopaedic Surgeons (AAOS) in 2009, with results summarized in 2010 [3], and updated in 2019 [2]. That group identified peer-reviewed literature to support the use of the Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) as screening tests,

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the evaluation of aspirated joint fluid and histology of periprosthetic tissue in selected cases, as well as microbiologic culture of fluid and tissue. Subsequent consensus meetings emphasized that not all factors are considered of equal value, so the contribution of any given test could be considered either "major vs minor" or given a numeric value [7,9]. A modification of the 2018 Musculoskeletal Infection Society (MSIS) criteria for the diagnosis of PJI is listed in Table 1 [5]. Based on these principles, several publications have recommended similar testing algorithms starting with serology tests of peripheral blood (ESR and/or CRP), joint fluid aspiration with cell count, differential and culture, serology of joint fluid (alpha-defensin), potential intraoperative frozen section and ultimately histology of periprosthetic tissue interpreted in conjunction with final microbiologic cultures [3,6,7,14,15]. Each of these tests will be discussed in more detail below.

2. Serologic tests

2.1. Peripheral blood tests

The peripheral blood **white blood cell count** (WBC) is commonly ordered as part of a Complete Blood Count (CBC) when considering the possibility of an infection in any site. Some studies have reported good specificity but poor sensitivity for peripheral blood WBC for diagnosing PJI [16], but others have not found WBC to be clinically useful [17]. The AAOS Practice Guidelines did not recommend routine use of peripheral blood leukocyte count [4], hence it appears that the peripheral blood WBC has a limited role in the routine workup of patients with suspected PJI.

Another index available from a CBC is the **neutrophil-to-lymphocyte ratio** (NLR). The NLR is calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, and the normal range is 1–2. The serum as well as synovial fluid NLR was recently reported to have prognostic value with respect to septic arthritis [18], and several studies have suggested that serum NLR may be useful for diagnosing PJI. For example, Yu and co-authors [17] reported a significantly higher NLR in 20 cases of early PJI compared with 101 aseptic cases. NLR was less accurate than IL-6 but more accurate than CRP and had the added convenience of being easy to calculate without extra cost. The NLR also normalizes more rapidly post-op than the ESR or CRP, offering a potential advantage for the diagnosis of early PJI [19,20]. Other studies, however, have suggested that that compared with traditional inflammatory biomarkers the value of NLR, either alone or combined with CRP and ESR, for diagnosing chronic PJI is limited [21], with only moderate sensitivity (63 %) and specificity (73 %) [16].

Another routinely available parameter from the CBC is the **ratio of platelet count to mean platelet volume** (PC/MPV). In the presence of inflammation and infection, markers of inflammation, such as ESR and CRP, and platelet production increase, while the MPV decreases, making it a negative acute phase reactant. The opposing patterns of platelet count and MPV lead to an elevated ratio between these two variables in cases of inflammation and infection. Paziuk et al. [22] evaluated PC/MPV in 5888 patients with revision total hip and knee arthroplasties including 949 (16 %) infected cases. They reported a mean ratio of 33.4 for diagnosed PJI cases and 25.7 for aseptic revision cases, with a sensitivity of 48 % and specificity of 81 % when using a cutoff of 31.7. This specificity was higher than that of both ESR and CRP for the same cohort of patients. However, other authors have suggested that while PC/MPV may be of value when combined with CRP, fibrinogen, or CRP, when used alone its specificity is similar but sensitivity is generally lower than that of ESR and CRP [16,23,24].

The integration of pathways between inflammation and coagulation suggests that commonly used **coagulation screening tests** may also be abnormal in PJI. The endotoxin and exotoxins production by pathogens of PJI stimulate phagocytic and endothelial cells to produce various proinflammatory cytokines, such as IL-1, IL-6, IFN-**γ** and TNF-α. These cytokines disrupt the normal coagulation cascade through induction of tissue factor (TF), with abnormal activation of the extrinsic coagulation pathway and fibrinolysis [25]. Saxena and co-authors noted that the mean INR (prothrombin time) of patients undergoing revision arthroplasty for infection was significantly higher than patients undergoing resection for aseptic loosening (1.24 vs 1.01 respectively) [26]. Another study found that the coagulation profile (including aPTT, INR, platelet count and fibrinogen) were higher before the first stage compared to at reimplantation in patients undergoing a two-stage operation for PJI [25].

Fibrinogen is an acute-phase reactant glycoprotein that, besides being a precursor to fibrin, impacts the inflammatory process by inducing the synthesis of proinflammatory cytokines, such as IL-6 and TNF-α. The sensitivity and specificity of fibrinogen in PJI diagnosis appears to be similar to those of the more classical PJI markers, CRP and ESR [27,28], suggesting that it may be a complementary test to indicate residual infection at reimplantation of a 2-stage operation for PJI [27,29], but the overall accuracy of this test alone is insufficient to confirm or exclude infection. Although not yet widely available, **thromboelastography** is an assay that evaluates blood clot development and elasticity in whole blood and can be used to help guide transfusion, especially in the context of trauma and organ transplantation. Preliminary studies suggest that several thromboelastography parameters, including blood clot kinetics ("K") (reflecting fibrinogen) and Maximum Amplitude (MA) may be helpful for diagnosing PJI and predicting optimum timing for re-implantation, especially when used in combination with ESR and CRP [30,31].

D-dimer is a degradation product of the fibrin monomer and a specific marker of fibrinolysis that is generally used as a screening test for venous thromboembolism. However, besides being an acute-phase reactant, it is also increased in systemic or local infections, including PJI [32]. For example, in a study of 245 patients, Shahi and co-authors found an optimal threshold level of 850 ng/mL, yielding sensitivity of 89 % and specificity of 93 % for D-dimer in diagnosing PJI. Yan et al. reported that D-dimer is an effective biomarker for PJI diagnosis as long as patients do not have a history of hypercoagulation or inflammatory arthritis [33]. Others have suggested that D-dimer provides little additional information beyond fibrinogen, ESR or CRP [25,27], but it has been adopted as a minor criterion for PJI in at least one of the consensus meetings, with a score equal to that of CRP, and twice that of ESR [5] (Table 1).

