Aus dem Centrum für Muskuloskeletale Chirurgie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Evaluation of infections after internal fixation of long bones – clinical characteristics and outcome analysis from a retrospective study

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von

Pia Carolin Vössing

aus Bornheim

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Abbreviations

ASA	American Society of Anesthesiologists
BMI	Body Mass Index
BMP- II	Bone Morphogenetic Protein 2
CDC	Center for Disease Control
CFU	Colony Forming Unit
CRIF	Closed Reduction Internal Fixation
CRF	Case Report Form
CRP	C-reactive protein
CT	Computed Tomography
DTT	Difficult To Treat
ESR	Erythrocyte Sedimentation Rate
FDG	Fluorodeoxyglucose
GHB	γ-Hydroxybutyric Acid
HTO	High Tibial Osteotomy
ID	Infection Disease
IEIF	Internal Extramedullary Fixation
IIIF	Internal Intramedullary Fixation
LCP	Locking Compression Plate
LFN	Lateral Femoral Nail
MRI	Magnetic Resonance Imaging
NPWT	Negative-Pressure Wound Therapy
ORIF	Open Reduction and Internal Fixation
PCR	Polymerase Chain Reaction

PET-CT	Positron Emission Tomography–Computed Tomography
PFN	Proximal Femoral Nail
PMMA	Polymethylmethacrylate
RIA	Reamer-Irrigator-Aspirator
Tc-99m MDP	Technetium-99m labeled Methylene Disphosphonate
UFN	Unreamed Femoral Nail
VAC	Vacuum-Assisted-Closure
WBC	White Blood Cell

Abstract

Background: Infection of long bones after surgery with internal fixation is a challenging complication and causes significant morbidity. However, there is still limited data available on clinical characteristics, valid treatment options and long-term outcome. The aim of this study is to analyze risk factors for internal fixation device-associated infections and to evaluate the newly implemented treatment concept at our center.

Methods: We performed a retrospective study of patients treated for an internal fixation device-associated infection from January 2010 to October 2017 in a tertiary healthcare center. All data was collected with a case report form (CRF). We compared the characteristics of infection after internal extra- (IEIF) and intramedullary fixation (IIIF). In 04/2013, a dedicated interdisciplinary team was established, and a standardized surgical and antimicrobial treatment concept was implemented. Outcome before and after establishment of the new treatment strategy was evaluated with chi-square test and Kaplan-Meier survival method was employed for outcome analysis.

Results: We reviewed 127 patients (89 males) with a median age of 53 years. In the two groups, IEIF and IIIF patient' characteristics were similar. Comparing the infection side, open fractures were significantly more common in the IIIF than in the IEIF-group (24 vs. 16 patients; $p<0.001$). In the IEIF group, retention of the implant ($p=0.026$) and inadequate antibiotic treatment ($p=0.023$) were significant risk factors for a failure. Relapsing or persistent infection was observed in 33 (30%) patients. In the patient cohort with the standardized treatment concept, significantly less patients showed a persisting or relapsing infection compared to the group with the non-standardized treatment (n=16 (22%) vs. 17 (46%); $p=0.015$). Among the 78 infection-free patients, 24 reported impaired functional outcomes (nonunion, Girdlestone situation or amputation of the limb).

Conclusion: Several factors for internal fixation device-associated infections have been identified, while the outcome was considerably better after implementation of a standardized treatment algorithm. A few risk factors for internal fixation device-associated infections were found. However, long-term treatment outcome of infections after internal fixation of the long bones is still improvable and further advancement of treatment concepts is needed.

Abstract

Hintergrund: Infektionen der langen Röhrenknochen nach Implantation von interner Fixation sind eine schwerwiegende Komplikation, welche zu einer Steigerung der Mortalität der betroffenen Patienten führen. Bisher mangelt es an Daten bezüglich des klinischen Krankheitsbildes, suffizienter Therapiekonzepte und der langfristigen Ergebnisse bei Infektionen assoziiert mit interner Fixation.

Methoden: Wir führten eine retrospektive Studie mit Patienten durch, die an einer Infektion der langen Röhrenknochen als Folge der Implantation von interner Fixation erkrankt waren. In die Studie eingeschlossen wurden Patienten, die im Zeitraum von Januar 2010 bis Oktober 2017 im Centrum für Muskuloskeletale Chirurgie der Charité behandelt wurden. Die Daten wurden in einem dafür angelegten Formular (CRF) erfasst. Zum einen verglichen wir Infektionscharakteristika bei interner extramedullärer (IEIF) versus interner intramedullärer Fixation (IIIF). Im April 2013 wurde, mit Hilfe eines interdisziplinären Teams, ein neues standardisiertes chirurgisches und antimikrobielles Therapiekonzept eingeführt. Somit verglichen wir zum anderen die Langzeitergebnisse der Patienten die vor bzw. nach der Einführung dieses Therapiekonzeptes behandelt wurden. Angewendet wurden der Chi-Quadrant-Test sowie die Kaplan-Meier-Schätzung.

Ergebnisse: Eingeschlossen in die Studie waren 127 Patienten (89 männliche) mit einem mittleren Alter von 53 Jahren. In den beiden Kohorten (IEIF vs. IIIF) waren die Patientencharakteristika vergleichbar. Im Vergleich der Indikation für die interne Fixation, waren offene Frakturen signifikant häufiger der Grund in der IIIF- als in der IEIF-Kohorte (24 vs. 16 Patienten; $p<0,001$). In der Patientenkohorte mit IEIF stellten sich die Beibehaltung des Implantats ($p=0,026$) und ein inadäquates antibiotisches Therapiekonzept ($p=0,023$) als signifikante Risikofaktoren für ein Therapieversagen heraus. Eine wiederkehrende oder persistierende Infektion wurde bei 33 (30%) Patienten

gesehen. In der Kohorte, welche nach der Einführung des standardisierten Therapiekonzepts behandelt wurden, zeigten sich signifikant weniger persistierende oder wiederkehrende Infektionen, als in der Kohorte vor der Einführung des Therapiestandards (n=16 (22%) vs. 17 (46%); p=0,015). Unter den 78 Patienten mit einer erfolgreichen Infektionseradikation, wiesen 24 eine Funktionsbeeinträchtigung (Pseudarthose, Gridlestone-Situation oder Amputation der betroffenen Extremität) vor.

Schlussfolgerung: Zum einen konnten Risikofaktoren für eine Infektion als Folge einer Implantation einer internen Fixation identifiziert werden. Zum anderen zeigte sich, eine Besserung der Langzeitergebnisse für Patienten, die nach der Einführung des standardisierten Therapiekonzeptes behandelt wurden. Insgesamt bleibt zu sagen, dass diagnostische und therapeutische Konzepte standardisiert und optimiert werden müssten, um Infektionen nach Implantation von interner Fixation häufiger erfolgreich zu therapieren und somit die Mortalität für diese Patienten zu senken.

1. Introduction

Internal fixation of long bones is a widely used procedure for different situations in orthopedic and traumatological practice. Infections after internal fixation of the long bones are a devastating complication and cause significant morbidity. Comprehensive treatment algorithms were established in recent years (1-8). However, data about clinical characteristics and treatment outcome is still limited.

1.1 Internal Fixation

Internal fixation of long bones is performed for different indications and can be accomplished with various types of materials such as intramedullary nails, screws and pins, generating different degrees of stability. Various indications for internal bone fixation are reviewed in the following paragraphs.

1.1.1 Fracture fixation

Posttraumatic internal fixation can be challenging due to the severity of bone and concomitant soft tissue damage (1). The methods of internal fixation after fracture are classified according to the grade of stability into techniques resulting in absolute stability and techniques with relative stability (9). Absolute stability is achieved with interfragmentary compression plating, which is used for articular, metaphyseal and diaphyseal fractures. It allows a direct fracture healing while taking off the strain on the fracture site. The downside to this approach is the compromised local blood supply due to the stiff contact of the plate to the surface of the bone. This may cause necrosis of the bone and thereby increase the risk of infection (10). A new type of plates, the locking compression plates (LCP) are noncontact plates, which lower the risk of impaired blood

flow and their inherent consequences. Techniques with relative stability such as intramedullary nailing or use of bridging plates allow for small interfragmentary movements to take place. The union of the bone is achieved through indirect bone healing.

Intramedullary nails exist in a reamed or unreamed type. Unreamed nails hold the advantage of not requiring widening of the medullary canal in advance. Reaming of the medullary canal bears its own risks, such as increase of the intracavity pressure and temperature which may eventually lead to damage of the cortical lamella and bone necrosis (9). Nevertheless, according to the current knowledge reamed nailing has a significantly lower risk of nonunion or implant failure (9, 11, 12).

In the upper extremity, plates and intramedullary nails are commonly used for internal fracture fixation. In fractures of the lower extremity, intramedullary nails are preferred because this technique allows for earlier weight bearing. In cases of shaft fractures or extended fractures involving the metaphysis or the joint, intramedullary nailing is not sufficient. For surgical treatment planning, the condition of the soft tissue, the quality of the fractured bone (e.g. osteoporosis) and the cause of the facture (e.g. pathologic fracture) should be considered (9, 12).

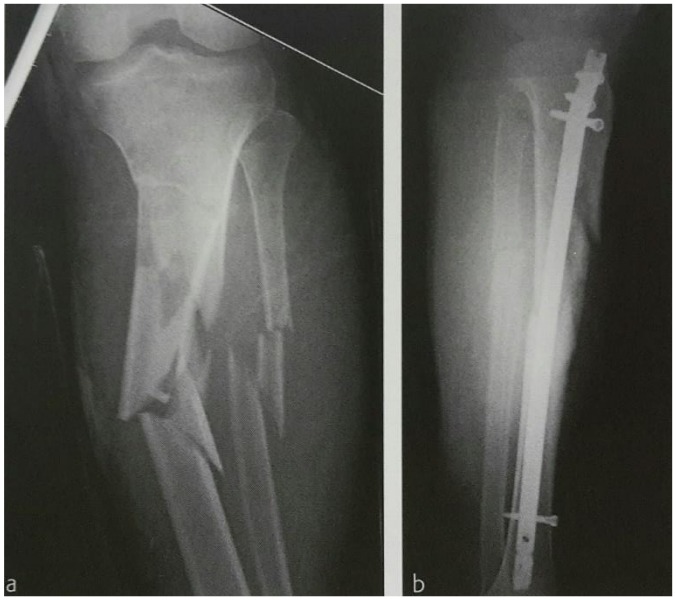


Figure 1: Patient with open segmental fracture of tibia and fibula (left), treated with intramedullary nailing (right) (13)

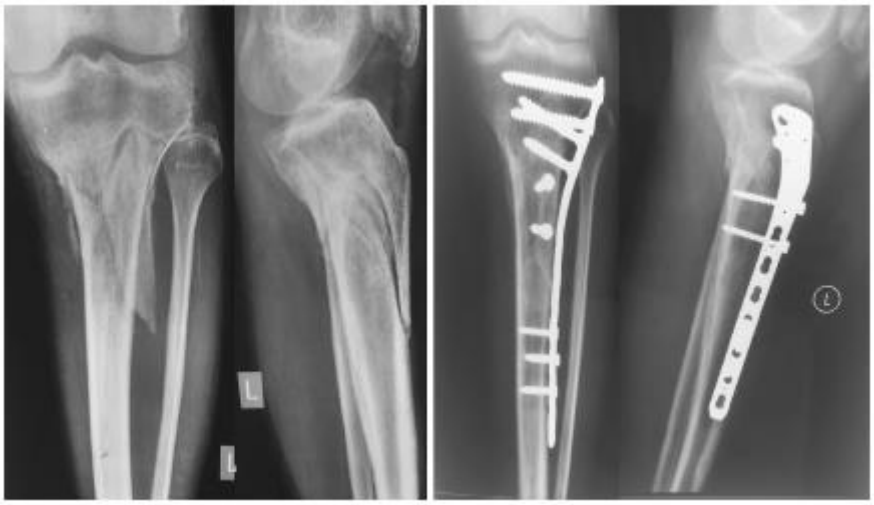


Figure 2: Patient with tibial fracture (left: preoperative x-ray) treated with an extramedullary plate (right) (14)

1.1.2 Arthrodesis

Arthrodesis of the knee is performed in cases of advanced destruction of the joint due to chronic infection, osteoarthritis or neuropathic arthropathy, where an endoprosthesis cannot be implanted or does not improve the joint function. It may also be performed as salvage procedure in cases of recurrent periprosthetic joint infection. The ankylosis of the knee is usually performed with plates, intramedullary nails or an external fixator (15).

Arthrodesis of the upper ankle joint is a surgical treatment for arthrosis if symptoms progress and the conservative therapy is no longer effective. In patients with upper ankle joint osteoarthritis pain, instability and deformity may be addressed with an arthrodesis. It is commonly done with screws, which can be placed through an open access or arthroscopically. Other surgical options are ankylosis via plates, intramedullary nails, pins or external fixator (16).

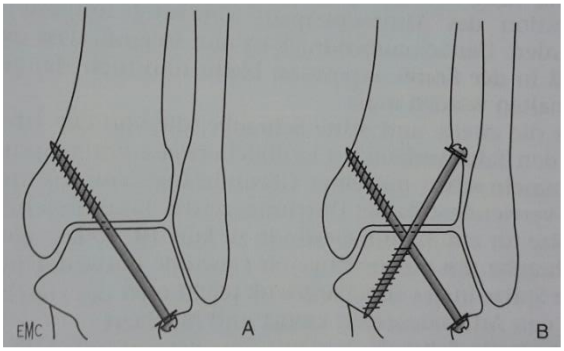


Figure 3: Arthrodesis of the ankle with screws (17)

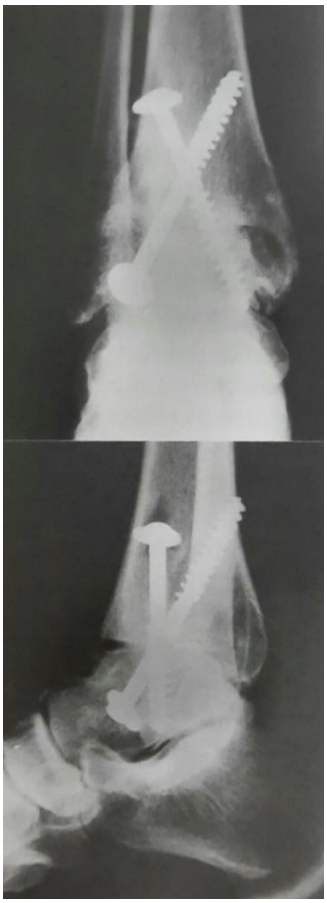


Figure 4: Patient with posttraumatic arthrosis of the ankle treated with a screw arthrodesis (17)

1.1.3 Corrective osteotomy

Correction of the leg axis may be restored by osteotomy. This procedure is mainly indicated for patients with unicompartimental osteoarthritis of the hip or knee due to a malposition of the axis. The indication of an osteotomy needs to be strictly evaluated as an alternative for the placement of an endoprothesis. The corrective osteotomy is mainly realized with plates rarely with intramedullary nails or external fixators (15).

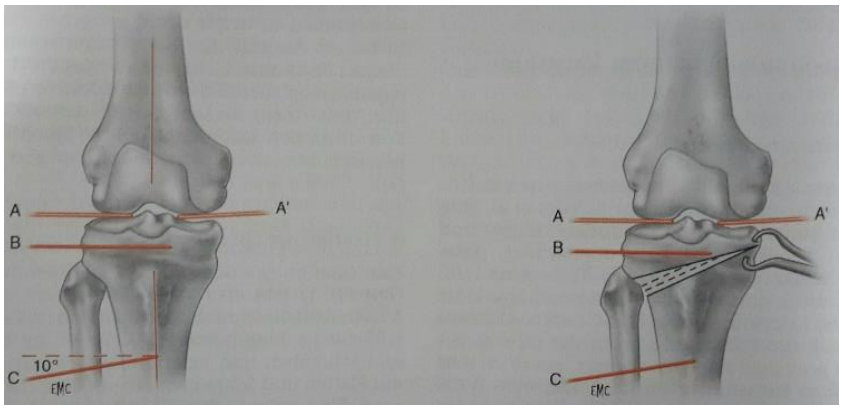


Figure 5: Angular deformity, osteotomy for correction of the axis (18)

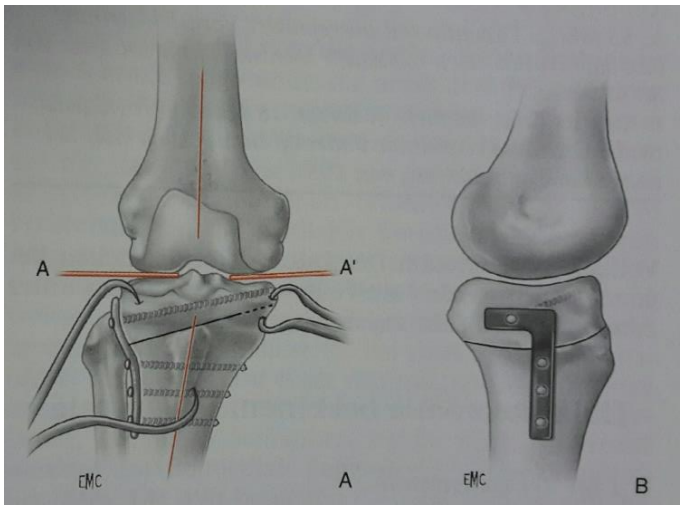


Figure 6: Fixation of the osteotomy with a plate (18)

1.1.4 Distraction osteogenesis

The technique of distraction is used for reconstruction of bone defects or deformities in patients with congenital or posttraumatic defects and in tumor surgery. Surgeries for distraction osteogenesis are mainly performed with external fixators, but can also be carried out with an intramedullary nail (19).

