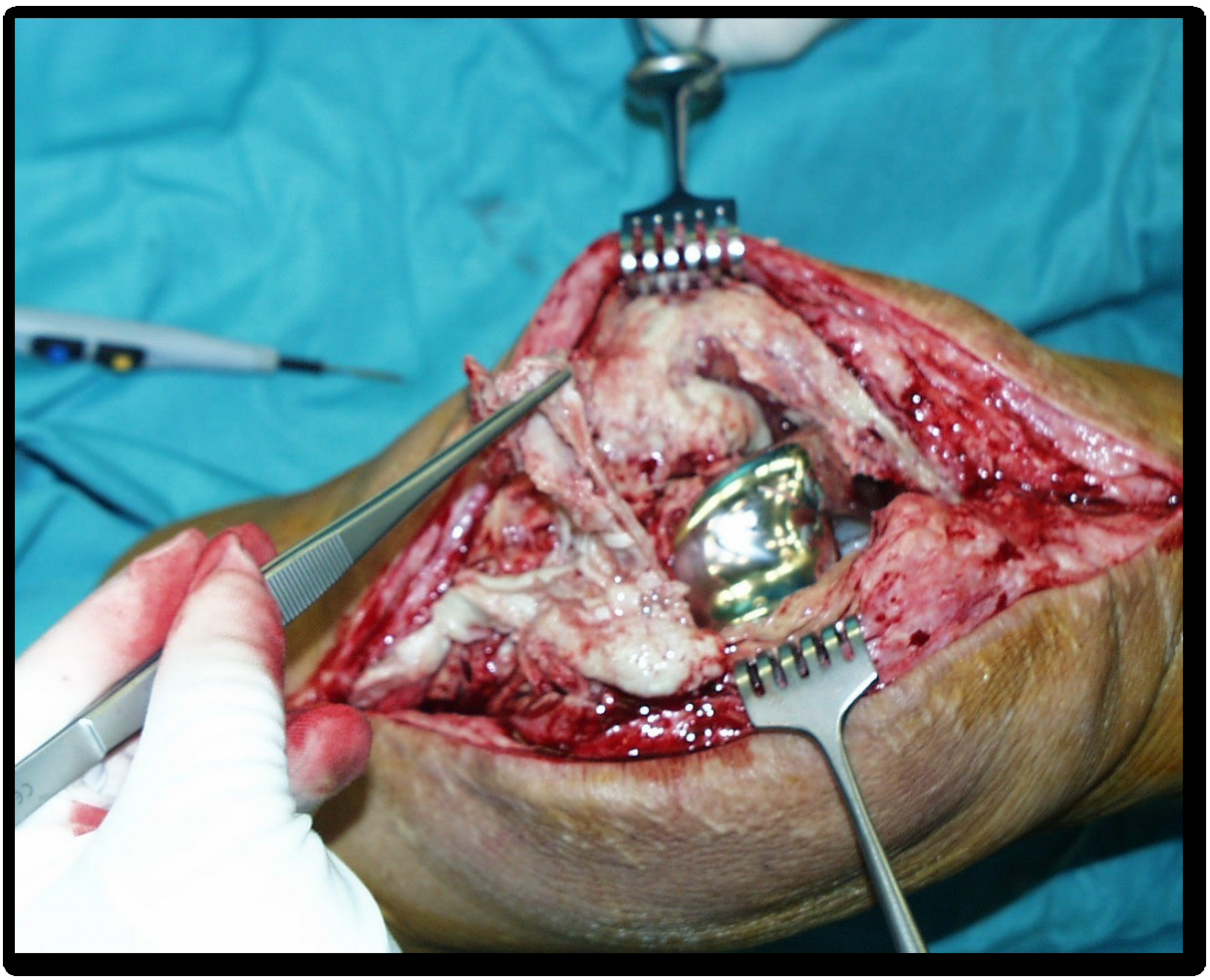
Sensitivity and specificity of new serum blood parameters like Procalcitonin, Interleukin-6, Fibrinogen and Interferon-alpha in the field of orthopaedic surgery



Alexandra Ujvari

Diploma Thesis

Sensitivity and specificity of new serum blood parameters like Procalcitonin, Interleukin-6, Fibrinogen and Interferon-alpha in the field of orthopaedic surgery

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for the degree of
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Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

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I, hereby, declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself.

