Advances in Experimental Medicine and Biology 971
Advances in Microbiology, Infectious Diseases and Public Health

Lorenzo Drago *Editor*

A Modern Approach to Biofilm-Related Orthopaedic Implant Infections

Advances in Microbiology, Infectious Diseases and Public Health Volume 5

Springer

Advances in Experimental Medicine and Biology

Advances in Microbiology, Infectious Diseases and Public Health

Volume 971

The Advances in Microbiology, Infectious Diseases and Public Health Series will provide microbiologists, hygienists, epidemiologists and infectious diseases specialists with well-choosen contributed volumes containing updated information in the areas of basic and applied microbiology involving relevant issues for public health, including bacterial, fungal and parasitic infections, zoonoses and anthropozoonoses, environmental and food microbiology. The increasing threat of the multidrug-resistant microorganisms and the related host immune response, the new strategies for the treatment of biofilm-based, acute and chronic microbial infections, as well as the development of new vaccines and more efficacious antimicrobial drugs to prevent and treat human and animal infections will be also reviewed in this series in the light of the most recent achievements in these fields.Special attention will be devoted to the fast diffusion worldwide of the new findings of the most advanced translational researches carried out in the different fields of microbiological sciences, with the aim to promote a prompt validation and transfer at clinical level of the most promising experimental results. The book series publishes review and original research contributions, short (data) reports as well as guest edited thematic book volumes. All contributions will be published online first and collected in (thematic) book volumes. There are no publication costs.This series is a subseries of Advances in Experimental Medicine and Biology 2015 Impact Factor: 1.953 Advances in Experimental Medicine and Biology has been publishing exceptional works in the field for over 30 years and is indexed in Medline, Scopus, EMBASE, BIOSIS, Biological Abstracts, CSA, Biological Sciences and Living Resources (ASFA-1), and Biological Sciences.

More information about this series at http://www.springer.com/series/13513

Lorenzo Drago
Editor

A Modern Approach to Biofilm-Related Orthopaedic Implant Infections

Advances in Microbiology, Infectious Diseases and Public Health Volume 5

Springer

Editor
Lorenzo Drago
Department of Biomedical Sciences Health
University of Milan
Milan, Italy

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-52273-9 ISBN 978-3-319-52274-6 (eBook)
DOI 10.1007/978-3-319-52274-6

Library of Congress Control Number: 2016935504

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 1–13
DOI 10.1007/5584_2016_158

Published online: 19 October 2016

The Concept of Biofilm-Related Implant Malfunction and "Low-Grade Infection"

Carlo Luca Romanò, Delia Romanò, Ilaria Morelli, and Lorenzo Drago

Abstract

Biofilms have a tremendous impact on industrial machines working in moist environments, while in biological systems their effect is further complicated by the host's response.

Implant-related infections are a complex process, starting with bacterial adhesion and biofilm formation, followed by the variable interaction between host, implant, microorganisms and their by-products. Depending on the balance of these factors, different clinical presentations are observed, which may eventually, at times, shift from one into the other.

–"Implant malfunction" displays only mild clinical signs/symptoms – light pain and/or slight soft tissue contracture or functional impairment – with negative infection/inflammatory markers; it requires prolonged cultures, antibiofilm and eventually genomic investigations for pathogen detection;
–"Low-grade infection" features recurrent or persistent pain and/or soft tissue contracture with various functional impairment and mixed positive/negative markers of infection/inflammation; pathogen identification requires prolonged cultures and antibiofilm techniques;
–"High-grade infection" displays classical signs/symptoms of infection/inflammation with positive tests; pathogen identification is often possible with traditional microbiological techniques, but is better achieved with prolonged cultures and antibiofilm processing.

C.L. Romanò (✉)
Center for Reconstructive Surgery of Osteoarticular Infections (CRIO) and Milano Biofilm Center (MBM), I.R.C.C.S., Galeazzi Orthopaedic Institute, Milan, Italy
e-mail: carlo.romano@grupposandonato.it

D. Romanò and I. Morelli
Center for Reconstructive Surgery of Osteoarticular Infections (CRIO), I.R.C.C.S., Galeazzi Orthopaedic Institute, Milan, Italy
e-mail: delia-romano@libero.it; ilaria.morelli@gmail.com

L. Drago
Laboratory of Clinical Chemistry and Microbiology and Milano Biofilm Center (MBM), I.R.C.C.S., Galeazzi Orthopaedic Institute, Milan, Italy
e-mail: lorenzo.drago@unimi.it

Understanding biofilms-related clinical presentations is crucial for physicians, to implement the best diagnostic and therapeutic measures, and for regulatory bodies, to define the evaluation process of technologies aimed at reducing implants' malfunctions and infections, like anti-adhesive and antibiofilm coatings, that should be regulated as (part of) medical devices, requiring a suitable post-marketing surveillance.

Only an effective antibiofilm-targeted approach from all players will hopefully allow the medical community to mitigate the current unacceptable social and economical burden of implant-related infections and malfunctions.

Keywords

Biofilm • Implant • Malfunction • Infection • Low-grade infection

1 Introduction

Biofilms are defined as communities of microbial cells and intercellular matrix, attached to surfaces in moist environments, while biofouling or biological fouling is the accumulation of biofilms on wetted surfaces.

Leeuwenhoek (1684), using his primitive light microscope, found microbes attached to tooth surfaces, forming sessile communities, which could be considered as the first observation of microbial biofilms.

Biofilms are probably the prevalent mode of life for microorganisms in nature, but it was not until the 1920s that the concept of bacterial biofilms was formulated. Angst (1923) observed that the number of marine bacteria on the surface of ships hulls was higher than the surrounding floating cells, and proposed that bacterial biofilms led to serious corrosions of ships hulls. By the 1980s, bacteria were observed on the solid surfaces of many ecological environments including waste water treatment systems, industrial water systems, equipment used to manufacture vinegar, etc.

Now we know that the general principles of biofilm formation and factors leading to settlement on hard surfaces are similar in medical, marine and industrial applications (Bixler et al. 2014).

In the industrial and marine setting biofouling has a well known impact on performance, causing biodegradation, malfunction and finally energy loss (Fig. 1) and hundreds billion dollar costs every year.

In the industrial environment, biofouling is often associated with microbiologically influenced corrosion (MIC) or biocorrosion and inorganic fouling. Biocorrosion is the degradation of materials, usually metals, due to the activity of microorganisms (Table 1), while inorganic fouling is the accumulation of non biological particles, that may form in addition to or indipendently from biofouling.

On the other hand, in the medical setting, and particularly in implant-related infections, the effects of bacterial adhesion and biofilm formation on tissues and implanted biomaterials are complicated by the variable host's response, while consequences are equally devastating.

In fact, it is estimated that 20 % of fatalities worldwide are due to infectious diseases, of which approximately 80 % are biofilm-related (Harrison et al. 2005; Prentice et al. 2004), including the great majority of chronic bacterial and fungal infections and virtually all biomaterials-associated infections (Gristina et al. 1990; Romanò et al. 2014; Stoodley et al. 2011; Nana et al. 2016).

The presence of biofilms makes both the diagnosis and treatment particularly challenging (Drago et al. 2013; Romanò et al. 2013a, b), given the protection offered by the extra-cellular matrix to the microorganisms. In fact, bacterial

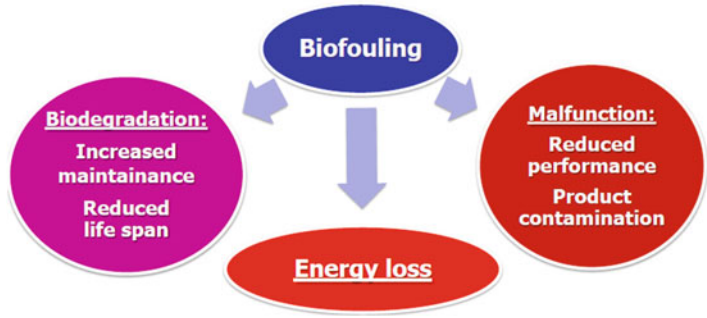


Fig. 1 Effect of biofouling on working machines and systems. Main detrimental effects on performance concern implants degradation and malfunction and energy loss. The economical impact in the industrial field ranges from 10 to >30 % of the operating costs, depending on different settings and reports (see text for more details)

Table 1 Mechanisms (Coetser and Cloete 2005) and processes (Lee et al. 1995) underlying Microbiological Influenced Corrosion (MIC) in the industrial environment

Mechanisms
Utilization of oxygen by aerobic organisms resulting in anodic areas. Localized differences in concentration shift the potential of metal surfaces resulting in the creation of localized corrosion cells.
Utilization of hydrogen by microorganisms via a cathodic reaction depolarizes the cathode which increases the rate of metal loss at the anode
Microbial degradation of protective coatings on metal surfaces
Microbial degradation of corrosion-inhibiting chemicals added to protect metals in industrial water systems – corrosion or scaling inhibitors
Microbial production of metabolites which are corrosive organic and inorganic acids are often end-products of the metabolism of microorganisms
Metabolic by-products such as H_2S can precipitate metal ions, such as iron to form FeS, which is corrosive itself.
Processes
Transport and accumulation of materials from the bulk liquid to the metal surface. These materials can be soluble (microbial nutrients and corrosive chemicals) or particulate (viable microorganisms or inorganic particles)
Microbial and electrochemical transformation processes within the biofilm and the metal surface. Microorganisms excrete extra-cellular polymers, which contribute to the biofilm deposit and promote adherence of corrosion products. Microbial transformation processes influence the corrosivity of the microenvironment at the biofilm-metal interface. Abiotic processes influence the rate, extent, and distribution of colonizing microbial species, as well as the chemical composition and physical properties of the resulting biofilm.
Erosion and detachment from the surface of the film. These processes limit the overall extent of fouling deposit accumulation.

slime not only reduces the immune system ability to fight infections, but may increase antibiotic resistance by more than 1000 times; in line with this observation, introducing antibiofilm strategies should probably be regarded as a better response than investing in new antibiotics in order to overcome the alarming increasing antibiotic resistance worldwide (WHO Report, 2014).

Both in the industrial and in the medical settings, the process from bacterial adhesion to the progressive loss of performance is influenced by many variables and the impact to the overall performance of the affected device or system may range from a difficult-to-detect light malfunction to a severe functional impairment.

Here, after a brief review of the impact of biofouling on industrial systems, we focus on implanted biomaterials, introducing the concept of biofilm-related implant malfunction, low- and high-grade infection, with its possible practical implications.

2 Biofouling in Industry and Working Machines

Industrial biofouling and biocorrosion is estimated to cost to governments and industries over two hundreds billion dollars per year (Colautti et al. 2006; Schmitt 2009; Schultz et al. 2011).

Microbial biofilms contaminate and clog water and water filtration units (affecting drinking water, wastewater, desalinization and industrial cooling water) (Chmielewski and Frank 2003), corrode and block pipelines and interfere with oil and gas extraction processes, affecting several industrial systems and manufactures (Table 2).

Industrial process water or potable water is not sterile, so there is biofilm in all systems that is inherently present without causing problems. Problems occur when the biofilm builds up, creating dead biomass and therefore a nutrient source that leads to re-growth of organisms in the water. Biofilm structures vary according to flow conditions in a water system, for example, a turbulent flow produces homogeneous and slimy biofilms, which are harder to inactivate than biofilm produced by laminar flows. Also the effectiveness of a disinfectant or biocide depends on the age of the biofilm as well as its particular physical and chemical structure. The present trend in industrial water systems is to minimize both water consumption and water discharge by recirculation. This results in the concentration of dissolved and suspended substances promoting growth of waterborne microbes, and shifting the microbial community to a more copiotrophic state.

Table 2 List of some of the main industrial and manufacturing sectors in which biofouling has a major impact on performance and efficiency

Water production and pipelines
Food and beverage industry
Petrochemical industry
Pharmaceutical and cosmetic manufacturing
Shipping industry
Heat exchanger and cooling systems
Paper production
Automotive industry
Steel production
Nuclear and hydro-electric plants

The oil industry has cited many problems resulting from biofilm formation by sulphate-reducing bacteria. Examples include pipe and rig corrosion, blockage of filtration equipment and oil spoilage (Voordouw et al. 1996).

The presence of biofilms is common in food industry. Biofilms can exist on all types of surfaces in food plants ranging from plastic, glass, metal, wood, to food products (Chmielewski and Frank 2003), causing serious engineering problems such as impeding the flow of heat across a surface, increases in fluid frictional resistance of surfaces and increases in the corrosion rate of surfaces leading to energy and production losses (Verran and Jones 2000). Pathogenic microflora grown on food surfaces and in processing environments can cross-contaminate and cause post-processing contamination (Ganesh and Anand 1998). If the microorganisms from food-contact surfaces are not completely removed, they can lead to mature biofilm formation and so increase the biotransfer potential. Examples of the food sectors that pay particular attention to the possibility of cross-contamination are the milk industry (Chye et al. 2004) and the slaughter industry (Petrak et al. 1999). Agricultural crops are also negatively impacted by certain pathogenic microbial biofilms, which cause "blights" and other agricultural disease that can ruin crops.

The most common foodborne biofilm producers belong to the genera *Pseudomonas spp.*, *Listeria*, *Salmonella spp.*, *Escherichia coli, Enterobacter*, *Flavobacterium, Alcaligenes, Staphylococcus, Bacillus spp., etc.* (Chmielewski and Frank 2003; Shi and Zhu 2009).

In the marine shipping industry, biofilms that form on ship hulls lead to corrosion and cause "drag," which results in much higher consumption of fuel during transport as well as higher ship hull maintenance costs (Flemming 2011; Kamino 2013). It is in fact estimated that 25–50 μm biofilms on a ship hull increase hydrodynamic drag by 8–22 % respectively, with an increase in fuel consumption that may raise up to

40 % and additional greenhouse gas production (estimated to be 384 million tonnes per annum) (Townsin 2003; Schultz et al. 2011).

The influence of biofouling on coastal and oceanographic measuring instruments, which are routinely used in marine and coastal research and monitoring programs, is very strong and the earliest stages of biofouling, within a few days of immersion, significantly affect data quality and instrument performance. There is a need to protect the instruments from biofouling so that they are able to gather better quality data and require less maintenance. Currently there are no effective coatings to control this problem, the only solution involves expensive manual cleaning by divers.

Biofouling of intake structures, screens, seawater piping systems and heat-exchanger tubes in desalination and power plants causes an overall decline in plant efficiency at great economic cost. For example the presence of a biofilm on transfer surfaces of heat exchangers cooled by seawater reduces the heat transfer rate by 20–50 % and incurs a global expenditure of over $15 billions per annum to control the problem. The majority of current measures to control biofouling involve the use of biocides.

In the area of membrane technology, microfiltration and ultrafiltration membranes are used for drinking water production and wastewater treatment. The primary limitation to the more widespread adoption of membrane filtration is fouling with microorganisms and organic molecules which leads to a significant decline of the permeate flux, higher energy consumption, and eventually, failure to meet the regulatory standards (Flemming and Schaule 1988). Frequent cleaning of the membranes is costly and may damage the membrane materials/barrier layers (Flemming 2009).

3 Biofilms and Biofouling in Implanted Biomaterials

The presence of a foreign body, such as an orthopedic implant, has been shown to significantly increase susceptibility to infection. While this is at least partially due to a locally acquired granulocyte defect, biofilm formation is of major importance (Zimmerli et al. 1982; Costerton et al. 1995).

Adherence of micro-organisms to the surface of the implant involves rapid attachment by specific (e.g. adhesins) or non-specific factors (e.g. surface tension, hydrophobicity, and electrostatic forces). This initial phase is followed by an accumulative phase during which bacterial cells adhere to each other and form a biofilm. Depletion of nutrients and/or waste product accumulation in biofilms causes micro-organisms to enter a slow or non-growing (stationary) state making them up to 1000 times more resistant to most antimicrobial agents than their planktonic (free living) counterparts and allow them to eventually persist for months or years (Donlan 2002).

Orthopedics is among the leading specialties for implanted biomaterials. In spite of the average excellent clinical results, implant-related infections is the first reasons for failure in the first 2 years after implant. Even if current perioperative infection prevention methods, like antibiotic prophylaxis, have significantly reduced the incidence of surgical site infections, up to 2.5 % of primary hip and knee joint replacement and to 10 % of revision arthroplasties can still be complicated by periprosthetic joint infection (PJI) (Lentino 2003) (Fig. 2).

Moreover, according to recent analysis, these figures could even be underestimated and are on the rise (Dale et al. 2009).

The presence of biofilms and of sessile bacteria on joint prosthesis makes pathogen (s) detection more difficult and often leads to treatment failures. In fact, the occurrence of PJI is considered a devastating complication, often requiring implant removal, prolonged hospitalization and long-lasting medical treatment, with high morbidity and possible long-term infection recurrence (Costerton et al. 1999; Scarponi et al. 2013; Romanò et al. 2014); PJI has been shown to be associated with mortality raise (Zmistowski et al. 2013) and elevated economical and social costs (Kurtz et al. 2012).

Fig. 2 Visible biofilm remnants on the titanium surface of a failed hip acetabular implant

A similar worrying impact is associated with biofilm-related infections after osteosynthesis for fracture fixation (Gomez and Patel 2011), pacemakers, catheters and cardio-vascular prosthesis (Baddour et al. 2010), maxillo-facial surgery (Prakasam et al. 2016), breast implants (Pittet et al. 2005) and virtually all surgeries involving implanted biomaterials, with an average risk of biofilm-related infection ranging from 0.5 to more than 20 %, depending, among other variables, on the type of implant, the length of the operation, the degree of surgical field contamination and host's co-morbidities and risk factors (Table 3).

4 Biofilm-Related Malfunction, Low- and High-Grade Infection and Thresholds for Clinical Interference

In the industrial setting, biofouling works as an operational definition, referring to that amount of biofilms development that interferes with technical, aesthetic or economical requirements.

For example, virtually all nonsterile technical water systems bear biofilms, but not all of them suffer from biofouling. The term biofouling is in fact related to the interference of biofilms with the efficiency and the performance of a given machine or a system and a threshold level exists above which biofouling begins.

In industry, this "level of interference", illustrated by the curve proposed more than two decades ago by Flemming et al. (1994) (rewritten in Fig. 3), is defined mostly by economical considerations, connected to the extent to which biofilm effects can be tolerated without unacceptable losses in process performance or product quality and quantity. Beyond this point, which can be quite different in various industries, biofouling begins.

This threshold of interference is a felt limit, which reflects the fouling tolerance of an operator. Flemming (2009) speculated that, although it may be felt differently in different technical fields, it may be assumed that a 30 % loss of productivity, product quality loss or process efficacy will alert any operator, who will try to identify and eliminate the reason.

We here hypothesize that, in the clinical setting, a similar threshold of interference can be traced for biofilm formation on implanted biomaterials. At variance with its industrial counterpart, the threshold of "clinical" interference is mostly defined by the clinical performance of the implanted device and by our ability to detect and interpret signs and symptoms of implant failure; in fact, here are inflammatory symptoms or infection markers

Table 3 List of most commonly used biomaterials by application

Cardiovascular
Stents – Pacemaker – Implantable cardiac defibrillators – Heart valves – Catheters – Guidewires – Vascular grafts – Sensors – Others (ventricular assist device (VAD), sternum closure devices, and introducer sheaths)
Orthopedic
Joint replacement (Knee-, Hip-, Shoulder-, Ankle-, Elbow-, Wrist-, Finger- arthroplasty)
Spine (spinal fusion, motion preservation/dynamic stabilization, interspinous spacers, disc arthroplasty)
Bioresorbable (Suture anchors, Interference screws, Meniscal repair tacks, mesh)
Orthobiologics (Allografts, bone substitutes, autografts)
Dental
Dental implants
Dental bone grafts & substitutes
Dental membranes
Plastic surgery
Acellular dermal matrices
Craniofacial surgery
Bioengineered skins
Breast implants
Trauma
Fracture fixation device (bone plates, screws, pins, rods, wires
Tissue engineering
Scaffolds for regenerative medicine
Ophthalmology
Contact lens
Intraocular lens
Functional replacements of ocular tissues
Synthetic corneas
Others
Neurological disorders/Central Nervous Systems
Shunting systems
Cortical neural prosthetics and implantable neurostimulators
Other applications
Drug delivery systems
Urinary catheters and prosthesis

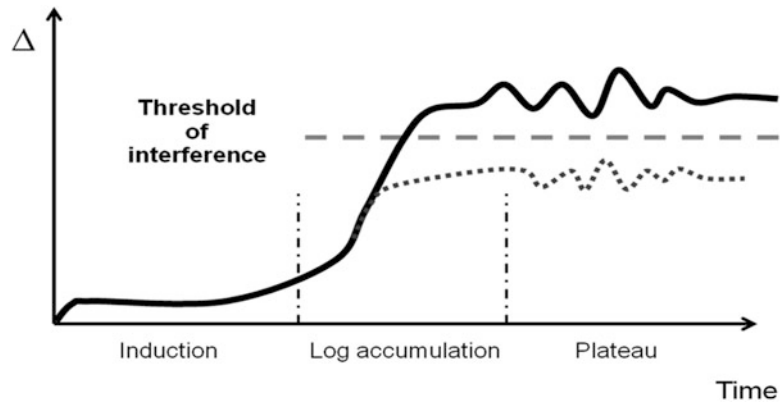


Fig. 3 Schematic biofilm development below and above the arbitrary "threshold of interference" (*dotted line*) (rewritten after Flemming et al. 1994). Δ represents the effect of biofilms development (e.g.: thickness, friction resistance, etc.)

that may trigger an "alert" to the involved "operators", the patient and his/her physician, raising the suspect of a biofilm-related impaired performance of the implanted medical device; this in turn will eventually elicit further investigations and an appropriate response or treatment.

In any given patient, the threshold of "clinical" interference depends on the net balance of different variables, including the type of microorganism(s) and of the implant, the antibacterial prophylaxis/treatment and, most importantly, the extent of the host's immune and inflammatory response.

According to the level set as an alert to define the presence of a pathological condition, the following clinical scenarios (Fig. 4), with respective thresholds of interference, can be distinguished (Fig. 5).

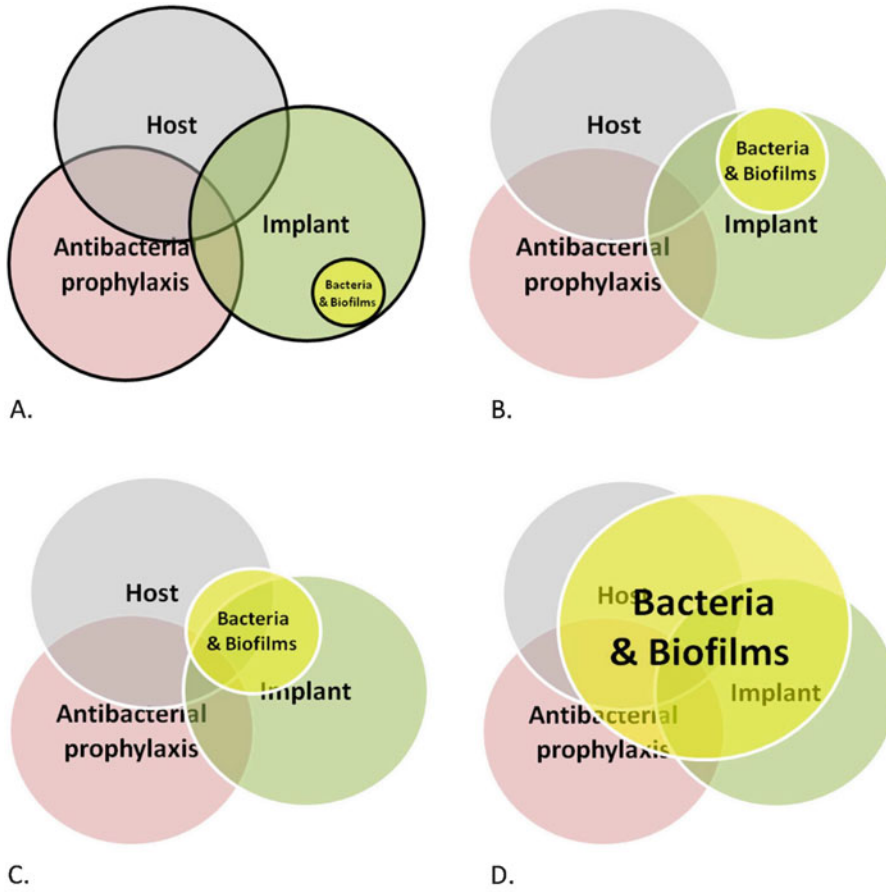


Fig. 4 Schematic representation of possible clinical scenarios, in the presence of a medical implant and adhering biofilm-producing microorganisms. (**a**) Subclinical presentation. Bacteria and biofilms do not interfere in any detectable way with implant function. Symptoms and markers of infection are absent and the device is felt as normally functioning. (**b**) Implant malfunction. Bacteria and biofilms induce only minor clinical signs and symptoms, but markers of infection remain negative. (**c**) Low-grade infection. Bacteria and biofilm interaction induce a mild host's reaction, with moderate clinical signs and symptoms and slight changes in inflammatory markers. (**d**) High-grade infection. A condition in which the classical signs and symptoms of infection and inflammation are present, with positive markers

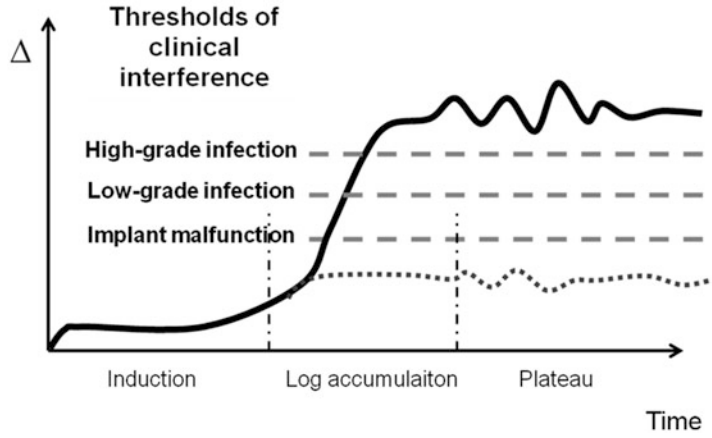


Fig. 5 Schematic biofilm development below and above arbitrary "thresholds of clinical interference" for implant malfunction, low- and high-grade infection. Subclinical presence of bacteria and biofilms is theoretically possible and is represented by the *dotted grey curve*. Δ represents the effect of biofilms development (clinical signs and symptoms, positive markers of infection/inflammation) and is the result of the interaction between the microorganisms and their by-products, the host response, the type of implant and the antibacterial prophylaxis/treatment

4.1 Subclinical Contamination and Implant Malfunction

While subclinical contamination is defined as the presence of bacteria and biofilms that do not interfere with the normal function of the implanted medical device, implant malfunction is associated with mild clinical signs/symptoms, that may be reported as mild but persistent or recurrent pain at the site of surgery and/or mild soft tissue contracture or functional impairment, with negative laboratory and imaging markers of infection/inflammation; the identification of the slow-growing microorganisms, that generally cause this condition, requires prolonged microbiological cultures, antibiofilm and eventually genomic or molecular techniques (Drago et al. 2013; Palmer et al. 2011).

4.2 Low-Grade Infection

Low-grade infection is a condition in which the patient complains about recurrent or persistent pain and/or soft tissue contracture with various functional impairment, with mixed positive/negative markers of infection/inflammation; pathogen identification requires prolonged cultures and antibiofilm techniques.

4.3 High-Grade Infection

High-grade infections display the classical signs/symptoms of infection/inflammation, including various degrees of redness, swelling, pain and local warmth and/or delayed wound healing or draining sinus, with positive laboratory and imaging investigations; in these cases, pathogen identification is often possible with traditional microbiological techniques, but is better achieved with prolonged cultures and antibiofilm processing, especially if patients underwent empirical antibiotic treatment prior to cultural examination.

On the average, the estimated incidence of high-grade infection lays in a range between approximately 0.5–2.5 % after clean surgery; on the other hand, low-grade infections and biofilm-related malfunctions probably occur in approximately 8–12 % of the patients receiving an implanted biomaterial, accounting for unexplained pain, soft tissue contractures, joint stiffness, delayed bone healing or non-union after

fracture fixation, etc., that may eventually be left untreated of managed only by medications or rehabilitation measures and never lead to implant removal.

Examples of implant malfunction or low-grade infections can be observed in various surgical fields.

In plastic surgery, Pajkos et al. (2003) reported a statistically significant positive, direct relationship between *Staph. epidermidis* sonication coltures in removed breast implants and the degree of capsular contracture, in the absence of clinical signs of infection.

Beswick et al. (2012), in a recent systematic review of prospective studies in patients undergoing total hip or knee replacement for osteoarthritis, found a proportion of people with long-term pain of unknown origin ranging from about 7–23 % after hip and from 10 to 34 % after knee replacement, while other studies have shown that "between 4 % and 13 % of patients with preoperative diagnosis of aseptic loosening were infected", when retrieved implants were analyzed with genomic identification methods (Moojen et al. 2010). Furthermore, *P. acnes* has been identified in recent years as an occult causative agent of pain after shoulder prosthesis (Millett et al. 2011).

In fracture fixation, an animal model showing the impact of low-grade infection of the rate of non-union due to *Staph. epidermidis* has been recently published (Lovati et al. 2016), while "aseptic" tibial non-union in 23 patients had been recently reported by Gille et al. (2012) to be associated in 2 cases (8.7 %) with pathogens that could only be detected by investigating bacterial rRNA with polymerase chain reaction (PCR).

Each one of the above mentioned clinical conditions may eventually, at times, shift from one to the other, depending on treatments, host's immune system, bacterial life cycle, etc. (Fig. 6).

For example, it is a common observation in the clinical setting that a well conducted antibiotic treatment may sometimes suppress inflammatory signs associated with a peri-prosthetic joint infection, thus changing a high-grade infection to a low-grade one or even to a mild

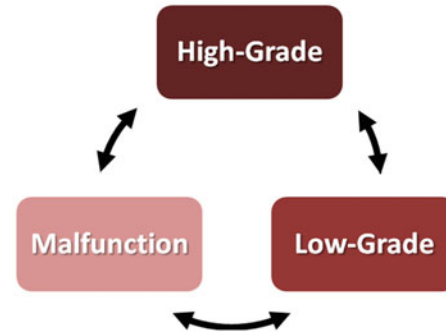


Fig. 6 The clinical presentation of biofilm-related infections of medical devices and biomaterials is influenced by many factors and shifts from one clinical condition to another may occasionally happen

implant malfunction, with only minor clinical signs and negative serum markers of inflammation. On the other side, a breach in the immune system competence, due for example to an concurrent chemotherapy, may eventually shift a previous low-grade infection to a high-grade, acute sepsis.

5 Conclusions

Biofilms and biofouling have a well known detrimental impact on most industrial and manufacturing processes.

Threshold of interference in industry depends mostly on economical considerations and is believed to be reached in any case when a 30 % loss of productivity, product quality loss or process efficacy occurs.

Implanted biomaterials also are greatly affected by possible bacterial contamination and biofilm formation, that may ultimately interfere with implant function, durability and performance or patient's well being.

Similar to the threshold of interference in industry, here thresholds of "clinical" interference can be imagined, which identify various possible clinical conditions, ranging from an implant malfunction to a high-grade infection. The occurrence of each of these clinical conditions depends on the relative balance between the type of implant, the antibacterial

prophylaxis, the behavior of the colonizing microorganisms and the host's response.

It a common observation that, even the medical field the perceived threshold of clinical interference is set at a rather high level and usually only the high-grade infections trigger some adequate response in the operators. However, a better understanding of biofilms-related clinical presentations and acknowledging the fact that biofilms on an implant can be associated with only minor or no signs of infection, is crucial for physicians, in order to implement the best diagnostic and therapeutic measures for all patients with low-performing implant; a practical example is the introduction in the surgical routine of systems that may allow cultural examination with antibiofilm processing of all failed implants (Fig. 7).

Fig. 7 Removed implant, a bone cement spacer, is sent for microbiological examination with chemical antibiofilm processing by means of dithiothreitol (DTT), a pure antibiofilm agent that does not interfere with bacterial growth. The disposable kit (microDTTect, 4i Srl, Monza, Italy) allows to collect samples at surgery and to transport and process them in a completely closed system, avoiding contamination, and increasing sensitivity of cultural examination

On the other hand, recognizing bacteria and biofilms as a possible reason of implant malfunction, should prompt regulatory bodies to consider anti-adhesive and antibiofilm implant coating technologies as a (part of) medical device aimed at reducing implant malfunction, thus adopting the relative evaluation process and certification procedure, also in consideration of the promising clinical outcomes reported in different clinical trials (Tsuchiya et al. 2012; Romanò et al. 2015, 2016).

An effective antibiofilm-targeted approach from all players is the only way the medical community may have to mitigate the current unacceptable social and economical burden of implant-related infections and malfunctions.

References

Angst EC (1923) The fouling of ship bottoms by bacteria. Report, Bureau Construction and Repair, United States Navy Department, Washington, DC

Baddour LM, Epstein AE, Erickson CC, et al., American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in Young, Council on Cardiovascular Surgery and Anesthesia, Council on Cardiovascular Nursing, Council on Clinical Cardiology, Interdisciplinary Council on Quality of Care, American Heart Association (2010) Update on cardiovascular implantable electronic device infections and their management: a scientific statement from the American Heart Association. Circulation 121:458–477

Bauer TW, Grosso MJ (2013) The basic science of biofilm and its relevance to the treatment of periprosthetic joint infection. Orthop Knowl Online J 11(9):12–20

Beswick AD, Wylde V, Gooberman-Hill R et al (2012) What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. BMJ Open 2:e000435. doi:10.1136/bmjopen-2011-000435

Bixler et al (2014) J colloid Interface Sci 419:114–133

Chmielewski RAN, Frank JF (2003) Biofilm formation and control in food processing facilities. Compr Rev Food Sci Food Saf 2:22–32. doi:10.1111/j.1541-4337.2003.tb00012.x

Chye FYF, Abdullah A, Ayob MK (2004) Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol 21:535–541

Coetser SE, Cloete TE (2005) Biofouling and biocorrosion in industrial water systems. Crit Rev Microbiol 31:213–232

Colautti RI, Bailey SA, Van Overdijk CD, Amundsen K, Macisaac HJ (2006) Characterised and projected costs of nonindigenous species in Canada. Biol Invasions 8:45–59. doi:10.1007/s10530-005-0236-y

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin -Scott HM (1995) Microbial biofilms. Ann Rev Micro 49:711–745

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322

Dale H, Hallan G, Hallan G, Espehaug B, Havelin LI, Engesaeter LB (2009) Increasing risk of revision due to deep infection after hip arthroplasty. Acta Orthop 80:639–645

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890

Drago L, Signori V, De Vecchi E, Vassena C, Palazzi E, Cappelletti L, Romanò D, Romano' CL (2013) Use of dithiothreitol to improve the diagnosis of prosthetic joint infections. J Orthop Res 31:1694. doi:10.1002/jor.22423

Flemming HC (2009) Microbial biofouling: unsolved problems, insufficient approaches, and possible solutions. In: Flemming HC et al. (eds) Biofilm highlights. Springer Series on Biofilms 5. Springer-Verlag, Berlin/Heidelberg. doi:10.1007/978-3-642-19940-0_5, 2011

Flemming HC (2011) Microbial biofouling: unsolved problems, insufficient approaches, and possible solutions. In: Flemming H-C, Wingender J, Szewzyk U (eds) Biofilm highlights. Springer, Essen, pp 81–109

Flemming HC, Schaule G (1988) Biofouling on membranes – a microbiological approach. Desalination 70:95–119

Flemming H-C, Schaule G, McDonogh R, Ridgway HF (1994) Mechanism and extent of membrane biofouling. In: Geesey GG, Lewandowski Z, Flemming H-C (eds) Biofouling and biocorrosion in industrial water systems. Lewis, Chelsea, pp 63–89

Ganesh CK, Anand SK (1998) Significance of microbial biofilms in food industry a review. Int J Food Microbiol 42:9–27

Gille J, Wallstabe S, Schulz AP et al (2012) Is non-union of tibial shaft fractures due to nonculturable bacterial pathogens? A clinical investigation using PCR and culture techniques. J Orthop Surg Res 7:20–25

Gomez E, Patel R (2011) Laboratory diagnosis of prosthetic joint infection, part I. Clin Microbiol Newsl 33 (8):55–60

Gristina AG, Naylor PT, Webb LX (1990) Molecular mechanisms in musculoskeletal sepsis: the race for the surface. Instr Course Lect 39:471–482

Harrison JJ, Turner RJ, Marques LLR, Ceri H (2005) Biofilms. A new understanding of these microbial communities is driving a revolution that may transform the science of microbiology. Am Sci 93:508–515

Kamino K (2013) Mini-review: barnacle adhesives and adhesion. Biofouling 29:735–749

Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J (2012) Economic burden of periprosthetic joint infection in the United States. J Arthroplast 27:61–65

Lee W, Lewandowski Z, Nielsen PH, Hamilton WA (1995) Role of sulfate-reducing bacteria in corrosion of mild steel: a review. Biofouling 8:165–194

Lentino JR (2003) Prosthetic joint infections: bane of orthopedists, challenge for infectious disease specialists. Clin Infect Dis 36:1157–1161

Lovati AB, Romanò CL, Bottagisio M, Monti L, De Vecchi E, Previdi S et al (2016) Modeling *Staphylococcus epidermidis*-induced non-unions: subclinical and clinical evidence in rats. PLoS One 11(1): e0147447. doi:10.1371/journal.pone.0147447

Millett PJ, Yen YM, Price CS, Horan MP, van der Meijden OA, Elser F (2011) Propionibacterium acnes infection as an occult cause of postoperative shoulder pain: a case series. Clin Orthop Relat Res 469(10):2824–2830

Moojen DJF, van Hellemondt D, Vogely HC et al (2010) Incidence of low-grade infection in aseptic loosening of total hip arthroplasty: a prospective multicenter study using extensive routine and broad-range 16S PCR with reverse line blot diagnostics. Acta Orthop 81(6):667–673

Nana A, Nelson SB, McLaren A, Chen AF (2016) What's new in Musculoskeletal infection: update on biofilms. J Bone Joint Surg Am 98:1226–1234

Pajkos A, Deva AK, Vickery K et al (2003) Detection of subclinical infection in significan breast implant capsules. Plast Recontr Surg 111:1605–1610

Palmer M, Costerton W, Sewecke J, Altman D (2011) Molecular techniques to detect biofilm bacteria in long bone nonunion: a case report. Clin Orthop Relat Res 469:3037–3042

Petrak T, Kalodera Z, Novakovic P et al (1999) Bacteriological comparison of parallel and counter flow water chilling of poultry meat. Meat Sci 53:269–271

Pittet B, Montandon D, Pittet D (2005) Infection in breast implants. Lancet Infect Dis 5:94–106

Prakasam S, Stein K, Lee MK, Rampa S, Nalliah R, Allareddy V, Allareddy V (2016) Prevalence and predictors of complications following facial reconstruction procedures. Int J Oral Maxillofac Surg 45 (6):735–742

Prentice T, Beaglehole R, Irwin A (2004) The World Health Report, 2004: changing history. World Health Organization, Geneva

Romanò CL, De Vecchi E, Vassena C, Manzi G, Drago L (2013a) A case of a late and atypical knee prosthetic infection by no-biofilm producer Pasteurella multocida strain identified by pyrosequencing. Pol J Microbiol 62(4):435–438

Romanò CL, Toscano M, Romanò D, Drago L (2013b) Antibiofilm agents and implant-related infections in

orthopaedics: where are we? J Chemother 25 (2):67–80
Romanò CL, Logoluso N, Drago L, Peccati A, Romanò D (2014) Role for irrigation and Debridement in Periprosthetic infections. J Knee Surg 27:267
Romanò CL, Scarponi S, Gallazzi E, Romanò D, Drago L (2015) Antibacterial coating of implants in orthopaedics and trauma: a classification proposal in an evolving panorama. J Orthop Surg Res 10:157–163
Romanò CL, Malizos K, Capuano N, Mezzoprete R, D'Arienzo M, Van Der Straeten C, Scarponi S, Drago L (2016) Does an antibiotic-loaded hydrogel coating reduce early post-surgical infection after joint arthroplasty ? Bone Joint Infect 1:34–41
Scarponi S, Drago L, Romanò D, Logoluso N, Peccati A, Meani E, Romanò CL (2013) Cementless modular intramedullary nail without bone-on-bone fusion as a salvage procedure in chronically infected total knee prosthesis: long-term results. Int Orthop 35:413
Schmitt G (2009) Global needs for knowledge dissemination, research, and development in materials deterioration and corrosion control. World Corrosion Organization, New York
Schultz MP, Bendick JA, Holm ER, Hertel WM (2011) Economic impact of biofouling on a naval surface ship. Biofouling 27:87–98. doi:10.1080/08927014.2010.542809
Shi X, Zhu X (2009) Biofilm formation and food safety in food industries. Trends Food Sci Technol 20:407–413
Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358(9276):135–138
Stoodley P, Ehrlich GD, Sedghizadeh PP, Hall-Stoodley-L, Baratz ME, Altman DT, Sotereanos NG, Costerton JW, Demeo P (2011) Orthopaedic biofilm infections. Curr Orthop Pract 22(6):558–563
Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, Engebretsen IL, Bayles KW, Horswill AR, Kielian T (2011) Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. J Immunol 186(11):6585–6596
Townsin RL (2003) The ship hull fouling penalty. Biofouling 19(Suppl):9–15
Tsuchiya H, Shirai T, Nishida H, Murakami H, Kabata T, Yamamoto N et al (2012) Innovative antimicrobial coating of titanium implants with iodine. J Orthop Sci 17(5):595–604
Verran J, Jones M (2000) Industrial biofouling. Wiley, New York
Voordouw G, Armstrong SM, Reimer MF et al (1996) Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. Appl Environ Microbiol 62:1623–1629
World Health Organization (2014) Antimicrobial resistance: global report on surveillance 2014. http://www.who.int/drugresistance/documents/surveillancereport/en/
Zimmerli W, Waldvogel FA, Vaudaux P, Nydegger UE (1982) Pathogenesis of foreign body infection: description and characteristics of an animal model. J Infect Dis 146(4):487–497
Zmistowski B, Karam JA, Durinka JB, Casper DS, Parvizi J (2013) Periprosthetic joint infection increases the risk of one-year mortality. J Bone Joint Surg Am 95:2177–2184

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 15–27
DOI 10.1007/5584_2016_173

Published online: 5 November 2016

Mechanisms of Bacterial Colonization of Implants and Host Response

Christof Wagner and G. Maria Hänsch

Abstract

The review focuses on the current knowledge and the most pertinent hypotheses regarding the local host immune response as the key factor for the pathogenesis of implant-associated infections. Although bacterial biofilms have long been recognized as causative agents, the link between the infection and the devastating inflammatory response, particularly the localized tissue destruction and bone degradation is less well understood. Understanding these consequences of infection, however, is of utmost importance, because suppressing inflammation and preventing bone destruction could be a novel, alternative therapeutic option in cases when eradicating the infections fails.

Keywords

Implant infection • Bacterial biofilm • Immune response • Neutrophils • Osteolysis

1 Introduction

Implant-associated infection is a very complex, interactive and multifactorial event. Consequently, when looking for means to detect, to treat or even better to prevent infection, an in-depth understanding of the pathophysiology and the underlying molecular processes is required. Since formation of bacterial biofilms on the implants have been recognized as the "ultimate cause of persistent infection" (Costerton et al 1999), a wealth of data have been accumulated analyzing the transition of free-swimming planktonic bacteria to biofilms (excellent reviews e.g. in O'Toole et al. 2000;

The authors state that there is no actual or potential conflict of interest regarding the study.

C. Wagner (✉)
Zentrum für Orthopädie und Unfallchirurgie, Klinikum Ingolstadt, Krumenauerstraße 25, 85049 Ingolstadt, Germany
e-mail: christof.wagner@klinikum-ingolstadt.de

G.M. Hänsch
Institut für Immunologie der Universität Heidelberg, Im Neuenheimer Feld 305, 69120 Heidelberg, Germany
e-mail: N50@ix.urz.uni-heidelberg.de

Donlan and Costerton 2002; Stoodley et al 2002; Hall-Stoodley et al 2004; Jefferson 2004; Wuertz et al 2004; Karatan and Watnick 2009; Bjarnsholt et al 2012). Of note, the majority of these data are derived from studying biofilm formation *in vitro* on inert carrier materials, which is justified, because biofilm infections are usually derived from the colonization of artificial surfaces (Agarwal et al 2010; Wagner et al 2011; McLean et al 2012). Although our knowledge of biofilm biology has greatly increased by the *in vitro* models, it is becoming increasingly apparent that comparability of the available data is very limited (Lourenco et al 2014). Moreover, the transfer from *in vitro* experiments to the *in vivo* situation is rather limited, among others due to the microenvironment, comprising the adjacent tissue cells and the local immune response (Bjarnsholt et al 2013; Roberts et al 2015, reviewed in Hänsch 2012a). This review will focus on the latter. A better understanding of the local host response explains not only the devastating consequences of implant infection, such as bone resorption and septic loosening, but it also might help designing alternative therapeutic options.