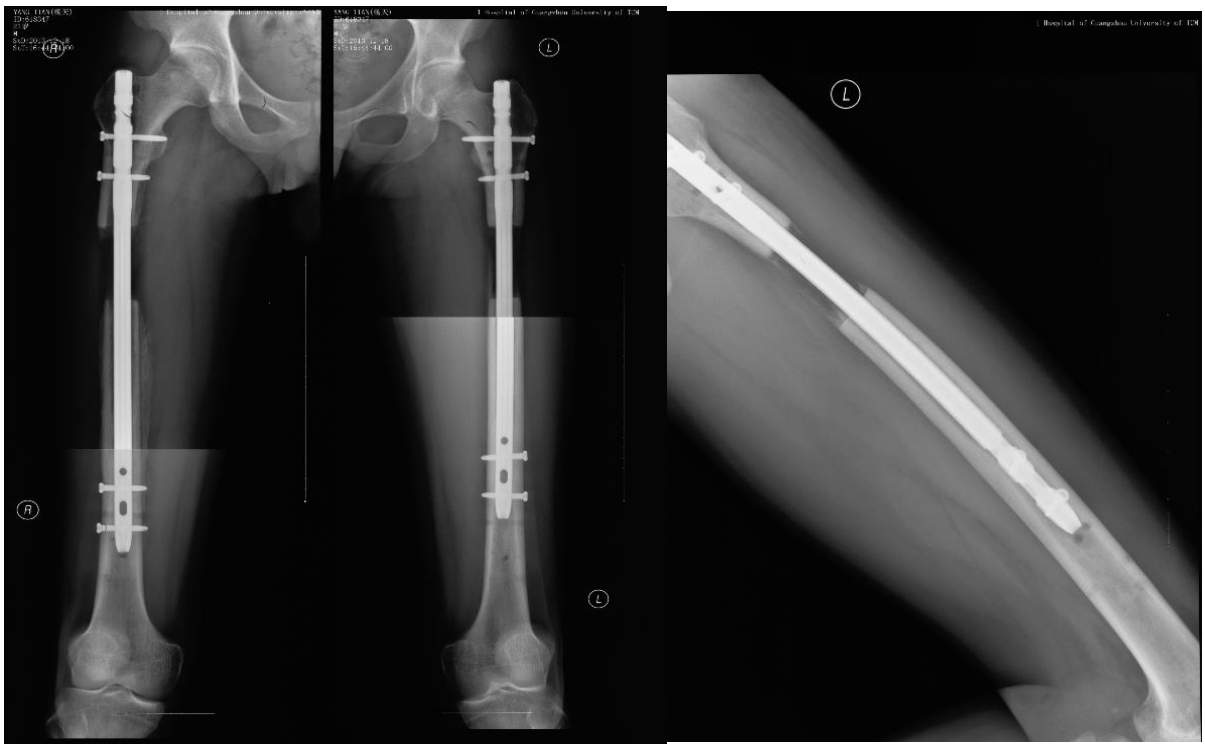


Figure 7: Distraction osteogenesis with an intramedullary nail, showing both femurs (left) and the left femur (right) of patient number 112 of our cohort

1.1.5 Internal fixation after bone tumor

Malignant tumors require complete resection and may cause bone defects of variable sizes. Different surgical treatments exist to address those bone defects. An option for bridging long bone defects is for example the fibula-pro-tibia reconstruction (19). Fibula-pro-tibia reconstruction, also known as fibula centralization, can be performed as a single-stage reconstruction technique to bridge the tibial defect and to achieve a functional outcome for the limp. The tibial lesion needs to be excised and a proportionate length of the fibula including the muscle and vessels is moved medially in the tibial gap. After centralization the fibula fragment can be stabilized with a combination of plates, screws and wires (20).

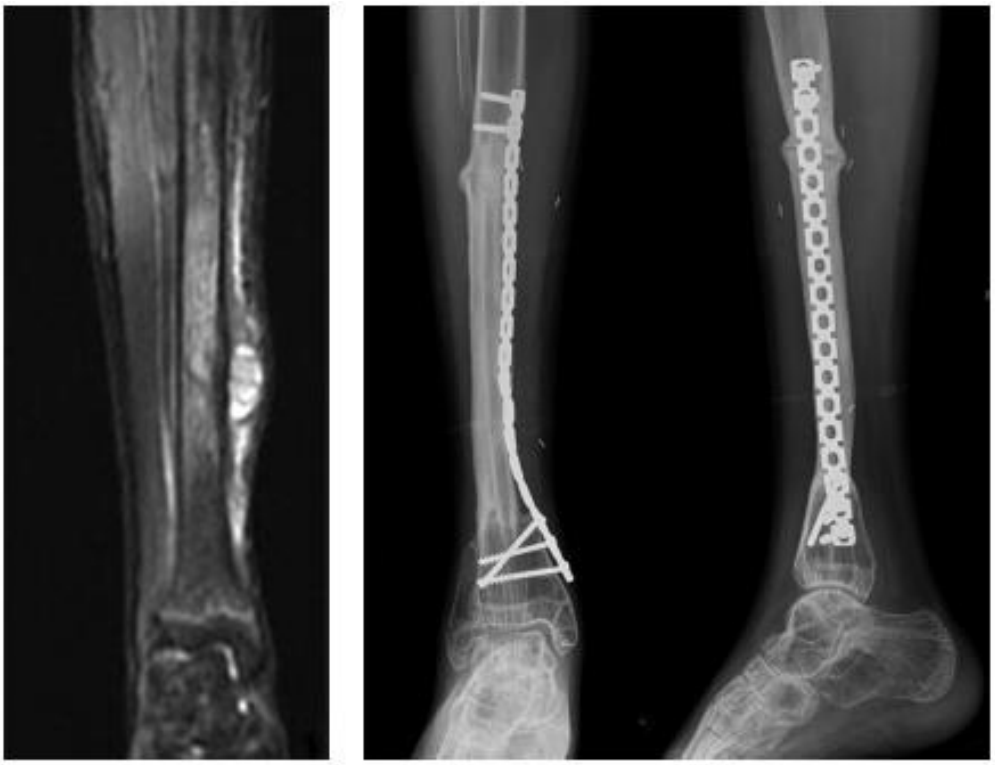


Figure 8: MRI of the lower leg showing an Ewing's sarcoma (left), x-ray showing a fibula-pro-tibia reconstruction (right) (20)

1.2 Infections after internal fixation

Infection after internal fixation is an earnest complication. The presence of an implant and the consecutive biofilm formation represent a considerable challenge regarding diagnosis and treatment of this specific entity.

1.2.1 Definition

In clinical and scientific practice, there was no uniform or standardized definition of infection after internal fixation worldwide and in different institutions until a few years ago (1). In a systematic review by Metsemakers et al. it was shown, that 70% of the randomized controlled trials reporting clinical practice approaches of infections after fracture fixation did not provide a description of the used definition (21). By the Centers

for Disease Control (CDC) guidelines the surgical site infections are classified into superficial, deep or organ/space infections, however osteomyelitis is not included (22). Bhandari et al. stated that there is not even a consensus when it comes to the definition of nonunion (23).

A widely used definition in Europe is extrapolated from the definition of periprosthetic joint infection. According to this classification system of Ochsner et al. (24), infection after internal fixation is confirmed if at least one of the following criteria applies:

- “Abscess with pus discharge following incision
- Presence of a sinus tract or pus
- Microbiological detection of the same pathogen in at least two samples (tissue samples, sonication fluid)
- Histological preparations containing a total of more than 20-25 granulocytes in 10 fields of view at 400x magnification (25).”

In an expert panel, a recent effort was made to develop a consensus definition. The consensus process was designed specifically to address the development of a definition for fracture-related infection. They proposed two groups of diagnostic criteria with different levels of certainty: confirmatory and suggestive criteria (26).

Extracted from “Fracture-related infection: a consensus on definition from an international expert group” by Metsemakers et al. (26):

<u>Confirmatory criteria for fracture-related infection</u>

- Sinus tract or wound breakdown
- purulent drainage or pus at the surgical site
- cultivation of a pathogen from at least two separate deep tissue samples or the implant (including sonication fluid)

- microorganisms in a histopathological examination of deep tissue

Suggestive criteria for fracture-related infection

- local or systemic clinical signs: pain, redness, swelling, warmth, fever
- new-onset of joint effusion
- wound discharge (persistent, increasing or new-onset)
- pathological inflammatory markers: erythrocyte sedimentation rate (ESR), white blood cell count (WBC), serum C-reactive protein (CRP) persistent high level or secondary increase, without other infectious foci identified
- radiological imaging signs: bone lysis, implant loosening, sequestration, nonunion or periosteal bone formation
- cultivation of a pathogenic organism from a single deep tissue or the implant (including sonication fluid)

1.2.2 Epidemiology

The incidence of infection after internal fixation varies widely and depends on the underlying pathology or indication for fixation, the anatomic location, the soft tissue involvement and the employed procedural precautions such as antimicrobial prophylaxis. Overall about 5% of the primary internal fixation devices become infected (27). Depending on the fracture types and the anatomic location the infection rate after fracture fixation varies from 1% in closed up to 30% in open fractures (28-31). For osteotomy with internal fixation an infection rate of less than 3% is described (32). However, due to hampered diagnosis in implant-associated infections owing to the biofilm formation, low grade infections are easily missed and the infection rates are probably widely underestimated.

1.2.3 Pathogenesis

1.2.3.1 Biofilm

The high susceptibility of implanted devices to infection is explained by a local defect of host defense, and persistence is mainly caused by formation of a biofilm, which is resistant to host defense and antimicrobial agents due to a lack of vascularisation (33). The establishment of a biofilm includes initial microbial adherence to the implant surface upon the first contact, which is mediated by host-derived adhesins (including fibrinogen, fibronectin and collagen). The biofilm then recruits additional planktonic (free-floating) organisms and also secretes bacterial products (34). Further development results in organized structures with numerous microorganisms surrounded by a self-produced matrix (exopolysaccharides, DNA and proteins) (33). The microbes of the biofilm are in a slow- or non-growing (stationary) state, which makes them 1,000 times more resistant to antimicrobial treatment than their planktonic counterparts (35, 36).

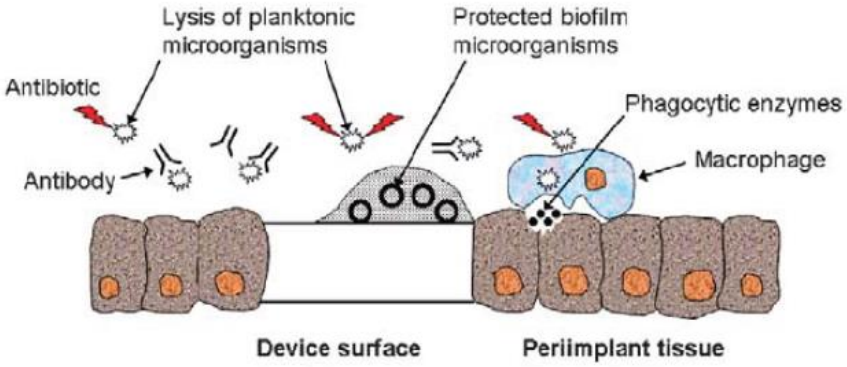


Figure 9: Biofilm microorganisms are attached to the implant surface and protected by an extracellular matrix, planktonic microorganisms are eradicated by the immune system and antibiotics (2)

1.2.3.2 Route of infection

Colonization of an internal fixation device occurs preoperatively during the initial injury, perioperatively during the implantation of the device or postoperatively in case of a persistent wound dehiscence (37, 38). A secondary hematogenous infection of the osteosynthesis following bacteremia is rare. A prospective study of Murdoch et al. did show a 7% infection rate of osteosynthesis in patients with *Staphylococcus aureus*-bacteremia (39). The primary infection focus may be located in the pulmonary, gastrointestinal or urogenital tract, in the cardiovascular system (heart valves, intravascular catheters or cardiac implantable electronic devices), oral cavity or in the skin and soft tissue. Furthermore orthopedic implants get infected due to a contiguous contamination from an adjacent infection focus affecting the skin, soft tissue or intraabdominal/ -pelvic region (40).

1.2.3.3 Osteomyelitis

The term osteomyelitis implies that the cortex and the medulla of a bone are infected. Periosteal stripping, medullary ischemia and inflammatory cells lead to bone death. Those sequesters can either stay trapped in the bone (involucrum) or migrate to the surface through a sinus tract. The infection may be silent over years and be reactivated at a later stage or persist with constant drainage via fistula for a long period (41). Cierny and Mader classified chronic osteomyelitis due to the anatomic types of the disease and additionally took into account the physiological status of the patient regarding the capacity of the host’s defense (42).

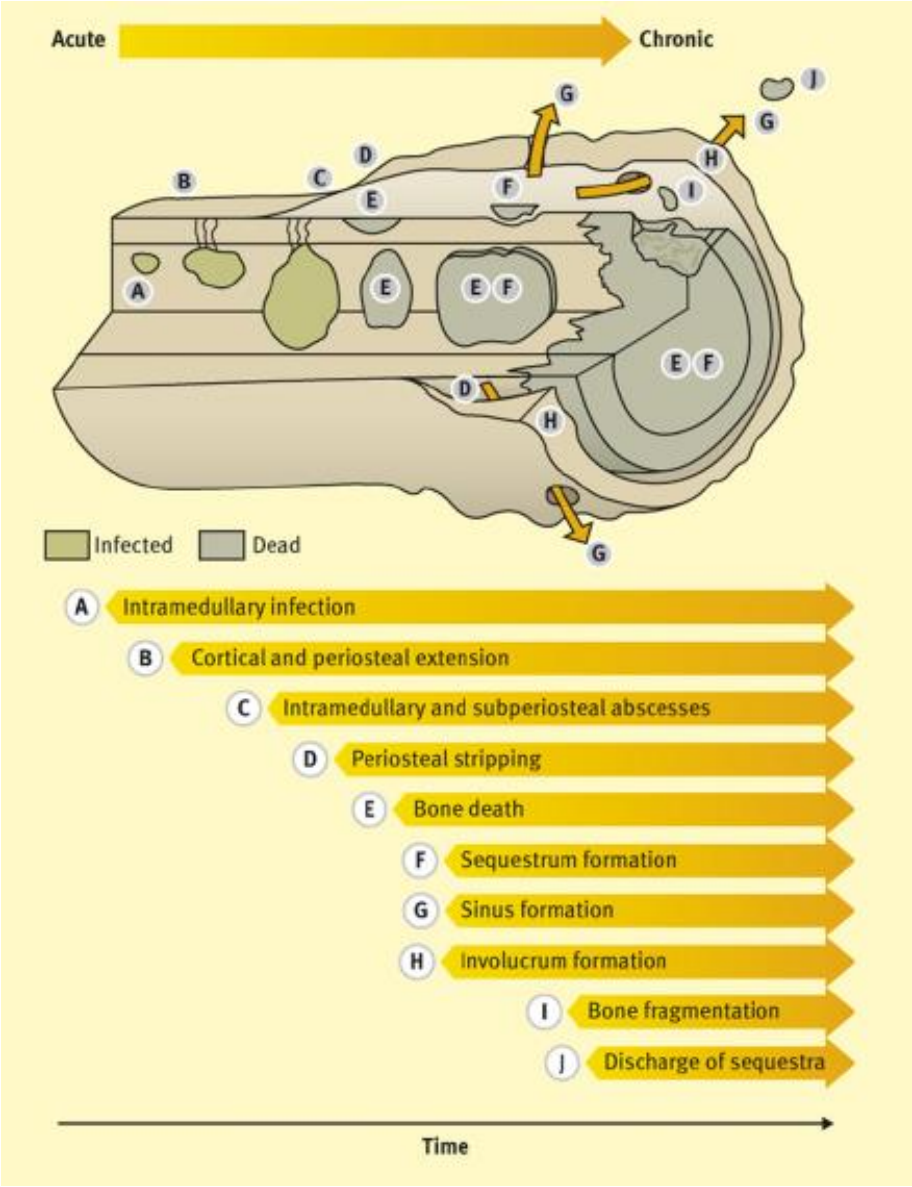


Figure 10: Evolution of bone infection from acute medullary infection to chronic osteomyelitis (41)

1.2.3.4 Microbiology

The pathogenesis of infection determines the spectrum of pathogens. Mainly *Staphylococcus aureus* (30%) and coagulase-negative staphylococci (22%) are found in osteosynthesis-associated infections (43). In cases of infections due to a hematogenous spread from a soft tissue infection or an intravascular foreign body *Staphylococcus*

species are most common, in secondary infections originating from the pulmonary tract streptococci are expected and from abdominal infections gram-negative bacteria and enterococci are usually found. When the osteosynthesis-associated infection is caused by the invasion of pathogens through the initial trauma, the type of fracture, soft tissue damage and environment of the accident is considerably influencing the spectrum of pathogens (43). Especially in trauma with severe soft tissue damage gram-negative pathogens and mixed infections with anaerobes have to be expected (44). A retrospective study including 132 patients showed that 27% of the infected internal fixations were caused by more than one pathogen (mixed infections), 30% of the cases had a *Staphylococcus aureus* infection, 22% infections caused by coagulase-negative staphylococci, 10% by gram-negative bacilli, and a minority by other pathogens (45). An epidemiologic study revealed a pathogen shift from gram-positive to gram-negative strains with high incidence of *Pseudomonas aeruginosa* and polymicrobial infections in sub-/total major traumatic amputations (44).

