Aus dem Centrum für Muskuloskeletale Chirurgie

der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Establishment of a checklist for the identification of patients with postoperative spinal implant infections

Einrichtung einer Checkliste für die Identifizierung von Patienten mit postoperativen spinaler Implantat Infektionen

zur Erlangung des akademischen Grades

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List of abbreviations

American Society of Anesthesiologists (ASA)

Adjacent segment degeneration (ASD)

Body mass index (BMI)

Centers of Disease Control and Prevention (CDC)

Confidence interval (CI)

Colony forming units (CFU)

Computed tomography (CT)

C-reactive protein (CRP)

Extracellular matrix (ECM)

Erythrocyte sedimentation rate (ESR)

Hazard ratio (HR)

International Consensus Meeting Criteria (ICMC)

Infectious Diseases Society of America (IDSA)

Intravenous injection (IV)

Kilogram (kg)

Milligram (mg)

Magnetic resonance imaging (MRI)

Nanogram (ng)

Nanoliter (nl)

Negative predicative value (NPV)

Polymerase chain reaction (PCR)

Periprosthetic joint infection (PJI)

Positive predicative value (PPV)

Postoperative spinal implant infection (PSII)

Receiver operating characteristic (ROC)

System Analysis and Software Development (SAP)

Standard deviation (SD)

Square meter (m^2)

Surgical site infections (SSI)

Total hip arthroplasty (THA)

Visual analog scale (VAS)

Abstract (auf Deutsch)

Einleitung. Die Inzidenz postoperativer spinaler Implantat Infektionen (PSII) wurde mit bis zu 20% angegeben. Bisher gibt es keine konsensbasierten Kriterien für die Diagnose von PSII. Ziel dieser Studie ist es, Patienten mit dem Nachweis einer PSII mit Patienten ohne Infektionsnachweis zu vergleichen. Darauf basierend wird eine Checkliste mit diagnostischen Parametern entworfen, um Patienten mit einem erhöhten PSII Risiko präoperativ zu identifizieren.

Methodik. Eine retrospektive Studie wurde durchgeführt. Patienten mit dem Nachweis einer PSII nach Wirbelsäulenrevisionseingriffen wurden retrospektiv identifiziert und mit einer nach Alter, Geschlecht und Indikation gepaarten Kontrollgruppe ohne PSII verglichen. Es wurden klinische, radiologische, laborchemische, intraoperative und mikrobiologische Befunde erhoben. Die verwendeten Parameter wurden auf der Basis der Empfehlungen vom Center for Disease Control and Prevention, der International Consensus Meeting Criteria und der Infectious Diseases Society of America ausgewählt. Der gepaarte t-Test und der Wilcoxon Test wurden entsprechend der Abhängigkeit der Variablen zum Vergleich der beiden Gruppen herangezogen.

Ergebnisse. Es wurden 61 Patienten mit PSII zu 61 Patienten in der Kontrollgruppe gematched. Patienten mit PSII zeigten eine signifikant erhöhte Inzidenz eines lokal suspekten Hautbefundes (P = 0,008), eine erhöhte Körpertemperatur (P = 0,003), das Vorhandensein eines Portkatheters (P = 0,002), einen Erregernachweis in der Blutkultur (P = 0,008) und eine Harnwegsinfektion (P = 0,032). Patienten mit einer PSII zeigten eine niedrigere Rate an Implantatversagen (P = 0,031) und Schmerzen in Ruhe bzw. Bewegung (P = 0,006; P = 0,002). Zusammengefasst zeigten die Parameter des Routinelabors eine geringe Sensitivität in der Diagnostik von PSII. Die histologische Analyse hatte eine geringe Sensitivität von 48,84% und eine gute Spezifität von 100%. Die mikrobiologische Kultur von periimplantärem Gewebe hatte ebenfalls eine gute Spezifität von 94,23%, jedoch eine moderate Sensitivität von 68,63%. Die Sonikation zeigte sowohl eine hohe Sensitivität als auch Spezifität (92,45% und 95,08%).

Schlussfolgerung. Einzelne Risikofaktoren für das Auftreten von peri-implantären Infektionen können bereits vor dem Revisionseingriff identifiziert werden. Suspekte Hautbefunde, eine erhöhte Körpertemperatur, das Vorhandensein eines Portkatheters, ein Erregernachweis in Blutkulturen und eine Harnwegsinfektion konnten als

Risikofaktoren für peri-implantäre spinale Infektionen identifiziert werden. Radiologische und laborchemische Befunde, sowie vermehrte Schmerzen sind für die Diagnostik einer PSII als nachrangig zu betrachten. Die Sonikation sollte aufgrund der höchsten Sensitivität und Spezifität zum Ausschluss einer peri-implantären Infektion gewählt werden. Auf der Grundlage dieser Ergebnisse wurde eine präoperative Checkliste für Patienten vor Wirbelsäulenrevisionseingriffen entworfen.

Abstract

Introduction. The incidence of postoperative spinal implant infections (PSII) has been reported to be up to 20%. Many PSIIs are caused by low virulent pathogens and thus the diagnosis rate of PSII is relatively low. There is no scientific consensus regarding the diagnostic criteria for PSII. This study therefore aimed to compare PSII patients with patients without evidence of infection. Based on the results, we aimed at formulating a comprehensive checklist with all important diagnostic parameters to help surgeons identify patients with an increased risk prior to revision surgery.

Method. A retrospective study was carried out. Patients with evidence of peri-implant infections after spinal revision surgery were identified. Patients without PSII (control group: matched according to age, gender and surgical indications) were included. Digital and physical patient files were examined for collecting of clinical, radiological, laboratory, intraoperative and microbiological findings in both groups. The parameters collected were identified based on the recommendations of the Center for Disease Control and prevention, the International Consensus Meeting Criteria, and the Infectious Diseases Society of America. The paired t-test and Wilcoxon test were used to compare the two groups according to the dependence of the variables.

Results. In total, 61 patients with PSII and 61 controls were included. Patients with PSII showed a significantly higher incidence of local skin findings (P = 0.008) and a raised body temperature (P = 0.003). In addition, significant risk factors for the occurrence of PSII were the presence of a port catheter (P = 0.002), positive blood culture (P = 0.008), and urinary tract infections (P = 0.032). Furthermore, patients with PSII showed a lower rate of implant failure (P = 0.031) and pain at rest or in motion (P = 0.006; P = 0.002). In summary, routine laboratory parameters showed a low sensitivity in the diagnosis of PSII. The histological analysis showed a low sensitivity of 48.84%, yet a good specificity of 100%. The microbiological culture of peri-implant tissue also showed a good specificity of 94.23%, but a moderate sensitivity of 68.63%. The sonication showed both a high sensitivity and specificity (92.45% and 95.08%).

Conclusion. Individual risk factors for the occurrence of peri-implant infections already exist before the revision surgery. In summary, radiological analysis, laboratory findings and the presence of pain have been regarded as secondary to the presence of PSII. Suspicious skin findings, raised body temperature, the presence of a port catheter, blood

and urinary tract infections could lead to an increased risk of peri-implant spinal infections. Considering these risk factors, sonication should be chosen to exclude a peri-implant infection due to it having the highest sensitivity and specificity. Based on the present results, a preoperative checklist for patients before spinal revision interventions was drawn up.

1. Introduction

1.1. Spinal fusion

Spinal fusion, also known as spondylodesis, was originally introduced in the 1910s by Dr. Russell A. Hibbs [1] for treating a nine-year-old child with lumbar tuberculosis (Pott disease). Over the past few decades, the indication for spinal fusion has broadened and is applied today in a variety of degenerative disorders, spinal fractures, infections, deformities and tumors of the spine. Numerous surgical techniques have been described, and any spinal segment can be addressed in order to fuse two or more vertebra. Spinal surgeons insert autograft, allograft or artificial materials in between the adjacent vertebrae to accelerate anterior bony consolidation. In addition, depending on the surgical strategy, in various techniques, accessorial screws are placed to stabilize the spinal segment and to help the spinal vertebrae heal into a solid unit [2].

In Germany, due to the expected growth of the geriatric population and the consequent increase of degenerative spinal diseases, it is expected that from 2018 to 2026, the spinal surgery device market will witness a compound 5.2% growth rate annually [3]. In the United States, lumbar fusion procedures significantly increased and the associated hospital costs, which increased by approximately twofold (177%) during 2004-2015, exceeded ten billion dollars in 2015 [4]. In the past eight years in mainland China, spinal operations numbers have grown by more than threefold, from about 46,000 in 2013 to about 146,000 in 2020. The cost value of spinal operational in China also increased from 186 million euros to 548 million euros by 2020, with a 16.7% compound annual growth rate and the fastest growing fusion market worldwide. In the future, through the continuous progress of health care and the abandoning of the traditional Chinese culture of ‘avoiding impairment Qi of physical body’, the number of spinal surgeries and the use of spinal fusion is predicted to potentially have significant growth [5].

Nevertheless, spinal fusion remains a technique with high risk and the surgical complications resulting from this procedure can lead to devastating outcomes and increased patient morbidity and mortality. Thus, reducing the spinal fusion related complication rate is of outstanding importance. Intraoperatively, patients are at risk of, for example, blood loss, nerve and surrounding tissue damage, dural laceration and instrument insertion complications. Various improvements over the past few decades, such as spinal navigation or intraoperative neuro-monitoring, have been developed to

decrease intraoperative complications. Postoperatively, the emergence of various infections (superficial, deep site, implant related, as well as early and late onset infection), hardware failure, neurologic injury, pseudarthrosis, deep vein thrombosis, adjacent segment degeneration (ASD), and many more can occur. Among these complications, surgical related infection after spinal fusion accounts for heavy social and economic burden for patients, hospitals as well as the healthcare security system because it accounts for prolonged hospital stays, increased reoperation rates and a decreased daily working productivity rate of the patient [6]. It was reported that the rate of infection after spinal fusion was between two to five percent, and additional implant insertion further increases this infection rate. Furthermore, for patients who have previous fusion history, diabetes and smoking history, the infection risk was significantly higher [7]. Therefore, preventive measures should be taken for patients who have received a spinal fusion procedure in a meticulous way in order to reduce infection as well as improve surgical outcomes. At this point, it is important to state that spinal surgery has one of the highest infection rates of all surgical fields in orthopedics, which is why great attention should be placed on its morbidity and the related complications management.

1.2. Surgical site infections in spinal surgery

Surgical site infections (SSIs) have been considered as one of the most frequently reported hospital-acquired infections. Development of an SSI causes a substantial clinical and economic burden [8]. According to the Centers of Disease Control and Prevention (CDC), SSI accounts for about 16.2% of the all-nosocomial infections in USA and is responsible for increased hospital length of stay, patient morbidity and mortality as well as re-operation rates [9].

As defined by the CDC, SSI is referred to as an infection that happens in one part of the body where surgeries took place, or which involves tissues under the skin, muscles, organs, or implanted instruments. It can be classified, according to the WHO, into three types, i.e., superficial incisional SSI, deep incisional SSI and organ / space SSI [10]. However, standardizing the definition of SSI in spinal surgery remains a challenge due to the lack of uniform diagnosis and it requires multidisciplinary expertise as well as cooperation.

The incidence of postoperative spinal SSI has been reported ranging widely from 0.7% to 16% due to the various intervention types on the spine [11-14]. In order to reduce poor

patient outcomes as well as hospital and social economic burdens, primary prevention and the recognition of risk factors are crucial. Aging, diabetes, long-term use of steroids, excessive BMI, smoking, alcohol abuse, an American Society of Anesthesiologists (ASA) score greater than two, repeated revision surgeries, transfusion history and preoperative anemia, etc., have been identified as associated risk factors leading to SSI in spine operations [15].

Careful diagnosis and appropriate therapy demand serious attention from both clinicians and microbiologists. Various strategies such as microorganism screening, wound drainage and/or debridement, individualized antibiotics therapy, careful patient comorbidity moderation as well as blood glucose control have been recommended aiming to reduce the morbidity and mortality of SSI [13, 16, 17]. The high incidence of SSI, and this therefore being a possible cause of implant-related infections in spinal surgery, illustrates the importance of ongoing investigations in this matter.

1.3. Implant-related infections in spinal surgery

Postoperative spinal implant-related infections (PSIIs) are the result of microorganism adhesion and subsequent biofilm formation to a spinal implant surface. Recently, this special type of infection has been investigated as a major issue in orthopedic and trauma-associated surgeries with serious outcomes for the patients involved. Recent studies have found the incidence of PSII to be as high as 20% [18]. This high incidence rate might reflect the lack of standardized diagnostic procedures and comprehensive management criteria for PSII. Investigation of primary sources of infections that directly or indirectly caused PSII is complex yet crucial. It has been reported that factors such as patients with multiple previous operations, prolonged operation periods, blood transfusion records as well as intraoperative iatrogenic contamination contributed to the risk of infection following instrumented spinal surgeries [19].

The main problem with implant-related infections in spine surgery is that they are hard to diagnose. Biofilm formation on the surface of implants has posed serious challenges, especially for those cases with indolent microorganisms that were confined to the biofilm. Since these microorganisms do not induce an obvious body inflammatory response, infection-related symptoms such as pain, skin redness or swelling and fever are occult [20]. The biofilm formation is a multistep process beginning with the adhesion of microorganisms to the implant surface, where the organisms initiate the production of

specific proteins which cause neutralizations (e.g., cephalosporinase AmpC enzymes). This feature makes biofilms beyond the access of antibiotics and body's immune system [21]. The spectrum of pathogens forming biofilm in infectious implants is broad. The most common causative pathogens are identified as Staphylococcus aureus, Propionibacterium acnes, Coagulase-negative staphylococci (as shown in Figure 1), Gram-negative bacilli, Streptococci and Enterococci [22]. Notably, the Staphylococcal species have been observed as a diverse group of gram-positive bacteria that generally inhabit the human skin and mucous membranes, of which Staphylococcus aureus and Staphylococcus epidermidis have been regarded as the leading causes of hospital-acquired SSI [23].

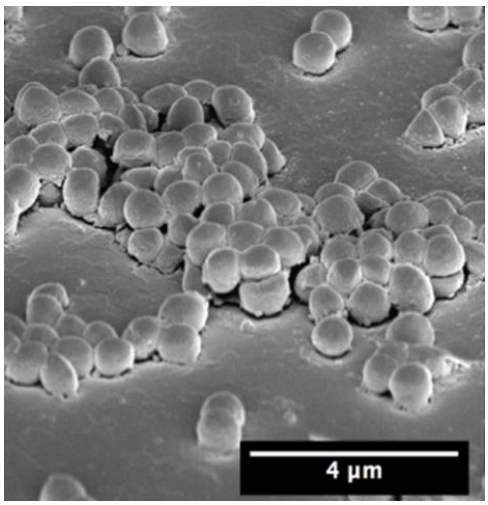

Figure 1. Staphylococcus epidermidis cells resting on a polished titanium implant surface [22]. Authorized for use by Prof. Jinju Chen (School of Dental Sciences, Newcastle University, Newcastle Upon Tyne, NE2 4BW, UK).

Detecting the exact infectious pathogen is, however, time and energy consuming. Even though many existing diagnostic approaches have been observed to be contributory to some extent, the diagnosis of implant-related infections is somewhat strenuous. It might not be possible to retain infected implants with mature biofilms formed, which significantly increases the healthcare costs. Compared to a preoperative joint puncture that is performed regularly and acts as a central element in diagnosing periprosthetic joint infection (PJI), preoperative invasive examinations applied to the diagnosis of spinal implant-related infection seem far-fetched. Even though various diagnostic approaches, including clinical examinations, imaging, microbiology, histopathology of implant surrounding tissue as well as sonication of explanted materials have been widely

suggested, making a diagnostic decision does not only involve routine checks, but also the ratification of proposed answers as well as formalized diagnostic algorithms [24].

How to increase the PSII detection rate has posed major diagnostic and therapeutic challenges to spinal surgeons and physicians. Some arguments have stated that a possible implant iatrogenic contamination during the spinal operation should be held responsible for the PSII occurrence [25]. Surgical glove perforation and airflow defects have been investigated as major sources for intraoperative implant contamination. In addition, intraoperative uncovered implants were found presenting the bioburden of virulent bacterial species (e.g., Staphylococcus and Micrococcus), which further increased the postoperative infection rate [26]. Surprisingly, a range of 105 to 107 colony-forming units of dangerous species such as indole Staphylococcus epidermis and aggressive Staphylococcus aureus were detected on unguarded pedicle screws.