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1. Abbreviations

CRP: C-reactive protein

ESR: Erythrocyte sedimentation rate

FBG: Fibrinogen

H: hours

IFN´s: Interferons

IFN-alpha: Interferon-alpha

IFN-beta: Interferon-beta

IFN-gamma: Interferon-gamma

IFN-lambda: Interferon-lambda

IL-6: Interleukin-6

IL-8: Interleukin-8

PCT: Procalcitonin

PJI: Periprosthetic joint infection

ROC: Receiver operating characteristic

SCV: Small colony variants

TNF-alpha: Tumour necrosis factor-alpha

VAC-system: Vacuum assisted closure-system

WBC: White blood cell count

2. Abstract

Background: Periprosthetic joint infection (PJI) is still a severe problem after total hip and knee arthroplasty. One of the most important steps in the diagnostic process of detecting PJI remains the analysis of laboratory infection parameters. The aim of this study was to investigate the sensitivity and specificity of the serum parameters procalcitonin (PCT), interleukin-6 (IL-6), fibrinogen (FBG) and interferon-alpha (IFN-alpha) in comparison to conventional parameters (leukocyte count, C-reactive protein [CRP]) in the field of orthopaedic revision surgery.

Methods: Eighty-four patients (124 operations) were prospectively included. The blood parameters of interest were PCT, IL-6, FBG, IFN-alpha, leukocyte count and CRP. Samples were taken preoperatively and on the first, the third and the seventh postoperative days. Samples of FBG were taken only preoperatively. The sensitivity and specificity of these parameters were calculated.

Results: Considering all 124 operations, PCT ($p=0.038$), IL-6 ($p=0.012$), FBG ($p<0.001$), the leukocyte count ($p<0.001$) and CRP ($p<0.001$) had a statistically significant correlation with a positive histology for infections, while IFN-alpha ($p=0.432$) did not. All infection parameters were considered to indicate an infection if they were elevated over their normal range. PCT of 0.35 ng/ml revealed a sensitivity of 90% and a specificity of 67%. IL-6 of 2.55 pg/ml had a sensitivity of 92% and a specificity of 59%. Fibrinogen of 519 mg/dl had a sensitivity of 90% and a specificity of 34%. The leukocyte count of 5.68 G/l revealed a sensitivity of 90% and a specificity of 39%. The CRP value of 11.00 mg/dl had a sensitivity of 90% and a specificity of 74%.

Discussion: Conventional parameters of infection (CRP and leukocyte count) were superior to PCT, IL-6 and FBG in detecting joint infection. It might be helpful to additionally analyse PCT, IL-6 and FBG in special cases, i.e. when it is unclear whether there is a bacterial joint/implant infection or an aseptic inflammation and when it is important to analyse the impact of antibiotic therapy.

3. Zusammenfassung

Hintergrund: Protheseninfekte sind noch immer ein ernst zunehmendes Problem nach Hüft- und Knie-total-Endoprothesen. Einer der wichtigsten Schritte in der Diagnostik des Protheseninfektes ist die Analyse von Laborparametern. Das Ziel der Studie war es die Sensitivität und Spezifität der Serumparameter Procalcitonin (PCT), Interleukin-6 (IL-6), Fibrinogen (FBG) und Interferon-alpha (IFN-alpha) mit den herkömmlichen Parametern (Leukozytenzahl, C-reaktives Protein [CRP]) im Bereich der orthopädischen Revisionschirurgie zu vergleichen.

Methoden: 84 Patienten (124 Operationen) wurden prospektiv eingeschlossen. Die zu untersuchenden Blutwerte waren PCT, IL-6, FGB, IFN-alpha, die Leukozytenzahl und CRP. Proben wurden präoperativ und am 1., 3. und 7. postoperativen Tag abgenommen. Proben von FBG wurden nur präoperativ abgenommen. Die Sensitivität und Spezifität dieser Parameter wurde berechnet.