Additionally, in terms of treatment options the presence of pathogens causing difficult-to-treat infections must be considered. Those pathogens are resistant to biofilm active antimicrobials and thus eradication of the infected foreign body is not possible (40). Pathogens evoking difficult-to-treat infections are rifampin-resistant staphylococci, ciprofloxacin-resistant gram-negative pathogens and fungi (e.g. *Candida spp*.) (8).

1.2.4 Classification

There are different classification systems for infections after internal fixation (46). With regards to the time interval from primary implantation of the fixation device until the infection diagnosis, infections are considered as early (less than 2 weeks), delayed (2-10 weeks) or late infection (more than 10 weeks) (1, 47-49). An early-onset infection is

usually characterized by prominent local or systemic signs of infection or wound dehiscence. It is often caused by high-virulent pathogens such as *Staphylococcus aureus* or gram-negative pathogens. The delayed and late infections are predominantly caused by less virulent pathogens, such as coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis*) and *Cutibacterium* (formerly known as *Propionibacterium*) species. In the course of time the biofilm matures and gets more resistant to antimicrobial treatment and host defense (1, 2). Therefore, the infection is considered to be chronic after 6 weeks, which has relevant impact on the choice of treatment. Clinically the delayed and late infections often present with local signs of infection, pain, compromised functionality and present in some cases with a draining fistula. In cases of with nonunion, movement-induced pain and functional impairment, an infection with low-virulent pathogens should be taken into account (50). Persisting bone instability is a sign for delayed and late infections. Even if bone healing had taken place, osteolysis and inflammation eventually lead to instability of the osteosynthesis. A chronic osteomyelitis may even cause a new bone formation within the medulla or under the elevated periosteum producing an involucrum (9, 41).

According to the anatomic extension, Cierny and Mader classified osteomyelitis in medullary, superficial, local and diffuse infections (Figure 11) (42). A different classification system categorizes the pathogenesis of the infection into exogenic, hematogenous and continuous (see above).

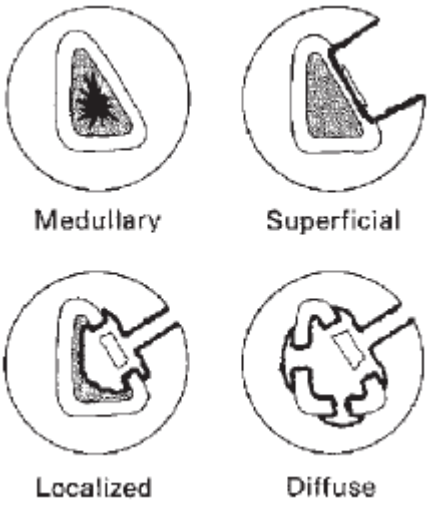


Figure 11: Anatomic classification of adult osteomyelitis by Cierny and Mader (42)

1.2.5 Diagnosis

The diagnosis of an implant-associated infection is made with a synopsis of findings, which include patient's history, clinical presentation, laboratory, imaging studies, microbiology and histopathology.

1.2.5.1 Patient's History and Clinical Presentation

Reports of antimicrobial therapy prescribed in the early course after the primary fracture fixation and performed revision surgeries after index surgery are suggestive for an infection of the osteosynthesis although infection was formally not diagnosed at that point. The clinical presentation of an osteosynthesis-associated infection varies depending on the initial trauma, the type of fracture fixation, the anatomic location, condition of the soft tissue, virulence of the pathogen and onset of the infection (5). In anatomic locations with thin soft tissue and in early-onset infections with high-virulent pathogens, local signs of infection are more common and more prominent.

In cases of osteomyelitis after plating the complications occurs at the interface between plate and bone and between plate and soft tissue. The contact with the surface of the

plate may lead to devascularized areas, necrotic bone and in the later course to sequestration and delayed union/ nonunion. If soft tissue is compromised, a subcutaneous plate will cause earlier local sings of infection than a submuscular or subfascial plate (2). In cases of infection after intramedullary nailing the inner part of bone cortex is affected and will cause impaired fracture healing and nonunion (51, 52). In cases of infection after fracture fixation a weaker callus formation is expected (53). Lovati et al. showed in experimental studies that *Staphylococcus epidermidis* inoculation into a fracture gap leads to nonunion rates of 83-100% in rats (54).

Postoperatively after the initial fracture fixation a persistent wound drainage or dehiscence is suspicious for infection. Definitive signs of infection are pus drainage or draining sinus tract which communicates with the implant, a positive probe-to-implant and internal fixation material on view (see Figure 12 and 13) (1, 55). In chronic infections with low-virulent pathogens, the clinical presentation is often less prominent. Systemic signs of infection such as fever and sepsis are rare. In contrast, patients with acute hematogenous infection secondary to a distant infection focus may present with systemic signs and sepsis (40).

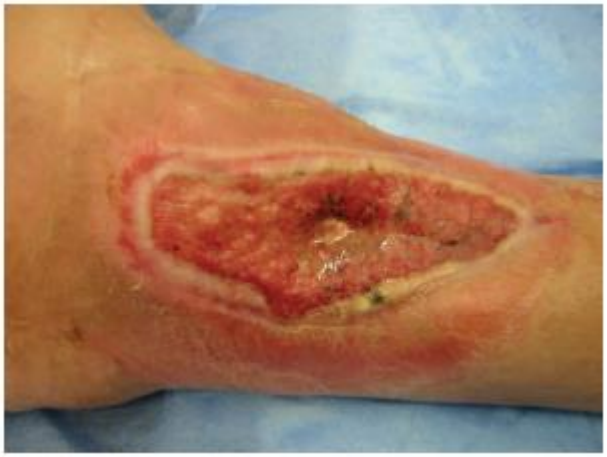


Figure 12: Patient with a previously open tibial fracture, treated with plating, now showing a wound breakdown and a discharging sinus tract (56)

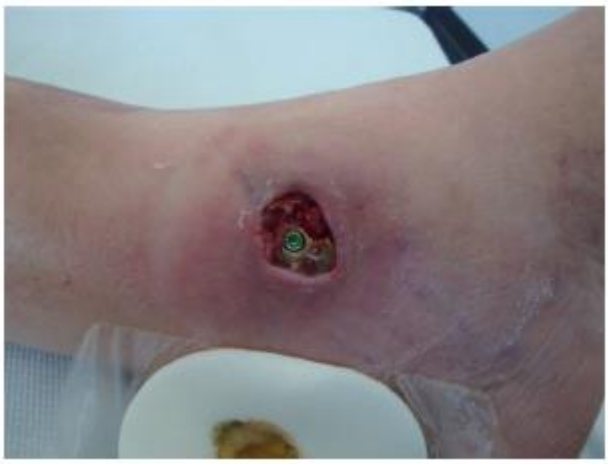


Figure 13: Patient with a wound breakdown and material on view (i.e. exposed plate) (57)

1.2.5.2 Laboratory values

Systemic inflammatory markers, such as serum C- reactive protein (CRP) are widely used in the setting of a suspected infection after fracture-fixation. However, due to low sensitivity and specificity they are not helpful to exclude or confirm an infection (58). They are therefore considered a suggestive criterion for infection and not confirmatory. In contrast, the relative changes of the CRP level after internal fixation is a helpful diagnostic marker (59, 60). Suggestive for an early-onset infection is a persistent high level of CRP or a secondary increase of the CRP level, after an initial decline postoperatively (2). White blood cell count, procalcitonin and erythrocyte sedimentation rate are also not sufficiently sensitive (3, 61). In patients with chronic infections or a sinus tract, the inflammatory markers may be normal (3).

If the internal fixation is close to an adjacent joint and a septic arthritis is suspected, an arthrocentesis is recommended to clarify, if the joint is involved in infection. A leukocyte count in synovial fluid of >2000/µl or >70% granulocytes is highly suggestive for an infection (with some exceptions such as early postoperative phase, after trauma, in patients with underlying rheumatologic disease etc.) (62).

1.2.5.3 Imaging studies

Imaging studies are helpful to make the diagnosis of an implant-associated infection, especially in cases of delayed or late infections (2). Even though conventional x-rays are not very sensitive nor specific for bone infections, they are often obtained as an initial imaging study to rule out other pathologies, such as tumor and fracture. In subacute or chronic stages of osteomyelitis, specific diagnostic signs, like abscesses, sequestrum, sinus tract, nonunion and implant loosening can identified (63).

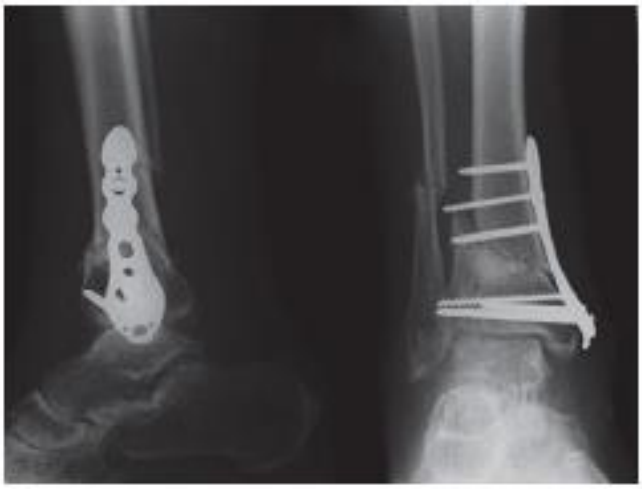


Figure 14: Conventional x-rays showing a nonunion of an open tibial fracture treated with plating (56)

Computed tomography (CT) imaging with intravenous contrast depicts changes in the surrounding soft tissue in addition to the modification in the bone (as described above). Furthermore, computed tomography and magnetic resonance imaging (MRI) are helpful for treatment planning. In comparison to other imaging studies, magnetic resonance shows edema and exudates in the medullary space, changes of the soft tissue or sinus tracts (63). An enhancement with gadolinium in magnetic resonance imaging helps to differentiate between abscess and cellulitis (64). However, metallic artefacts have a major negative impact on image quality of computed tomography and magnetic resonance (2).

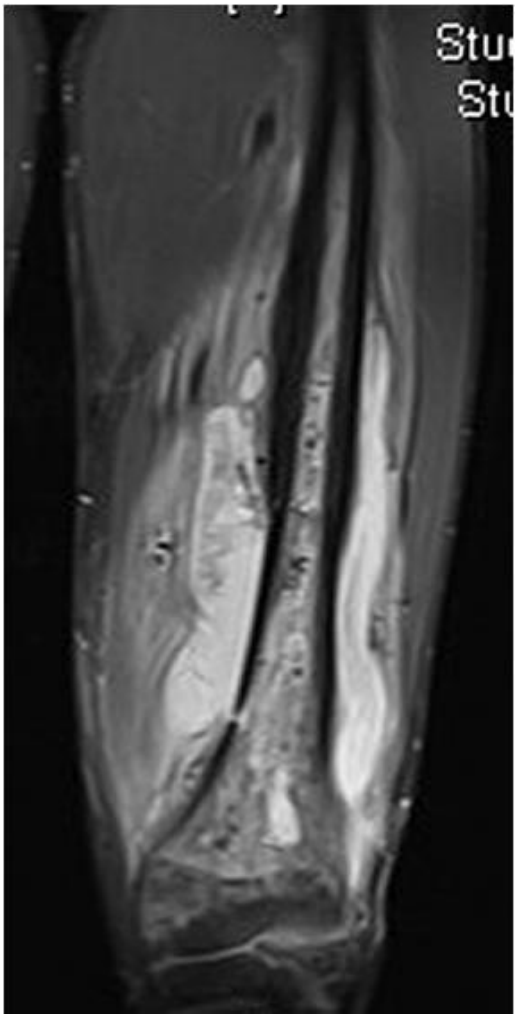


Figure 15: MRI study of a patient with chronic osteomyelitis of the femur; signs of infection are medullary necrosis, abscess formation and sequestrum (41)

Nuclear imaging studies have a high sensitivity but a low specificity. They can be used in cases of multifocal infections and for patients with metallic hardware in place where the infection diagnosis is not confirmed. The different types of nuclear imaging such as technetium-99m labeled methylene diphosphonate (Tc-99m MDP), gallium-67 citrate, indium-111-labeled WBCs and fluorine-18 fluorodeoxyglucose positron-emission tomography computed tomography (FDG-PET CT) all detect early stages of musculoskeletal infections before they can be detected in x-rays (63). The FDG-PET CT is a relatively new tool in this field and is expected to be able to detect biofilms and antimicrobial peptides in the future (65). However, due to interference with the normal

healing process after fracture and fixation surgery, it may be false positive at an early stage.

Sonography is a useful method to diagnose abscesses and to estimate their extent. Supplementary ultrasound is helpful for performing a diagnostic aspiration (66).

1.2.5.4 Microbiology and histopathology analysis

Knowledge of the causing pathogen is essential to confirm the diagnosis and to guide the antimicrobial treatment. At least three to five intraoperative tissue samples should be harvested to increase the detection rate of infecting microorganism. Superficial swabs from an open wound or a sinus tract are not recommended, as they usually show normal skin microbiome, do not correlate with the pathogens found in the deep tissue and are, therefore not representative. For discrimination between contaminant and real pathogen in case of a typical skin microbiome organism, at least two specimens yielding the same pathogen are required to confirm infection. For virulent species such as *Staphylococcus aureus* and *Escherichia coli* one positive tissue sample is sufficient (67). It is of utmost importance to take the specimens from representative areas with the most inflammatory changes, i.e. the nonunion zone or the interface between implant and bone (2). Antimicrobial therapy should preferably be withheld or discontinued at least two weeks prior to taking the tissue samples (68). Prolonged culture incubation up to 14 days is recommended in order to detect slowly growing pathogens such as *Cutibacterium* spp., usually involved in implant-associated infections (69). However, the prolonged incubation bears the risk of culturing microbiological contaminants (1).

As bacteria reside in high density in the biofilm in implant-associated infections, novel diagnostic methods to dislodge the biofilm and embedded bacteria from the implant were developed. A well-established technique is sonication, which has been shown to improve

the pathogen detection rate in implant-associated infections in different medical fields (70-72). Especially in cases of painful internal-fracture fixation implants with no clinical signs of infection, sonication is a useful tool to support diagnostic and treatment decisions (73). The polymerase chain reaction (PCR) is an additional method to detect pathogens causing infection. In studies for prosthetic infections it could be shown that PCR is especially useful in cases with negative cultures and in patients undergoing antimicrobial therapy (74-76). The limitations to PCR are a high risk of false positive results and that it does not provide comprehensive information about the susceptibility to antibiotics (77-79).

