FACULTY OF MEDICINE AND HEALTH SCIENCES

Periprosthetic Joint Infections: Literature Review on Location of Infection, Value of Tissue Sampling and Next-Generation Sequencing

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Inhoudsopgave

Abstract (EN)

Background. Up to one out of five patients are not satisfied after their joint replacement. An important cause of joint replacement failure is the development of a periprosthetic joint infection (PJI). The incidence of PJI after primary arthroplasties is estimated to be 1% for hip arthroplasties and 1-2% for knee arthroplasties every year. Despite a wide variety of available diagnostic tests and definitions, diagnosing a PJI remains challenging. Up to 50% of the suspected PJIs could have negative cultures despite clear clinical signs of infection. To confront these diagnostic challenges, better diagnostic testing and a universally accepted definition, even in an obvious clinical setting, are necessary. Recently, next-generation sequencing (NGS) has attracted much attention and could be a possible game changer. The purpose of this dissertation is to compare the diagnostic value of NGS with standard culture-based methods, to review the recent literature on the concept of infection mapping and to evaluate the diagnostic value of preoperative tissue biopsy techniques.

Methods. A detailed search of the MEDLINE, Embase and Web of Science databases was performed to identify studies involving the value of NGS in the diagnosis of PJI, the concept of infection mapping and the diagnostic value of preoperative sampling techniques. Papers published between January 1st, 2017 until July 31st, 2023 in the English, French or German language were eligible for inclusion.

Results. A total of 7,627 potential papers were identified and 72 were included in this review. First, the diagnostic value of NGS was reviewed. Secondly, an attempt was made to define the PJI topography based on bacterial adherence and colonisation of the prosthetic joint. Thirdly, the value of different sampling techniques was evaluated.

Discussion. NGS appears to demonstrate a high diagnostic value in the diagnosis of PJI. This method could be useful to diagnose PJI, especially in culture negative cases. Knowledge about the PJI infection topography might prevent unnecessary open major revision, but instead support a partial exchange of genuinely infected components. Distinct techniques of preoperative tissue sampling might provide promising diagnostic accuracy in case of a suspected, but difficult to diagnose PJI.

Abstract (NL)

Achtergrond. Tot één op vijf patiënten zijn niet tevreden na hun gewrichtsprothese. Een belangrijke oorzaak van een gefaalde gewrichtsprothese is de ontwikkeling van een periprothetische gewrichtsinfectie. De incidentie van infecties bij een primaire gewrichtsprothese is naar schatting 1% voor heupprothesen en 1-2% voor knieprothesen per jaar. Ondanks de variatie aan beschikbare diagnostische tests en definities blijft de diagnose van een periprothetische gewrichtsinfectie een uitdaging. Tot wel 50% van vermoede infecties kunnen negatieve cultuurresultaten krijgen, ondanks de aanwezigheid van duidelijke klinische tekens. Om deze uitdagingen aan te gaan, zijn er betere diagnostische testen en een universeel geaccepteerde definitie noodzakelijk. Next-generation sequencing (NGS) heeft de laatste jaren veel aandacht opgeëist en is een potentiële meerwaarde in de diagnosestelling van periprothetische gewrichtsinfecties. Het doel van deze masterthesis is om de diagnostische waarde van NGS te vergelijken met de standaard cultuurmethode, de recente literatuur over het concept van infectietopografie te onderzoeken en de diagnostische waarde van preoperatieve weefselbiopsietechnieken te evalueren.

Methodologie. De MEDLINE, Embase en Web of Science databases werden op een gedetailleerde en systematische manier doorgenomen om studies te identificeren die de diagnostische waarde van NGS in de diagnose van periprothetische gewrichtsinfecties, het concept van infectietopografie en de diagnostische waarde van preoperatieve biopsietechnieken behandelen. Studies geschreven in het Engels, Duits of Frans en gepubliceerd tussen 1 januari 2017 en 31 juli 2023 waren geschikt voor inclusie.

Resultaten. In totaal werden 7,627 papers geïdentificeerd en 72 studies werden geïncludeerd in deze review. De diagnostische waarde van NGS werd beoordeeld. Vervolgens werd een poging ondernomen om de topografie van periprothetische gewrichtsinfecties te definiëren. Ten derde werd de waarde van verschillende biopsietechnieken geëvalueerd.

Discussie. NGS lijkt een hoge diagnostische accuraatheid te hebben in de diagnose van periprothetische gewrichtsinfecties. Deze methode is mogelijks een waardevol onderzoek in de diagnostiek van periprothetische gewrichtsinfecties, vooral wanneer de cultuurresultaten negatief zijn. In de toekomst zijn bijkomende onderzoeken noodzakelijk om de beschrijving en de rol van infectietopografie verder in kaart te brengen. De verschillende preoperatieve weefselbiopsietechnieken leveren een goede diagnostische accuraatheid in geval van een vermoede, maar moeilijk aantoonbare periprothetische gewrichtsinfectie.

Introduction

Joint replacement surgery with artificial endoprostheses has dramatically altered the treatment of patients with degenerative osteoarthritis. These procedures appear to be promising in terms of reduction of pain and recovery of function, resulting in improvement of daily activities and increasing the overall quality of life (1). Despite recent innovations and optimisation of prosthetic implants and surgical techniques, some patients still have a bad outcome with these procedures. Up to 1 out of 5 patients are not satisfied after their joint replacement (2, 3) and up to 12% of total knee and hip arthroplasties are revised within ten years (4).

One of the main causes of joint replacement failure is the development of periprosthetic joint infections (PJIs) (5). Periprosthetic joint infection (PJI) is a devastating complication after prosthetic surgery. The incidence of PJI after primary arthroplasties is estimated to be 1% for hip arthroplasties and 1-2% for knee arthroplasties each year (2, 6). As more joint replacements are performed annually, the incidence of PJI is expected to increase (2). Moreover, treating PJIs results in a major burden on healthcare economics. One-stage or two-stage exchange arthroplasties are commonly performed in the management of PJI (2). Hence, PJI is the most common reason for revision of total knee arthroplasty and the third leading cause for revision total hip arthroplasty (1, 7). It is expected that the need for revision surgery will increase because of the increasing prevalence of risk factors for PJI, such as obesity, diabetes and other comorbidities (2). The financial cost of a PJI is estimated to be 3 to 5 times the cost of a primary arthroplasty (1, 2, 8). In addition to the economic implications, PJI diminishes the quality of life and overall life expectancy of patients (2, 8). Zmistowsky et al. (9) report a 1-year mortality rate of 10.6% and a 5-year mortality rate of 25.9%.

Infections can occur on the basis of different mechanisms. Firstly, direct inoculation around the prosthetic components is possible. These infections can occur during implantation and are attributed to exogenous sources from the operating theatre or endogenous skin flora. Secondly, there is possible hematogenous spreading from an infection site located elsewhere in the body. Thirdly, infection can also occur as a recurrence of an indolent infection (2). In arthroplasties, the susceptibility for infection is increased due to the insertion of artificial components into the joint. Microbial inoculation usually results in biofilm formation as a sessile platform on the prosthetic components. Bacteria attach to the prosthesis and multiply to create microcolonies encased in a glycocalyx (2, 10). The biofilm formation starts within seconds after contamination (11). The architecture and composition of the biofilm create firm defences against the host's immune system and the antimicrobial therapy (2, 10). Besides the presence of components in the joint, other risk factors increasing the risk of PJIs can be patient related factors (e.g. uncontrolled diabetes, morbid obesity, smoking and malnutrition), demographic

factors and surgery associated risk factors (e.g. blood transfusions and postoperative drainage) (2).

The clinical presentation of PJI varies from clear signs of infection to more non-specific symptoms, such as pain and reduced range of movement. The clinical presentation of PJI is dependent on the mechanism of infection and the microbiological aetiology (1, 12). It is reported that especially PJIs with less virulent organisms might present without clear symptoms (2, 13). A classification can be made based on the time between arthroplasty and onset of symptoms. (Fig. 1) Early acute postoperative infections present within 3 months after surgery. Delayed acute infections present between 3 and 12 months after surgery. In delayed occurring infections the pathogen is mostly less virulent. Infections diagnosed after 12 months are mainly caused by hematogenic spreading from a different site of the body (1, 2). Chronic infections may present with vague and often intermittent symptoms (13). It is important to differentiate periprosthetic joint infection from aseptic failure, as both can present with similar symptoms, but require different therapeutic approaches (1, 2).

Type of Infection	Time to Presentation	Mechanism of Infection	Organisms	Clinical Presentation	
Early	<3 mo	Intraoperative contamination	Virulent bacteria (ie, *Staphylococcus aureus*)	Acute	Sudden onset erythema, edema, warmth, and tenderness
Delayed	3–12 mo	Intraoperative contamination	Low virulent bacteria (coagulase-negative staphylococci)	Chronic	Joint pain and stiffness
Late	>12 mo	Hematogenous seeding	Virulent bacteria (ie, *S.aureus*)	Acute	Sudden-onset erythema, edema, warmth, and tenderness
		Intraoperative contamination	Low virulent bacteria (ie, *Propionibacterium acnes*)	Chronic	Joint pain, sinus tract

Figure 1. Classification and clinical presentation of prosthetic joint infections based on time of symptom onset after arthroplasty. Common findings in physical examination, culture, and mode of pathogenic entry are presented. Adapted from Gomez-Urena et al. (1).

The variability in clinical presentation complicates the diagnosis of PJI. Many attempts have been made in the past to define diagnostic criteria for PJI. In 2011, the Musculoskeletal Infection Society (MSIS) published the first definition (14). In 2013, the International Consensus on Musculoskeletal Infection (ICM) adjusted the definition to international consensus (15). Also in 2013, the Infectious Diseases Society of America (IDSA) published diagnostic guidelines based on an international expert group (16). More recently in 2018, a new definition for hip and knee PJIs was proposed using a weight-adjusted scoring system with major and minor criteria (17). In 2021, the European Bone and Joint Infection Society (EBJIS) published “The EBJIS definition”, which was supported by the MSIS and the European

Society of Clinical Microbiology and Infectious Disease (ESCMID) Study Group for Implant-Associated Infections (ESGIAI) (12). To date, most definitions are based on a combination of clinical signs, laboratory analysis of peripheral blood and synovial fluid and microbiology or histology of aspirated synovial fluid or intraoperative tissue samples. Imaging can also be performed, such as nuclear imaging and radiological investigations (12, 18). Nowadays it appears that the validated EBJIS definition might become a classification more frequently used, even outside Europe (12, 19).

The EBJIS definition of periprosthetic joint infection introduces a three-level definition for PJI (Infection unlikely, Infection likely and Infection confirmed). (Fig. 2) Binary definitions (infected or not infected) are difficult to use as no available diagnostic test can exclude PJI. Current diagnostic tests still have significant false-positive and false-negative rates (12). In addition, low grade infections can be missed in the classical bimodal definitions, since the specificity of their positive diagnostic test can be insufficient to confirm diagnosis of PJI. Therefore, introducing a middle group (infection likely) can include patients with a significant risk of infection and for whom further investigation should be considered (12). The EBJIS definition includes clinical signs and several diagnostic tests, as illustrated in figure 2. It is important to note that infection can be confirmed based on one major criterion, such as the presence of a communicating sinus tract or by direct visualisation of the prosthesis, on cytological analysis of synovial fluid or by the presence of synovial fluid biomarkers or by microbiological or histological investigations. Two positive cultures with the same microorganism are a major criterion to confirm a PJI as well. Furthermore, the diagnosis of infection is only likely when e.g. there is a minor positive clinical parameter or a raised C-reactive protein (CRP) value, together with another positive test (e.g. synovial fluid, microbiology, histology or nuclear imaging) (12).

Despite current diagnostic tests and definitions, diagnosing a periprosthetic joint infection remains challenging. Culture-based methods are still considered standard tests for detecting causative organisms in infection (8), but approximately 7-50% of patients have negative cultures despite clear clinical sings of infection and positive laboratory tests (18). Common causes of culture-negativity in PJI can be classified into patient factors, organism factors and laboratory findings. Instillation of saline or local anaesthesia into the joint during synovial fluid aspiration or antibiotic treatment previous to aspiration can potentially result in negative cultures (8). Furthermore, the organisms that cause PJI are characterised by the ability to develop a biofilm (8). Despite optimisation of laboratory culturing methods to detect planktonic (free-floating) bacteria (1), infections might not be detected due to the pathogens' ability to conceal themselves in biofilm (8, 20). In addition, the behaviour of these sessile bacteria is different from planktonic organisms since the sessile bacteria have a reduced growth rate and

are less stable in various microenvironments, making it more difficult to facilitate the growth and replication on culture agar plates (8). PJIs can also be caused by mycobacteria, fungi and more rare organisms that cannot be detected on routine bacterial cultures and require more specialised tests (1).

	Infection Unlikely (all findings negative)	Infection Likely (two positive findings)[a]	Infection Confirmed (any positive finding)
Clinical and blood workup			
Clinical features	Clear alternative reason for implant dysfunction (e.g. fracture, implant breakage, malposition, tumour)	1) Radiological signs of loosening within the first five years after implantation 2) Previous wound healing problems 3) History of recent fever or bacteraemia 4) Purulence around the prosthesis[b]	Sinus tract with evidence of communication to the joint or visualization of the prosthesis
C-reactive protein		> 10 mg/l (1 mg/dl)[c]	
Synovial fluid cytological analysis[d]			
Leukocyte count[c] (cells/µl)	≤ 1,500	> 1,500	>3,000
PMN (%)[c]	≤ 65%	> 65%	> 80%
Synovial fluid biomarkers			
Alpha-defensin[e]			Positive immunoassay or lateral-flow assay[e]
Microbiology[f]			
Aspiration fluid		Positive culture	
Intraoperative (fluid and tissue)	All cultures negative	Single positive culture[g]	≥ two positive samples with the same microorganism
Sonication[h] (CFU/ml)	No growth	> 1 CFU/ml of any organism[g]	> 50 CFU/ml of any organism
Histology[c,i]			
High-power field (400x magnification)	Negative	Presence of ≥ five neutrophils in a single HPF	Presence of ≥ five neutrophils in ≥ five HPF
			Presence of visible microorganisms
Others			
Nuclear imaging	Negative three-phase isotope bone scan[c]	Positive WBC scintigraphy[j]	

Summary Key

a. Infection is only likely if there is a positive clinical feature or raised serum C-reactive protein (CRP), together with another positive test (synovial fluid, microbiology, histology or nuclear imaging).

b. Except in adverse local tissue reaction (ALTR) and crystal arthropathy cases.

c. Should be interpreted with caution when other possible causes of inflammation are present: gout or other crystal arthropathy, metallosis, active inflammatory joint disease (e.g. rheumatoid arthritis), periprosthetic fracture, or the early postoperative period.

d. These values are valid for hips and knee periprosthetic joint infection (PJI). Parameters are only valid when clear fluid is obtained and no lavage has been performed. Volume for the analysis should be > 250 µL, ideally 1 ml, collected in an EDTA containing tube and analyzed in <1h, preferentially using automated techniques. For viscous samples, pre-treatment with hyaluronidase improves the accuracy of optical or automated techniques. In case of bloody samples, the adjusted synovial WBC= synovial $WBC_{observed}$ – [WBC_{blood} / RBC blood x $RBC_{synovial\ fluid}$] should be used.

e. Not valid in cases of ALTR, haematomas, or acute inflammatory arthritis or gout.

f. If antibiotic treatment has been given (not simple prophylaxis), the results of microbiological analysis may be compromised. In these cases, molecular techniques may have a place. Results of culture may be obtained from preoperative synovial aspiration, preoperative synovial biopsies or (preferred) from intraoperative tissue samples.

g. Interpretation of single positive culture (or < 50 UFC/ml in sonication fluid) must be cautious and taken together with other evidence. If a preoperative aspiration identified the same microorganism, they should be considered as two positive confirmatory samples. Uncommon contaminants or virulent organisms (e.g. *Staphylococcus aureus* or Gram negative rods) are more likely to represent infection than common contaminants (such as coagulase-negative staphylococci, micrococci, or *Cutibacterium acnes*).

h. If centrifugation is applied, then the suggested cut-off is 200 CFU/ml to confirm infection. If other variations to the protocol are used, the published cut-offs for each protocol must be applied.

i. Histological analysis may be from preoperative biopsy, intraoperative tissue samples with either paraffin, or frozen section preparation.

j. WBC scintigraphy is regarded as positive if the uptake is increased at the 20-hour scan, compared to the earlier scans (especially when combined with complementary bone marrow scan).

Figure 2. The EBJIS definition of periprosthetic joint infections. This classification introduces three-level definition for PJIs (Infection unlikely, Infection likely and Infection

confirmed) based on clinical features, laboratory findings and nuclear imaging. Adapted from McNally et al. (12).

Furthermore, if the patient received antibiotic therapy in the last two weeks prior to the diagnostic tests, the diagnostic accuracy of synovial fluid and perioperative tissue cultures are compromised, increasing the risk of false-negative results (21).

If an Infection remains undiagnosed, revision surgery is often based on “aseptic” treatment options (12), which - in light of an existing infection - might result in higher treatment failure rates (22). In culture-negative PJI cases, an empiric antibiotic treatment might perhaps be needed, but actually it should be avoided because of higher failure rates compared with targeted antibiotic treatment (8, 18). Therefore, antimicrobial treatment based on microbiological diagnosis is recommended, unless in the presentation of a severe sepsis where fast life-saving treatment is needed. In addition, empirical antimicrobial treatment often requires administration of broad-spectrum antibiotics, which can induce adverse drug reactions and antimicrobial resistance in the long term. Moreover, in case of fungal or yeast-based infections antibiotic treatment will not be effective (20).