In the following sections, a concise overview of all existing relevant clinical, radiographic, laboratory, and microbiological parameters or tools will be introduced regarding their investigated or potential effectiveness in the diagnosis of PSII.

1.4. Preoperative examinations

1.4.1. Anamnesis

The incidence of orthopedic implant-associated infection ranges from two to 20%, and increased infection can lead to poorer outcomes, cause high levels of morbidity, higher healthcare costs and even a higher death rate [27]. A meticulous medical history should therefore be scrutinized to obtain the first hint of the possible infection type. Any possible transmission route of microorganisms should be recorded in detail, such as perioperative contamination, transfusion, installed ports, pacemakers and implanted prostheses. Additionally, other records like previous operated spinal level as well as indication of index surgery and radical focal debridement could possibly indicate increases to the potential risk of infection, especially low virulent organisms (e.g., Propionibacterium acnes and Staphylococcus epidermidis). Furthermore, some researchers observed that the presence of preoperative antibiotic therapy could modify the nature of the body's inflammatory response and alter the results of the peri-implant tissue histopathological examinations [28]. Strong consensus has also been achieved on the medical community

abstaining from antibiotic use in patients with suspected PJI, unless the patient has significant systemic instability due to sepsis [29]. Therefore, any information (e.g., antibiotic types, timing, dosage and duration) about previous antimicrobial therapy should be carefully collected.

Demographic factors such as old age, an American Society of Anesthesiologists (ASA) grade greater than two, low body mass index (BMI) (malnutrition) or excessive BMI (obesity), long-term steroid usage, diabetes mellitus, chemotherapy/radiotherapy, smoking and alcohol abuse have been identified as risk factors predisposing patients for the occurrence of postoperative SSI in orthopedic surgeries [13, 30, 31]. Therefore, considerable attention should be addressed in isolating associated factors for the purpose of decreasing surgery-related morbidity and, most essentially, improving patients' surgical outcomes.

1.4.2. Clinical examinations

Physical examinations are beneficial for examining signs such as local site tenderness, warmth, skin ulcers, erythema, edema and drainage as they are suggestive of a possible infection in the area of the implant. However, in low-grade infection cases, the clinical signs are discreet. The expression of local signs of inflammation such as redness, swelling and overheating might not be impressive. Patients sometimes present with complaints of chronic, unspecific back pain and radiological loosening signs [32]. For early onset infection, systemic signs of inflammation such as fever and chills are present up to the clinical presentation of sepsis. Therefore, the body and local site temperature should also be carefully monitored.

More concerns were raised by some researchers about the possible routes of bacteria from the mouth (e.g., tooth cavity, damaged or loosened tooth), lung and urinary tract to the orthopedic implants. Patients may get hip or knee endoprosthesis infections after dental procedures (e.g., decayed teeth, extraction and periodontal disease) no matter whether this was with/without antibiotic prophylaxis. Streptococci, for example, is the most common bacterial species in the human mouth and causes a three times higher rate of late-onset PJI than early-onset PJI [33]. Hematogenous infections can occur even years after the placement of implant material due to primary or secondary bacteremia, even though the hematogenous spread route accounts for less than ten percent of all causes in peri-implant infections [34]. Other possible routes such as the urinary tract and

chest infection, whether causing bacteremia or not, should also be taken into consideration as the microorganisms could reach and attach to the implant through hematogenous seeding. Therefore, it is important to perform preoperative blood culture for patients with suspect dental, chest and urinary infections.

1.4.3. Radiological examinations

Although around 50 percent of implant infections cases present as normal in the imaging examinations, commonly used radiological examinations such as plain radiographs, computed tomography (CT) and magnetic resonance imaging (MRI) are indispensable.

The X-ray may lack early sensitivity in diagnosing postoperative spine infection, yet it has been widely utilized for assessing implant failure, spondylodiscitis, vertebral endplate erosion and disc height loss. It has been stated that serial X-rays have a sensitivity of 14% and a specificity of 70% in detecting joint implant-associated infections [35]. Hence, they should be set as a part of the initial workups. In order to analyze peri-implant vertebral structure and soft tissue, CT scans are regarded as the modality of choice for evaluation. Early changes such as bone structure (e.g., erosion, osteolysis and periosteal reactions), reduced disc space, pseudarthrosis as well as the lucency sign of implant-to-vertebrae gap can be detected as presentations that are suggestive of implant-related infections. Abnormal soft tissue swelling, sinus tracts formation, fluid collections as well as inflammatory edema may also be detected through CT scan. MRI was determined as the most advantageous and reliable modality in diagnosing spondylodiscitis and intervertebral abscess [36] due to its high sensitivity, high specificity, and its reliable capability of providing structural information on paraspinal tissues as well as the epidural space [37]. In addition, intravenous gadolinium contrast techniques also provide extra evidence, improving the visibility of micro soft tissue infection. The drawback is that implant-associated (especially cobalt-chromium-molybdenum material) magnetic susceptibility artifacts have been detected after surgery, which has also raised concerns when applying this technique [38].

Other novel imaging modalities such as Single-Photon Emission Computed Tomography (SPECT) / CT and Positron-Emission Tomography (PET) / CT have both been indicated with high sensitivities and specificities. But they have not yet been widely used and accepted as routine diagnostic tools for spinal surgeons in PSII workups.

1.4.4. Laboratory examinations

Inflammatory markers such as leucocyte count and C-reactive protein (CRP) are easy to measure and employed as indicators of spinal implant-related infection. CRP, as an acute-phase protein that is synthesized by hepatocytes, reaches its peak on the third day and is found to be decreased to the normal level over five to fourteen days in patients after instrumented posterior lumbar interbody fusion operations [39]. It has been observed as one of the most accurate laboratorial markers in diagnosing patients with total hip arthroplasty, with a sensitivity of 96% and a specificity of 92% [40].

However, there is little existing available data on the performance of CRP in the diagnosis of spinal implant-related infection. For PJI, CRP currently serves as the first-line screening tool, due to its high sensitivity and routine accessibility [41], yet it is not specific for the presence of other inflammatory as well as systemic immune diseases. Particularly in diagnosing late-onset infection with the presence of a high incidence of low-virulent pathogens (e.g., Propionibacterium acnes and coagulase-negative staphylococci), CRP showed a low specificity even after optimized cutoffs were employed [42]. There was a diagnostic 64% sensitivity and 68% specificity with a CRP cutoff of 4.05 mg/l, and sensitivity and specificity of 79% and 68% when the threshold was set at 4.6 mg/l [42, 43].

Unlike synovial fluid aspiration with the determination of white blood cell count, which is widely used as a main diagnostic criterion for PJI, it is not practical to apply this technique to diagnose spinal implant-related infection. Serum leucocyte count is of limited use, and achieved poor sensitivity of from 21% to 70% in diagnosing PJI [44, 45], yet it remains as an invasive, first-line screening tool. If all above-mentioned preoperative tests are negative, yet clinical suspicion of spinal implant-related infection persists, intraoperative tissue sampling should be performed.

1.5. Intraoperative analysis

Histopathological and microbiological examinations of the intraoperative peri-implant tissue as well as sonication techniques of the suspected implants are considered as the gold standard for diagnosing and ascertaining pathogens of spinal peri-implant infections [46]. In addition, these examinations provide direct evidence on patients with a presumed aseptic spine surgery but who in fact have a subclinical infection.

1.5.1. Histopathological examination

The histological analysis of peri-implant tissue has always been recommended as a significant component of the efforts to confirm or exclude implant-related infections. According to the histopathological criteria described by the Musculoskeletal Infection Society for diagnosing PJI, an observation of more than five neutrophils per high power field from the periprosthetic tissue in five high-power fields at a magnification of 400x was recommended [47].

According to Morawietz and Krenn, a more widely accepted system for PJI histological diagnosis is based on the classification of the periprosthetic interface membrane which located at the interface between the focal bone and prosthesis [48]. They recommended four types of periprosthetic membrane, which are defined as wear particle induced type (Type I), infectious type (Type II), combined type (aspects of Type I and Type II occur simultaneously; Type III), and indeterminate type (Type IV). A histopathological sample was considered as positive if the findings were described as Type II or III. In a prospective multicenter study, the sensitivity and specificity of histopathological diagnosis with a cutoff value of 23 neutrophils in ten high-power fields were reported as 82.0% and 90.0%, respectively [49]. The histopathological examination helps to define early or later onset infection, but is limited for determining causative pathogens.

1.5.2. Microbiological culture

Intraoperative peri-implant tissue microbiological examination is crucial and considered as the diagnostic benchmark in diagnosing spinal implant-related infection. Isolation of causative pathogens and further antimicrobial susceptibility testing were described as critical in targeting antimicrobial therapy, thus improving patient outcomes.

The intraoperative tissue samples were obtained either directly from the implant (attached or inside) or from the hardware surrounding sites. Intraoperative removed samples were transferred into standard sterile receptacles and sent to the specialized testing laboratory within four hours in order to minimize contamination rates. At least three peri-implant tissue samples should be taken from each patient and incubated for at least seven days in both aerobic and anaerobic culture plates [50, 51]. Sensitivity and specificity of microbiological tissue culture in examining spinal implant-related infection were observed as 65.7% and 96.4%, respectively [18]. Due to the low sensitivity detection rate of this method, the recommended culture period must be extended by at least 15 days because

of slow growing nature of organisms such as Staphylococcus epidermidis, Pseudomonas aeruginosa and Escherichia coli. Therefore, it was recommended to suspend any use of antimicrobial greater than two weeks before the microbiological culture, as it was reported that the sensitivity of tissue culture would, in diagnosing PJI, decrease from 76.9% to 47.8% to 41.2% as the preoperative antimicrobial-free interval period decreased from greater than fourteen days, to four - fourteen days, to zero - three days, respectively [52].

1.5.3. Sonication

As a novel diagnostic tool, sonication fluid culture of the explanted spinal implants (e.g., screws, nuts or rods) has been proposed and employed aiming to remove biofilm embedded bacteria from the explanted prosthetics. Routine use of sonication in revision spinal surgery could significantly improve the accuracy in detecting subclinical infection in patients with suspected aseptic failure [53], [20]. The central problem remains the biofilm that attached to the implant, which leads to the failed detection of microbiological culture as well as pathological staining, and especially so in cases with delayed implant-related spine infections, where surgeons expect to eradicate the infection while maintaining the stability of the spine. Studies observed that bacteria in the biofilm cannot be eradicated by either antimicrobial therapy alone or debridement with retention of implants, and that low-grade infection signs such as persistent pain, loss of deformity correction and increased secondary debridement/operation rate might possibly occur [14], [52].

Furthermore, it has been suggested that implanted pedicle screw loosening is related not only to the exposure to imbalanced/excessive stress but also to the implant-associated infection [54]. Theoretically, the microbial particle from the biofilm can be agitated and released when the sound energy waves are applied. The sensitivity of sonicate-fluid culture was found to be significantly superior to that of tissue culture, with 78.5% versus 60.8% in diagnosing PJI and 94.3% versus 65.7% in diagnosing spinal implant-related infection. In addition, the testing specificity of this new approach does not compromise when the sensitivity is increased, with 98.8% versus 96.4% and 98.8% versus 99.2% in diagnosing PJI and PSII, respectively [18], [55]. With its reliable testing results, sonication has been recommended to be integrated into the clinic as a routine instrument in the diagnostic workup for spinal-implant related infection [56].

1.6. Aims and hypothesis

Implant-related infections remain one of the most devastating complications in spinal revision surgery due to their high occurrence rate and poor outcomes for the patients. However, the diagnostic algorithms of PSII, as compared to periprosthetic joint infections, have not yet been intensively studied. Unfortunately, most patients cannot be identified by the usual clinical and laboratory parameters, and especially those infected with low-virulent microorganisms.

Therefore, the aim of this doctoral thesis was to study all relevant risk factors for the development of PSII in patients undergoing revision surgery. To the best of our knowledge, we have performed for the first time a matched-cohort study of patients with and without PSII and included clinical, radiographic, laboratory, intraoperative tissue and implant analysis to identify risk factors. Our hypothesis was that similarly to PJI, certain risk factors for the development of PSII, such as suspicious clinical features, previous device implantation or hematogenous infections in the present study could lead to an increased risk of peri-implant spinal infections. The overall goal was to establish a pre-operative checklist to identify patients at risk for the development of a PSII. With this tool in hand, spine surgeons could modify their diagnostic approach to rule out any infection due to new tools like sonication.

2. Methods

2.1. Ethical approval

This retrospective study was reviewed and approved by the Ethical Committee of the Charité – Universitätsmedizin Berlin (application number: EA2/128/19).

2.2. Population and study design

From October 2015 to October 2018 at the Charité – Universitätsmedizin Berlin Campus Mitte, the medical records of a total of sixty-four patients (assigned as the septic group) who underwent a revision surgery after instrumental spinal operations were retrospectively identified and enrolled. Then aseptic patients (assigned as the aseptic group) were identified and matched in parallel for age (a gap of within five years is acceptable), gender and surgical indications with each septic case. With three septic patients failing to match, in total there were 61 septic and 61 aseptic patients eventually enrolled. The patients' data were obtained in detail by reviewing information on a digital patient document system - System Analysis and Software Development (SAP) - and on physical/written medical records as well. The detailed patient diagnostic parameters were collected and shown in Table 1.

Table 1 The diagnostic items and their specific parameters.

Main items	Specific parameters
Patient anamnesis	• Indication for the index surgery • Duration from index until the revision surgery • No. previous surgeries in the segment • Operation time of the second last surgery • Operated spinal levels of the second last surgery • With/without radical debridement of surrounding tissue

	• With/without complete instrumentation exchange of the second last surgery • Transfusion record of the second last surgery • With/without preoperative antibiotic use
Patient demographics	• Age • Gender • BMI • ASA grade • Operation time • Operated area
Risk factors	• Diabetes • Smoke • Alcohol abuse • Chemo/radiotherapy • Long-term corticosteroid use
Clinical examinations	• Local site skin findings • Pain at rest • Pain under motion • Body temperature • With/without port catheter implantation • With/without pacemaker • With/without other inserted implants • Blood microorganism cultures • Urinary tract infection • Dental status • With/without thoracic infection

Radiological examinations	• X-ray signs of any implant loosening, dislocation and/or breaking • CT examination for any implant loosening, dislocation and/or breaking • MRI examination with or without infection signs
Laboratory examinations	• Serum leucocyte count • Serum CRP level • Kidney function • Liver function
Intraoperative tissue analysis	• Histopathological examination using Morawietz and Krenn diagnostic system • Microbiological examination for specific microorganisms • Sonication of the explanted instruments

As listed in Table 1, we covered several diagnostic items, including: 1) patient anamnesis including the index and the second last surgery; 2) patient demographics; 3) risk factors; 4) clinical examinations; 5) radiological examinations; 6) laboratory examinations; and 7) intraoperative peri-implant tissue analysis as well as sonication of the explanted instruments. By listing detailed parameters in these seven categories, this thesis managed to study all relevant risk factors for the development of PSII.

2.3. Literature search for relevant checklists

Due to the lack of literature on the diagnostic consensus or criteria in defining postoperative spinal-implant infection, we managed to integrate and adapt various diagnostic checklists for musculoskeletal SSI and PJI in order to inform the evaluation and diagnose postoperative spinal implant-related infection. We searched all the studies

in PubMed and Google Scholar with the keywords “spinal implant related infection OR spinal implant infection OR peri-spinal implant infection OR spinal instrument infection”. Diagnostic checklists with clinical, radiological, laboratory, intraoperative and microbiological parameters were observed and reviewed. The authoritative definition of orthopedic surgery infection adapted from CDC [57] as well as the diagnosis of PJI from the International Consensus Meeting Criteria (ICMC) [58] and the Infectious Diseases Society of America (IDSA) [59] were proposed as references (as shown in Table 2).

Table 2. The definition of SSI adapted from CDC and the criteria of PJI of different classification systems.