For a histopathologic diagnosis, multiple biopsies of different sites are needed. Ochsner et at. showed that there are typical histological characteristics found in patients with osteosynthesis-associated infections (80). Bone necrosis and sequestration are a regular finding and additionally helpful for classifying the duration of the infection. A centralization of bone necrosis and sequester is a sign for a chronic infection. Other signs which are indicative for infection are abscess membranes and periosteal new bone formations. The surrounding soft tissue may also show signs of infection. Extensive granulocytes are indicative for acute infections, plasma cells and lymphocytes are typical for chronic infections (80). A recent study by Morgenstern et al. showed that histopathological samples with more than five neutrophil polymorph counts per high power field have a sensitivity of 80% and a specificity of 100% regarding the diagnosis of fracture- related infections in patients with nonunion (81). The histopathology results can also exclude other diagnosis, such as a malignancy (50).

1.2.6 Therapy

Aim of the therapy of osteosynthesis-associated infections is the adequate healing of the bone and to prevent osteomyelitis and chronification of infection (2). If the fixation device is only needed until the bone healed, suppression of the infection until removal of the implant may be a feasible alternative to the eradication of implant-associated infections (1). The key to success in implant-associated infections is a concerted treatment concept consisting of surgical debridement (if applicable with removal or exchange of the implant) followed by an antimicrobial therapy.

1.2.6.1 Surgical Therapy

The decision on surgical therapy is based on the consolidation of the bone (6). If the infection occurs after the bone is well consolidated, the surgical procedure of choice is debridement and removal of the implant, if in place (3). If the bone is not consolidated yet, the surgical treatment algorithm differentiates between early-onset and late-onset infections based on the age of the biofilm (see Figure 16). In an early-onset infection the internal fixation device can be retained and a sound debridement should be performed (3). This treatment is only possible if the implant is stable and the reduction is adequate, the soft tissue is in good condition (i.e. absence of an abscess and sinus tract) and the microorganisms are susceptible to biofilm-active antimicrobial therapy (4). In case of a late-onset infection the implant needs to be exchanged, either in a one-stage or two-stage procedure. A one-stage surgical procedure is possible if the soft tissue is in a good condition, there is no sinus tract or extensive bone defect and the causing pathogen is preferably known (2, 5). A two-stage surgical procedure needs to be considered, if there is a major soft tissue defect or in the presence of a sinus tract. The bridging stabilization

of the bone during the implant-free interval can be managed with an external fixator, (e-g- Ilizarov-fixator), a spacer or a cast.

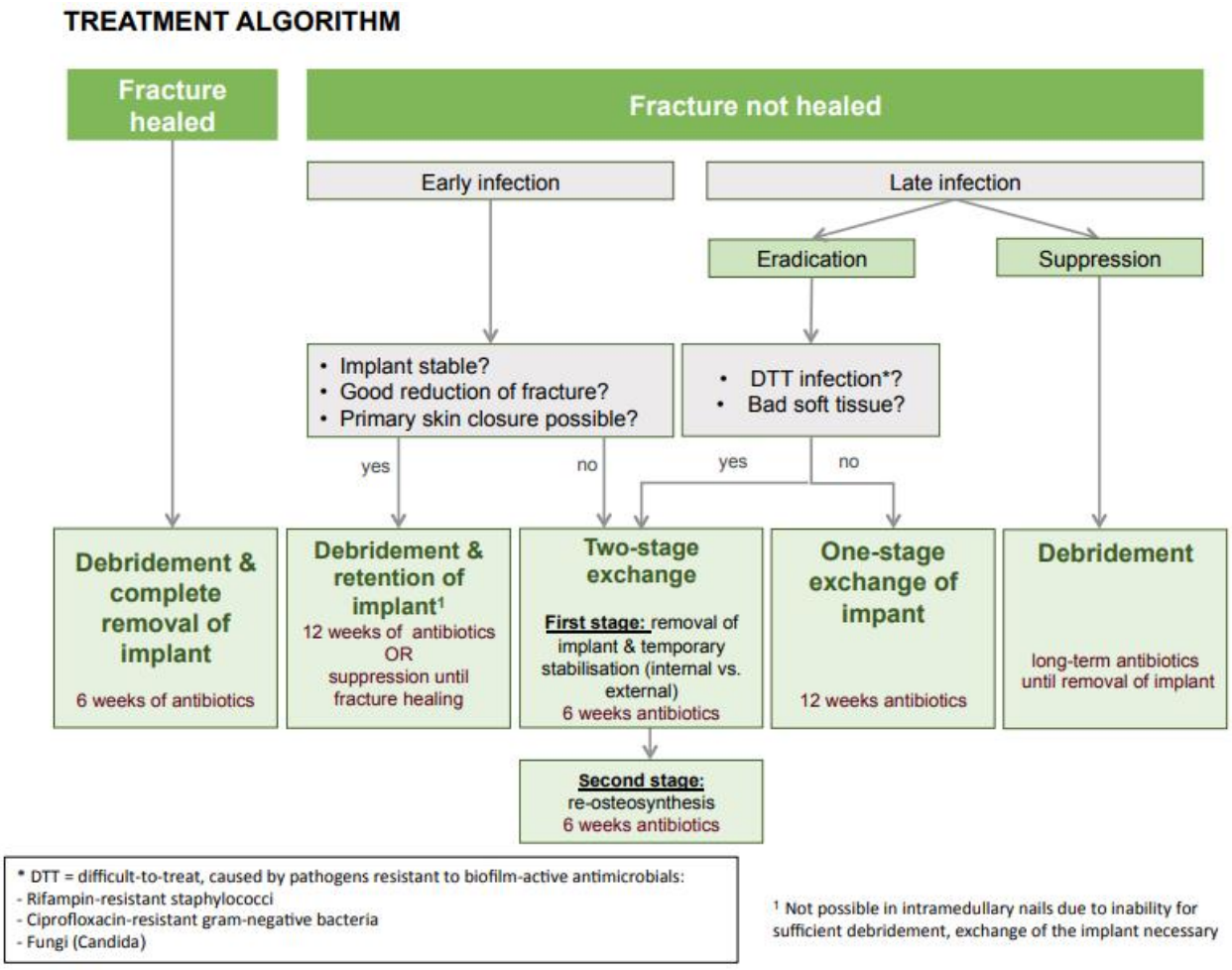


Figure 16: Treatment Algorithm, extracted from “Pocket Guide to Diagnosis and Treatment of implant-associated infections after fracture fixation”, PRO-IMPLANT Foundation, N. Renz, A. Trampuz”

Regarding the soft tissue defect, a skin grafting might be necessary. If the infection is difficult to treat, i.e. caused by a pathogen which is not susceptible to biofilm-active antibiotics (see above), a two-stage surgical procedure is recommended, as the infection cannot be eradicated in presence of an implant. The re-osteosynthesis is then performed

after an implant-free interval in which the pathogen will be definitely eradicated (6). In the first stage surgery all implants and dead tissue (including sequestrum and necrosis) need to be removed (1, 82). In large bone defects, antibiotic loaded cement or bone substitutes may be placed, for a local antimicrobial treatment and to enable Masquelet technique in the second surgery (82). Complicating factors for the surgical treatment are multifragmentary fractures and involvement of a joint (6). Negative pressure wound therapy (NPWT) is not a preferred treatment for infection. It may be used to address a challenging soft-tissue condition only (83, 84). In case of using a vacuum-assisted-closure-therapy (VAC), the bone and the implant need to be covered by tissue and should never be in direct contact with the VAC-system. Otherwise the risk of superinfection caused by multidrug-resistant pathogens, additional colonization with gram-negative bacteria (including *Pseudomonas aeruginosa*) or fungi is increased (6).

1.2.6.2 Antimicrobial Therapy

The antimicrobial therapy needs to be coordinated with the surgical treatment regimen. A considerable reduction of the bacterial count through a meticulous surgical debridement a prerequisite for a successful antimicrobial treatment (7). Antimicrobial substances with a bactericidal effect, good bone penetration, high bioavailability and -in case of implant retention- with biofilm activity, should be used. Only in cases of resistant pathogens bacteriostatic drugs, such as Clindamycin or Linezolid represent an alternative (6) (see Table 3). Empirical antibiotic therapy usually is ampicillin/sulbactam 3 x 3 g i.v. or amoxicillin/clavulanate 3 x 1.2 g i.v. In cases of Gustilo type III open fracture or sinus tract piperacillin/tazobactam 3 x 4.5 g i.v is preferable, to also cover the gram-negative pathogens (6). Once the microbiology results are available the treatment can be changed

to pathogen specific therapy (see Table 1 and 2). The systemic antimicrobial treatment can be combined with a local antibiotic therapy.

Table 1: Targeted eradication therapy (6)

Pathogen	**Antibiotics**
***Staphylococci* spp.**	
Oxacillin-/ methicillin-susceptible	▪ Flucloxacillin 4 x 2 g i.v. For two weeks followed by ▪ Rifampin 2 x 450 mg p.o. plus ▪ Levofloxacin 2 x 500 mg p.o. or ▪ Cotrimoxazole 3 x 960 mg p.o. or ▪ Doxycyclin 2 x 100 mg p.o.
Oxacillin-/ methicillin-resistant	▪ Daptomycin once 8mg/kg body weight i.v. or ▪ Vancomycin 2 x 1 g i.v. For two weeks followed by oral rifampin- combination (see above)
Rifampin-resistant	Intravenous therapy for two weeks (see above) plus, long-term suppression for ≥ 1 year
***Streptococci* spp.**	▪ Penicillin G 4 x 5 Mio. I.U. i.v. or ▪ Ceftriaxone 1 x 2 g i.v. For two to four weeks, followed by ▪ Amoxicillin 3 x 1000 mg p.o. or ▪ Doxycycline 2 x 100 mg p.o.

***Enterococci* spp.**	
Penicillin-susceptible	▪ Ampicillin 4 x 2 g i.v. plus ▪ Gentamicin 1 x 120 mg i.v. or ▪ Ceftriaxon 2 x 2 g i.v. (if *E. faecalis*) For two to three weeks, followed by ▪ Amoxicillin 3 x 1000 mg p.o.
Penicillin-resistant	▪ Vancomycin 2 x 1 g i.v. or ▪ Daptomycin 1 x 10 mg/kg body weight i.v. plus ▪ Gentamicin 1 x 120 mg i.v. For two to four weeks followed by ▪ Linezolid (maximal four weeks) 2 x 600 mg p.o.
Vancomycin-resistant (VRE)	Individual; removal of implant or long- term suppression necessary
Gram-negative pathogens	
Enterobacteriaceae (*E. coli, Klebsiella, Enterobacter* etc.)	▪ Ciprofloxacin 2 x 750 mg p.o.
Non-fermenting (*Pseudomonas aeruginosa*, *Acinetobacter* spp.)	▪ Piperacillin/tazobactam 3 x 4.5 g i.v. or ▪ Meropenem 3 x 1 g i.v. or ▪ Ceftazidim 3 x 2 g i.v. plus ▪ Tobramycin 1 x 300 mg i.v. For two to three weeks, followed by ▪ Ciprofloxacin 2 x 750 mg p.o.
Ciprofloxacin-resistant	Depending on the sensitivity: ▪ Meropenem 3 x 1 g

	▪ Colistin 3 x 3 Mio. I.U. and/ or ▪ Fosfomycin 3 x 5 g i.v. Followed by oral suppression
Anaerobes	
Gram-positive (*Cutibacterium, Peptostreptococcus, Finegolida magna*)	▪ Penicillin G 4 x 5 Mio.I.U. i.v. or ▪ Ceftriaxon 1 x 2 g i.v. For two weeks, followed by ▪ Rifampin 2 x 450 mg p.o. plus ▪ Levofloxacin 2 x 500 mg p.o. or ▪ Amoxicillin 3 x 1000 mg p.o.
Gram-negative (*Bacteroides*)	▪ Ampicillin/sulbactam 3 x 3 g i.v. For two weeks, followed by ▪ Metronidazole 3 x 400 or 500 mg p.o.
Candida spp.	
Fluconazole-susceptible	▪ Caspofungin 1x 70 mg i.v. for two weeks, followed by ▪ Fluconazole once 400 mg p.o. (suppression for ≥1 year)
Fluconazole-resistant	Individual (e.g. Voriconazol 2 x 200 mg p.o.) Removal of implant or long- term suppression
Negative microbiology result (culture-negative)	▪ Ampicillin/sulbactam 3 x 3 g i.v. for two weeks followed by ▪ Rifampin 2 x 450 mg p.o. plus ▪ Levofloxacin 2 x 500 mg p.o.

Usually the antimicrobial therapy is given over a time period of 12 weeks if an implant is involved in the infection (2, 85). In case of a consolidated fracture and performed bone debridement and implant removal, the treatment duration can be shortened to 6 weeks. The intravenous treatment allows for a high tissue concentration and therewith a quick reduction of bacterial count. The oral treatment should only be switched to oral formulations, if the wound is dry and the CRP level is declining (6).

Another concept of antimicrobial treatment relies on suppression of the infection until fracture healing and implant removal (see Table 2). This is not only an option for multifragment fractures but also for difficult-to-treat infections (6). As long as the implant is still in place, a discontinuation of the suppressive treatment may lead to a recurrence of the infection (8).

Table 2: Suppressive therapy during implant free interval or after removal of the implant (6)

Pathogen	**Substance**
Staphylococci	Cotrimoxazole or doxycycline or clindamycin
Streptococci	Amoxicillin or clindamycin or doxycycline
Enterococci	Amoxicillin (or linezolid)
Anaerobes (gram-positive)	Clindamycin or amoxicillin
Anaerobes (gram-negative)	Metronidazole or clindamycin
Gram-negative pathogens	Ciprofloxacin or cotrimoxazole

1.2.7 Prevention

Perioperative antibiotic prophylaxis reduces the risk of postoperative infections and is therefore well established in orthopedic surgery (86). A single dose of a cephalosporine

30-60 minutes prior to skin incision is most efficient and needs to be repeated if the procedural time is longer than 3 hours or if the blood loss exceeds 1500 ml. In cases of a severe cephalosporine allergy, vancomycin can be used instead. If there is a severe soft tissue defect, the antibiotic therapy needs to be extended according to the expected pathogens. In case of a skin colonization with methicillin resistant *Staphylococcus aureus* (MRSA) or preceding antibiotic therapy, vancomycin should be given additionally to a cephalosporine (6). For a patient with a Gustilo type III open fracture a combination of ampicillin/sulbactam or piperacillin/tazobactam should be given for five further days to prevent transition from colonization to infection (preemptive therapy) (87, 88). There was no benefit shown for antibiotic prophylaxis in surgeries of implant removal or in surgery with implantation of screws or pins (88).

Table 3: Perioperative antibiotic prophylaxis and preemptive therapy (6)

Indication	**Antibiotic of first choice**	**Alternative in case of penicillin allergy**	**Duration**
Implantation of surgical devices	Cefazolin 2 g i.v. or Cefuroxim 1.5 g i.v.	Vancomycin 1 g i.v.	Single shot
Open fracture, type I and II	Cefazolin 2 g i.v. or Cefuroxim 1.5 g i.v.	Vancomycin 1 g i.v.	Single shot, max. 24 hrs.
Open fracture, type III	Ampicillin/sulbactam 3 g i.v. 8 hourly or Piperacillin/tazobactam 4.5 g i.v. 8 hourly	Vancomycin 1 g i.v. 12 hourly plus ciprofloxacin 400 mg i.v. 12 hourly	5 days

1.3 Aim of this study

Aim of this study is to analyze epidemiological, clinical and diagnostic characteristics of infections of long bones after internal fixation. Furthermore, it aims at assessing treatment approaches and outcome of these infections with a special focus on the impact of the current standardized treatment of the interdisciplinary infectious diseases and surgical team at the Charité Universitätsmedizin.

2 Methods

2.1 Study Design

This retrospective cohort study was conducted in the orthopedics and traumatology facility at Charité Universitätsmedizin Berlin, a tertiary healthcare center providing advanced specialty care to a population of four million inhabitants. Patients with infections after internal-fixation of a long bone were identified in the electronic medical record system based on the ICD-diagnosis M.86 (Osteomyelitis) and the ICD- diagnosis T86.4 (Infection and inflammatory reaction due to internal fixation device). In addition, the institutional database of patients with musculoskeletal infections was screened. The study protocol was reviewed and approved by the institutional ethics committee and was performed in accordance with the Declaration of Helsinki. This study was conducted as subproject of the institutional implant infection cohort and the need for informed consent was waived (application number EA2/132/15).

The patients' data was collected from the electronical records using a standardized protocol and was classified in a case report form (CRF) specifically designed for this purpose (see Figure 17).