Unfortunately, reported studies of D-dimer in PJI diagnosis have not used consistent specimen types. Many have used serum D-dimer, but others used either plasma D-dimer or a combination of the two. Li and

Table 1
A "scoring based" definition of PJI [5].

Major Criteria (at least one of the following)		Decision
Two positive cultures of the same organism		Infected
Sinus tract with evidence of communication to the joint		Infected
Preoperative Diagnosis		
Minor Criteria	Score	Decision
Elevated Serum CRP >1 mg/dL or D-Dimer >860 ng/mL	2	
Serum ESR >30 mm/h	1	➢6 Infected
Synovial WBC Count >3,000 cells/uL or Leukocyte Esterase ++	3	
Synovial alpha-defensin +	3	2-5 Possibly Infected
Synovial PMN >80 %	2	
Synovial CRP >6.9 mg/L	1	0–1 Not Infected
Intraoperative Diagnosis:		
Inconclusive pre-op score or dry tap	Score	Decision
Preoperative Score	–	
Positive Histology*	3	➢6 Infected
Positive Purulence	3	4-5 Inconclusive
Single Positive Culture	2	≤3 Not Infected

• 5 or more neutrophils in each of 5 or more high power fields.

co-authors found that serum D-dimer had a better diagnostic value for PJI than plasma D-dimer [34]. Overall, like many biomarkers, it appears that D-dimer is best used in combination with other markers, and there is a need for more research, with particular reference to specimen type (serum vs plasma).

The **erythrocyte sedimentation rate (ESR)** test is a sensitive, but non-specific metric that reflects inflammation in general. Although Hunter first noted the influence of an inflammatory state on blood sedimentation in the late 18th century, Swedish physicians Fahraeus and Westergren developed the reproducible method of quantifying sedimentation now known as the Westergren method [35]. The test measures the rate at which red blood cells in sodium citrate anti-coagulated blood aggregate and descend in an open-ended, vertical tube after 1 h. That rate is influenced by the properties of the erythrocytes (including net charge) as well as the viscosity of the plasma, which in turn is influenced by fibrinogen and other plasma proteins. In the Westergren method, anti-coagulated blood is diluted 4:1 in a sodium citrate solution and placed in a glass or plastic tube of at least 2.5 mm inner diameter and 200–300 mm length. The tube is placed in a vertical position and the distance from the top of the plasma to the top of the sedimented erythrocytes (excluding buffy coat) is measured after 60 min. Sedimentation occurs in three stages: a preliminary stage of at least a few minutes as rouleaux formation occurs and aggregates form; then a period in which the descent of the aggregates takes place at approximately a constant speed; and finally a phase of slower sedimentation as the aggregated cells pack at the bottom of the tube [36].

The ESR is influenced by anything that alters plasma viscosity, such as plasma albumin, immunoglobulins and fibrinogen, as well as red blood cell shape and hematocrit. The ESR rises within 24–48 h of the onset of inflammation, then slowly decreases with resolution of the inflammation.

As noted by Alijanipour [37], the upper limit of reference ranges (i.e. thresholds) that maximize predictive value of ESR to diagnose PJI are controversial, with some investigators suggesting different thresholds for acute vs chronic PJI, or differences based on location (e.g. hips vs knees or shoulders). Examples of recommended ESR thresholds are shown in Table 2.

Most of the Consensus Conferences have adopted the threshold of 30 mm/h for chronic PJI, but studies using Receiver Operator Characteristic Curves have often suggested either higher [38,39] or lower [40] diagnostic thresholds, and the Consensus Conferences have not recognized differences in ESR testing methodology. Piper et al. [41] reviewed 8 studies of PJI, and noted diagnostic thresholds of ESR ranging from 22.5 to 50 mm/h. Using the traditional Westergren test, they evaluated 64 of their own patients (19 infections), and the commonly used threshold of 30 mm/h yielded sensitivity and specificity of 16 % and 98 % respectively. Lowering the threshold to 26 mm/h increased sensitivity but decreased specificity. Complicating comparing ESR rates in the literature is the observation that most studies do not describe the method of determining ESR, with the assumption that the Westergren method was used. For example, in a study of several different serologic markers predicting PJI, Berbari and co-authors [42] reviewed 25 studies that reported ESR and noted that reported thresholds ranged from 12 to 40 mm/h but in many of the studies the diagnostic threshold was apparently arbitrary, and there was no assessment of the methods used to determine ESR.

The Westergren method is considered the "gold standard" for quantifying ESR, but it is manual and slow (1 h "run time"). In 2016 a working group of the International Council for Standardization in Hematology (ICSH) [36] surveyed more than 6000 laboratories, and found that only 28 % used the unmodified Westergren method, while 62 % used alternate methods, with results differing up to 142 % when compared to the Westergren method. Mainly intended to accelerate turnaround time and reduce costs, alternate methods use other techniques to detect whole blood viscosity, such as photometric rheology, vacuum extraction into glass tubes, and reduced duration of sedimentation to 20 or 30 min with transformation of results to equivalent Westergren values (so-called "Modified Westergren"). Based on these findings, the Working Group provided recommendations to manufacturers concerning labeling and validation of new methods with reference to the Westergren test. Although some examples of systemic bias have been reported [43], most validation studies of ESR testing methods have shown fairly consistent results in mid-ranges, with the largest discrepancies occurring at very low, or very high sedimentation rates [44]. Therefore, while these differences many not be clinically significant for most cases of suspected PJI, the topic deserves further investigation, and future studies of PJI diagnosis should include the specific methods used to measure ESR.