2 Bacteria and Host: A Complex and Multifaceted Relationship

Bacteria are ubiquitous. Our entire environment, including food and water, is not sterile, which means that we are constantly exposed to bacteria, some of which having the potential to destroy the integrity of the body. We survive within that hazardous and threatening environment, because numerous protective mechanisms have been developed during evolution, notably mechanical barriers such as skin or mucous membranes, as well as the adaptive and non-adaptive (innate) immune system. The crucial importance of these defense systems become obvious, when considering infections in patients with large skin defects, compromised mucous membranes, or acquired or congenital malfunctions of the immune system (e.g. Robins 1989; Bowler 2002; Berliner et al 2004; Percival et al 2015). Furthermore, in addition to environmental bacteria, it is important to realize, that the human body is also colonized with bacteria, particularly the skin and the mucous membranes, including the intestinal mucosa. The "healthy" human body hosts about 10^{14} bacteria (approx. 1–2 kg of body weight) with an estimated 10-fold higher number of bacteria compared to tissue cells. In this *per se* "highly infected" environment bacteria and host coexist in mutual acquiescence without adverse effect or even benefiting the host. Basic principles of this friendly co-existence are hiding from or silencing of the immune response, and the non-invasion policy – the bacteria do not cross the barriers (reviewed in Hänsch 2012a). Disturbance or injury of the physiological barriers – as it occurs e.g. during surgery – allows invasion of bacteria, resulting in activation of the host immune system and transition of the symptomless colonization to infection. In this context, by creating surgical incisions allowing entry of bacteria at the wound site, and by compromising the local and possible also the systemic immune response due to the underlying iatrogenic tissue damage, surgery can be considered as a major perturbation of the protective shield. Thus, despite ongoing attempts to achieve "sterile" conditions (reviewed in Busscher et al 2012; Dumville et al 2015; Levy et al 2016; Tanner et al 2016), the perioperative risk of infection remains a key factor, a presumption supported by the observation that the majority of implant infections occurs within the first 2 years (reviewed in Tande and Patel 2014). Furthermore, the causative microorganisms found most commonly at the infected site are staphylococci species, frequently the same as those colonizing the outer surface as "opportunists", as part of the physiological skin flora (Schierholz and Beuth 2001; Otto 2009, reviewed in Tande and Patel 2014). Taken together, by these facts an infection by the patient's "own" opportunistic bacteria becoming "accidental pathogenic" is suggested.

3 Colonization of Implants: The First Step of Implant Infection

After breakage of the physiological barriers invading bacteria rapidly seek adequate surfaces to settle down and form biofilms, a life style, in which – in contrast to their planktonic counterparts – they are protected against host defense mechanisms. According to the phrase "race to the surface" introduced by Gristina (Gristina 1987) microbial adhesion compete with tissue cell integration for colonization of the biomaterial's surface. Gristina's concept is still valid, and is now supported by an abundance of data, particularly regarding the suitable surfaces, means of bacterial attachment to the said surfaces, and signals controlling biofilm formation. It is now apparent, that biofilms formation is a rather complex, genetically driven process, mediated by number of bacteria-derived signaling molecules, also known as "quorum sensing (QS) molecules" or autoinducers. The basic steps of biofilm formation are quite similar among the bacteria species: bacteria attach to a surface by means of specialized adhesion molecules, then signaling molecules are released, which in turn drive the biofilm formation by inducing the production of the extracellular matrix (extracellular polymer substances, EPS), the name-giving, in some instances visible, film or slime, and also by altering bacteria-inherent features and properties, for example the loss of flagella. The bacteria are then embedded in the extracellular matrix, the most conspicuous feature of the biofilm, yielding a well-organized bacterial community. Of note, although the basic mechanisms appear to be similar, the adhesion molecules as well as the signaling quorum-sensing molecules differ greatly among the species, as does the quality and composition of the extracellular matrix (Heilmann 2011; Van der Mei and Busscher 2012; Foster et al 2014, reviewed in Dickschat 2010; Garg et al 2014; Büttner et al 2015). This is important to realize when attempting to interfere with attachment or biofilm formation as a preventive regimen (Drago et al 2013, reviewed in Arciola 2009; Shunmugaperumal 2010; Beloin et al 2014; Wilkins et al 2014).

Biofilms are considered as an interactive symbiotic "city of microbes". Biofilms are an efficient and protective survival strategy in a potentially aggressive environment (for in-depth understanding of the molecular mechanisms of biofilm formation and resistance resp. tolerance please see e.g. the following references and reviews: Costerton et al 1999; O'Toole et al. 2000; Donlan and Costerton 2002; Greenberg 2003; Wuertz et al 2004; Costerton et al 2005; Williams et al 2007; Karatan and Watnick 2009; Agarwal et al 2010; Hänsch 2012a; Wolcott et al 2013; Olsen 2015) (Fig. 1).

Colonization of the implant is the decisive step in implant-related infections (reviewed in Hall-Stoodley et al 2004), and depends on the ability of bacteria to adhere to a given surface. Adhesion is influenced by a great variety of components, including bacteria species, properties of the implant surface, such as microarchitecture, roughness, or electrical charge, as are environmental parameters, including flow conditions, rheology, or temperature. Adhesion involves the classical physicochemical forces (Van der Waals attraction, electrostatic charges, gravitational forces and/or hydrophobic interactions), and specialized adhesion molecules on the bacteria (reviewed e.g. in Pavithra and Doble 2008; Harmsen et al 2010; Otto 2014; Persat et al 2015). *In vitro*, colonization appears within a few hours, slime production within several days, depending on experimental conditions, particularly e.g. species, initial number of bacteria, or flow conditions. However, very little is known about the *in vivo* situation, and insights provided by studies in animal models are also limited.

Biofilm formation is a dynamic process. Single bacteria can leave or maybe are released from the biofilm, and also the organization of bacteria within the biofilm and the quality of the extracellular matrix is subject to modifications, reconstruction or self-inhibition (Nagar and Schwarz 2015). The components of the extracellular matrix vary among species (reviewed in Sutherland 2001; Flemming and Wingender

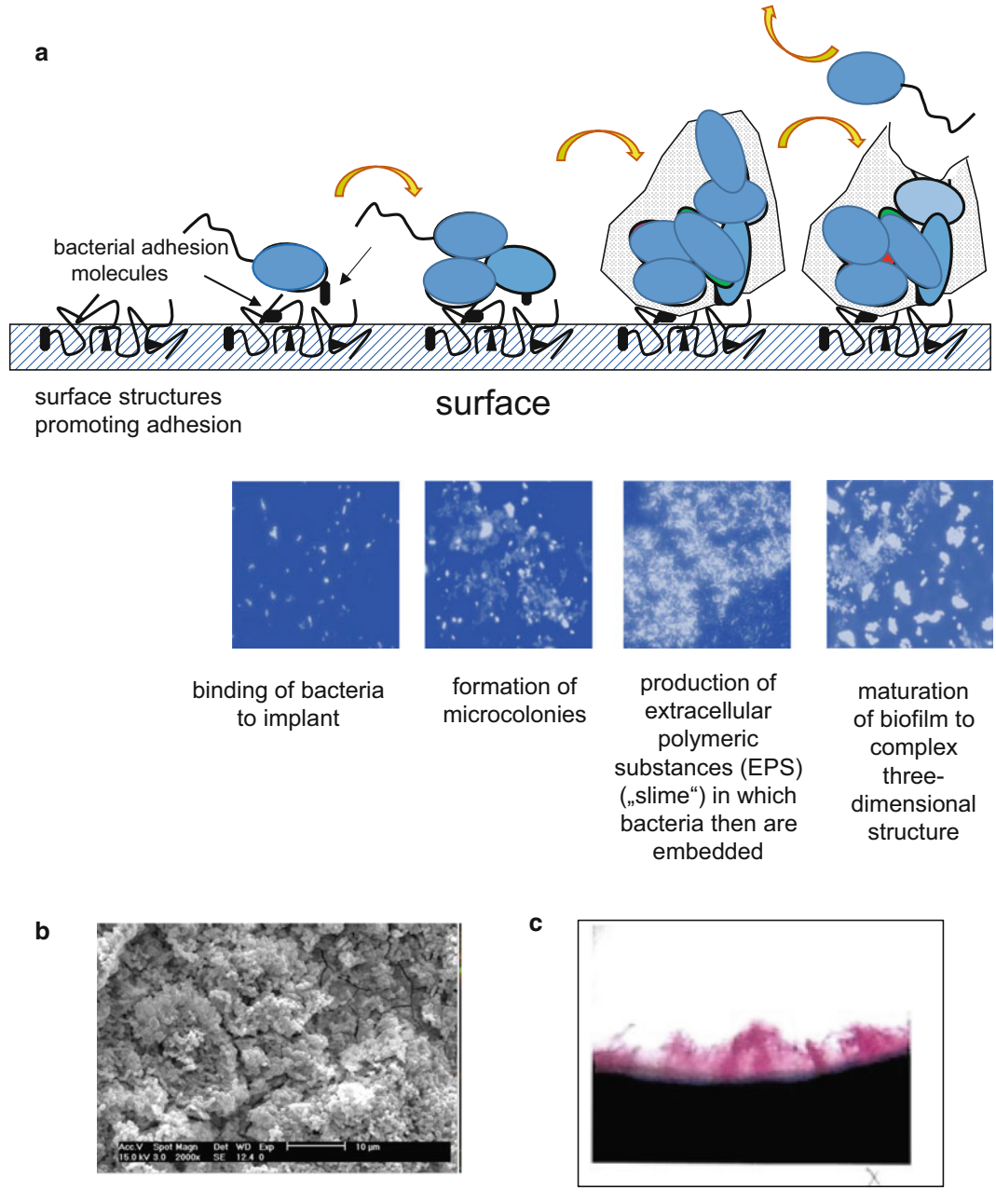


Fig. 1 Biofilm formation on implant surface. (**a**) Sequence of biofilm formation: From *right* to *left*: Binding of bacteria to implant (adhesion, colonization), formation of bacterial microcolonies and production of extracellular polymeric substances (EPS, slime) in which the bacteria are embedded, finally biofilm maturation to a complex three-dimensional structure. *Top*: schematic cartoon; *below*: illustration of step-by-step formation of biofilms (*white*: bacteria) using laser scan microscopy (b + c). Detection of biofilm formation on implant by scanning electron microscopy (SEM) (**b**) (Courtesy of Prof. Ursula Obst, Karlsruher Institut für Technologie) (**c**) and by staining with mira-ton (**c**) (Figure adapted from Wagner and Hänsch 2015)

2010), which is also important to take into account when attempting to sanitize or to disrupt biofilms. On implants, bacterial biofilms may be single-species, or may comprise multiple species and also fungae, such as *Candida albicans,* can form biofilms (Rendueles and Ghigo 2012;

Sherry et al 2014, reviewed in Lynch and Robertson 2008).

Artificial surfaces are excellent substrata for biofilm formation; however, it remains still elusive why they, compared to host tissue, are preferentially colonized by bacteria. A likely explanation is that artificial surfaces are inert and hence lack defense mechanisms as there are found on tissue cells that prevent or fend off colonization (Chun et al 2004; Hastings 2004). Moreover, after implantation, biomaterials are readily covered by blood and serum proteins (e.g. fibrinogen, fibronectin, vitronectin) resulting in the formation of a so-called conditioning film or layer, which in turn promotes adhesion of bacteria by providing exclusive receptor sites (Rochford et al 2012). Consistently, mimicking the *in vivo* microenvironment by coating non-biological surfaces with human serum or plasma, it could be shown that bacterial adherence and biofilm formation is increased (Wagner et al 2011). Taking into account that immersion in blood occurs immediately after placing the implant *in situ*, strategies modifying the implant surface, e.g. by potentially antibacterial substances (e.g. silver or copper) – although showing promising results in the *in vitro* experiments or in animal models (reviewed in Schmidmaier et al 2006; Goodman et al. 2013; Gbejuade et al 2015; Francolini et al 2015; Romano et al 2015) – are not really promising in a long-term setting.

4 Inflammation: An Essential and Telling Response to Infection

Entry of bacteria into tissue signals "danger" to the host. Via messenger molecules, systemic and local means of host defense are activated, and also the tissue at the infected sites is altered, a process collectively addressed as "inflammation". Major alterations are the increased blood flow, the enhanced permeability of blood vessels and the expression on the endothelial cells of molecules, attracting and binding phagocytic cells, which consequently squeeze through the vessel wall and migrate actively towards the infected site. Additionally, also serum and blood seep through the openings. Cytokines control the motility and the directed migration of the cells, as well as the surface molecules on the phagocytic cells and on the endothelium, which are essential for adhesion and orientation. The enhanced permeability of blood vessels is usually restricted to the infected area and accounts for the traditional symptoms of inflammation known as "rubor, calor, dolor, tumor, and function laesa" (Fig. 2). The generation of the so-called

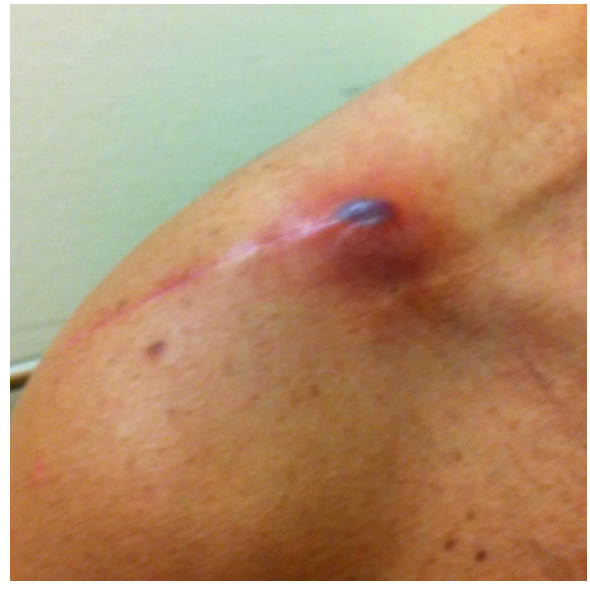


Fig. 2 Characteristic clinical signs of local inflammation following osteosynthesis of the right lateral clavicula

"pro-inflammatory environment" is an essential, indispensable response of the host to injury and a crucial prerequisite for an efficient host defense.

For clinicians, inflammation is an indicator for infection, and determining inflammation-associated alterations such as increased number of leukocytes in the peripheral blood or enhanced serum concentrations of the C-reactive protein have a long tradition. In fact, bacterial infections are primarily recognized by the inflammatory response they have created, rather than by detection and identification of the bacteria themselves. However, symptoms of inflammation or a lack thereof, do not necessarily prove or disprove bacterial infections, because also sterile irritations of tissues cause inflammation. Moreover, localized bacterial infection do not inevitably induce a notable systemic inflammatory response.

5 The Host Response to Bacterial Infection

As described above, when bacteria cross the defending barriers, for example following damage to the skin or the mucous membranes, respectively, the local cells signal "danger" and alert the immune response. The exact pathway that links the local danger signal to a systemic response is still under investigation. Cytokines have been identified that induce the increase of the blood C-reactive protein concentration, and that mobilize granulocytes from the bone marrow, resulting in leukocytosis, an important indicator of infection.

The first cells to respond are phagocytic cells, especially granulocytes (polymorphonuclear neutrophils, PMN) as "first line defense", which, to efficiently combat bacteria, infiltrate the infected site in a complex and well-regulated controlled manner (the sequence of the neutrophil-mediated inflammatory host response to bacteria is illustrated in Fig. 3). Briefly, due to chemokines (e.g. complement C5), generated at the site of infection and diffused into the tissues, the nearby endothelial cells become "sticky" by up-regulation of specialized adhesion proteins, that capture PMN from the peripheral blood. After being activated and attached firmly, PMN then squeeze between the endothelial cells (so-called diapedesis) and migrate actively towards the bacteria via chemotaxis. As phagocytic cells PMN bind and take up planktonic bacteria, which then, after being engulfed into a plasma membrane-derived vacuole, the phagosome, are killed intracellularly, predominantly by cytotoxic oxygen radicals, generated by a sequential reduction of oxygen. In addition to oxidative killing, granulocytes carry a large arsenal of bactericidal entities, among those e.g. lysozyme, defensins, collagenase and elastase (an overview is shown in Table 1) (reviewed in Faurschou and Borregaard 2003), which are stored preformed in the cells, and are released in response to bacteria-derived agents or to cytokines either into the cell or into the environment. Successful phagocytosis initiates apoptosis of the neutrophils, which then in turn are cleared by invading macrophages; thereby spilling of PMN's cytotoxic and proteolytic content is prevented. In summary, ideally, phagocytosis results in the clearance of the offending bacteria, the termination of the inflammatory response, and the restoration of the tissue, the wound healing. In that, the host response is limited in a time-, and in many instances also in a space-dependent manner (reviewed in e.g. Savill 1997; Kobayashi et al 2003; Lee et al 2003; Wagner and Hänsch 2005).

Invading bacteria also alert the adaptive immune response (Wagner et al 2008; Karauzum and Datta 2016). B- and T-lymphocytes are activated, and the generation of antibodies is induced. Basically, an increased blood antibody concentration is an indicator of an ongoing adaptive immune response, and can be also used as a diagnostic tool. However, because the majority of bacteria found in implant infections are the same that are permanently colonizing the skin or the mucous membranes as opportunists, antibodies are present at any time, and therefore are not useful as diagnostic tool for device-associated infections.

Because we are constantly exposed to bacteria, the immune system is permanently in action,

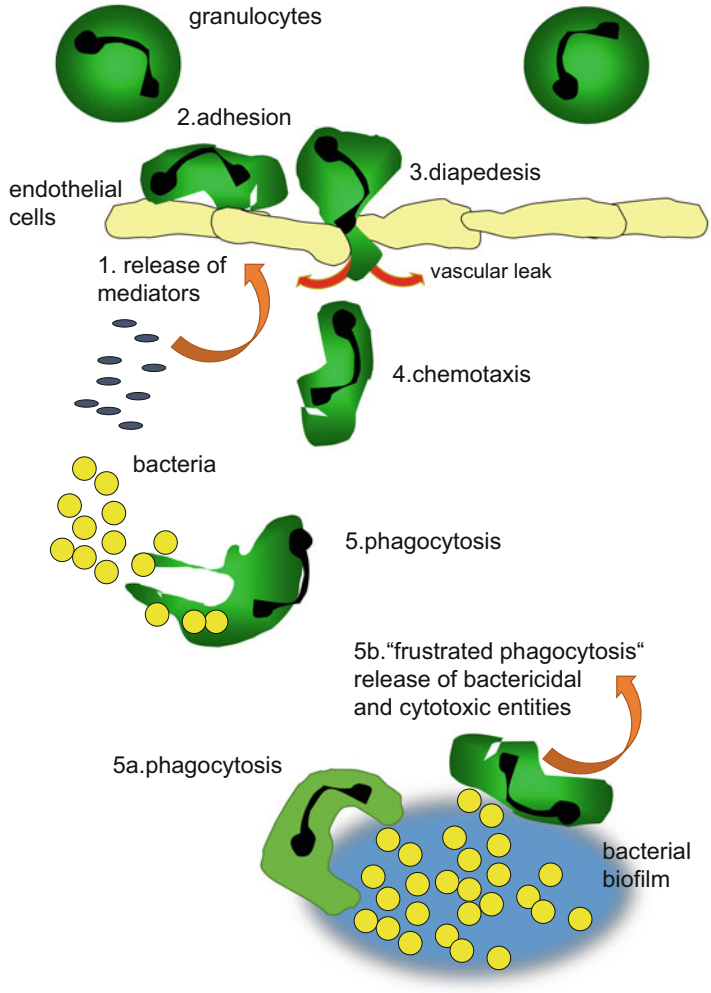


Fig. 3 The role of granulocytes in host response to bacteria: (1) Mediators generated and released at the infected site, act on the close-by endothelium. (2) By up-regulation of adhesion proteins, the endothelial cells become “sticky” and capture circulating granulocytes from the peripheral blood. (3 + 4) After binding granulocytes transmigrate in between the endothelial cells (diapedesis) towards the site of infection in a direct manner (chemotaxis). (5) Having reached the site they take up bacteria (phagocytosis) which then are killed intracellularly. (5a) Depending on maturation state bacterial biofilms can also be phagocytosed by granulocytes (successful phagocytosis); the site of infection will be cleared. (5b) In case that the biofilm resist the attack of the granulocytes, PMN, not able to take up bacteria (“frustrated phagocytosis”), are further activated and consequently release their proteolytic and cytotoxic entities into the surroundings causing progressive tissue destruction

Table 1 Bactericidal and proteolytic content of neutrophil granules (selection)

Azurophil granules	Specific granules	Gelatinase granules
α_1-antitrypsin	Collagenase	Leukolysin
α-mannosidase	Gelatinase	Gelatinase
Cathepsins	Histaminase	Lysozyme
Defensins	Heparanase	
Elastase	Lactoferrin	
Lysozyme	Lysozyme	
Proteinase-3	Sialidase	

Reviewed in Faurschou and Borregaard (2003)

though mainly unperceived. The latter becomes obvious when dealing with patients on immunosuppressive therapy, or with congenital or acquired immunodeficiency syndromes (e.g. reviewed in Armengaud 1976; Doria et al 2008; Shadyab and Crum-Cianflone 2012).

The extent and the efficiency of the local host response depends on various factors, particularly the number of bacteria, the bacteria species, the ability of the bacteria to invade the tissue, and their virulence, the latter defined as the propensity of the bacteria to damage host cells, e.g. by producing cytotoxic substances or toxins that interfere with the cellular signaling or the cell metabolism (e.g. pertussis toxin). On the other hand, also the extent and quality of the immune response varies widely among individuals. The genetically determined repertoire of immune cells determines the recognition of foreign and potentially dangerous materials (such as bacteria); moreover, the also genetically imprinted capacity to produce messenger molecules (such as interleukins) that regulate, support or control the individual immune response varies among individuals, as does the density of cytokine receptors, or the number of molecules that sense "danger". Consequently, the efficiency of eliminating a given bacteria varies among individuals, as does the accompanying inflammatory response. Thus, the host defense can occur "virtually unnoticed by the host", or lead to moderate local symptoms like swelling and redness, or even to extreme systemic reactions, the sepsis.

6 Host Defense Against Bacterial Biofilms

How the host reacts to biofilms is not really known (reviewed in Zimmerli and Sendi 2011; Hänsch 2012a). In patients with implant-associated infections we see –most likely – only an extreme situation with fulminant inflammation and more or less extensive tissue damage. On the other hand, on routinely removed implants, bacterial biofilms are found without signs of inflammation or adverse tissue reactions (Neut et al 2003; Trampuz et al 2007; Obst et al 2012; Yano et al 2014; Dapunt et al 2014b). To reconcile these two extremes, the following scenarios are feasible:

1. The bacteria are recognized by the immune response, and are eliminated, probably even before a mature biofilm is formed. Because quorum-sensing molecules are recognized by cells of the host response, e.g. by phagocytes, it is a distinct possibility (Vikström et al 2005; Zimmermann et al. 2006; Maurer et al 2015, reviewed in Hänsch 2012b). Such an early and efficient host response would go unnoticed by the host
2. Alternatively, the bacteria form a biofilm that escapes recognition by the immune response. The biofilm then persists, but without eliciting an immune response or an inflammatory response. This is a distinct possibility, because there is the claim that bacteria in biofilms have a limited metabolism and do no not divide or at least not that as rapidly as their planktonic counterparts. Moreover, the surrounding extracellular matrix might not be recognized as dangerous.
3. Bacterial biofilms are recognized by the immune system, phagocytic cells infiltrate, but are unable to eliminate the biofilm. This would have two important consequences: (a) the biofilm continuously activates the host response. More phagocytic cells and eventually also T-lymphocytes infiltrate the sites (Wagner et al 2003; Wagner et al 2006), pro-inflammatory mediators are produced which in turn cause more cell infiltration, but eventually also activation of local cells, for example osteoblasts. The inflammatory immune response is thus not self-limited, but rather progresses and expands. (b) When phagocytic cells are further activated but are unable to take up bacteria, they release their cytotoxic and bactericidal entities into the surroundings. However, even in this case, there is still a chance for the neutrophils to attack or destroy a biofilm, because *in vitro* data show clearly that biofilms are not inherently protected against the attack by

phagocytic cells (Wagner et al 2004; Günther et al 2009; Meyle et al 2010; Hänsch 2012a). In case that the biofilm resists the attack by the phagocytic cells ("frustrated phagocytosis" or "attempt without success") (Fig. 3), the host reaction at the local site proceeds and even progresses, resulting in an ongoing release of cytotoxic and proteolytic entities with subsequent progressive tissue destruction (Dallegri and Ottonello 1997; Ward and Lentsch 1999) (Fig. 4) as dire collateral damage by the local host defence (Wagner et al 2005). By generation of a proinflammatory microenvironment with increased cytokine levels (e.g. tumor necrosis factor alpha, TNF alpha; interleukin 8, IL-8, MRP-14) the differentiation of bone resorbing osteoclasts (osteoclastogenesis) from myeloid precursor cells is induced (Fig. 5), perpetuating the self-inflicted tissue damage, eventually

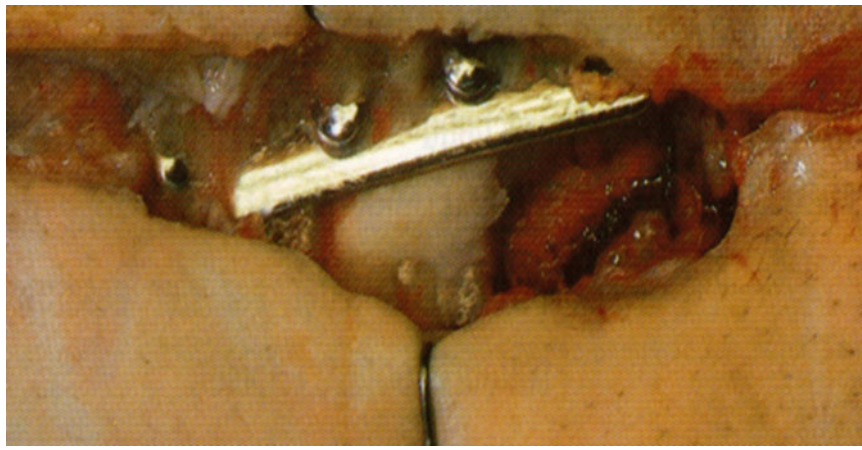


Fig. 4 Progressive tissue destruction in implant-associated infection following plate osteosynthesis

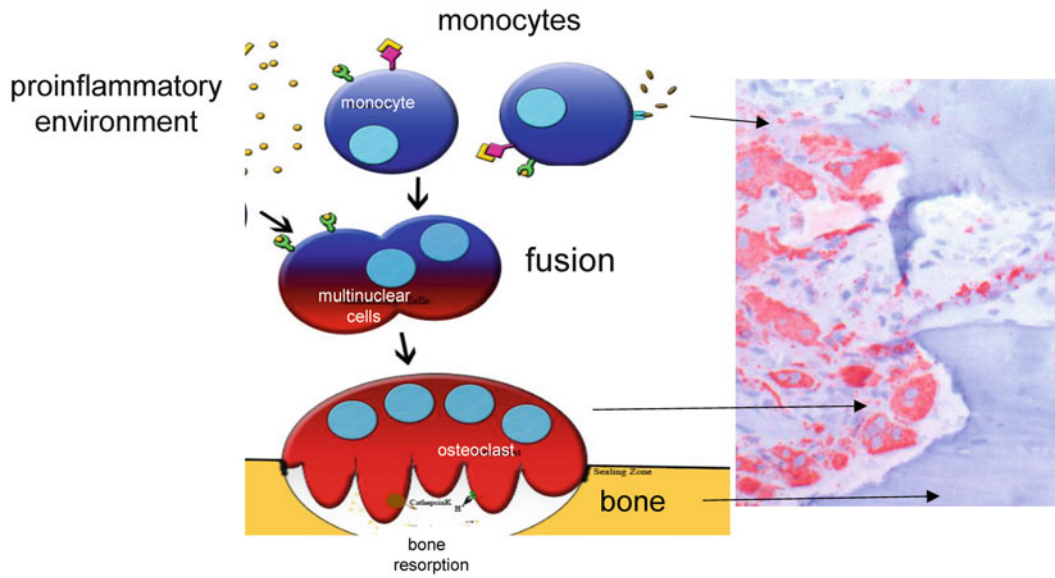


Fig. 5 Link between inflammation and osteolysis. *Left*: Schematic illustration of differentiation and fusion of monocytes by pro-inflammatory mediators with formation of giant cells and finally multi-nucleated bone-resorbing osteoclasts. *Right*: bone biopsy showing osteoclasts (*red* kathepsin K, *blue* multiple nuclei) and infiltrated phagocytic cells (*blue*) in the surrounding (Figure adapted from Wagner and Hänsch 2015)

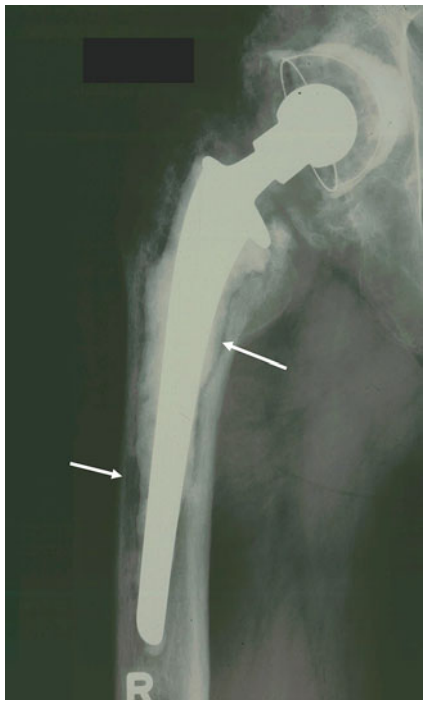


Fig. 6 X-ray (anteroposteral view) of patient with "septic loosening" of hip prosthesis (osteolysis marked by *arrows*)

leading to osteolysis and successional "septic loosening" of the implant, a prominent feature seen in long-term chronic biofilm infections (Gaida et al 2012; Dapunt et al. 2015) (Fig. 6). Consistently, *ex vivo* analysis of the tissue at the infected sites shows massive infiltration of granulocytes, evidence for the local generation of pro-inflammatory cytokines, and a co-localization of neutrophils with osteoclasts, by this, in summary, providing a link between biofilm infection, inflammation and pathological bone resorption (Wagner et al 2005; Moermann et al 2008; Gaida et al 2012; Hänsch 2012a; Dapunt et al 2014a).

Potentially important further players in the local host response are tissue cells, particularly osteoblasts. Although their contribution *in vivo* has not yet been established, there is good evidence that osteoblasts respond to bacterial-derived products and inflammatory mediators, and also produce pro-inflammatory mediators after stimulation (Sanchez et al 2013; Dapunt et al 2016, reviewed in Marriott 2004).

7 When and Why Biofilms Cause Implant-Associated Infections?

The factors deciding which of the three routes outlined above are taken, are still elusive. Because biofilm formation as well as the host response are dynamic processes, either self-limiting or progressing, the three scenarios described above are not mutually exclusive. Our hypothesis is that there is a time-line, along which colonization might proceed to a full-blown inflammatory reaction. Triggers could be derived from the biofilm itself, for example an increase of the bacterial load, or the release from the biofilm of planktonic bacteria, which – in contrast to the mature biofilm – might be elicit a host response. Alternatively, a compromised immune response as it occurs in association with an unrelated infection, might weaken the host defense.

References

Agarwal A, Singh KP, Jain A (2010) Medical significance and management of staphylococcal biofilm. FEMS Immunol Med Microbiol 58:147–160

Arciola CR (2009) New concepts and new weapons in implant infections. Int J Artif Organs 32:533–536

Armengaud M (1976) Bacterial infections and immunosuppression. Sem Hop 52(2):91–97

Beloin C, Renard S, Ghigo JM et al (2014) Novel approaches to combat bacterial biofilms. Curr Opin Pharmacol 18:61–68

Berliner N, Horwitz M, Loughran TP (2004) Congenital and acquired neutropenia. Hematology Am Soc Hematol Educ Program, 63–79

Bjarnsholt T, Høiby N, Donelli G et al (2012) Understanding biofilms – are we there yet? FEMS Immunol Med Microbiol 65(2):125–126

Bjarnsholt T, Alhede M, Eickhardt-Sørensen SR et al (2013) The in vivo biofilm. Trends Microbiol 21 (9):466–474

Bowler PG (2002) Wound pathophysiology, infection and therapeutic options. Ann Med 34(6):419–427

Busscher HJ, van der Mei HC, Subbiahdoss G (2012) Biomaterial-associated infection: locating the finish

line in the race for the surface. Sci Transl Med 4(153):153rv10
Büttner H, Mack D, Rohde H (2015) Structural basis of Staphylococcus epidermidis biofilm formation: mechanisms and molecular interactions. Front Cell Infect Microbiol. doi:10.3389/fcimb.2015.00014
Chun CK, Ozer EA, Welsh MJ et al (2004) Inactivation of a pseudomonas aeruginosa quorum-sensing signal by human airway epithelia. Proc Natl Acad Sci U S A 101 (10):3587–3590
Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1326
Costerton JW, Montanaro L, Arciola CR (2005) Biofilm in implant infections: its production and regulation. Int J Artif Organs 28:1062–1068
Dallegri F, Ottonello L (1997) Tissue injury in neutrophilic inflammation. Inflamm Res 46:382–391
Dapunt U, Giese T, Lasitschka F et al (2014a) Osteoclast generation and cytokine profile at prosthetic interface: a study on tissue of patients with aseptic loosening or implant-associated infections. Eur J Inflamm 12:147–159
Dapunt U, Lehner B, Burckhardt I et al (2014b) Evaluation of implant sonication as a diagnostic tool in implant-associated infections. J Appl Biomat Funct Mater 12:135–140
Dapunt U, Giese T, Maurer S et al (2015) Neutrophil-derived MRP-14 is up-regulated in infectious osteomyelitis and stimulates osteoclast generation. J Leukoc Biol 98(4):575–582
Dapunt U, Giese T, Stegmaier S et al (2016) The osteoblast as an inflammatory cell: production of cytokines in response to bacteria and components of bacterial biofilms. BMC Musculoskelet Disord. doi:10.1186/s12891-016-1091-y
Dickschat JS (2010) Quorum sensing and bacterial biofilms. Nat Prod Rep 27(3):343–369
Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193
Doria A, Canova M, Tonon M et al (2008) Infections as triggers and complications of systemic lupus erythematosus. Autoimm Rev 8(1):24–28
Drago L, De Vecchi E, Mattina R et al (2013) Activity of N-acetyl-L-cysteine against biofilm of Staphylococcus aureus and Pseudomonas aeruginosa on orthopaedic prosthetic materials. Int J Artif Organs 36 (1):39–46
Dumville JC, McFarlane E, Edwards P et al (2015) Preoperative skin antiseptics for preventing surgical wound infections after clean surgery. Cochrane Database Syst Rev. doi:10.1002/14651858.CD003949.pub4
Faurschou M, Borregaard N (2003) Neutrophil granules and secretory vesicles in inflammation. Microbes Infect 5(14):1317–1327
Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633
Foster TJ, Geoghegan JA, Ganesh VK et al (2014) Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol 12(1):49–62
Francolini I, Donelli G, Crisante F (2015) Antimicrobial polymers for anti-biofilm medical devices: state-of-art and perspectives. Adv Exp Med Biol 831:93–117
Gaida MM, Mayer B, Stegmaier S (2012) Polymorphonuclear neutrophils in osteomyelitis: link to osteoclast generation and bone resorption. Eur J Inflamm 10:413–426
Garg N, Manchanda G, Kumar A (2014) Bacterial quorum sensing: circuits and apllications. Antonie Van Leeuwenhoek 105(2):289–305
Gbejuade HO, Lovering AM, Webb JC (2015) The role of microbial biofilms in prosthetic joint infections. Acta Orthop 86:147–158
Goodman SB, Yao Z, Keeney M et al (2013) The future of biologic coatings for orthopaedic implants. Biomaterials 34:3174–3183
Greenberg EP (2003) Bacterial communication and group behavior. J Clin Invest 112(9):1288–1290
Gristina AG (1987) Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 237:1588–1595
Günther F, Wabnitz G, Stroh P et al (2009) Host defence against Staphylococcus aureus biofilm infection: phagocytosis of biofilms by polymorphonuclear neutrophils (PMN). Mol Immunol 46:1805–1813
Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2(2):95–108
Hänsch GM (2012a) Host defence against bacterial biofilms: "mission impossible"? ISRN Immunol. doi:10.5402/2012/853123
Hänsch GM (2012b) Molecular eavesdropping: phagocytic cells spy on bacterial communication. J Leukoc Biol 91:3–5
Harmsen M, Yang L, Pamp SJ et al (2010) An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal. FEMS Immunol Med Microbiol 59:253–268
Hastings JW (2004) Bacterial quorum-sensing signals are inactivated by mammalian cells. Proc Natl Acad Sci U S A 101(12):3993–3994
Heilmann C (2011) Adhesion mechanisms of staphylococci. Adv Exp Med Biol 715:105–123
Jefferson KK (2004) What drives bacteria to produce a biofilm? FEMS Microbiol Lett 236(2):163–173
Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347
Karauzum H, Datta K (2016) Adaptive immunity against staphylococcus aureus. Curr Top Microbiol Immunol. doi:10.1007/82_2016_1
Kobayashi SD, Voyich JM, DeLeo FR (2003) Regulation of the neutrophil-mediated inflammatory response to infection. Microbes Infect 5(14):1317–1327

Lee WL, Harrison RE, Grinstein S (2003) Phagocytosis by neutrophils. Microbes Infect 5(14):1299–1306
Levy DM, Wetters NG, Levine BR (2016) Prevention of periprosthetic joint infections of the hip and knee. Am J Orthop 45(5):E299–E307
Lourenco A, Coenye T, Goeres DM et al (2014) Minimum information about a biofilm experiment (MIABiE): standards for reporting experiments and data on sessile microbial communities living at interfaces. Pathog Dis 70(3):250–256
Lynch SA, Robertson GT (2008) Bacterial and fungal biofilm infections. Ann Rev Med 59:415–428
Marriott I (2004) Osteoblast responses to bacterial pathogens. Immunol Res 30(3):291–308
Maurer S, Wabnitz GH, Kahle NA et al (2015) Tasting Pseudomonas aeruginosa biofilms: human neutrophils express the bitter receptor T2R38 as sensor for the quorum sensing molecule N-(3.oxododecanoyl)-I-homoserine lactone. Front Immunol. doi:10.3389/fimmu.2015.00369
McLean RJ, Lam JS, Graham LL (2012) Training the biofilm generation – a tribute to J.W. Costerton. J Bacteriol 194:6706–6711
Meyle E, Stroh P, Günther F et al (2010) Destruction of bacterial biofilms by polymorphonuclear neutrophils: relative contribution of phagocytosis, DNA release, and degranulation. Int J Artif Organs 33:608–620
Moermann M, Thederan M, Nackchbandi I et al (2008) Lipopolysaccharides (LPS) induce the differentiation of human monocytes to osteoclasts in a tumour necrosis factor (TNF) α-dependent manner: a link between infection and pathological bone resorption. Mol Immunol 45:3330–3337
Nagar E, Schwarz R (2015) To be or not to be planktonic? Self-inhibition of biofilm development. Environ Microbiol 17(5):1477–1486
Neut D, van Horn JR, van Kooten TG (2003) Detection of biomaterial-associated infections in orthopaedic joint implants. Clin Orthop 413:261–268
Obst U, Marten SM, Niessner C et al (2012) Diversity of patients microflora on orthopaedic and dental implants. Int J Artif Organs 35:727–734
Olsen I (2015) Biofilm-specific antibiotic tolerance and resistance. Eur J Clin Microbiol Infect Dis 34:877–886
O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Annu Rev Microbiol 54:49–79
Otto M (2009) Staphylococcus epidermidis – the "accidental" pathogen. Nat Rev Microbiol 7:555–567
Otto M (2014) Physical stress and bacterial colonization. FEMS Microbiol Rev 38(6):1250–1257
Pavithra D, Doble M (2008) Biofilm formation, bacterial adhesion and host response on polymeric implants – issues and prevention. Biomed Mater. doi:10.1088/1748-6041/3/3/034003
Percival SL, McCarty SM, Lipsky B (2015) Biofilms and wounds: an overview of the evidence. Adv Wound Care (New Rochelle) 4(7):373–381
Persat A, Nadell CD, Kim MK et al (2015) The mechanical world of bacteria. Cell 161(5):988–997
Rendueles O, Ghigo JM (2012) Multi-species biofilms: how to avoid unfriendly neighbors. FEMS Microbiol Rev 36(5):972–989
Roberts AE, Kragh KN, Bjarnsholt T (2015) The limitations of in vitro experimentation in understanding biofilms and chronic infection. J Mol Biol 427(23):3646–3661
Robins EV (1989) Immunosuppression of the burned patient. Crit Care Nurs Clin North Am 1(4):767–774
Rochford ET, Richards RG, Moriarty TF (2012) Influence of material on the development of device-associated infections. Clin Microbiol Infect 18(12):1162–1167
Romano CL, Scarponi S, Gallazzi E et al (2015) Antibacterial coating of implant in orthopaedics and trauma: a classification proposal in an evolving panorama. J Orthop Surg Res. doi:10.1186/s13018-015-0294-5
Sanchez CJ Jr, Ward CL, Romano DR et al (2013) Staphylococcus aureus biofilms decrease osteoblast viability, inhibits osteogenic differentiation, and increases bone resorption in vitro. BMC Musculoskelet Disord. doi:10.1186/1471-2474-14-187
Savill J (1997) Apoptosis in resolution of inflammation. J Leukoc Biol 61(4):375–380
Schierholz JM, Beuth J (2001) Implant infections: a haven for opportunistic bacteria. J Hosp Infect 49:87–93
Schmidmaier G, Lucke M, Wildemann B et al (2006) Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review. Injury 37(Suppl2):S105–S112
Shadyab AH, Crum-Cianflone NF (2012) Methicillin-Resistant Staphylococcus Aureus (MRSA) infections among HIV-infected persons in the era of highly active antiretroviral therapy. A review of the literature. HIV Med 13(6):319–332
Sherry L, Rajendran R, Lappin DF et al (2014) Biofilms formed by Candida albicans bloodstream isolates display phenotypic and transcriptional heterogeneity that are associated with resistance and pathogenicity. BMC Microbiol. doi:10.1186/1471-2180-14-182
Shunmugaperumal T (2010) Microbial colonization of medical devices and novel preventive strategies. Recent Pat Drug Deliv Formul 4(2):153–173
Stoodley P, Sauer K, Davies D et al (2002) Biofilms as complex differential communities. Ann Rev Microbiol 56:187–209
Sutherland IW (2001) The biofilm matrix – an immobilized but dynamic microbial environment. Trends Microbiol 9(5):222–227
Tande AJ, Patel R (2014) Prosthetic joint infection. Clin Microbiol Rev 27:302–345
Tanner J, Dumville JC, Norman G et al (2016) Surgical hand antisepsis to reduce surgical site infection. Cochrane Database Syst Rev. doi:10.1002/14651858.CD004288.pub3

Trampuz A, Piper KE, Jacobson MJ et al (2007) Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 357:654–663

Van der Mei HC, Busscher HJ (2012) Bacterial cell surface heterogeneity: a pathogen's disguise. PLoS Path. doi:10.1371/journal.ppat.1002821

Vikström E, Magnusson KE, Pivoriunas A (2005) The Pseudomonas aeruginosa quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone stimulates phagocytic activity in human macrophages through the p38 MAPK pathway. Microbes Infect 7 (15):1512–1518

Wagner C, Hänsch GM (2005) Born to kill – bound to die: the role of leukocytes in host defence. Quaderni di Infezioni Osteoarticolari April 3–9

Wagner C, Hänsch GM (2015) Pathophysiology of implant-associated infections. From biofilm to osteolysis and septic loosening. Orthopade 44:967–973

Wagner C, Kondella K, Bernschneider T et al (2003) Post-traumatic osteomyelitis: analysis of inflammatory cells recruited into the site of infection. Shock 20:501–510

Wagner C, Kaksa A, Müller W et al (2004) Polymorphonuclear neutrophils (PMN) in posttraumatic osteomyelitis: cells recovered from the inflamed site lack chemotactic activity but generate superoxides. Shock 22:108–115

Wagner C, Obst U, Hänsch GM (2005) The implant-associated posttraumatic osteomyelitis: collateral damage by the local host defence? Int J Artif Organs 28:1172–1180

Wagner C, Heck D, Lautenschläger K et al (2006) T-lymphocytes in implant-associated posttraumatic osteomyelitis: identification of cytotoxic T-effector cells at the site of infection. Shock 25:241–246

Wagner C, Kotsougiani D, Pioch M et al (2008) T lymphocytes in acute bacterial infection: increased prevalence of $CD11b^{+}$ cells in the peripheral blood and recruitment to the infected sit. Immunology 125:503–509

Wagner C, Aytac S, Hänsch GM (2011) Biofilm growth on implants: bacteria prefer plasma coats. Int J Artif Organs 34:811–817

Ward PA, Lentsch AB (1999) The acute inflammatory response and its regulation. Arch Surg 134:666–669

Wilkins M, Hall-Stoodley L, Allan RN et al (2014) New approaches to the treatment of biofilm-related infections. J Infect 69(Suppl1):S47–S52

Williams P, Winzer K, Chan WC et al (2007) Look who's talking: communication and quorum sensing in the bacterial world. Phil Trans R Soc B 362:1119–1134

Wolcott R, Costerton JW, Raoult D et al (2013) The polymicrobial nature of biofilm infection. Clin Microbiol Infect 19:107–112

Wuertz S, Okabe S, Hausner M (2004) Microbial communities and their interactions in biofilm systems: an overview. Water Sci Technol 49(11–12):327–336

Yano MH, Klatau GB, da Silva CB et al (2014) Improved diagnosis of infection associated with osteosynthesis by use of sonication of fracture fixation implants. J Clin Microbiol 52:4176–4182

Zimmerli W, Sendi P (2011) Pathogenesis of implant-associated infection: the role of the host. Semin Immunopathol 33(3):295–306

Zimmermann S, Wagner C, Müller W et al (2006) Induction of neutrophil chemotaxis by the quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone. Infect Immun 74(10):5687–5692

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 29–50
DOI 10.1007/5584_2016_157

Published online: 9 October 2016

Animal Models of Implant-Related Low-Grade Infections. A Twenty-Year Review

Arianna Barbara Lovati, Marta Bottagisio, Elena de Vecchi, Enrico Gallazzi, and Lorenzo Drago

Abstract

The demand for joint replacement and surgical treatment is continuously increasing, thus representing a clinical burden and a cost for the healthcare system. Among several pathogens involved in implant-related infections, staphylococci account for the two-thirds of clinically isolated bacteria. Despite most of them are highly virulent microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*), low virulent bacteria (*Staphylococcus epidermidis*, *Propionibacterium acnes*) are responsible for delayed, low-grade infections without specific clinical signs and hardly distinguishable from aseptic prosthetic failure. Therefore, there is a real need to study the pathogenesis of orthopedic infections through *in vivo* animal models. The present review of the literature provides a 20-year overview of animal models of acute, subclinical or chronic orthopedic infections according to the pathogen virulence and inocula. Through this analysis, a great variety of conditions in terms of bacterial

Authors Contribution: Arianna Barbara Lovati and Marta Bottagisio contributed equally with all other contributors.