Fixation device- associated infection - Charité university hospital Berlin
retrospective study (2010 - 2017)

Patient data: **No.:**_____
Last name, first name: _____ DOB: _____
Sex: M □ F □ Age (Admission): _____ BMI: _____ ASA: _____

Coexisting medical conditions: □ DM □ RF □ HF □ MA □ Immunosupp. □ Immune Deficieny

Infected bones: □ humerus □ radius □ ulna □ femur □ tibia □ fibula
Infected joints: □ septic arthritis: □ shoulder □ elbow □ wrist □ hip □ knee □ ankle

Date of injury:_____ **Fracture:** □ upper arm □ forearm □ thigh □ lower leg □ ankle
Cause:□traffic acci. □fall □path.f. **Type :** □**closed** □**open**:Grade □I □II □III **polytrauma**: □no □yes

Prior operations of the infected bone:

□ **1. surgery (date)**:_____
diagnosis:_____
procedure:_____
□ debridement □ lavage □ VAC
□ removal of material: □ total □ partial_____
□ material:_____
□ other:_____

□ **2. surgery (date)**:_____
diagnosis:_____
procedure:_____
□ debridement □ lavage □ VAC
□ removal of material: □ total □ partial_____
□ material:_____
□ other:_____

□ **3. surgery (date)**:_____
diagnosis:_____
procedure:_____
□ debridement □ lavage □ VAC
□ removal of material: □ total □ partial_____
□ material:_____

□ **4. surgery (date)**:_____
diagnosis:_____
procedure:_____
□ debridement □ lavage □ VAC
□ removal of material: □ total □ partial_____
□ material:_____

□ **5. surgery (date)**:_____
diagnosis:_____
procedure:_____
□ debridement □ lavage □ VAC
□ removal of material: □ total □ partial_____
□ material:_____

□ **6. surgery (date)**:_____
diagnosis:_____
procedure:_____
□ debridement □ lavage □ VAC
□ removal of material: □ total □ partial_____
□ material:_____

Prior microbiology: date: _____ germ: _____
Preoperative laboratory: date: _____ CRP (mg/dl): _____

Signs/symptoms: Fever:□y/□n □pain □red □warm □swelling □secretion □fistula □material vis □woundd

Current hospitalization: □ ER/ □ Polyclinic/ □ transfer
1.stay:_____,____days; diagnosis:_____

Radiology (X-ray, MRI, CT): Suggestive signs of infection:
□ X-ray _____ diagnosis_____ □ no specific signs for osteomyelitis
□sequestrum □soft tissue oedema □fistula □implant loosening □delayed union □non- union
□other: _____
□ MRI _____ diagnosis: _____ □ no specific signs for osteomyelitis
□sequestrum □soft tissue oedema □fistula □implant loosening □delayed union □non- union
□other: _____
□ CT _____ diagnosis:_____ □ no specific signs for osteomyelitis
□sequestrum □soft tissue oedema □fistula □implant loosening □delayed union □ non- union
□other: _____
□ other: _____

Surgeries: □ pus visible □ sequestrum visible

1. surgery: _____ Diagnosis: _____

Procedure: _____ □debridement □fistula excision □intramedullary drill □sequestrectomy □arthrodesis □amputation □bone grafting.:_____ □skin grafting:_____ □VAC

material: □ Fixateur externe □ Ilizarow fixateur □ ORIF (□ intramedullary nail □ plate osteosynthesis) □ Pins □ screws □ PMMA (□ chains □ Spacer)

removal of material □ total □ partially _____

Microbiology: intraoperative specimen positive:___ total:___

germ:_____ pos.:__; DTT- Resistenz:_____

germ:_____ pos.:__; DTT- Resistenz:_____

germ:_____ pos.:__; DTT- Resistenz:_____

Sonication: □ ND / □ neg. □ pos. :germ:_____ □ ____ KBE /ml □ n. A.

Pathology: diagnosis:_____ □ no specific signs for OM □ sequester □ inflammation: _____

2. surgery: _____ Diagnosis: _____

Procedure: _____ □debridement □fistula excision □intramedullary drill □sequestrectomy □arthrodesis □amputation □bone grafting.:_____ □skin grafting:_____ □VAC

material: □ Fixateur externe □ Ilizarow fixateur □ ORIF (□ intramedullary nail □ plate osteosynthesis) □ PMMA (□ chains □ Spacer)

removal of material □ total □ partially _____

Microbiology: intraoperative specimen positive:___ total:___

germ:_____ pos.:__; DTT- Resistenz:_____

germ:_____ pos.:__; DTT- Resistenz:_____

germ:_____ pos.:__; DTT- Resistenz:_____

Sonication: □ ND / □ neg. □ pos. :germ:_____ □ ____ KBE /ml □ n. A

Pathology: diagnosis:_____ □ no specific signs for OM □ sequester □ inflammation: _____

3. surgery: _____ Diagnosis: _____

Procedure: _____ □debridement □fistula excision □intramedullary drill □sequestrectomy □arthrodesis □amputation □bone grafting.:_____ □skin grafting:_____ □VAC

material: □ Fixateur externe □ Ilizarow fixateur □ ORIF (□ intramedullary nail □ plate osteosynthesis) □ Pins □ Screws □ PMMA (□ chains □ Spacer)

removal of material □ total □ partially _____

Microbiology: intraoperative specimen positive:___ total:___

germ:_____ pos.:__; germ:_____ pos.:__

germ:_____ pos.:__; germ:_____ pos.:__

germ:_____ pos.:__; germ:_____ pos.:__

Sonication: □ ND / □ neg. □ pos. :germ:_____ □ ____ KBE /ml □ n. A

Pathology: diagnosis:_____ □ no specific signs for OM □ sequester □ inflammation: _____

Antibiotic therapy (>48h):	Application	Dates	Days	Weeks
	□ i.v. □ p.o.			
	□ i.v. □ p.o.			
	□ i.v. □ p.o.			
	□ i.v. □ p.o.			
	□ i.v. □ p.o.			

Figure 17: Case report form

2.2 Study Population

We included all consecutive patients ≥ 18 years of age from January 1, 2010 to November 17, 2017, who were treated at our institution for infection of a long bone after internal fixation due to a fracture, an osteotomy or an arthrodesis and who fulfilled the inclusion criteria (see below). It was not mandatory that the implant was still in place at time of admission to our institution.

Exclusion criteria were osteomyelitis of hematogenous origin or secondary to vascular origin, osteomyelitis of the pelvis, head, spine, hands or feet, presence of joint prosthesis in the anatomic site of the infection. In addition, patients with infections limited to the soft tissue and with incomplete dataset were excluded from the analysis.

2.3 Definitions

2.3.1 Definition of infection after internal fixation

Infection after internal fixation of a long bone was confirmed, if the patient presented with clinical symptoms and at least one of the following criteria applied:

- intraoperatively visible purulence, sequestrum or sinus tract
- positive microbiology: significant growth of a microorganism (definition see below)
- acute or chronic inflammation in intra-operative tissue histopathology.

2.3.2 Significant microbiology results

Specimen were considered representative if the tissue or deep swab was obtained intraoperatively. We excluded samples from superficial or fistula swab and microbiology results from drainage systems from our analysis. If the detected pathogen was highly virulent one positive tissue culture and any growth in the sonication was sufficient. For low virulent pathogens more than one tissue culture had to grow the identical pathogen

and only sonication results showing more than 50 colony forming units (CFU)/ml were considered significant. Otherwise, the result was judged as contamination.

2.3.3 Adequate antimicrobial treatment

The antimicrobial treatment was considered as adequate if the criteria of the current standardized comprehensive treatment algorithm as described in 1.2.6 were fulfilled. This means the susceptibility testing considered, the type of application regarding bioavailability and the duration of the therapy had to be individually matched for each patient and the pathogens causing the infection. Furthermore, an adequate antimicrobial treatment needed to be adapted according to the surgical treatment, e.g. retention or removal of the implant. In case of retention of the implant, the antimicrobial substance needed to be biofilm-active.

2.3.4 Outcome definitions

Failure was defined as a recurrent, persistent, or new infection caused by another pathogen in the clinical course. A recurrent, persistent, or new infection was diagnosed with microbiological results, tissue histopathology and clinical presentation such as implant on view, wound dehiscence and sinus tract at the surgical site.

Infection success was defined as infection-status without microbiological, histopathological nor clinical signs of infection at time of follow-up.

Functional failure was diagnosed, if functional impairment such as persistent nonunion was present, amputation of the limb or resection arthroplasty (Girdlestone situation) was performed.

2.4 Data collection

The following patient's data were collected in a case report form (Figure 17): Demographic information including age, sex, height, weight, BMI, ASA- classification, coexisting- medical conditions such as, diabetes mellitus type 1 and 2, chronic renal failure with creatinine level >220 µmol/l [> 2.5 mg/dl], active malignancy, immunosuppression [HIV infection or use of >25 mg prednisone-equivalent/day or other immunosuppressive medication in the preceding month], exposure to radiotherapy or chemotherapy, hepatic failure (Child-Pugh B or C) were collected. Documented was the anatomic side of the infection and if in addition to the long bone a joint was infected as well. Information about a fracture and prior surgeries to the infected bone were recorded. If the patient had growth of a microorganism in >1 intraoperative tissue from a surgery prior to the actual infection episode we captured that information. At time of admission to our institution signs and symptoms for a local infection such as pain, redness, excess heat, swelling, secretion, sinus tract, visible material and wound dehiscence and signs for a systemic infection such as fever and increased serum level of C-reactive protein (CRP) were noted. Preoperative radiographic findings such as loosening of the implant, nonunion (at > 6 months after fracture fixation), delayed union (at 4-6 months after fracture fixation), sequestrum, soft tissue edema and sinus tract were recorded. The type of surgical therapy and the intraoperative aspect (pus or sequestrum) were noted. The results from the tissue cultures, implant sonication and results of pathology were recorded for all patients. Data on type and duration of an antimicrobial was collected.

2.4.1 Follow-up evaluation

The follow-up was ascertained with the computerized medical charting system. A failure or success of the therapy was evaluated with clinical findings, radiological imaging,

surgical reports, tissue cultures, sonication results and intraoperative tissue histopathology, according to the aforementioned outcome definitions.

2.5 Implementation of a standardized comprehensive treatment concept

In the year 2013, a standardized interdisciplinary treatment was introduced in our institution. The above described treatment algorithm of surgical and antimicrobial therapy (1.2.6) was adjusted for each patient individually based on the present clinical and microbiological features. A dedicated interdisciplinary team consisting of internal medicine and infectious diseases specialists and traumatologists was responsible for the patients treated for infections of the musculoskeletal system. Before 2013, patients were treated at the treating traumatologists decision without standardized concept and without collaboration of internal medicine or infectious disease specialists.

2.6 Statistical analysis

For comparison of categorical variables Fisher's exact test was applied. The probability of infection- free survival and the respective 95% confidence intervals (CI) were estimated using the Kaplan-Meier survival method. Survival curves between groups were compared by the Log-rank Mantel-Cox test. A univariate analysis was used to determine the predictors of treatment failure, followed by a multiple logistic regression model for significant predictors in the univariate analysis. A two-sided P value of <0.05 was considered significant. For statistical analyses, the program package R (version 3.1.3.) and the software Prism (version 7.03; GraphPad, La Jolla, CA, USA) were used.

3 Results

Hundred twenty-seven patients with device-associated infection after internal fixation of long bones met the criteria for inclusion. Based on the type of the first internal fixation device patients were stratified into two groups. The first group contained all patients, who received an intramedullary nail for internal fixation. The second group included all patients, in whom extramedullary internal fixation devices such as screws, plates, pins or a combination of them were used for bone fixation.

3.1. Patient Characteristics

The cohort consisted of 89 male (70%) and 38 female (30%) patients with an age ranging from 19 to 89 years and a median of 53 years. The median body mass index was 26.5 kg/m² with a range from 18.4 to 55.6 kg/m². The ASA physical status classification ranged from 1 to 4 (median 2). Most patients were otherwise healthy, only a minority of patients had coexisting medical conditions such as diabetes mellitus in 14%, active malignancy in 6%, immune deficiency in 3%, renal failure in 2% and hepatic failure in 1% of the cases. The patient characteristics were similar in both groups (see Table 4).

Table 4: Patients characteristics

	All patients (n=127)	**Intramedullary fixation (n=47)**	**Extramedullary fixation (n=80)**	**p-value**
Sex, male	89 (70)	35 (47)	54 (68)	0.431
Age, median (range) - years	53 (19-89)	53 (19-89)	53 (19-86)	0.352

BMI, median (range) - kg/m²	26.5 (18.4-55.6)	26.5 (18.5-36.6)	26.5 (18.4-55.6)	0.201
ASA, median (range)	2 (1-4)	2 (1-4)	2 (1-4)	0.617
Comorbidities				
Diabetes mellitus	18 (14)	7 (15)	11 (14)	1.000
Malignancy	8 (6)	2 (4)	6 (8)	0.709
Immune deficiency [1]	4 (3)	-	4 (5)	0.296
Renal failure	3 (2)	-	2 (4)	0.295
Hepatic failure	1 (1)	1 (2)	-	0.370

NOTE. Data are no. (%) of episodes, if not indicated otherwise

[1] Among them 2 patients with immunosuppression due to kidney-transplantation, 2 patients with HIV infection

3.2 Baseline characteristics of the infection site

The baseline characteristics of the index surgeries and the implant history are summarized in Table 5. In 111 cases (87%) the lower extremity was affected and in 16 cases (13%) the upper extremity was involved. The main cause of internal fixation was fracture (115 patients). Significantly more open factures were reported in the group with intramedullary fixation devices than with extramedullary fixation devices (56% vs. 22%, p <0.001). Among all fractures, 38 out of 78 (49%) were caused by a fall, 26 (33%) by traffic accidents, 6 (8%) through other accidents, 5 (6%) due to a bomb explosion or bullet injury and 4 (5%) were pathological fractures due to bone metastases. In 18 cases (26%) the patient experienced a polytrauma. Four patients received internal fixation to perform arthrodesis, 3 patients to perform corrective osteotomy, 2 patients for distraction osteogenesis and 2 patients to bridge bone defects because of bone tumors. In most

cases (n=110, 85%), the initial stabilization of the bone was carried out with an internal fixation. In 25 cases, cultures from fixation surgery were positive at the index surgery, 68% of them were monomicrobial and 32% polymicrobial. The clinical consequence of the positive cultures is unknown. Of 23 patients we do not have any information about revision surgeries after the implementation of the internal fixation until the diagnosis of an infection. Of the 104 patients who underwent revision surgeries of the bone at the implant site, in average two surgeries were done (range from 1 to 11).

Table 5: Baseline Characteristics of the infection site

	All patients (n=127)	**Intramedullary fixation (n=47)**	**Extramedullary fixation (n=80)**	**p-value**
Anatomic location				
Lower extremity	111 (87)	46 (98)	65 (81)	0.005
Femur	34	21	13	
Tibia	62	19	43	
Fibula	2	0	2	
Femur and tibia	1	0	1	
Tibia and fibula	12	6	6	
Upper extremity	16 (13)	1 (2)	15 (19)	0.005
Humerus	9	1	8	
Radius	4	0	4	
Ulna	3	0	3	
Cause for fixation				
No fracture [1]	12 (9)	4 (9)	8 (10)	1.000

Fracture	115 (91)	43 (91)	72 (90)	1.000
Open	40 (35)	24 (56)	16 (22)	<0.001
Closed	33 (29)	10 (23)	23 (32)	0.396
Not classified	42 (37)	9 (21)	33 (46)	0.009
Cause of fracture				
Fall	38/78 (49)	9/33 (27)	29/45 (64)	0.001
Traffic accident	26/78 (33)	18/33 (55)	8/45 (18)	0.001
Other accidents (e.g. sporting)	6/78 (8)	3/33 (9)	2/45 (4)	0.645
Bullet wound, bomb explosion	5/78 (6)	2/33 (6)	3/45 (7)	1.000
Pathological fracture [2]	4/78 (5)	1/33 (3)	3/45 (7)	0.634
Polytrauma	18/69 (26)	8/26 (31)	11/36 (31)	1.000
Initial external fixation	27/110 (25)	8/39 (21)	19/71 (27)	0.497
Positive microbiology at index surgery	25/45 (55)	7/17 (41)	18/28 (64)	0.216
Monomicrobial	17 (68)	3 (43)	14 (78)	0.156
Polymicrobial	8 (32)	4 (57)	4 (22)	0.156

NOTE. Date are no. (%) of episodes, if not indicated otherwise

[1] Among them 4 cases with arthrodesis, 3 cases with corrective osteotomy, 2 cases with distraction osteogenesis; [2] among them 2 cases with bone tumors (one Ewing's sarcoma, one osteosarcoma) and 2 cases with bone metastases due to renal cell carcinoma

3.3 Infection characteristics

Clinical characteristics of infections after fracture fixation are shown in Table 6. On admission to our hospital, in 96 patients (76%) the implant was still in place and implant-associated infections were present. In the remaining 31 patients, predominantly chronic osteomyelitis was diagnosed. The median time from implantation of the internal fixation until the onset of the infection was 10.9 months (range from 0.2 to 618.8 months). Notably, infections after extramedullary fixation occurred considerably earlier than those after intramedullary fixation (i.e. after 7.0 months versus 24.9 months, p=0.027). Acute infection occurring within 6 weeks after fixation was reported in 21 of 125 patients (17%), 104 of 125 (83%) were late-onset infections. The predominant clinical feature of infection was local signs such as erythema, excess heat, swelling, wound dehiscence or sinus tract and was noted in 96 patients (75%). Whereas sinus tracts were more common in infections after intramedullary fixation, wound dehiscence and material on view was documented predominantly in infections after extramedullary fixation. Four patients (3%) presented with fever and 54 (43%) complained of pain. In 39 cases (31%) a concomitant septic arthritis was diagnosed, mostly in the ankle (13 patients), hip (10 patients) and knee (9 patients). At admission 64 patients (58%) presented with an elevated CRP (>10 mg/l). The median CRP was 15.4 mg/l with a range of 0.3 to 334.5 mg/l. Radiological imaging showed nonunion in 53 cases (43%), a loose implant in 22 (23%), sequestration in 4 (3%) and other signs such as edema, sinus tract, chronic osteomyelitis and abscess in 11 cases (9%).