C-Reactive Protein (CRP) is an acute phase reactant, has a higher sensitivity to inflammation than ESR, and is a direct measure of the inflammatory response. It increases in infectious diseases as well as non-infectious inflammatory disorders, such as rheumatoid arthritis, systemic lupus erythematous, kidney and liver disease. The main function of CRP is to help promote phagocytosis and the immune response against foreign infectious pathogens. CRP also activates complement via the classical C1q pathway. In the presence of acute inflammation, the CRP levels start to rise within 4–6 h and peak by 36–50 h. After the inflammation has resolved, concentrations fall rapidly. Although CRP can be elevated in a variety of inflammatory conditions, CRP trends are still helpful in screening for PJI as well as monitoring response after the first stage of a two-stage operation for PJI. The 2018 MSIS criteria suggested a serum CRP threshold of >1 mg/dL (10 mg/L) as a minor criterion for PJI diagnosis [5], although CRP may show little elevation in the presence of an infection with low virulent pathogens. Like ESR, CRP levels normally increase after joint arthroplasty, although CRP decreases more rapidly than ESR. Recognizing an unexpected trend, such as failure of CRP to decrease after 2 months post-op, may be of more diagnostic value than absolute values. When both ESR and CRP are negative, periprosthetic infection is unlikely, hence their value as screening tests in all patients being assessed for hip and knee PJI [13].

Table 2
Examples of ESR methods and recommended diagnostic thresholds.

Authors	ESR Test	Joints	ESR by Stage (mm/h)			Recommended Threshold (mm/hr)*		
			Acute	Chronic	Late	Acute	Chronic	Late
Alijanipour [37]	ESR-Auto Plus1; Streck	Hip	80 [51–100]		80 [50–95]	54.5		48.5
		Knee	78 [44–91]		90 [61–104]	54.5		46.5
DiCesare [38]	Westergren	Hip		76.1 (±38 SD)			30	
		Knee		86.1 (±30.7 SD)				
Ghanem [39]	Mini-Ves, Plymouth, MN	Hip		77			31	
Piper [41]	Westergren	Hip		30 (3–137)			13	
		Knee		53.5 (6–128)			19	
		Shoulder		9 [1–71]			26	
		Spine		48.5 [1–83]			45	

• The upper limit of the reference range in non-infected patients (mm/hr).

3. Aspirated joint fluid

It is well known that the concentration of **white blood cells (WBC)** in aspirated joint fluid, and the proportion of WBC that are neutrophils are also increased with infection. For example, Trampuz and co-authors [45] prospectively studied cell counts of aspirated fluid before revision knee arthroplasty of 133 patients, and calculated that a WBC count greater than 1,700 cells/μL, had sensitivity and specificity of 94 % and 88 % respectively, while a differential of >65 % PMNs had 97 % sensitivity and 98 % specificity. In a study of similar design (but using different units of measure), Ghanem [46] recommended thresholds of 1, 100 cells/$10^{-3}cm^3$ (equivalent to 1,100 cells/μL) and 64 % PMNs. These studies made no reference to the duration between arthroplasty and synovial fluid aspiration (i.e., acute vs chronic PJI). However, synovial fluid WBC and the PMN differential also increase after arthroplasty in the absence of infection, so understanding the natural history of these reactive changes is necessary before defining thresholds suggestive of periprosthetic infection. From a series of 571 patients who presented within the first two years after primary knee arthroplasty, Christensen and co-authors [47] identified 452 samples not associated with infection. The synovial fluid WBC count and %PMN all increased immediately post-op, and then decreased after the first 90 days, with total cell count decreasing somewhat more rapidly than the %PMN. These authors emphasized that the use of diagnostic thresholds that had been based on diagnosing chronic PJI would yield a false-positive rate of 25–41 % if used in the first 6 weeks after arthroplasty.

Similarly, Bedair [48] noted that thresholds to diagnose acute PJI (within 6 weeks of arthroplasty) should be much higher than those used to diagnose chronic PJI (after 6 weeks). Based on the use of receiver operator characteristics curves, Bedair suggested that for acute PJI, a threshold of >10,700 cells/μL would provide high sensitivity, while 27, 800 cells/μL with 89 % PMNs would decrease sensitivity but increase specificity. For this reason, other publications [49] and recent consensus meetings have suggested different diagnostic thresholds for acute PJI (e.g. > 10,000 WBC/μL and >90 % PMN) vs chronic PJI (e.g. > 3,000 WBC/μL and >80 % PMN) [50].

Another factor to consider when reviewing published thresholds for either peripheral blood or synovial WBC counts is variability in **units of measure**. For example WBC counts have been expressed as either cells/μL [51], cells/mL [52], cells/mm^3 [18], cells/L [17], or cells/$10^{-3}cm^3$ [46]. These values become problematic when reference ranges are incorrectly transcribed when citing previous work. For example, one group of authors [46] mistakenly cited two other studies as using cells/$^{10-3}cm^3$ when the original reports were actually reported as cells/mL (a 1000-fold error in units). As first recognized in 2006 [13] and again more recently [53], widely discrepant recommended thresholds may be based on inconsistent units of measure. We recommend WBC results be expressed as cells/μl.

Pagliaccetti et al. [54] recently discussed the variability of cell counts in different surgical settings. For example, cell counts and % PMNs are often higher after hip arthroplasty compared to knee arthroplasty. There may be differences between failed unicompartmental knees compared to total knee arthroplasty, cell counts may be influenced by the presence of a cement spacer, and thresholds should be different based on the duration since arthroplasty. There is also evidence that low virulent organisms such as coagulase-negative Staphylococcus may be associated with false negative synovial fluid WBC [49,52].

Adding to the complexity of synovial fluid cell counts is the issue of **manual vs automated cell counts**. In general, automated cell counts are more precise and much faster than manual counting [55], although accuracy can be compromised by necrotic tissue or intracellular particles of metal debris, especially in patients with metal-on-metal implants. For example, Wyles and co-authors [56] reviewed synovial fluid metrics from 39 patients who had undergone revision hip arthroplasty for failed metal-on-metal implants. Only 4 were culture positive; the threshold of >3,000 WBC/μL yielded 100 % sensitivity but only 57.1 % specificity. The %PMN threshold of >80 % however, was 100 % sensitive and 97 % specific. The authors suggested that automated synovial fluid WBC had poor predictive value, but the %PMN had good predictive value in this patient population. Similarly, Abdelaziz and co-authors retrospectively reviewed the synovial fluid cell counts of 702 patients who had undergone aseptic revision hip arthroplasty [57]. Patients with polyethylene wear or metallosis had a wide range of WBC counts, with 25 of 42 patients (60 %) with WBC counts above the 3000 cells/μL threshold often used to suggest infection. Ten of 47 (21 %) patients with metallosis (defined as stained tissue) had more than 70 % PMNs. The authors noted that in revision hip patients with polyethylene or metal wear, an elevated WBC count alone should not be considered strongly suggestive of infection, but the PMN% is more reliable, especially when using an automated analyzer. The authors suggested that using manual cell counts for patients with PE or metal wear might be considered, although they noted that doing so would be time consuming and expensive, and manual cell counts were not performed for any of their patients.