A.B. Lovati (✉)
Cell and Tissue Engineering Laboratory, IRCCS Galeazzi Orthopedic Institute, via R. Galeazzi 4, 20161 Milan, Italy
e-mail: arianna.lovati@grupposandonato.it

M. Bottagisio
Cell and Tissue Engineering Laboratory, IRCCS Galeazzi Orthopedic Institute, via R. Galeazzi 4, 20161 Milan, Italy

Department of Veterinary Medicine, University of Milan, via Celoria 10, 20133 Milan, Italy

E. de Vecchi
Laboratory of Clinical Chemistry and Microbiology, IRCCS Galeazzi Orthopedic Institute, via R. Galeazzi 4, 20161 Milan, Italy

E. Gallazzi
Department of Reconstructive Surgery of Osteo-articular Infections C.R.I.O. Unit, IRCCS Galeazzi Orthopedic Institute, via R. Galeazzi 4, 20161 Milan, Italy

L. Drago
Laboratory of Clinical Chemistry and Microbiology, IRCCS Galeazzi Orthopedic Institute, via R. Galeazzi 4, 20161 Milan, Italy

Department of Biomedical Science for Health, University of Milan, via L. Mangiagalli 31, 20133 Milan, Italy

strains and inocula emerged, thus encouraging the development of more reproducible *in vivo* studies to provide relevant information for a translational approach to humans.

Keywords
Animal models • Staphylococcus • Propionibacterium acnes • Low virulence • Low inocula

List of Abbreviations

CFU	colony forming unit
MRSA	methicillin-resistant *S. aureus*
MRSE	methicillin-resistant *S. epidermidis*
IL	interleukin
MCP-1	monocyte chemoattractant protein-1
MDSC	myeloid-derived suppressor cells
Ag^+	silver ions
HA	hydroxyapatite
K-wire	Kirschner wire

1 Introduction

The increasing percentage of elderly and trauma patients continuously demands for both joint replacement and surgical treatment of complex fractures. According to recent data from the United States, the number of patients with osteoarthritis – requiring total hip and knee joint replacements – reached 905.000 cases in 2009 (Song et al. 2013). Moreover, failures of arthroplasty lead to surgical revisions, reaching about 59.000 per year (Song et al. 2013). The incidence of periprosthetic joint infections ranges from 9 to 40 % after primary or revision procedures, respectively (Arciola et al. 2015). Similarly, long bone fractures requiring a fixation have been estimated to account for 73 % of the total number of fractures (Ensrud 2013), and the rate of infections after internal/external fixation of fractures ranges from 2 to 30 % in closed and open fractures, respectively (Trampuz and Widmer 2006). Overall, in these cases, the presence of implant devices (metals, polymers or ceramics) promotes the risk of microbial infections (Ribeiro et al. 2012).

Among several pathogens involved in implant-related infections, staphylococci account for the two-thirds of clinically isolated bacteria (Arciola et al. 2015; Campoccia et al. 2005). Most of them are highly virulent pathogens – *Staphylococcus aureus* (35.5 %), *Escherichia coli* (3 %), *Pseudomonas aeruginosa* (4–6 %) – and determine both perioperative and early infections characterized by severe clinical signs. Differently, low virulent pathogens – such as *Staphylococcus epidermidis* (27.5 %) and *Propionibacterium acnes* (≈10 %) – are responsible for delayed, low-grade infections without specific clinical signs and hardly distinguishable from aseptic prosthetic failure (Trampuz and Widmer 2006; Phillips et al. 2006; Portillo et al. 2013). These bacteria responsible for the implant-related infections are typically biofilm-producers and induce both a poor host response and multidrug resistance to antimicrobial agents, thus leading to the development of resistant pathogens (e.g. MRSA and MRSE). Moreover, the diagnosis of these infections is often complicated due to the difficult isolation of such bacteria on common culture media. In addition, low virulent pathogens often require specific growth conditions such as the prolonged incubation period (up to 15 days) which is not routinely carried out in clinical laboratories (Drago et al. 2015).

Furthermore, the presence of additional comorbidities (e.g. diabetes, autoimmune diseases and immunodeficiency) increases the patients' risk to establish infections after orthopedic surgery (Ribeiro et al. 2012). This dreadful scenario inspires the scientific community to deeply investigate the mechanisms of infection development as well as the prevention and

treatment of bacterial colonization of implant devices. Due to the complex interaction between the host and microorganisms, *in vitro* studies cannot fully resemble the *in vivo* environment. Therefore, there is a real need to study the pathogenesis of orthopedic infections through *in vivo* animal models (Tatara et al. 2015). These models are also useful to explore both preventive or therapeutic strategies against infections (antimicrobial agents, antimicrobial implants and implant coating, etc.) and diagnostic methods for an early detection. Despite it is difficult to generate a human clinical onset in animals, several aspects must be considered to obtain the most appropriate model able to answer specific questions, for example, the vehicle of infections (hematogenous, contiguous, implant-related and contaminated post-traumatic infections), the bacterial strain and inoculum, and the time-dependent establishment of acute, subclinical or late chronic infections.

This review provides a 20-year overview of animal models of acute, subclinical or chronic orthopedic infections according to the pathogen virulence and inocula.

2 Methods: Inclusion Criteria and Search Strategy

The literature search was performed on PubMed database, by considering English articles published from 1996 until May 2016. The database was searched by including the following keywords: animal model(s) OR preclinical model(s) AND orthopedic infection(s) OR osteomyelitis OR prosthetic infection(s). The inclusion criteria considered the use of animal models to reproduce orthopedic infections. Specifically, we evaluated studies that employed either highly virulent pathogens at low bacterial inocula (Table 1) or low virulent pathogens (Table 2) to induce acute, subclinical or chronic infections. A total of 764 studies were identified, of which 118 duplicate and 38 no English studies were excluded. Two hundred and sixty two articles based on non-orthopedic infections (cardiovascular or gastrointestinal infections, etc.) were excluded. Of the 346 studies assessed for eligibility, 29 studies were excluded because concerning infections developed vertebral, mandibular, sternal or calvarial osteomyelitis. Again, 18 studies were excluded because concerning hematogenous-induced osteomyelitis and 12 studies were not included because describing *in vitro* procedures without the involvement of animals. Finally, 10 studies were removed from this analysis because of describing septic arthritis. Sixty-eight review articles were also excluded. The remaining 209 studies were analyzed for bacterial load, of which 157 were excluded due to the injection of highly virulent pathogens at a high load and 7 studies were not included due to the undeclared bacterial load. In conclusion, a total of 45 articles were considered: 39 regarding highly virulent pathogens at low bacterial load and 6 regarding low virulent pathogens.

The pie chart reports the selection process used in this review Fig. 1.

3 Results and Discussion

3.1 Animal Species, Bacterial Strain, Site of Inoculum and Type of Implants

Our analysis of the literature revealed that rats represent the most employed animal models (53 %) followed by mice (25 %), rabbits (20 %) and large animals (2 %). Laboratory animals take the advantages to be inexpensive and easy to handle. Moreover, the widespread availability of adequate facilities for their housing and management permits the establishment of infections. Again, these small species allow to directly investigate the distribution of bioluminescent bacteria within the organism through sophisticated imaging techniques (Sanchez et al. 2013; Heim et al. 2014, 2015; Pribaz et al. 2012; Del Pozo et al. 2009). Finally, rats and mice can be genetically modified to evaluate the host response to infections in particular diseases like diabetes (Lovati et al. 2013, 2014).

Table 1 Animal models of orthopedic infections determined by high virulent pathogens at low bacterial inocula

Species	Strain and inoculum	Site of inoculum	Type of implants	Time points	Study aim	Results	Reference
Rats (Sprague-Dawley)	*S. aureus* (KCTC16121) 10^3 CFU	Femoral canal	Absence of implants	3, 6 weeks	To generate an ion-complexed antibiotic to allow for the continuous release of drug to treat osteomyelitis.	The ion-complexed doxycycline has a greater therapeutic effect for osteomyelitis compared to the untreated control and free doxycycline hyclate treatment.	Oh et al. (2016a)
Rats (Sprague-Dawley)	*S. aureus* (KCTC1621) 10^3 CFU	Femoral canal	Absence of implants	6 weeks	To investigate the role of a vancomycin-loaded bone cement to treat bone osteomyelitis.	The antibiotic-laded bone cement is successful in treating osteomyelitis.	Oh et al. (2016b)
Rats (Sprague-Dawley)	*S. aureus* (subsp. Rosenbach) 10^2 CFU	Femoral canal	K-wire coated with gentamicin/palmitate	2, 4, 6 weeks	To evaluate the efficacy of controlled local release of gentamicin base from a highly lipophilic gentamicin/palmitate compound to reduce bone bacterial growth.	Implants coated with gentamicin/palmitate significantly reduce periprosthetic bacterial growth and signs of systemic inflammation compared to uncoated implants.	Fölsch et al. (2015)
Rats (Sprague-Dawley)	*S. aureus* (ATCC 49230) 10^3 CFU	Tibial fracture	Titanium K-wire	5 weeks	To develop a new animal model for fracture delayed union due to osteitis.	Biomechanical testing shows a significant higher maximum torque in the non-infected compared with the infected group. A significantly higher callus formation and lower leukocyte count are found in the non-infected group than in the infected one.	Helbig et al. (2015)
Rats (Sprague-Dawley)	*S. aureus* (ATCC 25923) 10^3, 10^4, 10^5 and 10^6 CFU	Unicortical hole in tibial metaphysis	Titanium conical implant	6 weeks	To evaluate a novel animal model to generate an implant-associated infections.	A significantly high viable bacterial count in bone samples of animals inoculated with 10^6 CFU was found. Pus and abscess formation is only observed for implants inoculated with at least 10^5 CFU. Comparison between low and high bacterial inocula.	Haenle et al. (2013)
Rats (Sprague-Dawley)	MRSA (clinical isolated from sepsis) 10^2 CFU	Tibial canal	Titanium rods (Ø 1 mm) coated with HA added or not with Ag	1, 2, 3 days	To clarify the Ag-HA coating antibacterial activity against MRSA in the medullary cavity.	Ag-HA coatings may help prevent surgical-site infections associated with joint replacement.	Akiyama et al. (2013)

Rats (Sprague-Dawley)	*S. aureus* (MN8 and UAMS-1 wild type) 10^2, 10^3, 10^4 and 10^5 CFU *S. aureus* (MN8 and UAMS-1 mutants *ica*::tet) 10^3 CFU	Femoral and tibial canal	Metal alloy and polyethylene implant	2, 6 weeks	To develop a new rat model of implant-related osteomyelitis.	An inoculum of at least 10^4 CFU results in signs of osteomyelitis with loosening of the prosthesis. An inoculum of 10^3 CFU gives signs of osteomyelitis but the prosthesis remains in situ. Bacterial inocula of 10^2 CFU show no signs of osteolysis.	Søe et al. (2013)
Rats (Sprague-Dawley)	*S. aureus* (ATCC 49230 and Xen 36 ATCC49525) 10^2 CFU	Femoral fracture	Polyacetyl plate and K-wire	2 weeks	To evaluate whether local delivery of D-Amino Acids, a biofilm dispersal agent, protects scaffolds from contamination and reduces microbial burden in segmental defects.	The local delivery of D-Amino Acids reduces bacterial contamination by targeting bacteria within biofilm.	Sanchez et al. (2013)
Rats (Sprague-Dawley)	*S. aureus* 10^2 CFU	Femoral fracture	Stainless steel K-wire	3 weeks	To evaluate the infection rates among rats with MCP-1 and IL-12 coated implants using an open fracture infection model.	Local applications of cytokines (MCP-1, IL-12) prevent infections and the use of cytokines relevancies useful to circumstances of impaired wound/fracture healing with suboptimal macrophage function.	Li et al. (2010)
Rats (Sprague-Dawley)	*S. aureus* (clinical wound isolate) 10 and 10^2 CFU	Femoral fracture	Stainless steel K-wire (Ø 0.045 mm)	1,2,3 weeks	To develop a model that includes trauma (blunt fracture in the fashion of Bonnarens and Einhorn), surgical stabilization (standardized intramedullary K-wire fixation), and infection.	An inoculum of 10^2 CFU produces a 90–100 % infection rate. An immunosuppression (decreased IL-12 levels) is demonstrated after fracture fixation versus non-fractured controls.	Lindsey et al. (2010a)
Rats (Sprague-Dawley)	*S. aureus* (clinical wound isolate) 10^2 CFU	Femoral fracture	Stainless steel K-wire	1, 2, 3 weeks	To study the effect of IL-12 systemic therapy on a previously established open fracture model.	The overall infection rate is not changed by IL-12 supplementation. Bacterial qualitative growth scores are significantly lower in the IL-12 treated group at day 10, which corresponds to the lowest level of systemic IL-12 in the fracture group.	Lindsey et al. (2010b)
Rats (Sprague-Dawley)	*S. aureus* (clinical strain isolated from infected prosthesis) 10^3, 10^4, 10^5 and 10^6 CFU	Femoral critical defects	Bovine collagen, polyacetyl plate, K-wire	1, 2, 3, 4 weeks	To characterize a new model of chronic osteomyelitis in rats.	An inoculum of 10^4 CFU over 2 weeks is found to consistently create an infection without severe lysis and loss of fixation stability.	Chen et al. (2005)

(continued)

Table 1 (continued)

Species	Strain and inoculum	Site of inoculum	Type of implants	Time points	Study aim	Results	Reference
Rats (Sprague-Dawley)	*S. aureus* (ATCC 49230) 10^2 CFU	Tibial canal	Stainless steel K-wire (Ø 0.8 mm)	6 weeks	To evaluate the efficacy of locally (poly(D,L-lactide) (PDLLA) coating of titanium implants versus one-shot systemically applied gentamicin in a rat model.	Uncoated or PDLLA-coated rods without systemic treatment develop osteomyelitis and are positive on *S. aureus*. Osteomyelitis can be prevented by prophylaxis of systemically applied gentamicin in 15 % of animals. Onset of infection can be prevented in 90 % of animals treated with gentamicin PDLLA-coated implants, and in 80 % of animals treated with a combination of local and systemic application.	Lucke et al. (2005)
Rats (Sprague-Dawley)	*S. aureus* (ATCC 49230) 10^2, 10^3 and 10^6 CFU	Tibial canal	Titanium K-wire (Ø 1 mm)	1, 2, 3, 4 weeks	To investigate the pathology of infection in orthopedic surgery by developing a new rat model of implant-related osteomyelitis.	All animals inoculated with *S. aureus* in either concentration develop signs of osteomyelitis in correlation to the amount of inoculated bacteria.	Lucke et al. (2003a)
Rats (Sprague-Dawley)	*S. aureus* (ATCC 49230) 10^3 CFU	Tibial canal	Titanium K-wire (Ø 1 mm)	6 weeks	To evaluate the efficacy of a new biodegradable, gentamicin-loaded poly(D,L-lactide) (PDLLA) coating of orthopedic devices in preventing implant-related osteomyelitis.	Local application of 10 % gentamicin-coated orthopedic devices containing PDLLA significantly reduced implant-related infection.	Lucke et al. (2003b)
Rats (Wistar)	*S. aureus* (ATCC 25923) 10^2 and 10^3 CFU	Unicortical hole in tibial metaphysis	Titanium screws coated with HA added or not with Ag	6 weeks	To evaluate a novel animal model mimicking infection with low amounts of bacterial inocula and to study the device osseointegration.	Development of a model of implant-related osteomyelitis in rats with low amounts of bacteria to better mimic clinical constellations. The addition of Ag-HA coated implant cannot reduce the infection rates.	Harrasser et al. (2016)
Rats (Wistar)	*S. aureus* (ATCC 29213) 4×10^2, 10^4 and 10^6 CFU	Tibial canal	Fibrin glue	4, 8, 12, 16 days	To observe the pain condition in a rat model of osteomyelitis induced by *S. aureus* and the alleviating effect of Celecoxib.	Rats with osteomyelitis induced by *S. aureus* display a chronic pain state, being an appropriate model for bone inflammation pain.	Yang et al. (2012)
Rats (Wistar)	*S. aureus* (ATCC 25923) 10^3 CFU	Tibial fracture	Stainless steel K-wire (Ø 0.8 mm)	6 weeks	To compare the effectiveness of different surgical modalities in the treatment of implant-related infection: surgical debridement, teicoplanin-loaded cement and teicoplanin-loaded autogenous bone.	The teicoplanin-loaded cement group reveals superior results compared with the other groups in terms of reduction of bacterial colonies, despite three animals reveal extensive infection.	Sener et al. (2010)

Rats (Wistar)	*S. aureus* (ATCC 25923) 10^3 CFU	Femoral canal	Titanium K-wire (Ø 1.25 mm) coated with vancomycin-sol-gel	4 weeks	To evaluate the efficacy of a vancomycin-coated titanium rods in treating implant-related infections.	The vancomycin-containing sol-gel film on titanium rods can successfully treat bacterial infections. The untreated control group shows extensive bone degradation, abscesses and periosteal reaction, and bone resorption.	Adams et al. (2009)
Rats (Wistar)	*S. aureus* (ATCC 25923) 10^3, 10^5 and 10^7 CFU	Femoral canal	Titanium K-wire coated (Ø 1 mm) with vancomycin	2, 3, 4 weeks	To generate an animal model of infection and to evaluate the efficacy of a vancomycin-coated titanium rods in treating implant-related infections.	The infection is detected in all animals receiving 10^3 CFU. A similar result is observed using 10^5 CFU at 3 weeks, and the high infectious doses 10^7 CFU after 10 days. The 10^3 CFU model is used to test vancomycin-coated implants that show superior inhibition of bacterial attachment and proliferation compared to control titanium surfaces.	Antoci et al. (2007)
Rats (Wistar)	MRSA (clinical osteomyelitis isolate) 10 CFU	Tibial canal	K-wire (undefined alloy) (Ø 1 mm)	3 months	To evaluate the efficacy of poly(D, L-lactide-co-glycolide) microspheres containing sodium fusidate for the local treatment of chronic osteomyelitis.	The implanted poly(D,L-lactide-co-glycolide) microspheres containing sodium fusidate are effective for the treatment of chronic osteomyelitis.	Cevher et al. (2007)
Rats (Wistar)	*S. aureus* (bovine mastitis strain) 6×10, 10^2, 10^3 and 10^4 CFU	Unicortical hole in tibial metaphysis	Absence of implants	1 week	To assessed the relationship between inoculation dose and histological, radiological, and microbiological changes in the acute phase using this rat osteomyelitis model.	The development of significant histological and radiological signs of osteomyelitis requires an inoculum of at least 6×10^3 CFU.	Fukushima et al. (2005)
Mice (C57BL6)	*S. aureus* (subsp. Rosenbach) 2×10^3 CFU	Unicortical hole in tibial metaphysis	Absence of implants	4 weeks	To evaluate the efficacy of debridement and subsequent antibiotic therapy or antibiotic treatment with gentamicin alone in osteomyelitis.	A reduction or eradication of *S. aureus* is found within debrided bones treated with antibiotic, whereas sole antibiotic therapy does not provide sufficient treatment of the osteomyelitis.	Wagner et al. (2016)
Mice (C57BL6)	MRSA (USA300 LAC::*lux*) 10^3 CFU	Femoral canal	Titanium K-wire (Ø 0.6 mm)	1–4 weeks	To study the inflammatory events and cytokines associated with *S. aureus* periprosthetic joint infection.	Several cytokines are significantly elevated in the mouse model, as well as an increase of MDSC infiltrates, reduced monocyte, macrophage, and T-cell influx compared with uninfected animals. These findings reveal a critical role for IL-12 in	Heim et al. (2015)

(continued)

Table 1 (continued)

Species	Strain and inoculum	Site of inoculum	Type of implants	Time points	Study aim	Results	Reference
						shaping the anti-inflammatory biofilm milieu by promoting MDSC recruitment.	
Mice (C57BL6)	MRSA (USA300 LAC::*lux*) 10^3 CFU	Femoral canal	Titanium K-wire (Ø 0.6 mm)	4 weeks	To investigate the role and function of MDSCs in *S. aureus* periprosthetic joint infection.	MDSCs are key contributors to the chronicity of *S. aureus* biofilm infection, as their immunosuppressive function prevents monocyte/macrophage pro-inflammatory activity, which facilitates biofilm persistence.	Heim et al. (2014)
Mice (C57BL6)	MRSA (USA300-NRS384) 10^3 CFU	Tibial canal	Stainless steel pins (Ø 0.25 mm)	3 weeks	To test purified, recombinant HlaH35L in three mouse models of *S. aureus* infection: systemic infection, skin and soft tissue infection, and chronic prosthetic implant infection.	In this prosthetic implant model of chronic biofilm infection, there is no significant difference in bacterial levels when compared to controls. These results demonstrate that vaccines may confer protection against one form of *S. aureus* disease.	Brady et al. (2013)
Mice (C57BL6)	*S. aureus* (ALC2906, Xen29, Xen36, Xen40) 10^2, 10^3 and 10^4 CFU	Femoral canal	Stainless steel (Ø 0.6 mm) or titanium (Ø 0.8 mm) K-wire	6 weeks	To develop a model of a chronic post-arthroplasty infection.	Xen29, Xen40 and Xen36 are feasible for long-term in vivo monitoring of bacterial burden and biofilm formation to study chronic post-arthroplasty infections and potential antimicrobial interventions.	Pribaz et al. (2012)
Mice (C57BL6)	*S. aureus* (ALC2906) 5×10^2, 10^3 and 10^4 CFU	Femoral canal	Stainless steel K-wire (Ø 0.6 mm)	2 weeks	To develop a model of a post-arthroplasty infection to measure the bacterial burden in real-time by means of luminescence.	Mice inoculated with 5×10^3 and 5×10^4 CFUs develop increased bacterial counts consistent with an acute joint infection. Mice inoculate with 5×10^2 CFUs develop a low-grade infection, resembling a chronic infection.	Bernthal et al. (2010)

Mice (BALB/c)	*S. aureus* (ATCC 49230-UAMS1) 10^3 CFU	Femoral canal	Absence of implants	2 days	To provide evidence for the in vivo production of inflammatory chemoattractant molecules by osteoblasts during bacterial infection of bone tissue.	Demonstration of in vivo expression of the chemokines (MCP-1) by murine osteoblasts in *S. aureus*-infected bone under in situ-like conditions.	Marriott et al. (2005)
Mice (BALB/c)	*S. aureus* (ATCC 49230-UAMS1) 10^3 CFU	Femoral canal	Absence of implants	2, 4 days	To provide the first evidence for the in vivo production of inflammatory mediators by osteoblasts during bacterial infection of bone tissue.	Demonstration of the in vivo expression of the key inflammatory cytokine (interleukin-6) by murine osteoblasts in *S. aureus* infected bone under in situ-like conditions.	Marriott et al. (2004)
Mice (NOD/ShiLtJ type I diabetes and CD1)	*S. aureus* (ATCC 25923) 10^3 CFU	Femoral canal	Stainless steel needle (Ø 0.5 mm)	4 weeks	To validate a model of implant-related infection in the diabetic mouse.	Unlike the controls and the CD1 mice, all the diabetic mice receiving a single inoculum of *S. aureus* display severe osteomyelitis changes around the implant.	Lovati et al. (2013)
Mice (NOD/ShiLtJ type I diabetes)	*S. aureus* (ATCC 25923) 10^3 CFU	Femoral canal	Stainless steel needle (Ø 0.5 mm)	4 weeks	To investigate the effect of a PGE_1 vasodilator on the incidence of implant-related infections in diabetic mice.	Increased host response to implant-related infection in diabetic mice treated with the combination of a PGE_1 and antibiotic, with restrained signs of infections. The diabetic mice treated with the antibiotic alone show a severe infection and inability to successfully respond to the antimicrobial treatment.	Lovati et al. (2014)
Mice (CD1)	*S. aureus* (ATCC 49230) 10^3 CFU	Tibial canal	Silk suture	2 h–4 weeks	To evaluate the cellular response to trauma and infection in a murine model of posttraumatic osteomyelitis.	Cytokines, applied at the time of injury, may be able to alter the host response to trauma with contamination, promote better elimination of the necrotic and infected tissue, and reduce the risk of development of chronic osteomyelitis.	Pesanti and Lorenzo (1998)
Rabbits (New Zealand)	*S. aureus* (JAR060131) 6×10^2, 10^3 and 10^6 CFU	Humeral fracture	Stainless steel nail and plate	4, 10 weeks	To create human standardized and repeatable preclinical models of implant-related bone infection after osteosynthesis in the rabbit humerus.	Higher bacterial doses lead to an increasing infection rate. In infected groups, there is a complete lack of osteotomy closure at 4 weeks. In the 10-week infection group, healing does not occur in the plated rabbits.	Arens et al. (2015)

(continued)

Table 1 (continued)

Species	Strain and inoculum	Site of inoculum	Type of implants	Time points	Study aim	Results	Reference
Rabbits (New Zealand)	MRSA (ATCC 43300) 5×10^2 CFU	Femoral canal	Titanium rods (Ø 2.5 mm) coated with HA added or not with Ag	6 weeks	To compared the bacterial colonization resistance in Ag-HA-, HA-coated titanium prostheses and uncoated prostheses.	Ag-HA coated titanium implants lead to an increased resistance to bacterial colonization compared to uncoated implants.	Kose et al. (2013)
Rabbits (New Zealand)	*S. aureus* (P1 strain variant ATCC 25923) 5×10^2 CFU	Femoral canal	Titanium pins (Ø 2.8 mm)	1 week	To assess the efficacy of a coating with minocycline and rifampin to prevent colonization of a grit-blasted titanium implant and subsequent osteomyelitis.	The antimicrobial-coated devices have a significantly lower rate of colonization than the uncoated devices and are associated with significantly lower rates of device-related osteomyelitis.	Darouiche et al. (2007)
Rabbits (New Zealand)	MRSA (strains-021) 10^2, 10^3 and 10^4 CFU	Unicortical hole in femoral epiphysis	Stainless steel screw	7 days	To generate an arthroplasty infection model in rabbits.	The uninfected group does not demonstrated evidence of biomaterial infection. 40 % of 10^2 CFU injected rabbits develops a biomaterial infection. 70 % of 10^3 and 10^4 CFU injected rabbits develops a biomaterial infection.	Craig et al. (2005)
Rabbits (New Zealand)	*S. aureus* (strain V 8189–94) 4×10^3 up to 4×10^7 CFU	Tibia	Stainless steel or titanium plates	1 week	To evaluate a radiographic-microbiological correlation in rabbit tibiae after local inoculation of different doses of *S. aureus*.	An association between the amount of inoculated bacteria and the extent of radiographic changes is found.	Kraft et al. (2001)
Rabbits (New Zealand)	*S. aureus* (ATCC 49230; ATCC 13709) 2×10^3 to 2×10^6 CFU	Radial canal	Absence of implants	4 weeks	To evaluate a rabbit model of *S. aureus* osteomyelitis.	An infection rate of 75 % is found with an inoculum of 2×10^3 CFU *S. aureus* ATCC 49230, despite radiographic and histological signs of infection are seen with at least 2×10^4 CFU after 3 weeks. A minimum inoculum of 2×10^6 CFU *S. aureus* ATCC 13709 needs to have similar osteomyelitis signs, showing a lower virulence than ATCC 49230.	Smeltzer et al. (1997)

CFU colony forming unit, *MRSA* methicillin-resistant *S. aureus*, *HA* hydroxyapatite, *Ag* silver, *MDSC* myeloid-derived suppressor cells, *IL-12* interleukin 12, *PGE* prostaglandin E1, *MPC-1* monocyte chemoattractant protein-1, *HlaH35L* genetically inactivated form of alpha hemolysin

Table 2 Animal models of orthopedic infections determined by low virulent pathogens

Species	Strain and inoculum	Site of inoculum	Type of implants	Time points	Study aim	Results	References
Rats (Wistar)	MRSE (clinical strain) 10^3, 10^5 and 10^8 CFU	Femoral fracture	Stainless steel plates	8 weeks	To evaluate the development of biofilm-related nonunion in rats.	Dose-dependent effect between bacterial inoculum and nonunion rate. Role of subclinical infections in orthopedic.	Lovati et al. (2016)
Rats (Sprague–Dawley)	*S. epidermidis* (ATCC 35984) 10^5 CFU	Femoral canal	Polymethylmethacrylate cement and K-wire (Ø 1 mm)	2, 4 weeks	To evaluate the effectiveness of a proteolytic enzyme, serration peptidase to eradicate a periprosthetic infection.	At two weeks, inoculated bacteria grow on 63.2 % culture of untreated specimens. The infection persisted in 5.6 % in the serratiopeptidase-and-antibiotic group, whereas it is present in 37.5 % in the antibiotic-only group.	Mecikoglu et al. (2006)
Rabbits (New Zealand)	*S. epidermidis* (Xen43) 10^4 CFU	Tibial canal	Stainless steel electrode (Ø 3 mm)	7 weeks	To evaluate the efficacy of a treatment with low-amperage electrical current compared to intravenous doxycycline treatment or no treatment in osteomyelitis.	Treatment with electrical current was statistically significantly more efficacious than doxycycline treatment in osteomyelitis.	Del Pozo et al. (2009)
Rabbits (New Zealand)	*S. epidermidis* (RP62A) 4×10^5 CFU	Femur canal	Stainless steel and titanium K-wire (Ø 2 mm)	48 h	To assess the biofilm adhesion to metal implants. A direct bacterial inoculation or biofilm-coated implants were compared.	The direct inoculation model gives larger and more reproducible biofilm adhesion to implanted metals. *S. epidermidis* shows lower adhesion ability to metals, and biofilm adheres greater to stainless steel over titanium.	Sheehan et al. (2004)

(continued)

Table 2 (continued)

Species	Strain and inoculum	Site of inoculum	Type of implants	Time points	Study aim	Results	References
Rabbits (New Zealand)	*P. acnes* (RMA13884) 6×10^6 to 2×10^8 CFU	Tibial canal	Absence of implants	4 weeks	To establish a rabbit model of implant-associated infection with *P. acnes*.	Low dose of *P. acnes* could be cleared by the host immune response. 10^8 CFU is needed for *P. acnes* to establish a chronic infection due to the reduced virulence associated with this bacterial species.	Achermann et al. (2015)
Goats (Saanen)	*S. epidermidis* (HBH276) 3×10^5 CFU	Tibiae	External fixation stainless steel pins (Ø 3 mm)	3 weeks	To determine whether a direct electric current of 100 μA can prevent clinical infection around percutaneous pins implanted in tibia.	The infection develops in 89 % of the control pin sites, whereas only 11 % of the pin sites in the current group shows infection. The low amperage electric current prevents infections of percutaneous pin sites of external fixators.	van der Borden et al. (2007)

CFU colony forming unit, MRSE = MRSA = methicillin-resistant *S. epidermidis*

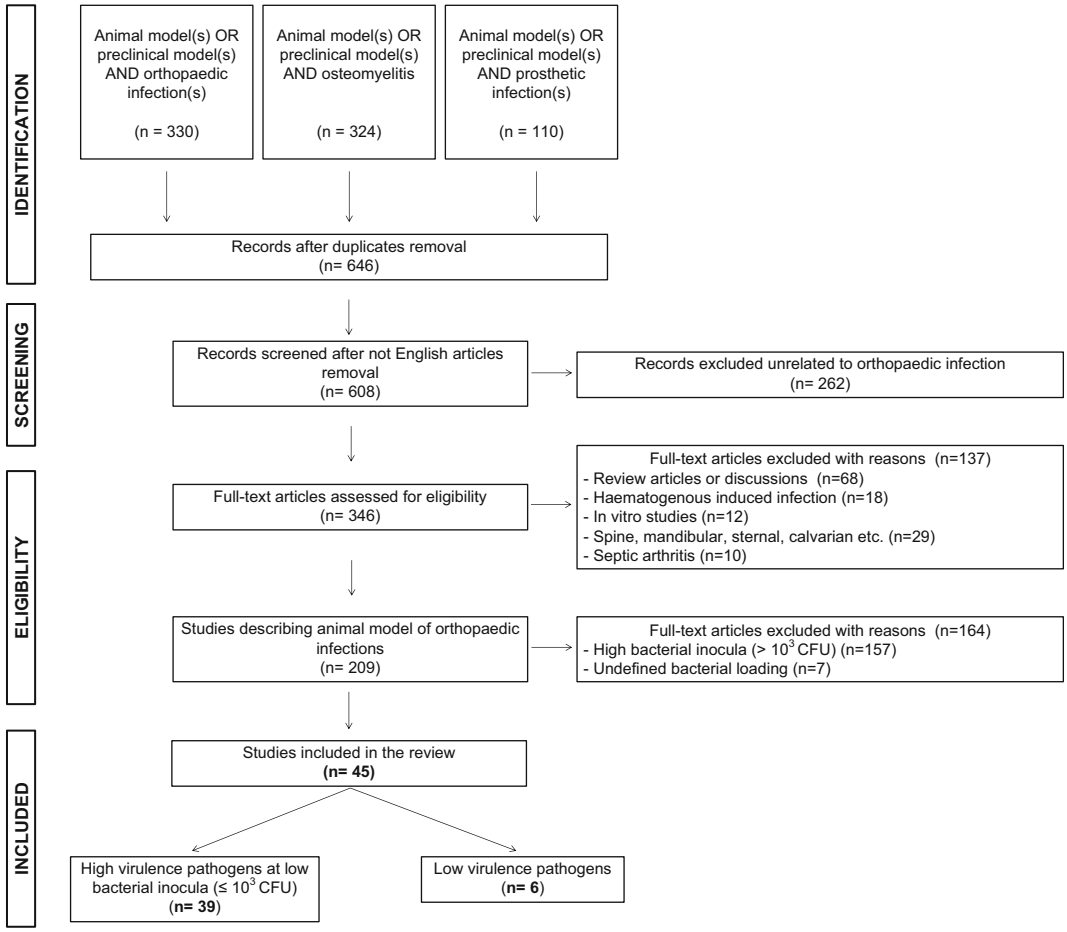


Fig. 1 Research strategy. Flow chart of the selection process

Differently, despite large animals offer suitable dimensions, weight-bearing similar to humans and tolerate multiple procedures, they are more expensive and require specific infrastructures and practiced personnel to be managed (Tatara et al. 2015).

Overall, all the analyzed studies regarding the use of highly virulent pathogens at low bacterial inocula used different *S. aureus* strains derived from clinical isolates, genetically modified or methicillin-resistant. Among studies that induced infections by means of low virulent pathogens, *S. epidermidis* is the main employed pathogen, while only one study described the use of *P. acnes* (Achermann et al. 2015).

Several techniques are described to reproduce an animal model of infection. In particular, the most frequent site of inoculum is represented by the medullary canal of long bones (69 %), followed by fractures (18 %), and only a few studies described the generation of bone defects within the long bone epiphysis/metaphysis in which bacteria were grafted (13 %).

Most of the models proposed implant-related infections by using metallic implants (titanium or stainless steel) (73 %) or other materials (collagen, fibrin glue, and sutures) (9 %). Only seven studies determined the infections without implant materials to investigate the development of osteomyelitis (18 %).

3.2 Animal Models of Orthopedic Infections Induced by Highly Virulent Pathogens at Low Bacterial Inocula

In the panorama of animal models able to mimic the establishment of orthopedic infections, *S. aureus* is the major causative pathogen. *S. aureus* is characterized by the high bone affinity, the capability to develop osteomyelitis lesions and to induce bone resorption. Furthermore, both the antimicrobial resistance and evasion of the host immune system of the *S. aureus* make this pathogen clinically relevant. Indeed, its virulence factors are responsible for forming the biofilm that generates a favorable microenvironment for the bacterial growth masking them from the host response. To better understand the pathogenesis of *S. aureus*-induced osteomyelitis, the dose–response relationship of single strains together with the time course of the infection need to be deeply investigated *in vivo*. This series is summarized in Table 1.

3.2.1 Models of Post-traumatic Osteomyelitis by Direct Injection of Bacterial Suspensions

In our review, several studies generated animal models of orthopedic infections by injecting bacteria directly into the long bone medullary canal. In 1997, Smeltzer and colleagues characterized a model of staphylococcal osteomyelitis in rabbits. They demonstrated that a small inoculum (2×10^3 CFU) of a highly virulent *S. aureus* isolated from human chronic osteomyelitis (ATCC 49230, UAMS-1) could establish a bone infection in 75 % of animals during the 4-week time course, resembling the amount of bacteria that might be delivered during a traumatic injury in the absence of any implants. Differently, the development of significant signs of osteomyelitis required an inoculum of at least 2×10^4 CFU. The same *S. aureus* strain and dose of inoculum – resembling a trauma-induced osteomyelitis – were employed in mice to provide evidence of the role of osteoblasts in the production of specific inflammatory cytokines, such as interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1), that promote the host immune response (Marriott et al. 2004, 2005). Wagner et al. (2016) described another post-traumatic model of osteomyelitis with the aim to translate to clinical approaches. A mouse model of low-grade *S. aureus*-related infection was developed, and treated with the combination of surgical debridement and antibiotic therapy as a gold standard in clinical practice. Similarly to Smeltzer et al. (1997), another group evaluated the relationship between the bacterial dose and the clinical signs of osteomyelitis in rats (Fukushima et al. 2005). They demonstrated that a minimal dose of 6×10^3 CFU was causative of acute clinical changes when directly injected within the tibial canal. However, the obtained results were not strictly comparable with those of Smeltzer et al. (1997), because Fukushima et al. (2005) employed a strain of *S. aureus* isolated from a case of bovine mastitis that was chosen for its high virulence in rodents.

Other two recent studies investigated the response to locally-delivered antimicrobial agent within the femoral canal in a rat model of low dose *S. aureus*-induced osteomyelitis in the absence of implants (Oh et al. 2016a, b). In particular, they tested an ion-complexed antibiotic (Oh et al. 2016a) or antibiotic-loaded bone cement (Oh et al. 2016b) to evaluate their therapeutic efficacy in the osteomyelitis.

3.2.2 Models of Osteomyelitis by Bacterial-Loaded Carriers

Three studies determined bone infections by means of carriers to firmly enclose bacteria within the long bone canals or critical defects. Pesanti and Lorenzo (1998) loaded a low dose (1×10^3 CFU) of *S. aureus* (ATCC 49230) into a silk-based suture to be directly introduced within the tibial canal of mice. Through this approach, they aimed to study the activity of IL-4 in inhibiting the osteoclasts function during the bone repair process in chronic osteomyelitis. To locally carry bacteria, Yang et al. (2012) employed fibrin glue loaded with different

concentrations of S. *aureus* (ATCC 29213) ranging from 4×10^2 to 10^9 CFU. They demonstrated that a stable and persistent pain behavior associated with signs of bone osteomyelitis was induced by loading 10^4 or 10^6 CFU. Otherwise, 10^2 CFU of the used strain were not sufficient to determine a bone inflammation able to produce any pain state. In the study of Chen et al. (2005), a critical size femoral defect was synthesized with polyacetyl plates and infected by means of collagen type I wetted with different doses of a clinical isolated *S. aureus*. They did not detect any signs of bone lysis in any animal inoculated with 10^3 CFU either after 1 or 2 weeks. Differently, the 10^4 CFU inoculum represented the lower *S. aureus* concentration that resulted in an infection in all rats in the shortest time (2 weeks).

3.2.3 Models of Implant-Related Orthopedic Infections: Intramedullary Devices

To better reproduce the clinical onset of infections, most of the analyzed studies employed metallic alloy implants (stainless steel and titanium) either to deliver bacteria or to act as a surface for the bacterial adhesion and biofilm formation. Consequently, the presence of biofilm makes difficult the eradication of microorganisms.

Recently, an innovative model of implant-related infection was developed in type I diabetic mice (Lovati et al. 2013). The authors determined the infection by means of a low-grade inoculum (10^3 CFU) of *S. aureus* (ATCC 25923) within the femoral canal implanted with a stainless steel needle. After the validation of this model, the same group tested the efficacy of systemically-injected prostaglandin E1 in preventing the development of osteomyelitis. The use of a vasodilator enhanced the local blood flow and improved the intake of the antibiotic therapy within the bone (Lovati et al. 2014).

Bernthal et al. 2010 developed a dose-related model of post-arthroplasty infection combining the use of bioluminescent *S. aureus* and genetically-engineered mice expressing fluorescent neutrophils. In particular, they infected the femoral canal after placing a stainless steel K-wire with 5×10^2, 10^3 and 10^4 CFU of ALC2906 strain in order to track both the bacteria and the host neutrophils through an *in vivo* imaging technique. This study verified that mice inoculated with 5×10^2 CFU developed a low-grade infection resembling a chronic onset. The group of Brady et al. (2013) implanted a stainless steel K-wire within the tibia of mice, then infected with a low bacterial load of methicillin-resistant *S. aureus,* to investigate the efficacy of a novel vaccine derived from *S. aureus* antigen (inactivated form of alpha hemolysin). This immunization strategy did not confer protection in case of prosthetic implant-related infection.

With the most recent techniques to genetically engineer bacteria with bioluminescent genes, titanium implants permitted also the evaluation of brightest bioluminescent strains (i.e. Xen36) avoiding any artifacts associated with the use of stainless steel tools. Pribaz et al. (2012) compared the biofilm formation derived from bioluminescent strains (Xen36 and Xen40) on both stainless steel and titanium K-wires when implanted within the femoral canal of mice.

Other two models in mice determined a periprosthetic joint infection by using titanium K-wires, then injected with a low dose (10^3 CFU) of a bioluminescent *S. aureus* strain (USA300; LAC::*lux*), thus representing a realistic bacterial exposure that might occur in patients (Heim et al. 2014, 2015). Thanks to these models, the authors evaluated the role of myeloid-derived suppressor cells (MDSC) and their pro-inflammatory activity associated with *S. aureus* biofilm. Moreover, they identified the IL-12 role in the recruitment of MDSC at the site of periprosthetic infection, in which MDSC impaired the phagocyte activity and reduced the pro-inflammatory events in this type of infections.

Other authors used titanium implants to be as close as possible to the clinical situation. In particular, Lucke et al. (2003a) were the first to introduce the use of titanium intramedullary nails in a rat model of acute osteomyelitis. They demonstrated that the signs of osteomyelitis

occurred in correlation to the amount of the bacterial inocula, finding that also a low dose (10^2 CFU) of *S. aureus* ATCC 49230 was sufficient to determine typical signs of bone infection, despite significantly lower compared to the 10^3 and 10^6 CFU inocula.

Two more studies in rat models of implant-related osteomyelitis employed undefined metallic alloys as intramedullary nails (Cevher et al. 2007) or non-constrained knee prosthesis (Søe et al. 2013). After determining a chronic bone infection with a very low dose (10 CFU) of clinically isolated methicillin-resistant *S. aureus*, Cevher et al. (2007) tested the effectiveness of a local treatment with poly(D, L-lactide-*co*-glycolide) microspheres containing sodium fusidate. Differently, Søe et al. (2013) established an acute model of osteomyelitis associated with metallic implants to evaluate the dominant features of two different *S. aureus* strains (MN8 and UAMS-1) in their wild-type or genetically-modified forms in developing implant-related infections. In their study, the authors sustained the 10^3 CFU inoculum as a suitable experimental condition, supporting the findings of Fukushima et al. 2005.

3.2.4 Models of Intramedullary Antimicrobial Treatment of Implant-Related Infections

In the panorama of several antimicrobial agents used against bacterial infections in orthopedics, vancomycin, gentamicin and rifampin are the most efficient antibiotics. In particular, vancomycin is commonly used for its proven efficacy against methicillin-resistant gram-positive cocci. However, there are some drawbacks related to the systemic administration (poor local intake, unpredictable kinetics, etc.), thus, the need to locally deliver the antibiotic molecules encouraged to identify innovative implant-coating systems or bioabsorbable materials that permitted a controlled release of antibiotics.

A periprosthetic rat model of infection was established by Antoci et al. 2007 to examine the activity of chemically-bonded vancomycin on titanium rods implanted within the rat femoral canal in inhibiting the implant colonization of a low inoculum (10^3 CFU) of *S. aureus* ATCC 25923. Another group coated titanium rods with a sol-gel film of vancomycin verifying the complete release of the antibiotic within 14–21 days in a rat model of *S. aureus* (ATCC 25923) femoral infection (Adams et al. 2009).

It is well known that gentamicin-loaded bone cements are effective to contain the infection rates in endoprosthetic surgery. Moreover, the systemic injection of gentamicin was recognized to be related to side effects such as ototoxicity and severe nephrotoxicity, especially in critically ill patients. Thus, novel strategies to carry this antibiotic directly at the site of implant have been investigated. By using the model proposed in a previous study (Lucke et al. 2003a), Lucke et al. 2003b evaluated the efficacy of a titanium K-wire coated with 10 % gentamicin-loaded poly(D, L-lactide) in preventing implant-related osteomyelitis and they demonstrated a significantly reduced infection in rats. The same group investigated the activity of the aforementioned coated implants able to locally deliver the 10 % gentamicin also in an acute model of osteomyelitis determined by the injection of 10^2 CFU *S. aureus* ATCC 49230 and compared to a systemic treatment with the same antibiotic (Lucke et al. 2005). In this study, they demonstrated that the 90 % of animals treated with 10 % gentamicin-loaded poly(D, L-lactide) on titanium implants did not establish a bone infection. A similar result was obtained in rats treated with the combination of local and systemic delivery of gentamicin. Another study by Fölsch et al. 2015 established a rat model of acute implant-associated osteomyelitis with 10^2 CFU *S. aureus* (sbsp. Rosenbach) subsequently implanted with a gentamicin/palmitate-coated K-wire. They showed that coated-implants significantly reduced the bacterial growth as well as the inflammatory response compared to the uncoated implants.