The development of accurate microbial diagnostic tests is essential to improve the diagnosis of PJI and optimise the treatment of patients (23). The introduction of molecular methods in the diagnosis of PJI could improve the diagnostic process. In 1970 it was demonstrated that molecular techniques, such as polymer chain reaction (PCR) could reveal the presence of more pathogens compared with the culture methods (8). Even more, it is suggested that less than 2% of all existing pathogens can be cultured in laboratory settings (24). Therefore, all patients with negative cultures could potentially benefit from molecular diagnostic testing (17). In addition, molecular methods could detect pathogens despite previous antimicrobial treatment without compromising the sensitivity, which can be difficult for culture-based methods. It is also suggested that molecular methods might avoid time-related logistical issues associated with culture-based methods, such as prolonged transport time or insufficient incubation time (8).

The PCR technology has become a frequently used diagnostic technique in the past decade (25). This technology is designed to detect specific species of microbes by using primers that are complementary to DNA sequences, unique to a given species (26). Several authors demonstrated a good sensitivity, but especially a high specificity for PCR analysis to diagnose PJIs (25, 27). However, PCR-based techniques have a major limitation. The use of primers to detect specific organisms requires prior knowledge of the epidemiology and microbial profile and limits the possible scope of detecting all microbes (8). To overcome this limitation of the PCR technology, a more recent method called multiplex PCR has been introduced. This

method combines multiple primers, which gives the opportunity to detect more species, but may still overlook uncommon or atypical organisms (21). Therefore, an entire screening of the bacterial load cannot be achieved (26).

Next-generation sequencing (NGS) has shown promising results and it has been suggested that it could serve as a potential additional diagnostic test for culture-negative PJIs (8). Furthermore, NGS cannot only identify microbial species, but might also detect genes associated with antibiotic resistance whilst predicting antibiotic susceptibility (23). Besides finding bacteria, NGS can also detect viruses, fungi and parasites (28). According to the American Society of Microbiology, NGS would have the potential to replace current techniques with “a single all-inclusive diagnostic test” (29). The World Health Organization (WHO) also recognises the potential of the NGS technology as the WHO advises the use of NGS for testing infectious diseases (23). Due to increasing availability of the NGS technology, the diagnostic testing for infectious diseases based on NGS has become a more feasible option in the standard clinical setting (23).

To conclude, periprosthetic joint infections have a huge impact on patients and healthcare systems. Over the last few decades there has been a great advancement in the prevention, diagnosis and treatment of PJIs, but the incidence is still rising due to the increasing numbers of arthroplasties, the increasing volume of risk factors for PJIs and the progressive antimicrobial resistance in micro-organisms. In addition to this, culture-based methods cannot always identify the causative organism in all patients, resulting in higher treatment failure rates. To meet these challenges, better diagnostic tests are necessary and NGS might present itself as potential game changer.

The scope of this dissertation has the following objectives:

1) To evaluate the diagnostic value of next-generation sequencing compared with standard culture-based methods.
2) To outline intra- or extra-articular locations of periprosthetic joint infection based on location samples.
3) To evaluate the diagnostic value of preoperative tissue sampling techniques compared with synovial fluid aspiration and open biopsies.

Methods

Data sources and search strategy

The search strategy of this review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We systematically searched the databases of MEDLINE using the PubMed interface, Embase and Web of Science. The inclusion of search terms was based on the patient, intervention, comparison, outcome (PICO) model. The search strategy combined search terms related to infection and prosthesis to analyse the population of patients with periprosthetic joint infections. Then we combined these search terms with other search terms related to NGS, fluid sampling, tissue sampling and terms related to the topography of the infection. In the MEDLINE database, both [Mesh]-terms and [Title/Abstract]-terms were included to retrieve also the most recently published papers. Thereafter, the search strategy was translated to the Embase and Web of Science databases to screen for additional papers. The two authors of this dissertation independently screened titles and abstracts of the retrieved citations. Differences were discussed and agreements were made. Both authors read the full text of potentially suitable papers. The search was completed by manual screening of the reference list of relevant papers. All included papers were saved in Endnote 20. Appendix A provides a detailed overview on the search strategy.

Eligibility Criteria

Papers written in English, French or German and published between January 1st, 2017 until July 31st, 2023 were eligible for inclusion. Furthermore, in order to evaluate the diagnostic value of NGS and preoperative tissue sampling techniques, the literature was screened in accordance with additional inclusion and exclusion criteria. The inclusion criteria were: (1) human studies related to NGS or preoperative tissue sampling in periprosthetic hip, knee or shoulder infections; (2) a clear definition of PJI in the manuscript; (3) provision of the numerical values of sensitivity and specificity. Exclusion criteria were: (1) animal studies, case reports, conference papers, systematic reviews and meta-analyses; (2) details about the sampling technique were not clearly stated; (3) the full text was not available.

Data extraction

Two independent reviewers extracted data to evaluate the diagnostic value of NGS and the preoperative tissue sampling techniques. Extracted data included data on study characteristics, study population, demographic characteristics, clinical characteristics, and statistical analyses. We used the Methodological Index for Non-randomized Studies (MINORS) tool to assess the risk of bias of the studies (30).

Results

1. Next-generation sequencing

In recent years, molecular diagnostic methods have rapidly evolved and they are currently starting to play an important role in the diagnosis of cancers, genetic and infectious diseases (21). Current molecular techniques in daily practice rely on PCR and NGS (8, 31).

The NGS technology overcomes the limitations of PCR by allowing to simultaneously sequence millions of small DNA fragments and to differentiate polymicrobial samples (31, 32). The universal principle of NGS is that DNA or RNA sequences, extracted from cells (human, bacterial or fungal) and viruses, present in the samples can be amplified and compared with an available database. In addition, this method can differentiate human cell types or bacterial species based on DNA or RNA characteristics in the detected sequences from the sample (33). For example, the presence of ribosomal RNA (rRNA) operons in bacteria - specifically the 16S rRNA genes - have been used as the primary tool for classification for many years, because a 16S rRNA gene is present in at least one copy in any bacterial genome and its sequence provides reliable information on bacterial family, genus, or species in most cases (34).

The NGS technology has been applied in the diagnostic process of several infectious diseases. Several papers were published about the impact of NGS results to diagnose infections of the central nervous system (35-37), respiratory system (38-41), cardiac system (42), uveitis (43), sepsis (44), gastrointestinal tract infections (45, 46), hepatitis (47) and urinary tract infections (48). In line with this, it is suggested that NGS might also improve the diagnosis of PJI. Applying NGS in the diagnostic work-up of PJIs could aid in detecting bacterial or fungal DNA or RNA in the samples extracted from the joint and periarticular area (33).

NGS technology requires the conversion of the nucleic acid material from the sample under investigation into standard libraries suitable for loading onto a sequencing instrument. (Fig. 3) There is a wide variety of NGS library preparation protocols available, but they all have in common that DNA or RNA fragments are fused with adapters that contain the necessary elements for immobilisation on a solid surface. In addition, size selection steps are often performed to remove free adapters and to select molecules in the desired size rage (approximately 200 base pairs). Next, PCR amplification of the molecules is often performed to generate sufficient quantities of template DNA to allow accurate quantification (33).

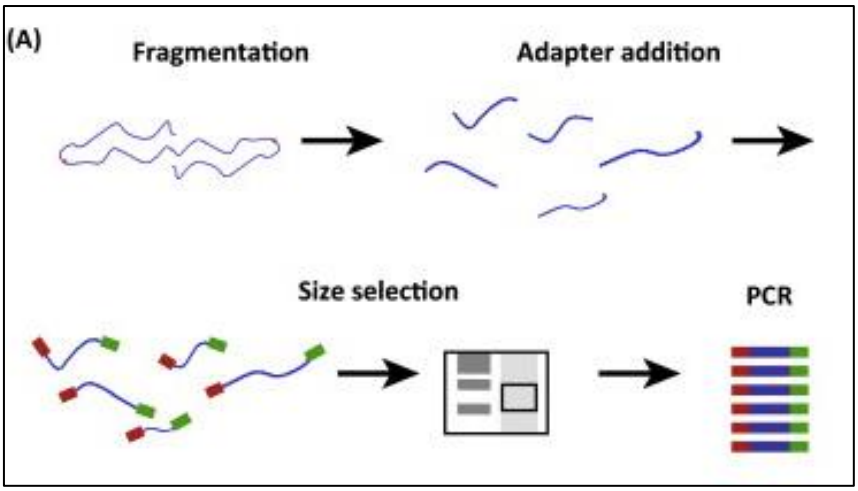

Figure 3. DNA or RNA preparation for a sequencing library. DNA or RNA is fragmented into smaller sized sequences, adapters are added on both ends and after size selection PCR-amplification is performed. Adapted from Van Dijk et al. (33).

Currently, there are two main types of NGS frequently used to diagnose PJI: shotgun metagenomic next-generation sequencing (sNGS) and targeted next-generation sequencing (tNGS) (31). (Fig.4) Shotgun metagenomic next-generation sequencing (sNGS), also referred to as metagenomic next-generation sequencing (mNGS), sequences all nucleic acids present in a specimen in parallel, including those deriving from the host or any other microorganism present (49). This high-throughput sequencing technique is combined with bioinformatics analysis to directly detect all nucleic acids in a test sample. The results are then compared with alignment tools to identify the species and abundance of all known microorganisms in the sample. Prior knowledge of a specific primer is thus unnecessary (49-51). On the other hand, tNGS, also called 16SrRNA amplicon sequencing (8, 32), targets the bacterial 16SrRNA gene with a primer (52). Next, these genes are amplified prior to sequencing (32, 52). This approach can reduce the laboratory noise induced by the presence of human host DNA in the sample (52). Therefore, a significant increase in sequencing cycles might be required to overcome host DNA contamination and to adequately characterise the microbes present (52), yielding potentially more easily interpretable results (32). However, using 16S gene-based tNGS obviously limits detection to bacterial pathogens only (32). Besides sNGS and tNGS, several research groups have also investigated the potential use of meta-transcriptomic NGS (MT-NGS), which analyses gene expression and could distinguish between active and inactive genetic pathways based on the transcriptome of the microbial community (8, 53, 54).

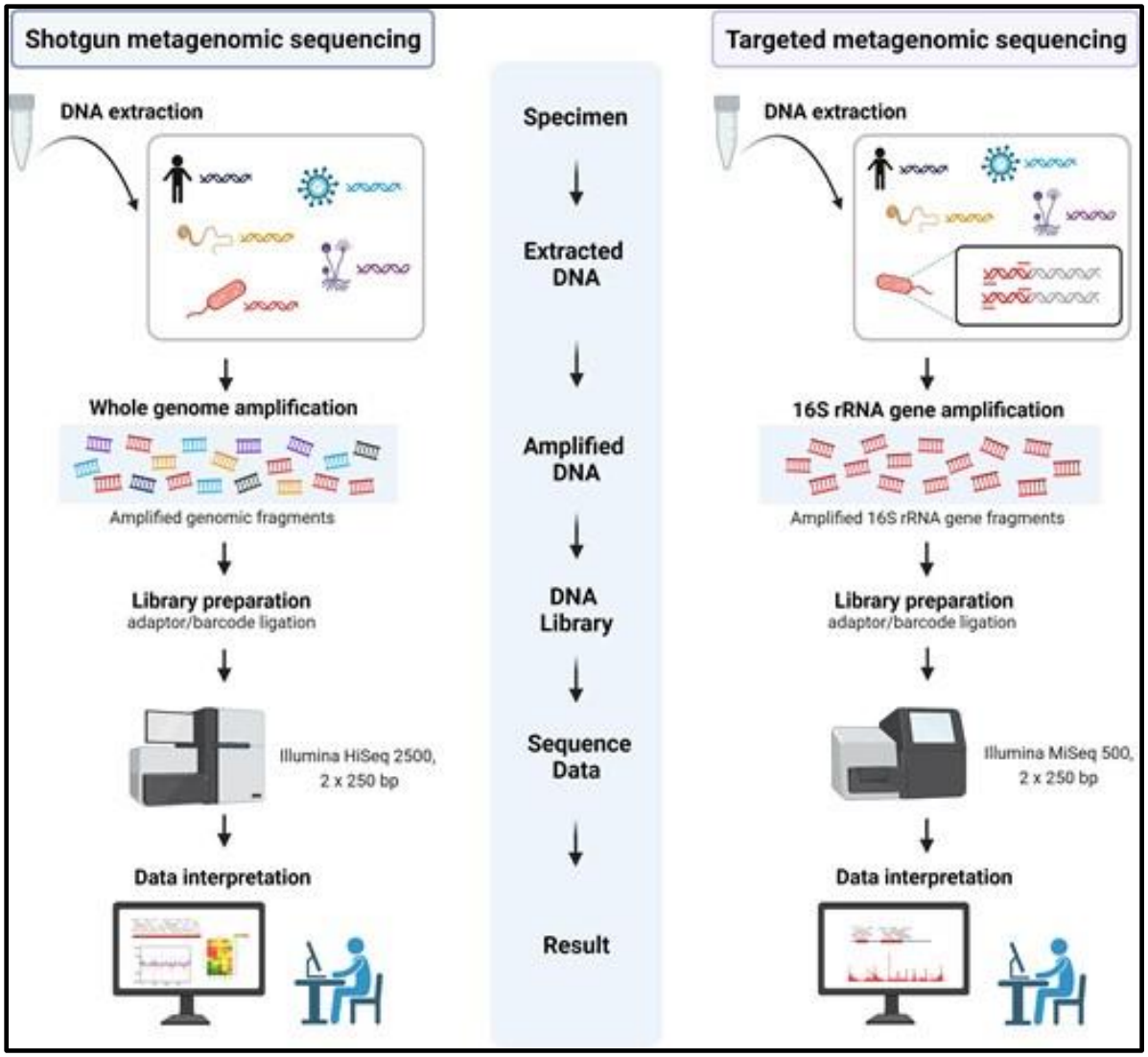

Figure 4. Workflow of shotgun metagenomic sequencing (left) and targeted metagenomic sequencing (right). While shotgun metagenomic sequencing amplifies the whole genome, targeted metagenomic sequencing amplifies bacterial specific 16S rRNA gene fragments using primers. Adapted from Hong et al. (31).

1.1. Diagnostic value of next-generation sequencing

Several authors have investigated the diagnostic value of NGS to detect PJIs. This chapter reviews the diagnostic value of the NGS methods in comparison to the standard culture-based methods. The search strategy yielded 264 potential papers about the diagnostic value of NGS. After more detailed screening of titles and abstracts, 223 papers were excluded. Of the remaining 41 papers, 22 were included in this review. Details about the study characteristics from the included papers are presented in appendix B.

Eleven studies were included which evaluated the sensitivity and specificity of NGS compared with culture methods in the diagnosis of PJIs (32, 50, 51, 55-62). (Table I) The sensitivity of NGS ranged from 60.9% to 100% and the sensitivity of culture methods ranged between 47%

and 79.6%. Ten of the eleven studies showed that the NGS technique has a higher sensitivity compared with conventional culture techniques. The sensitivity in these ten studies ranged from 85% to 100% for NGS and 47% to 79.6% for cultures (32, 50, 51, 55-61). However, diagnostic superiority of NGS over culture methods was not universally reported. A recent larger study by Kildow et al. (62) showed a lower sensitivity for NGS (60.9%) compared with conventional culture techniques (76.9%). Furthermore, the specificity for NGS in all included studies ranged from 73% to 95.2% and for culture methods from 77.3% to 100% (32, 50, 51, 55-62). In two of the studies the reported specificity of NGS was higher compared with culture methods (50, 56). In three other studies was the specificity of NGS lower compared with culture methods (55, 59, 62). In the remaining six papers, the specificity of NGS and culture were similar (32, 51, 57, 58, 60, 61). In addition, it is also noteworthy that two studies showed that the sensitivity of cultures on preoperative fluid samples was remarkably lower than cultures on intraoperative fluid samples (51, 56), whereas the timing of sampling did not influence the sensitivity of NGS (51).

1.2. Next-generation sequencing in culture positive periprosthetic joint infections

There is a major agreement between NGS and culture results in culture positive PJI cases, even to the extent that culture positive results are virtually always confirmed by NGS methods. In the studies included, the positive diagnostic rate in culture-positive cases ranged between 94.1%-100% (51, 55, 58-61). This demonstrates that NGS might be a viable alternative laboratory method for detecting pathogens (63, 64).

1.3. Next-generation sequencing in culture negative periprosthetic joint infections

Multiple authors reported that NGS is capable to detect organisms in culture negative PJI cases (26, 49, 51, 55, 58-60, 63, 64). Tarabichi et al. (55) have shown that NGS is able to detect organisms in 81.8% (9/11) of culture negative PJIs. Surprisingly, in the study of Wang et al. (58), NGS could detect a single pathogen in all 10 culture negative PJI cases (100%). However, Flurin et al. (32) reported a lower detection rate of 36.4% (4/11) in culture negative PJIs with NGS, similar to the detection rate of 43.9% (43/99) reported by Thoendel et al. (64). In addition, Fang et al. (56) demonstrated that the NGS method applied on preoperative samples could be used to optimise culture techniques by modifying the AGAR-plates set up. This approach reduced the amount of culture negative PJIs. The combination of both methods increased the sensitivity with an insignificant loss of specificity (56). In summary, these results illustrate that NGS could be particularly useful in PJI cases where cultures are negative.

Table I. The sensitivity and specificity of NGS compared with culture methods.