3.1. Synovial fluid serology

Widely distributed in animals and plants are naturally occurring cationic polypeptides with antibiotic properties that appear to play a major role in innate immunity. After preliminary work in other mammals, Lehrner and colleagues in the 1980's isolated three such peptides from human neutrophils and coined the term "defensins" [58]. **Alpha-defensins** are primarily found in neutrophils, while beta-defensins are more prominent in epithelia. Both types of defensins appear to represent a rapid antimicrobial response from the innate immune system that, at the same time may activate the adaptive immune response [59].

With respect to PJI, Deirmengian and co-authors reported 100 % sensitivity and specificity for an immunoassay designed to detect the three alpha-defensin molecules in a study that included 29 PJIs and 66 aseptic joints [60]. Originally available only from a single commercial laboratory, other studies have also demonstrated overall good results [61], although some have reported false-positive results in the presence of orthopaedic wear debris and false-negative results related to low-virulence pathogens [62]. Compared to the ELISA version of the alpha-defensin test ("Synovasure®"), a lateral-flow version has been reported to have equivalent specificity but lower sensitivity [63], although a subsequent study reported equivalent sensitivity [64] and was used to support clearance of the test by the FDA. Those findings, in part, led to incorporation of a positive alpha-defensin test as a minor criterion for a diagnosis of PJI in the 2018 update of the MSIS consensus [5]. Diagnosing PJI can be especially difficult in a patient with an underlying non-infectious inflammatory arthropathy such as rheumatoid arthritis, but Miyamae and co-authors reported higher accuracy of synovial fluid alpha-defensin (Synovasure®) when compared to serum ESR or CRP to diagnose PJI in patients with various inflammatory arthropathies [65]. The most appropriate application of this relatively expensive test is still controversial, but like many biomarkers, it may prove most useful in combination with other tests, especially in patients with equivocal findings [66].

Several of the serum analytes described above can also be measured in aspirated joint fluid, although the clinical significance of the results is controversial. For example, Tetreault and co-authors [67] compared serum CRP with synovial fluid CRP in 119 patients undergoing revision arthroplasty, and reported optimum diagnostic thresholds of 6.6 mg/L for synovial fluid and 11.2 mg/L for serum CRP, with very similar sensitivities and specificities. Parvizi and co-authors [68] also reported a strong correlation between serum and synovial fluid CRP in patients with PJI. As described above, the 2018 MSIS criteria for diagnosing PJI (Table 1) involves calculating scores of various tests, with a sum of ≥6 diagnostic of infection. In that scheme, a serum CRP ≥1 mg/dl is assigned a score of 2, and a synovial fluid CRP ≥6.5 mg/L is assigned a score of 1 [5]. It should be noted, however, that quantifying CRP from

synovial fluid may require manually adding hyaluronic acid to the sample to reduce viscosity.

A different approach to detecting pathogens involved with PJI is to use an immunologic assay to detect antigens in aspirated joint fluid. For example, a synovial fluid Microorganism Antigen Immunoassay Detection (MID) Panel has been developed to detect antigens from genera of Staph., Candida and Enterococcus. Recently reported results described good sensitivity and specificity, especially in samples that had been culture negative, although the authors modified the MSIS infection criteria by including synovial fluid CRP instead of serum CRP to define infection [69].

Additional studies are needed, but assays of synovial fluid for cytokines such as interleukin-6 may also be of value, especially in conditions such as painful shoulder arthroplasty, in which serum ESR, CRP, and microbiologic cultures are often equivocal [70].

3.2. Gram stain

Serologic tests and the results of WBC cell count and differential of aspirated joint fluid are important screening tests for PJI, and surgeons are often tempted to request a gram stain of fluid obtained at either preoperative or intra-operative aspiration. The 2010 AAOS Working Group evaluated the peer-reviewed literature available at that time [3] and identified three high quality studies indicating that a gram stain is a poor "rule out" test, with sensitivity values ranging from 19 to 44 %. Although specificity was much better, other studies have reported false positive gram stains, sometimes due to the presence of necrotic but stainable bacteria present in tissue processing and staining reagents [71]. Consensus conferences have also recommended against requesting a Gram stain to rule-out PJI [46,72,73]. Although sensitivity is still low, a better use of a Gram stain is to help select the most appropriate antibiotic to administer to a patient with a known, obvious septic arthritis while waiting for the results of microbiologic culture.