In orthopedic infections, a prolonged antibiotic therapy favors developing of antimicrobial resistance. For this reason, the association of different type of antibiotics could lessen this risk. Two interesting studies based on low dose inoculum (10^2 CFU) of *S. aureus*-induced

arthroplasty infections in mice (Bernthal et al. 2010) and rabbits (Darouiche et al. 2007) proposed the use of rifampin and minocycline associated and loaded onto metallic implants within the femoral canal for the treatment of this disease. In both studies, a reduced bacterial growth together with the prevention of biofilm formation and clinical signs of inflammation has been demonstrated.

Besides the aforementioned coating of implants with antibiotics, in the recent years, the mechanisms of antimicrobial action of silver ions (Ag^+) have been described. Indeed, the activity of Ag^+ is related to the interaction with thiol (sulfphydryl) groups, inhibiting the bacterial adherence and proliferation on implant devices. Due to the potential dose-dependent toxicity of silver, it has been investigated the embedding of Ag^+ within hydroxyapatite (HA), then loaded onto the implant surface. With this aim, Akiyama et al. 2013 were the first to confirm the antibacterial activity of Ag-HA coating of titanium rods against a 10^2 CFU methicillin-resistant *S. aureus* infection in the medullary canal of rat tibia. In this model, they evaluated the time-dependent Ag^+ release within the medullary cavity. The increase of the resistance to bacterial colonization of a titanium Ag-HA-coated rod was also demonstrated by Kose et al. 2013 in a rabbit model of a low dose (5×10^2 CFU) methicillin-resistant *S. aureus* (ATCC 43300) femoral infection. By embedding Ag^+ in HA-coated implant, this group sustained the efficacy of this treatment in preventing the development of bone infections while minimizing the total amount of silver and reducing its potential toxicity.

3.2.5 Models of Implant-Related Orthopedic Infections: Unicortical Bone Defects

A total joint arthroplasty animal model, including all components of a prosthetic implant does not exist in the literature. With the need to better mirror a human approach, some studies modeled unicortical bone defects in the joint, then implanted with different orthopedic devices, such as screws or conical implants. In the study by Craig et al. 2005, a model of knee arthroplasty was designed in rabbits by means of a stainless steel screw associated with polyethylene (UHMWPE) implanted in drilled femoral condyles and fixed with bone cement. In this preclinical model, the infection was determined with methicillin-resistant *S. aureus* at different concentration (10^2, 10^3 and 10^4 CFU) directly injected into the joint space before the wound closure. After 7 days, they found a biomaterial-related infection in the 70 % of animals infected with 10^3 and 10^4 CFU, but only 40 % of rabbits established a bone infection in the group inoculated with 10^2 CFU. On this basis, other authors determined unicortical defects in the tibial metaphysis of rats, infected with low amounts (10^2 and 10^3 CFU) of *S. aureus* (ATCC 25923) (Harrasser et al. 2016). Then, they introduced a titanium plasma spayed screw into the defects after being coated with HA alone or associate with a low concentration of silver to evaluate both the antimicrobial effect and the osseointegration activity of this coating. However, they demonstrated that the Ag-HA coated implants did not reduce the infection rates. A similar model of bone defects drilled in the rat tibial metaphysis was described by Haenle et al. 2013. After the defect generation, a custom-made canulated conical titanium implant was inserted and a bone infection was determined by injecting different concentrations (10^3, 10^4, 10^5 and 10^6 CFU) of *S. aureus* ATCC 25923. This study aimed to compare low and high bacterial inocula in establishing an implant-associated infection. The results confirmed that a strong infection could be induced by an initial small bacterial inoculum, since a constant bacterial growth can occur in the presence of an implant device.

3.2.6 Models of Osteomyelitis in Synthesized Fractures

Animal models able to resemble severe injuries, such as open or compound fractures are mandatory to study the risks of bacterial contaminations occurring as a result of immunosuppression in response to traumatic events. Moreover, contaminated fractures are frequently followed by complications like the increased risk of

nonunion development. In clinical practice, complicated fractures of long bones are commonly approached by means of intramedullary fixations or synthesized with plates. Helbig et al. 2015 established a new model of tibia and fibula fracture in rats by means of a specific device that was able to create a closed fracture without any soft tissue damage, as differently occurs in animal models of osteotomy. Then, they synthesized the tibia with an intramedullary titanium K-wire and determined an infection with 10^3 CFU *S. aureus* (ATCC 49230). Thanks to this model, they monitored the course of the development of osteomyelitis through biomechanical and micro-CT analysis. The study by Sener et al. 2010 was the first to compare the efficacy of antibiotic-loaded autogenous bone and other surgical procedures (debridement, use of bone cement and systemic antibiotic therapy) to treat osteomyelitis in a rat model of infected fracture. They inoculated a low concentration (10^3 CFU) of *S. aureus* (ATCC 25923) within the fracture site of the osteotomized tibia, then synthesized by intramedullary stainless steel K-wire. They concluded that the association of debridement and systemic antibiotic therapy was the most effective and primary treatment for infected fractures. Otherwise, they suggested the use of antibiotic-loaded cements only after an unsuccessful attempt at debridement. Two more studies generated models of blunt traumatic open fractures of the rat femurs, then synthesized with an intramedullary K-wire (Lindsey et al. 2010a, b). Lindsey et al. 2010a proposed a model of osteomyelitis, in which an optimal infection rate (90–10 % of rats) was achieved in rats injected with 10^2 CFU clinical-derived *S. aureus*. This model was subsequently employed by the same group to determine the effects of IL-12 systemic therapy (Lindsey et al. 2010b). The rats treated with IL-12 showed significantly higher macrophage activation, thus containing the infection rate and enhancing the natural host immune response. The same approach using IL-12 therapy to treat contaminated open fractures was proposed by Li et al. 2010. In a rat midshaft femur fracture, the bone infection was caused by injecting 10^2 CFU of *S. aureus* and fixed with an intramedullary K-wire coated with MCP-1 and IL-12p70. The local application of MCP-1 played a key role in the macrophage recruiting, while exogenous IL-12p70 stimulated the activation of macrophages and enhanced a cell-mediated immune response. This group supported that the increased number of macrophages at the site of fracture ameliorates the fracture healing by stimulating the angiogenesis, collagen synthesis and wound debridement.

Other studies proposed the osteosynthesis of critical segmental defects by means of plates fixed to the surface of long bones. Indeed, to maximize the translational potentiality, these studies evaluated the impact of the fracture fixation stability on the infection rate. Kraft et al. 2001 compared radiological changes to microbiological findings in a rabbit model of infected tibial fracture with different concentrations (4×10^3 to 4×10^7 CFU) of *S. aureus* (V 8189-94). They verified that radiographic changes can be predictors of the severity of infection related to the increasing bacterial load. In this radiographic study, the analyzed parameters were periosteal new bone formation, bone structure, peri-implant reaction, plate-screw loosening and soft tissue swelling. Another study developed a rabbit model of infected humeral fracture stabilized with either locked plates or custom-designed interlocked intramedullary nails (Arens et al. 2015). In particular, they induced a bone infection by means of *S. aureus* (JAR060131) in a dose-dependent manner from 6×10^2 to 6×10^6 CFU. They concluded that the infection was not self-limiting and that the bone incurred in a lower healing process when synthesized with plates compared to the intramedullary nails. In a critical size model of femoral fractures in rats by Sanchez et al. 2013, a novel therapeutic approach using a scaffold augmented with amino acids was proposed. Specifically, the femurs were osteotomized, synthesized with a polyacetyl plate and infected by using type I bovine collagen as a carrier for 10^2 CFU *S. aureus* (ATCC 49230 and Xen36). After the contamination, a polyurethane scaffold augmented with D-isomers of amino acids was

implanted within the fracture site. Their results showed that the local delivery of D-amino acids reduced the bacterial growth by dislodging bacteria from the biofilm.

3.3 Animal Models of Orthopedic Infections Induced by Low Virulent Pathogens

Animal models describing the use of low virulent pathogens, such as *S. epidermidis* and *P. acnes*, are poorly described in the literature probably due to their fewer incidence of orthopedic infections. Although *S. aureus* is causative of the majority acute orthopedic infections because of its high virulence, *S. epidermidis* and *P. acnes*, commonly present as commensal inhabitants of human skin, can easily break through wound in case of orthopedic traumatic injuries and surgical procedures. Furthermore, their low virulence and great capability to form biofilm on implant devices are the main causes of sneaky and latent infections. According to these observations, the need to deeply understand the mechanisms related to this kind of infections and their pathogenesis encourages to create new animal models. This series is summarized in Table 2.

Recently, Lovati et al. 2016 validated a rat model of *S. epidermidis*-induced nonunion of femoral fractures. In this study, different concentrations (10^3, 10^5 and 10^8 CFU) of a biofilm-producing methicillin-resistant clinical *S. epidermidis* (MRSE) were injected within non-critical sized fractures, and then synthesized with stainless steel plates and screws. The results demonstrated a dose-dependent effect between the MRSE inoculum and the nonunion rate. Interestingly, this study identified a relevant preclinical model to assess the role of subclinical infections occurred in the animals infected with 10^3 CFU. Moreover, the authors were able to generate an acute model (10^5 CFU) of infected nonunion characterized by severe signs of osteomyelitis, whereas animals injected with 10^8 CFU showed a greater amount of cocci embedded in the biofilm on the implant surface that led to chronic osteomyelitis development. In the study by Mecikoglu et al. 2006, a rat model of periprosthetic infection was induced by the association of a 10^5 CFU *S. epidermidis* (ATCC 35984) inoculum and stainless steel K-wire fixed with bone cement into the medullary femoral canal. The aim of this study was to eradicate the biofilm-related infection with serratiopeptidase instilled into the joint space. Microbiological results showed that the bone infection persisted in 5.6 % in the serratiopeptidase-antibiotic treated animals, whereas, in the group treated with the antibiotic alone, signs of persistent infection were found in 37.5 % of the subjects. Nevertheless, the use of serratiopeptidase had degenerative effects on the articular cartilage. The study by Sheehan et al. 2004 compared the assessment of a femoral infection in rabbits by directly injecting 4×10^5 CFU *S. epidermidis* (RP62A) or delivering the bacteria through a loaded stainless steel or titanium K-wire. The authors had interesting results in terms of a standardized and reproducible model of biofilm-related infection by the direct inoculum of bacteria within the medullary canal. Furthermore, the *S. epidermidis* showed a lower ability to adhere to the titanium surface and to produce biofilm compared to the stainless steel implant. Two studies based on the development of tibial infections in caprine (van der Borden et al. 2007) and rabbit models (Del Pozo et al. 2009) by means of a *S. epidermidis* investigated the application of electrical current to impede the bacterial adhesion on the implanted stainless steel devices. In both cases, the application of a 100–200 μA direct electric current was able to significantly reduce the number of viable bacteria, thus preventing signs of clinical infections.

In our revision of the literature, we found only one recent study that described the use of *P. acnes* to determine a tibial infection in rabbits (Achermann et al. 2015). To perform this model, the authors delivered bacteria within the intramedullary canal by means of dextran beads, in which biofilm-forming *P. acnes* grown anaerobically. This group evaluated both the dose-dependent clinical and histological signs of osteomyelitis. They classified infections as acute (6×10^6 CFU) or chronic (2×10^8 CFU) based on the presence of polymorphonuclear

neutrophils or plasma cells lymphocytes and macrophages, respectively.

4 Conclusions

The purpose of the present review is to analyze the complex panorama of animal models employed in the study of orthopedic infections caused by high or low virulent bacteria. In the literature, several animal models have been established to study osteomyelitis or implant-related infections in terms of their pathogenesis, development and diagnosis, but also to investigate the host response to bacteria and to novel therapeutic or preventive approaches to fight this critical clinical burden. The serious clinical problem associated with the challenging diagnosis of biofilm-related infections characterized by frequent negative cultures inspired the main topic of this review article. According to this, the authors examined *in vivo* studies based on either highly virulent pathogens at low bacterial inocula or low virulent pathogens to induce acute, subclinical or chronic infections. In most articles, a single bacterium is used. Among many pathogens, *S. aureus* represents the most commonly used microorganisms to develop high infection rates in animals when injected at $\geq 10^4$ CFU. However, the use of a lower bacterial inoculum ($\leq 10^3$ CFU) better responds to the ordinary clinical scenario. Furthermore, in the orthopedic field, it cannot be overlooked the use of implant devices in case of joint prosthetic surgery or fracture synthesis. The presence of implants provides a suitable surface for bacterial attachment and colonization, thus supporting the biofilm formation. Indeed, it has been demonstrated that a strong infection can be induced by initial small bacterial inocula (10^2 CFU), since a constant bacterial growth occurred in presence of implant devices (Bernthal et al. 2010; Lucke et al. 2003a, 2005; Cevher et al. 2007; Fölsch et al. 2015; Darouiche et al. 2007; Akiyama et al. 2013; Kose et al. 2013; Lindsey et al. 2010a, b; Li et al. 2010; Sanchez et al. 2013). Otherwise, just a few studies were not able to induce a significant bone infection by injecting a low *S. aureus* inoculum (10^2 CFU) despite of the presence of metallic implants (Søe et al. 2013; Craig et al. 2005). Differently, it has been demonstrated that 10^2 CFU of *S. aureus* were not sufficient to provoke signs of osteomyelitis when inoculated in the absence of foreign bodies (Yang et al. 2012). This supports the failure of infection or infection rate < 50 % of animals when they received only bacterial inocula without implants. The importance of implant devices is particularly evident in order to develop infection by means of low virulent pathogens, such as *S. epidermidis* and *P. acnes*. It is well known that metallic alloys favor the attachment of biofilm-producing low virulent bacteria. In particular, stainless steel implants better support the bacterial adhesion compared to titanium surfaces, as demonstrated by Sheehan et al. 2004. Among studies that implanted stainless steel devices, Lovati et al. 2016 were the only authors that investigated low bacterial inocula (10^3 CFU) of *S. epidermidis*, thus developing a subclinical rat model of low virulent pathogens. This model is mostly relevant because of establishing a useful tool to study the real clinical occurrence in case of orthopedic infections and future therapeutic approaches. Considering the low virulent pathogens, *P. acnes* has emerged as a major microorganism involved in prosthetic joint infections in both young and old patients being normally present on the skin flora (Saper et al. 2015; Song et al. 2013). However, only one recent study developed a rabbit model of *P. acnes*-related bone infection in the tibia (Achermann et al. 2015). On these bases, the authors recognize the need for additional animal models to study the influence of low virulent bacteria in the orthopedic field.

Despite the large number of animal models, there are several limitations in properly comparing the results obtained by using different bacterial strains and inocula. This variety of conditions encourages the development of more reproducible *in vivo* studies to provide relevant information for a translational approach to humans. In this regard, the main aspect is to determine the right dose to induce the infection with respect to the virulence of the inoculated bacteria. Thus, the evaluation of different bacterial load in a pilot study could be an effective strategy to establish the desired infection rate.

Thanks to most appropriate animal models, a deeper knowledge will be obtained on the role and dynamic interactions between pathogens and the host environment that will allow the analysis of polymicrobial infections closer to the clinical scenario.

Conflict of Interests The authors declare that there is not conflict of interests regarding the publication of this paper.

References

Achermann Y, Tran B, Kang M et al (2015) Immunoproteomic identification of in vivo-produced propionibacterium acnes proteins in a rabbit biofilm infection model. Clin Vaccine Immunol 22:467–476

Adams CS, Antoci V Jr, Harrison G et al (2009) Controlled release of vancomycin from thin sol–gel films on implant surfaces successfully controls osteomyelitis. J Orthop Res 27:701–709

Akiyama T, Miyamoto H, Yonekura Y et al (2013) Silver oxide-containing hydroxyapatite coating has in vivo antibacterial activity in the rat tibia. J Orthop Res 31:1195–1200

Antoci V Jr, Adams CS, Hickok NJ et al (2007) Vancomycin bound to Ti rods reduces periprosthetic infection: preliminary study. Clin Orthop Relat Res 461:88–95

Arciola CR, Campoccia D, Ehrlich GD et al (2015) Biofilm-based implant infections in orthopaedics. Adv Exp Med Biol 830:29–46

Arens D, Wilke M, Calabro L et al (2015) A rabbit humerus model of plating and nailing osteosynthesis with and without Staphylococcus aureus osteomyelitis. Eur Cell Mater 30:148–161

Bernthal NM, Stavrakis AI, Billi F et al (2010) A mouse model of post-arthroplasty Staphylococcus aureus joint infection to evaluate in vivo the efficacy of antimicrobial implant coatings. PLoS One 5:e12580

Brady RA, Mocca CP, Prabhakara R et al (2013) Evaluation of genetically inactivated alpha toxin for protection in multiple mouse models of Staphylococcus aureus infection. PLoS One 8:e63040

Campoccia D, Montanaro L, Arciola CR (2005) The significance of infection related to orthopaedic devices and issues of antibiotic resistance. Biomaterials 27:2331–2339

Cevher E, Orhan Z, Sensoy D et al (2007) Sodium fusidate-poly(D, L-lactide-co-glycolide) microspheres: preparation, characterisation and in vivo evaluation of their effectiveness in the treatment of chronic osteomyelitis. J Microencapsul 24:577–595

Chen X, Tsukayama DT, Kidder LS et al (2005) Characterization of a chronic infection in an internally-stabilized segmental defect in the rat femur. J Orthop Res 23:816–823

Craig MR, Poelstra KA, Sherrell JC et al (2005) A novel total knee arthroplasty infection model in rabbits. J Orthop Res 23:1100–1104

Darouiche RO, Mansouri MD, Zakarevicz D et al (2007) In vivo efficacy of antimicrobial-coated devices. J Bone Joint Surg Am 89:792–797

Del Pozo JL, Rouse MS, Euba G et al (2009) The electricidal effect is active in an experimental model of Staphylococcus epidermidis chronic foreign body osteomyelitis. Antimicrob Agents Chemother 53:4064–4068

Drago L, De Vecchi E, Cappelletti L et al (2015) Prolonging culture to 15 days improves bacterial detection in bone and joint infections. Eur J Clin Microbiol Infect Dis 34:1809–1813

Ensrud KE (2013) Epidemiology of fracture risk with advancing age. J Gerontol A Biol Sci Med Sci 68:1236–1242

Fölsch C, Federmann M, Kuehn KD et al (2015) Coating with a novel gentamicinpalmitate formulation prevents implant-associated osteomyelitis induced by methicillin-susceptible Staphylococcus aureus in a rat model. Int Orthop 39:981–988

Fukushima N, Yokoyama K, Sasahara T et al (2005) Establishment of rat model of acute staphylococcal osteomyelitis: relationship between inoculation dose and development of osteomyelitis. Arch Orthop Trauma Surg 125:169–176

Haenle M, Zietz C, Lindner T et al (2013) A model of implant-associated infection in the tibial metaphysis of rats. ScientificWorldJournal 2013:481975

Harrasser N, Gorkotte J, Obermeier A et al (2016) A new model of implant-related osteomyelitis in the metaphysis of rat tibiae. BMC Musculoskelet Disord 17:152

Heim CE, Vidlak D, Scherr TD et al (2014) Myeloid-derived suppressor cells contribute to Staphylococcus aureus orthopedic biofilm infection. J Immunol 192:3778–3792

Heim CE, Vidlak D, Scherr TD et al (2015) IL-12 promotes myeloid-derived suppressor cell recruitment and bacterial persistence during Staphylococcus aureus orthopedic implant infection. J Immunol 194:3861–3872

Helbig L, Guehring T, Rosenberger S et al (2015) A new animal model for delayed osseous union secondary to osteitis. BMC Musculoskelet Disord 16:362

Kose N, Otuzbir A, Pekşen C et al (2013) A silver ion-doped calcium phosphate-based ceramic nanopowder-coated prosthesis increased infection resistance. Clin Orthop Relat Res 471:2532–2539

Kraft CN, Schlegel U, Pfluger D et al (2001) Radiological signs of osteitis around extramedullary metal implants. A radiographic-microbiological correlative analysis in rabbit tibiae after local inoculation of Staphylococcus aureus. Arch Orthop Trauma Surg 121:338–342

Li B, Jiang B, Dietz MJ et al (2010) Evaluation of local MCP-1 and IL-12 nanocoatings for infection prevention in open fractures. J Orthop Res 28:48–54

Lindsey BA, Clovis NB, Smith ES et al (2010a) An animal model for open femur fracture and osteomyelitis: Part I. J Orthop Res 28:38–42

Lindsey BA, Clovis NB, Smith ES et al (2010b) An animal model for open femur fracture and osteomyelitis-Part II: immunomodulation with systemic IL-12. J Orthop Res 28:43–47

Lovati AB, Drago L, Monti L et al (2013) Diabetic mouse model of orthopaedic implant-related Staphylococcus aureus infection. PLoS One 8:e67628

Lovati AB, Romanò CL, Monti L et al (2014) Does PGE_1 vasodilator prevent orthopaedic implant-related infection in diabetes? Preliminary results in a mouse model. PLoS One 9:e94758

Lovati AB, Romanò CL, Bottagisio M et al (2016) Modeling staphylococcus epidermidis-induced non-unions: subclinical and clinical evidence in rats. PLoS One 11:e0147447

Lucke M, Schmidmaier G, Sadoni S et al (2003a) A new model of implant-related osteomyelitis in rats. J Biomed Mater Res B Appl Biomater 67:593–602

Lucke M, Schmidmaier G, Sadoni S et al (2003b) Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. Bone 32:521–531

Lucke M, Wildemann B, Sadoni S et al (2005) Systemic versus local application of gentamicin in prophylaxis of implant-related osteomyelitis in a rat model. Bone 36:770–778

Marriott I, Gray DL, Tranguch SL et al (2004) Osteoblasts express the inflammatory cytokine interleukin-6 in a murine model of Staphylococcus aureus osteomyelitis and infected human bone tissue. Am J Pathol 164:1399–1406

Marriott I, Gray DL, Rati DM et al (2005) Osteoblasts produce monocyte chemoattractant protein-1 in a murine model of Staphylococcus aureus osteomyelitis and infected human bone tissue. Bone 37:504–512

Mecikoglu M, Saygi B, Yildirim Y et al (2006) The effect of proteolytic enzyme serratiopeptidase in the treatment of experimental implant-related infection. J Bone Joint Surg Am 88:1208–1214

Oh SH, Nam BR, Lee IS et al (2016a) Prolonged antibacterial activity of ion-complexed doxycycline for the treatment of osteomyelitis. Eur J Pharm Biopharm 98:67–75

Oh EJ, Oh SH, Lee IS et al (2016b) Antibiotic-eluting hydrophilized PMMA bone cement with prolonged bactericidal effect for the treatment of osteomyelitis. J Biomater Appl 30:1534–1544

Pesanti EL, Lorenzo JA (1998) Osteoclasts and effects of interleukin 4 in development of chronic osteomyelitis. Clin Orthop Relat Res 355:290–299

Phillips JE, Crane TP, Noy M et al (2006) The incidence of deep prosthetic infections in a specialist orthopaedic hospital: a 15-year prospective survey. J Bone Joint Surg (Br) 88:943–948

Portillo ME, Corvec S, Borens O et al (2013) Propionibacterium acnes: an underestimated pathogen in implant-associated infections. Biomed Res Int 2013:804391

Pribaz JR, Bernthal NM, Billi F et al (2012) Mouse model of chronic post-arthroplasty infection: noninvasive in vivo bioluminescence imaging to monitor bacterial burden for long-term study. J Orthop Res 30:335–340

Ribeiro M, Monteiro FJ, Ferraz MP (2012) Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatter 2:176–194

Sanchez CJ Jr, Prieto EM, Krueger CA et al (2013) Effects of local delivery of D-amino acids from biofilm-dispersive scaffolds on infection in contaminated rat segmental defects. Biomaterials 34:7533–7543

Saper D, Capiro N, Ma R et al (2015) Management of Propionibacterium acnes infection after shoulder surgery. Curr Rev Musculoskelet Med 8:67–74

Sener M, Kazimoglu C, Karapinar H et al (2010) Comparison of various surgical methods in the treatment of implant-related infection. Int Orthop 34:419–423

Sheehan E, McKenna J, Mulhall KJ et al (2004) Adhesion of Staphylococcus to orthopaedic metals, an in vivo study. J Orthop Res 22:39–43

Smeltzer MS, Thomas JR, Hickmon SG et al (1997) Characterization of a rabbit model of staphylococcal osteomyelitis. J Orthop Res 15:414–421

Søe NH, Jensen NV, Nürnberg BM et al (2013) A novel knee prosthesis model of implant-related osteomyelitis in rats. Acta Orthop 84:92–97

Song Z, Borgwardt L, Høiby N et al (2013) Prosthesis infections after orthopedic joint replacement: the possible role of bacterial biofilms. Orthop Rev (Pavia) 5:65–71

Tatara AM, Shah SR, Livingston CE et al (2015) Infected animal models for tissue engineering. Methods 84:17–24

Trampuz A, Widmer AF (2006) Infections associated with orthopedic implants. Curr Opin Infect Dis 19:349–356

van der Borden AJ, Maathuis PG, Engels E et al (2007) Prevention of pin tract infection in external stainless steel fixator frames using electric current in a goat model. Biomaterials 28:2122–2126

Wagner JM, Zöllner H, Wallner C et al (2016) Surgical debridement is superior to sole antibiotic therapy in a novel murine posttraumatic osteomyelitis model. PLoS One 11:e0149389

Yang CJ, Li Q, Wu GC et al (2012) A practical model of osteomyelitis-induced bone pain by intra-tibial injection of Staphylococcus aureus in rats. Neurosci Lett 513:198–203

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 51–67
DOI 10.1007/5584_2016_154

Published online: 5 November 2016

Microbiological Diagnosis of Implant-Related Infections: Scientific Evidence and Cost/Benefit Analysis of Routine Antibiofilm Processing

Lorenzo Drago and Elena De Vecchi

Abstract

Prosthetic joint infection is one of the most severe complication following joint arthroplasty, producing a significant worsening of patient's quality of life. Management of PJIs requires extended courses of antimicrobial therapy, multiple surgical interventions and prolonged hospital stay, with a consequent economic burden, which is thought to markedly increase in the next years due to the expected burden in total joint arthroplasties. The present review summarizes the present knowledge on microbiological diagnosis of prosthetic joint infections, focusing on aethiological agents and discussing pros and cons of the available strategies for their diagnosis.

Intra-operative clinical diagnosis and pathogen identification is considered the diagnostic benchmark, however the presence of bacterial biofilm makes pathogen detection with traditional microbiological techniques highly ineffective. Diagnosis of PJIs is a rather complex challenge for orthopedics and requires a strict collaboration between different specialists: orthopaedics, infectivologists, microbiologists, pathologists and radiologists. Diagnostic criteria have been described by national and international association and scientific societies. Clinicians should be trained on how to use it, but more importantly they should know potential and limitation of the available tests in order to use them appropriately.

Keywords

Prosthetic joint infection • Microbiological diagnosis • Synovial fluid culture • Dithiothreitol • Sonication

The original version of this chapter has been revised. An erratum to this chapter can be found at DOI 10.1007/5584_2017_21

L. Drago (✉)
Laboratory of Clinical-Chemistry and Microbiology, IRCCS Galeazzi Institute, Milan, Italy

Department of Biomedical Science for Health, University of Milan, Milan, Italy
e-mail: lorenzo.drago@unimi.it

E. De Vecchi
Laboratory of Clinical-Chemistry and Microbiology, IRCCS Galeazzi Institute, Milan, Italy
e-mail: elena.devecchi@grupposandonato.it

1 Introduction

Implant-related infections, including periprosthetic joint infection (PJI), infected osteosynthesis and other biomaterials, are biofilm-related. Intra-operative clinical diagnosis and pathogen identification is considered the diagnostic benchmark, however the presence of bacterial biofilm makes pathogen detection with traditional microbiological techniques highly ineffective. Replacement of native joints contributes to significantly improve quality of life of millions of patients, allowing pain relief recovery of joint function. Unfortunately, in some cases, the implant may fail leading in most of patients to additional surgery with notable costs in terms of individual quality of life and of the health system. Causes of implant failure may be aseptic (loosening of some components of the prosthesis, dislocation, instability, adverse reaction of the host to the implant material, periprosthetic fracture, material consumption) or infective. Although affecting a minority of patients being estimated incidence 1–9 % after primary total arthroplasty, depending on joint (Huotari et al. 2015), PJIs require long hospital stay, prolonged antibiotic therapy and additional surgery (Kapadia et al. 2016). Considering that the amount of joint arthroplasties has been estimated to burden in the next 20 years, especially because of the growth of world population and of increase in population age and prevalence of comorbidities such as diabetes and obesity, the absolute number of prosthetic joint infections is predicted to inevitably increase, with a sustained economic impact on the health care system (Kurtz et al. 2012). Therefore an accurate recognition of these infection is essential not only to ensure appropriate treatment to patients with PJI, but also to avoid unnecessary therapies for patients with aseptic failure. Diagnosis is further complicated by the wide spectrum of manifestations of PJIs which may depend on the pathogenicity and virulence of the causative organisms, the host conditions (age, comorbidities etc), the joint involved, the time of infection. Usually PJIs are classified by the time to infection as early (within 3 months), delayed (after 3 months but before 24 months) and late infections (after 24 months) (Tande and Patel 2014).

Most PJIs are caused by intra-operative contamination which cause either early or delayed infection while hematogenous seeding is less common, being, instead, seen in late infections. Although the different pathogenesis, both early postoperative and hematogenous infections usually present an acute onset. In contrast, chronic late infections may be also caused by less virulent microorganisms, and although they are considered to be caused by intraoperative contamination, symptoms develop very slowly. Therefore, their appearance is quite similar to that of aseptic failure and they pose major issues in the diagnosis.

Although a series of efforts have been made in the recent past to improve diagnosis of PJIs, till now a microbiological gold standard has not been yet established, so that international consensus meetings and national guidelines have been also proposed (Zmistowski et al 2014a, b; Caola and Drago 2013).

2 Microbial Etiology of PJIs

2.1 Biofilm Related Infections

Biofilm is intrinsic to the pathogenesis of all kinds of prosthetic infections, including joints ones. Microbial biofilm can be found on hardware components, cement, bone and fibrous tissue, and detached clumps of biofilm have been also recovered in the joint fluid (Stoodley et al 2011) and infected tissues. Formation of biofilm on a prosthetic implants begins with adhesion of microbes through their surface structures such as pili, fimbriae, flagella, and glycocalyx (Renner and Weibel 2011). This is the reason why the presence of biofilm notably complicates isolation of bacteria when conventional sampling and culture methods are used, significantly lowering sensitivity of microbiological analysis. This issue has lead in the recent past to development

of novel strategies to approach microbiological diagnosis of PJIs with the aim to remove biofilm embedded bacteria from prosthetic implants and periprosthetic tissues. (Drago et al. 2013; Janz et al. 2015; Roux et al. 2011).

2.2 Microorganisms Responsible of PJIs

2.2.1 Staphylococci

Staphylococcus aureus, *Staphylococcus epidermidis* and other coagulase negative staphylococci are universally recognized as the major responsible of PJIs, being isolated in about 50–60 % of cases in similar rates (Langvatn et al. 2015; Drago et al. 2014; Holleyman et al. 2016). Risk factors for *S. aureus* PJIs include indwelling prosthetic devices, rheumatoid arthritis, diabetes, nasal colonization by *S. aureus* (Bouaziz et al. 2012; Campbell et al. 2015; Tande et al. 2016).

Although *S. aureus* has been isolated from infections occurring at any time after implantation, early or late infections occur more frequently than delayed ones (Senneville et al. 2011) Moreover, *S. aureus* has been associated with a significantly higher risk of failure when compared with other microorganisms, where the failure was associated to early infection and presence of bacteremia (Tande et al. 2016; Tornero et al. 2012).

The term coagulase negative staphylococci (CNS) defines a variegated group of staphylococci usually retrievable as normal components of the human skin, which have gained considerable attention as pathogens only in the recent past. Pathogenicity and virulence factors of CNS have been only partially explored, but biofilm production has been recognized in majority of *S. epidermidis* strains. Globally they represent the main cause of PJIs, although their role as pathogen has to be accurately evaluated for each patient. Among them, the most frequently isolated CNS from PJIs is *Staphylococcus epidermidis*, while the real isolation rate of the other CNS species varies widely, mainly due to technical difficulties of laboratory test in discriminating one species from another. In the last years, particular relevance has been attributed to *Staphylococcus lugdunensis* which is increasingly isolated not only from PJIs but also from osteomyelitis, endocarditis and bacteremia (Becker et al. 2014). Other species encountered in PJIs are *Staphylococcus simulans*, *Staphylococcus caprae*, *Staphylococcus hominis* and *Staphylococcus warneri*. CNS are isolated at any time after prosthetic implants, although the majority of the infections due to CNS are in revision arthroplasties (Tande and Patel 2014). It could be hypothesized that some implant failure classified as aseptic may be unrecognized low-grade infections due to CNS (Lovati et al. 2016), and revision surgery may favor development of an acute infection. In respect to *S. aureus* infections, those caused by CNS are characterized by a lower inflammatory response and by the fact that CNS infections are more frequently diagnosed later, probably due to the lower virulence factors produced by CNS (Langvatn et al. 2015). However, if there are specific risk factors or different clinical manifestations for the different CNS species remains unclear. Route of infection of CNS seems mainly related to contamination at time of surgery by skin contamination, while data on airborne intraoperative transmission are conflicting (Månsson et al. 2015).

S. aureus and CNS infections are often complicated by development of the so-called "small colony variants" (SCV) which are naturally occurring subpopulations of staphylococci characterized by peculiar phenotype and pathogenic traits isolated with variable frequency in PJIs. Phenotypically, SCVs have a slow growth rate, atypical colony morphology, unusual biochemical features and, even if clonally related to co-isolated normal phenotype bacteria, up to one-third had discordant susceptibilities using E-test or disc diffusion testing. SCVs are associated with persistent or relapsing infections probably because they are able to invade and survive inside non-professional phagocytes, where they are protected against the host immune system and antimicrobial agents (Maduka-Ezeh et al. 2012; Proctor et al. 2006). However,

clinically no difference in the likelihood of treatment failure between patients with PJI caused by SCVs and those with PJI caused by staphylococci with normal phenotype has been shown (Tande et al. 2014) In the same study, SCV staphylococci were more likely isolated from subjects who have received prior surgical and chronic antimicrobial therapy for their infection.

2.2.2 Enterococci

Enterococci are involved in about 3–15 % of PJIs, where they are often part of early polymicrobial infections in association prevalently with staphylococci followed by *Escherichia coli* and *Pseudomonas aeruginosa*. Their isolation is usually associated with a bad outcome, especially in the case of *Enterococcus faecium*. A possible explanation is the high tolerance to different classes of antimicrobials which characterizes enterococci but it is also possible that co-morbidities often present in these patients (diabetes mellitus, coronary disease, chronic renal failure, chronic obstructive pulmonary disease, malignancy or liver cirrhosis) may affect their outcome. Moreover, the high rate of polymicrobial infections, the severity of the infection and the ability of enterococci to form strong biofilms may equally influence treatment results (Tornero et al. 2014). In a large multicentric study on patients with an enterococcal PJI, localization was the hip in 63 %, the knee in 34 % while in the rest other joints (shoulder or elbow) were involved (Tornero et al. 2014). The most frequent specie isolated was *Enterococcus faecalis*, followed by *E. faecium*. Patients with a monomicrobial infection present a late onset but prolonged symptoms, which correlate with the limited virulence of enterococci.

2.2.3 Streptococci

Streptococci are the causative agents of about 10 % of PJIs, with most of them at delayed or late onset and no differences between hip and knee. A wide variety of streptococci have been identified, being Lancefield Group A, B, C and G the most prevalent, while viridans streptococci and *Streptococcus pneumoniae* are rarely isolated (Langvatn et al. 2004; Murillo et al. 2015). Infections are usually acute with patients frequently presenting fever and/or systemic symptoms (Sendi et al. 2011). Co-morbidities such as diabetes, obesity and malignancy are often associated with these infections. Route of transmission is generally haematogenous starting from gastrointestinal or genito-urinary tracts and from skin (Everts et al. 2004).

2.2.4 Gram Negative Bacilli

Gram-negative bacteria are responsible for about 5–23 % of all PJIs especially among the elderly (Murillo et al 2015; Rodríguez-Pardo et al. 2014; Fernandes and Dias 2013) but their isolation rate may increase up to 60 % in early PJIs where they may be retrieved as co-pathogens in polymicrobial infections. *Escherichia coli* and *Pseudomonas aeruginosa* are the most frequently pathogens followed by other Enterobacteriacecae such as *Klebsiella pneumoniae* and Salmonella species (de Sanctis et al. 2014; Gupta et al. 2014). Acquisition of infection is generally hematogenous and the virulence of these bacteria contributes to a common acute presentation. Clinical outcomes of PJI caused by Gram-negative bacteria are reportedly less favorable than those of infection caused by Gram-positive bacteria (Zmistowski et al. 2011) Moreover, emergence of Gram negative bacilli, particularly *K. pneumoniae* and *P. aeruginosa*, resistant to a wide spectrum of antibiotics has raised some concerns on antimicrobial treatment of these infections (de Sanctis et al. 2014).

2.2.5 Anaerobes

About 3–6 % of PJIs are caused by anaerobes with *Propionibacterium acnes* being the most prominent species (Tande and Patel 2014). PJIs caused by anaerobes often present late after implant surgery, and have a subtle clinical presentation. *P. acnes* is most frequently anaerobe isolated from shoulders. Acute presentation is quite rare (Shah et al. 2015), being pain the only symptom, so that no alterations in inflammatory serum markers are observable. Also histology is unable to evidence acute inflammation. Most of *P. acnes* PJIs occur through surgical

contamination, since *P. acnes* resides in sebaceous follicles of the skin, conjunctiva, external ear, oral cavity and gastrointestinal tract. Risk factors include male gender, younger age, trauma-associated surgery and duration of surgery (Shah et al. 2015). Among other anaerobes isolated from PJIs, especially as part of polymicrobial infections, there are Clostridia, *Bacteroides fragilis*, *Peptostreptococcus species* and *Actinomyces species*. *Clostridium difficile* and *Clostridium perfringens* are the most frequently isolated Clostridia. They are mostly responsible of early infections, but late PJIs have been also described. Differently from *P. acnes* infections, inflammatory markers are often elevated and traditional clinical signs (redness, warmth and swelling) are present in the majority of patients (Pearle et al. 2003). Underlining malignancy, presence of necrotic tissue, preexisting musculoskeletal infections, recent hospitalization have been identified among risk factors for Clostridia PJIs. Anaerobic Gram-positive cocci (*Peptostreptococcus spp.* and *Finegoldia magna*) are commensal of the gastrointestinal and genito-urinary tracts and skin, that have been uncommonly isolated from delayed and late PJIs. They mainly affect hip and knee prostheses that they reach through hematogenous or contiguous spread or directly during surgery. *Actinomyces* and *Bacteroides fragilis* are responsible of a limited number of generally monomicrobial infections. Hematogenous seeding from a distant site has been hypothesized for both bacteria, while possible risk factors include dental and periodontal disease and treatment for *Actinomyces spp* and immunosuppression, intra-abdominal infections and rheumatoid arthritis for *Bacteroides fragilis* (Shah et al. 2015).

2.2.6 Uncommon Microorganisms

Corynebacteria, *Pasteurella multocida*, *Mycobacterium tubeculosis*, have been occasionally reported as cause of PJIs. *Corynebacterium amycolatum*, *Corynebacterium aurimucosum*, *Corynebacterium jeikeium*, and *Corynebacterium striatum* are the most common *Corynebacterium* species causing PJI, though their real incidence may be underestimated since their identification is not routinely performed in all laboratories (Cazanave et al. 2012).

Zoonotic infections, particularly caused by *Pasteurella multocida* may occur in patients with exposure risk, such as living with pets (Romanò et al. 2013). These infections are generally at late onset and may have an acute presentation with regional lymphoadenopathy occurring after scratch, bite or lick from dogs or cats.

Mycobacterium tuberculosis complex is responsible of more than 10 % of infections of native bone and joint while it accounts for less than 0.5 % of PJIs (Berbari et al. 1998). However, when arthroplasty is performed in joints with a previous tubercular septic arthritis, the risk increases up to 31 %. Although infections mainly occur in patients with a history of active or latent tuberculosis, they may affect also patients without previous contacts with *M. tuberculosis* (Carrega et al. 2013). Non tuberculous mycobacteria are rarely identified as cause of PJIs.

Fungi have been isolated in less than 1 % of PJIs and *Candida spp.* are responsible of 80 % of these infections. (Dutronc et al. 2010; Bartalesi et al. 2012). Geographical distribution is observed in isolation rate of the different Candida species. Dimorphic fungi, *Aspergillus spp* and other filamentous fungi have been occasionally reported. Fungal PJIs are often subacute and occur more frequently after revision arthroplasty.

3 Microbiological Diagnosis

Laboratory diagnosis occupies a prominent role in the diagnosis of PJIs. In particular, the main aim is to discriminate between infection and other causes of implant failure. As previously outlined, the lack of test characterized by optimal sensitivity and specificity has led to development of a multidisciplinary approach that integrates results from microbiological, biochemical, histological analyses with clinical and radiological examination. Anyway, isolation of the pathogen

Table 1 Most frequently used laboratory test for diagnosis of prosthetic joint infections

Specimen	Available analysis
Synovial fluid	**Microbiological diagnosis**
Additional tests	Leukocyte count and neutrophil percentage
	Leukocyte esterase
	CRP
	Alpha-defensin
Periprosthetic tissue	**Microbiological diagnosis**
Additional tests	Histology of frozen section or paraffin embedded tissue
Prosthetic implants	**Microbiological diagnosis**
Peripheral blood	**Blood culture**
Additional tests	ESR
	CRP
	Interleukin-6

and antimicrobial susceptibility testing is still considered critical to target antimicrobial therapy, thus improving patient outcome. Available laboratory tests are summarized in Table 1. Microbiological diagnosis can be conducted at different levels and stages of the infection.

3.1 Preoperative Cultures

Synovial fluid culture is critical for diagnosis of PJIs allowing early identification of the causative organisms with evaluation of antibiotic susceptibility in order to establish the appropriate therapy and selection of therapeutic strategy. Arthrocentesis should be performed in all patients with suspected PJIs excepted in subjects with contraindications or when diagnosis has been already previously established. However, if aspiration of the knee is a relatively simple procedure, aspiration of the hip frequently requires ultrasonographic guide. Unfortunately, in some cases hip aspiration fail to yield a sufficient volume of liquid for further analyses, so that injection and aspiration of normal saline in the joint is needed. Aspiration must be performed under aseptic conditions. The main risks associated to the procedure are represented by contamination of the sample by skin organisms or by the inoculation of organisms into the joint. Nonetheless, joint aspiration is an invasive procedure and may cause complications (Barrack and Harris 1993).

Culture may be performed by directly inoculating the fluid into blood culture bottles or onto solid and/or liquid medium. Data on use of blood culture bottles shows a high variability in sensitivity ranging from 85 to 90 % and specificity of 95–100 % (Levine and Evans 2001; Hughes et al. 2001; Font-Vizcarra et al. 2010). Culture on solid and liquid media shows a highly variable sensitivity (50–80 %) but lower than that of culture in blood bottle though maintaining a sustained specificity. Prolonged incubation of liquid media may improve sensitivity but may be also associated with a higher isolation rate of contaminants (Font-Vizcarra et al. 2010). Consumption of antibiotics in the days before sampling negatively affects culture yield; a significant decrease in sensitivity has been observed in patients receiving prior antibiotics in respect to untreated patients (41.6 % vs. 75 %) (Barrack et al. 1997). Moreover, the probability of negative culture is 4.7 folds higher if the patient has received antibiotics within the last 3 months. Therefore to increase culture sensitivity, a minimum of 2 weeks after stopping of antibiotics is recommended before joint aspiration (Yee et al. 2013).

Moreover, other factors such as bacterial load or the type of germ may affect synovial culture. This may explain the higher sensitivity observed in acute versus chronic infections (91 vs 79 %), which can also be related to the high number of planktonic bacteria present in acute infection (Font-Vizcarra et al. 2010).

Gram staining of synovial fluid has a limited role according to most authors (Zywiel et al. 2011; Ghanem et al. 2009). Despite good specificity and positive predictive value, sensitivity is so scarce to make Gram staining unreliable. Conclusions drawn by a meta-analysis performed on 18 studies for a total of 4647 patients suggest that available data do not support the routine use of Gram staining without additional proof of infection (Ouyang et al. 2015).

Culture of pre-operative arthroscopy tissue biopsies may be a valid alternative or an additional test to synovial fluid cultures. In a retrospective review of a series of patients with shoulder PJIs, results of culture periprosthetic biopsies obtained preoperatively completely overlapped those obtained from intraoperative cultures. Sensitivity and specificity were higher than those observed with fluoroscopically guided glenohumeral aspiration (Dilisio et al. 2014). Similar results were obtained for knee pre-operative biopsies with a sensitivity of 100 % and a specificity of 98.1 % (Fink et al. 2008). By contrast, in a large population of hip PJIs patients, positive and negative predictive values were 81.4 % and 93.1 % for aspiration and 73.8 % and 93.8 % for tissue biopsy (Williams et al. 2004). Therefore, due to the higher costs of collecting tissue biopsies and related possible complications, preoperative biopsy is not routinely advisable.

The presence of a sinus communicating with the implant is considered in the MSIS criteria as one the index of PJIs. Nonetheless, despite swabs from the sinus tract are still considered by some clinicians a suitable specimen for PJI diagnosis, their use is unadvisable. In fact, only a 50 % concordance between cultures from the sinus and deep cultures and a higher probability to identify polymicrobial infections than deep cultures because of isolation of contaminants (particularly CNS) have been reported (Tetreault et al. 2013).

Finally, synovial fluid culture may provide a significant contribute to pre-operative diagnosis of PJIs, while culture from sinus tract is less useful at this stage.