References	Criteria	NGS type	NGS Sample type	Population size (PJI/Non-PJI)	NGS		Culture	
					Sensitivity	Specificity	Sensitivity	Specificity
Tarabichi et al., 2018 (55)	MSIS	tNGS	Synovial fluid; Deep-tissue; Swabs	65 (28/37)	89.3%	73.0%	60.7%	97.3%
Zhang et al., 2019 (61)	MSIS	mNGS	Sonicated fluid	37 (24/13)	100%;	92.3%	70.8% (sonicated culture) 62.5% (conventional culture)	92.3% (Sonicated culture) 100% (conventional culture)
Cai et al., 2020 (50)	MSIS	mNGS	Periprosthetic tissue	44 (22/22)	95.5%	90.9%	72.7%	77.3%
Fang et al., 2020 (51)	MSIS	mNGS	Synovial fluid, preoperative	37 (24/13)	92.0%	92.3%	52.0%	92.3%
	MSIS	mNGS	Synovial fluid, intraoperative	37 (24/13)	96.0%	100%	72.0%	100%
Huang et al., 2020 (60)	MSIS	mNGS	Synovial fluid	70 (49/21)	95.9%	95.2%	79.6%	95.2%
Wang et al., 2020 (58)	MSIS	mNGS	Synovial fluid; Sonicated fluid; Homogenised tissue	63 (45/18)	95.6 %	94.4%	77.8%	94.4%
Fang et al., 2021 (56)	MSIS	mNGS	Synovial fluid, preoperative	56 (35/21)	91.4%	95.2%	34.3%	81.0%
	MSIS	mNGS	Synovial fluid, intraoperative	56 (35/21)	NR	NR	60.0%	81.0%
Flurin et al., 2021 (32)	IDSA	tNGS	Sonicated fluid	105 (47/58)	85.0%	98.0%	77.0%	100%
He et al., 2021 (57)	MSIS	mNGS	Synovial fluid; Sonicated fluid; Tissue	59 (40/19)	95.0%	94.7%	85.0%;	95.0%
Kildow et al., 2021 (62)	MSIS	tNGS	Synovial fluid	116 (48/68)	60,9%	89.9%	76.9%	95.3%
Yin et al., 2021 (59)	MSIS	mNGS	Synovial fluid	35 (15/20)	93.3%	90.0%	47.0%	95.0%

MSIS: MusculoSkeletal Infection Society; IDSA: Infectious Diseases Society of America; NGS: next-generation sequencing; mNGS: metagenomic next-generation sequencing; tNGS: targeted next-generation sequencing; PJI: periprosthetic joint infection; NR: not reported

1.4. Next-generation sequencing in aseptic revision procedures

Five studies reported the detection of organisms in preoperatively assumed “aseptic” cases. In these cases, an infection was not suspected since the diagnostic criteria for PJI were not fulfilled (49, 51, 55, 58, 64). The reported detection rate of organisms in assumed aseptic joints ranged between 3.6% and 25.0% (55, 64). The detection of microorganisms in presumably “aseptic” patients may indicate that many unrecognised or occult infections of prosthetic joints are considered to be “aseptic” based on the available classification systems. Alternatively, this could also raise the issue that NGS sequencing may be associated with false positive or non-clinically significant findings in some samples.

1.5. Next-generation sequencing and prior antibiotic administration

An important limiting factor in the detection of organisms with culture-based methods is the influence of antibiotic treatment prior to sampling. Indeed, Hong et al. (31) compared NGS with culture methods in patients whom had received antibiotics prior to surgery and found a higher detection rate with NGS (96/128 cases) compared with culture methods (64/128) cases. In addition, He et al. (57) detected pathogens with NGS in 8 out of 9 patients who had received antibiotics within one month before surgery and culture methods only detected pathogens in 5 patients. (57) Furthermore, Larsen et al. (65) reported that NGS did not appear to be influenced by antibiotic treatment before surgery. This study showed that in 21 out of 24 patients NGS had positive results and only 19 of the matched culture results were positive. Overall, NGS methods appear to increase the detection rate of pathogens in cases where antibiotics were not stopped two weeks before sampling. Zhang et al. (61) and Huang et al. (60) both describe NGS as a useful tool to detect pathogens in particularly culture negative cases were antibiotics were administrated within two weeks before sampling.

1.6. Effect of sample type on next-generation sequencing results

There seems to be a lot of controversy regarding the optimal sampling type to detect PJIs with NGS. Part of the problem is that not all studies report results on their types of sampling, making the comparison between sampling types more difficult. Goswami et al. (24) compared NGS results in tissue samples, swab samples and synovial fluid. They found that tissue samples were superior to swab samples and synovial fluid, respectively detecting 46.6%, 34.1% and 19.4% of the NGS positive results. Larsen et al. (63) compared NGS on tissue samples, sonicated fluid, synovial fluid, bone and swab samples. In contrast to Goswami et al. (24), Larsen et al. (63) found tissue sampling actually a more inferior sampling method, detecting a pathogen in only 8 out of 32 (25.0%) PJIs. Sonicated fluid however seems the superior method detecting a pathogen in 32 out of 37 (86.5%) PJIs. The same research group found a pathogen in 25 out of 35 (71.4%) cases with synovial fluid, and 15 out of 34 (44.1%) cases with bone and swab samples (63). In aseptic cases, NGS of bone and swab samples, however, could detect a pathogen in 4 out of 54 (7.4%) cases (63). He et al. (55) studied the effect of sampling methods by comparing the sensitivity and specificity of sonicated fluid, synovial fluid and tissue samples. Similar to Larsen et al. (63), they found that sonicated fluid samples were superior to synovial fluid and tissue samples with a sensitivity of respectively 92.5%, 87.5% and 65.0%. The specificity for all three sample methods was similar (55). Overall, sampling of sonicated fluid appears to be superior, but can obviously only be performed on extracted implants.

1.7. Value of next-generation sequencing to prove clinical infection

The positive and negative predicted values of NGS have been calculated in several papers. (Table I) Five of them showed a high positive predictive value (PPV) for NGS, ranging between

91.3% to 100% (32, 50, 51, 56, 57). The same authors also showed high PPV for culture methods, ranging between 75.0% to 100%. These results suggest that both methods are good indicators for PJI. The negative predictive value (NPV) for NGS was also high, ranging between 85.7% to 95.2%. Culture, on the other hand, had a much lower NPV, ranging between 42.5% to 84.0%. This signifies that negative NGS results would indicate absence of PJI in contrast to culture methods, in which a negative result could not rule out a PJI.

1.8. Differences between shotgun and targeted next-generation sequencing

As described above, there are different NGS techniques available of which sNGS and tNGS are the most commonly used to diagnose PJI. To our knowledge, Hong et al. (31) were the only authors so far who investigated the differences between both methods. They found that tNGS and sNGS showed concordant results in 83.6% of culture positive cases. In culture negative PJIs, both techniques showed concordant results in 77.6% of the cases (31).

However, three papers used tNGS as the preferred diagnostic method (32, 55, 66). These papers demonstrated a lower sensitivity (range 60.9% to 89.3%) compared with studies using sNGS (range 91.3% to 100%) (50, 51, 56-61, 67). The specificity of tNGS compared with sNGS was similar in one study (32) and lower in the two of the studies included (55, 66), especially in the study of Tarabichi et al. (55). However, these comparisons are only observations and future research comparing sNGS and tNGS is warranted.

1.9. Next-generation sequencing versus polymerase chain reaction

When molecular techniques are used, PCR based methods are by far the most utilised techniques. However, Kildow et al. (66) demonstrated that PCR methods lack in sensitivity. In their study, the PCR technique had a sensitivity of only 18.4% and a specificity of 100%, compared to a sensitivity and specificity of respectively 60.9% to 89.9% for tNGS. Furthermore, Wang et al. (58) investigated if broad-range PCR (BR-PCR) could be a used as a verification method of sNGS in PJI. They found that the value of joint fluid BR-PCR had a sensitivity of 82.2%, which was not significantly different from the sensitivity of mNGS (95.6%) or culture (77.8%). The specificities of the 3 methods appeared all to be 94.4%.

1.10. Does the type of arthroplasty affect the next-generation sequencing results?

Tarabichi et al. (55) demonstrated that the NGS method was capable to detect an organism in 6 out of 17 (35.3%) patients undergoing primary arthroplasties, in which samples were taken intraoperatively. However, al positive samples derived from tissue samples. Swabs and fluid samples were negative in all cases. On the other hand, Huang et al. (60) could not detect a single pathogen in ten primary arthroplasties. Kildow et al. (62) showed that the sensitivity and specificity of the NGS method were lower in primary arthroplasties (58.3% and 85.7%) compared with revision arthroplasties (70.0% and 100.0%). Torchia et al. (68), while trying to

characterise the microorganisms of the native knee by NGS, detected at least one organism in twelve out of forty primary total knee arthroplasties of patients with osteoarthritis. Nine of those were polymicrobial. It is important to mention that they aspirated joint fluid prior to arthrotomy. The research group states that the presence of organisms in primary arthroplasty suggest a possible existence of a native microbiome. However, contamination remains a possibility, raising more questions than answers (69).

1.11. Challenges for next-generation sequencing

1.11.1 Interpretation of the detected pathogens

A critical appraisal of research findings is necessary before NGS could be implemented as the standard diagnostic technique. First of all, correctly distinguishing whether detected pathogens represent a true PJI or should be considered as contaminants, remains open to research. Due to the fact that mNGS sequences all nucleic acids present in a specimen, this technique might be highly prone to contamination bias, making it more difficult to know whether the joints are truly infected (26, 32, 49, 50, 53, 62, 64, 70). To reduce the influence of contamination by bacteria and host DNA, Ivy et al. (49) used filtering strategies such as microbial enrichment and DNA isolation, resulting in a reproducible method to properly detect pathogenic microbial DNA.

Besides contamination, there is also controversy about a potential existence of a native microbiome in the joint (53, 55, 62, 70, 71). However, it is very difficult to evaluate the “native microbiome” since it is necessary to invade the joint through a soft tissue envelope in order to aspirate the joint fluid. Moreover, detailed information about preceding injections or arthrocentesis procedures was not stated by the authors.

1.11.2 Antibiotic treatment based on next-generation sequencing results

Wang et al. (20) examined the potential impact of mNGS targeted antibiotic treatment compared with empirical antibiotic treatment. They observed a slightly better infection control in the group where NGS targeted antibiotic treatment was used with a rate of 100% (12/12) compared to an infection control rate of 83.33% (10/12) in the group with empiric antibiotic treatment. The difference was not significant. However, targeted antibiotic treatment based on mNGS showed potential. Huang et al. (60) also came to the conclusion that mNGS results led to good infection control without treatment failures. Multiple authors mention that modification of antibiotic treatment based on NGS could be useful, especially in cases where cultures are negative (26, 50, 55, 61).

In addition, antibiotic resistance patterns might explain persistence of infections. Subsequently, identification of antibiotic resistance might aid in determining optimal antibiotic treatment in a clinical setting. In this suspect, mNGS could help to identify genes related to antibiotic

resistance (54, 72). Larger studies are needed to indicate the clinical outcome of the implementation of NGS-based antibiotic treatment in PJIs.

1.11.3 Cost-effectiveness

Until now, only Torchia et al. (68) compared the cost-effectiveness of culture and NGS. They evaluated the cost-effectiveness based on hospital records, located in the United States, as well as published data. The group concluded that NGS could be cost-effective in comparison to culture methods. They also stated that NGS should be reserved for clinical contexts with high pre-test probability (68). On the other hand, Larsen et al. (65) highlight that NGS may be used in cases where sequencing is critical (for example long-term chronic cases and cases with prior antibiotic administration) due to its cost.

Multiple papers mention that the high cost of NGS is a major limiting factor in the clinical application of NGS (49, 51, 57, 64, 65). However, others describe how the cost of NGS is decreasing over the years (51, 58, 63, 64), making the use of NGS clinically feasible. Moreover, two papers state that NGS technology is a cost-effective method (60, 63). Interestingly, Wang et al. (20) showed that the antibiotic cost was lower in a group in which targeted antibiotic treatment was based on NGS, compared to a group where empirical antibiotic treatment was used. Furthermore, He et al. (57) describe combining NGS with culture on sonicated fluid samples as a highly cost-effective method. Hong et al. (31) even described how tNGS could be a more cost-effective way to introduce NGS in clinical laboratories compared to mNGS which is more labour intensive and has a higher reagent cost. It is important to note that all above mentioned cost issues are influenced by a variation of multiple factors such as health care systems, transport time, laboratory availability or degree of analysis depth of the samples (68).

If NGS would be applied in Belgium for PJI, extrapolated figures based on reliable sources reveal that the cost for NGS samples at present would be around €50 to €100 per sample, in comparison to €20 per sample for culture methods.

2. Infection topography

The location of bacteria in PJIs has been described in the past (73-75). Nevertheless, few studies have this topic as their main study target. This chapter will discuss the currently available literature about the anatomic distribution of the infection in attempt to further develop the concept of infection topography. The hypothesis is that better understanding of the distribution of the infection in and around the infected joint could play a critical role to improve

accurate diagnosis and treatment of PJIs. In addition, the value of preoperatively performed targeted sampling will be determined to improve the diagnosis of PJI.

The search strategy yielded 1,505 potential papers related to infection topography. After more detailed screening of titles and abstracts, 1,483 papers were excluded. Of the remaining 22 papers, 19 were included in this review based on the full text and four additional papers were identified by screening their references. Five additional studies, published before 2017, were included as well due to their added value to this review.

2.1. The introduction of infection topography

Several classification systems of PJIs have been introduced in the past (12, 14, 15, 17, 76). These classifications are usually based on variables such as onset of symptoms, the severity of clinical presentation and the virulence of the causative pathogen. However, these classifications might present some shortcomings and their usefulness in clinical practice is not universally applicable (77).

The concept of infection topography was introduced in 2019 by Pellegrini et al. (77) in an attempt to critically review the classification systems of PJIs. To our knowledge, it appears these authors are the first ones to describe this concept as such. They introduced a new topographic principle in their modified classification. This was based on the theory that identifying the exact location of the bacterial colonisation may help to decide the best therapeutic approach. Further, they stated that the timing of the PJI onset should not account as the only factor to predict the clinical outcome of the treatment of PJI. Indeed, the infection topography was absorbed in their proposal, discriminating between three distinctive patterns: 1) infection located only in the joint space; 2) infection located at the bone-implant interface; and 3) infection involving both compartments (77).

Subsequently, the topographic information might help to predict which implant components could be infected and this could help to decide the optimal treatment strategy. (Fig. 5) The authors postulate that implant removal might not be required in PJI cases where bacterial colonisation has not reached the bone-implant interface yet (e.g. late infections). In contrast, implant removal is indicated when the bone-implant interface is invaded by the microbiome. Hence, their research suggests to limit DAIR (Debridement, antibiotics and implant retention) procedures in the former situation, but a more radical approach with extraction of components in the latter (77). Rosinsky et al. (78) and Shi et al. (79) found that, outlining the PJI topography beforehand, partial prosthetic component replacement might be indicated in selective patients, without negatively affecting the surgical outcome.

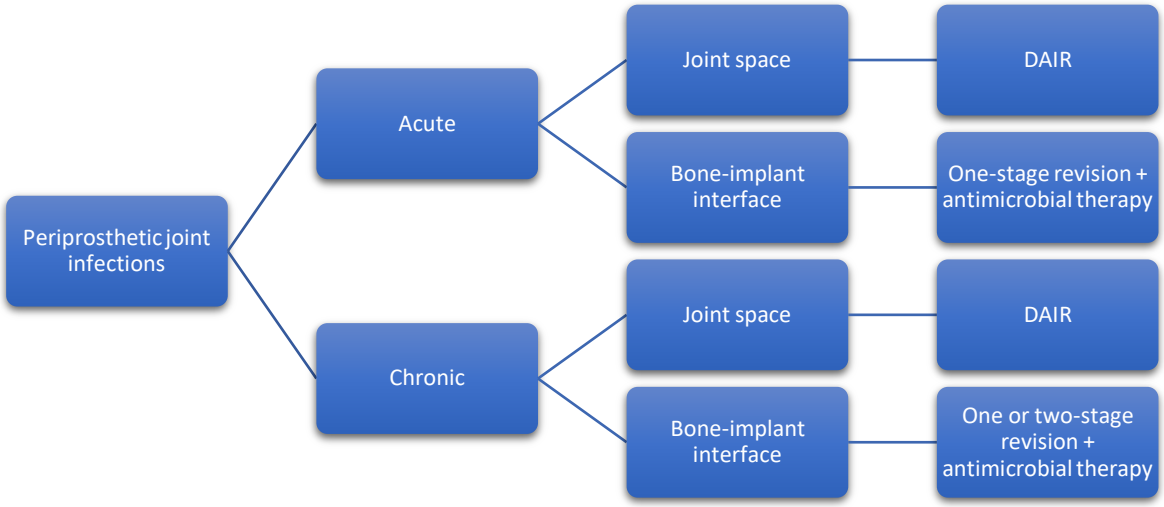

Figure 5. New treatment algorithm including the topography of the infectious process. Debridement, antibiotics and implant retention (DAIR) is suggested when only the joint space is infected. Revision surgery and antimicrobial therapy is recommended when the bone-implant interface is infected. Modified from Pellegrini et al. (80).

Alt et al. (81) introduced another classification system based on the oncological TNM-classification for malignant tumours. Their motives to introduce a new classification system were similar to Pellegrini et al. (77) They state that currently available classification systems consider the timing of onset of symptoms as the most important variable. However, PJI is a multifactorial process and therefore more parameters such as the host, the implant with the surrounding soft tissue and bone and the causative microorganisms should be included to predict the best treatment strategy and the final outcome (81).

In this new PJI-TNM classification system, they grade the local situation of the tissue and the indwelling implant (T), the causative non-human bacterial or fungal organisms (N) and the morbidity of the patient (M). (Fig. 6) To evaluate the tissue and implant conditions, they take into account the soft tissue defect, implant stability and implant type (standard or revision). The presence of a biofilm influences the N-staging, but the authors do not differentiate between the anatomical location of the biofilm. Therefore, it appears that the concept of topography is not really absorbed in the PJI-TNM classification.