4. Tissue histology

The histology of tissue obtained at revision arthroplasty commonly provides clues concerning the mechanism(s) of arthroplasty failure (Table 3). Of particular importance with respect to infection is the extent of acute inflammation characterized by neutrophils (polymorphonuclear leukocytes, PMNs). PMNs are common in periprosthetic infection, and rare in most other mechanisms of arthroplasty failure. However, there has been considerable variability in the threshold of inflammation thought to suggest infection [72,74–78]. The 2010 AAOS Working Group evaluated the peer-review literature to identify a threshold of acute inflammation that would support a diagnosis of infection, especially when used in the context of an intraoperative frozen section. High-quality peer-reviewed literature was found to support either of two thresholds: 1) 10 or more PMN in each of 5 or more high power fields (HPF) (78 % sensitivity and 97 % specificity), or 2) 5 or more PMN in each of 5 or more HPF (80 % sensitivity and 91 % specificity) [3] (Fig. 1). Most subsequent consensus meetings and reviews have endorsed the second of those thresholds [5–7,13]. However, although orthopaedic surgery residents are taught and are tested on the tissue concentration of neutrophils suggesting infection, the use of frozen sections during revision arthroplasty has not been widely adopted, in part because the diagnosis is admittedly more complicated than simply counting neutrophils/HPF. First, the concentration is not based on 5 or more "average" high power fields, but instead, like counting mitoses in a sarcoma, is based on the 5 fields with maximum tissue concentration of inflammation. Acute inflammation is also expected to accompany a recent fracture, so a frozen section is of dubious value when associated with a periprosthetic fracture. Similarly, neutrophils entrapped in superficial fibrin or blood clot adherent to the fibrous membrane (Fig. 2) or within blood vessels are not predictive of infection, and granulocytes present in hematopoietic bone marrow should not be interpreted as acute inflammation. Soft tissue injured by a recent dislocation can also have increased neutrophils for a few days, and pathologists should recognize that the cytoplasmic granules characteristic of neutrophils and eosinophils are often lysed when tissue is frozen, so one must pay attention to the number and shape of the lobes in granulocyte nuclei to avoid misinterpreting eosinophils as neutrophils (Fig. 3), especially in frozen sections. Similarly, the use of cautery to obtain biopsy tissue in the OR also distorts nuclei increasing the likelihood of a false positive interpretation of infection (Fig. 4). Surgeons should be encouraged to use sharp dissection instead of cautery in this context. Criteria supporting a histologic diagnosis of probable infection in a patient with an underlying inflammatory arthropathy such as rheumatoid arthritis are not well-defined, but several articles have concluded that the thresholds for tissue inflammation are similar in the presence or absence of an underlying inflammatory arthropathy [79,80]. The sensitivity of frozen sections will always be limited by sampling, but careful attention to the above details will help minimize false positive frozen section interpretations.

Once a periprosthetic infection has been diagnosed with certainty, the current "gold standard" for treatment is a two-stage revision. The implant is removed, local tissue is debrided and a spacer containing antibiotic bone cement is inserted. The patient then receives systemic antibiotics. Clinical and serologic features are followed, and when the results suggest "clearance" of the infection, the spacer is removed and a

Table 3
Morphologic classification of periprosthetic tissue at revision arthroplasty.

Classification[a]	Histology	Likely Pathogenesis
Fibrous Membrane	Fibrous or pseudosynovial membrane with no inflammation and few particles	Aseptic, mechanical loosening
Adaptive Immune Reaction (ALVAL)[b]	Diffuse and perivascular chronic inflammation. Absent (or rare) PMNs	Idiosyncratic adaptive immune response to CoCr particles or ions from articular surfaces or modular connections
Macrophage Particle Reaction	Numerous macrophages associated with particles	Innate macrophage and giant cell reaction to wear, usually of articular surface(s)
Probable Infection	5 or more PMNs in each of 5 or more 400X fields	Periprosthetic Infection
Extensive necrosis	Necrosis	Necrosis precludes further classification

[a] Modified from Ref. [86], and similar to a classification of Krenn et al. [116].
[b] Aseptic Lymphocyte Dominant Vasculitis-Associated Lesion ([87,88]).

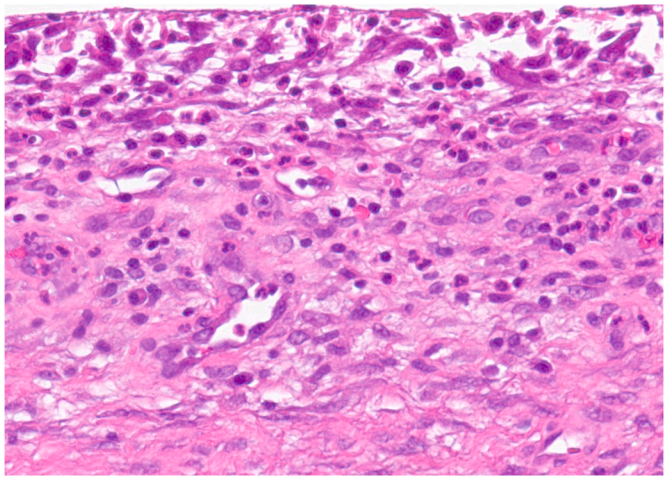

Fig. 1. Typical histology that is highly suggestive of periprosthetic infection (>5 PMN in each of 5 or more 400X microscopic fields). H&E, 320X

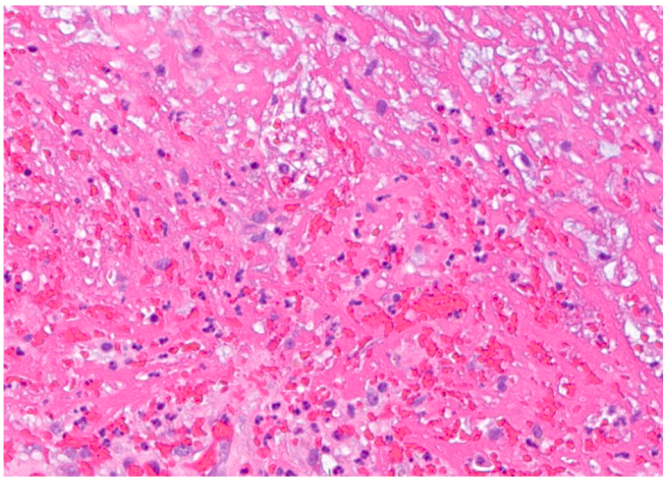

Fig. 2. Neutrophils can become entrapped in superficial fibrin or blood clot, and can accompany a recent periprosthetic fracture. In this clinical context, the apparent high concentration of neutrophils by itself is not predictive of infection. H&E, 200X

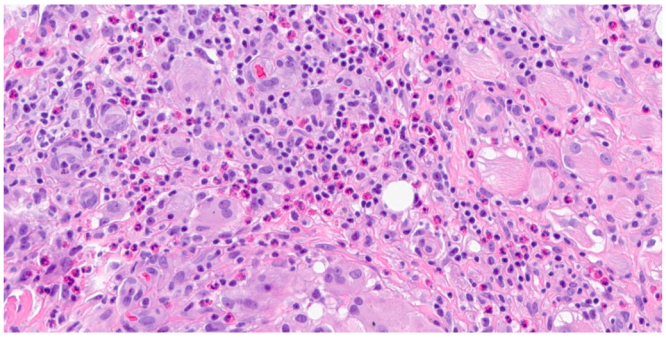

Fig. 3. Sometimes misinterpreted as neutrophils (PMNs), eosinophils are not predictive of infection. H&E, 250X