3.2 Intra-operative Cultures

3.2.1 Periprosthetic Tissues

Culture of periprosthetic tissues is a valid tool for diagnosis of PJIs and represents the predominant analysis performed in laboratory for PJIs diagnosis. Samples should be collected from visible inflamed or abnormal tissue accordingly to the surgeon opinion by using separate scalpel. Nonetheless, culture methods are not-standardized and sample processing used media and incubation times which widely varied between laboratories, so that different issues have to be considered. Despite these differences, there is a general agreement on the number of samples to send to the microbiology laboratory. In fact, given the limited sensitivity of tissue culture and the possibility of contamination during sample collection, interpretation of results from culture of a single sample could not provide useful information. Generally accepted guidelines (Infectious Diseases Society of America, Musculo Skeletal Infectious Society, International Consensus Meeting of Philadelphia) recommend collection of 5–6 samples with a minimum of 3 (Parvizi et al. 2011; Osmon et al. 2013; Zmistowski et al. 2014a, b).

This sample amount was initially proposed by Kamme and Lindberg (1981) and further corroborated by other studies such as that of Atkins and colleagues (1998) that calculate the optimal number of samples to be collected by using a mathematical model based on data from 297 patients undergoing revision surgery. According to a multicentric study involving 264 patients with suspected PJIs, the number of tissue samples to confirm diagnosis of PJIs may be decreased to four (Bemer et al. 2016).

As regards the preparation of tissue samples for microbial culture, various methods have been adopted: cutting bigger samples into smaller pieces with surgical knives, grinding with mortar and pestle, homogenization using Ballottini beads or using a Seward Stomacher. Tissue homogenization results in release of bacteria for the subsequent culture. Such method, however, is a predominantly manual technique which is labour-intensive and may depend on individual skills and, as such, it is at high risk of contamination (Saeed 2014). In fact, the probe used for disrupting tissues must be substituted or decontaminated between samples in order to avoid cross-contamination, thus making the method not applicable in laboratories with high workloads, because of the excessive time required for treating one sample. Use of bead-mills represents a valid alternative to

homogenization process, allowing the operator to process multiple samples (up to 24) simultaneously (Roux et al. 2011). The method is characterized by a good sensitivity (83 %) and a limited contamination rate (8.7 %) if compared to traditional homogenization where contaminants may be isolated in up to 20 % of cases. The main limitation is represented by the costs related to acquisition of a dedicated instrumentation that could be not affordable by all laboratories and by the need to recover and sterilize beadmills after use. An alternative treatment may be represented by the use of DL-Dithiothreitol (DTT) to remove microorganisms from tissues. DTT is a sulfhydryl compound which is routinely used in clinical microbiology for liquefying specimens from the respiratory tract. Initially evaluated for its ability to dislodge microorganisms from infected implants (Drago et al. 2013), it has been subsequently tested for treatment of tissue samples, showing good sensitivity and specificity (De Vecchi et al. 2016) (Fig. 1).

As far as culture media are concerned, few studies have addressed this issue. Most studies on tissue cultures employ blood or chocolate agar for growth of aerobes and a media for anaerobes, while the use of selective media (i.e. for Gram positive cocci and Gram-negative bacilli) is optional and variable. The use of enrichment broth is still matter of debate; several studies have shown an increase in culture sensitivity when they are used with an improvement particularly in isolation of propionibacteria and CNS staphylococci (Drago et al. 2015; DeHaan et al. 2013). Moreover, broths may preserve microbial vitality and, when added to samples directly in the operating theater, increase sensitivity from 83 to 95 % (Blackmur et al. 2014).

On the other hand, use of broth enrichment may affect specificity by contributing to increase isolate rate of contaminants (Jordan et al. 2015). An alternative strategy may be culture of tissue specimens in blood culture bottles, as described previously for synovial fluid (Peel et al. 2016). In this case, tissue must be homogenized before inoculation of the fluid into blood culture bottle. The method notably increases sensitivity of culture if compared to use of solid or liquid media (92.1 vs 62.6 %). However, it must be evidenced that enrichment broth was used only for anaerobes, while plates for aerobes were incubated for 5 days. This is in contrast with data evidencing the advantages of prolonged incubation, as detailed below. In fact, till now duration of incubation has not reach a widely accepted consensus. Traditionally, plates are incubated for 2–5 days for aerobic bacteria and

Fig. 1 Example of a device for Dithiothreitol teatment of tissues and implants

up to 7 days for anaerobes. However, some studies have evidenced that prolonging incubation to up to 15 days improves microbial detection with an increase of detection rate of about 20 % without significantly increasing isolation of contaminants which were isolated at a similar rate in the first 7 days of incubation (Schäfer et al. 2008; Butler-Wu et al. 2011; Drago et al. 2015). The need of prolonged time of incubation has been confirmed by the large study of Bemer and co-workers previously cited (Bemer et al. 2016). This means that about one fifth of PJIs patients could be missed when short incubation time is used.

3.2.2 Sampling by Swabs

Use of swabs to sampling periprosthetic tissue as well as synovial fluid is not recommended and must be avoided. Increased risk of contamination, decreased volume of specimen for culture, and inhibition of pathogen growth have been recognized as potential risks associated with use of swabs (Rasouli et al. 2012). Moreover, a lower sensitivity has been reported for swabs if compared with sampling of tissue (53–76 % vs. 63–94 %) (Aggarwal et al. 2013; Font-Vizcarra et al. 1999).

3.2.3 Prosthetic Implants

Due to the limited sensitivity of periprosthetic tissue culture, culture of the prosthesis may be considered as the method of choice for PJIs diagnosis. The main issue related to cultures of implants is related to dislodge bacteria from the implant. In fact as previously described, bacterial growth on these devices led to production of biofilm, which strongly encases bacteria, hampering their recovery for subsequent culture. Therefore, implants should be treated before plating to assure a high bacterial recovery. This may be obtained by mechanical, physical or chemical methods.

Mechanical methods such as scraping or vortexing have been rarely evaluated. One study using vortexing of implants for 1 min after addition of thioglycollate broth showed a sensitivity of only 40 %, though specificity was very high (99 %) when limit of 50 CFU/mL was used for considering a sample as positive (Portillo et al. 2013). Interestingly the same study reported an increase in sensitivity up to 69 % by lowering the cut-off to 1 CFU/mL. This value was quite similar to those observed by the same authors for more sophisticated techniques, such as sonication. The mechanism by which vortexing remove bacteria is based on the high shear forces generated on the interface between the prosthesis and the vortexing fluid. These shear forces could be increased by addition of detergents (e.g., polysorbate 80) or anticoagulants (e.g., EDTA) to the vortexing fluid (Portillo et al. 2013).

The first report on sonication (Fig. 2) was published in 1990 by Tunney and colleagues (Tunney et al. 1998). They analyzed the impact

Fig. 2 Sonication bath for treatment of prosthetic implants

of mild ultrasounds on release of bacteria from hip implants of 120 patients, demonstrating an increase in bacterial isolation rate, when compared with tissue culture. However, this study was limited by the lack of a precise definition of PJI, and no other studies appeared until 2006 (Trampuz et al. 2006). In that study involving 78 patients a superior sensitivity of sonication over tissue culture was reported despite a lower specificity. The lack of specificity was mainly due to the use of bags not completely sealed, which caused contamination of the bags by water bath. In the following years, the protocol was ameliorated by use of rigid sealable polypropylene containers and by vortex of samples before sonication. When these changes were applied sensitivity confirmed to be higher than tissue culture, and specificity increased to 99 % (Holinka et al. 2011). Use of sonication was then deeply investigated by several authors and results are summarized by a meta-analysis published in 2014 (Zhai et al. 2014). Conclusions were that sonication has adequate and clinically acceptable diagnostic values for detecting PJI, especially if associated with 14-day anaerobic culture, and centrifugation or vortexing of sonicated fluid. However, the same authors state that some factors may have affected data analysis. First, since a gold standard for diagnosing PJI, has not been yet established, the considered studies used different reference standards which may have led to misclassification bias, causing underestimation of diagnostic accuracy. Moreover, not all of the examined studies were performed prospectively, a fact that could reduce the strength of study conclusions. Finally, a number of the significant differences in the subgroup analyses were based on only two studies of the same research group. Therefore, the authors evidenced the need for further studies to reinforce data analysis. By time, some limitation of sonication were reported by different authors and comprised the risk of contamination during sample processing, the need for dedicated instrumentation, and a lower detection rate of *S. epidermidis* in respect to other methods (Trampuz et al. 2006; Esteban et al. 2008; Bjerkan et al. 2009). To avoid misinterpretations due to the presence of contaminants, a cut-off for colonies count have been calculated, corresponding to 2–10 CFU/mL for unconcentrated fluid, and 200 CFU/mL for 100-fold concentrated fluid (Trampuz et al. 2007; Piper et al. 2009). The need to perform a semi-quantitative analysis impacts on culture media, since liquid media may not be used. The lack of an enrichment step could be reduced to isolate slow growing microorganisms. Therefore, results should be carefully interpreted to discriminate between real pathogens and contaminants, considering the microorganism involved (low-grade pathogen) and patients history.

The risk for isolation of contaminants (particularly of CNS) has been also evidenced in the unique study evaluating use of blood bottles for culture of sonicated fluid (Shen et al. 2015). In this study, inoculation of concentrated sonicated fluid into blood bottles showed a better sensitivity when compared to use of blood bottle for culture of synovial fluids. The authors also evidenced that the yield was not affected by antibiotic therapy, which in some cases was not discontinued before surgery. Although showing promising results, a comparison with traditional culture was not performed, so that no conclusions can be drawn on the superiority of the use of blood bottles.

An alternative approach to dislodge bacteria from infected implants may be also obtained by use of chemical agents. Sulfhydryl compounds are well known for their ability to reduce disulphide bounds between polysaccharides and neighboring proteins and to interfere with biofilm formation.

Efficacy of DTT in removing bacteria from prosthetic materials has been initially studied in an in vitro model (Drago et al. 2012). In this study, amount of bacteria grown in biofilm on titanium discs was comparable to that obtained after sonication of the same material and superior to bacterial cells recovered after treatment with N-acetylcysteine and after scraping of disk surfaces. Subsequently the method was applied to prosthetic implants and compared with sonication and tissue cultures (Drago et al. 2013). After addition of a 0.1 % w:v DTT solution,

samples were vortexed, mechanically stirred for 15 min and centrifuged. The pellet was plated onto solid media and inoculated in enrichment broths which were incubated for 15 days. DTT showed a higher sensitivity than sonication (85.7 vs 71.4 %) and the same specificity of sonication (94.1 %). Differences in sensitivity were mainly due to a higher frequency of isolation of *S. epidermidis* obtained with DTT in respect to sonication.

Rather few data are available on the applicability of the method described above for microbiological analysis of spacers when revision procedure is completed in two stages.

3.2.4 Gram Staining

Similarly to what has been discussed for synovial fluid, Gram staining of periprosthetic tissue or fluid from prostheses is characterized by sensitivity even lower than staining of synovial fluid (0–45 %) despite a considerable specificity. Therefore, it is not recommended, also considering that other tests characterized by higher sensitivity are available for a prompt intra-operative diagnosis (Tande and Patel 2014).

3.2.5 Susceptibility Testing

The aim of microbiological analysis is to provide the clinician with data on antimicrobial susceptibility of the causative organism. Susceptibility testing are usually performed automatically together with pathogen identification. Usually susceptibility is expressed as Minimum Inhibitory Concentration (MIC) values, defined as the lowest antimicrobial concentration able to inhibit bacterial growth. However, MIC values are calculated by using bacteria grown freely in planktonic form, while bacteria responsible of PJIs usually growth in biofilm. Therefore, in vitro susceptibility testing could not correlate with susceptibility of bacteria adhered on prosthetic implants and embedded in a biofilm. In this context, it must be underlined that biofilm bacteria may be even 1000 fold more resistant to antibiotics than their planktonic counterparts. Therefore, susceptibility should be assessed in a different way. The term Minimum Biofilm Eradication Concentration (MBEC) indicates the lowest antimicrobial concentration able to prevent regrowth of the bacteria from the treated biofilm. It can be determined using specific developed assays such as the Calgary biofilm device, that allows formation of 96 identical biofilms on plastic pegs placed on the lid of the device. Once formed, the biofilms are exposed to the test antibiotics for a defined period of time and then transferred in a fresh medium in a second microwell plate which is further incubated overnight. The assay requires prolonged incubation and it is not easy to perform, so it can not be applied to all isolates, isolated in routine work.

Another issue concerns the possibility to assess antibiotic susceptibility of both SCV and of normal phenotype colonies. Data suggest the importance of performing susceptibility testing on all observed isolates of variant colony morphotype, due to the possibility of obtaining discordant susceptibilities for the different colony types when antimicrobial susceptibility testing is performed using disc-diffusion test or E-test (Tande et al. 2014).

3.3 Molecular Analysis

Characteristics of molecular methods, high sensitivity and specificity, rapid turnaround time, should theoretically perfectly suit to diagnosis of PJIs. Nonetheless, their use has not been universally accepted and some comparative studies with culture have not highlighted significant differences between molecular and cultural methods (Rak et al. 2013; Zegaer et al. 2014). The main advantage of molecular methods is that they do not need growth of microorganisms to detect their presence. By contrast, they detect also DNA of microorganisms dead or inadvertently left on the sample during its collection.

Essentially two approaches have been used in molecular diagnosis of PJIs: broad range PCR and specific PCR. These latter are tailored to detect single bacterial species or a group of closely related microorganisms. Real-time PCR assays may produce results in a few hours, and have a sufficient limit of detection, allowing to quantitatively detect only microorganisms which

are considered significant. In this way, contamination may be kept under control. The main limitation of specific PCR is that it detects only microorganisms for which primers have been designed. This means that panel must include primers for microorganisms such as Propionibacteria and Corynebacteria, which are usually considered as contaminants in other infections. Multiplex or multipanel PCR have been used for analysis of sonicated fluids, thanks to their higher specificity, but their sensitivity may be affected by inhability to detect infections caused by the above mentioned bacteria (Vasoo et al. 2015). When panel specifically dedicated to PJIs pathogens are used, PCR of sonicated fluid proved to be more sensitive than culture in patients who received antibiotic therapy in the 2 weeks before surgery (88 % vs 70 %), while no significant differences were observed for the other patients (Cazanave et al. 2013). As far as synovial fluids are concerned, PCR specific assays have shown a lower sensitivity than culture (Hischebeth et al. 2016; Melendez et al. 2016). The low sensitivity for this kind of specimen may be caused by the presence of aggregated bacteria, rather than by inhibitory compounds.

Broad range PCR permits detection of a wide variety of microorganisms. The majority of broad range PCR are based on amplification of the 16S rDNA, which codifies for the small ribosomal unit. This gene is composed by conserved regions common to all bacteria, and more variable region which are specific for bacterial species. Usually detection of nucleic acid is followed by amplicon sequencing. The main limitation of these assays is the high risk of detect contaminating DNA. Methods developed to reduce contamination dramatically affect test sensitivity, therefore they are not used. An alternative may be represented by the use of interpretative criteria similar to those existing for culture methods. In fact, the best combination of sensitivity and specificity is reached when PJI is defined by detection of the bacteria from at least two samples (Rak et al. 2015).

The same limits described for broad range PCR have also reported for PCR associated to mass spectrometry ESI-MS (Greenwood-Quaintance et al. 2014).

Finally, although not recommended for routine use as alternative to cultures, molecular methods may represent an additional tool in select negative cultures from patients for whom strong suggestion of PJI exists.

4 National Recommendations for Diagnosis of PJIs

Due to the complexity of PJIs diagnosis and the wide variability in analytical methods used by the various centers even in the same country, definition of a uniform diagnostic protocol is advisable. Most of national recommendations for evaluation and treatment of PJIs, such as those published in France and Italy are rather similar to those of the ICM document (Société de Pathologie Infectieuse de Langue Française et al. 2010).

The Italian Association of Clinical Microbiologists (AMCLI) in 2013 defined a diagnostic workflow for PJIs. In particular, the protocol addressed microbiological diagnosis which was considered the most critical point of the whole diagnostic flow. The protocol is freely available at association website (www.amcli.it). The workflow for PJIs diagnosis was divided in pre and intraoperative analysis and describes in details the pre-analytic, analytic and post-analytic steps. The pre-analytical phase comprises details on the specimens of choice (synovial fluids, periprosthetic tissues, implants) and modalities for their collection and transport to the laboratory. In the section dedicated to the analytical phase, processing of synovial fluid, periprosthetic tissues and implants are described. When more options are available (for instance use of blood bottles or enrichment broths for synovial fluids, sonication or DTT treatment for prostheses) both the possibilities are described. Finally, interpretation of results and how to report them to clinicians are also discussed. In particular, synovial fluids can be directly inoculated in blood bottles and/or transferred to sterile tubes for delivery to the laboratory.

In both cases, aliquots in sterile tubes are centrifuged and the resuspended pellet plated onto chocolate agar, blood agar and Schaedler agar. If blood bottles are not used, use of broth enrichment is needed. For periprosthetic tissues homogenization is recommended, but alternatively, since not all laboratories are equipped with an homogenizer, reduction of biopsies in small pieces, vortexing of the sample or streaking of tissue onto agar plates together with inoculation of the tissue into broths are also described. For implants both sonication and DTT treatment are recommended. Prolonged incubation up to 14 days for broths (for aerobes and for anaerobes) with daily inspection are recommended. Colonies identification is based on biochemical and molecular (sequencing) methods and on mass spectrometry. Recommendations to identify and to test antibiotic susceptibility of small colony variants are also reported. Use of molecular assays to detect pathogens is discussed, but their use must be very careful. Finally, details on reporting microbiological results are provided, due to difficulties in data interpretation. Considering that all positive samples must be identified and reported, it is advisable, in presence of potential contaminants to add a warning suggesting the hypothesis that the isolate could be a contaminant. Moreover, due to prolonged incubation a preliminary report after 5 days is recommended. Update of the protocol is planned every 3 years.

5 Cost/Benefit Analysis of Microbiological Diagnosis

Currently it has been estimated that the total annual cost for PJI revisions in the USA exceeds $566 million, and it is expected to exceed $1.6 billion by 2020 (Kurtz et al. 2012). Moreover, in case of treatment failure a 156 % increment of costs has been reported (Peel et al 2013) Therefore, an accurate discrimination between septic and aseptic failures is essential to ensure appropriate treatment to infected patients and to avoid unnecessary therapies and costs for patients with aseptic failure. PJI diagnosis is further complicated by the lack of an universal diagnostic test (Drago et al. 2016). However, current knowledge in PJI diagnosis does not allow for diagnostic recommendations incorporating considerations of the benefits, opportunities, costs and risks presented by the different diagnostic strategies and tests. Therefore, the development of a diagnostic algorithm which allows with sufficient potency to discriminate among septic and aseptic implant failures, thus reducing economic costs due to excessive laboratory analyses and wrong treatments will be mandatory for an innovative and comprehensive strategy in the management of PJIs diagnosis. With the perspective of minimizing costs and non-value added activities, it is also equally important to reduce the turnaround time (TAT), which is related to more effective hospital care, increased efficiency of hospital performance and decreased hospital-stay.

6 Conclusions

In summary, diagnosis of PJIs is a rather complex challenge for orthopedics and requires a strict collaboration between different specialists: orthopaedics, infectivologists, microbiologists, pathologists and radiologists. Diagnostic criteria have been described by national and international association and scientific societies. On the whole, they are rather similar though presenting some peculiarities. Clinicians should be trained on how to use it, but more importantly they should know potential and limitation of the available tests in order to use them appropriately.

References

Aggarwal VK, Higuera C, Deirmengian G et al (2013) Swab cultures are not as effective as tissue cultures for diagnosis of periprosthetic joint infection. Clin Orthop Relat Res 471:3196–3203

Atkins BL, Athanasou N, Deeks JJ et al (1998) Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS collaborative study group. J Clin Microbiol 36:2932–2939

Barrack RL, Harris WH (1993) The value of aspiration of the hip joint before revision total hip arthroplasty. J Bone Joint Surg Am 75:66–76

Barrack RL, Jennings RW, Wolfe MW et al (1997) The Coventry award. The value of preoperative aspiration before total knee revision. Clin Orthop Relat Res 345:8–16

Bartalesi F, Fallani S, Salomoni E et al (2012) Candida glabrata prosthetic hip infection. Am J Orthop 41:500–505

Becker K, Heilmann C, Peters G (2014) Coagulase-negative staphylococci. Clin Microbiol Rev 27:870–926

Bemer P, Leger J, Tande D et al (2016) How many samples and how many culture media to diagnose a prosthetic joint infection: a clinical and microbiological prospective multicenter study. J Clin Microbiol 54:385–391

Berbari EF, Hanssen AD, Duffy MC et al (1998) Prosthetic joint infection due to Mycobacterium tuberculosis: a case series and review of the literature. Am J Orthop 27:219–227

Bjerkan G, Witsø E, Bergh K (2009) Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro. Acta Orthop 80:245–250

Blackmur JP, Tang EYH, Dave J et al (2014) Use of broth cultures peri-operatively to optimize the microbiological diagnosis of musculoskeletal implant infections. Bone Joint J 96-B:1566–1570

Bouaziz A, Uçkay I, Lustig S et al (2012) Microbiological markers suggesting high inoculum size at time of surgery are risk factors for relapse in patients with Staphylococcus aureus prosthetic joint infection. J Infect 65:582–584

Butler-Wu SM, Burns EM, Pottinger PS et al (2011) Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. J Clin Microbiol 49:2490–2495

Campbell KA, Cunningham C, Hasan S et al (2015) Risk factors for developing Staphylococcus aureus nasal colonization in spine and arthroplasty surgery. Bull Hosp Jt Dis 73:276–281

Caola I, Drago L (2013) Percorso diagnostico: infezioni delle protesi articolari e dei mezzi di osteosintesi. Available at http://www.amcli.it/wp-content/uploads/2015/10/PercorsodiagnosticoprotesiarticolariAMCLI2013.pdf. Accessed 6 June 2016

Carrega G, Bartolacci V, Burastero G et al (2013) Prosthetic joint infections due to Mycobacterium tuberculosis: a report of 5 cases. Int J Surg Case Rep 4:178–181

Cazanave C, Greenwood-Quaintance KE et al (2012) Corynebacterium prosthetic joint infection. J Clin Microbiol 50:1518–1523

Cazanave C, Greenwood-Quaintance KE et al (2013) Rapid molecular microbiologic diagnosis of prosthetic joint infections. J Clin Microbiol 51:2280–2287

de Sanctis J, Teixeira L, van Duin D et al (2014) Complex prosthetic joint infections due to carbapenemase-producing Klebsiella pneumoniae: a unique challenge in the era of untreatable infections. Int J Infect Dis 25:73–78

De Vecchi E, Bortolin M, Signori V et al (2016) Treatment with Dithiothreitol improves bacterial recovery from tissue samples in osteoarticular and joint infections. J Arthroplasty. doi:10.1016/j.arth.2016.05.008

DeHaan A, Huff T, Schabel K et al (2013) Multiple cultures and extended incubation for hip and knee arthroplasty revision: impact on clinical care. J Arthroplasty 28(8 Suppl):59–65

Dilisio MF, Miller LR, Warner JJ et al (2014) Arthroscopic tissue culture for the evaluation of periprosthetic shoulder infection. J Bone Joint Surg Am 96:1952–1958

Drago L, Romanò CL, Mattina R et al (2012) Does dithiothreitol improve bacterial detection from infected prostheses? A pilot study. Clin Orthop Relat Res 470:2915–2925

Drago L, Signori V, De Vecchi E et al (2013) Use of dithiothreitol to improve the diagnosis of prosthetic joint infections. J Orthop Res 31:1694–1699

Drago L, De Vecchi E, Cappelletti L et al (2014) Role and antimicrobial resistance of staphylococci involved in prosthetic joint infections. Int J Artif Organs 37:414–421

Drago L, De Vecchi E, Cappelletti L et al (2015) Prolonging culture to 15 days improves bacterial detection in bone and joint infections. Eur J Clin Microbiol Infect Dis 34:1809–1813

Drago L, Lidgren L, Bottinelli E et al (2016) Mapping of microbiological procedures by the members of the International Society of Orthopaedic Centers (ISOC) for diagnosis of periprosthetic infections. J Clin Microbiol 54:1402–1403

Dutronc H, Dauchy FA, Cazanave C et al (2010) Candida prosthetic infections: case series and literature review. Scand J Infect Dis 42:890–895

Esteban J, Gomez-Barrena E, Cordero J et al (2008) Evaluation of quantitative analysis of cultures from sonicated retrieved orthopedic implants in diagnosis of orthopedic infection. J Clin Microbiol 46:488–492

Everts RJ, Chambers ST, Murdoch DR et al (2004) Successful antimicrobial therapy and implant retention for streptococcal infection of prosthetic joints. ANZ J Surg 74:210–214

Fernandes A, Dias M (2013) The microbiological profiles of infected prosthetic implants with an emphasis on the organisms which form biofilms. J Clin Diagn Res 7:219–223

Fink B, Makowiak C, Fuerst M et al (2008) The value of synovial biopsy, joint aspiration and C-reactive protein in the diagnosis of late peri-prosthetic infection of total knee replacements. J Bone Joint Surg (Br) 90:874–878

Font-Vizcarra L, Spangehl MJ, Masri BA et al (1999) Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am 81:672–683

Font-Vizcarra L, García S, Martínez-Pastor JC et al (2010) Blood culture flasks for culturing synovial fluid in prosthetic joint infections. Clin Orthop Relat Res 468:2238–2243

Ghanem E, Ketonis C, Restrepo C et al (2009) Periprosthetic infection: where do we stand with regard to Gram stain? Acta Orthop 80:37–40

Greenwood-Quaintance KE, Uhl JR et al (2014) Diagnosis of prosthetic joint infection by use of PCR-electrospray ionization mass spectrometry. J Clin Microbiol 52:642–649

Gupta A, Berbari EF, Osmon DR et al (2014) Prosthetic joint infection due to Salmonella species: a case series. BMC Infect Dis 14:633

Hischebeth GT, Randau TM, Buhr JK et al (2016) Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. J Microbiol Methods 121:27–32

Holinka J, Bauer L, Hirschl AM et al (2011) Sonication cultures of explanted components as an add-on test to routinely conducted microbiological diagnostics improve pathogen detection. J Orthop Res 29:617–622

Holleyman RJ, Baker PN, Charlett A et al (2016) Analysis of causative microorganism in 248 primary hip arthroplasties revised for infection: a study using the NJR dataset. Hip Int 26:82–89

Hughes JG, Vetter EA, Patel R et al (2001) Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. J Clin Microbiol 39:4468–4471

Huotari K, Peltola M, Jamsen E (2015) The incidence of late prosthetic joint infections. A registry based study on 112,708 primary hip and knee replacement. Acta Orthop 86:321–325

Janz V, Wassilew GI, Kribus M et al (2015) Improved identification of polymicrobial infection in total knee arthroplasty through sonicate fluid cultures. Arch Orthop Trauma Surg 135:1453–1457

Jordan RW, Saithna A, Smith N et al (2015) Does intraoperative tissue sample enrichment help or hinder the identification of microorganisms in prosthetic joint infection? Eur J Orthop Surg Traumatol 25:731–736

Kamme C, Lindberg L (1981) Aerobic and anaerobic bacteria in deep infections after total hip arthroplasty: differential diagnosis between infectious and non-infectious loosening. Clin Orthop Relat Res 154:201–207

Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA (2016) Periprosthetic joint infection. Lancet 387:386–394

Kurtz SM, Lau E, Watson H et al (2012) Economic burden of periprosthetic joint infection in the United States. J Arthroplasty 27(Suppl 8):61–65e1

Langvatn H, Everts RJ, Chambers ST et al (2004) Successful antimicrobial therapy and implant retention for streptococcal infection of prosthetic joints. ANZ J Surg 74:210–214

Langvatn H, Lutro O, Dale H et al (2015) Bacterial and hematological findings in infected total hip arthroplasties in Norway assessment of 278 revisions due to infection in the Norwegian Arthroplasty Register. Open Orthop J 9:445–449

Levine BR, Evans BG (2001) Use of blood culture vial specimens in intraoperative detection of infection. Clin Orthop Relat Res 382:222–231

Lovati AB, Romanò CL, Bottagisio M et al (2016) Modeling Staphylococcus epidermidis-induced non-unions: subclinical and clinical evidence in rats. PLoS One 11:e0147447

Maduka-Ezeh AN, Greenwood-Quaintance KE, Karau MJ et al (2012) Antimicrobial susceptibility and biofilm formation of Staphylococcus epidermidis small colony variants associated with prosthetic joint infection. Diagn Microbiol Infect Dis 74:224–229

Månsson E, Hellmark B, Sundqvist M et al (2015) Sequence types of Staphylococcus epidermidis associated with prosthetic joint infections are not present in the laminar airflow during prosthetic joint surgery. APMIS 123:589–595

Melendez DP, Greenwood-Quaintance KE, Berbari EF et al (2016) Evaluation of a genus- and group-specific rapid PCR assay panel on synovial fluid for diagnosis of prosthetic joint infection. J Clin Microbiol 54:120–126

Murillo O, Grau I, Lora-Tamayo J et al (2015) The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century. Clin Microbiol Infect 21:254.e1–254.e8

Osmon DR, Berbari EF, Berendt AR et al (2013) Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 56: e1–e25

Ouyang Z, Zhai Z, Qin A et al (2015) Limitations of Gram staining for the diagnosis of infections following total or hip arthroplasty. Exp Ther Med 9:1857–1864

Parvizi J, Zmistowski B, Berbari EF et al (2011) New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res 469:2992–2994

Pearle AD, Bates JE, Tolo ET et al (2003) Clostridium infection in a knee extensor mechanism allograft: case report and review. Knee 10:149–153

Peel TN, Cheng AC, Lorenzo YP et al (2013) Factors influencig the cost of prosthetic joint infection treatment. J Hosp Infect 85:213–219

Peel TN, Dylla BL, Hughes JG et al (2016) Improved diagnosis of prosthetic joint infection by culturing periprosthetic tissue specimens in blood culture bottles. MBio 7:e01776

Piper KE, Jacobson MJ, Cofield RH et al (2009) Microbiologic diagnosis of prosthetic shoulder infection by

use of implant sonication. J Clin Microbiol 47:1878–1884
Portillo ME, Salvadó M, Trampuz A et al (2013) Sonication versus vortexing of implants for diagnosis of prosthetic joint infection. J Clin Microbiol 51:591–594
Proctor RA, von Eiff C, Kahl BC et al (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4:295–305
Rak M, Barlic-Maganja D, Kavcic M et al (2013) Comparison of molecular and culture method in diagnosis of prosthetic joint infection. FEMS Microbiol Lett 343:42–48
Rak M, Barlič-Maganja D, Kavčič M et al (2015) Identification of the same species in at least two intraoperative samples for prosthetic joint infection diagnostics yields the best results with broad-range polymerase chain reaction. Int Orthop 39:975–979
Rasouli MR, Harandi AA, Adeli B et al (2012) Revision total knee arthroplasty: infection should be ruled out in all cases. J Arthroplasty 27:1239–1243
Renner LD, Weibel DB (2011) Physicochemical regulation of biofilm formation. MRS Bull 36:347–355
Rodríguez-Pardo D, Pigrau C, Lora-Tamayo J et al (2014) Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. Clin Microbiol Infect 20:O911–O919
Romanò CL, De Vecchi E, Vassena C et al (2013) A case of a late and atypical knee prosthetic infection by no-biofilm producer Pasteurella multocida strain identified by pyrosequencing. Pol J Microbiol 62:435–438
Roux AL, Sivadon-Tardy V, Bauer T et al (2011) Diagnosis of prosthetic joint infection by beadmill processing of a periprosthetic specimen. Clin Microbiol Infect 17:447–450
Saeed K (2014) Diagnostics in prosthetic joint infections. J Antimicrob Chemother 69(Suppl 1):i11–i19
Schäfer P, Fink B, Sandow D et al (2008) Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin Infect Dis 47:1403–1409
Sendi P, Christensson B, Uçkay I et al (2011) Group B streptococcus in prosthetic hip and knee joint-associated infections. J Hosp Infect 79:64–69
Senneville E, Joulie D, Legout L et al (2011) Outcome and predictors of treatment failure in total hip/knee prosthetic joint infections due to Staphylococcus aureus. Clin Infect Dis 53:334–340
Shah NB, Tande AJ, Patel R et al (2015) Anaerobic prosthetic joint infection. Anaerobe 36:1–8
Shen H, Tang J, Wang Q et al (2015) Sonication of explanted prosthesis combined with incubation in BD Bactec bottles for pathogen-based diagnosis of prosthetic joint infection. J Clin Microbiol 53:777–781
Société de Pathologie Infectieuse de Langue Française (SPILF), Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT), Groupe de Pathologie Infectieuse Pédiatrique (GPIP) et al (2010) Recommendations for bone and joint prosthetic device infections in clinical practice (prosthesis, implants, osteosynthesis). Med Mal Infect 40:185–211
Stoodley P, Conti SF, Demeo PJ et al (2011) Characterization of a mixed MRSA/MRSE biofilm in an explanted total ankle arthroplasty. FEMS Immunol Med Microbiol 62:66–74
Tande AJ, Patel R (2014) Prosthetic joint infection. Clin Microbiol Rev 27:302–345
Tande AJ, Osmon DR, Greenwood-Quaintance KE et al (2014) Clinical characteristics and outcomes of prosthetic joint infection caused by small colony variant staphylococci. MBio 5:e01910–e01914
Tande AJ, Palraj BR, Osmon DR et al (2016) Clinical presentation, risk factors, and outcomes of hematogenous prosthetic joint infection in patients with Staphylococcus aureus bacteremia. Am J Med 129:221
Tetreault MW, Wetters NG, Aggarwal VK et al (2013) Should draining wounds and sinuses associated with hip and knee arthroplasties be cultured? J Arthroplasty 28(8 Suppl):133–136
Tornero E, Garcia-Oltra E, Garcia-Ramiro S et al (2012) Prosthetic joint infections due to Staphylococcus aureus and coagulase negative staphylococci. Int J Artif Organs 35:884–892
Tornero E, Senneville E, Euba G et al (2014) Characteristics of prosthetic joint infections due to Enterococcus sp. and predictors of failure: a multinational study. Clin Microbiol Infect 20:1219–1224
Trampuz A, Piper KE, Hanssen AD et al (2006) Sonication of explanted prosthetic components in bags for diagnosis of prosthetic joint infection is associated with risk of contamination. J Clin Microbiol 44:628–631
Trampuz A, Piper KE, Jacobson MJ et al (2007) Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 357:654–663
Tunney MM, Patrick S, Gorman SP et al (1998) Improved detection of infection in hip replacements. A currently underestimated problem. J Bone Joint Surg (Br) 80:568–572
Vasoo S, Cunningham SA, Greenwood-Quaintance KE et al (2015) Evaluation of the film-array blood culture ID panel on biofilms dislodged from explanted arthroplasties for prosthetic joint infection diagnosis. J Clin Microbiol 53:2790–2792
Williams JL, Norman P, Stockley I (2004) The value of hip aspiration versus tissue biopsy in diagnosing infection before exchange hip arthroplasty surgery. J Arthroplasty 19:582–586
Yee DK, Chiu KY, Yan CH et al (2013) Review article: joint aspiration for diagnosis of periprosthetic infection. J Orthop Surg 21:236–240
Zegaer BH, Ioannidis A, Babis GC et al (2014) Detection of bacteria bearing resistant biofilm forms, by using the universal and specific PCR is still unhelpful in the diagnosis of periprosthetic joint infections. Front Med 1:30

Zhai Z, Li H, Qin A, Liu G et al (2014) Meta-analysis of sonication fluid samples from prosthetic components for diagnosis of infection after total joint arthroplasty. J Clin Microbiol 52:1730–1736

Zmistowski B, Fedorka CJ, Sheehan E et al (2011) Prosthetic joint infection caused by gram-negative organisms. J Arthroplasty 26(6 Suppl):104–108

Zmistowski B, Della Valle C, Bauer TW et al (2014a) Diagnosis of periprosthetic joint infection. J Arthroplasty 29:77–83

Zmistowski B, Della Valle C, Bauer TW et al (2014b) Diagnosis of periprosthetic joint infection. J Orthop Res 32(Suppl 1):S98–S107

Zywiel MG, Stroh DA, Johnson AJ et al (2011) Gram stains have limited application in the diagnosis of infected total knee arthroplasty. Int J Infect Dis 15: e702–e705

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 69–79
DOI 10.1007/5584_2017_11

Published online: 28 February 2017

The Role of Biomarkers for the Diagnosis of Implant-Related Infections in Orthopaedics and Trauma

Abtin Alvand, Maryam Rezapoor, and Javad Parvizi

Abstract

Diagnosis of implant-related (periprosthetic joint) infections poses a major challenge to infection disease physicians and orthopaedic surgeons. Conventional diagnostic tests continue to suffer from issues of accuracy and feasibility. Biomarkers are used throughout medicine for diagnostic and prognostic purposes, as they are able to objectively determine the presence of a disease or a biological state. There is increasing evidence to support the measurement of specific biomarkers in serum and/or synovial fluid of patients with suspected periprosthetic joint infections. Promising serum biomarkers include interleukin (IL)-4, IL-6, tumour necrosis factor (TNF)-α, procalcitonin, soluble intercellular adhesion molecule 1 (sICAM-1), and D-dimer. In addition to c-reactive protein and leucocyte esterase, promising biomarkers that can be measured in synovial fluid include antimicrobial proteins such as human β-defensin (HBD)-2 and human β-defensin (HBD)-3, and cathelicidin LL-37, as well as several interleukins such as IL-1β, IL-6, IL-8, IL-17, TNF- α, interferon-δ, and vascular endothelial growth factor.

Keywords

Biomarkers • Diagnosis • Periprosthetic joint infection • Serum • Synovial fluid

1 Introduction

Accurate and timely diagnosis of implant related orthopaedic infections is essential as it has a profound influence on the management and outcome of this potentially catastrophic complication. Despite significant progress in the multidisciplinary management of periprosthetic joint infections (PJI) in recent years, a 'gold-standard' diagnostic test has not yet been developed. In addition to a detailed clinical history and physical examination, clinicians have to utilise a

A. Alvand (✉), M. Rezapoor, and J. Parvizi
The Rothman Institute, 925 Chestnut Street, Philadelphia, PA 19107, USA
e-mail: abtin.alvand@ndorms.ox.ac.uk

Table 1 International consensus meeting criteria for defining periprosthetic joint infection

Periprosthetic joint infection is present if one of two major criteria or three of five minor criteria exists			
Major criteria	1. A sinus tract communicating with the joint or		
	2. Two positive periprosthetic cultures (tissue or synovial fluid) with phenotypically identical microorganism		
Minor criteria		*Acute PJI (<90 days)*	*Chronic PJI (>90 days)*
	1. Elevated ESR or CRP	ESR: no threshold	ESR > 30 mm/h
		CRP > 100 mg/L	CRP > 10 mg/L
	2. Elevated SF WBC count or	10,000 cells/μL	3000 cells/μL
	Changes in the leukocyte esterase strip	+ or ++	+ or ++
	3. Elevated SF PMN%	90%	80%
	4. Positive histologic analysis of the periprosthetic tissue	>5 neutrophil per high-power field in 5 high-power fields (×400)	>5 neutrophil per high-power field in 5 high-power fields (×400)
	5. A single positive culture		

CRP C-reactive protein, *ESR* sedimentation rate, *SF WBC* synovial fluid white blood cell, *SF PMN* synovial fluid polymorphonuclear neutrophil

combination of serological, microbiological, histological and radiological investigations in order to diagnose PJI. In order to address these diagnostic challenges, the Musculoskeletal Infection Society (MSIS) recommended a number of criteria for better defining PJI (Parvizi et al. 2011b). Following modification at the International Consensus Meeting (ICM) on PJI in 2013, this definition is now the most widely adopted throughout the world (Zmistowski et al. 2014). Based on the ICM criteria, the presence of one of two 'Major', or three of five 'Minor' criteria confirms the diagnosis of PJI (Table 1). In addition to defining PJI, the ICM also recommended an algorithmic approach for diagnosing PJI based on all the aforementioned investigations (Fig. 1).

In this review, we will discuss some of the shortcomings of traditional tests for diagnosing PJI, and summarize recent advances, which highlight the increasingly important role of novel serum and synovial biomarkers in this field.

2 Conventional Diagnostic Tools for PJI Diagnosis

Routine 'work-up' of patients with suspected PJI continues to involve the measurement of serum white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and c-reactive protein (CRP). These can be classified under techniques that measure the host response to infection. However, several studies now suggest that serum WBC count and neutrophil differential have little role to play in the diagnosis of PJI (Toossi et al. 2012; Zmistowski et al. 2012; Di Cesare et al. 2005). This is further supported by a Meta-Analysis which calculated the pooled sensitivity of WBC count to be 45% and the specificity to be 87% (Berbari et al. 2010).

ESR is a non-specific measure of inflammation and tissue injury that can take between three to twelve months to normalize following surgery (Zlonis 1993; Aalto et al. 1984). CRP is an acute phase protein synthesized by the liver in order to activate the complement system but its levels normalize more rapidly between two to four weeks post-operatively (Niskanen et al. 1996). Although more accurate and sensitive than WBC count, serum ESR and CRP levels have a poor specificity for diagnosing PJI. In their Meta-Analysis of studies evaluating the accuracy of these two serum markers, Berbari and colleagues calculated the pooled estimate for the sensitivity of ESR to be 75% and that of CRP to be 88%, whilst the pooled specificity was 70% and 74% respectively (Berbari et al. 2010). Furthermore, based on receiver operating characteristic (ROC) curve analysis, other studies suggest that the

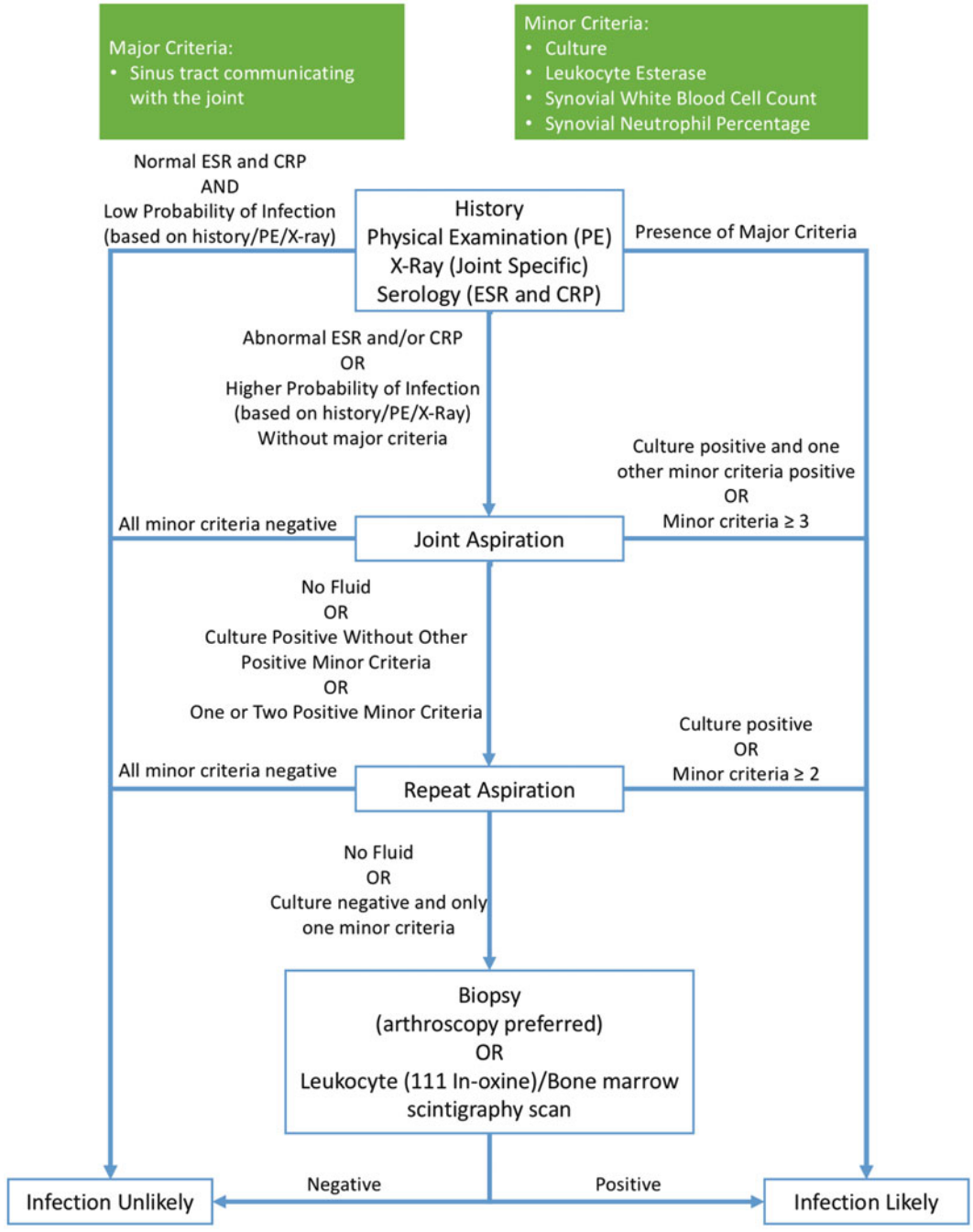


Fig. 1 Algorithm for diagnosing periprosthetic joint infection using history and physical examination followed by serological, microbiological, histological and radiological investigations (Reprinted with permission from Proceedings of the International Consensus Meeting on Periprosthetic Joint Infection. Chairs Javad Parvizi and Thorsten Gehrke. Data Trace Publishing Company; 2013, p. 160)

serum ESR and CRP cannot accurately predict the persistence of infection following revision surgery for PJI (Bejon et al. 2011; Ghanem et al. 2009b).

It is also well recognised that ESR and CRP may be raised as a result of a number of comorbidities such as inflammatory disorders and even obesity (Lee and Pratley 2005; Liu et al. 2014). More importantly, serum ESR and CRP levels may not be elevated in several commonly encountered clinical scenarios. One example is infections with low virulence organisms such as coagulase negative *Staphylococcus, Propionibacterium acnes, Candida, Corynebacterium, Mycobacterium, and Actinomyces* (McArthur et al. 2015). Further inaccuracies in diagnosis can occur during chronic or low grade infections (Sanzen and Sundberg 1997) and in cases where inadvertent antibiotic therapy has been administered (Shahi et al. 2015). However, when used together, they can form a useful screening tool for PJI with reports of almost 98% sensitivity for diagnosing PJI (Ghanem et al. 2009a; Schinsky et al. 2008). As such, thresholds for their levels have been recommended (Alijanipour et al. 2013) and their combined use has been proposed by the ICM guidelines (Table 1) as a minor criteria for PJI (Zmistowski et al. 2014).