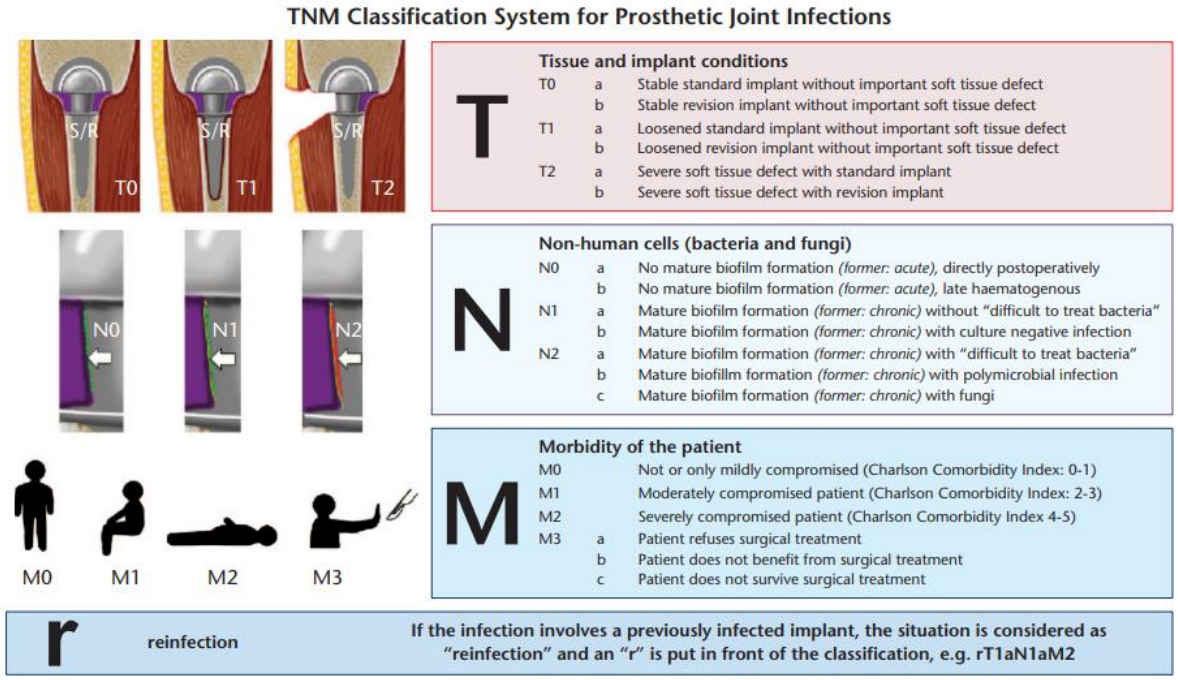

Figure 6. PJI-TNM classification system introduced by Alt et al. (82).

The two papers by Pellegrini et al. (2019) and Alt et al. (2020) seem to at least introduce the concept of infection landscape in the classification of PJIs. The classification of Alt et al. seems to highlight the impact of local conditions, but actually it is the research group of Pellegrini et al. that introduced the concept of infection mapping as an important variable in PJI. Despite all this, there is paucity of details about the anatomic zonal spread in the infected joint.

2.2. Bacterial colonisation and adherence to orthopaedic implants

In order to understand the topography, one should understand the processes of bacterial colonisation, biofilm formation and attachment to orthopaedic implants and the surrounding tissue. Indeed, biofilms do grow on implant components, in surrounding tissue and in fibrous sheaths (83-85). Each of these environments have their own characteristics and can be considered separate niches. (Fig. 7) Additionally, the prosthetic joint fluid might contain planktonic cells or clumps of detached biofilm, leading to further invasion of the periprosthetic tissue. However, these microenvironments could interact. In particular, bacterial eradication by debridement or antimicrobial therapy might be futile in light of a potential recolonisation by pathogens from the other microenvironments not affected by the therapeutic measures (83).

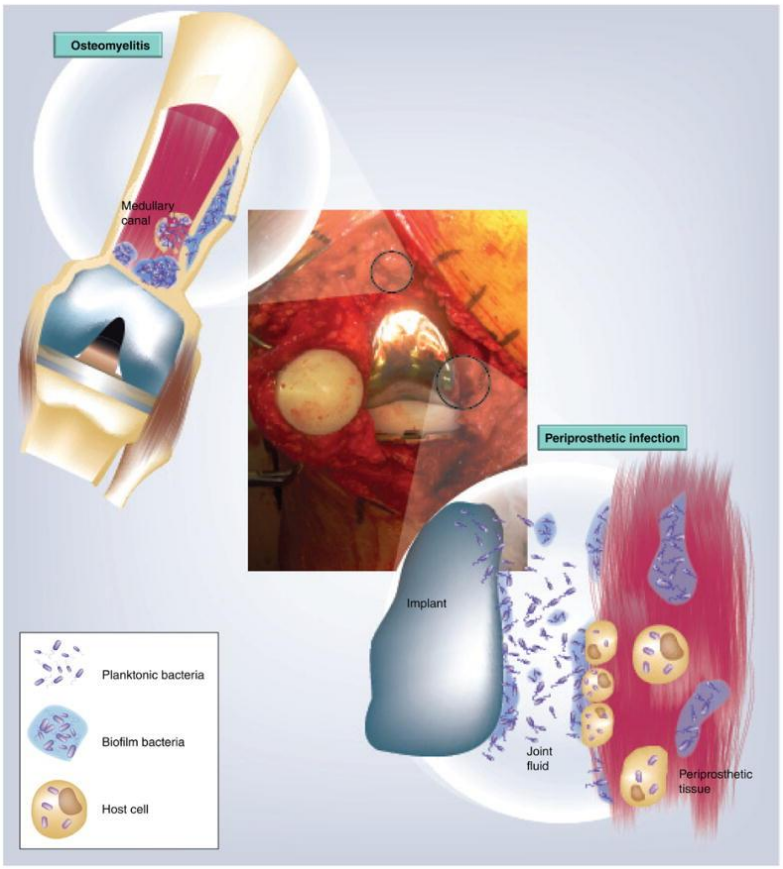

Figure 7. Illustration of possible bacterial adherence in PJI after total knee arthroplasty (TKA). The joint fluid, the implant surface and the surrounding tissue represent individual reservoirs of infection, each containing bacteria in different phenotypic states, from which pathogens could possibly repopulate if they are not completely eradicated through means such as washing, antibiotic therapy or surgical debridement. Adapted from McConoughey (83).

The bacterial colonisation is influenced by multiple factors. First of all, it is suggested that the surface of implants might play an important role in the biofilm formation. Moore et al. (86) studied the in vitro biofilm formation of *S. Aureus* on multiple orthopaedic implants using an in vitro imaging system and scanning electron microscopy. Their data suggest that implant roughness and large-scale surface features may be at greater risk of biofilm colonisation. Indeed, it appeared that the femoral hip stem and the total knee systems with the roughest surfaces showed the highest luminescence. Bacteria might benefit from the shelter that rough surfaces provide. There was also a significant bacterial attachment to edges of implants. The hypothesis is that attachment to an implant edge may allow delivery of nutrients from multiple sides of the biofilm, which might result in a nutritional benefit and enhanced growth (86).

However, more recent studies assessing the ex vivo bacterial adherence to explanted components in confirmed PJIs, reported other findings. In a study performed by Holinka et al. (75), the bacterial load on components from knee prostheses in patients with PJI was assessed

using sonication culture methods. The most important outcome was that polyethylene (PE) components and tibial components were mostly affected by microorganisms, but the ultra-high-molecular-weight PE (UHWMPE) components did show a much higher load of colony-forming units (CFU) per component than on cobalt chromium (CoCr) components (75). These results are comparable to the findings in total hip prosthetic replacements. In the latter, the highest bacterial load was found in sonication fluid dislodged from PE-liners, followed by ceramic heads, metal shell cups and femoral stems. The highest CFU loads per component were detected on PE liners, followed by the cup and head (74). When the results in total knee arthroplasty (TKA) and total hip arthroplasty (THA) are compared, in THA there are more isolated microorganisms per component surface unit found compared with TKA (74). In contrast, a clinical study of Gómez-Barrena et al. (87) showed no significant preference of bacteria to the different components in PJI-affected TKA and THA. They explained their findings by referring to the individual patient characteristics (host susceptibility, immune reaction, perfusion status and underlying local conditions) and the variability in microorganism adherence.

More recent studies confirm the findings from Holinka et al. (75) and Lass et al. (74). Karbysheva et al. (88) found that bacteria in sonication-fluid cultures grew in all PE-components (100%), followed by titanium alloy (79%) and cobalt-chromium components (71%). Larger bacterial counts were found on PE than on titanium or CoCr alloy. Janz et al. (89) reported a comparable outcome with significantly higher rates of bacterial isolation on PE-components compared with non-PE components.

To conclude, the unequal distribution of the bacterial load and CFU between different components of prosthetic joints show that location and make-up of the prosthetic components could influence the different bacterial load. It seems that biomaterials have variable intrinsic affinity for bacterial adhesion and biofilm formation. In addition, it is suggested that the toxic properties of metal-wear particles of CoCr alloys might also impact the bacterial viability of the microbiome, but the exact effect remains unclear (88). However, utilising the technique of sonication, it is not possible to determine the actual in vivo biofilm biomass on the prosthetic component, subject to extraction of the latter. In addition, other factors might also play a role, such as destruction of the biofilm during extraction of the prosthetic implant, the fixation method (cemented or non-cemented) and the biomaterial surface type (87). Appraising biofilm presence, volume or characteristics are not possible as long as the implant components remain in situ (88).

2.3. In vivo bacterial adherence in prosthetic joints

Studies on the in vivo bacterial adherence in prosthesis joints are scarce. Sonication fluid and tissue specimens obtained after implant extraction are frequently cultured to diagnose PJI. Besides the diagnostic value, the information might also be used to map the spreading of the infection. Ahsan et al. (73) characterised the bacterial load in *Cutibacterium*-infected (formerly known as *Propionibacterium*) revision shoulder arthroplasties based on material obtained from the stem explant, head explant, glenoid explant, humeral membrane, collar bone, soft tissue and fluid. At least 4 distinct specimen types were collected for culture.

In their study, only 32.6% of the joint fluid cultures were positive in comparison to 66.5% of the soft-tissue cultures and 55.6% of the cultures of the explant specimens. The higher rates of culture positivity of soft-tissue and explant specimens compared to joint fluid are consistent with the tendency of *Cutibacterium* to reside in a biofilm rather than existing in planktonic form in joint fluid that is accessible by aspiration (73).

The specimen *Cutibacterium* Value (SpCuV), representing the semiquantitative value of the bacterial load on specimens, was determined for each specimen. The average SpCuV for fluid (0.35 ± 0.89) was significantly lower than that for soft tissue (0.92 ± 1.50) and explant specimens (0.66 ± 0.90). However, a clear threshold above which a result can be considered as true-positive could not be defined (73).

The authors state that conclusions about the presence of bacteria in joint arthroplasties should be based on the amount and types of the specimens submitted for culture (73). In fact, a major criterion to define PJI is based on the of number of positive cultures of samples taken intraoperatively, with the presence of ≥2 positive cultures with phenotypically identical virulent organisms considered a major criterion to diagnose PJI (17, 90). However, one positive culture with a high-virulent organism should be considered as a possible PJI as well (90). The value of this criterion is somehow limited by the observation that culture positivity might be influenced by other factors, particularly the origin of the specimen sample, since soft-tissue and explant specimens had higher rates of culture positivity than joint fluid (73).

Furthermore, knowing the uneven distribution of the microbiome in an infected prosthetic joint appears to have an impact on the diagnostic approach. According to Garrigues et al. the recommendations are to sample multiple sites (at least 5) (90), although recommended sites for sampling have not been defined. Better understanding of the topography could help to define the location of the best diagnostic sample sites. Nhan et al (91) were the first to compare the value of shoulder explant cultures with standard tissue cultures in patients undergoing revision shoulder arthroplasty. They investigated the bacterial distribution in shoulder revision arthroplasties by sampling 3 explant locations and 3 tissue specimen locations (91). (Fig. 8)

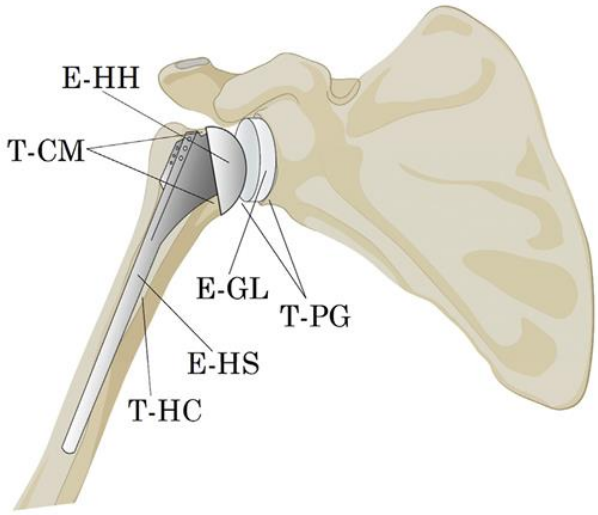

Figure 8. Locations of implant and tissue cultures as proposed by Nhan et al. Three explant types and three tissue types were defined:1) E-HH (explant, humeral head); 2) E-GL (explant, glenoid); 3) E-HS (explant, humeral stem); 4) T-CM (tissue, collar membrane); 5) T-PG (tissue, periglenoid); 6) T-HC (tissue, humeral canal). Adapted from Nhan et al. (91)

As illustrated in figure 8, the explants were subdivided into the following 3 regions: (1) the humeral head (E-HH), defined as the component adjoined to the humeral stem and articulating with the glenoid; (2) the humeral stem (E-HS), defined as the component removed from the humeral canal; and (3) the glenoid (E-GL). Tissue samples were divided into the following 3 categories: (1) collar membrane (T-CM), defined as the soft tissue between the humeral head and stem components; (2) humeral canal tissue (T-HC), defined as the tissue found around the humeral stem within the canal; and (3) periglenoid tissue (T-PG), defined as the tissue around the glenoid or glenoid component (91). To quantify the bacterial density, Nhan et al. (91) used the SpCuV, identical to Ahsan et al. (73). However, they did not calculate the average SpCuV.

The cultured explants had a higher proportion of positive specimens compared to the adjacent tissue, but the difference was not statistically significant. However, the proportion of culture-positivity for each implant was low, ranging from 15% of the glenoid component to 30% of the humeral stem. The individual culture-positivity rate of the tissue specimens varied from 11% of the periglenoid tissue to 22% of the collar membrane tissue. It should be noted that the number of specimens from the glenoid component and periglenoid tissue was very low (91).

The proportion of culture-negative tissue samples when the explant samples were positive ranged from 25-43% and was higher than the proportion of culture-negative explant samples when the corresponding tissue specimen was positive, ranging 0-21%. The bacterial density, based on the SpCuV, was generally higher in the explant samples compared with tissue

specimens. Based on tissue samples alone, only 17 out of 107 cases met the criterion of ≥2 positive cultures. When the results of explant samples were included, an additional 15 patients (14%) crossed the treatment threshold (91). In the study of Ahsan et al., the bacterial density was slightly higher on tissue samples than explant samples (73).

Similar to Ahsan et al., Nhan et al. and Hsu et al. recommend to sample tissue from 6 separate and distinct sites that might show a higher rate of positivity (92).

2.4. Unexpected positive intraoperative cultures

In light of the observation that infections could be unevenly distributed in the infected prosthetic joint or might be only present extraarticular around the implant, there could be an explanation for the finding that “aseptic” and culture-negative revision arthroplasties turn out to actually be a PJI on the basis of unexpected positive intraoperative cultures (UPICs). Many authors have recently described the prevalence of UPICs in shoulder (93-95), knee (96-98) or hip (98-101) arthroplasties. It remains open for debate whether UPICs would represent a genuine PJI or rather refer to contamination. Indeed, distinguishing between contamination and true colonisation remains elusive in term of universally accepted guidelines (96).

Kloos et al. (96) performed a systematic review on the prevalence and outcome of unexpected positive cultures in revision total knee arthroplasty. The estimated prevalence of at least 1 UPIC in revision TKA was 8.3% and 10.6% in revision TJA. Individual findings from the included studies varied significantly from 5.9% to 62.1%. Neufeld et al. (97) reported a comparable prevalence of at least 1 UPIC in 9.8% of their cases. However, both research groups did not link the presence of a UPIC to the prevalence of an unsuspected PJI. Jacobs et al. (98) reported a prevalence of 7.9% of unsuspected PJI in revision TKA, defined by ≥2 positive cultures with the same organism.

In revision total hip arthroplasties, the prevalence of UPICs was 10% when using tissue cultures alone and 28% with combined tissue cultures and sonication fluid cultures (100). The prevalence of tissue cultures alone is in line with the prevalence found by Milandt et al. (101) (12.2%) and Neufeld et al. (99) (9.2%). The prevalence of unsuspected PJI in revision hip arthroplasties, based on the criteria of ≥2 positive cultures with the same organism, was only 3% (100). This is again comparable with the registry-based study from Milandt et al. (101) and Neufeld et al. (99), which reported respectively 4.9% and 2.9% of unsuspected PJIs. However, the reported prevalence of unsuspected PJI in presumed aseptic hip revisions is remarkably lower than reported 12.1% by Jacobs et al. (98).