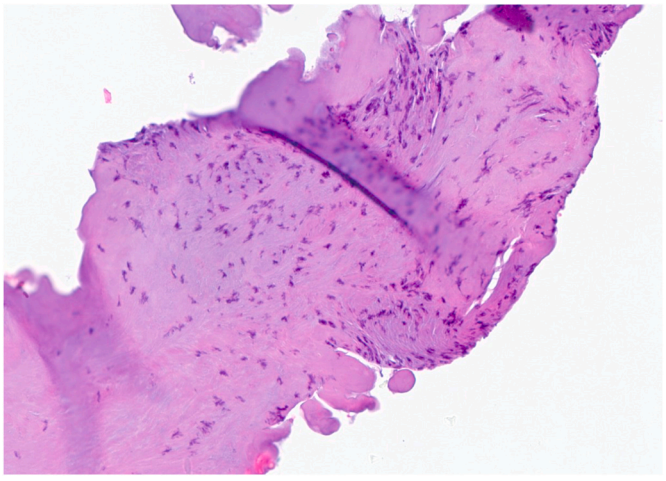

Fig. 4. Thermal artifact from electrocautery distorts the nuclei of many cell types. Misinterpretation of cautery artifact as acute inflammation is a common explanation for a false positive interpretation of infection at revision arthroplasty. H&E, 160X

new implant inserted (the second stage). However, it can be difficult to determine if the infection has resolved to the point needed for successful implant re-insertion. In part because the patient has been on prolonged antibiotics, microbiologic cultures may be negative. At the same time, molecular testing may detect nucleic acid of necrotic bacteria, so some surgeons request a frozen section at the time of re-implantation. One might question the threshold of acute inflammation to suggest persistent infection in that context, but in a study of 21 patients undergoing two-stage reimplantation, Bori and co-authors [81] concluded that using the standard 5 PMNs in 5 HPF threshold yielded only 28 % sensitivity but 100 % specificity. Reducing the threshold to an average of 1 PMN in 10 HPF increased sensitivity to 71 %, but reduced specificity to 64 %. In a similar study of 97 patients undergoing the second stage revision, George et al. [82] reported 50 % sensitivity but 94 % specificity diagnosing persistent infection. Several other studies have also reported relatively low sensitivity but good specificity for frozen sections at second stage re-implantation [83,84], leading to the conclusion that in that context, a negative frozen section has high predictive value to rule out infection, but has low sensitivity for detecting persistent infection.

There is also some evidence to suggest that pathogens of low virulence might induce less acute inflammation than the bacteria more commonly involved with PJI, indicating that if a low-virulent organism is likely, perhaps a different threshold would improve the sensitivity of frozen section diagnosis. For example, Grosso and co-authors [85] retrospectively reviewed 45 patients who had undergone revision shoulder arthroplasty, and who had had intraoperative frozen sections as well as other tests for infection. Clinical follow-up was used to determine the presence or absence of infection, and all microscope slides were reviewed and graded using four different PMN thresholds. A receiver operating characteristics curve was used to determine an optimal diagnostic threshold. The results showed frozen section sensitivity of 50 % for detecting *P. acnes* (now *C. acnes*) infection when using conventional thresholds, with 67 % sensitivity for patients with other pathogens. Reducing the tissue concentration threshold to between 7 and 10 PMNs in a total of 10 HPF improved the sensitivity in *P. acnes* cases to 72 % while maintaining 100 % specificity. This study supports the hypothesis that frozen sections (and possibly other tests of inflammation) may have lower sensitivity to detect infections caused by low virulence pathogens compared to more aggressive bacteria.

Although not practical at the time of frozen sections, there may be a role of traditional histochemistry or immunohistochemistry to highlight neutrophils, as was demonstrated by Moraweitz and co-authors [78], although we do not recommend the specific use of CD15 staining, since it stains eosinophils as well as neutrophils. It is anticipated that the combination of specific stains for neutrophils along with semi-automated morphometry and whole slide digital imaging of microscope slides should improve the sensitivity histology to diagnose PJI.

4.1. Unusual histologic findings at revision arthroplasty

As described in detail elsewhere, occasional patients develop solid or cystic soft-tissue masses associated with clinically unsatisfactory arthroplasty, usually either metal-on-metal hips or implants with fretting corrosion at modular connections. Often referred to as “pseudotumors”, these lesions can be associated with infection, an innate macrophage reaction to debris particles, or a chronic inflammation reaction thought to represent an adaptive immune response to metal particles or ions Table 2 [86,87]. The adaptive immune response to metal is almost always dominated by diffuse and perivascular lymphocytes, sometimes with lymphoid aggregates, and often with plasma cells [88]. Rare neutrophils have been reported in a few cases, but it has been difficult to rule out coexisting infection with low-virulent pathogens, so as a general rule, acute inflammation, even in the presence of other features of an immune reaction around a failed metal-metal implant, favors infection.

Most pathologists recognize the association between granulomas and Mycobacteria or fungal infections, although the identification of acid-fast bacilli around a failed implant is extraordinarily rare. Periprosthetic granulomas more commonly reflect a foreign-body reaction

to debris, sarcoidosis, or other rare granulomatous disorder (Fig. 5). Characteristic granulomas with distinctive myxoid contents, usually around the knee, are a consequence of previous "viscosupplementation" injections of hyaluronic acid preparations such as Synvisc®, and do not indicate a granulomatous infection (Fig. 6).