Joint arthrocentesis and measurement of synovial WBC count and neutrophil differential is commonly performed in suspected cases of PJI with good evidence to support their use (Mason et al. 2003; Trampuz et al. 2004; Bedair et al. 2011; Zmistowski et al. 2012; Dinneen et al. 2013). The ICM guidelines therefore include them as part of the minor diagnostic criteria for PJI. However, as with other conventional tests, the diagnostic value of synovial WBC count and neutrophil differential can be negatively affected by previous antibiotic use (Shahi et al. 2015). Synovial fluid and tissue culture form part of the routine protocol for managing PJI as they can both diagnose the infection and guide antibiotic use. Multiple samples are recommended in order to increase sensitivity and reduce false-positive results (Atkins et al. 1998; Pandey et al. 2000; Meermans and Haddad 2010; Mikkelsen et al. 2006). However, the utility of this technique can be hampered by prior antibiotic use thereby giving rise to culture-negative infections (Berbari et al. 2007; Malekzadeh et al. 2010; Parvizi et al. 2014). Hence, it is also recommend that antibiotics be stopped at least 2 weeks prior to joint aspiration or specimen sampling. Another problem with conventional culture techniques is that they may not be able to isolate low-virulence organisms such as *Propionibacterium acnes* (Zeller et al. 2007).

Histological analysis is included as one of the minor criteria for diagnosing PJI and its value has been well documented in multiple studies (Tsaras et al. 2012; Krenn et al. 2014). However, the main drawback lies in the fact that not all units have the histopathology expertise to perform this type of analysis.

Given some of the drawbacks of the conventional diagnostic techniques, there has recently been an increased focus on the identification of novel serum and synovial 'biomarkers' in order to improve the diagnosis and ongoing management of implant related infections.

3 Biomarkers in Medicine

According to the National Institute of Health (NIH) Biomarkers Definition Working Group, biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"(Biomarkers Definitions Working 2001). Due to advances in molecular technology and knowledge of biomarker discovery, the applications of biomarkers in patient screening, diagnosis, prognosis, and targeted therapy are growing. Biomarkers have shown promising results in rapid and accurate diagnosis of diseases such as cancers, cardiovascular disorders, infections, and genetic and immunological disease, which lead to improved outcome and reduced mortality (Mayeux 2004). For instance, in the field of cancer diagnosis, prostate specific antigen (PSA) is used for prostate cancer diagnosis (Henry and Hayes 2012), and detecting the combination of three different biomarkers, including Thomsen-Friedenreich (TF), urokinase-dependant plasminogen activator system (uPA), and the plasminogen activator

inhibitor (PAI), in nipple aspirate fluid is for early diagnosis of breast cancer with accuracy level of 97–100% (Dos Anjos Pultz et al. 2014). Brain natriuretic peptide (BNP) —released from the myocardium in response to myocardial stretch in the event of heart failure (Miller et al. 2007) – cardiac troponin I or T (cTnI/T), interlukin-6 (IL-6), IL-1, low-density lipoprotein (LDL), and tumour necrosis factor alpha (TNF-α) are examples of biomarkers used for diagnosis and risk stratification of cardiovascular disease (Qureshi et al. 2012).

Biomarkers also play an imperative role in the diagnosis of inflammation and infection (Ciriello et al. 2013; Pierrakos and Vincent 2010). A growing body of evidence now demonstrates the role of procalcitonin (PCT) in the diagnosis of different infectious diseases such as urinary tract, surgical site, and respiratory tract infections in addition to post-traumatic sepsis (Nanda and Juthani-Mehta 2009; Pierrakos and Vincent 2010; Schuetz et al. 2011; Ciriello et al. 2013). Specifically in sepsis diagnosis, CD11b, CD64, group II phospholipase 2 (PLA2-II), Interferon-induced protein 10 (IP-10), IL-12 have been shown to be practical with sensitivity and specificity of more than 90% (Pierrakos and Vincent 2010).

4 Biomarkers of Periprosthetic Joint Infection

In addition to 'conventional' biomarkers such as serum ESR/CRP and synovial fluid WBC count, a growing number of more specific antimicrobial proteins and pro-inflammatory cytokines are now being discovered. These novel markers have the potential to surpass the diagnostic accuracy of traditional tests (Jacovides et al. 2011; Deirmengian et al. 2014b). This has largely come about as our understanding of the molecular pathways governing joint infections has grown. Genomic studies using microarray techniques have demonstrated that specific WBC gene expression patterns exist in infected joints and these have been used to further understand the role of biomarkers in PJI (Deirmengian et al. 2005).

4.1 Serum Biomarkers

Measurement of serum biomarkers is more desirable than that of synovial fluid because it is less invasive and carries little risk of iatrogenic joint infection. Furthermore, there is the added advantage that serial serum measurements can allow monitoring of infection and response to treatment. This is particularly desirable between the stages of two-stage surgery in order to determine whether or not it is safe to re-implant prostheses. Currently, serum ESR and CPR are the two most commonly used biomarkers and form one of the minor criteria recommended by the ICM (Zmistowski et al. 2014). Their advantages and drawbacks have already been alluded to earlier.

A number of pro-inflammatory cytokines have so far been identified. The most widely investigated amongst these is serum IL-6 and multiple studies have shown that levels are elevated in PJI cases (Di Cesare et al. 2005; Berbari et al. 2010; Worthington et al. 2010). IL-6 is a major regulator of the acute phase response and is secreted by cells such as macrophages and T-cells. It stimulates the secretion of many acute-phase proteins including CRP from hepatocytes. This means that serum IL-6 levels rise quicker than CRP in response to sepsis or trauma (Heinrich et al. 1990). An advantage in using IL-6 is that it may be able detect early PJI more accurately as the serum levels return to normal much faster after index surgery (48–72 h after index surgery) whilst that of CRP and ESR stay elevated for a longer period (Wirtz et al. 2000). It has also been recommended to combined testing of serum IL-6 and CRP improves diagnostic accuracy (Bottner et al. 2007; Glehr et al. 2013; Berbari et al. 2010). Gollwitzer and colleagues also found that serum levels of IL-4, as well as IL-6, were significantly higher in septic hip and knee arthroplasty revisions compared to aseptic revisions (Gollwitzer et al. 2013). Both were very specific for PJI with IL-4 having a

specificity of 90.0% and IL-6 a specificity of 95.0%. The corresponding sensitivities were 60.0% and 46.7% respectively.

TNF-α, which is released by monocytes in response to infection, is another cytokine that has been identified as a serum biomarker for PJI with a high specificity of 94% but low sensitivity of 43% (Bottner et al. 2007). The problem with this biomarker is that it must be analysed within an hour of sampling and the processing time for samples is very long.

Soluble intercellular adhesion molecule 1 (sICAM-1) is another biomarker with significantly elevated levels in PJI (Worthington et al. 2010). In addition, Drago et al. demonstrated that sICAM-1 is a useful serum biomarker in confirming eradication of PJI (Drago et al. 2011).

Secreted by the mononuclear phagocyte system, procalcitonin (PCT) has received significant attention as a serum biomarker for sepsis in general (Selberg et al. 2000; Whicher et al. 2001; Simon et al. 2004), in addition to being able to discriminate between inflammatory and septic joint conditions (Arkader et al. 2006; Talebi-Taher et al. 2013; Hugle et al. 2008). Studies have advocated the role of PCT as a PJI biomarker because its serum levels are not increased following arthroplasty (Ali et al. 2009). However, the value of serum PCT as a diagnostic test has been questioned by several studies due to its low sensitivity (Drago et al. 2011). Bottner and colleagues demonstrated that despite a high specificity of 98%, PCT had a low sensitivity of 33% (Bottner et al. 2007). This is further supported by other studies which found no evidence to support the role of PCT in PJI diagnosis (Worthington et al. 2010). In contrast to the previous studies, a more recent study by Glehr and colleagues has demonstrated sensitivity of 80% and specificity of 37% but the authors still only recommended it as an adjuvant to conventional diagnostic tests (Glehr et al. 2013).

Given the costs and difficulties of routinely measuring some of the aforementioned serum biomarkers, researchers are now focusing on the identification of more feasible and cost-effective alternatives for screening patients for suspected PJI. A promising biomarker that has demonstrated high sensitivity in pilot studies performed at our institution is serum D-dimer (Shahi and Parvizi 2016). This fibrin degradation product reflects inflammation of the symposium within an infected joint and our preliminary results demonstrate that D-dimer can act as an excellent screening tool for PJI diagnosis.

The main drawback of serum biomarkers is that systemic sepsis and disease states may still affect their circulating levels, and that such, measurements may not accurately reflect the infection state at a local level (Gollwitzer et al. 2013).

4.2 Synovial Biomarkers

The main advantage of using a synovial biomarker is that it can reflect the host response to pathogens within the joint. Joint arthrocentesis and determination of WBC count and polymorphonuclear neutrophil percentage is the commonest example of synovial biomarker measurement. It is one of the minor criteria recommended by the ICM (Zmistowski et al. 2014) and has been shown to have a sensitivity of 84% and specificity of 93% (Deirmengian et al. 2010). As with many of the other conventional tests, the accuracy of WBC count and polymorphonuclear neutrophil percentage can be adversely affected by pre-mature antibiotic administration (Shahi et al. 2015).

Another one of the ICM's minor diagnostic criteria involves the measurement of leucocyte esterase (LE) enzyme in the synovial fluid. This enzyme is produced by activated neutrophils at the site of infection and can be measured using a simple colorimetric strip (urine dipstick) test (Parvizi et al. 2011a). When the synovial fluid is added to the strip, a detergent within the strip lyses the neutrophils in the fluid that release the esterase enzyme thereby resulting in the test strip color change. LE has a very high diagnostic accuracy and a recent Meta-Analysis estimated the pooled sensitivity and specificity of LE for diagnosing PJI to be 81% and 97% respectively (Wyatt et al. 2016). It is also an extremely simple and cheap test, which costs approximately $0.17

per test and can be performed as a point-of-care test. One disadvantage of LE is that blood contamination of the synovial fluid can mask the color change on the test strip (Deirmengian et al. 2015a; Wetters et al. 2012). However, this problem can be easily overcome by spinning the fluid using a mini centrifuge (Aggarwal et al. 2013).

It has been found that in addition to *serum* CRP, synovial fluid CRP can be used to diagnose PJI with a sensitivity of 85% and specificity of 95% (Parvizi et al. 2012a, b). Despite the good diagnostic accuracy, synovial CRP has been shown to have lower sensitivity and sensitivity when compared to LE (De Vecchi et al. 2016). Furthermore, recent work by Deirmengian et al. suggests that synovial CRP may not be as accurate in diagnosing PJIs caused by less virulent organisms such as *Staphylococcus epidermidis* (Deirmengian et al. 2016). One other practical disadvantage is that the laboratory machines in many institutions are not calibrated for measuring CRP levels in synovial fluid.

The only biomarker that has so been commercialized specifically for use as a test for PJI is α-defensin (Deirmengian et al. 2014a, b). This biomarker is a naturally occurring antimicrobial peptide which is released from activated neutrophils and destroys the cell membrane of pathogens (Lehrer and Ganz 1992). Studies suggest that in addition to responding to low virulence organisms (Deirmengian et al. 2015b), its sensitivity is not affected by prior antibiotic administration (Shahi et al. 2016). It has an impressive accuracy with a recent Meta-Analysis estimating its pooled diagnostic sensitivity at 100% and specificity at 96% (Wyatt et al. 2016). The main drawback for its routine use is financial with each test costing approximately $760.

Human β-defensin-2 (HBD-2) and HBD-3 (which are also secreted by neutrophils) have been investigated for use in the diagnosis of PJI. Gollwitzer and colleagues showed that HBD-2 had a sensitivity of 86.7% and specificity of 40%, whilst HBD-3 had a sensitivity of 60% and specificity of 85% (Gollwitzer et al. 2013).

Human host defense peptide LL-37 is another antimicrobial peptide that induces cytokines and prevents the formation of biofilm (Overhage et al. 2008; Nijnik and Hancock 2009). Its use for the diagnosis of PJI is supported by a sensitivity of 80% and specificity of 85% (Gollwitzer et al. 2013).

Proinflammatory cytokines, which are released by macrophages in infected joints, have also received considerable attention. IL-1β, IL-6, IL-8, IL-17and TNF-α were identified by Deirmengian and colleagues as having particularly good diagnostic accuracy for PJI (Deirmengian et al. 2010). In addition to these cytokines, the investigators found that the levels of the protein SKALP (skin derived antileukoproteinase) were also significantly elevated in PJI cases.

Other investigators have since also identified IL-1β, IL-4, IL-6, IL-17, TNF-α, and interferon (INF-δ) as promising diagnostic synovial biomarkers (Gollwitzer et al. 2013). In addition to IL-6, IL-8 and CRP levels, Jacovides and colleagues found that vascular endothelial growth factor (VEGF) had excellent diagnostic value when comparing synovial fluid samples from septic and aseptic revision arthroplasty cases (Jacovides et al. 2011).

As with serum cytokines, a drawback of many of the pro-inflammatory cytokines in synovial fluid is that their levels may also be elevated in inflammatory condition. Some investigators have therefore advocated increasing the diagnostic accuracy by using the cytokines in combination with certain antimicrobial peptides e.g. using IL-4 or IL-6 with LL-37 or HBD-3 (Gollwitzer et al. 2013).

5 Conclusion and Future Needs

Serum and synovial biomarkers not only have the potential to improve the diagnosis of PJI, but can also guide on-going antimicrobial therapy and response to surgical treatment. Despite the identification of several promising biomarkers, the search for a highly accurate, cost-effective and

feasible test for diagnosing implant related infections continues. Until such a biomarker is found, existing biomarkers should be used alongside conventional techniques in order to further delineate their role in the management of this devastating complication. Microbiological culture techniques will therefore have to continue to paly an important role in the management of implant related infections as they can guide antimicrobial treatment. However, traditional culture techniques may one day be superseded by molecular diagnostics such as metabolomics and next-generation DNA sequencing (Choe et al. 2015; Goldberg et al. 2015).

References

Aalto K, Osterman K, Peltola H, Rasanen J (1984) Changes in erythrocyte sedimentation rate and C-reactive protein after total hip arthroplasty. Clin Orthop Relat Res 184:118–120

Aggarwal VK, Tischler E, Ghanem E, Parvizi J (2013) Leukocyte esterase from synovial fluid aspirate: a technical note. J Arthroplast 28(1):193–195. doi:10.1016/j.arth.2012.06.023

Ali S, Christie A, Chapel A (2009) The pattern of procalcitonin in primary total hip and knee arthroplasty and its implication in periprosthetic infection. J Clin Med Res 1(2):90–94. doi:10.4021/jocmr2009.04.1236

Alijanipour P, Bakhshi H, Parvizi J (2013) Diagnosis of periprosthetic joint infection: the threshold for serological markers. Clin Orthop Relat Res 471 (10):3186–3195. doi:10.1007/s11999-013-3070-z

Arkader R, Troster EJ, Lopes MR, Junior RR, Carcillo JA, Leone C, Okay TS (2006) Procalcitonin does discriminate between sepsis and systemic inflammatory response syndrome. Arch Dis Child 91(2):117–120. doi:10.1136/adc.2005.077446

Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, McLardy-Smith P, Berendt AR (1998) Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. J Clin Microbiol 36(10):2932–2939

Bedair H, Ting N, Jacovides C, Saxena A, Moric M, Parvizi J, Della Valle CJ (2011) The Mark Coventry Award: diagnosis of early postoperative TKA infection using synovial fluid analysis. Clin Orthop Relat Res 469(1):34–40. doi:10.1007/s11999-010-1433-2

Bejon P, Byren I, Atkins BL, Scarborough M, Woodhouse A, McLardy-Smith P, Gundle R, Berendt AR (2011) Serial measurement of the C-reactive protein is a poor predictor of treatment outcome in prosthetic joint infection. J Antimicrob Chemother 66(7):1590–1593. doi:10.1093/jac/dkr182

Berbari EF, Marculescu C, Sia I, Lahr BD, Hanssen AD, Steckelberg JM, Gullerud R, Osmon DR (2007) Culture-negative prosthetic joint infection. Clin Infect Dis 45(9):1113–1119. doi:10.1086/522184

Berbari E, Mabry T, Tsaras G, Spangehl M, Erwin PJ, Murad MH, Steckelberg J, Osmon D (2010) Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systematic review and meta-analysis. J Bone Joint Surg Am 92(11):2102–2109. doi:10.2106/JBJS.I.01199

Biomarkers Definitions Working G (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69 (3):89–95. doi:10.1067/mcp.2001.113989

Bottner F, Wegner A, Winkelmann W, Becker K, Erren M, Gotze C (2007) Interleukin-6, procalcitonin and TNF-alpha: markers of peri-prosthetic infection following total joint replacement. J Bone Joint Surg (Br) 89(1):94–99. doi:10.1302/0301-620X.89B1.17485

Choe H, Deirmengian CA, Hickok NJ, Morrison TN, Tuan RS (2015) Molecular diagnostics. J Am Acad Orthop Surg 23(Suppl):S26–S31. doi:10.5435/JAAOS-D-14-00409

Ciriello V, Gudipati S, Stavrou PZ, Kanakaris NK, Bellamy MC, Giannoudis PV (2013) Biomarkers predicting sepsis in polytrauma patients: current evidence. Injury 44(12):1680–1692. doi:10.1016/j.injury.2013.09.024

De Vecchi E, Villa F, Bortolin M, Toscano M, Tacchini L, Romano CL, Drago L (2016) Leucocyte esterase, glucose and C-reactive protein in the diagnosis of prosthetic joint infections: a prospective study. Clin Microbiol Infect 22(6):555–560. doi:10.1016/j.cmi.2016.03.020

Deirmengian C, Lonner JH, Booth RE Jr (2005) The Mark Coventry Award: white blood cell gene expression: a new approach toward the study and diagnosis of infection. Clin Orthop Relat Res 440:38–44

Deirmengian C, Hallab N, Tarabishy A, Della Valle C, Jacobs JJ, Lonner J, Booth RE Jr (2010) Synovial fluid biomarkers for periprosthetic infection. Clin Orthop Relat Res 468(8):2017–2023. doi:10.1007/s11999-010-1298-4

Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J (2014a) Combined measurement of synovial fluid alpha-Defensin and C-reactive protein levels: highly accurate for diagnosing periprosthetic joint infection. J Bone Joint Surg Am 96(17):1439–1445. doi:10.2106/JBJS.M.01316

Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J (2014b) Diagnosing periprosthetic joint infection: has the era of the biomarker arrived? Clin Orthop Relat Res 472 (11):3254–3262. doi:10.1007/s11999-014-3543-8

Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Booth RE Jr, Parvizi J (2015a) The

alpha-defensin test for periprosthetic joint infection outperforms the leukocyte esterase test strip. Clin Orthop Relat Res 473(1):198–203. doi:10.1007/s11999-014-3722-7

Deirmengian C, Kardos K, Kilmartin P, Gulati S, Citrano P, Booth RE Jr (2015b) The alpha-defensin test for periprosthetic joint infection responds to a wide spectrum of organisms. Clin Orthop Relat Res 473(7):2229–2235. doi:10.1007/s11999-015-4152-x

Deirmengian CA, Citrano PA, Gulati S, Kazarian ER, Stave JW, Kardos KW (2016) The C-reactive protein may not detect infections caused by less-virulent organisms. J Arthroplast. doi:10.1016/j.arth.2016.01.060

Di Cesare PE, Chang E, Preston CF, Liu CJ (2005) Serum interleukin-6 as a marker of periprosthetic infection following total hip and knee arthroplasty. J Bone Joint Surg Am 87(9):1921–1927. doi:10.2106/JBJS.D.01803

Dinneen A, Guyot A, Clements J, Bradley N (2013) Synovial fluid white cell and differential count in the diagnosis or exclusion of prosthetic joint infection. Bone Joint J 95-B(4):554–557. doi:10.1302/0301-620X.95B4.30388

Dos Anjos PB, da Luz FA, de Faria PR, Oliveira AP, de Araujo RA, Silva MJ (2014) Far beyond the usual biomarkers in breast cancer: a review. J Cancer 5(7):559–571. doi:10.7150/jca.8925

Drago L, Vassena C, Dozio E, Corsi MM, De Vecchi E, Mattina R, Romano C (2011) Procalcitonin, C-reactive protein, interleukin-6, and soluble intercellular adhesion molecule-1 as markers of postoperative orthopaedic joint prosthesis infections. Int J Immunopathol Pharmacol 24(2):433–440

Ghanem E, Antoci V Jr, Pulido L, Joshi A, Hozack W, Parvizi J (2009a) The use of receiver operating characteristics analysis in determining erythrocyte sedimentation rate and C-reactive protein levels in diagnosing periprosthetic infection prior to revision total hip arthroplasty. Int J Infect Dis 13(6):e444–e449. doi:10.1016/j.ijid.2009.02.017

Ghanem E, Azzam K, Seeley M, Joshi A, Parvizi J (2009b) Staged revision for knee arthroplasty infection: what is the role of serologic tests before reimplantation? Clin Orthop Relat Res 467(7):1699–1705. doi:10.1007/s11999-009-0742-9

Glehr M, Friesenbichler J, Hofmann G, Bernhardt GA, Zacherl M, Avian A, Windhager R, Leithner A (2013) Novel biomarkers to detect infection in revision hip and knee arthroplasties. Clin Orthop Relat Res 471(8):2621–2628. doi:10.1007/s11999-013-2998-3

Goldberg B, Sichtig H, Geyer C, Ledeboer N, Weinstock GM (2015) Making the leap from research laboratory to clinic: challenges and opportunities for next-generation sequencing in infectious disease diagnostics. MBio 6(6):e01888–e01815. doi:10.1128/mBio.01888-15

Gollwitzer H, Dombrowski Y, Prodinger PM, Peric M, Summer B, Hapfelmeier A, Saldamli B, Pankow F, von Eisenhart-Rothe R, Imhoff AB, Schauber J, Thomas P, Burgkart R, Banke IJ (2013) Antimicrobial peptides and proinflammatory cytokines in periprosthetic joint infection. J Bone Joint Surg Am 95(7):644–651. doi:10.2106/JBJS.L.00205

Heinrich PC, Castell JV, Andus T (1990) Interleukin-6 and the acute phase response. Biochem J 265(3):621–636

Henry NL, Hayes DF (2012) Cancer biomarkers. Mol Oncol 6(2):140–146. doi:10.1016/j.molonc.2012.01.010

Hugle T, Schuetz P, Mueller B, Laifer G, Tyndall A, Regenass S, Daikeler T (2008) Serum procalcitonin for discrimination between septic and non-septic arthritis. Clin Exp Rheumatol 26(3):453–456

Jacovides CL, Parvizi J, Adeli B, Jung KA (2011) Molecular markers for diagnosis of periprosthetic joint infection. J Arthroplast 26(6 Suppl):99–103 . doi:10.1016/j.arth.2011.03.025e101

Krenn V, Morawietz L, Perino G, Kienapfel H, Ascherl R, Hassenpflug GJ, Thomsen M, Thomas P, Huber M, Kendoff D, Baumhoer D, Krukemeyer MG, Natu S, Boettner F, Zustin J, Kolbel B, Ruther W, Kretzer JP, Tiemann A, Trampuz A, Frommelt L, Tichilow R, Soder S, Muller S, Parvizi J, Illgner U, Gehrke T (2014) Revised histopathological consensus classification of joint implant related pathology. Pathol Res Pract 210(12):779–786. doi:10.1016/j.prp.2014.09.017

Lee YH, Pratley RE (2005) The evolving role of inflammation in obesity and the metabolic syndrome. Curr Diab Rep 5(1):70–75

Lehrer RI, Ganz T (1992) Defensins: endogenous antibiotic peptides from human leukocytes. CIBA Found Symp 171:276–290. ; discussion 290–273

Liu JZ, Saleh A, Klika AK, Barsoum WK, Higuera CA (2014) Serum inflammatory markers for periprosthetic knee infection in obese versus non-obese patients. J Arthroplast 29(10):1880–1883. doi:10.1016/j.arth.2014.07.005

Malekzadeh D, Osmon DR, Lahr BD, Hanssen AD, Berbari EF (2010) Prior use of antimicrobial therapy is a risk factor for culture-negative prosthetic joint infection. Clin Orthop Relat Res 468(8):2039–2045. doi:10.1007/s11999-010-1338-0

Mason JB, Fehring TK, Odum SM, Griffin WL, Nussman DS (2003) The value of white blood cell counts before revision total knee arthroplasty. J Arthroplast 18(8):1038–1043

Mayeux R (2004) Biomarkers: potential uses and limitations. NeuroRx 1(2):182–188. doi:10.1602/neurorx.1.2.182

McArthur BA, Abdel MP, Taunton MJ, Osmon DR, Hanssen AD (2015) Seronegative infections in hip and knee arthroplasty: periprosthetic infections with normal erythrocyte sedimentation rate and C-reactive protein level. Bone Joint J 97-B(7):939–944. doi:10.1302/0301-620X.97B7.35500

Meermans G, Haddad FS (2010) Is there a role for tissue biopsy in the diagnosis of periprosthetic infection? Clin Orthop Relat Res 468(5):1410–1417. doi:10.1007/s11999-010-1245-4

Mikkelsen DB, Pedersen C, Hojbjerg T, Schonheyder HC (2006) Culture of multiple peroperative biopsies and diagnosis of infected knee arthroplasties. APMIS 114 (6):449–452. doi:10.1111/j.1600-0463.2006.apm_428.x

Miller VM, Redfield MM, McConnell JP (2007) Use of BNP and CRP as biomarkers in assessing cardiovascular disease: diagnosis versus risk. Curr Vasc Pharmacol 5(1):15–25

Nanda N, Juthani-Mehta M (2009) Novel biomarkers for the diagnosis of urinary tract infection-a systematic review. Biomark Insights 4:111–121

Nijnik A, Hancock RE (2009) The roles of cathelicidin LL-37 in immune defences and novel clinical applications. Curr Opin Hematol 16(1):41–47

Niskanen RO, Korkala O, Pammo H (1996) Serum C-reactive protein levels after total hip and knee arthroplasty. J Bone Joint Surg (Br) 78(3):431–433

Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE (2008) Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 76(9):4176–4182. doi:10.1128/IAI.00318-08

Pandey R, Berendt AR, Athanasou NA (2000) Histological and microbiological findings in non-infected and infected revision arthroplasty tissues. The OSIRIS Collaborative Study Group. Oxford Skeletal Infection Research and Intervention Service. Arch Orthop Trauma Surg 120(10):570–574

Parvizi J, Jacovides C, Antoci V, Ghanem E (2011a) Diagnosis of periprosthetic joint infection: the utility of a simple yet unappreciated enzyme. J Bone Joint Surg Am 93(24):2242–2248. doi:10.2106/JBJS.J.01413

Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, Garvin KL, Mont MA, Wongworawat MD, Zalavras CG (2011b) New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res 469(11):2992–2994. doi:10.1007/s11999-011-2102-9

Parvizi J, Jacovides C, Adeli B, Jung KA, Hozack WJ, Mark B (2012a) Coventry Award: synovial C-reactive protein: a prospective evaluation of a molecular marker for periprosthetic knee joint infection. Clin Orthop Relat Res 470(1):54–60. doi:10.1007/s11999-011-1991-y

Parvizi J, McKenzie JC, Cashman JP (2012b) Diagnosis of periprosthetic joint infection using synovial C-reactive protein. J Arthroplast 27(8 Suppl):12–16. doi:10.1016/j.arth.2012.03.018

Parvizi J, Erkocak OF, Della Valle CJ (2014) Culture-negative periprosthetic joint infection. J Bone Joint Surg Am 96(5):430–436. doi:10.2106/JBJS.L.01793

Pierrakos C, Vincent JL (2010) Sepsis biomarkers: a review. Crit Care 14(1):R15. doi:10.1186/cc8872

Qureshi A, Gurbuz Y, Niazi JH (2012) Biosensors for cardiac biomarkers detection: a review. Sensors Actuators B Chem 171:62–76

Sanzen L, Sundberg M (1997) Periprosthetic low-grade hip infections. Erythrocyte sedimentation rate and C-reactive protein in 23 cases. Acta Orthop Scand 68 (5):461–465

Schinsky MF, Della Valle CJ, Sporer SM, Paprosky WG (2008) Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. J Bone Joint Surg Am 90(9):1869–1875. doi:10.2106/JBJS.G.01255

Schuetz P, Albrich W, Mueller B (2011) Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. BMC Med 9:107. doi:10.1186/1741-7015-9-107

Selberg O, Hecker H, Martin M, Klos A, Bautsch W, Kohl J (2000) Discrimination of sepsis and systemic inflammatory response syndrome by determination of circulating plasma concentrations of procalcitonin, protein complement 3a, and interleukin-6. Crit Care Med 28(8):2793–2798

Shahi A, Parvizi J (2016) Serum D-dimer levels may be a reliable test for diagnosis of periprosthetic joint infection. Orthopaedics Today

Shahi A, Deirmengian C, Higuera C, Chen A, Restrepo C, Zmistowski B, Parvizi J (2015) Premature therapeutic antimicrobial treatments can compromise the diagnosis of late periprosthetic joint infection. Clin Orthop Relat Res 473(7):2244–2249. doi:10.1007/s11999-015-4142-z

Shahi A, Parvizi J, Kazarian GS, Higuera C, Frangiamore S, Bingham J, Beauchamp C, Valle CD, Deirmengian C (2016) The alpha-defensin test for periprosthetic joint infections is not affected by prior antibiotic administration. Clin Orthop Relat Res 474 (7):1610–1615. doi:10.1007/s11999-016-4726-2

Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J (2004) Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis 39 (2):206–217. doi:10.1086/421997

Talebi-Taher M, Shirani F, Nikanjam N, Shekarabi M (2013) Septic versus inflammatory arthritis: discriminating the ability of serum inflammatory markers. Rheumatol Int 33(2):319–324. doi:10.1007/s00296-012-2363-y

Toossi N, Adeli B, Rasouli MR, Huang R, Parvizi J (2012) Serum white blood cell count and differential do not have a role in the diagnosis of periprosthetic joint infection. J Arthroplast 27(8 Suppl):51–54 . doi:10.1016/j.arth.2012.03.021e51

Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R (2004) Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med 117(8):556–562. doi:10.1016/j.amjmed.2004.06.022

Tsaras G, Maduka-Ezeh A, Inwards CY, Mabry T, Erwin PJ, Murad MH, Montori VM, West CP, Osmon DR,

Berbari EF (2012) Utility of intraoperative frozen section histopathology in the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. J Bone Joint Surg Am 94 (18):1700–1711. doi:10.2106/JBJS.J.00756

Wetters NG, Berend KR, Lombardi AV, Morris MJ, Tucker TL, Della Valle CJ (2012) Leukocyte esterase reagent strips for the rapid diagnosis of periprosthetic joint infection. J Arthroplast 27(8 Suppl):8–11. doi:10.1016/j.arth.2012.03.037

Whicher J, Bienvenu J, Monneret G (2001) Procalcitonin as an acute phase marker. Ann Clin Biochem 38 (Pt 5):483–493

Wirtz DC, Heller KD, Miltner O, Zilkens KW, Wolff JM (2000) Interleukin-6: a potential inflammatory marker after total joint replacement. Int Orthop 24 (4):194–196

Worthington T, Dunlop D, Casey A, Lambert R, Luscombe J, Elliott T (2010) Serum procalcitonin, interleukin-6, soluble intercellular adhesin molecule-1 and IgG to short-chain exocellular lipoteichoic acid as predictors of infection in total joint prosthesis revision. Br J Biomed Sci 67(2):71–76

Wyatt MC, Beswick AD, Kunutsor SK, Wilson MJ, Whitehouse MR, Blom AW (2016) The alpha-defensin immunoassay and leukocyte esterase colorimetric strip test for the diagnosis of periprosthetic infection: a systematic review and meta-analysis. J Bone Joint Surg Am 98(12):992–1000. doi:10.2106/JBJS.15.01142

Zeller V, Ghorbani A, Strady C, Leonard P, Mamoudy P, Desplaces N (2007) Propionibacterium acnes: an agent of prosthetic joint infection and colonization. J Infect 55(2):119–124. doi:10.1016/j.jinf.2007.02.006

Zlonis M (1993) The mystique of the erythrocyte sedimentation rate. A reappraisal of one of the oldest laboratory tests still in use. Clin Lab Med 13 (4):787–800

Zmistowski B, Restrepo C, Huang R, Hozack WJ, Parvizi J (2012) Periprosthetic joint infection diagnosis: a complete understanding of white blood cell count and differential. J Arthroplast 27(9):1589–1593. doi:10.1016/j.arth.2012.03.059

Zmistowski B, Della Valle C, Bauer TW, Malizos KN, Alavi A, Bedair H, Booth RE, Choong P, Deirmengian C, Ehrlich GD, Gambir A, Huang R, Kissin Y, Kobayashi H, Kobayashi N, Krenn V, Drago L, Marston SB, Meermans G, Perez J, Ploegmakers JJ, Rosenberg A, Simpendorfer C, Thomas P, Tohtz S, Villafuerte JA, Wahl P, Wagenaar FC, Witzo E (2014) Diagnosis of periprosthetic joint infection. J Arthroplast 29(2 Suppl):77–83. doi:10.1016/j.arth.2013.09.040

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 81–92
DOI 10.1007/5584_2016_156

Published online: 4 January 2017

Antibacterial Bioactive Glass, S53P4, for Chronic Bone Infections – A Multinational Study

Nina Lindfors, Jan Geurts, Lorenzo Drago, J.J. Arts, Vesa Juutilainen, Pekka Hyvönen, Arnold J. Suda, Aloj Domenico, Stefano Artiaco, Chingiz Alizadeh, Adrian Brychcy, Jertzy Bialecki, and Carlo L. Romanò

Abstract

Osteomyelitis is an infectious process in bone that occasionally leads to bone destruction. Traditionally, the surgical treatment procedure is performed in combination with systemic and local antibiotics as a two-stage procedure that uses autograft or allograft bone for filling of the cavitary defect. Bioactive glass (BAG-S53P4) is a bone substitute with proven antibacterial and bone bonding properties.

One hundred and sixteen patients who had verified chronic osteomyelitis was treated using BAG-S53P4 as part of the treatment. Most of the patients

The original version of this chapter has been revised. An erratum to this chapter can be found at DOI 10.1007/5584_2017_13

N. Lindfors (✉) and V. Juutilainen
Department of Orthopaedic and Plastic Surgery, Helsinki University Central Hospital, Helsinki University, Helsinki, Finland
e-mail: nina.c.lindfors@hus.fi

J. Geurts
Department of Orthopaedic Surgery, Research School CAPHRI, Maastricht University Medical Centre, Maastricht, The Netherlands

L. Drago
Laboratory of Clinical Chemistry and Microbiology, I.R.C.C.S. Galeazzi Orthopaedic Institute, Milan, Italy

J.J. Arts
Department of Orthopaedic Surgery, Research School CAPHRI, Maastricht University Medical Centre, Maastricht, The Netherlands

Orthopaedic Biomechanics Group, Department of Biomedical Engineering, Eindhoven University of Technology (TU/e), Eindhoven, The Netherlands

P. Hyvönen
Department of Orthopaedic and Traumatology, Oulu University Hospital, Oulu, Finland

A.J. Suda
Department for Septic Surgery, BG Trauma Center Ludwigshafen, Ludwigshafen, Germany

A. Domenico and S. Artiaco
I Orthopaedic Clinic, University of Turin, Orthopaedic and Trauma Center - CTO Hospital, Turin, Italy

C. Alizadeh
Azerbaijan Scientific Research Institute of Traumatology and Orthopaedics, Baku, Azerbaijan

A. Brychcy and J. Bialecki
Department of Bone and Joint Infections, Orthopedic Clinic of Postgraduate Medical Education Centre, Otwock, Poland

C.L. Romanò
Center for Reconstructive Surgery of Osteoarticular Infections (CRIO), I.R.C.C.S. Galeazzi Orthopaedic Institute, Milan, Italy

had previously undergone numerous procedures, sometimes for decades. A register of patient data obtained from 11 centers from Finland, Italy, the Netherlands, Germany, Azerbaijan and Poland was set-up and continuously maintained at Helsinki University Central Hospital.

The location of the osteomyelitis was mainly in the tibia followed by the femur and then the calcaneus. The median age of the patients was 48 years (15–87). The patients were either treated according to a one-stage procedure without local antibiotics (85 %) or by a two-stage procedure using antibiotic beads in the first procedure (15 %). The minimum follow-up was 1 year (12–95 months, median 31).

The cure rate was 104/116, the total success rate 90 % and most of the patients showed a rapid recovery.

The study shows that (BAG-S53P4) can be used in a one-stage procedure in treatment of osteomyelitis with excellent results.

Keywords
Bioactive glass • S53P4 • Osteomyelitis • Antibacterial • Bone substitute

1 Introduction

Osteomyelitis is an inflammatory and infectious process in bone that occasionally leads to bone destruction (Lazzarini et al. 2004). Osteomyelitis in adults is predominantly related to open fractures or surgical procedures that affect the bone or its adjacent soft tissues. Host related factors such as malnutrition, alcoholism, smoking, or systemic diseases i.e. diabetes or peripheral vascular disorders may also contribute to an unfavorable outcome (Cierny et al. 2003). Many patients undergo numerous surgical procedures and in some cases still do not achieve successful treatment.

The successful treatment outcome of chronic osteomyelitis relies on the proper debridement of the necrotic and infected bone. Traditionally, the surgical procedure is performed in combination with systemic and local antibiotics as a two-stage procedure. After debridement the cavitary bone defect is filled with antibiotic loaded polymethylmetacrylate (PMMA) beads or a spacer. The beads or the spacer is removed later and the cavitary bone defect is filled with autograft or allograft bone.

Problems related to PMMA include possible thermal damage or biofilm formation on the bone cement, or a second procedure to remove the PMMA. These problems can be avoided by using synthetic antibiotic-loaded bone substitutes such as hydroxyapatite, calcium phosphate and -sulphate based products as a part of the treatment (Romano et al. 2014). Unfortunately, the frequent use of antibiotics has its dark side. Antimicrobial resistance has become a growing health threat around the world, and is today a serious global problem gaining more and more attention.

Bioactive glass BAG-S53P4 is an antibacterial synthetic bone substitute that has a long and well-documented clinical history as a bone graft substitute in ENT (ear, nose, and throat) (Sarin et al. 2012; Silvola 2011), craniomaxillofacial (Peltola et al. 2006; Stoor et al. 2015), orthopedic (Lindfors et al. 2009; Lindfors et al. 2010a), trauma (Heikkilä et al. 2011; Pernaa et al. 2011), spine (Frantzén et al. 2011; Rantakokko et al. 2012) surgery.

The intrinsic antibacterial property of the BAG-S53P4 is due to the ion dissolution process that starts immediately after the bone substitute has been implanted into the body. The ion release at the BAG surface induces an increase of pH and also an osmotic pressure around the BAG: phenomena that have been shown to kill both planktonic bacteria and bacteria in biofilm *in vitro* (Coraca-Huber et al. 2014; Drago et al. 2014, 2015; Leppäranta et al. 2008; Munukka et al. 2008). Although the antibacterial property has been demonstrated to be detrimental to

prokaryotic structures (Virolainen et al. 1997), the same effects are not seen for eukaryotic cells (Virolainen et al. 1997; Välimäki and Aro 2006). The surface of the bioactive glass is osteoconductive, and it is also osteostimulative in promoting, migration, replication, and differentiation of osteogenic cells and their matrix production (Virolainen et al. 1997).

In this multinational, multicenter cohort study, which is a result of true collaboration between colleagues involved in the treatment of septic surgery, the outcome of 116 patients with a clinically and radiologically verified chronic osteomyelitis, requiring surgical debridement and bone void filling using the antibacterial BAG-S53P4 as bone graft substitute, is reported.

2 Materials and methods

One-hundred-and-sixteen patients with verified chronic osteomyelitis was treated using the BAG-S53P4 bone substitute as part of the treatment. The inclusion criteria were all patients who had been treated in the 11 participating centers with BAG-S53P4 as part of the osteomyelitis treatment who also had a minimum follow-up of 1 year. The exclusion criteria were segmental bone defects or septic arthritis. A register on patient data comprised 11 centers from Finland, Italy, the Netherlands, Germany, Azerbaijan and Poland was set-up and continuously up-dated at Helsinki University Central Hospital (HUCH). Approval for the study and the data register was obtained from the local ethics committee of HUCH (366/13/03/02/2012) and HUCH (TYH2013226), in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), informed consent was obtained.

Data recorded included gender, age, medical history, location of the osteomyelitis, pathogen, one- or two stage procedure, flap, total follow-up and clinical outcome and complications. The patients were graded according to the anatomical pathology Cierny classification (Cierny et al. 2003) and the McPherson host type classification (McPherson et al. 2003).

Pre-operatively all patients (median age 48, 15–87, male n = 84/female n = 32) underwent clinical and radiological examination, including X-ray, MRI or CT, in addition to laboratory investigation in compliance to their pre-operative medical history and in accordance with the treatment protocol in every single participating hospital.

Ninety-eight patients (84.5 %) were treated with a one-stage procedure: after the debridement, the cavitary defect was immediately and completely filled with granules of BAG-S53P4, (BonAlive® granules, BonAlive Biomaterials Ltd., Finland). The BAG-S53P4 received EU approval for the indication of treatment of osteomyelitis in 2011. Eighteen patients (15.5 %) were treated with a two-stage procedure that used antibiotic beads (Septopal®) in the first stage. A two-stage procedure was chosen by some of the surgeons shortly after the introduction of BAG-S53P4 for the treatment of osteomyelitis. The second stage of surgery occurred 1–4 months at which time the antibiotic beads were removed and the cavitary defects were filled with BAG-S53P4 without local antibiotics. Muscle flaps were used in 15 patients (12.9 %) and in three patients skin transplantation was performed. In the rest of the patients the wound was primarily closed. All patients received systemic antibiotic therapy.

The postoperative outcome of the treatments were evaluated by the surgeon according to the four following clinical scores/categories: (1) excellent – no complications and no sign of infection within 15 days, (2) good – a small complication i.e. in muscle flap or wound healing achieved within 15 days, with some drainage, (3) fair – a wound showing prolonged sterile drainage or serum leakage, with time to healing and less than 6 weeks, (4) poor – a temporary stable situation with sign of infection or a complication involving a re-infection.

Patients were followed-up at the outpatient department in compliance with the protocol of the respective hospital, at 2 weeks, 1, 2, 3, 6, 9, 12, 18, 24 and 36 months postoperatively. Patient data were gathered from hospital patient

records until December 2014. All patients had a follow-up of at least 1 year, which according to the literature is the minimum follow-up period for clinical trials of osteomyelitis treatment (Lazzarini et al. 2005).

3 Statistics

Descriptive statistics were used to summarize the data. Data were not normally distributed. Between group differences were evaluated by using Wilcoxon rank sum tests and the Mann Whitney U test. When three or more groups were compared Kruskall-Wallis tests were used followed by Wilcoxon rank sum tests for pairwise comparisons. Categorical data were analyzed using Pearson chi-square tests or Fisher's exact tests. Statistical analyses were performed using R version 3.2.0 (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/). P-values of less than 0.05 were considered statistically significant.

4 Results

4.1 Patients

The total follow-up for 101 successfully treated patients was a median of 31 months (12–95 months). The distribution of number of patients and follow-up time is shown in Fig. 1. Twelve patients with a re-infection were excluded (Table 1), in addition to one patient who had an arthrodesis and acquired a new separate postoperative infection in the same foot. Two patients died, one of pneumonia and the other of carcinoma of the esophagus.

The cure rate was 104/116, which resulted in a total success rate of 90 %. Outcome classifications at 1–2 months postoperatively were as follows: 66.4 % of the patients were excellent, 18.1 % were good, 6.0 % were fair and 9.5 % were poor. Two excellent treatment outcomes are shown in Figs. 2a–e and 3a–d.

Osteomyelitis was most often a consequence of trauma (83 %). In 12 % of the patients the osteomyelitis was classified as haematogenous and in 5 % the infection occurred after elective orthopedic surgery. All patients had a long treatment history sometimes for decades. The location of the osteomyelitis is shown in Table 2.

According to the Cierny classification 11 % of patients were of type 1, 15 % of type 2, 66 % of type 3 and 8 % of type 4. The McPherson classification was: A (62 %), B (35 %) and C (3 %).