CDC	ICMC	IDSA
Infection occurs within one year, with the implant and infection involved affecting parts of the body that lie below the fascia/muscle layer and in the operating area and match up to at least one of the following criteria: 1. Purulent drainage from a drain that communicates with the implant; 2. Pathogens detected from an aseptic liquid or tissue surrounding the implant;	**Major criteria:** 1. Two positive peri-prosthetic cultures detected with identical organisms; 2. At least one sinus tract communicating with the prosthesis. **Minor criteria:** 1. Elevated serum CRP and ESR level; 2. Elevated level of synovial fluid leukocyte count; 3. A single positive tissue culture;	1. A sinus tract that communicates with the prosthesis; 2. Purulence without other recognized etiology surrounding the prosthesis; 3. a) ≥ Two Intraoperative cultures or combination of preoperative aspiration liquid and intraoperative cultures that produce the same organism; b) Growth of a virulent microorganism observed in a single tissue biopsy or in synovial fluid;

3. An abscess or other infection evidence in the surgical area by macroscopic, histopathological or radiologic examination.	4. A positive leukocyte esterase reagent strip test (++ or +++); 5. Elevated polymorphonuclear neutrophil percentage of the synovial fluid; 6. Positive histological examinations of periprosthetic tissues.	4. Acute infection evidence based on histopathologic examination of peri-prosthetic tissues.
Infection presence if confirmed ≥ one of the above criteria	*Infection presence if examined: at least one of the two Major criteria OR at least three of six Minor criteria exist)*	*Infection presence if one confirmed criterion from 1 to 3; criterion No. four is suggested as highly possible*

2.4. Definition of diagnostic parameters

For the medical history, indications for the index surgery were classified as fracture, deformity, degenerative spinal diseases, inflammation, tumor and others. Operations of the same segment(s) were recognized if one or more segments were involved in previous operations. Radical debridement occurred in infected and tumor cases and involved thoroughly cleaning, and removing all hyperkeratotic, infected, non-viable tissue, and foreign debris as well as dressing residual materials. Complete instrumentation exchange was defined as thorough spinal implant replacement (e.g., all kinds of screws, cages, rods, plates and artificial discs) in previous surgeries. Any preoperative antibiotic use before the revision surgery was recorded, including oral and venal injection. An ASA grade greater than two was considered as a risk factor.

For clinical examinations, blood cultures of serum samples in suspected hematogenous cases and the exact detected organisms were recorded. The dental status was recorded as normal or with infection suspected (e.g., damaged or loosened). The local site skin findings included any skin tenderness, fluctuation or warmth, as well as ulcers, erythema,

edema or drainage formation. The blood temperature elevation was defined as being greater than 37.5°. VAS scores of two modes (VAS at rest and VAS under motion) were used and scored from zero to ten, which represented a continuum between “no pain” to “worst pain” in the spinal region. Inspections such as ports (e.g., portals and catheters), cardiac pacemakers and other embedded implants/prostheses were recorded. For examining any extra-spinal source of infection, lung infections were examined by thorax X-ray and urinary infection were investigated by urine tests.

For radiological parameters, spine regional X-rays and CT data were observed for reviewing implant/prosthesis status, and they were classified as normal, loosening, dislocation and/or breaking, which would be evaluated as possible indications of an infection. MRI results were examined for the possible implant-related infection.

For laboratory blood serum test, two inflammation parameters, i.e., leucocyte count and C-reactive protein, were assessed. The normal reference value for the leucocyte count was set at 3.90-10.50 /nl. A value of less than 5.0 mg/l of inflammation marker C-reactive protein was regarded as normal.

The histological analysis of intraoperative tissue was classified into four types according to Morawietz and Krenn’s grading system [48], where the wear particle induced Type I and indeterminate Type IV were determined as non-infected, while infectious Type II and combined Type III (Type I plus Type II occurred) were considered as positively infected.

At least three pieces of peri-implant tissue sample were obtained either directly from the implant site or from the immediately adjacent tissue, and then transferred into special sterile tubes and sent directly to the microbiological laboratory for tissue microorganism analysis. According to surgical site infection diagnostic criteria adapted from CDC[57], the PJI diagnostic checklist from ICMC [58], IDSA [59] and Bürger, et al. [18], a peri-implant tissue culture was determined positive in the case of:

- 1) detection of low-virulent microorganisms (e.g., Propionibacterium, Coagulase-negative Staphylococci and Enterococcus spp.) in more than two specimens;
- 2) detection of low-virulent microorganisms in no less than one specimen, if patient was under regular antimicrobial therapy 30 days prior to the revision surgery;
- 3) detection of low-virulent microorganisms in one tissue sample when confirmed with the identical microbial growth in the sonicated fluid culture;

- 4) detection of high-virulent microorganisms (e.g., Escherichia coli, Staphylococcus aureus, etc.).

For sonication evaluation, as shown in Figure 2, the removed implants were inserted into a sterile sonication receptacle and vortexed for 30 seconds using a Vortex-Genie device. Then, implants were subjected in an Aqua-sonic ultrasound bath (BactoSonic 14.2 unit, Bandelin, Germany) to sonication (40 kHz, 5mins), followed by an additional 30 seconds vortex. The resulting sonicated fluid was finally centrifuged and plated for both aerobic and anaerobic cultures. Routine microbiological techniques were employed for microorganism enumeration as well as classification [55], [53]. A cutoff value of 50 colony forming units (CFU) per milliliter of sonication fluid was applied [60]. Culture was defined as positive in the case of:

- 1) detection of less than 50 CFU of low-virulent microorganisms with antimicrobial treatment 30 days prior to the revision;
- 2) detection of less than 50 CFU of low-virulent microorganisms, together with a same organism detection in peri-implant tissue culture;
- 3) detection of high-virulent microorganisms of any amount.

The detection of less than 50 CFU of a low-virulent pathogen without the influence of antibiotics and without simultaneous detection of the same pathogen in at least one tissue sample was therefore classified as negative. The detected growth of greater than 50 CFU of Ralstonia pickettii or Micrococcus luteus pathogens was classified as contamination.

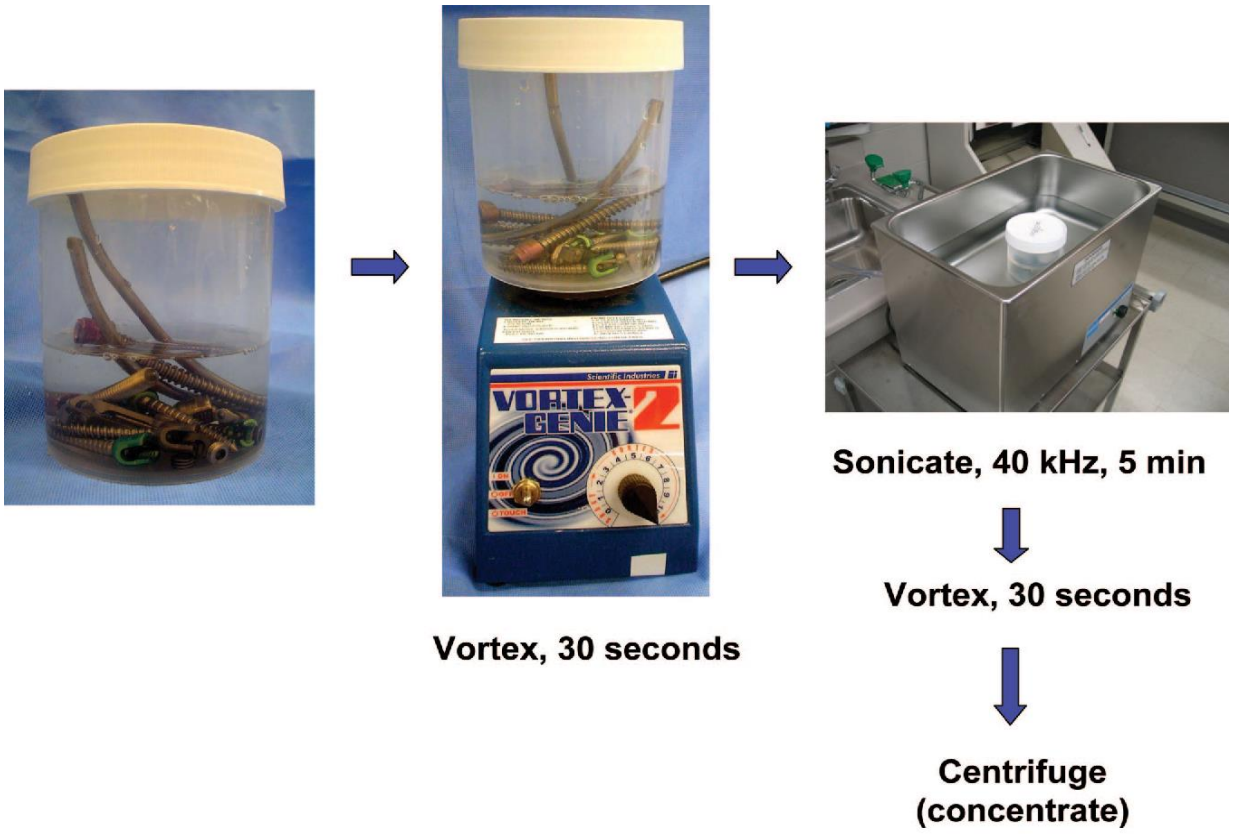

Figure 2. Sonication procedure of spinal implants [53]. Authorized for use by Dr. Robin Patel (Department of Internal Medicine, Division of Infectious Diseases, Mayo Clinic College of Medicine, Rochester, MN 55905, USA).

For risk factors, diabetes mellitus was diagnosed if the fasting plasma glucose was greater or equal to 126 mg/dl, or two-hour plasma glucose greater or equal to 200 mg/dl was observed according to the WHO recommendation [61]. Obesity was defined if BMI was greater or equal to 30 kg/m^2. Long-term corticosteroid use was identified if patients had taken corticosteroids at ≥5 mg per day and it lasted ≥ 3 months. ASA classification with five scores was defined as: 1) ASA1 normal healthy patient; 2) ASA2 patient with mild systemic disease; 3) ASA3 patient with severe systemic disease; 4) ASA4 patient with severe systemic disease so that life was threatened constantly; and 5) ASA5 dying patients who were not expected to survive without the surgery [62].

2.5. Statistical analysis

Data were displayed as mean ± standard deviation (SD), median (interquartile range, 25% – 75%) or in number count (n) with a percentage (%).

McNemar and marginal homogeneity tests were used for examining differences between categorical variables. The paired sample t-test was used for analyzing differences of parametric variables; Wilcoxon's signed rank test was used for analyzing differences of non-parametric variables. The software package SPSS version 24 (SPSS Inc, Chicago, Illinois, USA) was used for the statistical analyses. A P value of less than 0.05 was considered as statistically significant.

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of examinations were described as:

- Sensitivity = (True positive cases) / (True positive cases + False negative cases)
- Specificity = (True negative cases) / (True negative cases + False positive cases)
- PPV = (True positive cases) / (True positive cases + False positive cases)
- NPV = (True negative cases) / (True negative cases + False negative cases)
- Accuracy = (Correct assessed cases) / (All cases)

The statistical evaluation of the collected data was carried out after a consultation with, and the confirmation of, the Institute for Biometry and Clinical Epidemiology of Charité - Universitätsmedizin Berlin.

3. Results

3.1. Study population

In this thesis, a total of 64 PSII patients were enrolled. They were matched one-by-one for age, gender and surgical indication with each non-PSII case. After three PSII patients failed to find a matched control pair and were hence excluded, the remaining 61 septic patients were identified as the septic group, while the matched 61 control cases were identified as the aseptic group. All subjects were diagnosed by senior surgeons of the Center for Musculoskeletal Surgery, Campus Mitte of Charité – Universitätsmedizin Berlin.

3.2. Anamnesis

The characteristics of the included patients are summarized in Table 3. The mean age in the septic group was 58.57 ± 22.16 and 58.13 ± 22.47 in the aseptic group, P = 0.949. Both groups were consisted of 26 females (42.63%) and 35 males (57.38%), P = 0.999. The BMI in the septic group was 25.96 ± 6.38 kg/m^2 and 26.96 ± 6.87 kg/m^2 in the controls, P = 0.414. There were 25 (40.98%) and 27 (44.26%) cases with an ASA grade larger than two in the septic and aseptic groups, respectively, which yielded a P value equal to 0.890. The operation time of the septic group was 191.59 ± 112.52 minutes, compared with 204.31 ± 84.52 minutes in the aseptic group (P = 0.288). The operated spinal regions for the septic group were three in cervical, six in thoracic, eleven in thoracolumbar, 16 in lumbar, 22 in lumbosacral, one in sacral and two in thoraco-lumbo-sacral spine, compared with one in cervical, one in thoracic, 14 in thoracolumbar, 16 in lumbar, 16 in lumbosacral and 13 in thoraco-lumbo-sacral spine in the aseptic group, respectively (P = 0.022).

For the comparison of other risk factors, there were eleven (18.03%) patients in the septic group and 16 (26.23%) in the controls with diabetes mellitus, respectively, with P = 0.359. Seven of 61 (11.48%) infected cases and ten of 61 (16.39%) non-infection cases stated smoking; no significant difference was found (P = 0.581). No patient in either group was found to have alcohol abuse experiences. Four cases (6.56%) with spinal metastases in the septic group experienced chemoradiotherapy, while there was no counterpart

examined in the aseptic group, P = 0.125. There were two cases in the septic group with prolonged corticosteroids use (Fluticasone 17-propionate) versus none in the aseptic group, preoperatively (P = 0.496).

Table 3. Demographics and risk factors of the matched study populations (mean ± standard deviation).

Characteristics	**Septic patients (n = 61)**	**Aseptic patients (n = 61)**	**P value**
Demographics & risk factors			
Age (years)	58.57 ± 22.16	58.13 ± 22.47	0.949
Gender, n (%)			0.999
Female	26(42.62)	26(42.62)	
Male	35(57.38)	35(57.38)	
BMI, kg/m^2	25.96 ± 6.38	26.96 ± 6.87	0.414
BMI < 30 kg/m^2, n (%)	46(75.41)	42(68.85)	
BMI ≥ 30 kg/m^2, n (%)	15(24.59)	19(31.15)	0.355
ASA grade	2.32 ± 0.67	2.36 ± 0.66	0.890
ASA grade > 2, n (%)	25(40.98)	27(44.26)	
ASA grade ≤ 2, n (%)	36(50.02)	34(55.74)	
Diabetes mellitus, n (%)	11(18.03)	16(26.23)	0.359
Smoking, n (%)	7(11.48)	11(16.39)	0.581
Alcohol abuse, n (%)	0	0	-
Long-term corticosteroid use, n (%)	2(3.28)	0	0.496
Chemoradiotherapy, n (%)	4(6.56)	0	0.125
Operation time (mins)	191.59 ± 112.52	204.31 ± 84.52	0.288

Operated region, n (%)			0.022
Cervical	3(4.92)	1(1.64)	
Thoracic	6(9.84)	1(1.64)	
Thoracolumbar	11(18.03)	14(22.95)	
Lumbar	16(26.23)	16(26.23)	
Lumbosacral	22(36.07)	16(26.23)	
Sacral	1(1.64)	0	
Thoraco-lumbo-sacral	2(3.28)	13(21.31)	

For the anamnesis data, as shown in Table 4, indications for the index surgery in the septic group were four (4.92%) due to fracture, eleven (16.92%) deformity, 42 (64.62%) degenerative spinal diseases (e.g., disc herniation, spinal canal stenosis), one (1.54%) superficial would inflammation, and three (4.62%) tumor cases (spinal metastases). In comparison, there were three (4.92%), ten (16.39%) and 48 (78.69%) cases with indications as fracture, deformity and degenerative spinal diseases; no significant difference was found (P = 0.332). Durations from index surgery until revision (months) were 58.60 ± 85.50 in the septic and 74.99 ± 80.10 in the aseptic group, P = 0.221. The total number of previous operations were 1.93 ± 1.23 in the septic group and 1.90 ± 1.51 in the aseptic group; no significant difference was found (P = 0.906).

For the surgery prior to the latest revision, when comparing the septic versus the aseptic group, 1) the operation times were 212.36 ± 103.88 minutes and 207.40 ± 85.40 minutes, P = 0.810;

2) the operated segments were 3.80 ± 3.51 versus 3.90 ± 3.64, with a P value equal to 0.857;

3) the number of cases with radical debridement of surrounding tissue were four (6.56%) versus zero in controls, P = 0.119;

4) the number of complete implant exchanges were eight (13.79%) and five (8.20%), P = 0.379;

5) the numbers with a transfusion record during the operations were 14 (29.79%) and 17 (33.33%).