5. Microbiology

False-positive and false-negative microbiologic cultures of periprosthetic tissue or joint fluid are relatively common [13], such that cultures are no longer considered the "gold standard" for PJI diagnosis, but culture results are undoubtedly extremely important both to identify the organism and determine its susceptibility. Recent consensus conferences [6] recommend submitting at least 3, and optimally 5 or 6 periprosthetic tissue samples for aerobic and anaerobic culture at the time of tissue debridement or revision arthroplasty, although fewer samples may be satisfactory if specimens are inoculated into an automated blood culture bottle system [89]. In selected cases it may be appropriate for surgeons to submit up to two samples for mycobacterial and fungal cultures, but evidence does not support routine mycobacterial or fungal cultures of periprosthetic tissue or synovial fluid [4].

Antibiotics should be withheld for at least 2 weeks prior to tissue collection. Once in the microbiology lab, tissue or fluid samples are usually inoculated on blood agar, chocolate agar, MacConkey medium, CDC agar plate (anaerobe blood agar), and CAN agar. Although no firm consensus exists regarding the duration samples should be incubated, incubating for up to 14 days may be needed to identify some organisms, for example. *C. acnes.* Most consensus meetings have concluded that two positive cultures from the same joint identifying the same organism represents a major criterion for diagnosing PJI, although a single positive culture with a virulent organism such as *S. aureus* may also be considered diagnostic. The interpretation of a single culture of an organism of low virulence often requires consideration of other clinical and laboratory features [5].

Periprosthetic infections are sometimes classified with respect to the duration after surgery. Early/acute infections present less than 3 months after surgery and are often attributed to high virulent organisms acquired at the time of surgery. Late/chronic infections from 3 to 12 or 24 months are also thought to have been acquired at surgery but often involve less virulent organisms, while delayed/late-onset infections recognized more than 12 or 24 months from the operation may be of hematogenous origin or due to very low virulent organisms at the time of surgery [12]. In chronic PJI, Gram-positive cocci are involved in the overwhelming majority of hip and knee PJI [90], with *S. aureus* and coagulase-negative staphylococci alone accounting for 50–70 % of PJI cases [42,90], while Gram-negative bacilli have been shown to cause 5–20 % of PJI's [90].

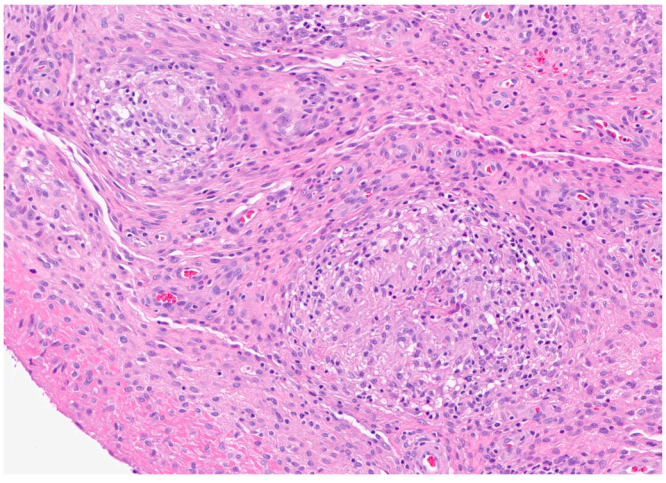

Fig. 5. Foreign-body granulomas are common in periprosthetic tissue, but non-necrotizing granulomas without debris can be associated with Mycobacterial or fungal infections, as well as other systemic granulomatous disorders such as sarcoidosis or, as in this case, idiopathic inflammatory bowel disease. H&E 220X

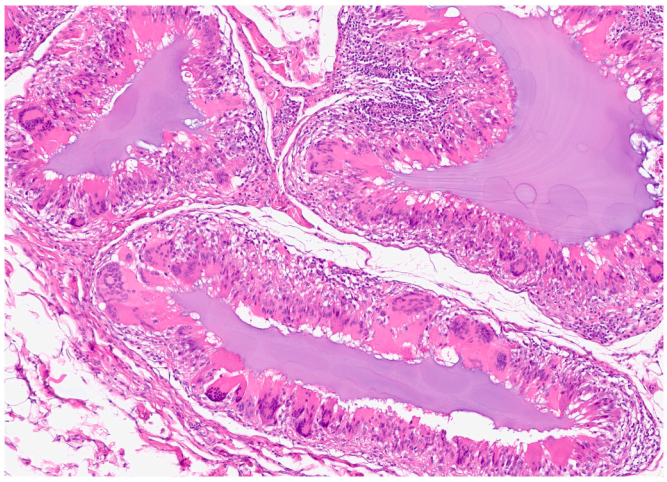

Fig. 6. Hyaluronic Acid granuloma associated with previous "viscosupplementation" injection. H&E 6.3X

Complicating the diagnosis and treatment of PJI is the observation that many pathogens produce **biofilms** that increase resistance to antimicrobial therapy when compared to planktonic cultures grown in liquid media. The mechanisms whereby a biofilm promotes resistance are incompletely understood. The extracellular polymeric matrix of the biofilm can enhance bacterial attachment to the implant and act as a barrier to protect bacteria [91]. Biofilms can also convert macrophages from a pro-inflammatory to anti-inflammatory state, such that the macrophages then decrease the efficacy of neutrophils, resulting in a less effective inflammatory response [92]. Pathogens hidden deep in the biofilm have low metabolic rates which also prevent accurate culture identification.

Sonication: One method to disrupt the biofilm on a retrieved implant and release bacteria into surrounding fluid is to use low-frequency ultrasound. The sonicate fluid can then be submitted for culture or for molecular methods of pathogen detection. Some studies have reported enhanced sensitivity compared to conventional culture alone, especially in patients who have received continuous antibiotics [93,94]. However, implementing sonication can be difficult in many hospital settings. It is logistically difficult and expensive to maintain an inventory of sterile and bacteria-free containers for retrieved implants in the operating room area, there are opportunities for contamination as implants are transported from the OR to the microbiology lab or during handling, and many microbiology laboratories do not have the space or personnel to operate one or more sonication instruments. The validation of sonication and maintaining quality sample consistency are technically difficult and time consuming, since the appropriate frequency and power are needed to de-clump the bacteria without lysing bacterial cell walls resulting in negative cultures [95]. In addition, other studies have reported no benefit of sonication over conventional culture [96], or have reported good or equivalent specificity but relatively poor sensitivity for the sonication process [97,98]. Sonication may also be less effective when applied to implants or spacers excised at the second stage re-implantation, since the sonication process may lead to antibiotic concentration in the sonicate fluid that inhibits bacterial growth in culture [99,100]. The current role of sonication in diagnosing PJI is controversial. In hospitals with adequate infrastructure to support the process, it might be most appropriate in cases that have been culture negative, especially when exposed to recent prolonged antibiotics. It is probably not appropriate at the second stage implantation for known

infection [100].