The statistical analyses showed no correlation between age and postoperative outcome at 1–2 months, $p = 0.376$, location of the osteomyelitis and postoperative outcome $p = 0.159$, Cierny classification and postoperative outcome $p = 0.375$, host type and postoperative outcome $p = 0.133$, or bacterial species and postoperative outcome $p = 0.136$. However, in cases of multiple bacteria the 1–2 months postoperative outcome

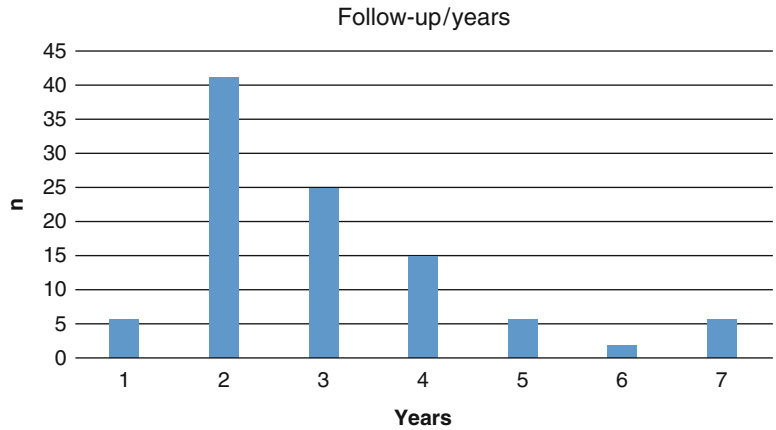


Fig. 1 Distribution of successfully treated patients and follow-up times

Table 1 Patient numbers. 1–9: A severe complication with an unfavorable outcome due to a persistence of infection less than 6 months postoperatively. Patient numbers 10–12: A re-infection sustained after a 6-month follow-up

No.	Age	Gender	Location	Pathogen	McPherson classification	Cierny classification	Soft tissue healing 1–2 months
1	42	M	Tibia	S. aureus	C Radiotherapy	3	Fair, sign of re-infection
2	33	F	Tibia	S. aureus, P. aeruginosa	B Smoker alcohol abuse	3	Poor
3	84	M	Tibia	S aureus, P. aeruginosa	A	2	Poor
4	62	F	Tibia	S. aureus, Acinetobacter spp., Candida spp.	B Reuma	3	Poor, wound wet
5	67	M	Tibia	S.epidermis, Enterococcus faecalis	A	3	Poor, fistular formation
6	52	M	Tibia	S.aureus	B Smoker	3	Fair
7	55	F	Tibia	S. aureus, E coli, Enterobacter spp., Streptococcus spp., Acinetobacter spp.	A	2	Poor
8	48	M	Femur	S. aureus, Streptococcus spp.	A	2	Poor
9	39	M	Femur	S. aureus	A	3	Poor
10	33	M	Cuneiforme lateralis	S aureus, S. epidermidis	A	3	Excellent
11	40	F	Femur	Negative	B Tyroideal tumour radiotherapy	3	Excellent
12	81	F	Tibia	S. aureus	B Smoker	3	Excellent

was less often categorized as "excellent" (46 %) compared to cases with no (73 %) or single bacteria (73 %), $p = 0.018$. In cases of multiple bacterial species the complications were also more often "severe" (21 %) compared to no (0 %) or a single bacterial species infection (4 %), $p < 0.001$.

A significant relationship was observed between the numbers of operation stages (single-stage vs dual-stage) at the 1–2 months postoperative outcome. A postoperative poor outcome was more often observed after the two-stage procedure (33 %) than after the one-stage procedure (5 %), $p = 0.008$.

A statistical association was also observed between soft tissue requiring a flap and a poor postoperative outcome. In cases in which a flap had been performed the outcome at 1–2 months was more often graded as poor (53 %) than cases in which no flaps had been used (3 %), $p < 0.001$.

A severe complication classified as persistence of an infection or a re-infection at less than 6 months postoperation were reported for nine patients (8 %). A re-infection was diagnosed for three patients (3 %) after a 6-month follow-up (Table 1). A statistical association between the complications and the outcome was obtained. The postoperative outcome at 1–2 months was more often categorized/classified as "excellent" (77 %) in successfully treated patients, compared to patients who had sustained a small (20 %) or a severe postoperative complication (25 %), $p < 0.001$. Patients with a good outcome, although having a small postoperative problem was seen in eight patients (Table 3).

The statistical analyses showed no correlation between persistence of infection or a re-infection and age ($p = 0.324$), gender ($p = 0.706$), location ($p = 0.199$), Cierny ($p = 0.686$) or McPherson ($p = 0.213$) classification, or bacteria ($p = 0.104$). However, a statistically significant association between multiple bacteria and persistence of infection

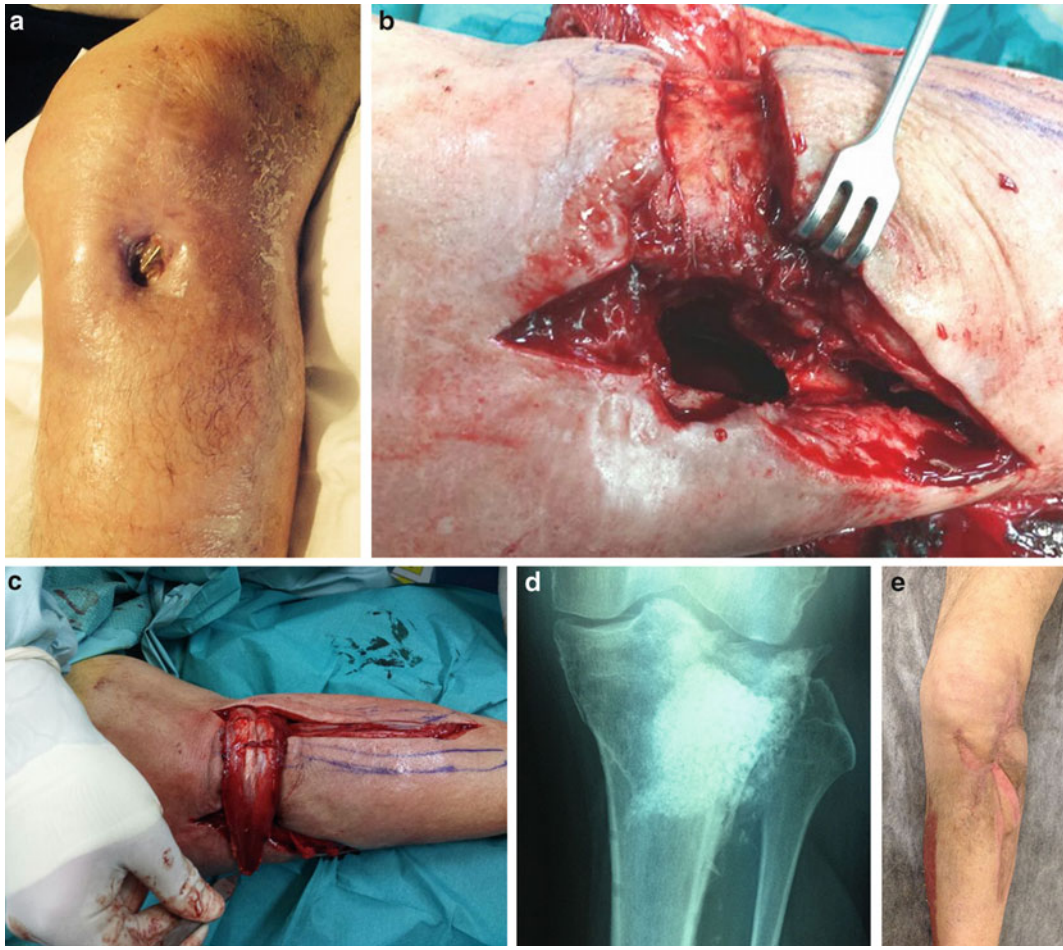


Fig. 2 Chronic osteomyelitis of the proximal third of the tibia with exposed osteosynthesis in a 63 year old male. (**a**) Pre-operative clinical aspect with soft tissue defect and the exposed plate. (**b**) Intra-operative picture, after hardware removal and bone and soft tissue debridement. Note the large bone and soft tissue defect, prepared to receive the lateral gastrocnemius muscular flap. (**c**) Rotation of the muscular flap. (**d**) Post-operative X-ray 1 year after surgery. (**e**) Clinical aspect at 1 year after surgery

was observed, $p = 0.014$. In cases of multiple bacterial species infections, persistence of infection or a re-infection was observed in 21 %, compared to no bacteria (0 %) or single bacterial species 4.3 %. A statistically significant association was also noted between a one- and two stage procedure and a re-infection, $p = 0.004$. The persistence of infection or a re-infection was observed in 28 % for the two-stage procedure compared to 4.1 % the one-stage procedure.

Other complications were: two non-unions, one pulmonary embolism, one patient with complex regional pain syndrome, and one spontaneous fracture.

4.2 Microbiology

Ninety-seven samples tested positive for microbial growth, whereas the remaining 19 did not yield positive cultures. Infections were caused by

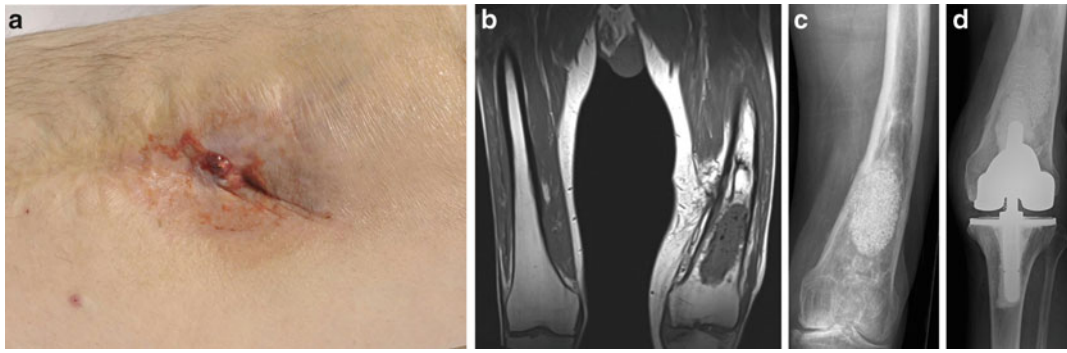


Fig. 3 Chronic osteomyelitis in femur in an 80 year old man, who had been suffering 21 years from a persistent fistula. The primary osteomyelitis was diagnosed when the patient was 8 years old. (**a**) Preoperative situation showing fistular formation. (**b**) Pre-operative Mri. Note the osteomyelitis in left femur. (**c**) Post-operative X-ray at 2 months follow-up. No clinical or radiological sign of infection. (**d**) Post-operative X-ray after arthroplasty performed 3 years later. Note the appearance of the S53P4 bone substitute in contact with the bone cement

Table 2 Location of infection. In four patients the osteomyelitis was observed in two bones, total n of infection =120

Location of infection	(n)	(%)
Tibia	62	51.6
Femur	28	23.3
Calcaneus	13	10.8
Fibula	7	6.0
Ulna	1	0.9
Metatarsal	3	2.6
Olecranon	2	1.7
Humerus	1	0.0
Cuneiform bone	1	0.9
Metacarpus	1	0.9
Phalang in finger	1	0.9

a single pathogen in 60 % of the patients, whereas a polymicrobial etiology was identified in 24 % of the patients.

Staphylococcus aureus was the prevalent isolated species (57 %) either as a single pathogen (68 %) or in combination with other microorganisms (67 %) upon analysis of microbial cultures Table 4.

Together with *S. aureus* also *Pseudomonas spp.* (5 %) and *Staphylococcus epidermidis* (15 %) were isolated with high frequency. The second combination of bacteria was associated with a worse treatment outcome. In particular *Pseudomonas spp.* was responsible for 7 % of the monomicrobial infections and for 29 % of the polymicrobial infections, whereas *S. epidermidis* was isolated in 9 % and 25 % in monomicrobial and polymicrobial infections, respectively. MRSA, was isolated in 14 patient and MRSE in three patients.

5 Discussion

The data obtained from the multicenter register show a 90 % success rate for the treatment methods described thus confirming that BAG-S53P4 can be used as bone substitute without local antibiotics in the treatment of chronic osteomyelitis with excellent results. This is the largest reported series on the use of antibacterial bone substitute BAG-S53P4 in the treatment of chronic osteomyelitis to our knowledge.

There are advantages in reporting the treatment outcome data of a multinational cohort study. Observations and experiences from several independent centers that participate in the collection of the data would be expected to provide more reliable and robust evaluations of the treatment outcome compared to reports from single centers only.

One of the main benefits of BAG-S53P4, is that it is an antibacterial bone substitute in itself, which has been demonstrated in several different studies

Table 3 Patients with a good outcome but who had a small postoperative problem

No.	Age	Gender	Loca-tion	Pathogen	Host type	Cierny classification	Soft tissue healing 1–2 months	Problem
1	34	M	Calcaneus	S. epidermis, E. faecalis	B Psoriasis	3	Poor, fistular formation	Psoriatic skin. A few BAG S53P4 granules secreted from the wound. No deep infection bact neg,. Superficial S aureus. Treatment with iv and po antibiotics. Good outcome.
2	54	F	Tibia	P. aeruginosa, S. aureus	B Periferal vasculopathy	2	Fair	Seroma 4 weeks
3	55	M	Tibia	S.aureus	A	3	Poor	Seroma, partial flap necrosis, excellent outcome 1 year
4	58	F	Tibia	S. aureus	A	3	Poor	Post-op infection, fistula, 1 year follow-up excellent
5	43	M	Femur	E coli, Prevoltella melaninogenica	A	3	Good	Dry scab in seroma leakage
6	53	M	Tibia	S. epidermidis, S. aureus	A	2	Fair	Flap infection
7	57	M	Tibia	Proteus mirabilis, E. faecalis	A	3	Fair	Athrophic wound edges
8	80	M	Tibia femur	P. aeruginosa, S aureus, Enterococcus spp.	B (psoriasis, corticosteroids)	3	Good	Spontaneous fracture at the site of previous knee fusion, requiring external fixation, required 2 surgeries to clear infection in tibia and femur.

Table 4 Pathogens and isolation rate/116 patients. Monomicrobial infections n total = 69, polymicrobial n total = 28

	Isolation rate	Monomicrobial infection	Polymicrobial infection
Microorganism	n (%)	n (%)	n (%)
Staphylococcus aureus	66	47	19
	(56.9)	(68.1)	(67.9)
Staphylococcus epidermidis	13	6	7
	(11.2)	(8.7)	(25.0)
Others Coagulase negative Staphylococci	5	2	3
	(4.3)	(2.9)	(10.7)
Streptococcus spp.	9	2	7
	(7.8)	(2.9)	(25.0)
Enterococcus spp.	7	3	4
	(6.0)	(4.3)	(14.3)
Pseudomonas spp.	13	5	8
	(11.2)	(7.2)	(28.6)
Serratia spp.	1	1	/
	(0.9)	(1.4)	
Corynebacterium spp.	1	/	1
	(0.9)	/	(3.6)
Acinetobacter spp.	3		3
	(2.6)	/	(10.7)
Escherichia coli	3		3
	(2.6)	/	(10.7)
Proteus mirabilis	1		1
	(0.9)	/	(3.6)
Enterobacter spp.	4		4
	(3.4)	/	(14.3)
Clostridium difficile	1	1	/
	(0.9)	(1.4)	/
Citrobacter freundii	1	1	/
	(0.9)	(1.4)	/

in vitro (Coraca-Huber et al. 2014; Drago et al. 2014, 2015; Leppäranta et al. 2008; Munukka et al. 2008). The literature indicates that BAG-S53P4 is the most effective of the bioactive glasses in inhibiting bacterial growth that have been under investigation *in vitro* so far. The antibacterial properties of the glass is ascribed to an elevation of pH and also of osmotic pressure that are caused by the chemical reactions at the glass surface, which take place as soon as the glass is implanted into the body. Resistance problems are not likely to occur (Drago et al. 2014, 2015) as the bacteria are killed by physically and inorganically induced chemical reactions, which is beneficial against the background of increasing bacterial resistance to antibiotics observed worldwide. BAG-S53P4 has been shown to have a high potential against multiresistant strains without selection for resistance (Drago et al. 2015). The isolated pathogen was either MRSA or MRSE in 15 % of the patients. Of these only one patient had a severe complication.

The antibacterial properties of BAG-S53P4 *in vitro* have been demonstrated but the antibacterial properties *in vivo* is still unclear. In a comparative study on BAG-S53P4, and two antibiotic-loaded calcium-based bone substitutes in the treatment of chronic osteomyelitis, patients treated with BAG-S53P4 without local antibiotics had an equally favorable outcome compared to patients treated with the antibiotic-loaded calcium-based bone substitutes (Romano et al. 2014).

Currently the gold-standard for the treatment of osteomyelitis is a two-stage procedure that involves the use of antibiotic containing PMMA beads in the first procedural stage. This method is open to criticism as the length of time the antibiotic release continues is not always known. A prolonged antibiotic release can foster resistance and after the antibiotic effect is dissipated, it is possible that the beads that represent a foreign material may provide a receptive surface for bacteria, which subsequently produce a biofilm. BAG-S53P4 has been shown not only to kill planktonic bacteria, but also to kill bacteria in biofilms (Coraca-Huber et al. 2014; Drago et al. 2014).

In this cohort study the BAG-S53P4 served two functions, namely: the prevention of bacterial growth in the bone defect and its functioning as an osteoconductive biomaterial for dead space management to treat the cavitary defect after debridement. The majority of the patients were treated according to the one-stage procedure and not according to the gold standard of the two-stage procedure. Interestingly, a significantly poorer outcome was more often observed after the two-stage procedure than after the one-stage procedure. One possible explanation for these observed results may depend on the fact that BAG-S53P4 was first used in the second operation of the two-stage procedure on severely infected patients, before the surgeon was completely familiar with the treatment method.

The most predictive factor of a treatment failure was a poor clinical outcome at 1–2 months of follow-up. The use of flaps was also statistically associated with a poorer outcome compared to those cases in which flaps were not used. This can be explained by the fact that a flap is more often used in severe cases including cases in which soft tissue damage has occurred. Vascularity is crucial in the treatment of osteomyelitis. At least one of the observed complications can be explained by problems during wound closure and the lack of a flap.

Interestingly no statistical correlation between the Cierny anatomical classification or the McPherson host type classification and the clinical outcome was observed. The Cierny classification is mainly used as an anatomical staging classification of the infection often combined to treatment protocols and not as a classification for the prognostic clinical outcome. The McPherson host type classification, in contrast, has been reported to show a strong correlation between systemic host grade and complication after infection treatment. Those findings were however reported on periprosthetic total hip infections, which differs from the patient group reported in this present study.

A weakness in this reported data is the lack of thorough preoperative treatment history. Many patients had been treated in other hospitals before they were sent to the final clinics and had undergone numerous procedures and antibiotic treatments, sometimes for as long as decades. Those data were collected to the fullest extent possible.

Bone substitutes i.e. calcium sulfate based bone substitutes are associated with postoperative seroma leakage. Seroma leakage was recorded for only three patients (2.6 %) in this study, which is an incidence comparable to previously reported results (Drago et al. 2013; Romano et al. 2014; Lindfors et al. 2010b). A recent study on 27 patients found that BAG-S53P4 was as effective as two different calcium-based antibiotic- loaded bone substitutes in the treatment of chronic osteomyelitis (Romano et al. 2014). In addition, BAG-S53P4 treated patients showed significantly lower prolonged wound serum leakage (3.7 %) compared to the two antibiotic-loaded calcium-based bone substitutes (29.6 % and 27.2 %): they also showed a trend towards reduction in hospital stay (Romano et al. 2014).

Another major benefit of using bioactive glass is the potential to use it in a one-stage setting, thereby avoiding additional surgery, thus reducing the burden for the patient and the risk for additional complications. There is also a potential financial advantage for health services to be able to prevent a second surgical procedure. This is, however, something that will have to be investigated in future health-economic studies.

6 In conclusion

The antibacterial, osteostimulative and osteoconductive bone substitute BAG-S53P4, is suitable as bone void filler in the treatment of chronic osteomyelitis. The treatment of osteomyelitis can be performed in a one-stage procedure with excellent results. This makes the treatment protocol cost-effective with a trend towards a reduction in the length of the hospital stay as well.

Acknowledgements We thank P Venesmaa, Kuopio University Hospital, Kuopio, Finland, M Nyyssönen, North Karelia Central Hospital, Joensuu, Finland, M Kirjavainen Helsinki University Central Hospital, Helsinki, Finland, and E Gullichsen, Turku University Hospital, Turku, Finland for their contributions of cases.

References

Cierny G 3rd, Mader JT, Pennick JJ (2003) A clinical staging system for adult osteomyelitis. Clin Orthop Relat Res 414:7–24

Coraca-Huber D, Fille M, Hausdorfer J, Putzer D, Nogler M (2014) Efficacy of antibacterial bioactive glass S53P4 against S. aureus biofilms grown on titanium discs in vitro. J Orthop Res 32:175–177

Drago L, Romano D, De Vecchi E, Vassena C, Logoluso N, Mattina R, Romano CL (2013) Bioactive glass BAG-S53P4 for the adjunctive treatment of chronic osteomyelitis of the long bone: an in vitro and prospective clinical study. BMC Infect Dis 10:584. doi:10.1186/1471-2334-13-584

Drago L, Vassena C, Fenu S, De Vecchi E, Signori V, De Francesco R, Romano CL (2014) In vitro antibiofilm activity of bioactive glass S53P4. Future Microbiol 9:593–601

Drago L, De Vecchi E, Bortolin M, Toscano M, mattina R, Romano CL (2015) Antimicrobila activity and resistance selection of different bioglass S53P4 formulations against multidrug resistance strains. Future Microbiol 10:1293–1299

Frantzén J, Rantakokko J, Aro H, Heinänen J, Kajander S, Koski I, Gullichsen E, Kotilainen E, Lindfors N (2011) Instrumented spondylodesis in degenerative spondylolisthesis with bioactive glass and autologous bone. A prospective 11-year follow-up. J Spinal Disorder Tech 24(7):455–461

Heikkilä JT, Kukkonen J, Aho AJ, Moisander S, Kyyrönen T, Mattila K (2011) Bioactive glass granules: a suitable bone substitute material in the operative treatment of depressed lateral tibial plateau fractures: a prospective, randomized 1 year follow-up study. J Mater Sci Mater Med 22(4):1073–1080

Lazzarini L, Mader JT, Cahoun JH (2004) Osteomyelitis in long bones. J Bone Joint Surg 86-A:2305–2318

Lazzarini L, Lipsky BA, Mader JT (2005) Antibiotic treatment of osteomyelitis: what have we learned from 30 years of clinical trials? Int J Inf Dis 9:127–138

Lindfors NC, Heikkilä J, Koski I, Mattila K, Aho AJ (2009) Bioactive glass and autogenous bone as bone graft substitutes in benign bone tumors. J Biomed Mater Res 90B(1):131–136

Lindfors NC, Koski I, Heikkilä JT, Mattila K, Aho AJ (2010a) A prospective randomized 14-year follow-up study of bioactive glass and autogenous bone as bone graft substitutes in benign bone tumors. J Biomed Mater Res 94B(1):157–164

Lindfors NC, Hyvönen P, Nyyssönen M, Kirjavainen M, Kankare J, Gullichsen E, Salo J (2010b) Bioactive glass S53P4 as bone graft substitute in treatment of osteomyelitis. Bone 47:212–218

Leppäranta O, Vaahtio M, Peltola R, Ziang D, Hupa L, Hupa M, Ylänen H, Salonen JI, Viljanen MK, Eerola E (2008) Antibacterial effect of bioactive glasses on clinically important anaerobic bacteria in vitro. J Mater Sci Mater Med 19:547–551

McPherson EJ, Woodson C, Holtom O, Roidis N, Shufelt C, Patzakis M (2003) Periprosthetic total hip infection. Outcome using a staging system. Vlin Orthop Relat Res 403:8–15

Munukka E, Leppäranta O, Korkeamäki M, Vaahto M, Peltola T, Zhang D, Hupa L, Ylänen H, salonen JI, Virtanen MK, Eerola E (2008) Bacterial effects of bioactive glasses on clinically important aerobic bacteria. J Mater Sci Mater Med 19:27–32

Peltola M, Aitasalo K, Suonpaa J, Varpula M, Yli-Urpo A (2006) Bioactive glass S53P4 in frontal sinus obliteration: A long-term clinical experience. Head Neck 28 (9):834–841

Pernaa K, Koski I, Mattila K, Gullichsen E, Heikkilä J, Aho AJ, Lindfors N (2011) Bioactive glass S53P4 and autograft bone in treatment of depressed tibial plateau fractures. A prospective randomized 11-year follow-up. J Long Term Eff Med Impl 21(2): 139–148

Rantakokko J, Frantzén J, Heinänen J, Kajander S, Kotilainen E, Gullichsen E, Lindfors N (2012) Posterolateral spondylodesis using bioactive glass S53P4 and autogenous bone in instrumented unstable lumbar spine burst fractures – a prospective 10-year follow-up study. Scand J Surg 101:66–71

Romano CL, Logoluso N, Meani E, Romano D, De Vecchi E, Vassena C, Drago L (2014) A comparative study of the use of bioactive glass S53P4 and antibiotic-loaded calcium-based bone substitutes in the treatment of chronic osteomyelitis. Bone Joint J 96-B:845–850

Sarin J, Grenman R, Aitasalo K, Pulkkinen J (2012) Bioactive glass S53P4 in mastoid obliteration surgery for chronic otitis media and cerebrospinal fluid leakage. Ann Otol Rhinol Laryngol 121:563–569

Silvola JT (2011) Mastoidectomy cavity obliteration with bioactive glass: a Pilot Study. Otolaryngol Head Neck Surg 145(2):P96–P97

Stoor P, Mesimäki K, Lindqvist C, Kontio R (2015) The use of anatomically drop-shaped bioactive glass S53P4 implants in the reconstruction of orbital floor fractures-A prospective long-term follow-up study. J Craniomaxillofac Surg 43:969–975

Virolainen P, Heikkilä J, Yli-Urpo A, Vuorio E, Aro HT (1997) Histomorphometric and molecular biologic comparison of bioactive glass granules and autogenous bone grafts in augmentation of bone defect healing. J Biomed Mater Res 35(1):9–17

Välimäki VV, Aro HT (2006) Molecular basis for action of bioactive glasses as bone graft substitute. Scand J Surg 95(2):95–102

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 93–100
DOI 10.1007/5584_2016_155

Published online: 21 March 2017

Prosthetic Joint Infections and Cost Analysis?

F.S. Haddad, A. Ngu, and J.J. Negus

Abstract

Prosthetic joint infection is a devastating complication of arthroplasty surgery that can lead to debilitating morbidity for the patient and significant expense for the healthcare system. With the continual rise of arthroplasty cases worldwide every year, the revision load for infection is becoming a greater financial burden on healthcare budgets. Prevention of infection has to be the key to reducing this burden. For treatment, it is critical for us to collect quality data that can guide future management strategies to minimise healthcare costs and morbidity / mortality for patients. There has been a management shift in many countries to a less expensive 1-stage strategy and in selective cases to the use of debridement, antibiotics and implant retention. These appear very attractive options on many levels, not least cost. However, with a consensus on the definition of joint infection only clarified in 2011, there is still the need for high quality cost analysis data to be collected on how the use of these different methods could impact the healthcare expenditure of countries around the world. With a projected spend on revision for infection at US $1.62 billion in the US alone, this data is vital and urgently needed.

Keywords

Periprosthetic infection • Septic arthroplasty • Joint infection • Cost analysis • DAIR

Abbreviations

DAIR	Debridement, Antibiotics and Implant Retention
THR	Total Hip Replacement
TKR	Total Knee Replacement

F.S. Haddad, A. Ngu, and J.J. Negus (✉)
University College London Hospitals, Euston road, London NW1 2BU, UK
e-mail: fsh@fareshaddad.net; Albert.Ngu@uclh.nhs.uk; Jonathan.negus@cantab.net

1 Introduction

Prosthetic joint infection remains a challenging problem in arthroplasty surgery. Its incidence remains steady at 1–2% but due to increasing numbers of arthroplasty surgeries each year, the number of revisions for infection is increasing around the world. It can be difficult to diagnose and often requires complex and costly surgery associated with significant patient morbidity. It is expensive to treat and with the increasing numbers of cases, its cost as a proportion of healthcare budgets is increasing. It is a devastating problem and the management options that we utilise and their costs, need focused attention using quality data as we deal with it over the next few decades.

2 Definition

Prior to 2011, the lack of a standard definition for prosthetic joint infections frustrated orthopaedic communities as well as surveillance authorities around the world. The interpretation of available literature became increasingly difficult due to investigators and centres adopting their own definition of prosthetic joint infections.

In 2011, the Musculoskeletal Infection Society (MSIS) convened a working group, which produced a standardised definition of prosthetic joint infection. (Parvizi et al. 2011) This was accepted by the International Consensus Group in August 2013 and modified slightly with the addition of leukocyte esterase test strip as a minor criterion, which has been endorsed by Centre for Disease Control.

Prosthetic joint infection is defined as having two positive periprosthetic cultures with phenotypically identical organisms; or a sinus tract communicating with the joint; or three of the following five criteria:

- Elevated serum C-reactive protein (CRP) and elevated erythrocyte sedimentation rate (ESR)
- Elevated synovial fluid white blood cell (WBC) count OR ++change on leukocyte esterase test strip
- Elevated synovial fluid polymorphonuclear neutrophil percentage (PMN%)
- Positive histological analysis of periprosthetic tissue
- A single positive culture

As this definition is still relatively recent, the data available to guide surgical decision-making and healthcare policy has been limited and much is of poor quality. It was difficult to perform an accurate cost analysis before 2011 and even more difficult to compare any results to other studies due to the heterogeneous mix of cases that would have been defined as infected. Using the consensus definition, the data that can be collected over the next decade will be critical to furthering our understanding of periprosthetic infection, including the most cost effective methods of management.

The mean incidence of prosthetic joint infections has decreased significantly from as high as 9% in the early days of arthroplasty surgery to 1.25% in the last few decades due to the advent of prophylactic antibiotics, laminar air flow operating rooms and various techniques. However, prosthetic joint infection still remains one of the leading causes of failure in arthroplasty surgery accounting for between 10 and 20% of all revisions. In addition, even though the incidence has remained stable there are ever increasing numbers of arthroplasties being performed which will lead to greater numbers of revisions for infection. (Australian Orthopaedic Association 2015) There were nearly 1,600 arthroplasty revisions in the UK in 2014 for infection (National Joint Registry 2015) & approximately 22,000 revisions for infection in the USA in 2009 (Kurtz et al. 2012).

3 Surgical Options

The surgical options for managing infected arthroplasty include 2-stage revision, 1-stage

revision, Debridement, Antibiotics & Implant Retention (DAIR) and in the rare worst-case scenarios, excision arthroplasty.

It is essential to first characterise the infection including whether it is acute or chronic based on duration of symptoms and time after surgery (Tsukayama et al. 1996):

1. Early post-operative: symptoms less than 4 weeks
2. Late chronic: a gradual onset of symptoms, >4 weeks
3. Acute haematogenous: acute onset in previously well functioning replacement.

While the preferred technique varies by country and even between centres within countries, the traditional option worldwide has long been the 2-stage approach. It can be used in cases of resistant organisms and regardless of whether the infection is acute or chronic. It also has the advantage of the highest success rates amongst the techniques available averaging 95%. (Garvin et al. 1993; Elson 1993; Klouche et al. 2010). However, it is the most expensive and leads to longer hospital stays, larger volumes of antibiotics, lengthy functional impairment and further procedures (Charnley 1964; Gulhane et al. 2012; Vanhegan et al. 2012b).

The 1-stage approach requires less hospitalisation and is cheaper than a 2-stage. The reported success rates have historically been lower than 2-stage revisions (Garvin et al. 1993; Elson 1993) but more recent studies in well selected patients or from specialist centres have reported success rates ranging from 70 to 95%. (Choi et al. 2013; Hansen et al. 2013; Zeller et al. 2014) In studies where the patients are carefully selected according to strict criteria, success rates of 100% have been reported using a 1-stage strategy for infected hip arthroplasty. (Klouche et al. 2012) It isn't clear if this strategy leads to a reduction in the overall amounts of antibiotics used. There are other factors to take into consideration such as the effects of implant choice. In the Endoklinic where approximately 85% of revisions for infection are performed as 1-stage procedures, the prostheses that are used can be substantially larger than in a standard 2-stage procedure. This can have implications upon bone stock (Gehrke et al. 2013).

Recent trends suggest single stage revision or even Debridement Antibiotics & Implant Retention (DAIR) are being employed more commonly for specific cohorts of patients (Byren et al. 2009; Gulhane et al. 2012; Kuiper et al. 2013b; Haddad et al. 2014).

Haddad and Bridgens further categorized the options available with a management protocol focused on implant retention (Table 1).

The patients appropriate for DAIR must be shown to have an early post-operative or acute haematogenous infection in a previously well functioning implant, diagnosed and managed within 4 weeks. This timeframe is to minimise the chance of the biofilm having formed which can happen between 36 h and 3 weeks. (Parvizi et al. 2010) They also need to have demonstrated prior to surgery, infection with an identifiable and non-resistant organism and have no significant co-morbidities. If employing the DAIR technique, the implant needs to be stable with no significant bone loss.

DAIR is the cheapest of the three strategies and has the lowest associated surgical morbidity. The risk of encouraging resistant organisms in those that fail the initial surgery must be considered. The success rates vary across the literature. This could be due to the different methods employed such as single or multiple debridements which can be dictated by whether the surgeon uses an antibiotic loaded cement or a resorbable antibiotic laden carrier. Establishing the success rates of DAIR is not easy. In the early days, the indications for DAIR were still being refined leading to retrospective analysis of groups between 1 and 41 patients with heterogenous inclusion criteria including chronic infection giving success rates from 0 to 89%.(Choi et al. 2011; Zimmerli et al. 1998) Prior to 1998, Crockarell et al. state a 31% success rate in their 1998 paper if the results of multiple studies of infected hips are pooled (Crockarell et al. 1998).

In publications from the mid-2000s onwards, there is often a greater than 70% success rate for DAIR. However, in the studies with smaller

Table 1 Infection classification system based upon clinical presentation (Haddad and Bridgens 2008)

Type 1	Positive intraoperative cultures (minimum of 2)
	Manage with appropriate antibiotics
Type 2	Acute post-operative infection
	Attempt DAIR
Type 3	Acute hamatogenous infection in a previously well functioning implant
	Attempt DAIR but removal of prosthesis may be necessary
Type 4	Late, chronic
	Removal of prosthesis

numbers ($n < 100$) of carefully selected patients, the success rate is often higher. In studies with over 100 patients, success rates range from 50 to 85% (Soriano et al. 2006; Byren et al. 2009; Vilchez et al. 2011; Sukeik et al. 2012; Osmon et al. 2013; Kuiper et al. 2013a; Klouche et al. 2011) However, there are no high-quality comparative studies between different DAIR strategies to guide future management.

4 Costs

Revision surgery for prosthetic joint infection is very expensive and represents a significant financial burden on healthcare systems. Bozic & Ries found the cost of revising a hip arthroplasty for infection in 2005 was 2.8 times greater than revision for aseptic loosening and 4.8 times greater than primary hip arthroplasty in the USA. (Bozic and Ries 2005) Klouche et al. also found the cost of revision for infected THA to be 2.6 times that of aseptic revisions and 3.6 times more than primary THA in France. (Klouche et al. 2010) An Irish study put the cost of revision for infection at 52% greater than revision for aseptic loosening. (Oduwole et al. 2010) They also noted a rising cost between two 5-year periods between 1997 and 2006. In Australia, Peel et al. found septic revisions to cost more than three times a primary arthroplasty.(Peel et al. 2013) Even analysing the less expensive technique of Debridement, Antibiotics and Implant Retention (DAIR) for infection, Peel et al. found it to be three times the cost of primary arthroplasty in Australia (Peel et al. 2013).

The cost per case of revision septic hip arthroplasty is approximately $90–100,000 US dollars in the United States (Kurtz et al. 2012; Bozic and Ries 2005), US$57,000 (£44,000) in the United Kingdom (Vanhegan et al. 2012a), and US$53,000 (AU$70,000) in Australia (Peel et al. 2013). These costs were for 2-stage revision hip arthroplasties. The cost of 2-stage knee revision for infection in the USA is approximately US$75,000 and in a large volume centre in the UK was US$40,000 (£30,011) (Kallala et al. 2015).

The estimated total cost incurred for treating PJI cases in the USA was US$320 million in 2001 and $566 million in 2009. We know that the number of hip and knee replacements being carried out per year is increasing and the rate of increase is accelerating. Even with a constant rate of infection, this is leading to an increasing revision load for infection. One study has projected the costs for revision of infected arthroplasties to be $1.62 Billion by 2020 in the USA alone (see Fig. 1) (Kurtz et al. 2012).

Merrollini et al. looked at the average cost of the different revision arthroplasty strategies for infection in a study of 114 Australian patients. (Merollini et al. 2013) They bundled the costs of the first revision and any subsequent operations for those who failed to eradicate infection after the first procedure. The 2-stage revision cost US $32,237 (AU$42,772) for successful cases but when the US$53,045 (AU$70,381) cost of the 1 failure (7%) was added in, the overall cost average was US$33,723 (AU$44,744). The 1-stage revision overall cost average was US $20,140 (AU$26,722) including the costs of subsequent procedure on the 10% of failures.

The patients on whom the DAIR strategy was employed had an overall cost average of US $14,838 (AU$19,688) with initial procedure

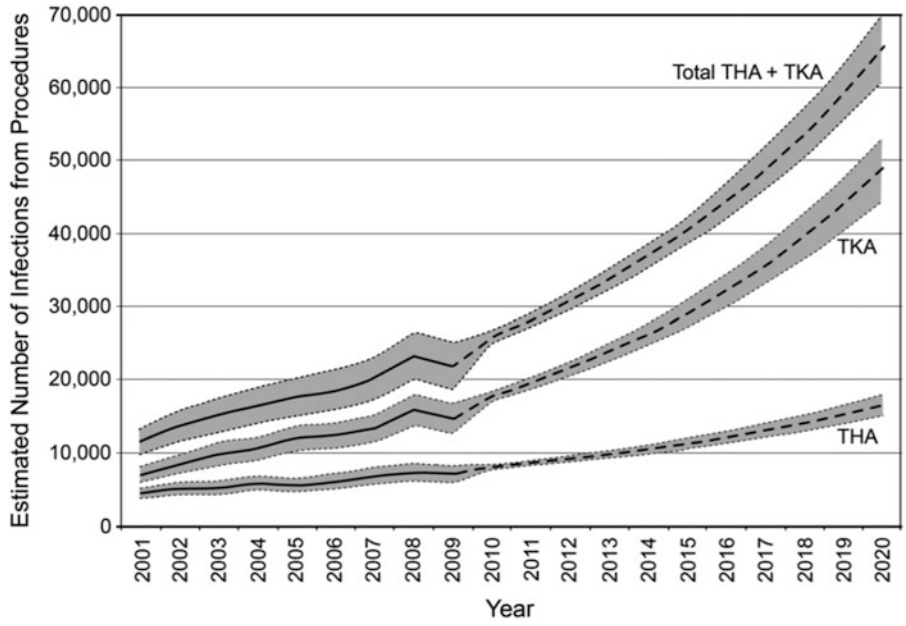


Fig. 1 Historical and projected number of infected THA, TKA, and total (THA + TKA) procedures in the United States (2001–2020). The *dashed lines* represent the projected values per surgery type, and the *dotted lines* represent the 95% CIs of the historical estimates (2001–2009) and the statistical projections (2010–2020) (Kurtz et al. 2012)

costs of US$9,938 (AU$13,187) and multiple treatment costs of US$22,279 (AU$29,560) for the 40% of failures.

Klouche et al. found a similar cost differential in France with 1-stage revision costing US $34,656 (EUR31,333) vs US$59,836 (EUR54,098) for a 2-stage revision. (Klouche et al. 2010) The 2-stage procedure costs 1.7 times that of the 1-stage.

It is worth considering as well that revision cases can lead to greater costs for providing hospitals and these costs are greatest in infected cases. (Kallala et al. 2015) The reimbursement varies from country to country dependent on the tariffs that are applied. If the same reimbursement tariff is applied for a revision irrespective of 1-stage or 2-stage, then the cost of the procedure is a significant factor to the hospital as the revision burden increases, such as in the United Kingdom (National Joint Registry 2015).

There is a cost implication to the population outside of hospital costs to consider. The 1-stage and DAIR approaches have demonstrated improved functional outcomes compared to 2-stage revisions for infection. (Winkler 2009; Theis 2008; Oussedik et al. 2010) This has implications for costs of care, assisted living and further medical interventions. There is also a reduction in mortality rates. Fisman et al. found DAIR resulted in an increased life-expectancy (2.2–2.3 quality adjusted life months) and an improved cost effectiveness ratio (Fisman et al. 2001).

When looking at the international practice of revision for infected arthroplasty, there are national approaches that differ significantly. This may be due to surgical training or financial pressures.

In the UK, the registry data from 2015 demonstrates that 36% of hip revisions for infection were single stage vs 64% 2-stage. There is no data for DAIR (National Joint Registry 2015).

There is a preference for 2-stage revisions in the USA and certain other countries such as Czechoslovakia and Switzerland. (Lentino 2003; Moyad et al. 2008; Landor 2005) According to the AJRR. In 2014, 7.7% of total hip arthroplasties performed in Australia underwent revision for infection hip arthroplasties (Australian Orthopaedic Association 2015).

For example, in Switzerland, Betsch et al. found that 75% of infected hip or knee arthroplasties were performed using a 2-stage strategy, 17.6% DAIR and only 5.9% were revised with a 1-stage (Betsch et al. 2008).

The 1-stage approach has been employed primarily in Europe in strictly selected patients, in particular the Endoklinik in Germany, where as many as 85% of all total joint infection were treated with a single stage approach. (Gehrke et al. 2013) They reported similar failure rates of between 9–20% compared with their two-stage approach after 8 years of follow-up. (Gehrke et al. 2013) These centres often rely on cemented fixation of the revision components in THR. This explains to some extent why this approach is not as popular in the USA where cemented components are used much less.

In Taiwan Hsieh et al. found that DAIR was the strategy in 51% of all infected THR and TKR revisions in their unit from 2000 to 2006, with 30% having a 2-stage revision and 19% having resection arthroplasties (Hsieh et al. 2009).

The protocol for the INFORM trial has been published this year. It is a randomised controlled trial comparing one-stage versus two-stage revision hip arthroplasty for infection and will provide more data on this tricky problem (Strange et al. 2016).

5 Conclusion

The 2-stage revision strategy has the highest success rates and is still essential for some patients with resistant organisms, significant comorbidities or poor soft tissue cover. However, along with significant morbidity and increased mortality, it is a very expensive technique compared to 1-stage or DAIR.

The use of 1-stage revision as a routine for large numbers of patients has been shown to be a successful strategy in certain specialist centres and could lead to a significant cost saving.

The DAIR technique depends on patient selection and is not appropriate for all patients but when utilised sensibly in appropriate hands, could have a significant impact on the cost of revision surgery for infected arthroplasty on a national basis, especially in countries where 2-stage is the majority strategy.

If we take an estimated US$20,000 reduction in cost for 1-stage or US$25,000 for DAIR over 2-stage revisions, then for every 10% of the 22,000 revisions performed in 2009 in the US alone performed as a DAIR rather than a 2-stage, there could be a cost saving of US$55,000,000. ($25,000 * (0.1*22,000)) This takes into account the lower success rate and repeat procedures needed when employing DAIR.

We have to continue to be vigilant to infection as a devastating complication of arthroplasty surgery that costs the patient and the healthcare system a huge amount. The best solution is always going to be prevention especially in light of the growing numbers of arthroplasty surgeries each year.

The best way to learn and adapt to the nature of this problem is to collect quality prospective data and review and incorporate the results into our practice. Registries do not currently give us this data but could be modified to do so in future (Haddad and George 2016) It is also apparent that we need better data on the cost implications of this problem and the various solutions available to us to enable comprehensive economic analyses to guide us in the future.

References

Australian Orthopaedic Association (2015) National Joint Replacement Registry (NJRR) Hip and Knee Arthroplasty Annual Report

Betsch BY, Eggli S, Siebenrock KA et al (2008) Treatment of joint prosthesis infection in accordance with current recommendations improves outcome. Clin Infect Dis 46:1221–1226. doi:10.1086/529436

Bozic KJ, Ries MD (2005) The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. J Bone Joint Surg 87:1746–1751. doi:10.2106/JBJS.D.02937

Byren I, Bejon P, Atkins BL et al (2009) One hundred and twelve infected arthroplasties treated with "DAIR" (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother 63:1264–1271. doi:10.1093/jac/dkp107

Charnley J (1964) A clean-air operating enclosure. Br J Surg 51:202–205

Choi H-R, Knoch von F, Zurakowski D et al (2011) Can implant retention be recommended for treatment of infected TKA? Clin Orthop Relat Res 469:961–969. doi:10.1007/s11999-010-1679-8

Choi H-R, Kwon Y-M, Freiberg AA, Malchau H (2013) Comparison of one-stage revision with antibiotic cement versus two-stage revision results for infected total hip arthroplasty. J Arthroplast 28:66–70. doi:10.1016/j.arth.2013.02.037

Crockarell JR, Hanssen AD, Osmon DR, Morrey BF (1998) Treatment of infection with débridement and retention of the components following hip arthroplasty. J Bone Joint Surg 80:1306–1313

Elson RA (1993) Exchange arthroplasty for infection. Perspectives from the United Kingdom. Orthopedic Clinics of NA 24:761–767

Fisman DN, Reilly DT, Karchmer AW, Goldie SJ (2001) Clinical effectiveness and cost-effectiveness of 2 management strategies for infected total hip arthroplasty in the elderly. Clin Infect Dis 32:419–430. doi:10.1086/318502

Garvin KL, Fitzgerald RH, Salvati EA et al (1993) Reconstruction of the infected total hip and knee arthroplasty with gentamicin-impregnated Palacos bone cement. Instr Course Lect 42:293–302

Gehrke T, Zahar A, Kendoff D (2013) One-stage exchange: it all began here. Bone Joint J 95-B:77–83. doi:10.1302/0301-620X.95B11.32646

Gulhane S, Vanhegan IS, Haddad FS (2012) Single stage revision: regaining momentum. J Bone Joint Surg Br 94:120–122. doi:10.1302/0301-620X.94B11.30746

Haddad FS, Bridgens A (2008) Infection following hip replacement: solution options. Orthopedics 31:907–908. doi:10.3928/01477447-20080901-14

Haddad FS, George DA (2016) Can National Joint Registries play a role in improving our understanding of periprosthetic infections? Bone Joint J 98-B:289–290. doi:10.1302/0301-620X.98B3.37841

Haddad FS, Sukeik M, Alazzawi S (2014) Is single-stage revision according to a strict protocol effective in treatment of chronic knee arthroplasty infections? Clin Orthop Relat Res 473:8–14. doi:10.1007/s11999-014-3721-8

Hansen E, Tetreault M, Zmistowski B et al (2013) Outcome of one-stage cementless exchange for acute postoperative periprosthetic hip infection. Clin Orthop Relat Res 471:3214–3222. doi:10.1007/s11999-013-3079-3

Hsieh P-H, Lee MS, Hsu K-Y et al (2009) Gram-negative prosthetic joint infections: risk factors and outcome of treatment. Clin Infect Dis 49:1036–1043. doi:10.1086/605593

Kallala RF, Vanhegan IS, Ibrahim MS et al (2015) Financial analysis of revision knee surgery based on NHS tariffs and hospital costs: does it pay to provide a revision service? Bone Joint J 97-B:197–201. doi:10.1302/0301-620X.97B2.33707

Klouche S, Sariali E, Mamoudy P (2010) Total hip arthroplasty revision due to infection: a cost analysis approach. Orthop Traumatol Surg Res 96:124–132. doi:10.1016/j.otsr.2009.11.004

Klouche S, Lhotellier L, Mamoudy P (2011) Infected total hip arthroplasty treated by an irrigation-debridement/component retention protocol. A prospective study in a 12-case series with minimum 2 years' follow-up. Orthop Traumatol Surg Res 97:134–138

Klouche S, Leonard P, Zeller V et al (2012) Infected total hip arthroplasty revision: one- or two-stage procedure? Orthop Traumatol Surg Res 98:144–150. doi:10.1016/j.otsr.2011.08.018

Kuiper JWP, Brohet RM, Wassink S et al (2013a) Implantation of resorbable gentamicin sponges in addition to irrigation and debridement in 34 patients with infection complicating total hip arthroplasty. Hip Int 23:173–180. doi:10.5301/HIP.2013.10612

Kuiper JWP, Vos SJC, Saouti R et al (2013b) Prosthetic joint-associated infections treated with DAIR (debridement, antibiotics, irrigation, and retention). Acta Orthop 84:380–386. doi:10.3109/17453674.2013.823589

Kurtz SM, Lau E, Watson H, Schmier JK (2012) Economic burden of periprosthetic joint infection in the United States. J Arthroplasty 27:61–65.e1. doi: 10.1016/j.arth.2012.02.022

Landor I (2005) General principles of infection treatment in joint replacements. Acta Chir Orthop Traumatol Cechoslov 72:183–190

Lentino JR (2003) Prosthetic joint infections: bane of orthopedists, challenge for infectious disease specialists. Clin Infect Dis 36:1157–1161. doi:10.1086/374554

Merollini KMD, Crawford RW, Graves N (2013) Surgical treatment approaches and reimbursement costs of surgical site infections post hip arthroplasty in Australia: a retrospective analysis. BMC Health Serv Res 13:91. doi:10.1186/1472-6963-13-91

Moyad TF, Thornhill T, Estok D (2008) Evaluation and management of the infected total hip and knee. Orthopedics 31:581–588. – quiz 589–90

National Joint Registry (2015) 12th annual report. National Joint Registry 1–183.