There were only 14 of 47 patients in the septic group versus 17 of 51 patients in the aseptic group with a transfusion record (no data available for 14 cases in the septic and 10 cases in the aseptic group, respectively). In cases with transfusion, there were twelve transfused with red cell concentration, one with platelet concentration and seven with fresh frozen plasma for the 14 infected patients, while for the controls, the numbers were 14, zero and six, respectively. However, no significant difference was found (P = 0.706). As to the preoperative antibiotic use, there were six cases in the septic group using Cefpodoxime plus Trimethoprim, Rifampicin plus Levofloxacin, Flucloxacillin plus Piperacillin / Tazobactam, Amoxicillin, Amoxicillin / Clavulanic acid and Doxycycline, respectively, while no case in the control group had prophylactic antibiotic treatment; P = 0.031.

Table 4. The anamnesis of the matched study populations (mean ± standard deviation).

Patient history before revision surgery	**Septic patients (n = 61)**	**Aseptic patients (n = 61)**	**P value**
Indication for the index surgery, n (%)			0.332
Fracture	4(6.56)	3(4.92)	
Deformity	11(18.03)	10(16.39)	
Degenerative spinal diseases	42(68.85)	48(78.69)	
Inflammation	1(1.64)	0	
Tumor	3(4.92)	0	
Duration from index surgery until revision (months)	58.60 ± 85.5	75.00 ± 80.1	0.221
Total number of previous operations in the segment (n)	1.93 ± 1.23	1.90 ± 1.51	0.906

Operation time of the second last surgery (mins)	212.36 ± 103.88	207.4 ± 85.4	0.810
Operated spinal levels of the second last surgery (n)	3.80 ± 3.51	3.90 ± 3.64	0.857
Cases with radical debridement of surrounding tissue, n (%)	4(6.56)	0	0.119
Complete instrumentation exchange of the second last surgery, n (%)	8(13.79)	5(8.20)	0.379
Transfusion of the second last surgery, n (%)			0.706
Without transfusion*	33(70.21)	34(66.67)	
With transfusion	14(29.79)	17(33.33)	
Red cell concentrate	12	14	
Platelet concentrate	1	0	
Fresh frozen plasma	7	6	
Preoperative antibiotic use (n)			0.031
Without	55	61	
With	6	0	
Cefpodoxime	1	0	
Rifampicin	1	0	
Levofloxacin	1	0	
Flucloxacillin	1	0	
Piperacillin/ Tazobactam	1	0	
Amoxicillin	1	0	
Amoxicillin/	1	0	

Clavulanic acid		
Doxycycline	1	0
Trimethoprim	1	0

*14 cases in the septic group and 10 cases in the aseptic group have no transfusion record.

3.3. Characteristics of clinical examinations

As shown in Table 5, local site skin findings including skin tenderness, fluctuation, warmth, ulcers, erythema, edema and drainage were found in 15 (24.59%) patients with PSII, while in the case of the aseptic group with observed preoperative skin disorders on the spine region, P = 0.008. The mean body temperature in the 61 infected cases was 36.75 ± 0.60°C vs. 36.43 ± 0.47°C in the controls, P = 0.003. In the infected cases, there were eight with elevated temperature (> 37.5°C) vs. none in the aseptic group. Pain complaints were shown using the VAS ten-point-scoring system, with the two modes being at rest and under motion. 51 of 61 cases with infection complained of pain at rest versus 55 of 61 in the non-infected group. More patients complained of pain status under motion in both groups, of which there were 56 and 59 cases in each group, respectively. The mean scores of VAS at rest for patients in the septic group were 3.35 ± 2.22 vs. 4.62 ± 2.56 for the aseptic group (P = 0.006). For VAS under motion, the mean scores in the septic group were 5.41 ± 2.52 versus 6.90 ± 2.41 in the aseptic grop (P = 0.002). The inspection findings mainly focused on port catheter, pacemaker and other prosthesis implanted of other body parts (extrinsic implants), from which possible infection routes were suspected. There were nine ports, five pacemakers and 15 extrinsic implants in the infected groups versus zero, one and 13 in the non-infected cohort, respectively. No significant differences can be observed in comparisons of pacemaker and extrinsic implant placement between the two groups (P = 0.219, P = 0.824). Notably, significant difference was found between two cohorts in port catheter (P = 0.002).

Blood cultures of patients in fever or with suspected hematogenous infection were observed. The blood samples of twelve patients with suspected PSII preoperatively were examined, with nine detected positive microorganisms (one with HIV). Six cases among the total eight bacteria infected cases (75%) were detected with identical bacterium in their implant surrounding tissues, as shown in Table 6. No blood culture was performed

in the cases of the aseptic group. Other possible hematological infection routes were also analyzed. In septic group, eight of the 61 had positive urinary infection, whereas one in 61 cases in noninfectious group was detected positive, P = 0.032. Possible tooth infection was documented, wherein there were 56 infected and 55 non-infected cases with normal dental status, and three versus four cases with suspected infectious signs (e.g., tooth defect, damaged, obvious loosen), respectively, and yielded a P value equal to 0.700.

Examination items	Septic patients (n = 61)	Aseptic patients (n = 61)	P values
Physical examinations			
Local site skin findings*	15	0	0.008
Body temperature (°C)	36.75 ± 0.60	36.43 ± 0.47	0.003
Elevated (> 37.5°C) #	8	0	
Pain at rest (VAS, from 0-10)	3.35 ± 2.22	4.62 ± 2.56	0.006**
With pain	51	55	
Without pain	10	6	
Pain under motion (VAS, from 0-10)	5.41 ± 2.52	6.90 ± 2.41	0.002**
With pain	56	59	
Without pain	5	2	
Inspections§			
Port catheter	9	0	0.002
Pacemaker	5	1	0.219
Other implants	15	13	0.824
Blood cultures			0.008
Without	3	61	
With	9†	0	
Urinary tract infection			0.032
With	8	1	
Without	53	60	
Thorax X-ray			0.500
With chest infection	4	0	
Without chest infection	57	61	

*e.g., tenderness, fluctuation and warmth, skin ulcers, erythema, edema and drainage, P = 0.004

Fever was defined as body temperature > 37.5°C, P = 0.004

** The control group indicated significantly higher values

§Port catheter instrument, P = 0.027

†Among nine cases, six (66.67%) detected with the same infected microorganisms

There were four cases with chest infection in the septic group, while no case in the aseptic group was observed, P = 0.500. Figure 3 shows a case in the septic group with an instrumented port and positive chest infection through plain film.

Table 5. Clinical examinations: characteristics of the matched two groups.

Table 6. Comparison of the blood culture and subsequent peri-implant microorganism species for the blood-positive cases in the septic group.

Patient	Blood culture	Intraoperative tissue analysis
1	Enterobacter cloacae	Enterobacter cloacae
2	Staphylococcus epidermidis	Candida albicans
3	Staphylococcus aureus	Staphylococcus aureus
4	Staphylococcus aureus	Staphylococcus aureus
5	HIV*	Streptococcus pyogenes
6	Escherichia coli and Enterococcus faecalis	Escherichia coli
7	Corynebacterium and Staphylococcus epidermidis	Propionibacterium avidum, Staphylococcus hominis and Staphylococcus epidermidis
8	Staphylococcus epidermidis	Staphylococcus epidermidis
9	Staphylococcus aureus	Propionibacterium acnes

*HIV stands for human immunodeficiency virus

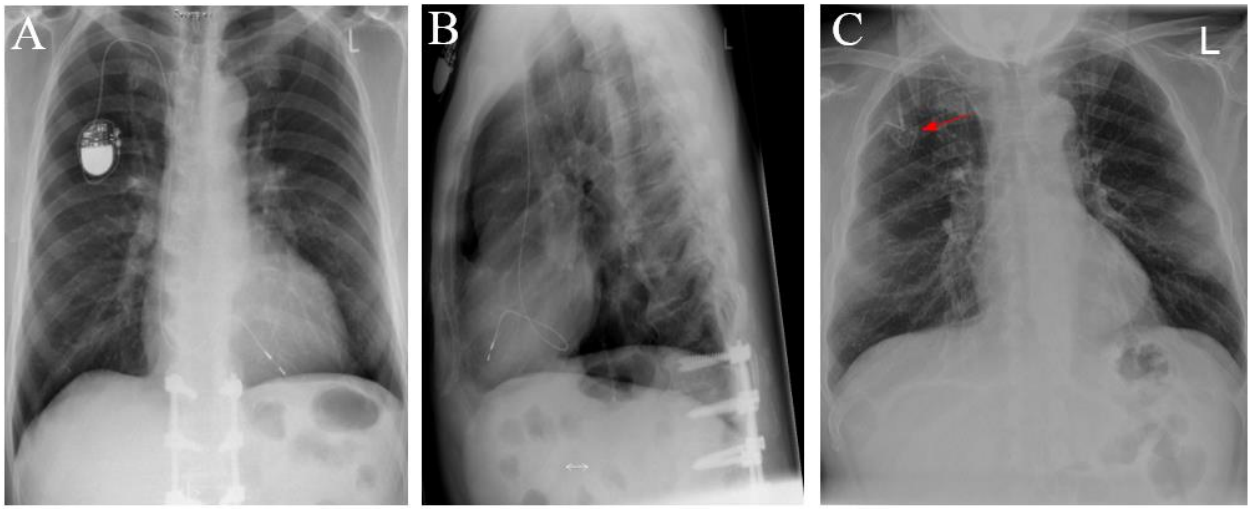

Figure 3. Chest X-ray of infected cases with pacemaker and port. A and B, a 72-year-old male patient; both the anterior-posterior and lateral planes indicated a pacemaker from the right pectoral region, whose probe ends in projection on the right ventricle; C, a 61-year-old female patient with PSII; a port was observed (shown by red arrow) from the right pectoral with projection of the tip onto the superior vena cava.

3.4. Characteristics of radiological examinations

Three preoperative radiological data were collected including X-ray, CT and MRI. Taking the X-ray and CT together into consideration, infectious radiological findings in the form of implant loosening, dislocation, and breaking were observed in 26 infected cases (42.62%) and 27 non-infected cases (44.26%). Materials loosening made up the largest percentage, which was observed in a total of 18 cases (29.51%) of septic group and in 22 cases (36.07%) in the aseptic group (see the case shown in Figure 4). Implant dislocation was found in a total of five cases (8.20%) in the septic group and two cases (3.28%) in the aseptic group. Material breakage was relatively rare, of which one case (1.64%) and four cases (6.56%) were detected in the infected and non-infected groups (an example shown in Figure 5), respectively. Separately, as shown in Table 7, X-rays indicated there were more cases in the aseptic group presenting implant failures (loosening, dislocation and breaking), with a P value equal to 0.031. However, no significant difference was observed in CT in this matter (P = 0.250).

MRI was performed in a limited number of cases for preoperative examination (n = 15 in both groups). The peri-implant infection MRI images of one case are shown in Figure 6, where peri-implant secretion can be observed. 13 infected cases with secretion signs were observed by MRI in the septic group, while none were observed in the aseptic group, P = 0.500.

Table 7. Radiological examination characteristics of the matched two groups.

Items	**Septic patients, n (%)**	**Aseptic patients, n (%)**	**P values**
X-ray			0.031
Normal	43 (71.67%)	33 (58.93%)	
Abnormal	17 (28.33%)	23 (41.07%)	
Implant loosening	14	18	
Implant dislocation	2	2	
Implant breaking	1	3	
CT			0.250
Normal	16 (55.17%)	9 (47.37%)	
Abnormal	13 (44.83%)	10 (52.63%)	
Implant loosening	9	7	
Implant dislocation	4	1	
Implant breaking	0	2	
MRI			0.500
Without peri-implant infection	13 (86.67%)	15	
With peri-implant infection	2 (13.33%)	0	

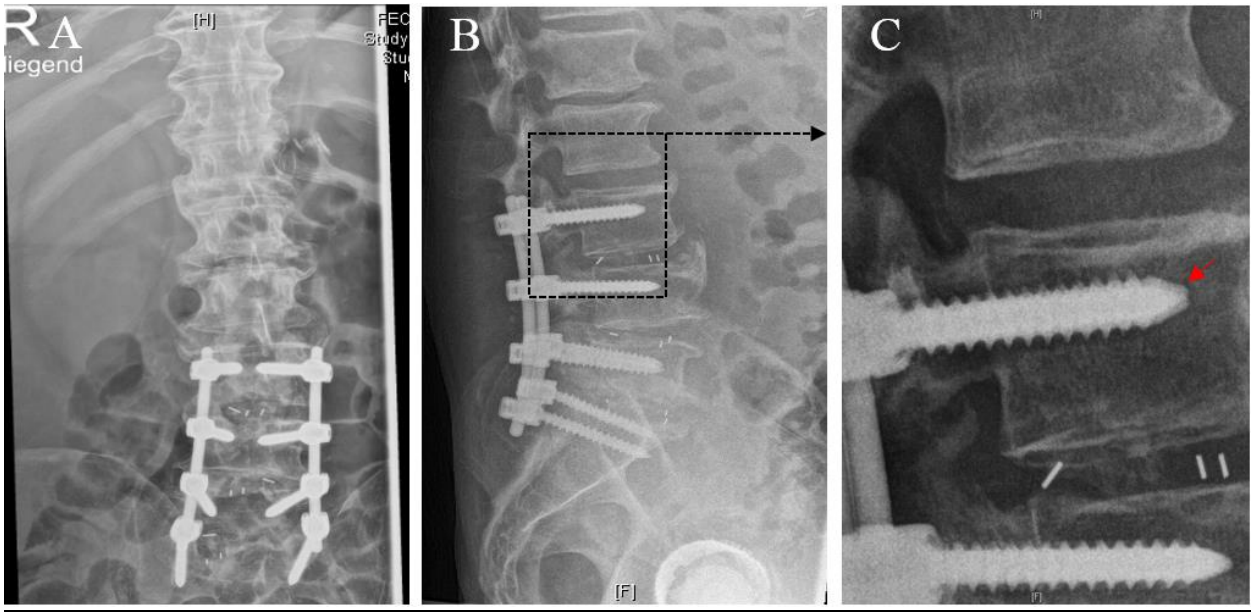

Figure 4. X-ray images of infection-related screw loosening. A 69-year-old male detected with Kocuria rhizophila and Pseudomonas oryzihabitans via implant sonication. The presence of a loosened screw on L3 was observed in the anterior-posterior (A) and lateral view (B, zoomed out in C).

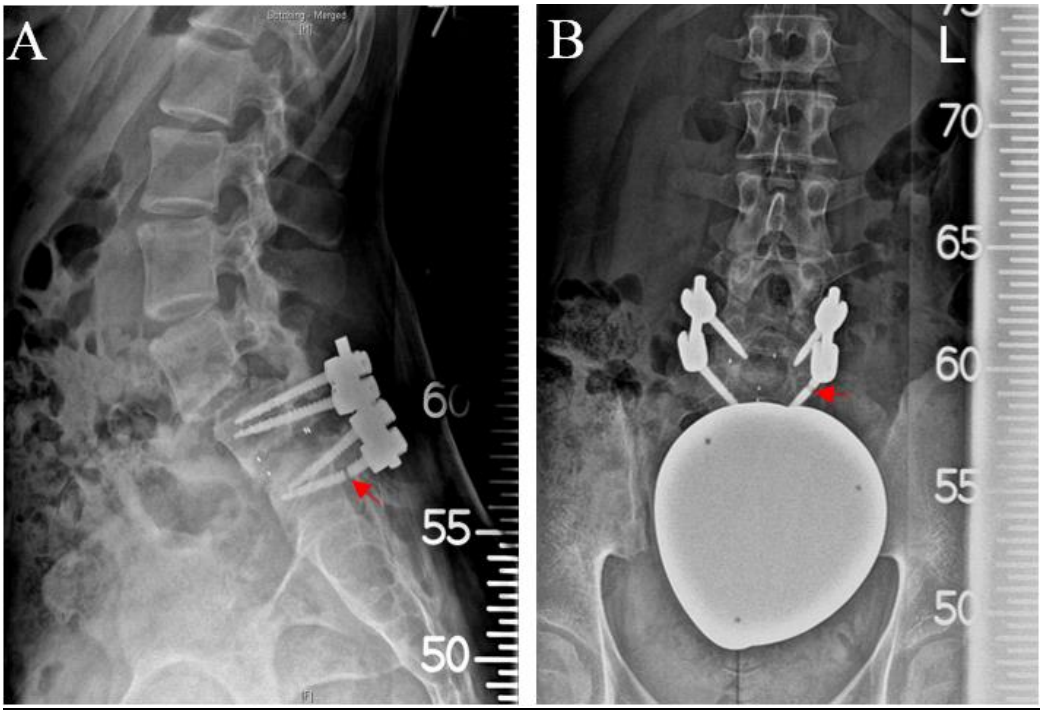

Figure 5. X-ray detection of material breakage in infected cases. A 17-year-old female complained of chronic lumbar pain syndrome with L4/5spondylolisthesis. S1 left-side screw breakage was indicated with red arrows in the sagittal (A) and coronal plane (B).