6. Molecular

Although microbiologic cultures are critically important for diagnosing PJI, culture-negative infections are relatively common (22 % in one recent study [101]), with negative cultures usually thought to reflect previous antibiotic use, slow-growing or low-virulent bacterial organisms, or protection of pathogens by adherent biofilm.

Recently developed molecular methods have shown promise in identifying pathogens in patients who are culture negative. Molecular diagnostic tests can be broadly grouped into those that target one specific pathogen (e. g. polymerase chain reaction, PCR), a group of specific pathogens (multiplex PCR, or MALDI), or organisms not limited to known sequences (e. g. Next-Generation Sequencing) [102].

Specific PCR assays can be performed on fluids such as from joint aspiration, and consist of several steps, including DNA extraction, amplification with the use of DNA primers and DNA polymerase, and extension of new complementary strands of DNA. PCR assays that target a single organism can confirm the diagnosis in the appropriate clinical context [103,104], especially if a specific pathogen is suspected at the time of sample collection, but can be difficult to perform and require technical resources not available in all hospitals. Commercial multiplex PCR kits that target the most likely group of pathogens for a clinical indication, such as PJI, have been developed and may be especially useful at revision arthroplasty and at the second stage re-implantation of known infection when a patient has been treated by antibiotics. Multiplex PCR assays, such as the BioFire® Joint Infection Panel target many of the pathogens commonly involved in musculoskeletal infections as well as a selection of antimicrobial resistance markers [105]. Multiplex PCR assays can only detect a limited number of pathogens, however, so a limitation of some assays is the current unavailability of specific probes for common low-virulent organisms such as *S. epidermiditis* or *C. acnes*. Another approach to molecular diagnosis is to use a broad range PCR that targets the 16S ribosomal RNA gene that is present in essentially all bacteria, followed by Sanger or next-generation sequencing (Targeted Metagenomic Sequencing) of the amplified DNA [106,107].

Next Generation Sequencing (NGS) refers to DNA sequencing methods that produce large amounts of genomic data from a single reaction that may require complex informatics for analysis. A potential advantage over multiplex PCR is the lack of required pathogen-specific primers, although a high-quality reference library is necessary for pathogen identification. Some studies have described favorable results of NGS in detecting culture negative PJI and in recognizing several different organisms in positive joint fluid samples [108]. However, molecular testing has occasionally identified unexpected organisms, including bacteria never reported in human infections [109], such that it may be difficult to distinguish an analytical false-positive from a clinically false-positive result. Some authors have even suggested that unexpected positive NGS results from clinically uninfected synovial fluid may represent normal “native microbiome” [110]. Interpreting a contaminant as a pathogen could lead to inappropriate clinical treatment, so work continues in defining thresholds to exclude background DNA from the host, other organisms, or from bacterial contaminants in reagents [111]. As far as we know, NGS has not been cleared by FDA for PJI diagnosis, although it can be available as a Laboratory Developed Test (LDT) with lab-dependent panels of genes. While early results of NGS are encouraging, some studies have reported no better sensitivity than conventional culture [112], and some have suggested that the most appropriate use of sequencing may be in culture-negative samples [106].

Matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) is another test that is becoming widely available. It cannot be used to directly test aspirated joint fluid or tissue, but can rapidly identify organisms that have grown in culture [113]. Samples of positive cultures are placed on a planchet, overlaid with matrix and subjected to a laser. The resulting ionized proteins are accelerated in an electromagnetic field, separated based on mass-to-charge ratio, and the resulting mass spectrometry profiles are compared to a reference database. Correct diagnosis depends on the quality of the reference database, and antibiotic sensitivities are not identified. Although the MALDI instrument itself is expensive, operating costs are not, so use of this technology is increasing.

Another relatively new test is to sequence cell-free DNA (cfDNA) from blood. This test has largely been used for cancer diagnosis or fetal genetic testing, but has also been applied to diagnosing blood-born infections [114] and PJI [115]. Although not cleared by the FDA, the Karius test is an LDT available through the CLIA-certified, CAP-accredited Karius laboratory (Redwood City, CA). Working with investigators from Karius, Donlin and co-authors [115] were able to identify pathogen cfDNA in 35 cases of PJI, including 4 that had been culture negative. The test also confirmed polymicrobial infection in one patient, and identified microorganisms not grown in culture in 14 cases. Limitations of the test include the inability to identify antimicrobial sensitivity, the short half-life of circulating cfDNA (minutes), and the necessity to define thresholds from clinically uninfected individuals that are used to classify a result as positive or negative. The detected pathogen could also reflect bacteria from anywhere in the body, including necrotic bacteria unrelated to PJI. Nevertheless, as an adjunct to other tests, blood cfDNA sequencing could increase confidence in culture results, and could help document the efficacy of treatment.

7. Conclusion

In conclusion, PJI diagnosis remains a complex challenge, demanding a multifaceted approach utilizing various tests and clinical expertise. Further research is needed to refine diagnostic accuracy and optimize patient outcomes especially as new molecular test methods become available. Pathologists hold a critical role in assisting with PJI diagnosis, maintaining laboratory test quality and interpreting test results. Continued collaboration between clinicians and pathologists will be essential in improving patient care and reducing the burden of PJI.

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CRediT authorship contribution statement

Fermina M. Mazzella: Methodology, Writing – original draft, Writing – review & editing. **Yaxia Zhang:** Writing – original draft, Writing – review & editing. **Thomas W. Bauer:** Conceptualization, Project administration, Writing – original draft, Writing – review & editing.

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