Oduwole KO, Molony DC, Walls RJ et al (2010) Increasing financial burden of revision total knee arthroplasty. Knee Surg Sports Traumatol Arthrosc 18:945–948. doi:10.1007/s00167-010-1074-8

Osmon DR, Berbari EF, Berendt AR et al (2013) Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 56:e1–e25

Oussedik SIS, Dodd MB, Haddad FS (2010) Outcomes of revision total hip replacement for infection after grading according to a standard protocol. J Bone Joint Surg Br 92:1222–1226. doi:10.1302/0301-620X.92B9.23663

Parvizi J, Zmistowski B, Adeli B (2010) Periprosthetic joint infection: treatment options. Orthopedics 33:659. doi:10.3928/01477447-20100722-42

Parvizi J, Zmistowski B, Berbari EF et al (2011) New definition for periprosthetic joint infection: from the workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res 469:2992–2994. doi:10.1007/s11999-011-2102-9

Peel TN, Dowsey MM, Buising KL et al (2013) Cost analysis of debridement and retention for management of prosthetic joint infection. Clin Microbiol Infect 19:181–186. doi:10.1111/j.1469-0691.2011.03758.x

Soriano A, García S, Bori G et al (2006) Treatment of acute post-surgical infection of joint arthroplasty. Clin Microbiol Infect 12:930–933. doi:10.1111/j.1469-0691.2006.01463.x

Strange S, Whitehouse MR, Beswick AD et al (2016) One-stage or two-stage revision surgery for prosthetic hip joint infection–the INFORM trial: a study protocol for a randomised controlled trial. Trials 17:90. doi:10.1186/s13063-016-1213-8

Sukeik M, Patel S, Haddad FS (2012) Aggressive early débridement for treatment of acutely infected cemented total hip arthroplasty. Clin Orthop Relat Res 470:3164–3170. doi:10.1007/s11999-012-2500-7

Theis JC (2008) Implant retention in infected joint replacements: a surgeon's perspective. Int J Artif Organs 31:804–809

Tsukayama DT, Estrada R, Gustilo RB (1996) Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg 78:512–523

Vanhegan IS, Malik AK, Jayakumar P et al (2012a) A financial analysis of revision hip arthroplasty. J Bone Joint Surg Br 94-B:619–623. doi:10.1302/0301-620X.94B5.27073

Vanhegan IS, Morgan-Jones R, Barrett DS, Haddad FS (2012b) Developing a strategy to treat established infection in total knee replacement: a review of the latest evidence and clinical practice. J Bone Joint Surg Br 94:875–881. doi:10.1302/0301-620X.94B7.28710

Vilchez F, Martínez-Pastor JC, García-Ramiro S et al (2011) Efficacy of debridement in hematogenous and early post-surgical prosthetic joint infections. Int J Artif Organs 34:863–869. doi:10.5301/ijao.5000029

Winkler H (2009) Rationale for one stage exchange of infected hip replacement using uncemented implants and antibiotic impregnated bone graft. Int J Med Sci 6:247–252

Zeller V, Lhotellier L, Marmor S et al (2014) One-stage exchange arthroplasty for chronic periprosthetic hip infection: results of a large prospective cohort study. J Bone Joint Surg 96:e1. doi:10.2106/JBJS.L.01451

Zimmerli W, Widmer AF, Blatter M et al (1998) Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group JAMA 279:1537–1541

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 101–111
DOI 10.1007/5584_2016_153

Published online: 19 October 2016

Algorithm to Diagnose Delayed and Late PJI: Role of Joint Aspiration

Olivier Borens, Pablo S. Corona, Lars Frommelt, Stergios Lazarinis, Michael Richard Reed, and Carlo Luca Romano

Keywords
Total Joint Arthroplasty (TJA) • Infection • Diagnosties • Algorithm • Joint aspiration • Alpha-defensin

1 Introduction

Total Joint Arthroplasty (TJA) continues to gain acceptance as the standard of care for the treatment of severe degenerative joint disease, and is considered one of the most successful surgical interventions in the history of medicine. A devastating complication after TJA is infection. Periprosthetic joint infection (PJI), represents one of the major causes of failure and remains a significant challenge facing orthopaedics today. PJI usually requires additional surgery including revision of the implants, fusion or amputations causing tremendous patient suffering but also a heavy health economics burden. PJI is at the origin of around 20–25 % of total knee arthroplasty (Bozic et al. 2010; de Gorter et al. 2015; Sundberg et al. 2015) and 12–15 % of total hip arthroplasty (Bozic et al. 2009; Garellick et al. 2014; de Gorter et al. 2015) failures.

In spite of the continuous technological advancements in implants and techniques, the incidence of PJI is increasing. This rise in PJI is multifactorial: First, the total number of TJAs being performed on an annual basis has increased dramatically and will continue to do so for the foreseeable future (Kurtz et al. 2008). Second, patient related factors such as the increasing number of comorbidities of patients undergoing total joint arthroplasty with higher rates of

O. Borens (✉)
Department of Septic Surgery, Orthopaedic-Trauma Unit, Department for the Musculoskeletal System, CHUV, Lausanne, Switzerland
e-mail: Olivier.borens@chuv.ch

P.S. Corona
Reconstructive and Septic Surgery Unit, Orthopaedic Surgery Department, Hospital Vall d'Hebron, Barcelona, Spain

L. Frommelt
Helios ENDO Klinik, Hamburg, Germany

S. Lazarinis
Uppsala University Hospital, Uppsala, Sweden

M.R. Reed
Northumbria HelathCare NHS Foundation Trust, North Shields, UK

C.L. Romano
Centre for Reconstructive Surgery and Osteoarticular Infections and Scientific Project Coordinator, Orthopaedic Research at Institute Galeazzi, Milan, Italy

obesity, diabetes, and cardiovascular diseases contributing to a greater risk of infection (Bozic et al. 2014; Vegari 2013; Wu et al. 2014). Third, the prevalence of antibiotic-resistant microorganisms responsible for PJI is also increasing (Parvizi et al. 2009).

The most common and main symptom of PJI is joint pain. In acute infection, the local signs and symptoms (e.g. severe pain, swelling, erythema, and warmth at the infected joint) of inflammation are generally present (Sia et al. 2005; Zimmerli 2006). On the other hand, delayed and late infections usually have a more subtle presentation, with pain alone, and may be accompanied by variable radiological signs of loosening of the prosthesis. In fact, the symptoms and signs of PJI are often nonspecific, making the diagnosis difficult (Del Pozo and Patel 2009). The ability to distinguish between septic and aseptic failure is very important as the treatment of these two entities is different and PJI necessitates unique surgical strategies to eradicate the infecting organism(s).

The diagnostic tests currently available individually fail to differentiate between aseptic and septic loosening of TJA and any given test may not be completely reliable on diagnosing PJI, if used alone (Dinneen et al. 2013; Ghanem et al. 2008); therefore, surgeons often rely on a wide spectrum of combined tests in order to diagnose PJI. These tests may include: measure of synovial fluid inflammation (e.g. synovial fluid white blood cells (WBC) count and differential, synovial tissue histology, etc.), measures of systemic inflammation (e.g. blood WBC count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), etc.), radiographic imaging (e.g. sonography, leukocyte bone scan, etc.), and tissue or synovial fluid culture. Due to the large number of tests, standard testing practices and their differential efficacy to diagnose PJI, the management of PJI differs substantially between different countries and institutions.

In an attempt to address these inconsistencies in diagnosing a PJI, the Musculoskeletal Infection Society (MSIS) convened a working group to evaluate the available literature and propose a standard definition of PJI that could be universally adopted (Parvizi et al. 2011b). This definition was then slightly modified and ratified during the International Consensus Meeting (ICM) on Periprosthetic Joint Infection in 2013 (Parvizi et al. 2013) and, although not clinically validated, it represents a useful tool of a common language in the medical and scientific community. Following the ICM definition, a joint prosthesis is considered as infected when at least 1 of the 2 major criteria or at least 3 out of 5 minor criteria (See Table 1 below) are present.

Table 1 Criteria for the diagnosis of Periprosthetic Joint Infection as defined by the International Consensus Meeting in Philadelphia in 2013

MSIS criteria	Major criteria	1. A sinus tract communicating with the joint
		2. Two positive periprosthetic cultures with phenotypically identical organisms
	Minor criteria	a. Elevated serum CRP (>10 mg/L) and ESR (>30 mm/h)
		b. Elevated synovial fluid WBC (>3'000 cells per μl) OR ++change on leukocyte esterase strip
		c. Elevated synovial fluid polymorphonuclear (PMN) neutrophil percentage (PMN% > 80 %)
		d. A single positive culture
		e. Positive histological analysis of periprosthetic tissue (>5 neutrophils per high-powered field in 5 high-power fields observed on periprosthetic tissue at 400x magnification)

While the ICM criteria take into account most of the presently commonly used tests, they do not consider more recent diagnostic tools such as those addressing the concentration of synovial fluid biomarkers.

Synovial fluid biomarkers are small proteins or peptides, which are found in the synovial fluid in the presence of pathogenic agents. Their existence was first demonstrated by a microarray study comparing the gene expression of synovial WBCs isolated from septic inflammation (*Staphylococcus aureus*) and aseptic inflammation (gout). This study reported a unique gene expression profile exhibited by the WBCs isolated from infected joints, characteristic of the innate host immune response to infection (Deirmengian et al. 2005). Later, these biomarkers were confirmed at the protein level (Deirmengian et al. 2010) and some of them were shown to exhibit

significantly greater diagnostic accuracy for infection when compared with standard tests (Deirmengian et al. 2010; Jacovides et al. 2011).

One of these biomarkers, called Alpha defensin, demonstrated superior performance on detecting infection. It is an antimicrobial peptide produced and stored in granules by neutrophils, its function being to provide antimicrobial support to the immune system. In response to pathogen phagocytosis the Alpha defensin granules fuse with phagocytic vacuoles and Alpha defensin integrates into the pathogen's cell membrane and causes its rapid killing (Ganz et al. 1985; Lehrer and Ganz 1992). Alpha defensin is released in the synovial fluid by apoptotic and necrotic neutrophils and further regulates the immune response (Brook et al. 2016). Additional studies have demonstrated that Alpha defensin represents an ideal biomarker for use in PJI diagnosis due to its distinct ability to differentiate between infected and aseptic cases (Bingham 2014; Deirmengian et al. 2014a, b, 2015; Frangiamore et al. 2015, 2016).

An immunoassay test has been recently developed to measure the concentration of the Alpha defensin peptide in the human synovial fluid (CD diagnostics 2013; Deirmengian et al. 2014a). The reported specificity and sensitivity for the detection of a PJI by this test were 96 % and 97 % respectively (CD diagnostics 2013; Deirmengian et al. 2014a). These results were further validated by independent studies (Bingham 2014; Bonanzinga et al. 2016; Frangiamore et al. 2016; Kasparek et al. 2016). In addition, the Alpha defensin test has been demonstrated not to be influenced by prior administration of antibiotics, it maintains its performance in patients with inflammatory disease such as rheumatoid arthritis and psoriasis, and it correctly identifies infection in a population of culture negative cases (CD diagnostics 2013; Deirmengian et al. 2014a). A lateral flow version of this test (Synovasure® Alpha Defensin, Zimmer Biomet EMEA) has recently been developed. The test is performed and reported in just over 10 min and it doesn't require any laboratory instrumentation for its interpretation (Aebischer; Kasparek et al. 2016). In this report, the authors propose a new algorithm for the diagnosis of PJI based on joint aspiration including the newly developed Alpha defensin test.

2 Algorithm to Diagnose Delayed and Late PJI – Role of Joint Aspiration

2.1 Goal of the Algorithm

The goal of this diagnostic algorithm is to provide the orthopaedic community with a support for the diagnosis of delayed and late PJI (Trampuz and Zimmerli 2005), i.e. infection onset after, respectively 3 or 24 months from implant, using joint aspiration as the main diagnostic intervention. The Alpha defensin test as a new, very accurate test in diagnosing PJI through joint aspiration, makes it an important part of the presented algorithm. This algorithm is not meant to replace the current clinical practice in different countries and hospitals but to help the reaching of a decision in cases suspected of PJI.

2.2 Development of the Algorithm

This algorithm represents a European view of the use of joint aspiration for PJI diagnosis, taking into consideration the clinical practice in different countries. The algorithm is based on the long-term clinical experience of the authors in diagnosing and treating PJI and takes into account the latest literature in the field. It recommends the use of Alpha defensin as one of the main tests for the diagnosis of PJI using joint aspiration. It is important to note that the sole function of this algorithm is to diagnose PJI using joint aspiration and therefore no advice on how patients should be treated after diagnosis is given.

2.3 When to Use the Algorithm

The first step was to define the conditions when this algorithm should be used. Only patients at a minimum of 3 months from their previous surgery would be considered. This would exclude

all cases of early PJI, thus putting the focus on late and delayed PJI. The reason for this exclusion is that within the first 3 months after surgery, early PJI can often be confused with a normal reaction to surgery and the cut-off value of included tests may be different. This has not currently been investigated.

To be considered as "suspected of PJI", the patient should show at least one of the following four signs: unexplained pain, joint stiffness, elevated serum CRP and/or elevated ESR, and/or early (within 2 years) implant loosening. In such a patient the procedures described in the next section, following the new step-by step algorithm (Fig. 1), is recommended to be performed. Subsequent sections will emphasize the most important points and discuss the open issues regarding the algorithm.

2.4 Pre-operative Phase

When PJI is suspected, a joint aspiration is recommended and the following tests of the synovial fluid should be performed: Cultures, WBCs counts with PMN percentage, Leukocyte Esterase and Alpha defensin. Importantly, the use of blood flasks for the cultures (Hughes et al. 2001) are preferred.

Whether the joint is considered to be infected depends upon the results obtained from the tests mentioned in the algorithm. After this initial pre-operative testing phase, 3 different outcomes could arise; for clarity they will be discussed separately.

2.4.1 Negative Results

The first possible outcome is that all of the tests of the synovial fluid give a negative result (Fig. 1, right side). As depicted in the Figs. 1 and 2, this would mean that:

- No bacterial growth is observed in the cultures
- WBC counts results below 1500 cells and the percentage of PMNs is below 65 % of the leukocytes counted
- Leukocyte esterase test is negative
- Alpha defensin test is negative

In this case, the patient will be considered as not infected and treated accordingly (as an aseptic loosening).

2.4.2 2 Or More Tests Positive (Figs. 1 and 2, left side)

In this case, the joint is considered to be infected and treated accordingly (Figure 1&2, left side). As discussed previously, no recommendations regarding the treatment procedure are given as this is outside the scope of this paper.

2.4.3 1 Test Positive (Figs. 1 and 2, middle)

In this case the infection cannot be ruled-out and the authors recommend performing a second joint aspiration, preferably after 2–6 weeks, and repeating the tests. If, after this second round of testing, 1 or more tests are positive, the patient will be considered to be infected and treated accordingly. Due to the high specificity of the individual tests, it is considered that obtaining positive tests from sequential aspirations is indicative of an infected state. If none of the tests are positive after the second aspiration, the patient should be considered to be non-infected and treated accordingly.

2.5 Intra-operative phase

After the initial pre-operative phase, and when the case is considered as non-infected, if any doubts of infection arise intra-operatively, the authors proposed to use an additional Alpha defensin test as a diagnostic tool. In light of the test's ability to provide a quick result which is not affected by antibiotics (CD diagnostics 2013; Deirmengian et al. 2014a), The Alpha defensin test can be a useful tool to rule out intra-operative doubts about possible infection. If the test gives a positive result, the authors recommend the patient to be considered as infected and treated accordingly (Figs. 1 and 3, INTRA-OP panel). If the result is negative, the patient can be considered as non-infected and can be treated accordingly.

2.6 Post-operative phase

Post-operatively all patients should be followed up for any signs of recurrent infection (Figs. 1 and 4, POST-OP panel). If doubts of infection

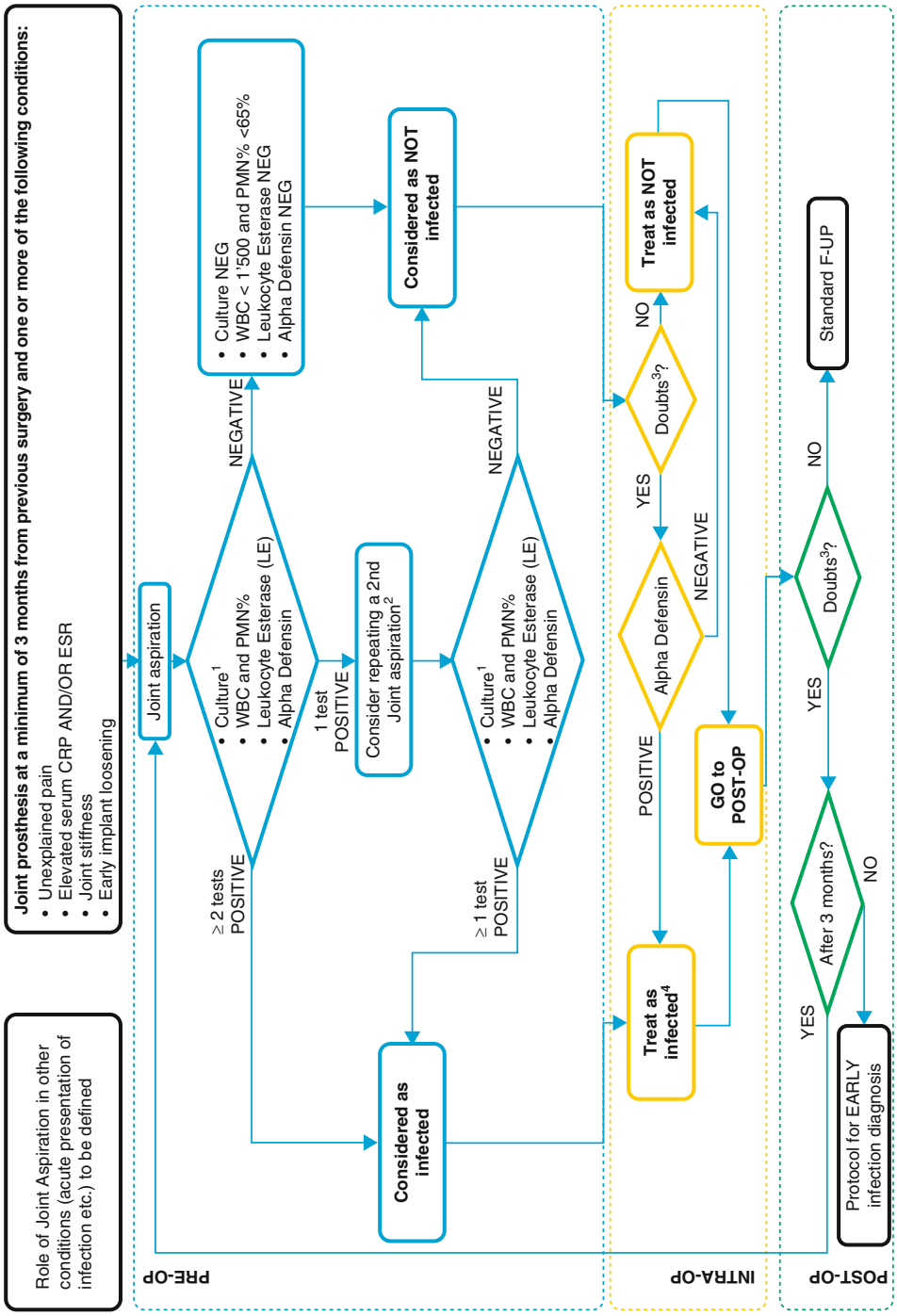
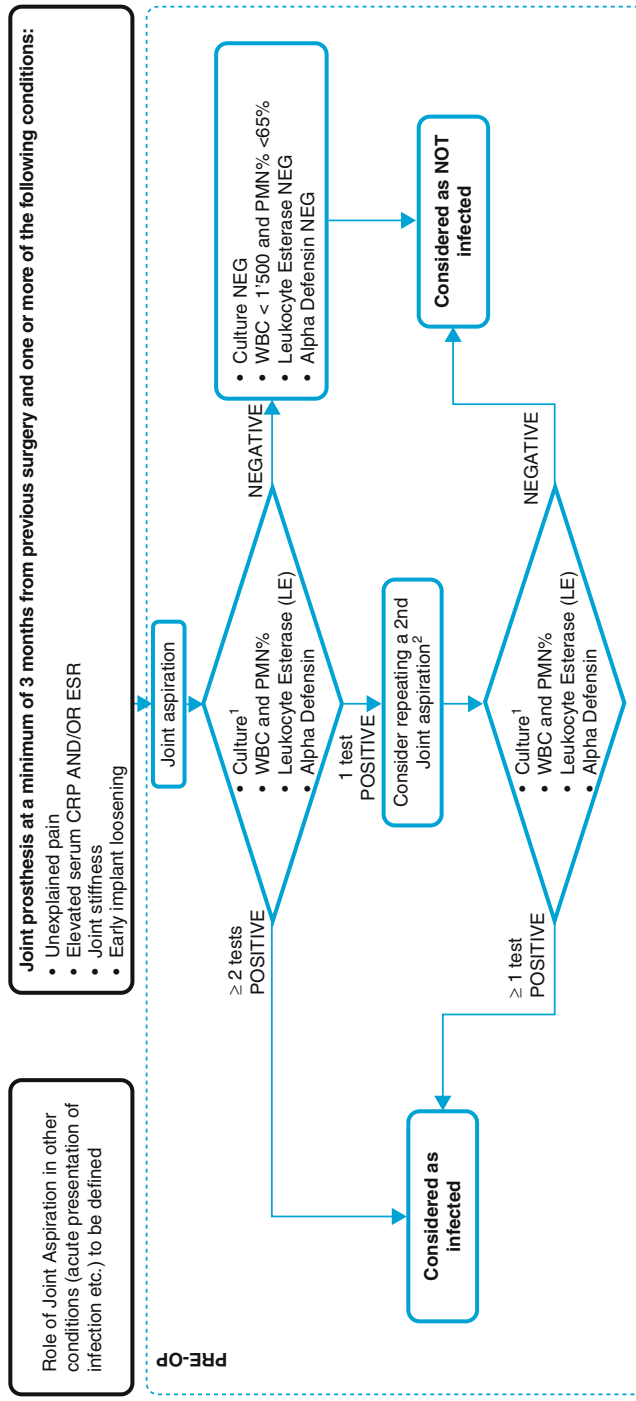


Fig. 1 The algorithm for the diagnosis of delayed and late PJI



[1] Preferentially performed in blood flasks
[2] Indicatevely 1 month after the 1st aspiration

Fig. 2 The algorithm for the diagnosis of delayed and late PJI: the pre-operative phase

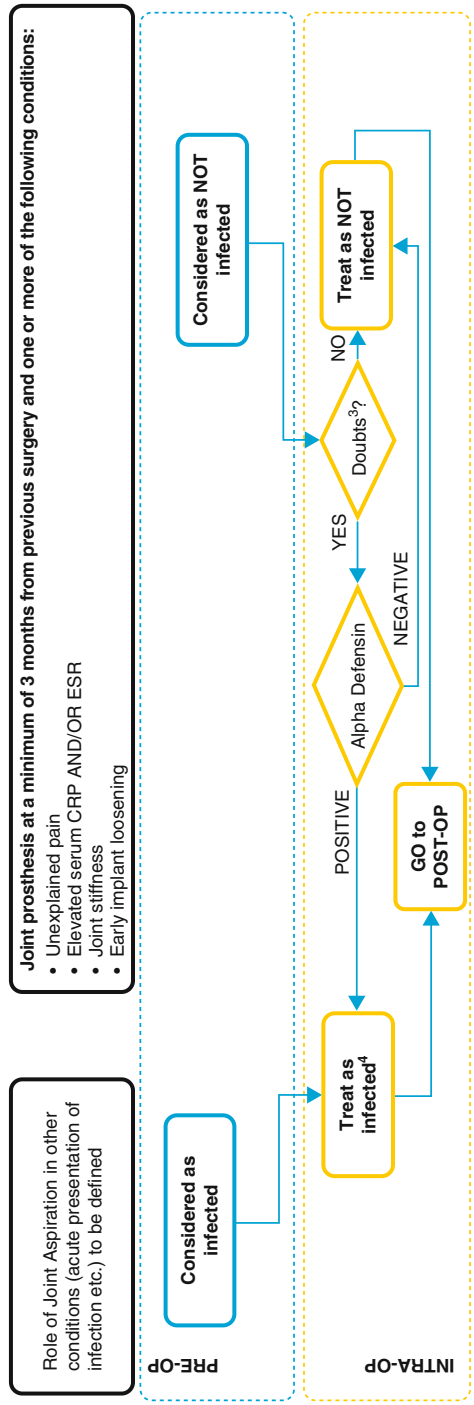


Fig. 3 The algorithm for the diagnosis of delayed and late PJI: the intra-operative phase

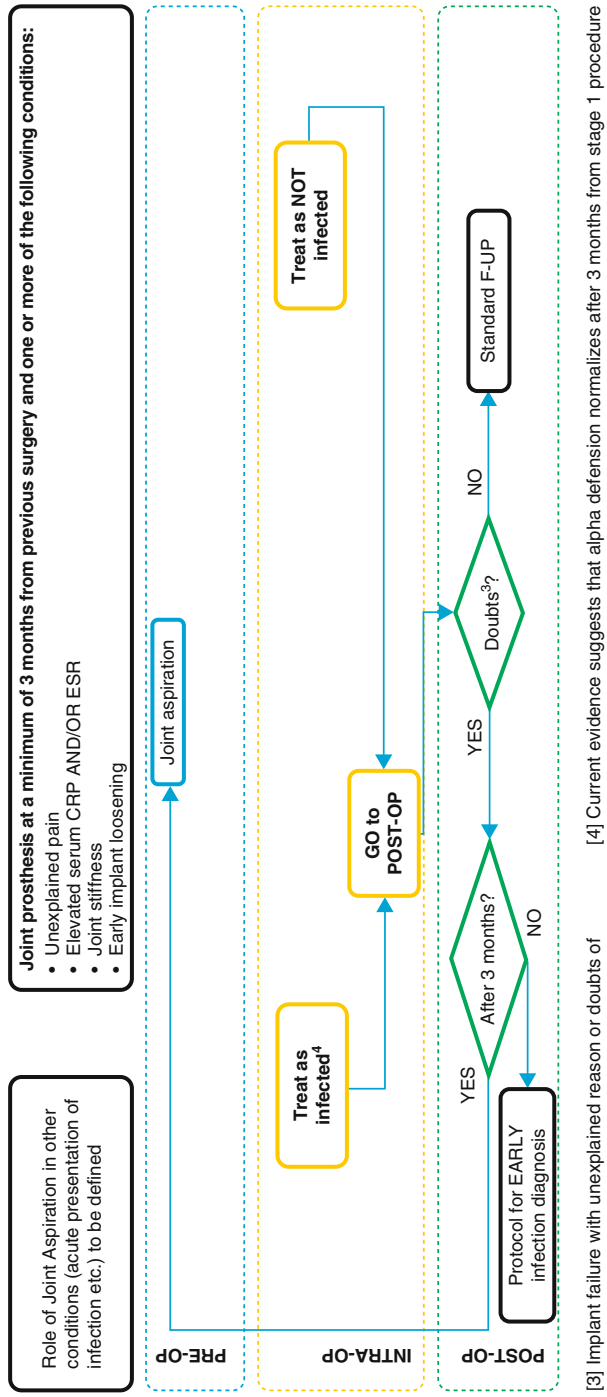


Fig. 4 The algorithm for the diagnosis of delayed and late PJI: the post-operative phase

appear 3 months after the operation, the authors recommend a joint aspiration following the procedure described above. In case suspicions arise within 3 months of surgery, the possible infection would fall into the category of early infection, which is outside the scope of this paper.

2.7 Discussion and Future Directions

Infection after joint replacement is a serious complication that requires complex medical and surgical intervention. The diagnosis of PJI has represented a challenge for the orthopaedic community since the advent of joint arthroplasty. There are various reasons for this diagnostic difficulty such as the variety of clinical signs and symptoms, the relative lack of accurate laboratory diagnostic tests and the difficulty of isolating the pathogens responsible for infection. The utility of serum and synovial fluid markers for the diagnosis of PJI has been evaluated in numerous studies (Berbari et al. 2010; Bottner et al. 2007; Ghanem et al. 2008; Qu et al. 2013; Schinsky et al. 2008; Wirtz et al. 2000).

This is, to our knowledge, the first attempt to include four of the main synovial fluid markers of PJI in an algorithm for delayed and late infection and to indicate the possible role of Alpha defensin in the diagnostic work-up in a suspected PJI.

In particular, cultural examination and white blood cell count have been traditionally used for many years to diagnose PJIs and both tests are part of the ICM criteria. In the lack of a clearly defined cut-off value for WBC (Qu et al. 2014), in the present algorithm, at variance with the final statement of the Philadelphia Consensus, it was decided to fix the cut-off value of WBC at 1500 cells/μL and $>$ 65 % PMN (Kersey et al. 2000; Trampuz et al. 2004) instead of 3000 cells/μL and $>$ 80 % PMN. These values, widely discussed among the authors, were finally chosen to increase the sensitivity of the test, since in the proposed algorithm this parameter is mainly used to exclude the presence of an infection.

Leukocyte esterase, another identified criterium in the ICM definition of PJI, although not originally designed for the diagnosis of peri-prosthetic infection, has been demonstrated to be a quick, easy-to-perform and reliable test in many studies (Aggarwal et al. 2013; De et al. 2016; Parvizi et al. 2011a; Wetters et al. 2012). Additionally, it has been recently proven effective even in diagnosing infection in metal-on-metal hip prostheses (Tischler et al. 2016), that usually pose high diagnostic challenges.

Alpha defensin, an antimicrobial peptide has been identified as a marker of microbial activity in the innate inflammatory response and has shown potential promise in diagnosing PJI in the hip and knee (Bingham 2014; Bonanzinga et al. 2016; Deirmengian et al. 2014a, b, 2015; Frangiamore et al. 2015, 2016; Gollwitzer et al. 2013; Jacovides et al. 2011; Kasparek et al. 2016) and this is the first time it is included in an algorithm to diagnose delayed and late PJI.

Although this algorithm is based on current scientific evidence and on the experience of the authors, the following limitations should be considered when applying it to the clinical setting.

Different cut-off values and techniques, used to evaluate synovial fluid samples, can significantly alter the clinical performance of this and any algorithm; a great effort appears necessary to standardize microbiological analysis and laboratory testing in order to provide comparable results (Drago et al. 2016).

Other synovial fluid markers, that may be potentially useful, such as C-reactive protein, glucose and others (De et al. 2016) were not included in the present algorithm, but should be considered for specific cases.

Clinical validation of the present algorithm, particularly concerning "low-grade" PJIs has not been performed and is currently under way; results should be available in the next 2 years. Until then, this algorithm should mainly work as a recommendation and be used in conjunction with the evaluation of the clinical history, clinical signs and other possible tests, including serum markers and imaging techniques, under the discretion of an expert practitioner.

References

Aebischer D, Ferrando M, Hasgall P, Rieker C. Synovasure PJI. Lateral flow diagnostic test: results from a multi-center multi-surgeon clinical survey:1–16. 16A.D

Aggarwal VK, Tischler E, Ghanem E, Parvizi J (2013) Leukocyte esterase from synovial fluid aspirate: a technical note. J Arthroplasty 28:193–195

Berbari E, Mabry T, Tsaras G, Spangehl M, Erwin PJ, Murad MH, Steckelberg J, Osmon D (2010) Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systematic review and meta-analysis. J Bone Joint Surg Am 92:2102–2109

Bingham J (2014) The alpha defensin-1 biomarker assay can be used to evaluate the potentially infected total joint arthroplasty

Bonanzinga T, Zahar A, Dutsch M, Lausmann C, Kendoff D, Gehrke T (2016) How reliable is the alpha-defensin immunoassay test for diagnosing periprosthetic joint infection? A prospective study. Clin Orthop Relat Res

Bottner F, Wegner A, Winkelmann W, Becker K, Erren M, Gotze C (2007) Interleukin-6, procalcitonin and TNF-alpha: markers of peri-prosthetic infection following total joint replacement. J Bone Joint Surg (Br) 89:94–99

Bozic KJ, Kurtz SM, Lau E, Ong K, Vail TP, Berry DJ (2009) The epidemiology of revision total hip arthroplasty in the United States. J Bone Joint Surg Am 91:128–133

Bozic KJ, Kurtz SM, Lau E, Ong K, Chiu V, Vail TP, Rubash HE, Berry DJ (2010) The epidemiology of revision total knee arthroplasty in the United States. Clin Orthop Relat Res 468:45–51

Bozic KJ, Ward DT, Lau EC, Chan V, Wetters NG, Naziri Q, Odum S, Fehring TK, Mont MA, Gioe TJ, Della Valle CJ (2014) Risk factors for periprosthetic joint infection following primary total hip arthroplasty: a case control study. J Arthroplasty 29:154–156

Brook M, Tomlinson GH, Miles K, Smith RW, Rossi AG, Hiemstra PS, Van't Wout EF, Dean JL, Gray NK, Lu W, Gray M (2016) Neutrophil-derived alpha defensins control inflammation by inhibiting macrophage mRNA translation. Proc Natl Acad Sci U S A 113:4350–4355

CD diagnostics (2013) A new paradigm for the diagnosis of periprosthethic joint infection [White Paper]:1–29

de Gorter JJ, Green M, Gregg P, Porter M, Price A, Tucker K, Wilkinson M, Wishart N (2015) 12th annual report 2015, National Joint Registry for England, Wales and Northern Ireland

De Vecchi VE, Villa F, Bortolin M, Toscano M, Tacchini L, Romano CL, Drago L (2016) Leucocyte esterase, glucose and C-reactive protein in the diagnosis of prosthetic joint infections: a prospective study. Clin Microbiol Infect 22:555–560

Deirmengian C, Lonner JH, Booth RE Jr (2005) The Mark Coventry Award: white blood cell gene expression: a new approach toward the study and diagnosis of infection. Clin Orthop Relat Res 440:38–44

Deirmengian C, Hallab N, Tarabishy A, Della VC, Jacobs JJ, Lonner J, Booth RE Jr (2010) Synovial fluid biomarkers for periprosthetic infection. Clin Orthop Relat Res 468:2017–2023

Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J (2014a) Combined measurement of synovial fluid alpha-Defensin and C-reactive protein levels: highly accurate for diagnosing periprosthetic joint infection. J Bone Joint Surg Am 96:1439–1445

Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J (2014b) Diagnosing periprosthetic joint infection: has the era of the biomarker arrived? Clin Orthop Relat Res 472:3254–3262

Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Booth RE, Parvizi J (2015) The alpha-defensin test for periprosthetic joint infection outperforms the leukocyte esterase test strip. Clin Orthop Relat Res 473:198–203

Del Pozo JL, Patel R (2009) Clinical practice. Infection associated with prosthetic joints. N Engl J Med 361:787–794

Dinneen A, Guyot A, Clements J, Bradley N (2013) Synovial fluid white cell and differential count in the diagnosis or exclusion of prosthetic joint infection. Bone Joint J 95-B:554–557

Drago L, Lidgren L, Bottinelli E, Villafane JH, Berjano P, Banfi G, Romano CL, Sculco TP (2016) Mapping of microbiological procedures by the members of the International Society of Orthopaedic Centers (ISOC) for diagnosis of periprosthetic infections. J Clin Microbiol 54:1402–1403

Frangiamore SJ, Saleh A, Grosso MJ, Kovac MF, Higuera CA, Iannotti JP, Ricchetti ET (2015) Alpha-Defensin as a predictor of periprosthetic shoulder infection. J Shoulder Elbow Surg 24:1021–1027

Frangiamore SJ, Gajewski ND, Saleh A, Farias-Kovac M, Barsoum WK, Higuera CA (2016) Alpha-defensin accuracy to diagnose periprosthetic joint infection-best available test? J Arthroplasty 31:456–460

Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI (1985) Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 76:1427–1435

Garellick G, Kärrholm J, Lindahl H, Malchau H, Rogmark C, Rolfson O (2014) The Swedish hip arthroplasty register, annual report 2014

Ghanem E, Parvizi J, Burnett RS, Sharkey PF, Keshavarzi N, Aggarwal A, Barrack RL (2008) Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. J Bone Joint Surg Am 90:1637–1643

Gollwitzer H, Dombrowski Y, Prodinger PM, Peric M, Summer B, Hapfelmeier A, Saldamli B, Pankow F,

von Eisenhart-Rothe R, Imhoff AB, Schauber J, Thomas P, Burgkart R, Banke IJ (2013) Antimicrobial peptides and proinflammatory cytokines in periprosthetic joint infection. J Bone Joint Surg Am 95:644–651

Hughes JG, Vetter EA, Patel R, Schleck CD, Harmsen S, Turgeant LT, Cockerill FR III (2001) Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. J Clin Microbiol 39:4468–4471

Jacovides CL, Parvizi J, Adeli B, Jung KA (2011) Molecular markers for diagnosis of periprosthetic joint infection. J Arthroplasty 26:99–103

Kasparek MF, Kasparek M, Boettner F, Faschingbauer M, Hahne J, Dominkus M (2016) Intraoperative diagnosis of periprosthetic joint infection using a novel alpha-defensin lateral flow assay. J Arthroplasty

Kersey R, Benjamin J, Marson B (2000) White blood cell counts and differential in synovial fluid of aseptically failed total knee arthroplasty. J Arthroplasty 15:301–304

Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, Parvizi J (2008) Infection burden for hip and knee arthroplasty in the United States. J Arthroplasty 23:984–991

Lehrer RI, Ganz T (1992) Defensins: endogenous antibiotic peptides from human leukocytes. Ciba Found Symp 171:276–290

Parvizi J, Azzam K, Ghanem E, Austin MS, Rothman RH (2009) Periprosthetic infection due to resistant staphylococci: serious problems on the horizon. Clin Orthop Relat Res 467:1732–1739

Parvizi J, Jacovides C, Antoci V, Ghanem E (2011a) Diagnosis of periprosthetic joint infection: the utility of a simple yet unappreciated enzyme. J Bone Joint Surg Am 93:2242–2248

Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, Garvin KL, Mont MA, Wongworawat MD, Zalavras CG (2011b) New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res 469:2992–2994

Parvizi J, Gehrke T, Chen AF (2013) Proceedings of the international consensus on periprosthetic joint infection. Bone Joint J 95-B:1450–1452

Qu X, Zhai Z, Wu C, Jin F, Li H, Wang L, Liu G, Liu X, Wang W, Li H, Zhang X, Zhu Z, Dai K (2013) Preoperative aspiration culture for preoperative diagnosis of infection in total hip or knee arthroplasty. J Clin Microbiol 51:3830–3834

Qu X, Zhai Z, Liu X, Li H, Wu C, Li Y, Li H, Zhu Z, Qin A, Dai K (2014) Evaluation of white cell count and differential in synovial fluid for diagnosing infections after total hip or knee arthroplasty. PLoS One 9:e84751

Schinsky MF, Della Valle CJ, Sporer SM, Paprosky WG (2008) Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. J Bone Joint Surg Am 90:1869–1875

Sia IG, Berbari EF, Karchmer AW (2005) Prosthetic joint infections. Infect Dis Clin North Am 19:885–914

Sundberg M, Lidgren L, Dahl AN, Robertsson O (2015) Swedish knee arthroplasty register, annual report 2015

Tischler EH, Plummer DR, Chen AF, Della Valle CJ, Parvizi J (2016) Leukocyte esterase: metal-on-metal failure and periprosthetic joint infection. J Arthroplasty

Trampuz A, Zimmerli W (2005) Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly 135:243–251

Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R (2004) Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med 117:556–562

Vegari D (2013) Predisposing factors for periprosthetic joint infection. In: Parvizi J (ed) Periprosthetic joint infection: practical management guide. Jaypee Brothers Medical Publishers, New Delhi, pp 59–63

Wetters NG, Berend KR, Lombardi AV, Morris MJ, Tucker TL, Della Valle CJ (2012) Leukocyte esterase reagent strips for the rapid diagnosis of periprosthetic joint infection. J Arthroplasty 27:8–11

Wirtz DC, Heller KD, Miltner O, Zilkens KW, Wolff JM (2000) Interleukin-6: a potential inflammatory marker after total joint replacement. Int Orthop 24:194–196

Wu C, Qu X, Liu F, Li H, Mao Y, Zhu Z (2014) Risk factors for periprosthetic joint infection after total hip arthroplasty and total knee arthroplasty in Chinese patients. PLoS One 9:e95300

Zimmerli W (2006) Infection and musculoskeletal conditions: prosthetic-joint-associated infections. Best Pract Res Clin Rheumatol 20:1045–1063

ERRATUM

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 113
DOI 10.1007/5584_2017_21

Published online: 3 March 2017

Microbiological Diagnosis of Implant-Related Infections: Scientific Evidence and Cost/Benefit Analysis of Routine Antibiofilm Processing

Lorenzo Drago and Elena De Vecchi

The original chapter was published with an incorrect subseries name Adv Exp Med Biol - Advances in Internal Medicine which is now corrected to Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health.

The updated online version of the original chapter can be found under DOI 10.1007/5584_2016_154

ERRATUM

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 115–116
DOI 10.1007/5584_2017_13

Published online: 3 March 2017

Antibacterial Bioactive Glass, S53P4, for Chronic Bone Infections – A Multinational Study

Nina Lindfors, Jan Geurts, Lorenzo Drago, J.J. Arts, Vesa Juutilainen, Pekka Hyvönen, Arnold J. Suda, Aloj Domenico, Stefano Artiaco, Chingiz Alizadeh, Adrian Brychcy, Jertzy Bialecki, and Carlo L. Romanò

The original chapter was corrected. Please find below the summary of the corrections made:

Author's name "Arnold J. Suda" was misspelt as Arnold Suda which has been corrected in this version.

Author's name "Carlo L. Romanò" was misspelt as Carlo Romano which has been corrected in this version.

Affiliations of authors were missing in the previous version. The affiliations have now been included as listed below.

Lindfors NC[1], Geurts J[2], Drago L[3], Arts JJ[4], Juutilainen V[1], Hyvönen P[5], Suda AJ[6], Aloj D[7], Artiaco S[7], Alizadeh Ch[8], Brychcy A[9], Bialecki J[9], Romanò CL[10]

1. Department of Orthopaedic and Plastic Surgery, Helsinki University Central Hospital, Helsinki University, Helsinki, Finland.
2. Department of Orthopaedic Surgery, Research School CAPHRI, Maastricht University Medical Centre, Maastricht, The Netherlands
3. Laboratory of Clinical Chemistry and Microbiology, I.R.C.C.S. Galeazzi Orthopaedic Institute, Milan, Italy
4. Department of Orthopaedic Surgery, Research School CAPHRI, Maastricht University Medical Centre, Maastricht and Orthopaedic Biomechanics Group, Department of Biomedical Engineering, Eindhoven University of Technology (TU/e), Eindhoven, The Netherlands

The updated online version of the original chapter can be found under DOI 10.1007/5584_2016_156

5. Department of Orthopaedic and Traumatology, Oulu University Hospital, Oulu, Finland.
6. Department for Septic Surgery, BG Trauma Center Ludwigshafen, Ludwigshafen, Germany
7. I Orthopaedic Clinic, University of Turin, Orthopaedic and Trauma Center - CTO Hospital, Turin, Italy
8. Azerbaijan Scientific Research Institute of Traumatology and Orthopaedics, Baku, Azerbaijan
9. Department of Bone and Joint Infections, Orthopedic Clinic of Postgraduate Medical Education Centre, Otwock, Poland
10. Center for Reconstructive Surgery of Osteoarticular Infections (CRIO), I.R.C.C.S. Galeazzi Orthopaedic Institute, Milan, Italy

The legends of Tables 1, 2, and 4 are divided into two parts one up and one down the Table. This is also not according to Springer Nature guidelines. This has also been corrected.

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2017) 5: 117–119
DOI 10.1007/5584_2017

Index