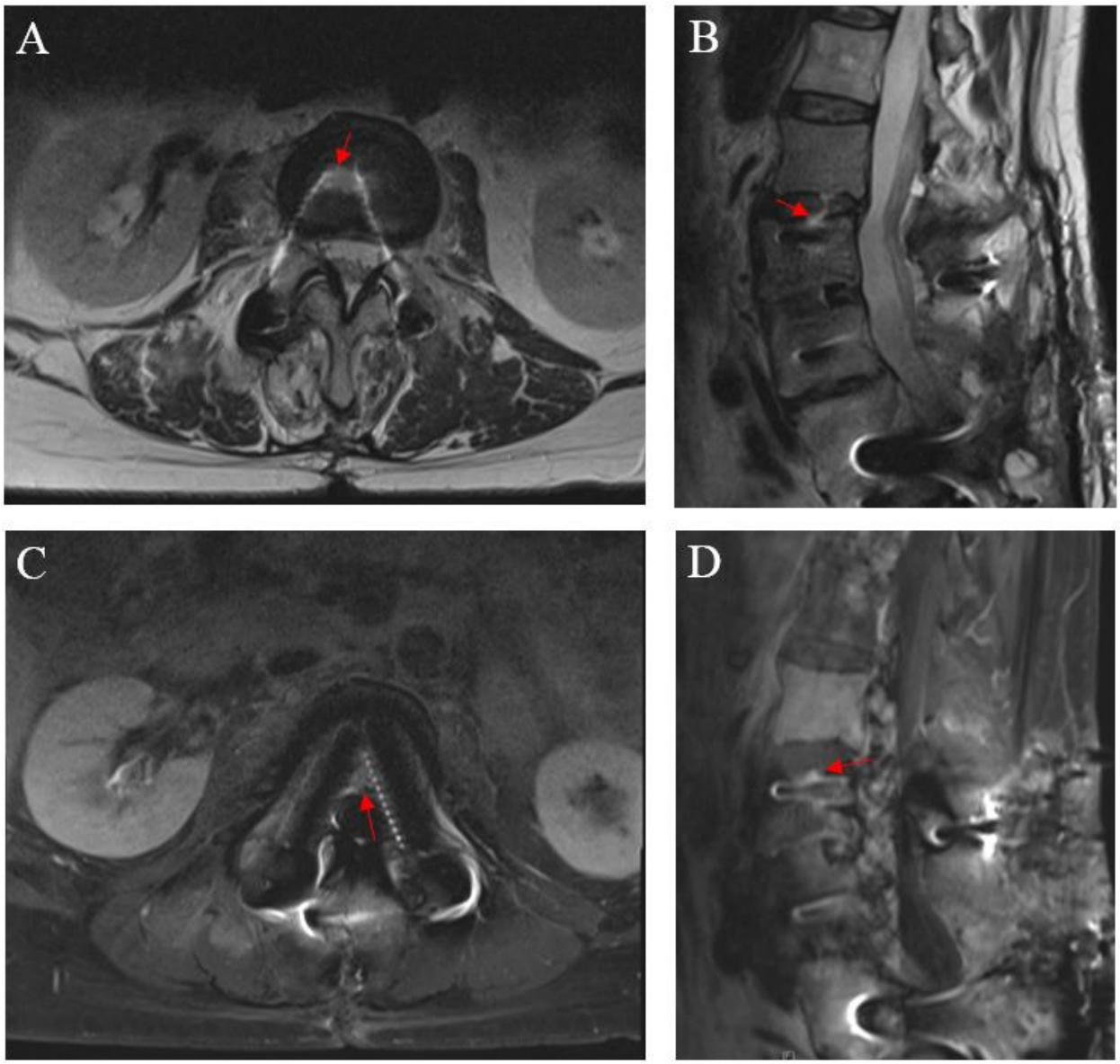

Figure 6. MRI for the diagnosis of PSII. A 60-year-old female diagnosed peri-implant infection with staphylococcus aureus and L1 spondylitis. A and B, T2-weighted image of transverse and sagittal plains indicated peri-implant infection, with red arrows showing the retained secretion; C and D, T1-weighted image of transverse and sagittal plains indicated the implant-surrounding infection (as shown by red arrows).

3.5. Characteristics of laboratory examinations

As shown in Figure 7 and Table 8, the leucocyte count for the infected cases was 7.85 ± 2.84 /nl in the septic group versus 7.92 ± 2.41 /nl in the aseptic group, with a P value equal to 0.784. There were eleven cases (18.03%) in the septic group with a leucocyte count level greater than 10.50 /nl, and three cases (4.92%) with a decreased leucocyte count (less than 3.90 /nl). In comparison, eight cases (13.11%) and one case (1.64%)

were observed with an elevated and lowered leucocyte count in the non-infected cohort, respectively.

A total of 45 patients were observed with an increased CPR level, of which 31 (50.82%) were in the infected cases and 14 cases (22.95%) in the controls, P = 0.022. The mean value of blood serum CRP was 33.80 ± 74.90 mg/l for the septic cases versus 8.20 ± 19.40 mg/l in the aseptic group, P = 0.014. Three cases were found with kidney dysfunction only for the infected group while no case was observed with impaired kidney function in the aseptic cohort, which yielded a P value equal to 0.500. There was one case versus no case in the septic and aseptic groups of observed liver dysfunction, respectively (P = 0.999).

As shown in Table 9 and Figure 8, the sensitivity, specificity, predictive positive value and predictive negative value and accuracy of the preoperative serum leucocyte count in the diagnosis of PSII were 22.98%, 85.24%, 60.87%, 52.53% and 54.10%, respectively. For the serum CRP investigation, the sensitivity, specificity, predictive positive value and predictive negative value and accuracy were 50.82%, 78.69%, 70.45%, 61.53% and 64.75%, respectively.

Table 8. Laboratory examination characteristics of the matched two groups.

Parameters	**Septic patients (n = 61)**	**Aseptic patients (n = 61)**	**P values**
Leucocyte count[*] (/nl)	7.85 ± 2.84	7.92 ± 2.41	0.784
No. of elevated leucocyte count	11 (18.03%)	8 (13.11%)	0.454
No. of decreased leucocyte count	3 (4.92%)	1 (1.64%)	0.619
CRP level (mg/l) [#]	33.8 ± 74.9	8.2 ± 19.4	0.014
No. of elevated CRP	31 (50.82%)	14 (22.95%)	0.022
Kidney function impairment			0.500
With	3 (4.92%)	1 (1.64%)	

Without	58 (95.08%)	60 (98.36%)	
Liver function impairment			0.999
With	1 (1.64%)	0 (0%)	
Without	60 (98.36%)	61 (100%)	

*Normal range 3.90 - 10.50 /nl, P = 0.772

#Normal value < 5.0 mg/l, P = 0.003

Table 9. Sensitivity, specificity, predictive positive value and predictive negative value and accuracy of preoperative leucocyte count and CRP of each sample.

Variables	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy
Leucocyte count*	22.95	85.24	60.87	52.53	54.10
CRP#	50.82	78.69	70.45	61.53	64.75

*Abnormal value as defined as < 3.0 nl or ≥10.5 /nl

#Abnormal value as defined as ≥ 5 mg/l

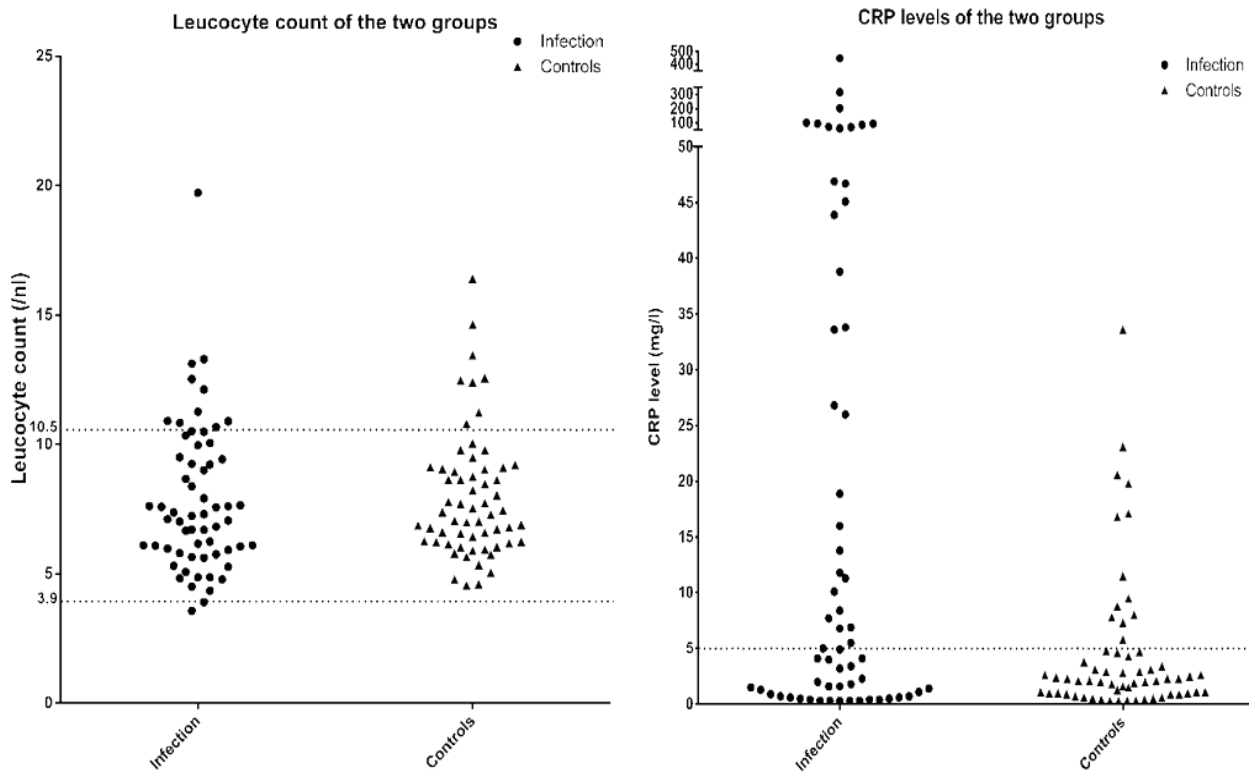

Figure 7. Distribution of preoperative serum leucocyte count and CRP level.

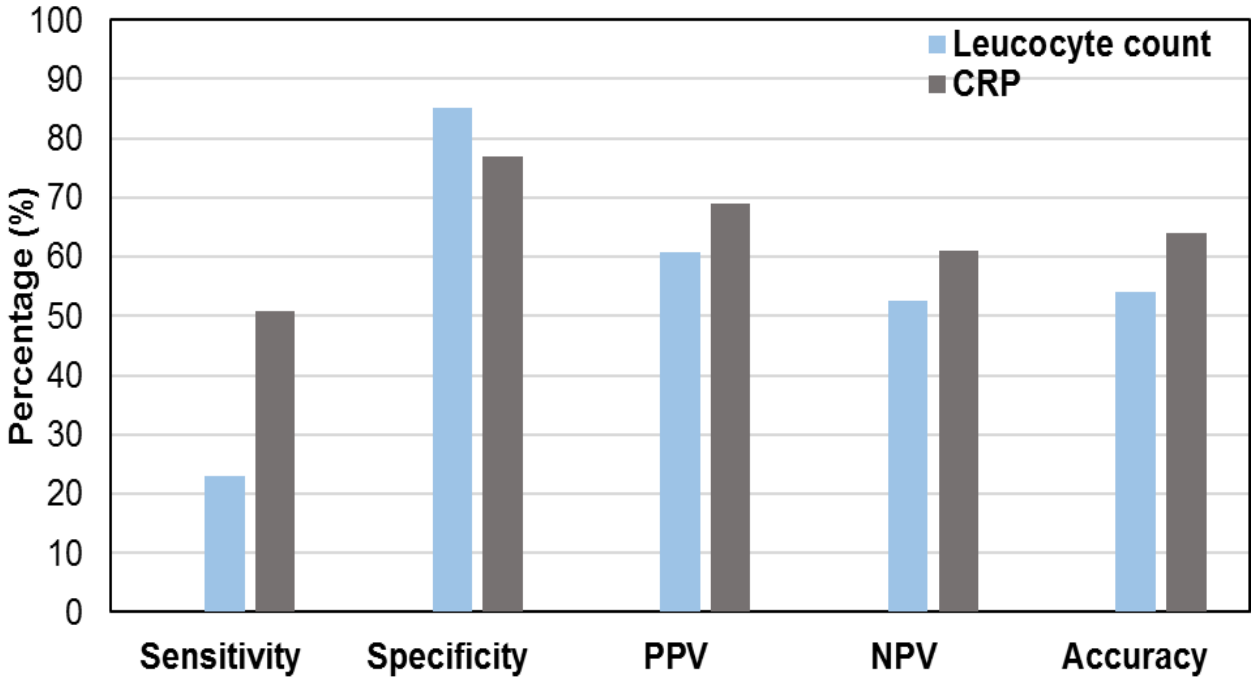

Figure 8. Comparison of diagnostic accuracy of serum leucocyte count and CRP. PPV=positive predicative value, NPV=negative predicative value.

3.6. Characteristics of intraoperative analysis

As shown in Table 10, there were a total of ten cases with intraoperative peri-implant purulence or fistula diagnosed by surgeon evaluation. They were both from the PSII group and accounted for 16.39% of all patients. Notably, four in ten cases were tested as carrying staphylococcus epidermidis. Regarding the histological analysis, there were 21 cases (48.84%) diagnosed and classified as infected types II and III. 22 cases (51.16%) were detected as negative (tissue with abrasion or indeterminate signs). In the aseptic group, the peri-implant tissue of 40 cases accepted histological staining as well as evaluation, of which nine cases were classified as abrasion-induced type I and 31 cases as indeterminate type IV. For microbiological tissue samples, in 44 cases in both groups a pathogen in at least one of the microbiological testing samples was detected, of which 39 (76.47%) were infected cases and five (9.62%) were in the control group. A positive pathogen was not detected in twelve and 47 cases in the septic and aseptic groups, respectively. Sonication of explanted foreign material showed that 71 cases were detected with pathogens attached on the implants, among which 49 cases (92.45%) were from the infected group and 20 cases (32.79%) from the aseptic group (two cases with Ralstonia pickettii and Micrococcus luteus were considered as contaminations).

Table 10. Intraoperative tissue analysis of the matched two groups.

Examination items	**Septic group**	**Aseptic group**
Peri-implant purulence and sinus tract	n = 61	n = 61
Without	51(83.61%)	61
With	10(16.39%)	0
Histopathological tissue samples*	n = 43	n = 40
Type I (abrasion induced)	9(20.93%)	9(22.50%)
Type II (infectious type)	13(30.23%)	0(%)
Type III (mixed type, abrasion-induced and infectious type)	8(18.60%)	0(%)
Type IV (indeterminate type, not abrasion-induced & not infectious)	13(30.23%)	31(77.50%)

Microbiological tissue samples	n = 51	n = 52
Without	16(31.37%)	49(94.23%)
With	35(68.63%)	3(5.77%)
Sonication of explanted foreign material	n = 53	n = 61
Without	4(7.55%)	58[#] (95.08%)
With	49(92.45%)	3(4.92%)

[*]Histopathological staining and scoring of the periprosthetic membrane of explanted implants by Morawietz and Krenn [48].

[#]Two cases with Ralstonia pickettii and Micrococcus luteus detections were considered as contaminations.

An overview of the detected microorganisms is shown in Table 11 and Figure 9. Among the infected microorganisms, Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus and Pseudomonas aeruginosa accounted for the highest proportion (86.89%), with 40.98%, 31.15%, 9.84% and 4.92%, respectively.

Table 11. Microorganisms overview of the septic group.