Eighty-six cases (68%) presented with a monomicrobial infection, 19 (15%) with a mixed infection and in 21 cases (17%) all examined samples were culture-negative. In 28 patients (48%) the pathology was positive for an implant-associated infection. The median duration of the hospital stay was 15 days with a range of 3 to 96 days.

Table 6: Infection characteristics

	All patients (n=127)	**Intramedullary fixation (n=47)**	**Extramedullary fixation (n=80)**	**p-value**
Implant involved	96 (76)	26 (62)	67 (84)	**0.010**
Time from implant to onset of infection, median (range) – months	10.9 (0.2 – 618.8)	24.9 (0.5-618.8)	7.0 (0.2-361.7)	0.027
Acute infection (≤6 weeks)	21/125 (17)	5/45 (11)	15/78 (19)	0.314
Chronic infection (>6 weeks)	104/125 (83)	40/45 (89)	63/78 (81)	0.314
Clinical findings				
Fever	5 (4)	4 (9)	1 (1)	**0.062**
Pain	54 (43)	24 (51)	30 (38)	0.143
Local signs of infection [1]	96 (75)	37 (79)	59 (74)	0.669
Sinus tract	46 (36)	23 (49)	23 (29)	**0.035**
Wound dehiscence	42 (33)	10 (21)	32 (40)	**0.033**
Material on view	6 (5)	1 (2)	5 (6)	0.412
Concomitant septic arthritis	39 (31)	12 (26)	27 (34)	0.426
Ankle	13	4	9	
Hip	10	7	3	
Knee	9	1	8	

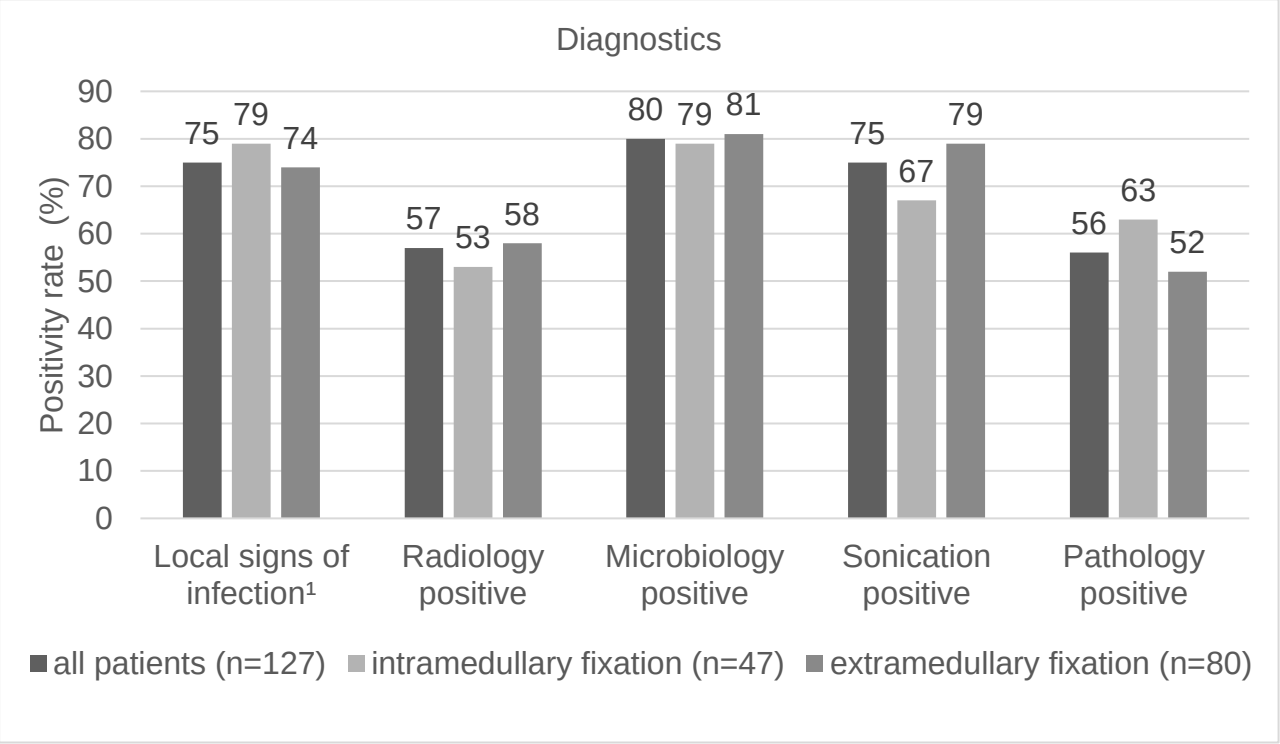
Shoulder	3	0	3	
Elbow	2	0	2	
Wrist	2	0	2	
Median CRP (range), mg/l	15.4 (0.3-334.5)	13.9 (0.7-255.7)	14.8 (0.3-334.5)	0.363
CRP >10mg/l	64/113 (58)	27/45 (60)	37/68 (54)	0.568
Radiology				
Nonunion	53/122 (43)	18/45 (40)	35/77 (45)	0.576
Loose implant	22/96 (23)	8/29 (28)	14/67 (21)	1.000
Sequestrum	4/122 (3)	1/45 (2)	3/77 (4)	1.000
Other [2]	11/122 (9)	4/45 (9)	7/77 (9)	1.000
Microbiology				
Culture-positive	105 (83)	38 (81)	67 (84)	0.809
Monomicrobial	86/126 (68)	31 (64)	55/79 (70)	0.558
Polymicrobial	19/126 (15)	7 (15)	12/79 (15)	1.000
Culture-negative	21/126 (17)	9 (19)	12/79 (15)	0.624
Positive histopathology	28/50 (56)	12/19 (63)	16/31 (52)	0.560
Hospital stay in days, median (range)	15 (3-96)	15 (3-80)	15 (4-96)	0.764

NOTE. Data are no. (%) of episodes, if not indicated otherwise

[1] Patient had at least one of the following symptoms: redness, swelling, sinus tract, wound dehiscence, material on view; [2] among them edema (n=6), sinus tract (n=3), chronic osteomyelitis (n=3), abscess (n=1)

Positivity rate of different definition criteria and diagnostic tools such as clinical presentation, radiology, microbiology, sonication of implant and pathology is shown in Figure 18. Each characteristic is evaluated for the complete cohort (grey), for patients with an intramedullary internal fixation (blue) and for patients with an extramedullary internal fixation (orange). Microbiological analysis in general and sonication of the explanted fixation device showed the highest positivity rate (99 patients, 80%). Local sings of infection confirming infection such as sinus tract and material on view were significantly more often present in infections after intramedullary fixation. Similarly, histopathology confirmed infection more often in infections after intramedullary fixation, however not reaching significance level.

Figure 18: Comparing the diagnostic tools



[1] Including all definitive confirmatory signs for infection such as sinus tract and/or visible purulence around the implant and/or implant on view

Table 7 and Figure 19 show the isolated causative pathogens of the cohort. In 45 cases (43%) *Staphylococcus aureus* was detected, coagulase-negative staphylococci in 29 (28%), gram-negative bacteria in 23 (22%), anaerobes in 11 (11%), enterococci in 10 (10%), streptococci in 6 (6%), fungi in 2 (2%) and others in 5 cases (5%). Comparing the microbiological results of samples harvested at infection site and at the fracture site during index surgery, 17 concordant pathogens and hence persistent infection were documented. The persisting infections were caused by *Staphylococcus aureus* (n=10), gram-negative bacteria (n=5). coagulase-negative staphylococci (n=1) and enterococci (n=1). No significant difference was shown between the two groups regarding persistent infection (57% in the group with intramedullary fixation vs. 67% in the group with extramedullary fixation). Of all implant-associated infections 14 were difficult-to-treat as pathogens resistant to biofilm-active antimicrobial agents or fungi were detected. In 12 of these 14 cases the implant was still in place at admission. Among them, we found 7 ciprofloxacin-resistant gram-negative bacteria, 4 rifampin-resistant staphylococci (3 coagulase-negative staphylococci and one *Staphylococcus aureus*) and 2 *Candida* spp.

Table 7: Causing pathogens

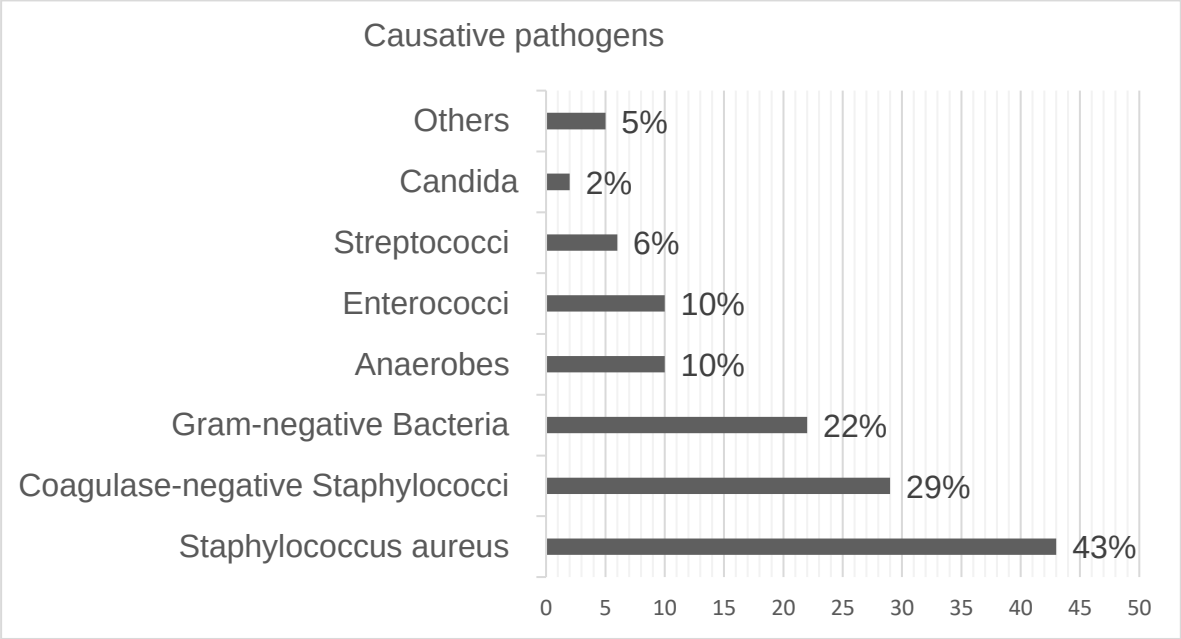
	All patients (n=127)	**Intramedullary fixation (n=47)**	**Extramedullary fixation (n=80)**	**p-value**
S. aureus [1]	45/105 (43)	16/37 (43)	29/67 (43)	1.000
Coagulase-negative staphylococci [2]	30/105 (29)	6/37 (16)	23/67 (34)	0.067
Gram-negative bacteria [3]	23/105 (22)	14/37 (38)	10/67 (15)	**0.014**
Anaerobes [4]	11/105 (10)	5/37 (14)	6/67 (9)	0.515

Enterococci [5]	10/105 (10)	3/37 (8)	7/67 (10)	1.000
Streptococci [6]	6/105 (6)	2/37 (5)	4/67 (6)	1.000
Candida parapsilosis	2/105 (2)	1/37 (3)	1/67 (1)	1.000
Others [7]	5/105 (5)	2/37 (5)	3/67 (4)	1.000

NOTE. Data are no. (%) of episodes, if not indicated otherwise. In patients with polymicrobial infections, more than one pathogen per patient was listed, therefore the sum exceeds 100%

[1] Including 1 strain resistant to rifampin; [2] Including *S. epidermidis* (n=20), *S. lugdunendis* (n=5), *S. caprae* (n=1), *S. haemolyticus* (n=1), *S. hominis* (n=1), *S. warneri* (n=1), *S. capitis* (n=1); among them 3 strains resistant to rifampin; [3] Including *E. coli* (n=6), *Pseudomonas* spp. (n=6), *Klebsiella* spp. (n=5), *Enterobacter* (n=5), *Actinobacter* spp. (n=3), *Proteus mirabilis* (n=2), *Aeromonas species* (n=1), *Serratia marescens* (n=1), *Morganella morganii* (n=1); among them 7 resistant to ciprofloxacin; [4] Including *Cutibacterium acnes* (n=4), *Clostridium* spp. (n=2), *Lactobacilus* species (n=1), *Paenipacillus* species (n=1), *Finegoldia magna* (n=1), *Peptoniphilus asacharolyticus* (n=1), *Anaerococcus praevotii* (n=1); [5] Including *E. faecialis* (n=6), *E. faecium* (n=3), *E. aerogenes* (n=2), *E. absuriae* (n=1), *E. casseliflavus* (n=1); [6] Including *S. pyogenes* (n=2), *S. mitis* (n=1), *S. dysgalacticae* (n=1), *S. vestibularis* (n=1), *S. parasanguinis* (n=1), *S. gordonii* (n=1), *S. sobrinus* (n=1); [7] Including *Bacillus cereus* (n=2), *Corynebacterium simulans* (n=1), *Leifsonia aquatica* (n=1), *Dermobacter hominis* (n=1)

Figure 19: Causative pathogens of the cohort



3.4 Treatment

The surgical and antimicrobial treatment of the patients is summarized in Table 8. All but one patient received surgical treatment. In one case no surgery was performed, due to the expected non-adherence in the post-surgical care. Out of the 126 surgical treatments 40 patients (32%) received debridement and retention of the implant, in 43 cases (34%) the implant was permanently removed, 36 patients received an exchange of the implant performing either a 1-stage (n=16, 13%) or 2-stage procedure (n=20, 16%). In the cases treated with a 2-stage procedure the median time between removal of the internal fixation and implantation of a new device was 70 days (range from 3 to 144 days). In 7 patients (6%) the affected extremity was amputated. When the cohort of patients with no implant (n=31) is seen separately, debridement was done in 28 patients, one patient received a two- staged procedure with a spacer and for two patients an amputation of the affected limb was necessary. The frequencies of the different surgical procedures performed in the two analyzed groups are shown in Figure 20. Whereas infections after intramedullary

fixation were treated more commonly with retentions of the device compared to infections after extramedullary fixation (47% versus 23%, p=0.006), plates and screws were significantly more often exchanged in one stage (16% vs. 6%, p=0.027). In median one surgical procedure was performed, with a range from 0 to 11 surgeries. Antimicrobial treatment was given in 120 cases (94%). The antimicrobial treatment was adequate in 85 (71%) and inadequate in 35 (29%) cases.