It is important to note that many authors state that a single UPIC or mixed growth from several cultures is currently regarded as clinically irrelevant or without impact on further treatment. Nevertheless, it is found that patients with presumed contaminants found during revision THA

were more likely to undergo re-revision due to subsequent PJI (100, 101). The incidence of implant survival after at least 2 years follow-up was 88% and 92% in TKA and THA respectively in the study of Jacobs et al. (98). The survival of the knee prosthesis was affected by the diagnosis of unexpected PJI. As previously stated, based on the virulence the pathogens found, a positive culture with a high-virulent organism should be considered as a possible PJI (90).

Intraoperative samples were harvested in the vast majority of the reviewed studies. However, the approach was not standardised and there was a substantial variability in the number of collected samples and the collection sites, based on the preference of the surgeon in TKA (96, 97) and THA (99). Besides tissue culturing, several studies performed microbiological analysis after explant sonication. It is remarkable that combining tissue cultures with synovial fluid cultures and sonication cultures could identify an additional 18% of positive cultures compared to using tissue cultures alone in revision THA (100). In revision TKA, Fernandez-Sampedro et al. (102) concluded that the diagnostic sensitivity of sonication fluid compared with periprosthetic tissue cultures is higher with 88% versus 67% respectively. This difference would suggest that a diagnostic approach with tissue cultures needs to be more optimised regarding the number and location of samples.

Falstie-Jensen et al. (93) found that the prevalence of UPICs in presumed aseptic shoulder revision arthroplasty was 22%. At least 5 separate samples were obtained in proximity to the bone-cement interface or synovium and from areas with visible membranes or necrotic tissue. In their study, cultures were defined as positive when bacteria were detected in at least 3 of the 5 biopsy specimens. Padegimas et al. (94) found UPICs in 23.9% of the patients, comparable to Falstie-Jensen et al. The number of collected specimens was not stated. Zmistowski et al. found a higher prevalence of 31.3% (95). In all of the reviewed papers, *Cutibacterium acnes* was the most frequently cultured microorganism, accounting for 57.1%-71.4% of the UPICs (93-95).

Detailed information about the sample sites with unexpected positive culture is missing in all of the reviewed studies. Nevertheless, having this information could assist in defining the topography of the PJI and could enhance the quality of tissue sampling methods.

3. Sampling techniques

Besides new microbiological techniques, such as NGS, new sampling methods might improve the diagnosis of PJIs. As previously described, preoperative joint fluid aspiration culture fails to detect microorganisms in up to 42% of the suspected PJIs (103). Consequently, many PJIs are suspected preoperatively, but can often only be confirmed on intraoperative samples during open surgery (18, 104). Therefore, preoperative diagnostic sampling techniques merit a fresh look.

It is clear that many studies have already shown the value of inflammatory parameters and synovial fluid biomarkers (e.g. alpha-defensin, LER strips, CRP, WBC count and PMN%) to increase the likelihood of diagnosing a PJI (105). However, it remains clinically important to identify the causative organism to guide the antibiotic therapy and define surgical treatment options. Therefore, culture-based tests remain important in PJI diagnostic algorithms (106). Preoperative arthrocentesis is a frequently used and minimal invasive technique to harvest joint fluid for bacterial culture. However, culturing synovial fluid might not yield any results to demonstrate a PJI. Perhaps sampling specimens other than synovial fluid should be obtained in the work-up to confirm a PJI. Based on the available knowledge of PJI topography and basic science information on bacterial adherence and biofilm characteristics, a case can be made for more targeted tissue biopsy methods. Besides arthrocentesis, other tissue samples such as synovial tissue specimens, bone biopsies and bone-prosthesis interface membrane samples could be obtained via minimally invasive techniques. The advantage of performing a biopsy procedure is the improved diagnostic yield from interpreting both bacteriological and histological results of the tissue (107). Furthermore, the importance of multiple tissue samples from different sites in the joint has already been described (108), but the best sampling method remains open to research (109). This chapter attempts to describe alternative preoperative sampling strategies and their potential role in diagnosing suspected and unsuspected PJI in failed total joint arthroplasty.

The search strategy yielded 1,314 potential papers related to infection topography. After more detailed screening of titles and abstracts, 1,263 papers were excluded. Of the remaining papers, 21 were included in this review after reading the full text. 16 papers evaluated the diagnostic value of preoperative tissue biopsy techniques. One additional study, published before 2017, was included due to the added value to this review. Details about the study characteristics from the included papers are presented in appendix C.

3.1. Joint fluid aspiration

Arthrocentesis or synovial joint fluid aspiration has been the most commonly used method in attempting to establish a preoperative PJI diagnosis (110). However, this technique has some important limitations. First of all, synovial fluid culture has a high specificity (95%), but a moderate sensitivity (72%) for detecting the causative microorganism preoperatively (27). The low sensitivity value in chronic PJI cases can be partly attributed to the fact that most microorganisms in chronic infections grow in biofilms with only a small percentage of free-floating bacteria (1). Secondly, a sufficient volume of synovial fluid cannot be obtained in up to 32% of the patients, especially in case of a dry tap (110). Furthermore, the diagnostic value of aspiration culture is significantly influenced by the synovial fluid volume, antibiotic use and specimen contamination (27).

Preoperative cultures based on synovial fluid alone poorly predict the pathogen of PJI and negative synovial fluid cultures do not exclude PJI, as approximately one third of intraoperatively culture-positive patients can be negative on preoperative synovial fluid culture (111). However, it has been shown that performing both a biopsy procedure and arthrocentesis can improve the diagnostic yield, since previous studies have described high accuracies for tissue biopsy combined with fluid aspiration in the diagnosis of PJI. Therefore, some authors recommend to routinely perform biopsies combined with arthrocentesis (112), but the role of tissue biopsies for the preoperative diagnosis of PJI remains undefined (7).

3.2. Tissue biopsy

Many methods for preoperative tissue biopsies have been described in the past. Feasible methods are biopsies from synovial tissue under fluoroscopic, ultrasound or arthroscopic guidance. In addition to synovial tissue biopsies, bone biopsies and interface biopsies could deliver important information as well.

As previously stated, an advantage of tissue biopsies is the ability to compare bacteriological findings with histological analysis (107). It is obvious that laboratory findings obtained by pathology are valuable since the observed presence of many leukocytes in the sampling site may assist in determining whether the body indeed interacts with either the presence of bacteria or with the process of aseptic loosening of the prosthetic components. Differentiating the one from the other is difficult. At least the criterion of having more than 5 leukocytes per high-power field in at least 5 high-power fields observed at x400 magnification is worldwide accepted and utilised as a criterion of PJI (12, 14, 113). Moreover, additional pathology might assist in clarifying whether a sample site is indeed infected (with many leukocytes present) versus contaminated (no leukocytes present). This is especially true in the diagnostic dilemma in which e.g. only one sample is culture positive with a low virulent pathogen.

3.2.1. Arthroscopic synovial tissue biopsy

We identified six studies performed on shoulder arthroplasty joints (114-119), followed by one study on knee arthroplasty joints (120) and two studies on hip arthroplasty joints (112, 121). The results of the reviewed studies concerning the diagnostic value of arthroscopic tissue biopsies are briefly presented in appendix C.

In shoulder arthroplasty joints, the sensitivity and specificity of arthroscopic biopsies to diagnose PJI ranged between 67%-100% and 60%-100% respectively (114-119). Moreover, Guild et al. (117) found a 100% correlation between arthroscopically obtained sample cultures and open biopsy cultures. All of the included studies describe arthroscopic biopsies in shoulder arthroplasties as a useful diagnostic tool in ambiguous cases with no objective sign of infection (114-119).

In the study of Akgün et al. (115) 16 out of 23 (70%) painful shoulder arthroplasties had at least one positive culture in diagnostic arthroscopy, whereas nine cases (39%) showed growth of microorganisms on intraoperatively collected tissues, but only five were considered as true with the presence of two or more positive cultures of the same microorganism obtained at open revision surgery. If the presence of at least one positive culture was considered as a true infection, diagnostic arthroscopy identified all infected cases correctly, but a false-positive result was obtained in 11 out of 23 (47.8%) cases. Under these criteria, diagnostics arthroscopy showed a 100% sensitivity, but a lower specificity of 39% and a positive predictive value of 31.3%. If growth of the same microorganism in at least two arthroscopic tissue samples was considered positive, the sensitivity dropped to 80%, whereas the specificity increased to 94.4% (115).

Tashjian et al. (114) evaluated the value of prerevision biopsies and differentiated between patients with abnormal or normal laboratory evaluation, including ESR and CRP, as well as fluoroscopically guided aspiration. The overall sensitivity, specificity, PPV and NPV for a positive prererevision sample to predict a positive culture by revision were 75%, 60%, 82% and 50% respectively. The overall sensitivity, specificity, PPV and NPV for a positive prererevision sample to predict an infection by revision were 90%, 86%, 90% and 86% respectively (114).

Pruijn et al. (118) analysed 12 cases with arthroscopically obtained tissue cultures. Nine out of 12 patients showed positive cultures, of which in four the diagnosis of infection was based on two or more cultures with the same microorganism and five had only one positive culture. Three of the infections diagnosed by arthroscopic culture were confirmed by revision tissue cultures. The sensitivity and specificity were 60.0% and 85.7%, with a PPV and NPV of 75.0% (118).

Doherty et al. (116) evaluated 14 patients with painful or stiff shoulder arthroplasty joints who underwent aspiration and arthroscopic biopsies. Arthroscopic tissue biopsies returned positive in three patients (21%). There were no unexpected positive cultures during revision surgery in the cases with negative biopsy cultures. Arthroscopy directed the next stage of treatment in all of the included patients (116).

Mederake et al. (119) retrospectively evaluated 56 patients receiving revision surgery on their shoulder arthroplasty. A standardised preoperative workup was performed including microbiological analyses from joint aspiration, and five synovial biopsy samples for bacteriologic and histologic analysis obtained by an arthroscopy procedure. 15 of 56 (27%) cases were diagnosed as PJI. Arthroscopic biopsy achieved a sensitivity and specificity of 90% and 83% respectively (119).

In addition, shoulder arthroscopy seems to be a useful diagnostic tool in evaluating for glenoid component stability, status of the rotator cuff, and metallosis to exclude mechanical reasons of failure (115-117). However, humeral stability could not be accurately evaluated with arthroscopy. Arthroscopically identified synovitis was also not accurate in detecting true infection (115). Guild et al. (117) performed a shoulder arthroscopy in 13 patients with painful shoulder arthroplasty and they were able to successfully treat 6 painful shoulder arthroplasties with arthroscopic procedures alone, preventing the need for major revision shoulder arthroplasty.

In total knee arthroplasties, Clarke et al. (120) reported that arthroscopic tissue sampling after a total knee arthroplasty in 65 suspected PJIs had a high diagnostic value with a reported sensitivity and specificity of 97.5% and 88% respectively when compared with the combination of aspiration culture and arthroscopic tissue culture results. Arthroscopic tissue cultures were positive in all cases with positive aspiration cultures. In 19 out of 65 (29%) cases, arthroscopic biopsies could identify new organisms in 7 patients with negative aspiration cultures and additional organisms in 12 patients that were not identified by aspiration. In these patients, arthroscopic biopsies influenced the treatment choice (120).

In total hip arthroplasties, Claassen et al. (121) assessed the diagnostic value of preoperative tissue biopsies obtained by hip arthroscopy to detect a PJI. The biopsies were microbiologically and histologically evaluated and compared with findings from intraoperative samples of the revision arthroplasty. They reported a sensitivity of 100% and a specificity of 83% with a PPV of 80% and a NPV of 100%. The authors concluded that arthroscopic biopsies might be a helpful tool to verify or rule out a PJI in suspected low-grade infections. However, Pohlig et al. (112) reported a lower sensitivity and higher specificity of 87.5% and 100%, as well as a PPV and NPV of 100% and 92% for arthroscopic biopsies of the hip joint, evaluated by a

combination of bacteriological and histological analysis. Bacteriological analysis alone delivered a sensitivity, specificity, PPV and NPV of 75.0%, 83.3%, 75.0% and 83.3% respectively. Similar to shoulder joints, during arthroscopic examination of the hip joint, mechanical failures could be identified (112).

Furthermore, arthroscopic biopsies are usually harvested after installing copious amount of saline fluid to visualise the joint (wet biopsy). It has been hypothesised that this activity could possibly reduce the bacterial load of the samples and decrease the detection rate of pathogens (122). Interestingly, Baumbach et al. (123) found that additional dry arthroscopic biopsies, when – rather unusually – the joint was filled with air, could increase the pathogen detection rate by 63% in suspected low-grade PJI following TKA. Interestingly, out of 23 patients with at least one positive microbiological biopsy, only 2 (9%) patients had both positive results on wet and dry biopsies. Moreover, the spectrum of the pathogens differed between wet and dry biopsies (123).

3.2.2. Fluoroscopic guided synovial tissue biopsy

Tissue specimens can be obtained using small approaches under fluoroscopic or dynamic X-ray imaging. We identified six studies concerning the diagnostic value of fluoroscopic guided synovial tissue biopsies (107, 110, 124-127). The study characteristics and outcomes are briefly presented in appendix C.

Several authors described different biopsy techniques under fluoroscopic guidance in hip and knee arthroplasties. First of all, arthroscopic biopsy forceps could be used to harvest periprosthetic tissue under fluoroscopic guidance. This technique showed a high accuracy in diagnosing intraoperatively confirmed THA and TKA PJI. Fink et al. (107) reported for the biopsy procedure for diagnosing a PJI a sensitivity and specificity of 93.8% and 97.3% respectively.

Rajakulasingam et al. (110) investigated the diagnostic value of simultaneous synovial aspiration and biopsy (SAB) and concluded that the diagnostic value of SAB was less than aspiration or biopsy alone. The sensitivity and specificity of biopsy was 70% and 97.7% and as slightly improved by combining biopsy with fluid aspiration, with a sensitivity and specificity of 70.0% and 100% (110).

Wimmer et al. (124) evaluated deep tissue sampling with a retrograde forceps technique, as previously introduced in 2014 (128), in 30 patients with suspected PJIs in painful total hip arthroplasties. The diagnostic value of their technique obtained a sensitivity of 85% and a specificity of 100% (124).

Enz et al. (127) compared the value of microbiological aspiration samples, histopathological biopsy samples or the combination of both in 102 hip or knee total arthroplasty joints with suspected PJI. They found that one preoperative biopsy alone, compared with intraoperative open biopsies, had a sensitivity and specificity of 51.9% and 100% respectively. The combination of one preoperative biopsy and one arthrocentesis obtained a sensitivity and specificity of 70.4% and 97.6% (127).

In shoulder arthroplasties, Lapner et al. (126) discussed capsular needle biopsy as a preoperative diagnostic biopsy technique for shoulder PJI. The specimens were obtained through an anterior approach under fluoroscopic guidance. Tissue samples were taken from the pericapsular tissue of the axillary recess, the rotator interval, and the inferior recess of the glenohumeral joint. 5 out of 17 patients had ≥2 positive culture samples and 4 of them had concordant positive culture results during revision shoulder arthroplasty, resulting in a sensitivity and specificity of 80% and 100% (126).

3.2.3. Ultrasound guided synovial tissue biopsy

We identified two studies concerning the diagnostic value of ultrasound guided synovial tissue biopsies (125, 129). The study characteristics and results of the reviewed studies are briefly presented in appendix C.

Ottink et al. (125) retrospectively analysed the diagnostic value of ultrasound guided thin needle (16G) biopsies and x-ray guided core needle (8G) biopsies in 16 patients with clinically suspected chronic infected THA. Cultures obtained by x-ray guided core needle biopsies had a sensitivity and specificity of 82% and 100% respectively and were superior compared to the ultrasound-guided biopsy cultures, with a sensitivity and specificity of only 33% and 85% respectively. However, it is hypothesised that the thin needle used in the ultrasound cohort could be the cause of the inferior result (125).

Sconfienza et al. (129) investigated the diagnostic value of ultrasound-guided periprosthetic biopsies in failed THA in patients with dry joints. They found that this technique is 100% feasible in patients with a dry tap, with similar diagnostic performance of ultrasound-guided joint aspiration. However, the sensitivity of both techniques was very low (41.7% and 52.2% respectively) (129).

3.2.4. Implant-interface and bone biopsy

As previously stated, the infected periprosthetic joint might potentially harbour separate compartments that do or do not communicate with each other. However, in the early stage, it might be possible that the extra-articular space around the implant is affected by microorganisms without affecting the synovial joint space. Therefore, obtaining biopsies from

the bone-implant interface or the bone itself might be a valuable option to evaluate the possibility of a localised bacterial spreading around the implant.

Our literature search did however not retrieve any eligible study about bone or interface sampling techniques. Therefore, we will discuss the previously published studies.

Corona et al. (130) investigated the value of percutaneous interface biopsies in dry-aspiration cases of chronic periprosthetic joint infections. They targeted the bone-prosthesis interface or bone-cement interface under fluoroscopic guidance and harvested at least two samples from each interface to culture. (Fig. 9) They found that the sensitivity and specificity of this technique was 88.2% and 100% respectively with in accuracy of 91.6% compared to intraoperative tissue cultures. No technique-related complications were recorded in any of the cases (130).

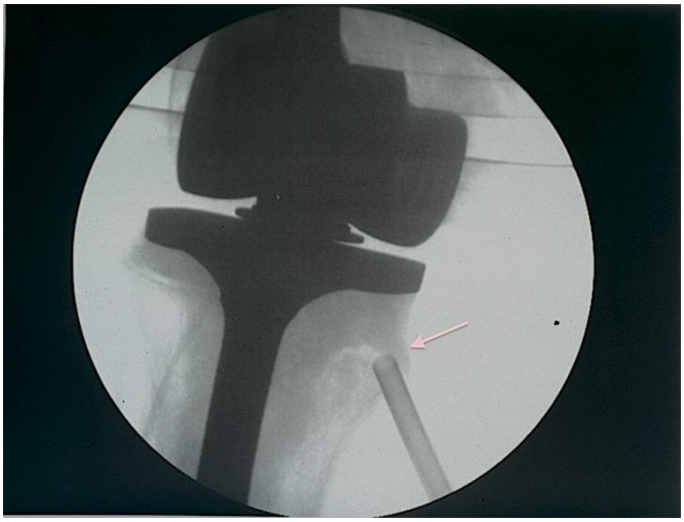

Figure 9. Percutaneous bone-prosthesis or bone-cement interface sampling technique, as described by Corona et al. (2012). Adapted from Corona et al. (130).