Microorganism	**Positive cultures (n)**	**Percentage (%)**
Propionibacterium acnes	25	40.98
Staphylococcus epidermidis	19	31.15
Staphylococcus aureus	6	9.84
Pseudomonas aeruginosa	3	4.92
Enterobacter cloacae complex	2	3.28
Streptococcus parasanguinis	2	3.28
Streptococcus pyogenes	2	3.28

Candida albicans	2	3.28
Escherichia coli	2	3.28
Staphylococcus saccharolyticus	2	3.28
Staphylococcus hominis	2	3.28
Corynebacterium tuberculostearicum	1	1.64
Propionibacterium granulosum	1	1.64
Staphylococcus warneri	1	1.64
Staphylococcus capitis	1	1.64
Proteus mirabilis	1	1.64
Kocuria rhizophila	1	1.64
Pseudomonas oryzihabitans	1	1.64
Streptococcus mitis/oralis	1	1.64
Streptococcus cristatus	1	1.64
Streptococcus sanguinis	1	1.64
Rothia aeria	1	1.64
Neisseria	1	1.64
Actinomyces viscosus	1	1.64
Eikenella corrodens	1	1.64
Enterococcus faecium	1	1.64
Corynebacterium simulans	1	1.64
Bacillus species	1	1.64
Micrococcus luteus*	1	1.64
Finegoldia magna	1	1.64
Candida krusei	1	1.64

Enterococcus faecalis	1	1.64
Propionibacterium avidum	1	1.64

*considered as contaminations (false positive result)

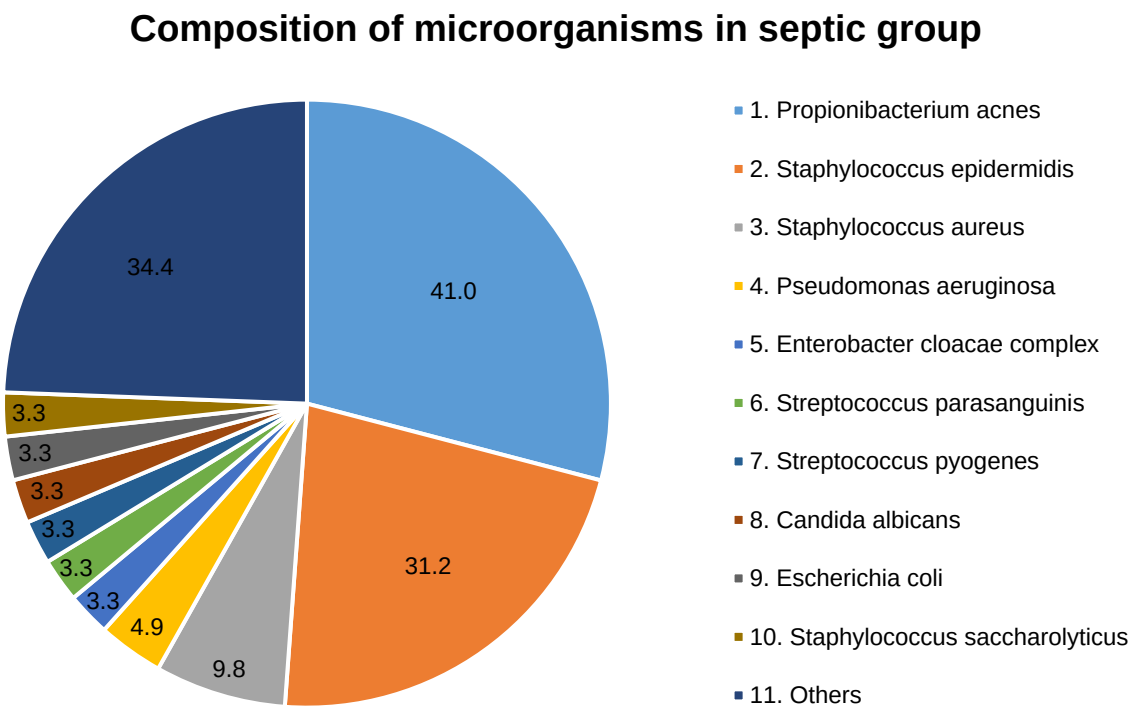

Figure 9. Composition of microorganisms in septic group.

The sensitivity, specificity, predictive positive value and predictive negative value and accuracy of histological, microbiological and sonication parameters in the diagnosis of PSII were investigated and are presented in Table 12 and Figure 10. Amongst three parameters, sonication yielded the most satisfactory results with 92.45% sensitivity, 95.08% specificity, 94.23% predictive positive value, 93.55% predictive negative value and 93.86% accuracy in predicting PSII. As shown in Table 13, there were twelve patients (twelve of fifty-one, 23.5%) examined as negative in the tissue culture test but who had a positive detection in sonication. An abnormal serum leucocyte count. Only two in twelve had an elevated body temperature. All presented negative results for blood culture and suspected skin conditions.

Table 12. Sensitivity, specificity, predictive positive value and predictive negative value and accuracy of histological, microbiological and sonication parameters of each sample.

Variables	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy
Histology*	48.84	100	100	64.52	73.49
Microbiology	68.63	94.23	92.11	75.38	81.55
Sonication	92.45	95.08	94.23	93.55	93.86

* Defining Morawietz and Krenn type II and III as peri-implant tissue infection

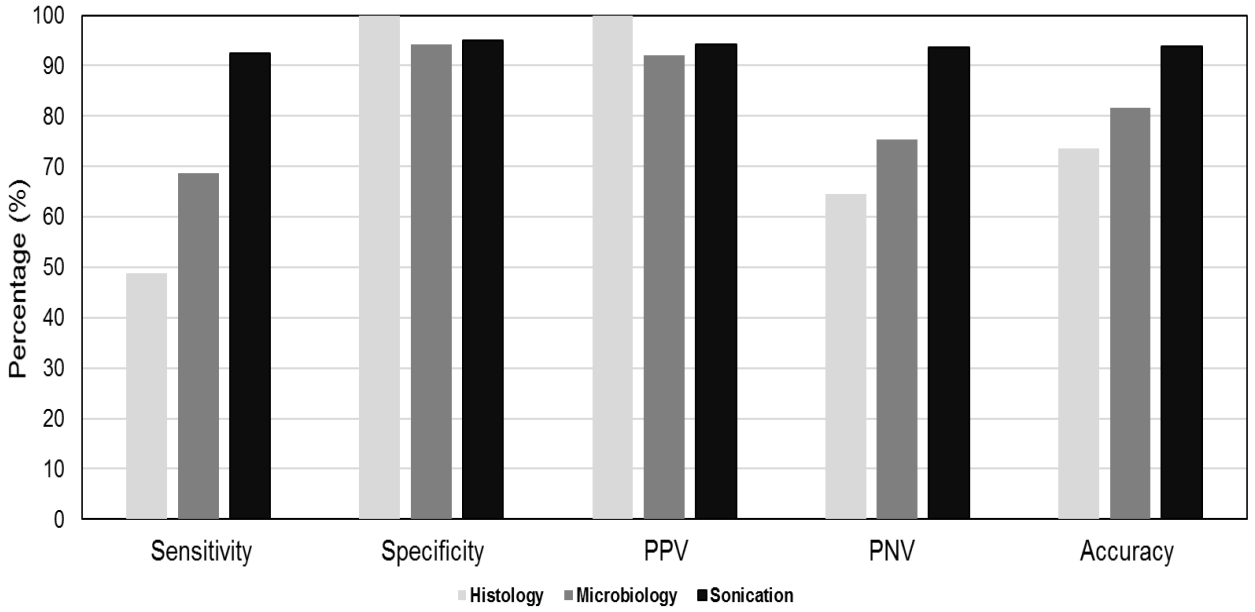

Figure 10. Comparison of diagnostic accuracy of intraoperative examination parameters. PPV = positive predicative value, NPV = negative predicative value.

Table 13. Information on ‘’sonication-positive’’ patients with negative detection in tissue culture analysis.

Cases (no.)	**Leucocyte count (/nl)**	**CRP (mg/l)**	**Body Temperature (°C)**	**Blood culture**	**Local skin finding**	**Radiology finding**	
						Xray & CT	*MRI*
1*	9.22	18.9	36.4	Negative	Negative	Negative	Negative
2	5.06	4	36.2	Negative	Negative	Implant loosening	Negative
3	5.64	2.3	37.6	Negative	Negative	Negative	Negative
4	6.66	0.6	36.5	Negative	Negative	Implant loosening	Negative
5#	6.16	5	36.6	Negative	Negative	Negative	Negative
6*	7.57	0.5	36.6	Negative	Negative	Negative	Negative
7#	13.29	96	37.2	Negative	Negative	Implant loosening	Negative
8*#	6.81	1.3	36.7	Negative	Negative	Implant dislocation	Negative
9#	5.61	0.4	36.6	Negative	Negative	Implant loosening	Negative
10*	4.82	1.8	36.4	Negative	Negative	Negative	Negative
11	7.02	16	36	Negative	Negative	Implant loosening +dislocation	Negative
12*	7.37	0.5	36.2	Negative	Negative	Implant loosening	Negative

* Propionibacterium acnes was detected in cases No. 1, 6,8,10 and 12;

Staphylococcus epidermidis was detected in cases No. 5, 7, 8 and 9.

4. Discussion

Over the past few decades, surgical complications following spinal fusion operations have aroused significant concerns. Successful operations are of paramount importance, yet the failure to manage the subsequent complications for PSII patients might impair their best outcomes as well as recovery, leading to high morbidity and mortality. Among the various complications, SSI has caused a heavy economic burden to patients as well as the hospital system by increasing inpatient stays and reoperation rates as well as decreasing productivity. In addition, applying instrumentations such as different kinds of plates, screws, rods and cages, etc. in the spinal operation further increases the infection incidence up to 20% [6, 7, 15, 19, 30, 37, 52]. In joint arthroplasty operations, for example PJI, the standardized diagnosis criteria and management have already been proposed and revised for decades. The pathophysiology of PJI, such as the issue of microorganism biofilm formation, has aroused substantial attention. Biofilm, as a solid complex attaching on the implant surface which requires strenuous eradication, plays an important role and poses serious challenges in the pathogenetic basic research as well as clinical management of PJI. However, PSII, which acts as an implant-associated issue as well, has not yet driven enough attention worldwide.

As mentioned, PSII is a serious complication following spinal arthroplasty, fusion or stabilization, which is also considered to be associated with high morbidity, and demands interdisciplinary therapeutic strategies [18], [20], [54]. The diagnosis of PSII is believed to be challenging, yet there is a lack of diagnostic criteria in the PSII workup, whereas multiple diagnostic algorithms and checklists have been proposed in the diagnosis of PJI [19, 58]. Therefore, we believe that the exploring of building a practical diagnostic checklist with all the possible parameters for the diagnosis of PSII is essential and emergent. Based on the expertise of the Center for Musculoskeletal Surgery, we adapted the existing PJI diagnostic checklists, and managed to design a retrospective study for investigating PSII patients with an age, gender and surgical indication matched control cohort, aiming to study all relevant risk factors for the development of PSII in patients undergoing revision surgery. Furthermore, all the existing risk factors were organized to establish a pre-operative checklist. This checklist should be published and therefore available for all spine surgeons in order to assess the risks for an implant infection preoperatively. With this tool in hand, spine surgeon could modify their diagnostic approach to rule out any infection according to the proposed parameters.

4.1. Summary results of diagnostic parameters

4.1.1. Anamnesis and risk factors

In total, 122 patients were enrolled in this study, of which 61 had PSII and 61 aseptic cases were assigned as the control cohort. They were matched by age, gender and surgical indications as mentioned above. There were no significant differences when comparing the mean age in the two cohorts (P = 0.949). Both groups were composed of 26 females (42.62%) and 35 males (57.38%), and the P value was 0.999. Obesity rates (BMI ≥ 30 kg/m^2) were 24.59% versus 31.15% for the septic and aseptic groups, and no significant difference was observed (P = 0.355). There were also no significant differences regarding ASA grade, although a study reported that patients with an ASA grade greater than two were at a higher risk for developing infection following total hip arthroplasty (THA) [62]. For the risk factors of diabetes mellitus and smoking, there were five and four more patients in the aseptic than the septic cohort, respectively. However, there were still no significant differences found (P = 0.359 and P = 0.581). No case with alcohol abuse was observed. There were four cases in the infected group that had received chemoradiotherapy due to metastases on the spine, while no case was presented in the control group and no significant difference was observed, P = 0.125. There was a significant difference on the operated region between the two groups, wherein more cases had undergone operations in the thoracic and lumbosacral regions in the septic group than the aseptic group. However, the possible influence of different operated regions on the increased incidence of PSII is less convincing. In addition, a meticulous medical history, with a wide range of parameters that may increase infection rates, was carefully scrutinized. From the index surgery to the revision, four specific variables were evaluated, such as operation time, operated spinal levels, radical debridement cases, complete material exchange cases and intraoperative transfusion, yet no significant differences were observed between the two cohorts. There were no significant differences found for index-to-revision duration, index-surgery indications or for the number of previous operations between the two cohorts. To date, no publication has systematically studied the risk factors and their associations to the presence of PSII yet.

Nevertheless, there has been various previous literature investigating the associations of risk factors to the PJI incidence. As shown in Table 14, we reviewed five retrospective

studies that had investigated various anamneses and risk factors to the PJI incidence, which might have been employed as a reference and comparison for the PSII investigation [63-68]. Although these retrospective studies had divergent sample sizes, they indicated respectively that surgical debridement, obesity, diabetes, smoking, BMI as well as operation time were not associated with an increased PJI rate, which is consistent with our findings. However, they were not consistent in every aspect with each other. There were several reasons that caused this discordance. First, the number of enrolled patients and follow-up periods ranged widely among these publications. Second, the interviewer and recall biases existing in the included retrospective studies were inevitable, which might have affected the associations between risk factors and PJI incidence as well. Thirdly, the measurements of various risk factors and even PJI diagnostic criteria also differed. Therefore, the presence of substantial heterogeneity was unavoidable among these studies. Overall, we observed a discordance regarding the associations of anamnestic- and demographic- factors to the PJI incidence in the literature. Future studies with large numbers of patients as well as powerful experimental evidence regarding the associations of these factors to the PSII incidence are urgently warranted.

Table 14. Comparison of literature regarding preoperative risk factors to PJI.

Literature	**Study design and sample size**	**Findings agreed with ours**
Schipper, 2015	Retrospective study with 307 diabetes cases and 2655 controls	No difference found in irrigation and debridement
Schipper, 2016	Retrospective study with 49 obese patients vs. 48 controls	No difference found in obesity
Gross, 2015	Retrospective case control study with 50 PJI cases and 55 controls	No difference found in diabetes
Lampley, 2016	Retrospective case control study with 34 current smokers, 249 former smokers and 359 controls	No difference found in smoking
Patton, 2015	Retrospective case control study with 29 PJI cases and 937 controls	No difference found in smoking, BMI, and length of surgery

One important finding regarding the preoperative antibiotic use required further attention. It has been shown that obvious differences were found between the two groups, with six more patients in the PSII group taking antibiotics before the revision surgery (P = 0.031). These 6 patients were either old with fever, or it concerned a subsequent systemic instability due to sepsis. This topic has been widely discussed, yet it yielded opposite points of view in the study of PJI. Some scientists believed that even a single dose of prophylactic antibiotic use would influence intraoperative tissue culture results [40, 69] and reported a 50-60% increased rate for culture negative PJIs even if sonication detection was applied [55]. Other researchers observed that preoperative antibiotic use did not influence the peri-implant tissue analysis. It was observed that identical preoperative pathogens collected from synovial fluid aspiration of PJI cases emerged in the intraoperative tissue culture plates [70, 71]. Nevertheless, it is essential to be alert to the possible avoidance of antibiotic prophylaxis until intraoperative peri-implant samples have been collected. However, whether - and to what extent - the preoperative antibiotic use before the revision would affect the blood culture and peri-implant tissue microorganism results in the PSII requires further investigations, and especially in spinal revision surgery, where there is no existing literature regarding the influence of pre-operative antibiotics on the sonication result.