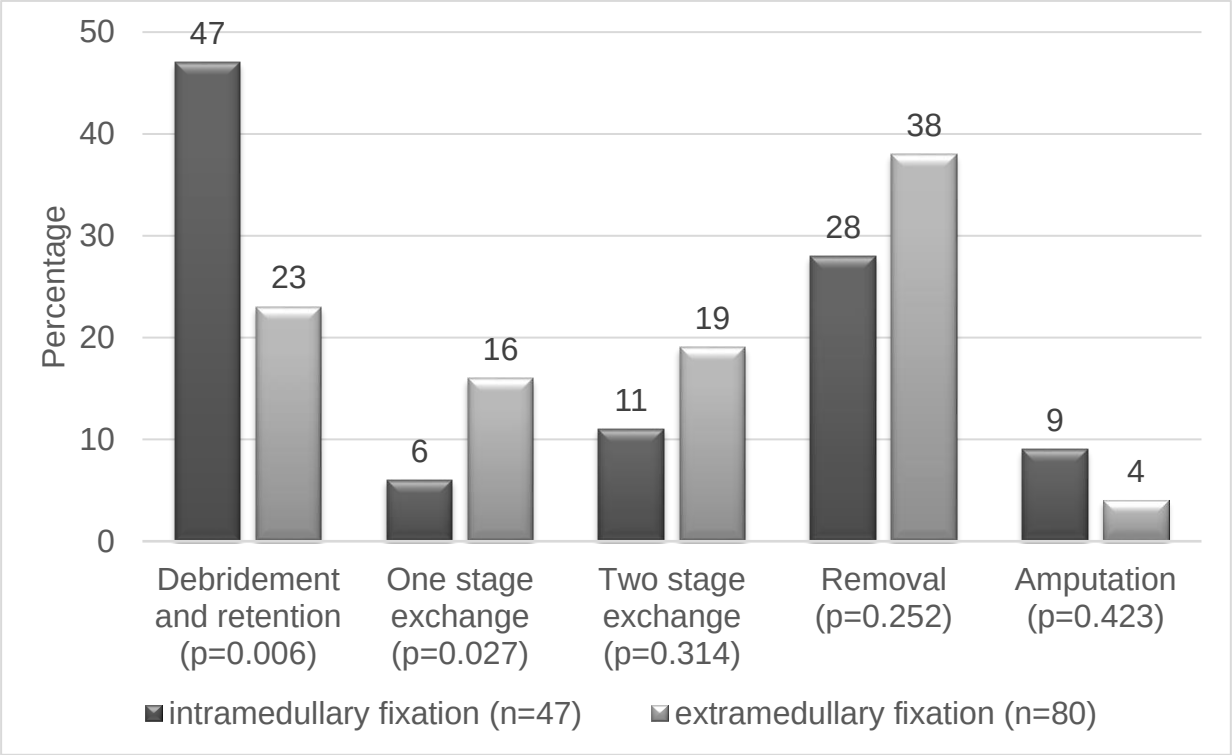
Table 8: Treatment

	All patients (n=127)	**Intramedullary fixation (n=47)**	**Extramedullary fixation (n=80)**	**p-value**
No surgery	1 (1)	-	1/80 (1)	1.000
Surgical treatment	126 (99)	47/47 (100)	79/80 (99)	1.000
Debridement (and implant retention)	40/126 (32)	22/47 (47)	18/79 (23)	**0.006**
Removal	43/126 (34)	13/47 (28)	30/79 (38)	0.252
1-stage	16/126 (13)	3/47 (6)	13/79 (16)	**0.027**
2-stage	20/126 (16)	5/47 (11)	15/79 (19)	0.314
Interval in days, median (range)	70 (3 - 144)	73 (44-117)	70 (3-144)	0.841
Amputation	7/126 (6)	4/47 (9)	3/79 (4)	0.423
Number of surgeries, median (range)	1 (0-11)	1 (1-7)	1 (0-11)	0.960
Antimicrobial treatment	120 (94)	45/46 (98)	75/79 (95)	0.651

Adequate	85/120 (71)	33/45 (73)	52/75 (69)	0.683
Inadequate	35/120 (29)	12/45 (27)	23/75 (31)	0.683

NOTE. Data are no. (%) of episodes, if not indicated otherwise

Figure 20: Performed surgical procedures in patients with intramedullary and extramedullary fixation.



3.5 Outcome analysis

3.5.1 Outcome

Follow-up was available for 111 patients (87%) and the median follow-up time was 5.2 months with a range of 0.2 to 85.9 months. In 78 cases (70%) the infection was cured at follow-up. Failure regarding infection cure occurred in 33 cases (30%), among them 19 had persistent (i.e. caused by the same pathogen plus possibly additional pathogens) and 14 patients relapsing infection. Sixteen (13%) patients were lost to follow-up.

Table 9: Outcome

	All patients (n=127)	**Intramedullary fixation (n=47)**	**Extramedullary fixation (n=80)**	**p- value**
Follow-up	111/127 (87)	41/47 (87)	70/80 (88)	1.000
Time, median (range) - months	5,2 (0,2 - 85,9)	5,2 (0,2-85,9)	5,2 (0,3-83,8)	0.503
Infection success	78/111 (70)	29/41 (71)	49/70 (70)	1.000
Failure	33/111 (30)	12/41 (29)	21/70 (30)	1.000
Persistence[1]	19/33 (58)	9/12 (75)	10/21 (48)	0.160
Reinfection[2]	14/33 (42)	3/12 (25)	11/21 (52)	0.160

NOTE. Data are no. (%) of episodes, if not indicated otherwise

[1] Including *S. aureus* (n=8) among them 1 resistant to rifampin strain, *Enterococcus* spp. (n=3), *Pseudomonas aeruginosa* (n=2), *Cutibacterium acnes* (n=1), *S. lugdunensis* (n=1);

[2] Including *S. aureus* (n=8) among them 1 resistant to rifampin strain, *Enterococcus* spp. (n=2), *Cutibacterium acnes* (n=1), coagulase-negative staphylococcus (n=1), *Prevotella disiens* (n=1), *Klebsiella pneumoniae* (n=1)

Subanalysis was performed to compare the outcome of patients treated before and after implementation of the standardized comprehensive treatment in April 2013 (see Table 10). Follow-up of patients before the implementation of the concept was available for 37 of 43 (90%) patients, among them successful treatment was documented for 20 (54%) patients. Failure regarding infection cure occurred in 17 cases (46%), among them 9 had persistent and 8 patients relapsing infection.

In the patient cohort between 2013 and 2017, 74 (86%) patients were available for a follow-up: 58 (78%) patients had a successful treatment and in 16 (22%) cases the treatment failed. The infection failure was due to a persistent infection in 10 cases and due to a reinfection in 6 cases.

Table 10: Comparison of infection outcome before and after implementation of a comprehensive diagnostic and therapeutic algorithm

	Before (2010-2012) (n=43)	**After (2013-2017) (n=84)**	**p- value**
Follow-up available	37/43 (90)	74/86 (86)	1.000
Infection success	20/37 (54)	58/74 (78)	**0.015**
Failure	17/37 (46)	16/74 (22)	**0.015**
Persistence	9/17 (53)	10/16 (63)	0.728
Reinfection	8/17 (47)	6/16 (38)	0.728

NOTE. Data are no. (%) of episodes, if not indicated otherwise

When comparing the success of the patients before and after the implementation of the standardized treatment concept a significant improvement was seen. (p=0.015) The overall success was defined as absence of an infection and at the same time no impaired function of the limb.

3.5.2 Analysis of treatment failures

Out of the 127 patients 33 (30%) had a treatment failure concerning infection eradication i.e. persistent or a new infection. In this group of treatment failure were 24 men (71%). In 28 cases (85%) the lower and in 5 cases (15%) the upper extremity was affected. 10

patients (30%) suffered of a concomitant septic arthritis. The reason for the internal fixation was for 22 patients (65%) a fracture (open fracture n=14, closed fracture n=8). Six patients (18%) suffered from a polytrauma. A chronic infection was present in 26 (79%) and an acute infection in 6 (18%) cases. Local signs of infection were seen in 26 patients (79%), the CRP was elevated (>10 mg/l) in 16 patients (47%). The microbiological results were positive in 29 cases (monomicrobial n=25, polymicrobial n=4). Detected pathogens were *Staphylococcus aureus* (n=12, 41%), coagulase-negative staphylococci (n=6, 21%), gram-negative bacteria (n=6, 21%), enterococci (n=4, 14%), anaerobes (n=2, 7%), streptococci (n=2, 7%), *Lactobacillus* (n=1, 3%) and among them were 3 were resistant to biofilm-active antimicrobials. Four cases (12%) remained culture-negative. The surgical treatment was debridement for 14 patients (42%), removal of the implant for 7 (21%), 1-stage procedure for 6 (18%) and 2-stage procedure for 5 (15%) patients. Of interest, in those 11 patients undergoing debridement and implant retention, the treatment failed in terms of infection eradication in 5 out of 6 cases with chronic infections, whereas the treatment was successful in 4 out of 5 patients with acute infection. The antimicrobial treatment was adequate in 7 and inadequate in 4 cases.

The univariate analysis of patient-, procedure-, infection- and treatment-associated risk factors showed immediate internal fixation, inadequate antimicrobial treatment and treatment before implementation of a standardized treatment concept to be a risk factor for infection failure (Table 11). Patients with an immediate external fixation showed a higher rate of treatment failure (44%), compared to the patients with immediate internal fixation (20%) (p=0.02). Among the patients with an infection failure, 16 (52%) received inadequate antimicrobial treatment. There was a significant difference (p=0.003) in the treatment outcome, whether the patient got an adequate or inadequate antimicrobial treatment. In the group treated before the implementation of a standardized treatment

concept, infection failure occurred in 46% of the patients and in those treated after implementation, infection failure was noted in 22% (p=0.02).

Table 11: Risk factor analysis of 33 cases with infection failure

	Factor present (No. of patients with factor + infection failure/ no. of patients with factor)	**Factor absent** (No. of patients without factor + infection failure/ no. of patients without factor)	**p-value**
Age >70 years	5/16 (31)	28/95 (29)	1.000
Diabetes mellitus	6/17 (35)	27/94 (29)	0.576
BMI >30 kg/m²	10/27 (37)	23/80 (29)	0.473
Concomitant septic arthritis	10/33 (26)	23/78 (29)	0.218
Upper extremity	5/13 (38)	28/111 (25)	0.329
Open fracture	14/40 (35)	10/38 (26)	0.467
Polytrauma	6/18 (33)	12/51 (24)	0.534
Immediate internal fixation	17/83 (20)	12/27 (44)	**0.023**
Positive microbiology at index surgery	8/25 (32)	5/19 (26)	0.749
Intramedullary fixation	12/43 (28)	21/84 (25)	0.256
Acute infection	6/21 (29)	26/104 (25)	0.786
Chronic infection	26/104 (25)	7/23 (30)	0.605
CRP >10 mg/l	16/64 (25)	10/49 (20)	0.655
Local signs of infection	15/52 (29)	18/75 (24)	0.545

Sinus tract	11/46 (24)	21/81 (26)	0.835
Intraoperatively visible pus	13/36 (36)	20/91 (22)	0.119
Surgical therapy			
Implant retention	6/10 (60)	27/101 (27)	0.062
One-stage exchange	7/14 (50)	26/97 (27)	0.115
Two-stage exchange	5/19 (26)	28/92 (30)	0.790
Debridement	8/24 (33)	25/87 (29)	0.801
Monomicrobial infection	25/74 (34)	8/35 (23)	0.273
Polymicrobial infection	4/18 (22)	29/91 (32)	0.776
Culture -negative infection	4/17 (24)	29/92 (32)	0.580
Infection with *S. aureus*	13/44 (30)	20/67 (30)	1.000
Infection with gram-negative bacteria	10/26 (38)	23/85 (27)	0.328
Difficult-to-treat infections	4/14 (29)	29/97 (30)	1.000
Inadequate antibiotic treatment	16/31 (52)	17/79 (22)	**0.003**
Before implementation of standardized treatment concept	17/37 (46)	16/74 (22)	**0.015**

NOTE. Data are no. (%) of episodes, if not indicated otherwise

In Tables 12 and 13 separate risk factors of treatment failure for patients with initial intramedullary and extramedullary fixation are shown. For the patients with treatment failure after intramedullary fixation no significant risk factors was found. The analysis showed two significant risk factors for the patient cohort with extramedullary fixation. In

this group a treatment failure was significantly more often if the fixation device remained in situ and if there was inadequate antimicrobial treatment. Patients with an extramedullary fixation and retention of the implant had a higher rate of failure (80%) than patients with a different surgical therapy (26%). The outcome was significantly different (p=0.03). Inadequate antimicrobial treatment was also a risk factor for patients with extramedullary fixation (p=0.02).

Table 12: Risk factor analysis of cases with infection failure (n= 12), intramedullary fixation

	Factor present	**Factor absent**	**p-value**
Age >70 years	3/5 (60)	9/36 (25)	0.140
Diabetes mellitus	3/6 (50)	9/35 (26)	0.334
BMI >30 kg/m²	2/7 (29)	10/31 (32)	1.000
Concomitant septic arthritis	2/9 (22)	10/32 (31)	0.702
Upper extremity	0	12/40 (30)	1.000
Open fracture	7/22 (32)	3/11 (27)	1.000
Polytrauma	2/8 (25)	3/14 (21)	1.000
Immediate external fixation	4/8 (50)	7/27 (26)	0.226
Immediate internal fixation	7/27 (26)	4/8 (50)	0.226
Positive microbiology at index surgery	2/6 (33)	2/9 (22)	1.000
Acute infection	1/5 (20)	11/36 (31)	1.000
Chronic infection	11/36 (31)	1/5 (20)	1.000
CRP > 10 mg/l	7/22 (32)	4/17 (23)	0.725

Local signs of infection	8/19 (42)	4/22 (18)	0.168
Sinus tract	5/22 (23)	7/19 (37)	0.493
Intraoperatively visible pus	6/14 (43)	6/27 (22)	0.278
Surgical therapy			
Implant retention	2/5 (40)	10/36 (28)	0.620
One-stage exchange	1/2 (50)	11/35 (31)	1.000
Two-stage exchange	1/5 (20)	11/36 (31)	1.000
Monomicrobial infection	10/27 (37)	2/14 (14)	0.170
Polymicrobial infection	2/7 (26)	10/34 (29)	1.000
Culture -negative infection	0	12/34 (35)	0.543
Infection with *S. aureus*	7/18 (39)	5/23 (22)	0.307
Infection with gram-negative bacteria	4/12 (33)	8/29 (28)	0.721
Difficult-to-treat infections	1/2 (50)	10/37 (27)	0.490
Inadequate antibiotic treatment	5/10 (50)	6/29 (21)	0.109
Before implementation of standardized treatment concept	6/12 (50)	6/29 (21)	0.128

NOTE. Data are no. (%) of episodes, if not indicated otherwise

Table 13: Risk factor analysis of cases with infection failure (n=21), extramedullary fixation

	Factor present	**Factor absent**	**p-value**
Age >70 years	2/11 (18)	19/59 (32)	0.485
Diabetes mellitus	3/11 (27)	18/59 (31)	1.000

BMI >30 kg/m²	8/20 (40)	14/50 (28)	0.397
Concomitant septic arthritis	9/25 (36)	14/47 (30)	0.606
Upper extremity	5/13 (38)	16/57 (24)	0.511
Open fracture	7/16 (44)	8/25 (32)	0.517
Polytrauma	4/9 (44)	9/28 (32)	0.691
Immediate external fixation	8/17 (47)	10/44 (23)	0.115
Immediate internal fixation	10/44 (23)	8/17 (47)	0.115
Positive microbiology at index surgery	6/18 (33)	3/7 (43)	0.673
Acute infection	5/13 (38)	15/55 (27)	0.503
Chronic infection	15/55 (27)	5/13 (38)	0.503
CRP >10 mg/l	9/33 (27)	6/26 (23)	0.771
Local signs of infection	7/25 (28)	14/45 (31)	1.000
Sinus tract	6/19 (32)	15/51 (29)	1.000
Intraoperatively visible pus	7/15 (47)	14/55 (25)	0.126
Surgical therapy			
Implant retention	4/5 (80)	17/65 (26)	**0.026**
One-staged exchange	6/12 (50)	15/58 (26)	0.163
Two-staged exchange	4/14 (29)	17/56 (30)	1.000
Monomicrobial infection	15/44 (34)	6/23 (26)	0.587
Polymicrobial infection	2/10 (20)	19/57 (33)	0.487
Culture -negative infection	4/13 (31)	17/54 (31)	1.000
Infection with *S. aureus*	6/26 (23)	15/44 (34)	0.422
Infection with gram-negative bacteria	6/14 (43)	15/56 (27)	0.329

Difficult-to-treat infections	3/12 (25)	19/60 (32)	0.744
Inadequate antibiotic treatment	11/21 (52)	11/50 (22)	**0.023**
Before implementation of standardized treatment concept	11/25 (44)	10/43 (23)	0.103

NOTE. Data are no. (%) of episodes, if not indicated otherwise

4 Discussion

4.1 Summary of results

We reviewed 127 patients (89 men, 38 women) with infection after internal fixation with a median age of 53 years (range, 19 to 89 years). In 87% the lower extremity was affected. An acute infection (≤6 weeks after internal fixation) was documented in 21 (17%), chronic infection was present in 104 patients (83%). At admission, 4% had fever, 43% pain and 75% local signs of infection (swelling, erythema, sinus tract, wound dehiscence, material on view). Infections were monomicrobial in 86 (68%), mixed in 19 (15%) and culture-negative in 21 patients (17%). Most common pathogens were *Staphylococcus aureus* (43%), coagulase-negative staphylococci (29%) and gram-negative bacteria (22%). Debridement (with retention of the implant, if applicable) was performed in 40 patients (32%), device removal in 43 (34%), one-stage exchange of osteosynthesis in 16 (13%) and two-stage exchange in 20 patients (16%). In one patient, no surgery and in 7 patients, amputation of the affected limb was performed. The median follow-up time was 5.2 months (range 0.2-86 months), 16 (13%) were lost to follow-up. Relapsing or persistent infection was observed in 33 patients (30%). Among the 78 infection-free patients, 26 (33%) reported impaired functional outcome (nonunion, Girdlestone situation or amputation of the limb).