Moreover, Bori et al. (131) found that the interface membrane is the best sample for histological study to diagnose PJI with significantly higher positive samples compared to synovial biopsies (83% vs. 42%), which further endorses the potential importance of this biopsy technique.

Percutaneous bone biopsies under image guidance can also be performed to obtain a diagnostic sample for confirmation of infection (132). However, in cemented joint prosthetic replacement, the cement layer surrounding the prosthetic components obviously presents a formidable obstacle to reach out to the biofilm on the prosthetic surface.

4. Study quality assessment

In order to assess the methodological quality of the included observational studies, we evaluated the papers using the MINORS tool, which was validated for non-randomized surgical studies, whether comparative or non-comparative (30). This tool consists of 12 items where each aspect is scored as 0 when not reported, 1 for reported but inadequate, and 2 for reported and adequate. For non-comparative studies, scores of ≤8 were considered to be low quality, 9-14 moderate quality and 15-16 good quality. For comparative studies, scores of ≤14 were considered to be low quality, 15-22 moderate quality, 23-24 good quality (133).

The mean MINORS score was 17,6 out of 24 for the studies about NGS, with a range of 14-21. Two studies had low quality and nine had moderate quality. The mean MINORS score for the studies on sampling techniques was 10,9 out of 16, with a range of 9-12. All studies had moderate quality. We did not identify randomised controlled trials. The results indicate that all studies had a low to moderate risk of bias. A detailed overview is presented in appendix D.

Discussion

Next-generation sequencing

NGS appears to be a promising diagnostic tool to detect the microbiologic profile in periprosthetic joint infections. Our findings suggest that NGS has a higher diagnostic accuracy in PJI compared with culture methods. The reported sensitivities range from 60.9% to 100% and are higher compared with the classic culture methods. However, stating NGS as diagnostic superior over culture methods is not universally accepted. Kildow et al. (62) showed an inferior sensitivity for NGS compared with culture techniques. It has to be noted this study was performed in a retrospective design and patients were not consecutively enrolled, which might affect the results. Also, there was no clear difference between patients who underwent revision arthroplasty and primary arthroplasty. Furthermore, the reported specificities for NGS ranged from 73% to 100% and again, there are variable results, suggesting that NGS has not been established as a standard for PJI yet.

Our literature search identified four systematic reviews about the diagnostic value of NGS (21, 134-136). All papers that we included in our review were also included over the systematic reviews and our findings about the diagnostic value were in line with results of these systematic reviews and meta-analyses. Tan et al. (134), Li et al. (21) and Hantouly et al. (136) reported a pooled sensitivity of respectively 93%, 81% and 94%, and a pooled specificity of respectively

95%, 94% and 89%. All four systematic reviews seem to agree in the potential role of NGS in detecting PJI due to its high sensitivity, specificity and accuracy.

Furthermore, we found a major agreement between NGS and culture results in culture positive PJIs, which indicates that NGS is a valuable alternative to culture methods. In culture negative PJIs, NGS seems to have a particular useful value. This was also reported by the study of Tan et al. (134) in which NGS detected potential pathogens in 54.2% of the culture negative PJI patients. So, in this sub-group of patients with culture negativity, NGS could definitely deliver a proper diagnostic tool. However, it is reported that NGS may still be unable to identify the causative organisms in up to 30% of culture-negative cases (137).

In aseptic revision procedures, it appears that assumed aseptic prosthetic joints could turn out to be a PJI on the basis of NGS methods. Several authors tried to explain these UPICs through the following hypotheses: first of all, several authors refer to the hypothesis that there might be a native microbiome in the joints (55, 62, 70, 71). Secondly, the currently available PJI criteria and definitions might be imperfect to diagnose all cases of PJI and lead to underdiagnosis of PJI (49, 51, 59). Thirdly, it is questioned whether the detected organisms could be contaminants in the sampling procedure (26, 32, 50, 67).

Does prior antibiotic treatment make a difference for both cultures and for NGS? Our results demonstrate somewhat variable results. However, overall antibiotic use prior to sampling did not negatively impact the diagnostic value of NGS and NGS seemed to detect more organisms in patients with prior antibiotic administration when compared with culture. NGS could be a useful tool to detect pathogens in particularly culture negative cases where antibiotics were administrated within two weeks before sampling. However, the antibiotic free interval varied between the included studies. Most of the included studies used a two to four weeks antibiotic free interval as an exclusion criterion for the sampled population. Only a few authors really determined how prior antibiotic use influences NGS and culture results. In these studies, the definition for prior usage of antibiotics was different as well. Hong et al. (31) and Larsen et al. (65) included all patients who received preoperative antibiotics. In contrast, He et al. (57) included all patients using antibiotics within a month before surgery. Others included all patients whom received antibiotics within two weeks before sampling. More research seems justified to study the use of NGS in patients with prior antibiotic treatment with a clearly defined antibiotic free interval.

Overall, the predictive value of NGS appeared to be similar for cultures with exception of a lower negative predictive value for the latter. When comparing NGS with classic PCR testing it appears that PCR could be used as a verification method in PJI, but again more research is warranted.

Furthermore, it appears that studies from China suggest that infection control in PJIs could be aided by NGS, instead of relying on empirical antibiotic treatment regimens in case of negative culture results (20, 60). However, there are no randomised studies on this topic and more research is required to investigate the clinical impact of NGS in PJIs.

In summary, NGS is an emerging technique. It is an established well-known analysis to detect genetic diseases and becomes an increasingly available diagnostic tool for diagnosing infection. For the diagnosis of PJIs, the technique is promising and will become more available, reducing the costs and providing validation. However, there are some remaining issues that need to be solved, such as the possibility of contamination and the challenge to differentiate between dead and living bacteria.

Interestingly, the more recent third-generation sequencing, such as Oxford Nanopore Technology, might further improve sequencing-based pathogen identification (54). The technique of Oxford nanopore sequencing (ONS) has been investigated in the diagnosis of PJI as well (138, 139). This technique allows to sequence longer read lengths compared with NGS (138, 139). Long read lengths might significantly increase the accuracy of species identification and functional gene mapping (139).

Infection topography and preoperative sampling techniques

Pellegrini et al. (77) were the first to introduce a topographic principle in their modified classification of PJI. In an attempt to further develop this concept, we reviewed the recent literature on bacterial adherence and colonisation of the prosthetic joint, the diagnostic value of sampling multiple tissue samples and the prevalence and clinical meaning of unexpected positive intraoperative cultures.

The bacterial load on the different components of the joint varied according to the implant location and material characteristics. Rough surfaces were associated with biofilm colonisation and the PE-components of an infected prosthetic joint were most likely to have the highest bacterial load. In contrast, Nhan et al. (91) found that in shoulder arthroplasties the highest rate of positive cultures from explants were from the humeral stem site.

The prevalence of unexpected positive intraoperative cultures in the reviewed studies were similar in TKA and THA and were approximately 10%. The reported rate of UPICs in shoulders were much higher. However, the rates unsuspected PJI based on UPICs varied between the reviewed studies. This might be explained by the different criteria that were stated by the authors.

Several authors have discussed the necessity of preoperative microbial detection. Some authors claim that preoperative microbial detection is not necessary and that the clinical

outcome is not significantly different compared to cases with preoperative microbial diagnosis (140, 141). They state that the indication for a septic revision can be based on clinical signs alone. However, others state that microbiological diagnosis before revision surgery may influence the antibiotic prescriptions and may also help the surgeon's decision to proceed with a single or two-stage approach (142, 143). Both treatment strategies have similar results in terms of eradication rates or functional outcomes (142-144), but single-stage exchange appears to have reduced morbidity and costs (142).

Furthermore, preoperative bacteriological examination should be carried out before a loosened or painful prosthetic joint is revised, because the presence of PJI typically has a significant impact on the subsequent management. Therefore, minimal invasive diagnostic tests, such as joint aspiration and/or biopsy of the periprosthetic tissue with culture analysis prior to revision surgery are recommended (107). Synovial fluid culture has a high specificity, but only a moderate sensitivity. Moreover, aspiration only yields a single sample for analysis. In case of negative synovial fluid culture and a remaining suspicion of PJI or in patients where fluid aspiration was not possible, alternative preoperative tissue biopsy techniques might be recommended.

Absence of effusion can be observed in both aseptic and septic failed total joint arthroplasties. In this scenario, minimal invasive biopsy techniques might be an adjunctive tool to diagnose PJIs (129). As an alternative technique to overcome dry joints, some authors have proposed culture analysis of intra-articular injected and reaspirated saline solution. However, up to 40% of the patients with prelavage positive cultures, might have negative postlavage cultures (145, 146).

In addition, performing biopsy and tissue analysis in addition to aspiration provides the opportunity to combine different diagnostic tools (culture and histology of the tissue in combination with bacteriological analysis of the synovial fluid, e.g. leucocyte count and/or Alpha defensin) with high accuracies (107).

Arthroscopic biopsies in shoulder arthroplasties showed to be a useful diagnostic tool in ambiguous cases with no objective sign of infection. If at least two positive cultures for the same microorganism are considered as infection, arthroscopically obtained tissue biopsies for cultures demonstrated a high sensitivity and specificity. However, previous studies have showed a 100% sensitivity, specificity, positive predictive value and negative predictive value of diagnostic arthroscopy by considering any microbiological growth as indicative for PJI (147). Akgün et al. (115) showed indeed a high sensitivity, but a much lower specificity of 39% and a positive predictive value of 31.3%. Therefore, they recommend to rely on multiple positive cultures to define true infection. A potential explanation for the differences in diagnostic value

between the included studies, is the fact that the antibiotic-free interval before obtaining the tissue samples was not clearly stated in several studies.

In total knee arthroplasties, Fuerst et al. (148) had previously reported similar results in the evaluation of 86 revision total knee arthroplasties. They showed that preoperative arthroscopic synovial biopsies were superior to joint aspiration alone, with a sensitivity, specificity, PPV and NPV of respectively 100%, 94.7%, 87.4% and 100% for synovial biopsies and 69%, 97%, 85% and 92% for aspiration. The results of Clarke et al. (120) were also similar to the previously reported results by Claassen et al. (149). They evaluated 56 suspected PJIs after TKA. The arthroscopic neosynovium biopsies had a sensitivity and specificity of 88% (149). Interestingly, Lavender et al. (150) described a technique in which synovial and bone tissue samples in a patient with prior total knee arthroplasty were collected under arthroscopic guidance, but the diagnostic value of this technique is not evaluated yet.

In total hip arthroplasties, several previous studies reported a beneficial diagnostic effect of combined histological and microbiological analysis of tissue biopsies (151), compared with microbiological analysis alone (152, 153).

To conclude, arthroscopic evaluation of the prosthetic joint seems a useful technique to obtain multiple biopsies and to assess mechanical causes of painful arthroplasties to some extent. However, arthroscopic tissue biopsy might have several disadvantages. First, studies have shown that patients who undergo an arthroscopic procedure following a TKA have higher chances of revision and an increased rate of subsequent PJI (154, 155). Secondly, Malik et al. (156) found a greater risk of PJI when a shoulder arthroplasty is necessary within 90 days after an arthroscopic procedure on the ipsilateral shoulder. Furthermore, there are several inaccessible areas in the joint from which obtaining a tissue specimen is not possible with arthroscopy. Specimens of these areas can be obtained during revision procedures and therefore, the predictive value may be lower (118). A fourth disadvantage is the fact that the patients require an anaesthetic, which carries a greater risk than aspiration alone. Furthermore, arthroscopic procedures have a higher cost, since this procedure requires an anaesthetist, occupation of an operating theatre and admission to the daycare ward (118).

We identified two systematic reviews and meta-analyses about the diagnostic value of arthroscopic biopsies in shoulder arthroplasties (109, 157). Both systematic reviews identified and included the same studies in comparison to our review. The most recent meta-analysis of Tat et al. (157) reported a pooled sensitivity and specificity of 76% and 91% for arthroscopic tissue cultures. This was superior to aspiration cultures, which had a pooled sensitivity and specificity of 15% and 93%.

Fluoroscopic guided tissue biopsies have the potential to identify the presence of an infection preoperatively with a sensitivity between 51.9%-93.8% and a specificity between 94.1%-100%. Four out of six included studies obtained a sensitivity ≥80% with a high sensitivity. The lower sensitivity of Enz et al. (127) could be explained by the fact that only one biopsy was obtained. When our results are compared to previous studies, Meermans et al. (158) performed a prospective analysis of 120 patients with suspected PJI of a hip or knee arthroplasty. All patients had an aspiration and biopsies performed under fluoroscopic guidance. They showed a sensitivity of 79% for biopsy and 90% for the combination of both biopsy and aspiration. The specificity was 100% in all of the three techniques. These results support the use of combined diagnostic testing.

In contrast to arthroscopic and fluoroscopic guided biopsies, studies on ultrasound guided tissue biopsies showed low sensitivities. Sconfienza et al. (129) only obtained one periprosthetic synovial tissue sample for each patient, which could have contributed to the low sensitivity of their technique. Furthermore, despite the lower reported sensitivity, this technique could be considered as a valuable alternative in patients with dry joints. In these patients, ultrasound-guided sampling showed to be feasible in most of the patients, but it might be harder to obtain samples in obese patients due to thicker subcutaneous fat mass around the hip joint, resulting in lower image quality and longer needle tracking (129). Furthermore, more and larger tissue samples result in a better culture yield, core needle biopsy cultures show clear superiority above thin needle biopsy cultures (125).

Further, implant-bone interface sampling showed a high sensitivity and specificity. Also, complications after percutaneous bone biopsies were rare, with an overall incidence of 0.52% (132). Therefore, percutaneous bone biopsies appear to reveal promising results, however, more studies about the diagnostic value of this technique are necessary.

To conclude, our literature search identified one recently published meta-analysis by Li et al. which evaluated the role of preoperative biopsies after hip and knee arthroplasty (7). The authors included arthroscopic, fluoroscopic and ultrasound guided tissue biopsy techniques as valuable prerevision biopsy techniques and found that synovial fluid cultures demonstrated better results than biopsy cultures, with a sensitivity and specificity of 78% and 96% respectively for synovial cultures versus 75% and 93% for biopsy cultures. However, they did not differentiate between the different biopsy techniques (7).

Limitations of this study

The limitations of this literature review are those inherent to all reviews. First of all, this study relies on accurate reporting in previous published studies. Moreover, many studies were retrospective in nature and the sample sizes were often very small. These factors could

potentially cause selection bias. Secondly, although many studies have similar study aims, the methodologies were not uniform. When comparing multiple studies on the value of a diagnostic test, it is especially important to review the study population, the PJI definition and the outcome measurements. As presented by Matter-Parrat et al. (141), the diagnostic value of aspiration culture was significantly lower when used for routine testing in unsuspected PJI, compared to a population with suspected PJI based on clinical, laboratory or radiological signs (141). Therefore, studies can only be compared when they have similar study populations. Next, the diagnostic criteria used to diagnose PJI differ among the reviewed studies. Hsu et al. (159) conducted a systematic review to summarise the reported definitions of shoulder PJI. Their research identified 22 studies and half of them defined shoulder PJI by an author-defined combination of clinical, laboratory and radiographic findings combined with aspiration and intraoperative cultures during revision arthroplasty. The differences in diagnostic definitions make it difficult to compare study findings. Thirdly, it is important to review the definitions that are stated regarding the outcome of the study. The diagnostic value of a test is usually compared to the golden standard. However, the golden standard varied between the reviewed studies. To determine the diagnostic value of preoperative biopsies, they are usually compared with aspiration cultures or intraoperative tissue cultures. Furthermore, there is a potential for reporting bias due to the limited number of studies on the discussed topics.

On critical appraisal of the reviewed papers on NGS, we found some shortcomings as well. Most studies appeared to have a limited sample size. Secondly, despite adherence to similar criteria, organising patient cases into infected versus non-infected groups might not be solid. Kildow et al. (62) remarked that using the MSIS criteria inherently introduces a bias to culture methods when comparing culture with NGS due to the inclusion of cultures in the MSIS criteria.

Concerning all studies published on the topic of periprosthetic joint infections, the antibiotic free interval period did not seem to be defined in most papers, let alone be standardised. Lonner et al. (160) and Fink et al. (161) recommend to withhold antibiotics four weeks preceding the diagnostic procedure to minimise the risk of antibiotic-induced false-negative results. However, in other papers antibiotics were withheld two weeks prior to arthrocentesis or biopsy, based on the guidelines by the Infectious Diseases Society of America (16) or the European Bone and Joint Infection Society (12). In addition, there is no need to delay antibiotic prophylaxis in relation to surgical incision timing (162). Furthermore, many authors consider intraoperative tissue cultures during open revision surgery as gold standard. Therefore, selection bias might be present in light of the authors' decision to exclude patients who indeed had arthrocentesis or biopsy procedures, but without subsequent validating revision surgery. This bias potentially could overestimate the sensitivity of preoperative sampling techniques.