4.1.2. Findings from clinical examinations

Notably, there were nine cases found with venous port implantation in the infected cohort versus none in the control cohort, which was a statistically significant difference. This finding indicated the possibility of a haematogenous route for obtaining PSII. Furthermore, among the 14 PSII cases with a port and pacemaker, definite blood infection was observed in six cases (42.86%). In these cases, the dislodged microorganisms were determined as Staphylococcus aureus (n = 3), Staphylococcus epidermidis (n = 2) and HIV (n = 1), of which three cases (two with Staphylococcus aureus and one with Staphylococcus epidermidis) observed the same pathogen with implant-tissue microbiological and/or sonication findings. There were also studies reporting that cardiac devices and venous port catheter implantation increased the risk of blood stream infections, and that the most common causative microorganisms were Staphylococcus species (46.15%) [72, 73]. Rakow et al. observed that the origin of hematogenous PJI was predominantly included infections of the cardiovascular system (intravascular devices or heart valves), followed by infectious skin, soft tissue and the urogenital tract,

wherein Staphylococcus aureus (41%) found was as the most noteworthy infecting microorganism [74]. Medina and his team members [75] retrospectively studied 845 patients in the previous 14 years that had PJI, and they enrolled six consecutive PJI cases with PJI secondary to venous-access device infection. Three coagulase-negative species, i.e., Staphylococcus epidermidis (66.7%), Staphylococcus capitis (16.7%) and Staphylococcus aureus (16.7%), were observed as haematogenous causative pathogen sources. Meanwhile, the six patients with port infection generated no local signs, with only joint pain, pseudarthrosis, limb swelling and prosthesis loosening. The authors recommended that for prevention, when patients had any inserted venous port, every prosthetic joint should be verified carefully. The surface of the venous port should be examined in detail by microbiological culture to determine a possible gram-positive Staphylococcus species infection. The results in the above findings also considerably endorsed ours, in that the coagulase-negative staphylococci species presented as the most prominently detected pathogen type. Therefore, spine surgeons should place intensive attention into the prevention and management of coagulase-negative staphylococci species for patients that have undergone revision implant spinal operations.

We observed that there were 15 cases (24.59%) in the infected group with local skin disorders on admission, while there was no counterpart in the aseptic group (P = 0.008). Skin problems on the focal spinal region, such as skin ulcer, edema and drainage, represented the potential portal of microbial entrance. In this study, microorganism tests of the skin tissue were not performed for specifying the exact microorganism(s). There are two reasons for this decision. First, some PSII patients had severe drainage caused by implant breakage with hardware pierced through the skin, so that the wound itself was contaminated already and emergent operations were required. Second, some normal flora such as Staphylococcus epidermidis are present and possibly widely distributed on the body surface of normal population. Therefore, it is relatively less meaningful to perform the skin pathogen test. However, conversely, for patients with Propionibacterium acnes, careful acne management and skin decolonization were highly recommended in the literature [76]. There were eight cases (13.11%) with a raised temperature in the infected cases, while no case was found in the aseptic group, and a significant difference was observed (P = 0.003). Interestingly, in seven of these eight cases a raised CRP level was observed, wherein five (72.43%) had a port implantation and four (57.14%) had

urinary tract infection. In addition, five in eight cases were detected with Staphylococcus epidermidis in either peri-implant tissue or sonication fluid culture.

The mean scores of VAS at rest for patients in the septic group were 3.35 ± 2.22 vs. 4.62 ± 2.56 for the aseptic group, and there was the same situation for VAS pain under motion with 5.41 ± 2.52 versus 6.90 ± 2.41, which shows that the pain of PSII patients may not present as an accurately detected syndrome for surgeon diagnosis. This may due to the high infection rate of low-virulent microorganisms in the septic group, where patients experienced occult symptoms compared to the aseptic cohort. This finding indicated the limited role of using pain complaints as a reliable risk factor in diagnosing PSII. One main consequence of a low-virulent microorganism infection (e.g., Escherichia coli and Staphylococcus epidermidis) would be the biofilm formation on implant surface. The biofilm complex structure that attached on inserted implants was able to evade immune tracking and evoke less body inflammatory response, which made infectious symptoms and signs occult. As a result, fever, pain or skin swelling symptoms would not show in large numbers of patients that had experienced delayed PSII, which has been reported in the literature to have increased incidences of hardware failures and to eventually lead to an increasing revision rate and worsening patient outcomes [54]. In our findings, over 70% of the detected microorganisms such as Propionibacterium acnes, Staphylococcus epidermidis, Escherichia coli, etc., in the PSII group have been observed to be able to form biofilm on the prosthesis surface [77]. Therefore, fighting against indolent microorganisms and their formed biofilm presents unique challenges for surgeons as well as microbiologists in the standardized management of PSII in the future.

Blood cultures of patients in fever or with suspected hematogenous infection were observed. In total, 6 of 122 cases (4.9%) were observed with hematogenous PSII and the most outstanding bacteria were Staphylococcus epidermidis and Staphylococcus aureus. As shown in Table 6, blood samples of twelve patients with suspected PSII preoperatively were collected. There were nine cases where positive microorganisms were detected (including one case with HIV), and six cases amongst the remaining eight bacterial infected cases (75%) were detected with the same pathogen in the subsequent peri-implant tissue or sonication fluid examinations. This finding provided valuable evidence for the possible hematogenous infection route to the occurrence of PSII. Our observation was considerably distinct for hematogenous infection in PJI cases, while a study observed that only seven cases were diagnosed with documented hematogenous PJIs in 551

remote seeding infections occurring in 6,101 hip and knee arthroplasties [78]. However, a consistency was also obvious where the Staphylococcus aureus bacteremia was noted to be associated with a 30 to 40% risk of hematogenous seeding of arthroplasties [33]. In this thesis, in two cases (22.22%) with Staphylococcus aureus and Staphylococcus epidermis hematogenous infections the same pathogens were detected in the subsequent tissue microbiology and/or in sonication fluid cultures, respectively. This finding indicated that for PSII patients with suspected preoperative hematological infection, the blood culture results and the specific microorganism species involved should be examined carefully regarding their probable spread to the spinal implants.

4.1.3. Findings from radiological examinations

In terms of X-ray and CT examinations, CT holds a relatively better positive material-failure detection rate (13 of 29 cases, 44.83%) than X-ray (17 of 43 cases, 39.53%) in the diagnosis of PSII. Nevertheless, material failures are not considered to confirm the presentation of infection according to our results, especially for X-ray, where 41.07% aseptic patients versus 28.33% septic patients presented implant failures, P = 0.031. Our findings indicate that implant failure (loosening, dislocation and breakage) in radiology may not be a reliable sign in predicting PSII. In a recent study published by Prinz et al., [54] 82 cases with sonication results that had undergone implant removal history were investigated. Two main reasons may lead to this discordance. First, in this thesis we investigated different cohorts, while in Prinz's study, 82 cases with implant removal history were enrolled, where there were only 22 infected yet 60 aseptic cases. In these 60 aseptic patients, 32 (53%) had screw loosening, which is even higher than our (38%) results. In their 22 septic cases, all (100%) had screw loosening, while in this thesis this rate is 30%. The discordance of septic subject enrollment could possibly cause the occurrence of selection bias. Second, the screw loosening in their paper was defined only by CT, while our study, most had an X-ray result, yet only 29 of 61 in the septic and 19 of 61 in the aseptic group had CT results. Therefore, this may also cause significant selection bias. Nevertheless, based on our results, implant loosening, dislocation or breakage detected by X-ray may not be applicable in predicting PSII.

MRI was evaluated as the one of the most advantageous and reliable modalities in diagnosing spondylodiscitis and intervertebral abscess with high sensitivity (96%) and high specificity (94%) [37]. In this study, MRI was observed to have low detection rates

(two in 15 cases, 13.33%), in which one case was infected with Staphylococcus aureus and the other one was infected with Staphylococcus epidermidis. These two cases both presented obvious secretions observed by the surgeon during the operation and also detected by histological examination. In the remaining 13 cases, no intraoperative secretion was identified, yet twelve cases (92.31%) were still detected with coagulase-negative Staphylococci and Propionibacterium acnes, which distinctly indicated the limited application of MRI in diagnosing PSII with indolent microorganisms. It has been reported that current medical imaging modalities lack the spatial resolution for the detection of biofilms which have aggregates as small as 10 to 100 μm on the implant surface [79]. In this thesis, low virulent microorganisms that do not generate any pus or sinus were detected in a large part of PSII patients. This finding has therefore indicated the limited merit of MRI in diagnosing PSII with low virulent microorganisms, especially when there was no obvious observed peri-implant secretion.

4.1.4. Findings from laboratory serum biomarkers

The sensitivity and specificity of the preoperative serum leucocyte count were 22.95% and 85.24%, respectively. For preoperative serum CRP, there was a significant difference between the two cohorts, with the P value equal to 0.003. However, the positive detection rate was only 50.82% with 78.68% specificity.

Synthesized by hepatocytes, CRP has been investigated as an acute-phase protein that presents within six hours of the onset inflammation. Abnormal CRP expression has been associated with many other diseases such as trauma, hepatitis, urinary tract infection, malignancy, as well as various inflammatory diseases. Another biomarker, the serum leucocyte count has also been elevated and rated as a hallmark for diagnosing various disorders with inflammation or infection. However, the current literature is not consistent regarding the determining of preoperative serum CRP and the leucocyte count in diagnosing orthopedic implant-related infections. A wide range of studies have been carried out on diagnostic power in the diagnosing of PJI - a devastating, intractable and costly complication following total joint arthroplasty. Xu et al. [80] observed that patients with preoperative raised serum ESR and CRP tend to have a significantly higher incidence of PJI compared to those with a normal serum inflammatory marker level (HR = 15.8, 95% CI: 2.57-96.7%, P = 0.003) even after confounding factors were adjusted. Bingham and his team colleagues [81] retrospectively investigated 81 PJI patients and

81 aseptic patients who experienced hip or knee joint arthroplasty. It was observed that the ESR setting at 10mm/h yielded a ≥ 95% (95% CI: 85.2-97.6%) sensitivity and a 68.6% (95% CI: 59.3-76.7%) specificity versus a sensitivity of ≥ 95% (95% CI: 87.1-98.4%) plus a specificity of 63.3% (95% CI: 54.2-71.5%) when the CRP cutoff was set at 5mg/l. An additional result also showed a surprising 100% sensitivity (95% CI: 94.1-100%) when the ESR and CRP tests were combined. Unfortunately, no ESR examination was carried out in our hospital, so that the value of ESR in the diagnosis of PSII is under further investigation. Most recently, there was another study conducted by Sigmund et al. [82] with findings that were highly consistent with ours. In this article, 75 of 177 patients who experienced joint arthroplasty were enrolled and defined as having PJI, demonstrating a very low sensitivity (36%) and a negative specificity (89%) of the leucocyte count in the diagnosis of PJI. They also observed that the sensitivities of CRP, the ratio of neutrophils to lymphocytes, and the percentage of neutrophils, fibrinogen and platelet count to mean platelet volume ratio were 68%, 66%, 63%, 69% and 43%, respectively, while the diagnostic specificities were 87%, 67%, 73%, 89% and 81%, respectively.

Meanwhile, studies on the effectiveness of serum leucocyte count and CRP examinations in diagnosing PSII have also been reported in the literature. Akgün et al. [42] enrolled 257 patients, within which 61 patients (24%) were confirmed with PSII. They reported that there was a significant difference of serum CRP levels between septic and aseptic cohorts, with a mean comparison value at 19.3 mg/l versus 4.8 mg/l, $P < 0.001$. However, according to the receiver operating characteristic curve (ROC) result, the serum CRP with a threshold of 4.05 mg/l yielded a 64% sensitivity and 68% specificity in diagnosing PSII. Chahoud and colleagues [17] also observed that less than 50% of spinal SSI cases experienced an elevated leucocyte count, while the CRP level was found to have only 31% PPV in the diagnosis of spinal SSI. Additionally, they also recommended that the comparison of pre-operation and post-operation CRP levels should be carried out when SSIs following a spine surgery were suspected. As shown in the above results, some PSII patients experienced exaggerated serum CRP, for example more than 300 or even 400 mg/l in the septic group, which was also responsible for the significant differences observed between the two cohorts. However, there were notably 30 cases (49.2%) in the septic group that presented with a normal serum CRP and 13 cases (21.3%) in the aseptic group who experienced an elevated serum CRP preoperatively. In the light of relatively

low sensitivity and specificity of serum CRP in diagnosing PSII, the recommendation of routine use for this tool remains cautious.

Therefore, based on the demonstration above, due to the poor performances of the serum biomarkers CRP and leucocyte count, it was considered that these two tools would only be recommended as supplementary criteria in the diagnosis of PSII, while other promising tests and biomarkers should be highly expected in the PSII workups.

4.1.5. The intraoperative analysis

In this thesis, histology analysis of intraoperative peri-implant tissues yielded a 48.84% sensitivity, 100% specificity, 100% positive predictive value, 64.52% negative predictive value, and 73.49% accuracy, respectively. Histopathology was considered as a gold standard in the diagnosis of SSI, yet its low sensitivity has long been discussed. Zagra et al. [83] investigated intraoperative samples from 119 patients who underwent revision THA and stated a sensitivity of 78.3% and a specificity of 96.9% for histology in the diagnosis of PJI. There are, however, studies that were harmonious with ours. Boettner's team [84] retrospectively studied 77 patients with revision THA, in which 21 patients were grouped as septic based on intraoperative tissue culture. Then, an experienced pathologist was invited and successfully identified twelve of 21 tissue samples, while wrongly classifying two out of 56 tissue samples, and eventually yielding a low sensitivity of 57% plus a high specificity of 96% in the diagnosis of PJI. Interestingly, they also changed the 'histology cut-off' where four more different histological diagnostic criteria were compared. The Banit cut off, whereby an infection can be diagnosed if one out of five most cellular high-power fields showed ≥ ten polymorphonuclear leucocytes, was identified. The diagnostic sensitivity increased to 90%, with a satisfactory 84% specificity, by using this criterion. In previous work by our team, Bürger et al. [85] enrolled 47 patients with PSII and observed only 24 with a positive histological result, which yielded a low 51.1% sensitivity. This finding is quite similar with the result (48.84%) in this thesis. Furthermore, the sensitivity even dropped to 36.4% in diagnosing PSII with low-virulent microorganisms. Three main reasons regarding this finding were proposed. First and foremost, the limited amount of the tissue obtained surrounding the spinal implants should take major responsibility. Taking screws as an example, very limited samples were allowed to be collected from pedicle screw cavities when compared to periprosthetic tissue samples in joint arthroplasty, where samples were mainly from the medullary cavity.

Inspired by this perspective, we highly recommend surgeons to expand the sample collection amount and scope around the suspected implants during the revision operation. The second would be the formation of biofilm which was able to escape the immune system and cause imperceptible inflammatory response. The third may result from the unstandardized tissue sampling methods taken by different personnel during the spinal operations.

In this thesis, microbiological analysis of intraoperative peri-implant tissues yielded a 68.63% sensitivity, 94.23% specificity, 92.11% positive predictive value, 75.38% negative predictive value, and 81.55% accuracy, respectively. The sensitivity of this method was relatively low in diagnosing PSII. In our view, the tissue sample size (only three peri-implant samples were taken) and the biofilm which firmly attached on the implant surface are the major reasons. In the current research, results regarding tissue culture in the diagnosis of PSII and PJI were discordant. In a prospective study [86], 117 patients who had undergone THA (63.2% hip and 36.8% knee) were included and analyzed using tissue culture for identifying PJI. It was observed that when compared to swab culture, tissue culture presented an obviously superior sensitivity (93% vs. 70%) and specificity (98% vs. 89%). However, one recent study was conducted at the Mayo Clinic, where 229 cases who had undergone revision THA were investigated. They observed a low sensitivity of tissue culture in the diagnosis of PJI (66.4%) when using the IDSA criteria, which is very comparable to our findings (68.6%). Recently, improved microbiological tissue culture methods have been introduced and have presented a significantly improved diagnostic power. Conventionally, solid culture media or the enrichment broth method were often used as the routine peri-implant tissue culture, yet with the concept of accepting Propionibacterium acnes and coagulase negative staphylococci (mainly Staphylococcus epidermidis) as causative agents instead of contaminants, not only should the incubation period be prolonged but also the microbiological culture method should be ameliorated. Hughes et al. [87] compared four microbiological culturing methods prospectively with direct plates, cooked meat broth, fastidious anaerobic and BACTEC blood culture bottles on a total of 178 peri-prosthetic tissue samples. BACTEC blood culture bottles were observed to have the highest sensitivity 87% (95% CI, 72-100) and specificity 98% (95% CI, 96-100), and were considered indicative in the diagnosis probably given that this works as a totally automated closed system with a continuous monitoring function. Most recently, Birdsall and colleagues [88] investigated 121

prosthetic joint specimens that were processed using three methods of microbiological culture, i.e, solid media, enrichment broth and BacT/ALERT paediatric bottles, and they observed that the paediatric bottle method was more effective (93.33% sensitivity and 87.8% specificity) than the solid media (60.00% sensitivity and 90.24% specificity) when applied independently. However, they highly recommended the combination use of BacT/ALERT paediatric blood culture bottle with solid media, which yielded a 100% sensitivity in the diagnosis of PJI.