4.2 Interpretation of results

4.2.1 Interpretation of patient and infection site characteristics

The described patient cohort depict a typical traumatological population with a predominance of male sex (70%), mostly otherwise healthy and with a median age of 53 years. In average, patients had two revisions on the affected extremity before they were

admitted because of infection to our hospital. A quarter of the patients suffered from comorbidities, only 18 (14%) of diabetes mellitus. The latter was not a significant risk factor, as opposed to the results of the study of Kuehl et al., who detected diabetes mellitus as a significant risk factor (89). Already in 55% of the index surgeries a positive microbiological result was present. Unfortunately, data on the taken consequences based on these results is missing and the impact on applied preemptive treatment could not be assessed.

4.2.2 Interpretation of infection characteristics

At diagnosis of infection, most of the patients (76%) still had the implant in place. The median time from the initial implantation of the internal fixation until the onset of infection was 10.9 months (327 days). In contrast to our results a similar study of Trampuz et al. from 2005 showed a median time interval of 44 days between the index surgery and the diagnosis (45). A possible explanation for this observation may be the better detection of low grade infections manifesting delayed or late by improved knowledge of diagnostic methods and available novel microbiological methods (e.g. sonication). In our study 104 patients (83%) were admitted with a chronic infection. The classification in acute (≤6 weeks) and chronic (>6 weeks) was applied because the implemented treatment algorithm included this gradation. In contrast to that Metsemakers et al. postulated that for the definition of fracture-related infections there should be no subdivision due to a lack of scientific evidence and for the reason of simplification. However, Metsemakers et al. described a consent regarding different entities in acute and chronic infections and therefore different treatment approaches (26). In the resent study of Kuehl et al. instead a classification in to early (0-2 weeks after implantation), delayed (3-10 weeks) and late (>10 weeks) was chosen to depict the different clinical presentations and variation of

pathogens (89). Overall, our study showed that acute or chronic infections are not a significant risk factor for a treatment failure. Further subanalysis were not reasonable due to the small cohort of patients with an acute infection and available follow-up (n=18).

Of note, infections after extramedullary fixation presented significantly earlier than the infections after intramedullary fixation (median of 7 vs 24.9 months, p=0.027). Reasons for this observation may be the extent of soft tissue involvement causing an earlier manifestation of the, in most cases perioperatively acquired infection, in infections after extramedullary fixation. In our study 75% of the patients had local signs of infection such as redness, swelling, wound dehiscence, sinus tract or material on view. Patients with an intramedullary fixation presented more often with a sinus tract, a wound dehiscence was more common when an extramedullary device was used. In the diagnostics of a periprosthetic joint infection sinus tract with material on view is a major criteria (90, 91). In the patient cohort with an extramedullary internal fixation the implant was significantly more often already explanted before the diagnosis of an infection, respectively referral to our institution, than in cases with an intramedullary fixation (84% vs. 62% p=0.010). Pain was not even mentioned in half of the cohort (43%). An elevated level of CRP (>10 mg/dl) was detected in 64 cases (58%). Due to the low sensitivity of a single CRP value, the dynamics of this laboratory test should be followed (40). The radiological imaging was postulated as important for the diagnosis of internal fixation-associated infection, for evaluation of the condition of the implant and for the healing process of the bone (40, 55). Our study detected nonunion in 43%, a loose implant in 23%, sequestrum in 3% and other signs in 9% of the cases. This supports the consent that radiological imaging is a supportive diagnostic tool, but mostly not able to detect the infection by itself. Histopathology was only done in 58 cases, 28 (48%) of those were positive. This low sensitivity in our small cohort of histopathological testing stays in contrast to the study of

Morgenstern et al., who postulated an 80% sensitivity of histopathological results (81). In comparison of the different diagnostic tools, microbiology was most sensitive, with a positive detection rate of 80%.

The infections were mainly caused by a single pathogen (n=86, 68%). In a few cases the infections were polymicrobial (15%) and in 21 (16%) cases no pathogen was detected. This is in line with the study of Kuehl et al. showing polymicrobial infections in 29.8% (89). Among the detected pathogens *Staphylococcus aureus* was found in 43%, coagulase-negative staphylococci in 39% and gram-negative bacteria in 22%. All other pathogens were less common. This correlates with other studies, which detected *Staphylococcus aureus* as the most frequent pathogen, coagulase-negative staphylococci as the second commonest, followed by gram-negative bacteria.(45, 92, 93) In the study of Torbert et al. the second commonest pathogen were gram-negative bacteria (94). Kuehl et al. showed a differentiation of the pathogen pattern in terms of early or late infections. In their study *Staphylococcus aureus* was as well the most common pathogen (42%) followed by Enterobacteriaceae (27%) in early infections, coagulase-negative staphylococci (39%), anaerobes (17%) and streptococci (11%) were more frequent in late infections (89).

4.2.3 Interpretation of treatment

The treatment of internal fixation device-associated infections consists of a surgical therapy on the one hand and an antimicrobial treatment on the other hand. The goal of the treatment is the consolidation of the bone and the prevention of a chronic infection. In contrast to periprosthetic joint infections, infection eradication is not the treatment target in all patients. The option of infection suppression until removal of the implant to allow for bone healing with subsequent explanation of the foreign material is a feasible strategy for specific situations (2, 5, 85, 95, 96). Even though conservative therapy is approached,

debridement with removal of dead tissue is necessary and needs to be performed in every case (2).

In our patient cohort 99% received a surgical treatment. Metsemakers et al. declare that sole antimicrobial treatment is an option in an early infection (0-2 weeks) (1). One patient of our cohort did not receive a surgical therapy due to expected non-compliance and non-adherence in the post-surgical care and follow-up. For this patient, the treatment plan was a suppression of the infection until the implant could be removed. Eventually the patient was lost to follow-up as expected.

If the infection is acute, the implant is stable and the bone is adequately repositioned but not consolidated yet, the treatment option is debridement and retention of the implant in addition to an antimicrobial treatment for 12 weeks (see Figure 16). In 40 cases of our study this regime was done; significantly more often in patients with an intramedullary fixation (47%) than in those with an extramedullary fixation (23%, p=0.006)). Retention of the implant might have been more often possible in cases with an intramedullary fixation due to less wound dehiscence and therefore a higher chance of primary skin closure. (see Figure 16) From pathogenetic point of view, no adequate debridement of the medullary canal is possible in this situation and therefore it is not considered first choice when an intramedullary implant is in place. In the prospective observational cohort study of Tschudin-Sutter et al. all patients received debridement, retention of the implant and antibiotic treatment. In their study it was possible to choose this treatment plan due to the strict inclusion criteria for the 233 patients, including acute symptoms (≤3 weeks), intact soft tissue and only causative pathogens which were susceptible to antimicrobial agents (93). No discrimination of intramedullary and extramedullary implants was done in this analysis. In our analysis regarding risk factors for failure, retention of the implant was seen to be associated with higher probability of infection failure, due to the low number of

patients it only reached statistically significant level in patients with extramedullary fixation and not in intramedullary fixation devices. These observations need to be interpreted carefully, as the biofilm age and its impact on treatment strategy is not taken in to account, i.e. also chronic infections with a mature biofilm were treated with retention.

If the bone is already consolidated, removal of the implant in combination with an antimicrobial therapy for 6 weeks is a convenient treatment strategy. In our study 43 patients received this treatment option. Metsemakers et al. describe that there is a high chance of clearing the infection once the implant with its biofilm is removed (1). Trampuz et al. also recommended a complete removal and external fixation in cases of resistant or difficult-to-treat pathogens (2). In contrast to that Al-Mayahi et al. did not see different results whether the implant was retained or removed (92).

Another treatment option is the exchange of the implant, which can be performed in a one-stage or two-stage procedure. A one-stage surgery was done for 16 and a two-stage procedure for 20 of our patients. The treatment algorithm recommends a one-stage exchange in a chronic infection, with the presence of good soft tissue condition and the absence of difficult-to-treat pathogens. The one-stage surgery was significantly more often performed in the cases with an extramedullary fixation (16% versus 6%, p=0.027). A two-stage exchange is to be done in an acute infection with an instable implant, a poor repositioning of the bone and a bad soft tissue condition and also in a chronic infection with compromised soft tissue and difficult-to-treat pathogens.

7 of our 127 patients received amputation of the infected limb. In rare cases with severe infections and repeated treatment failures, this might be the only option (1).

The antimicrobial treatment was classified into adequate and inadequate through the current knowledge of bioavailability and bactericidal effects of the antimicrobial agents and additionally regarding the duration of application. An adequate antimicrobial

treatment was performed in 71%. The implemented standardized treatment algorithm at our hospital advices to give antibiotics for 6 weeks when the implant is removed, otherwise for 12 weeks or in cases of suppressive treatment until the implant is removed (see Figure 16). Kuehl et al. used an antimicrobial treatment concept for 12 weeks which was derived from the treatment plan of prosthetic-joint infections by Zimmerli et al. (60, 89) and similar to the one applied in our cohort.

4.3 Outcome analysis

We had a follow-up of 111 patients (87%) with a median follow-up time of 5.2 months. The overall success rate was 48%, 41% in the cohort of patients with an intramedullary internal fixation and 51% in the cohort of patients with an extramedullary internal fixation. In comparison to that Tschudin-Sutter et al. had a higher success rate of 90% in the prospective observational cohort study. This significantly better result was amongst other things possible to achieve due to the strict inclusion criteria of the study (93). In the retrospective cohort study of Trampuz et al. a success rate of 88% after two years of follow-up was achieved (45). The success rate of 87-90% in the study of Kuehl et al. was amongst other things possible due to strict exclusion criteria. Patients who suffered of a septic nonunion or received an amputation were not included in their study (89). In contrast, in our study we assessed the functional outcome in patients who had a successful infection eradication. Among the patients of our study with an infection success (n=77), were 25 with a function failure which was defined as nonunion, a Girdlestone situation or amputation. In the patient cohort with an infection failure, 59% were due to a persistent infection and 41% due to a reinfection with other pathogens. Those stricter inclusion criteria of the above-mentioned studies can bereasons why the success rate of our study cohort is inferior compared to theirs.

When the follow-up was analyzed separately for the time period before and since the implementation of the comprehensive treatment algorithm the success rate varied significantly. It increased from a 33% success rate before 2013 to a 56% success rate since the implementation of the standardized treatment algorithm. This was a significant improvement of the outcome (p=0.03). The question is still, why the success rate is roughly above 50%. In our analysis, following risk factors were significant: initial external fixation (p=0.04), inadequate antibiotic treatment (p=0.03) and no standardized treatment concept (p=0.02). It can be discussed if the initial external fixation as itself is a risk factor, or if the circumstances leading to an initial external fixation are the risk factors. Probably patients with a more complicated fracture, a worse soft tissue condition and maybe a polytrauma do not receive an internal fixation right away, but an immediate external fixation. All these aspects may lead to a treatment failure. The risk factors of the inadequate antimicrobial treatment and the absence of a comprehensive treatment algorithm partly overlap and could be one risk factor. The comprehensive treatment concept considered the duration of the infection and the maturity of the biofilm. In cases of a young biofilm retention of the implant was a possible treatment concept and on the other hand exchange of the implant was recommended in chronic infections. These recommendations were leaned on the treatment concept of periprosthetic joint infections (60, 96). Of interest, one-staged exchange of the implant was seen to be a risk factor (p=0.03) and crystallized as one of the significant risk factors for failure in infections after extramedullary fixation (p=0.01). Overall, there was no significant difference in the overall and infection success between the patients of intramedullary an extramedullary internal fixation.

4.4 Evaluation of the new treatment concept

Since the implementation of the standardized treatment concept, the infection and functional outcome was improved significantly (55% overall success rate versus 35%, p=0.03). The implementation of the new concept might have shown a higher success rate due to the interdisciplinary cooperation in the team. A team of experienced and specialized experts was put together. The treatment concept included amongst other things knowledge of the maturity of the biofilm and suitable antibiotic treatment. Still the overall success rate was not as good as desirable. A reason for this might be, the distorted patient cohort. It is to be expected that there was a higher amount of severe and complex cases at our institution, as national referral center for musculoskeletal infections.

4.5 Impact for clinical practice

This study shows that it is necessary to reevaluate the diagnostic and treatment strategies of internal fixation-associated infections in the clinical practice. The diagnosis is difficult and therefor clinical, radiological, microbiological, laboratory and histopathological results have to be considered. Because of the lack of symptoms, the infection is often diagnosed delayed, especially in cases with an intramedullary fixation. The importance of microbiology and sonication must be emphasized. Therefor the samples must be collected adequately to achieve informative results.

The surgical treatment must be chosen, regarding to the state of the bone and its stability. Because inadequate antimicrobial treatment was detected as a risk factor, a correct antimicrobial treatment strategy is essential. This means that the antimicrobial susceptibility testing (antibiogram), the bioavailability and the duration of the application have to be taken into account. Furthermore, the antimicrobial treatment needs to fit the

surgical treatment plan. For example, in cases of retention of the implant the antimicrobial substances need to be biofilm active.

4.6 Limitations of this study

We acknowledge the following limitations to our study. Due to a retrospective study design, we face missing data.

Additionally, the patient cohort was heterogeneous regarding the cause for internal fixation with a predominance of fracture related indications and the infection type with a predominance of chronic infections.

The main limitation of this study is that there was only a short-term and passive follow-up. This needs to be considered due to the conclusion we make and the clinical recommendations we give, based on the follow-up data. The time span of the follow-up ended with the second incident in the patient's medical history after the initiation of the treatment at our institution. Therefor the time span was often short in cases of an immediate failure due to a new or recurrent infection. The follow-up also does not take into account possible long-term failures – recurrent infections - of patients, who were counted as infection free during our study. It also does not consider the possible low sensitivity of the diagnostic tools. The follow-up collected data on objective outcomes such as infections, nonunion, amputation and arthrodesis of the bone. A subjective functional impaired outcome was not considered. Only a passive follow-up was performed due to the assumption that patients with further complications or ongoing infections would return to our tertiary healthcare center due to the complexity of their cases. Nevertheless, we cannot make certain theses about the patients who were lost to follow-up.

Larger patient population size in each of the categories examined and an approximately equal distribution in both cohorts could have provided additional power to our conclusions.

Furthermore, we cannot rule out unmeasured variables as possible incompliance with the treatment regime. Treatment could only be assessed during hospitalization; after discharge of the patient we were not able to monitor the compliance.

5 Conclusion

We described clinical features and outcome of infections after fixation of long bones in a complex patient population of a referral center for septic surgery. Approximately half of the infections after internal fixation of long bones failed in terms of infection eradication or restoration of function. No significant differences between intramedullary and extramedullary internal fixation was observed in infection success. After implementation of an interdisciplinary team applying a standardized surgical and antibiotic treatment concept, the infection outcome improved significantly. Even if the infection free survival could be improved, the failure rate is still too high. More research for diagnostic tools and treatment options is needed.

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Statutory Declaration

“I, Pia Carolin Vössing, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Evaluation of infections after internal fixation of long bones – clinical characteristics and outcome analysis from a retrospective study”, 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.

[In the case of having conducted your doctoral research project completely or in part within a working group:] 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

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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

Curriculum vitae

For data protection reasons, my curriculum vitae will not be published in the electronic version of my work.

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