Conclusion

Based on this review, NGS has a potential role in diagnosing PJIs due to its high diagnostic sensitivity and specificity. Furthermore, it could serve as a potential additional diagnostic test for culture negative PJIs. In addition, NGS appeared to increase the detection rate of pathogens in cases where antibiotic treatment was not withheld two weeks before sampling. Furthermore, NGS might also detect genes associated with antibiotic resistance whilst predicting antibiotic susceptibility. However, challenges propose themselves regarding the interpretation of detected pathogens and more elaborate studies are necessary concerning the clinical impact of NGS.

Furthermore, combined efforts in the diagnostic work-up for PJI mapping and finetuning the microbiological profile by NGS particularly could prevent unnecessary open major revision procedures planned to find out whether a PJI is present or not. Also, knowledge about the PJI landscape potentially could lead to a partial exchange of genuinely infected components instead of destructive extraction of all components. In addition, mapping might guide preoperative sampling techniques and this in turn could improve the diagnosis of the microbial characteristics and sites of infection.

Moreover, a substantial group of patients with suspected chronic PJI have inconclusive investigations. In culture-negative painful prosthetic joints, the pros and cons have to be weighted whether there is an absence of PJI or not, to prevent unnecessary open procedures in complex revision cases. Preoperative tissue biopsies might be able to narrow down the infection to a particular location. Therefore, obtaining preoperative tissue biopsies by an arthroscopic procedure or percutaneous under fluoroscopic guidance might be a less invasive diagnostic tool compared to the current standard of open revision procedures. Multiple studies support the use of minimal invasive preoperative biopsy procedures and report an advantage of concurrent microbiologic and histologic examination of the biopsy specimens. These preoperative diagnostic techniques might contribute to the diagnosis of PJI in patients with a clinical suspicion of a chronic PJI and culture-negative synovial fluid or a dry tap.

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Appendix A: Search Strategy

1. Problem, Intervention, Comparison, Outcome model:

PICO	Study aim dissertation	Translation to search terms
Problem/Patient	Patients with periprosthetic joint infections	- Infection - Prosthesis - periprosthetic joint infection
Intervention	New sampling methods and analysis	- Biopsy, tissue and bone - NGS
Comparison	Standard methods	- Aspiration, open biopsy - Culture
Outcome	Diagnostic value	- Diagnosis - Topography

2. Search strategy: Included search terms to search the MEDLINE database

A) Basic structure: periprosthetic joint infection (Problem/Patient)

(("Infections"[Mesh:NoExp] OR "bone diseases, infectious"[MeSH Terms:noexp] OR "Osteomyelitis"[MeSH Terms:noexp] OR "Wound Infection"[Mesh] OR "Soft Tissue Infections"[MeSH Terms] OR "Persistent Infection"[MeSH Terms] OR "Latent Infection"[MeSH Terms] OR "Asymptomatic Infections"[MeSH Terms] OR "arthritis, infectious"[MeSH Terms] OR "Infection"[Title/Abstract] OR "Infections"[Title/Abstract] OR "prosthesis related infections"[Title/Abstract] OR "prosthesis related infections"[Title/Abstract] OR "prosthesis-related infection"[Title/Abstract] OR "prosthetic joint infection"[Title/Abstract] OR "joint infection"[Title/Abstract] OR "fungal infection"[Title/Abstract] OR "chronic prosthetic joint infection"[Title/Abstract] OR "prosthetic knee joint infection"[Title/Abstract] OR "prosthetic hip joint infection"[Title/Abstract] OR "periprosthetic joint infection"[Title/Abstract] OR "periprosthetic joint infections"[Title/Abstract] OR "periprosthetic infection"[Title/Abstract] OR "implant associated infection"[Title/Abstract] OR "persistent infections"[Title/Abstract] OR "long term infection"[Title/Abstract] OR "long term infections"[Title/Abstract] OR "chronic infection"[Title/Abstract] OR "chronic infections"[Title/Abstract] OR "latent infections"[Title/Abstract] OR "reactivated infection"[Title/Abstract] OR "reactivation infection"[Title/Abstract] OR "reactivation infections"[Title/Abstract] OR "infection reactivation"[Title/Abstract] OR "infection reactivations"[Title/Abstract] OR "asymptomatic infection"[Title/Abstract] OR "inapparent infections"[Title/Abstract] OR "inapparent infection"[Title/Abstract] OR "subclinical infections"[Title/Abstract] OR "subclinical infection"[Title/Abstract] OR "asymptomatic colonization"[Title/Abstract] OR "asymptomatic colonizations"[Title/Abstract] OR "culture-negative infection"[Title/Abstract] OR "culture negative infection"[Title/Abstract] OR "low-grade infection"[Title/Abstract] OR "low grade infection"[Title/Abstract]) AND ("Joint Prosthesis"[Mesh] OR "Arthroplasty, Replacement"[Mesh] OR "joint prosthesis"[Title/Abstract] OR "joint prostheses"[Title/Abstract] OR "hip prosthesis"[Title/Abstract] OR "hip prostheses"[Title/Abstract] OR "femoral head prosthesis"[Title/Abstract] OR "femoral head prostheses"[Title/Abstract] OR "knee prosthesis"[Title/Abstract] OR "knee prostheses"[Title/Abstract] OR "joint prosthesis implantation"[Title/Abstract] OR "joint prosthesis implantations"[Title/Abstract] OR "replacement arthroplasty"[Title/Abstract] OR "joint replacement"[Title/Abstract] OR "joint replacements"[Title/Abstract] OR "replacement arthroplasties"[Title/Abstract] OR "total joint replacement"[Title/Abstract] OR "total joint replacements"[Title/Abstract]) OR ("Prosthesis-Related Infections"[MeSH Terms] NOT ("Heart"[Title/Abstract] OR "Cardio*"[Title/Abstract] OR "endocarditis"[Title/abstract])))

B) Next-generation sequencing (Intervention)

("molecular diagnostic techniques"[MeSH Terms] OR "high throughput nucleotide sequencing"[MeSH Terms] OR "high throughput nucleotide sequencing"[Title/Abstract] OR "high throughput nucleotide sequencing"[Title/Abstract] OR "next generation sequencing"[Title/Abstract] OR "next generation sequencing"[Title/Abstract] OR "NGS"[Title/Abstract] OR "metagenomic sequencing"[Title/Abstract] OR "metagenomic shotgun sequencing"[Title/Abstract] OR "metagenomic analysis"[Title/Abstract] OR "Metagenomics"[Title/Abstract] OR "molecular diagnostic technique"[Title/Abstract] OR "molecular diagnostic techniques"[Title/Abstract] OR (("Molecular"[All Fields] OR "moleculars"[All Fields]) AND "diagnostic technics"[Title/Abstract]) OR (("Molecular"[All Fields] OR "moleculars"[All Fields]) AND "diagnostic technic"[Title/Abstract]) OR "molecular testing"[Title/Abstract] OR "molecular diagnostic testing"[Title/Abstract] OR "molecular techniques"[Title/Abstract] OR "molecular diagnostic strategies"[Title/Abstract] OR "molecular diagnostics"[Title/Abstract])

C) Sampling techniques (Intervention)

("Biopsy"[MeSH Terms:noexp] OR "biopsy, needle"[MeSH Terms] OR "Liquid Biopsy"[MeSH Terms:noexp] OR "Image-Guided Biopsy"[MeSH Terms] OR "Arthroscopy"[MeSH Terms] OR "Synovial Fluid"[MeSH Terms] OR "Arthrocentesis"[MeSH Terms] OR "Minimally Invasive Surgical Procedures"[MeSH Terms:noexp] OR "Biopsy"[Title/Abstract] OR "biopsies"[Title/Abstract] OR "needle biopsies"[Title/Abstract] OR "needle biopsy"[Title/Abstract] OR "aspiration biopsy"[Title/Abstract] OR "puncture biopsy"[Title/Abstract] OR "puncture biopsies"[Title/Abstract] OR "biopsy aspiration"[Title/Abstract] OR "Aspiration"[Title/Abstract] OR "Liquid Biopsy"[Title/Abstract] OR "liquid biopsies"[Title/Abstract] OR ("athroscopic"[All Fields] AND "Biopsy"[Title/Abstract]) OR "arthroscopic diagnosis"[Title/Abstract] OR "tissue sample"[Title/Abstract] OR "tissue samples"[Title/Abstract] OR "Synovial Fluid"[Title/Abstract] OR "synovial fluids"[Title/Abstract] OR "Arthrocentesis"[Title/Abstract] OR "Arthrocenteses"[Title/Abstract] OR "joint aspiration"[Title/Abstract] OR "synovial membrane"[Title/Abstract] OR "synovial membranes"[Title/Abstract] OR "Synovium"[Title/Abstract] OR "synovial biopsy"[Title/Abstract] OR (("arthroscoped"[All Fields] OR "arthroscopes"[MeSH Terms] OR "arthroscopes"[All Fields] OR "arthroscope"[All Fields] OR "Arthroscopic"[All Fields] OR "arthroscopical"[All Fields] OR "arthroscopically"[All Fields]) OR "guided synovial biopsy"[Title/Abstract]) OR ("Percutaneous"[Title/Abstract] AND ("biopsy"[Title/Abstract] OR "sampl*"[Title/Abstract])) OR “Implant-bone interface”[Title/Abstract] OR “Interface membrane”[Title/Abstract])

D) Topography (Outcome)

("Culture-negative"[Title/Abstract] OR "Culture-negative"[Title/Abstract] OR "negative culture"[Title/Abstract] OR "negative cultures"[Title/Abstract] OR "negative aspiration"[Title/Abstract] OR "Topography"[Title/Abstract] OR "Mapping"[Title/Abstract] OR "Staging"[Title/Abstract] OR "bacterial adherence"[Title/Abstract] OR "Distribution"[Title/Abstract] OR "colonization"[Title/Abstract] OR "bacterial load"[Title/Abstract] OR "components"[Title/Abstract] OR "infection imaging"[Title/Abstract] OR "infection distribution"[Title/Abstract]) OR ("positive culture*"[Title/Abstract] or "intraoperative culture*"[Title/Abstract]) AND ("revision"[Title/Abstract] or "aseptic"[Title/Abstract])

3. Search strategy: Included search terms to search the Embase database

A) Basic structure

('periprosthetic joint infection'/exp OR (('periprosthetic joint infection' OR 'arthroplasty'/exp OR 'joint prosthesis'/exp) AND 'infection'/exp) OR 'periprosthetic joint infection')

B) Next-generation sequencing

('high throughput sequencing'/exp OR 'molecular diagnosis'/exp OR 'metagenomics'/exp OR 'next-generation sequencing':ab,ti OR 'next generation sequencing':ab,ti OR 'targeted sequencing':ab,ti OR 'shotgun sequencing':ab,ti OR 'molecular diagnosis':ab,ti)

C) Sampling techniques

('biopsy technique'/exp OR 'joint biopsy'/exp OR 'bone biopsy'/exp OR 'tissue biops*':ab,ti OR 'preoperative biops*':ab,ti OR 'prerevision biops*':ab,ti OR 'bone biops*':ab,ti OR 'interface biops*':ab,ti)

D) Topography

(topography OR 'culture negative':ab,ti OR mapping:ab,ti OR 'bacterium adherence'/exp OR distribution:ab,ti OR 'bacterial load':ab,ti)

4. Search strategy: Included search terms to search the Web of Science database

A) Basic structure

(((TS=(periprosthetic joint infection)) OR (TS=(infection)) AND (TS=(arthroplasty)) OR TS=(joint prosthesis)))

B) Basic structure and Next-generation sequencing

(TS=(high throughput sequencing) OR TS=(molecular diagnosis)OR TS=(metagenomics) OR TS=(next-generation sequencing) OR TS=(next generation sequencing) OR TS=(targeted sequencing) OR TS=(shotgun sequencing))

C) Basic structure and Sampling techniques

(TS=(tissue biops*) OR TS=(biops*)OR TS=(arthroscopic biops*) OR TS=(percutaneous biops*) OR TS=(bone biops*) OR TS=(biopsy technique))

D) Basic structure and topography

(TS=(topography) OR TS=(bacterial load) OR TS=(bacterial adherence) OR TS=(mapping) OR TS=(distribution))

5. Retrieved papers

A) Periprosthetic joint infection and next-generation sequencing combined:

- ➔ MEDLINE: 134 results (83 between January 1st, 2017 - July 31st , 2023)
- ➔ Embase: 211 results (164 between January 1st, 2017 - July 31st , 2023)
- ➔ Web of science: 242 results (152 between January 1st, 2017 - July 31st , 2023)
- ➔ Total (without duplicates): 409 (264 between January 1st, 2017 - July 31st , 2023)

B) Periprosthetic joint infection and sampling techniques combined:

- ➔ MEDLINE: 1,796 results (826 between January 1st, 2017 - July 31st , 2023)
- ➔ Embase: 1,116 results (614 between January 1st, 2017 - July 31st , 2023)
- ➔ Web of Science: 324 results (150 between January 1st, 2017 - July 31st , 2023)
- ➔ Total (without duplicates): 2,733 results (1,314 between January 1st, 2017 - July 31st , 2023)

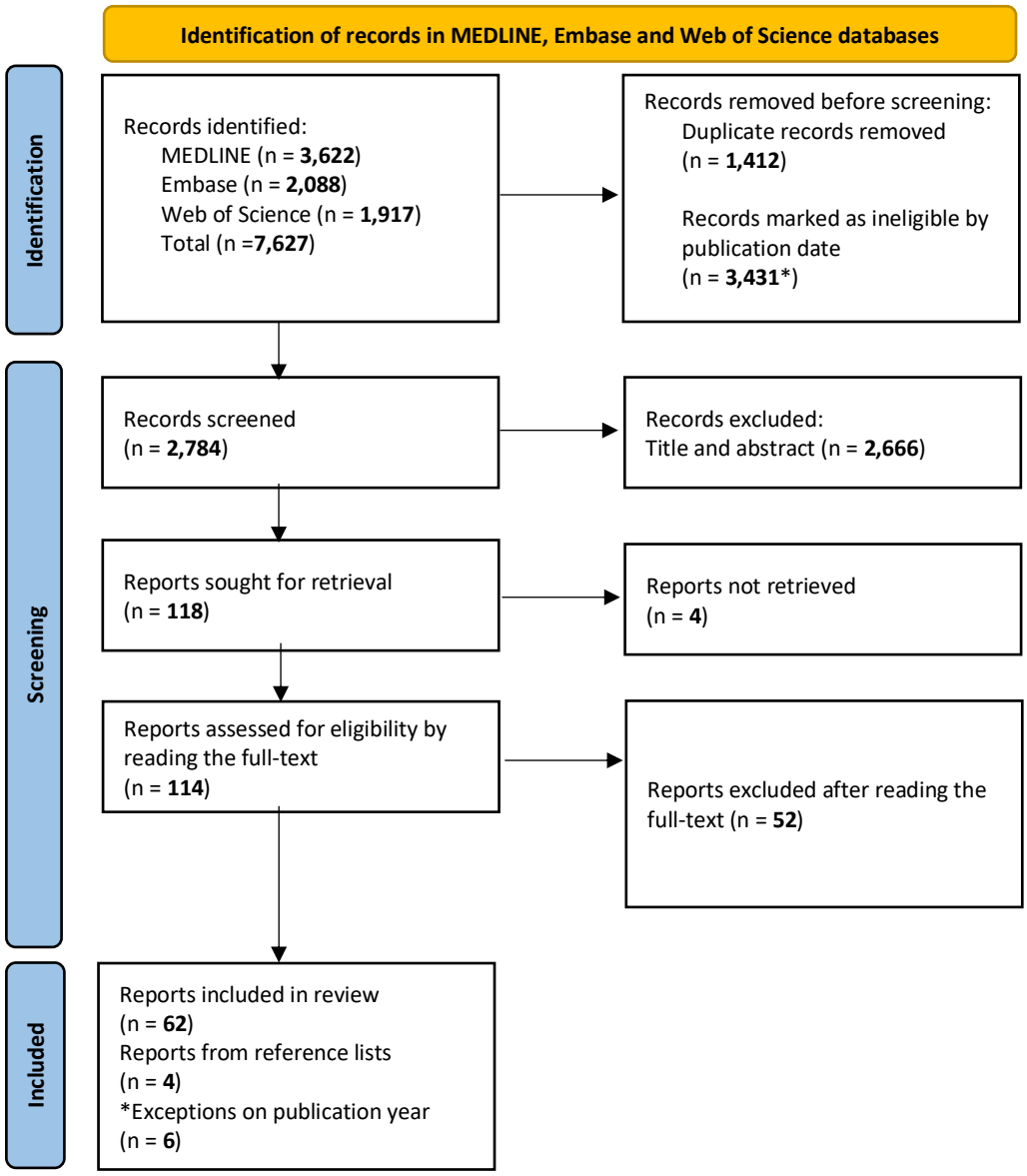
C) Periprosthetic joint infection and topography combined:

- ➔ MEDLINE: 1,692 results (687 between January 1st, 2017 - July 31st , 2023)
- ➔ Embase: 761 results (440 between January 1st, 2017 - July 31st , 2023)
- ➔ Web of Science: 1,351 results (588 between January 1st, 2017 - July 31st , 2023)
- ➔ Total (without duplicates): 3,492 results (1,505 between January 1st, 2017 - July 31st , 2023)

D) Periprosthetic joint infection combined with next-generation sequencing, sampling techniques and topography:

➔ Total (without duplicates): 6,215 results (2,784 between January 1st, 2017 - July 31st , 2023)

6. PRISMA flow diagram



Appendix B: Overview included studies on next-generation sequencing

Table II. Overview of the study characteristics of the included studies on next-generation sequencing (Part I)