The sonication fluid culture technique was observed as the most powerful diagnostic in this thesis. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 92.45%, 95.08%, 94.23%, 93.55%, and 93.86%, respectively. Notably, there were twelve patients (twelve of fifty-one, 23.5%) examined as negative in the tissue culture test but who had a sonication positive detection, as shown in Table 13. Among these twelve patients, ten had a single microorganism infection, two were infected with mixed species (cases number two and four in Table 13). None were observed with an abnormal serum leucocyte count. Only two in twelve had an elevated body temperature. All presented negative results for blood culture and suspected skin conditions. These basic tests and physical conditions showed the occult property of PSII patients with negative tissue culture. Furthermore, Propionibacterium acnes and Staphylococcus epidermidis were detected in five (41.7%) and four subjects (33.3%), respectively, which indicated that over 75% of sonicated pathogens were indolent species. Implant failures were presented in seven of twelve cases (58.3%), which is higher than the general septic group (17 of 60, 23.3%). It indicated the outstanding diagnostic value and complementary role of using sonication in diagnosing PSII with tissue-culture negative detection. To the best of our knowledge, there are no existing publications regarding the microorganism species analysis of tissue-culture negative yet sonication positive examinations in PSII. Nevertheless, there have been a large number of studies that were in concordant with our findings. Bürger et al. [18] performed a prospective cohort study where 35 PSII and 83 aseptic patients were analyzed and observed that microbiological tissue culture and sonication yielded sensitivities of 65.7% (95% CI, 48.6–80.0) versus 94.3% (95% CI, 85.7–100) and specificities of 96.4% (95% CI, 91.6–100) versus 98.8% (95% CI, 96.4–100), respectively. Bellova et al. [89] examined 145 PJI and 112 aseptic cases and observed sensitivities of 87.5%, 84.4% ($P = 0.63$) and specificities of 85.1%, 92.5% for peri-implant tissue culture versus sonication. Trampuz et al. [55] analyzed 404 patients

treated with a revision total hip or knee arthroplasty (61% knee and 39% hip) during two years at the Mayo Clinic and observed the most detected pathogen as coagulase-negative staphylococcus when the cutoff value setting was ≥ 5 CFU. The sensitivity and specificity were 78.5 (95% CI, 67.8–86.9) and 98.8 (95% CI, 96.6–99.8), respectively. Yan et al. [86] demonstrated a sensitivity of 88.7% using a Bayesian-latent-class-modeling modified IDSA criteria in diagnosing PJI. Notably, when they combined this with intraoperative tissue culture, the diagnostic sensitivity improved to 99.1%. They also underlined the importance of using sonicated fluid through centrifugation instead of gathering only implant membrane filtration. The special extracellular matrix (ECM) developed from attached bacteria cells can explain this issue. In many circumstances, this sophisticated compound that is made up of a mix of proteins, nucleic acids and exopolysaccharides was able to become fossilized and robust enough to bond tightly on the implant surface [90]. The above investigations presented a negative diagnostic power of sonication in diagnosing PJI, which was able to provide a valuable reference for PSII. Based on the above results, we recommended that sonication should be considered as a routine diagnostic method applying for spine surgeons in the PSII workups.

Based on our findings above as well as the adaptions from the PJI diagnostic guidelines referred by the CDC [57], ICMC [58] and the IDSA criteria [59] (see Table 2), we have proposed a sketched checklist containing important diagnostic parameters to help surgeons diagnose PSII, as shown in Figure 11. In this checklist, all the parameters that were analyzed in this thesis are presented, and those with a significant finding are marked with warning notations.

4.2. Limitations

There are several limitations in this study that need to be stated.

First, the sample size of this study is small, since only patients with submitted sonication and tissue samples were examined, which can lead to non-response errors. Therefore, further studies with larger number of matched cases would be required for validating results of this study. Nevertheless, the presented doctoral thesis presents the largest matched cohort in the field of PSII.

Second, because this study is a retrospective study, not all of the complete data in every parameter were able to be collected. Furthermore, information for patients who had surgeries at outer hospitals could not be followed up and obtained, therefore, selection bias has existed as a source of concern.

Third, not all of the patients with a suspected infection had blood samples taken for microorganism specification. Even though a portion of the patients (66.7%) with PSII had the same microorganism identified in both the blood sample and peri-implant tissue microbial analysis, whether a haematogenous approach can be ascertained as a determinate infection route requires further explorations. The same goes for the urinary test, where no information regarding the urinary microorganisms in patients with PSII were collected. Therefore, this is also an important research issue that our future studies should focus on.

Fourth, six PSII patients took antibiotics before the revision surgery in this study. The major concern regarding this issue is the potential effect of antibiotics in increasing the false positive rate of peri-implant tissue examinations. For enrolling PSII patients in future studies, the influence of preoperative antibiotic use should be highlighted.

Fifth, this study cannot give statements about the risk factors of age, gender and surgical indications because in the matching procedure, patients were matched exactly by these three parameters. Therefore, the possible influences of aging, gender distinction and surgical indications to the increased incidence of PSII has not yet been investigated.

4.3. Clinical relevance

A large proportion of PSII patients cannot be identified by the usual clinical and laboratory parameters, especially those with low-virulent microorganisms and where the collection of intraoperative peri-implant tissue samples should be performed in each case of revision spine surgery. We suggest the routine use of sonication of explanted materials due to its high sensitivity and specificity in diagnosing PSII; however, preoperative blood leucocyte count, CRP and MRI all have limited value in detecting PSII infection. For patients with port catheter implantation, urinary tract infection and a raised body temperature, the carrying of possible Staphylococcus species, especially Staphylococcus aureus and Staphylococcus epidermidis among these patients should be highlighted. Therefore,

preoperative focal screening and decolonization of the pathogens in PSII patients should be conducted in order to reduce infection rates.

Preventive strategies for PSII are crucial, as over 80% of the infected pathogens are indolent microbiomes. Hence, applying sensitive and biofilm-active antibiotics such as rifampicin with evidence-based treatment strategies against Staphylococci or Propionibacterium acne in patients with highly suspected skin, blood, port catheter as well as urinary tract infections should be highly recommended. For instance, any focal skin disorders on the spine such as edema, eczema and ulcers should be carefully examined and well managed before the revision surgery begins. Prophylactic antibiotic strategies such as vancomycin IV administration, wound powdered vancomycin dispersing prior to closure and antibiotic coating of spinal implants have shown negative antimicrobial effectiveness and are recommended for PSII incidences [91-93].

4.4. Outlook

Currently, there are many promising testing markers or tools that have been proven to have reliable effectiveness and satisfactory diagnostic accuracy in the diagnosis of PJI.

First, the alpha-defensin immunoassay test has been widely introduced. In the human body, alpha-defensin, mainly produced in neutrophils and Paneth cells, acts as a kind of microbicidal peptide that has an active broad-spectrum anti-bacterial and -fungal effect. Researchers collected synovial fluid from PJI patients and observed a significant elevated alpha-defensin expression. They also demonstrated the outstanding performance of this biomarker, with up to 97% in both diagnostic sensitivity and specificity [94]. A recent meta-analysis synthesized the diagnostic effect of alpha-defensin for diagnosing PJI. This work reviewed six studies with nearly 2000 enrolled patients and yielded a surprising pooled 96% (95% CI, 0.89-0.99%) specificity and a pooled sensitivity as high as 100% (95% CI, 0.82-1.00%). However, one obvious drawback for applying this test would be the expensive price which is over 4000 times greater than the traditional leucocyte esterase test [95]. Furthermore, follow-up evaluation with a large enrolled patient number is required to estimate its long-term performance. Nevertheless, whenever possible, the application of this promising biomarker in the diagnosis of PSII is highly anticipated due to its reliable effectiveness.

Second, the serum biomarker D-dimer, which detects fibrinolytic activities, has been recently demonstrated to have satisfactory sensitivity and specificity in the diagnosis of PJI. The elevated D-dimer is believed to be involved in mediating infection in the joint and its generation, just like other cytokines (e.g., tumor necrosis factors and interleukins), was observed as a byproduct of fibrinolytic activity [96]. It has been demonstrated that the inflamed tissue can secrete abundant fibrin and its degradation may lead to an increased D-dimer concentration subsequently in the serum [97]. In this study, Shahi and colleagues prospectively enrolled 245 patients who had undergone primary and revision arthroplasty. They observed that when setting the serum D-dimer value at 850 ng/ml as the optimal threshold value for diagnosing PJI, the average serum D-dimer level in the PJI cohort (1100 ng/ml, ranging from 243 to 8487 ng/ml) was 3.5-fold greater than the aseptic cohort (299 ng/ml, ranging from 106 to 2571 ng/ml). In addition, when compared to serum ESR and CRP, D-dimer has both better sensitivity (89%, with 95% CI from 77% to 95%) and specificity (93%, with 95% CI from 86% to 96%). Pannu et al. [98] observed that when the new evidence-based 2018 criteria were used [99] for PJI diagnosis, with a scoring system where minor criteria that is ≥ 6 defines infection, the overall sensitivity and specificity of serum D-dimer reached as high as 97.7% and 99.5%, respectively. Based on the above investigations, the accuracy of using serum D-dimer for the diagnosis of PJI has been observed. However, to the best of our knowledge, there are no data available regarding applying this useful tool in PSII diagnosis and, therefore, further research is warranted.

Third, various molecular diagnostics have been introduced recently. One method that targets the 16S rRNA of bacteria by polymerase chain reaction (PCR) has been reported to have an increased diagnostic rate for PJI. Larsen et al. prospectively investigated 111 patients that had undergone revision arthroplasty and observed an 83% sensitivity of the 16S rRNA sequencing method compared to a 65% sensitivity of conventional tissue [100]. Another study analyzed 41 patients that had undergone revision THA (71% hip vs. 29% knee) for suspected PJI and compared the diagnostic power of the amplification-based DNA technique with conventional intraoperative culture for diagnosing PJI [101]. A total of 64.7% of the septic patients were detected using the tissue culture, which is harmonious with our data (68.6%). However, the sensitivity increased to 91.2% using the DNA-based assay test and had the simultaneous benefit of less interference with existing sample collection procedures. Notably, the DNA sequencing method successfully

identified seven of nine negative-conventional culture patients who had preoperative antibiotic usage. They demonstrated that the molecular method substantially increased the pathogen detection rate in PJI, especially in the diagnosis of low-growing microorganisms as a result of recent or concomitant antibiotic therapeutics. Therefore, the future application of this technique in the PSII diagnostic workup is highly anticipated.

In general, several promising markers or tools have been identified above to be used in the prevention, diagnosis as well as treatment of PJI. All the above-mentioned techniques seem promising, yet further studies with a large number of subjects and powerful evidence levels are to be encouraged to clarify their potential in the diagnosis and management of PSII. In particular, for patients with low-virulent microorganisms, instant testing tools with a powerful and reliable diagnostic effect (e.g., applying preoperative serum alpha-defensin or D-dimer tests) is to be expected as a promising addition. In this thesis, even though the treatment of PSII was not the main topic, some highlights should be mentioned:

First, it has been recommended that spinal implant retention with radical debridement plus three months of sensitive antibiotic treatment is used for patients with early-onset PSII. Complete exchange of spinal implants, radical surgical debridement and three months of sensitive antibiotic treatment have been reported to have negative outcomes for patients who experienced the late-onset PSII [50, 102, 103].

Second, strategies of antibiotic treatment for PSII have also been proposed in the literature. For cases with removed spinal implants, a strategy of “two weeks of intravenous antibiotics followed by four weeks of oral intake” was proposed [91]. For patients with retained implants, prolonged oral antibiotic use with biofilm activity for at least ten weeks after the two-week intravenous therapy was suggested. In order to obtain successful pathogen eradication, appropriate broad-spectrum antibiotic agents were suggested and timely antibiotic adjustments according to the exact type of microorganism were also crucial [104, 105]. However, the resulting negative side effects of using antibiotics such as toxicity, fungal infection, microbial resistance as well as renal function deficiencies should be cautioned against.

4.5. Conclusions

In this study, we designed an age and gender matched retrospective study and identified 61 PSII versus 61 control patients. Detailed preoperative parameters were investigated, including anamnesis and risk factors as well as clinical, radiological and laboratory examinations. Additionally, intraoperative histopathological, microbiological parameters of peri-implant tissues and sonication results of implants were analyzed.

Based on the findings obtained, it can be observed that individual risk factors for the occurrence of peri-implant infections already exist before the revision surgery. In summary, radiological findings regarding implant failures and increased pain are less common in patients with PSII. In the present study, suspicious skin findings, increased body temperature, the presence of a port catheter, hematogenous infections and urinary tract infections were investigated as obvious symptoms of a possible peri-implant spinal infection. If these risk factors are present, sonication should therefore be chosen to exclude a peri-implant infection due to it having the highest sensitivity and specificity. Based on the present results, a preoperative checklist was sketched out (Figure 11) for patients before spinal revision interventions.

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Patient label

Coordinated analysis of spinal implant-related infection

Preoperative checks

1. Antibiotics use

Antibiotics	☐No	☐Yes:	Name	Dosage	Duration

2. Clinical examinations

Skin findings	☐Normal	☐Ulcer ☐Erythema ☐Edema ☐Drainage△;	☐Others:	
Ports	☐Yes△;	☐No		
Pacemakers	☐Yes	☐No		
Other implants/prosthesis	☐Yes	☐No		
Fever	☐Yes	☐No		
Blood culture△;	☐Staphylococcus species	☐Enterococcus species	☐Others:	
Other infection routes	☐Urinary tract infection△;	☐ Chest infection	☐Dental infection	☐Others

3. Radiological examinations

X-ray	☐Normal	☐Implants loosening	☐Implants dislocation	☐Implants breaking
CT	☐Normal	☐Implants loosening	☐Implants dislocation	☐Implants breaking
MRI	☐Normal	☐Superficial infection	☐Deep tissue infection	☐Peri-implant infection△;

4. Laboratory examinations

CRP	☐< 5.0mg/l	☐≥5.0mg/l△;	
Leucocyte count	☐3.90-10.50/nl	☐<3.90/nl	☐>10.50/nl

Intraoperative checks

1. Senor surgeon's observation ☐Normal ☐ Peri-implant secretion, drainage or sinus tract△;

2. Peri-implant tissue for microbiology and histology examination

1) 3 microbiological samples

No.	Region specification	Description of samples	Microorganisms△;
1	☐Cervical ☐Thoracic ☐Lumbar ☐Sacral		
2	☐Cervical ☐Thoracic ☐Lumbar ☐Sacral		
3	☐Cervical ☐Thoracic ☐Lumbar ☐Sacral		

2) 1 Histological sample

Region specification	☐Cervical	☐Thoracic	☐ Lumbar	☐ Sacral
Description of samples				
Morawietz and Krenn's classification	☐Type I (abrasion induced)			
	☐Type II (infectious type) △;			
	☐Type III (mixed type, abrasion-induced and infectious type) △;			
	☐Type IV (indeterminate type, not abrasion-induced & not infectious)			

3. Sonication for explanted foreign bodies△;

3 samples

No.	Region specification	Description of samples	Microorganisms△;
1			
2			
3			

Figure 11. A sketched checklist of coordinated analysis for the spinal implant-related infection diagnosis.

6. Statutory declaration

"I, Zhouyang Hu, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic Establishment of a checklist for the identification of patients with postoperative spinal implant infections and in German Einrichtung einer Checkliste für die Identifizierung von Patienten mit postoperativen spinaler Implantat Infektionen independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

7. Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection

8. Acknowledgements

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9. Confirmation by a statistician

The method and scope of the statistical processing is sufficient for this thesis and confirmed by a statistician accredited by the Doctoral Committee of Charité.