Study	Study design	Population	Diagnostic criteria	Type of NGS	Types of samples for NGS	Sample sites	Intraoperative or preoperative samples	Antibiotics prior to sampling?	Number of patients (PJI/Non-PJI)	Primary outcome(s) measured
Street et al. (2017) (67)	Cohort study, retrospective	PJI and orthopaedic device infections	IDSA	mNGS	Sonicated fluid and tissue samples	Knee, hip, ankle and shoulder	Intraoperative	Yes	97 (97/0)	Concordance with culture
Ivy et al. (2018) (49)	Case-control study	Aseptic failure and CP+CN PJI	IDSA	mNGS	Synovial fluid	Knee	Preoperative	Yes	168 (107/61)	Concordance with culture
Larsen et al. (2018) (65)	Cohort study, prospective	Revision arthroplasty	MSIS	tNGS	Joint fluid, soft tissue bio specimen, swabs and bone biopsy, sonicated fluid	Hip and knee	intraoperative	Yes	114 (71/43)	LR+; LR-
Namdari et al. (2018) (71)	Cohort study, prospective	Revision arthroplasty	Framiamore et al. (2015)	tNGS	Synovial fluid and tissue	Shoulder	Pre- and intraoperative	NR	44	Concordance with culture
Tarabichi et al. (2018) (63)	Prospective, single-blinded study	Routine aspiration	NR	tNGS	Synovial fluid	Hip and knee	Preoperative	NR	86	Concordance with culture
Tarabichi et al. (2018) (55)	Cohort study, prospective	Revision and primary arthroplasty	MSIS	tNGS	Synovial fluid, deep-tissue specimens and swabs	Hip and knee	Intraoperative	No	65 (28/37)	Concordance with culture, sensitivity and specificity
Thoendel et al. (2018) (64)	Cohort study	Aseptic failure and CP+CN PJI	IDSA	mNGS	Sonication fluid	Hip and knee	Intraoperative	Yes	408 (213/195)	Concordance with culture
Zhang et al. (2019) (61)	Cohort study, retrospective	Revision arthroplasty	MSIS	mNGS	Sonication fluid	Hip and knee	Intraoperative	Yes	37(24/13)	Sensitivity and specificity
Cai et al. (2020) (50)	Cohort study, prospective	Revision arthroplasty	MSIS	mNGS	Periprosthetic tissue	Hip and knee	Intraoperative	No	44 (22/22)	Sensitivity, specificity, PPV, NPV and accuracy
Carr et al. (2020) (70)	Cohort study	Revision arthroplasty	MSIS	tNGS	Swabs	Hip and knee	Intraoperative	No	41	Characterise polymicrobial communities, validity and optimization DNA extraction
Fang et al. (2020) (51)	Cohort study, prospective	Revision arthroplasty	MSIS	mNGS	Synovial fluid	Hip and knee	Pre- and intraoperative	Yes	37 (24/13)	Sensitivity, specificity, PPV, NPV and accuracy
Huang et al. (2020) (60)	Cohort study, prospective	Revision and primary arthroplasty	MSIS	mNGS	Synovial fluid	Hip and knee	Intraoperative	NR	70 (49/21)	Sensitivity, specificity

CP: Culture positive; CN: Culture negative; IDSA: Infectious Diseases Society of America; MSIS: MusculoSkeletal Infection Society; ICM: International Consensus Meeting; mNGS: metagenomic next-generation sequencing; tNGS: targeted next-generation sequencing; MT: metatranscriptomics; LR+: positive likelihood ratio; LR-: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value; PJI: periprosthetic joint infection; NR: not reported

Table III. Overview of the study characteristics of the included studies on next-generation sequencing (Part II)

Study	Study design	Population	Diagnostic criteria	Type of NGS	Types of samples for NGS	Sample sites	Intraoperative or preoperative samples	Antibiotics prior to sampling?	Number of patients (PJI/Non-PJI)	Primary outcome(s) measured
Wang et al. (2020) (58)	Cohort study, prospective	Revision arthroplasty	MSIS	mNGS	Synovial fluid, sonication fluid or homogenized tissue	Hip and knee	Pre- and intraoperative	No	63 (45/18)	Concordance with BR-PCR, sensitivity, specificity, PPV, NPV
Wang et al. (2020) (20)	Cohort study	Revision arthroplasty	MSIS	mNGS	synovial fluid, sonication fluid and homogenized tissue	Hip and knee	NR	Yes	124(97/27)	Infection control rate
Fang et al. (2021) (56)	Cohort study, prospective	Revision arthroplasty	MSIS	mNGS	Synovial fluid	Hip and knee	Preoperative	Yes	56(35/21)	Sensitivity, specificity, PPV and NPV
Flurin et al. (2021) (32)	Cohort study, Retrospective	Revision arthroplasty	IDSA	tNGS	Sonicated fluid	Elbow	Intraoperative	NR	105(47/58)	Sensitivity, specificity, PPV and NPV
Goswami et al. (2021) (53)	Cohort study, prospective	Revision arthroplasty and primary total joint arthroplasty	ICM	tNGS, mNGS, MT	Blood, synovial fluid	Hip and knee	Preoperative	NR	30(10/10/10)	Correlation, concordance with culture
He et al. (2021) (57)	Cohort study, Prospective	Revision arthroplasty	MSIS	mNGS	Synovial fluid, sonication fluid and tissues	Hip and knee	Intraoperative	Yes	59 (40/19)	Sensitivity, specificity, concordance with culture
Kildow et al. (2021) (62)	Cohort study, retrospective	Revision and primary arthroplasty	MSIS	tNGS	Synovial fluid	Hip and knee	Preoperative	No	116 (48/68)	Sensitivity, specificity, PPV, NPV and concordance
Yin et al. (2021) (59)	Cohort study, prospective	Revision arthroplasty	MSIS	mNGS	Synovial fluid	Hip and knee	Preoperative	NR	35 (15/20)	Accuracy, sensitivity and specificity
Goswami et al. (2022) (26)	Cohort study, Multicenter prospective	Revision arthroplasty	ICM	tNGS	Synovial fluid, deep-tissue and swabs	Hip and knee	Intraoperative	NR	301(216/85)	Profile organisms, summary incidences, abundances of organisms, polymicrobial detection; numerical dominance
Hong et al. (2022) (31)	Cohort study	THA or TKA removal	IDSA	mNGS and tNGS	Sonication fluid	Hip and knee	Intraoperative	Yes	395(208/187)	Performance comparison

CP: Culture positive; CN: Culture negative; IDSA: Infectious Diseases Society of America; MSIS: MusculoSkeletal Infection Society; ICM: International Consensus Meeting; mNGS: metagenomic next-generation sequencing; tNGS: targeted next-generation sequencing; MT: metatranscriptomics; LR+: positive likelihood ratio; LR-: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value; PJI: periprosthetic joint infection; NR: not reported

Table IV. Overview of the study characteristics of the included studies on arthroscopic tissue biopsies

Reference	Study design	Joint	Population	Number of cases (patients)	Sampling technique	Number of tissue specimens	Definition infection	AB-free interval	Sensitivity	Specificity	PPV	NPV
Arthroscopic tissue biopsy												
Tashjian et al. (2017) (114)	Retrospective	Shoulder	Painful shoulder arthroplasty without objective signs of infection and subsequent revision arthroplasty	17 (17)	Arthroscopic tissue cultures vs. intraoperative tissue cultures	≥2	≥1 positive	NR	75%	60%	82%	50%
Akgün et al. (2019) (115)	Retrospective	Shoulder	Painful shoulder arthroplasty without objective signs of infection and subsequent revision arthroplasty	23 (22)	Arthroscopic tissue cultures vs. intraoperative tissue cultures	≥3	≥2 positive	2 weeks	80%	94,4%	80%	94,4%
							≥1 positive		100%	39%	31,3%	100%
Doherty et al. (2019) (116)	Retrospective	Shoulder	Painful shoulder arthroplasty without objective signs of infection and subsequent revision arthroplasty	14 (14)	Arthroscopic tissue cultures vs. intraoperative tissue cultures	5	≥3 positive	Prophylactic preoperative antibiotics	100%	100%	100%	100%
Guild et al. (2020) (117)	Retrospective	Shoulder	Painful shoulder arthroplasty without objective signs of infection	13 (13)	Arthroscopic tissue cultures vs. intraoperative tissue cultures	5	≥1 positive	NR	67%	100%	100%	80%
Pruijn et al. (2022) (118)	Retrospective	Shoulder	Shoulder arthroplasties who underwent a synovial fluid aspiration, arthroscopic biopsies and revision arthroplasty	12 (12)	Arthroscopic tissue cultures vs. intraoperative tissue cultures	6	≥1 virulent or >2 non-virulent positive	NR	60%	85,7%	75%	75%
Mederake et al. (2021) (119)	Retrospective	Shoulder	Shoulder arthroplasties who underwent a synovial fluid aspiration, arthroscopic biopsies and revision arthroplasty	56 (56)	Arthroscopic tissue vs. intraoperative tissue, both evaluated with combined microbiological and histological analysis	5	≥2 positive	4 weeks	90%	83%	66%	96%
Clarke et al. (2021) (120)	Retrospective case series	Knee	Suspected PJI, evaluated with synovial fluid aspiration and arthroscopic biopsies and subsequent revision arthroplasty	65 (65)	Arthroscopic tissue culture vs. aspiration and tissue culture	5	≥2 positive	2 weeks	97,5%	88%	92,9%	95,7%
Pohlig et al. (2017) (112)	Prospective	Hip	Suspected PJI, evaluated with synovial fluid aspiration and arthroscopic biopsies and subsequent revision arthroplasty	20 (20)	Arthroscopic tissue vs. aspiration vs. intraoperative tissue, both evaluated with combined microbiological and histological analysis	5	≥2 positive	2 weeks	87,5%	100%	100%	92%
Claassen et al. (2018) (121)	Retrospective	Hip	Suspected PJI, evaluated with arthroscopic biopsies and subsequent revision arthroplasty	10 (10)	Arthroscopic tissue vs. intraoperative tissue, both evaluated with combined microbiological and histological analysis	5	≥2 positive	NR	100%	83%	80%	100%

PPV: positive predictive value; NPV: negative predictive value; AB: antibiotic; THA: total hip arthroplasty; TKA: total knee arthroplasty; NR: not reported

Table V. Overview of the study characteristics of the included studies on fluoroscopic and ultrasound guided tissue biopsies and bone-implant interface biopsies

Reference	Study design	Joint	Population	Number of cases (patients)	Sampling technique	Number of tissue specimens	Definition infection	AB-free interval	Sensitivity	Specificity	PPV	NPV
Fluoroscopic guided tissue biopsy												
Wimmer et al. (2017) (124)	Prospective case-control	Hip	Suspected PJIs in painful total hip arthroplasties	30 (30)	Retrograde synovial biopsy vs. intraoperative tissue cultures	≥2	≥2 positive	NR	85%	100%	NR	NR
Ottink et al. (2018) (125)	Retrospective	Hip	Clinically suspected chronic PJI	29 (29)	Fluoroscopic guided thick-bore needle tissue cultures vs. intraoperative tissue cultures	≥4	≥2 positive	NR	82%	100%	100%	90%
Lapner et al. (2019) (126)	Prospective	Shoulder	Painful shoulder arthroplasty with suspicion of PJI who required revision	17 (17)	Fluoroscopic guided capsular needle biopsy vs. intraoperative tissue cultures	≥3	≥2 positive	NR	80%	100%	100%	92%
Fink et al. (2020) (107)	Prospective	Hip and Knee	Intra-operatively confirmed PJI with preoperatively fluoroscopic guided tissue cultures	178 (113 THA and 65 TKA)	Fluoroscopic guided tissue cultures vs. intraoperative tissue cultures	5	≥2 positive or ≥1 positive with positive histologic analysis	4 weeks	All: 93.8% Hip: 93.8% Knee: 93.8%	All: 97.3% Hip: 94.1% Knee: 99.1%	All: 94.9% Hip: 93.8% Knee: 96.8%	All: 96.7% Hip: 94.1% Knee: 98.1%
Enz et al. (2020) (127)	Retrospective	Hip and Knee	Clinically suspected PJI	102 (102)	Histopathological biopsy samples vs. intraoperative tissue cultures	≥1	≥1 positive	NR	Biopsy: 51.9% Biopsy and aspiration: 70.4%	100% 97.6%	NR NR	NR NR
Rajakulasingam et al. (2021) (110)	Retrospective	Hip and knee	Clinically suspected PJI	111 (103)	Fluoroscopic guided needle tissue and aspiration cultures vs. intraoperative tissue cultures	NR	≥2 positive	2 weeks	Biopsy: 70% Aspiration + Biopsy: 70	97.7% 100%	87.5% 100%	93.3% 92.6%
Ultrasound guided tissue biopsy												
Ottink et al. (2018) (125)	Retrospective	Hip	Clinically suspected chronic PJI	16 (16)	Ultrasound guided thin needle tissue cultures vs. intraoperative tissue cultures	1-2	≥2 positive	NR	33%	85%	33%	85%
Sconfienza et al. (2021) (129)	Retrospective	Hip	Failed THA who underwent revision surgery	109 (35 PJI/74 aseptic)	US guidance percutaneous biopsy (US-PB) cultures vs. US guided joint aspiration (US-JA) cultures vs. intraoperative tissue cultures	1	1 positive	NR	US-JA: 52.2% US-PB: 41.7%	US-JA: 97.8% US-PB: 100%	US-JA: 92.3% US-PB: 100%	US-JA: 80.3% US-PB: 80%
Bone-implant interface and bone biopsy												
Corona et al. (2012) (130)	Retrospective	Hip and Knee	Suspected PJI with dry aspiration	24 (24)	Percutaneous bone-implant interface biopsy cultures vs. intraoperative tissue cultures	≥2 samples from each interface	50% positive	NR	88.2%	100%	100%	77.8%

PPV: positive predictive value; NPV: negative predictive value; AB: antibiotic; THA: total hip arthroplasty; TKA: total knee arthroplasty; NR: not reported

Tabel VI. Study quality assessment (MINORS score)

Next-generation sequencing	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**Total MINORS score**
Tarabichi et al. (2018) (55)	2	2	2	2	2	2	2	0	2	2	1	2	21 of 24
Zhang et al. (2019) (61)	2	2	2	2	0	1	2	0	2	2	1	2	18 of 24
Cai et al. (2020) (50)	2	2	2	2	0	2	2	0	2	2	1	1	18 of 24
Fang et al. (2020) (51)	2	2	2	2	0	2	2	0	2	2	1	2	19 of 24
Huang et al. (2020) (60)	2	2	2	2	0	2	2	2	1	2	1	2	20 of 24
Wang et al. (2020) (58)	2	2	2	2	0	0	2	0	2	2	1	2	17 of 24
Fang et al. (2021) (56)	2	1	2	2	0	0	2	0	2	2	1	0	14 of 24
Flurin et al. (2021) (32)	2	2	2	2	2	0	2	0	2	2	1	2	19 of 24
He et al. (2021) (57)	2	2	2	2	0	0	2	0	2	2	1	2	17 of 24
Kildow et al. (2021) (62)	2	1	1	2	0	0	2	0	1	2	1	2	14 of 24
Yin et al. (2021) (59)	2	2	2	2	0	0	2	0	2	2	1	2	17 of 24
Sampling techniques	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**Total MINORS score**
Corona et al. (2012) (130)	2	2	2	2	0	2	2	0	/	/	/	/	12 of 16
Tashjian et al. (2017) (114)	2	1	2	2	0	1	1	0	/	/	/	/	9 of 16
Pohlig et al. (2017) (112)	2	2	2	2	0	1	2	0	/	/	/	/	11 of 16
Wimmer et al. (2017) (124)	1	2	2	2	2	1	2	0	/	/	/	/	11 of 16
Ottink et al. (2018) (125)	2	2	1	2	0	1	2	0	/	/	/	/	10 of 16
Lapner et al. (2019) (126)	2	2	2	2	0	1	2	0	/	/	/	/	11 of 16
Akgün et al. (2019) (115)	2	2	2	2	0	1	2	0	/	/	/	/	11 of 16
Doherty et al. (2019) (116)	1	2	2	2	0	1	2	0	/	/	/	/	10 of 16
Guild et al. (2020) (117)	2	2	2	2	0	1	2	0	/	/	/	/	11 of 16
Fink et al. (2020) (107)	2	2	2	2	0	2	2	0	/	/	/	/	12 of 16
Enz et al. (2020) (127)	1	2	2	2	0	1	2	0	/	/	/	/	10 of 16
Clarke et al. (2021) (120)	2	2	2	2	0	1	2	0	/	/	/	/	11 of 16
Mederake et al. (2021) (119)	2	1	2	2	1	1	2	0	/	/	/	/	11 of 16
Rajakulasingam et al. (2021) (110)	2	2	2	2	0	2	2	0	/	/	/	/	12 of 16
Sconfienza et al. (2021) (129)	2	2	2	2	0	0	2	0	/	/	/	/	10 of 16
Pruijn et al. (2022) (118)	2	2	2	2	1	1	2	0	/	/	/	/	12 of 16

Criteria MINORS tool
I. A clearly stated aim
II. Inclusion of consecutive patients
III. Prospective collection of data
IV. Endpoints appropriate to the aim of the study
V. Unbiased assessment of the study endpoint
VI. Follow-up period appropriate to the aim of the study
VII. Loss to follow up less than 5%
VIII. Prospective calculation of the study size
Additional criteria in the case of comparative studies
IX. An adequate control group
X. Contemporary groups
XI. Baseline equivalence of groups
XII. Adequate statistical analyses

MINORS: Methodological Index for Non-randomized Studies.

Each item is scored 0 (not reported), 1 (reported but inadequate), or 2 (reported and adequate).

Total MINORS score non-comparative studies: ≤8 = low quality; 9-14 = moderate quality; 15-16 = good quality.

Total MINORS score comparative studies: ≤14 = low quality; 15-22 = moderate quality; 23-24 = good quality.