Ergebnisse: Berechnet für alle 124 Operationen, hatten PCT ($p=0.038$), IL-6 ($p=0.012$), FBG ($p<0.001$), die Leukozytenzahl ($p<0.001$) und CRP ($p<0.001$) einen statistisch signifikanten Zusammenhang mit einer positiven Histologie für Infekte, während IFN-alpha ($p=0.432$) diesen nicht hatte. Bei allen Entzündungsparametern wurde angenommen, dass sie eine Infektion anzeigten, wenn der Wert höher war als der Normbereich. Ein PCT Wert von 0.35 ng/ml hatte eine Sensitivität von 90% und eine Spezifität von 67%. Ein IL-6 Wert von 2.55 pg/ml hatte eine Sensitivität von 92% und eine Spezifität von 59%. Ein FBG Wert von 519 mg/dl hatte eine Sensitivität von 90% und eine Spezifität von 34%. Die Leukozytenzahl von 5.68 G/l hatte eine Sensitivität von 90% und eine Spezifität von 39%. Ein CRP Wert von 11.00 mg/dl hatte eine Sensitivität von 90% und eine Spezifität von 74%.

Diskussion: Die herkömmlichen Entzündungswerte (CRP und Leukozytenzahl) waren PCT, IL-6 und FBG darin überlegen Protheseninfekte nachzuweisen. Es könnte hilfreich sein PCT, IL-6 und FBG in speziellen Fällen zusätzlich zu analysieren, z.B.: wenn es unklar ist, ob ein bakterieller Protheseninfekt, bzw. eine

bakterielle Gelenksentzündung oder eine aspetische Entzündungsreaktion vorliegt und wenn es wichtig ist den Effekt einer antibiotischen Therapie zu analysieren.

4. Introduction

4.1 Infections in the field of orthopaedic surgery

4.1.1 The diagnostic dilemma

Bacterial infection of endoprosthesis is a common and severe complication after orthopaedic surgery; it is often associated with more risks for the patients and higher costs for the health care system. (1) Periprosthetic joint infection (PJI) after total knee arthroplasty has an incidence of 1-4% after primary surgery (2) and 3-20% after revisions. (3) After primary total hip arthroplasty PJI also has an incidence of 1-2%. (2) Therefore the diagnostic decision if a bacterial infection of the artificial joint is on hand or not has to be made as soon as possible to avoid harming the patient. Still today there is no consensus on a gold standard for diagnosing PJI. (2)

Nowadays orthopaedic surgeons trust in conventional inflammation markers like C-reactive protein (CRP) or leukocyte count, and on the clinical picture of an infected joint (swelling, pain, erythema and fever). (4,5) The medical history and the clinical examination are an important diagnostic factor, but the symptoms of infections are similar to other prosthesis-related complications like “hematoma, instability, and aseptic loosening”. (6)

Conventional markers of infection can also be elevated because of other reasons like obesity, metabolic syndrome, diabetes mellitus, insulin-resistance and in persons of lower socioeconomic status. (4,7,8) Yet in chronic and low grade infections CRP and leukocytes can be in a normal range. (4) Furthermore the sensitivity and specificity of erythrocyte sedimentation rate (ESR) and CRP are rather low. (2)

For further confirmation of the diagnosis aspiration of synovial fluid for microbiological cultures and gram staining can be performed. But until the results of the cultures are available it takes between 3 to 14 days and the gram staining is rather unspecific. (5,9,10) If the cultures turn out to be negative a surgical

intervention (e.g. explantation of endoprosthesis and implantation of antibiotic cement spacer) or an antibiotic treatment was performed unnecessarily. Besides with every surgical intervention the risk of endoprosthesis infection rises. (11)

Also “the appearance of the joint during surgery” (2) can give a hint for on-going infection, but that is dependent on the experience of the surgeon. And the analysis of frozen sections obtained during surgery is dependent on the experience of the pathologist in attendance. (6)

Furthermore surgery itself and the healing-process afterwards can induce the release of proinflammatory cytokines and can therefore cause a nonspecific inflammatory response syndrome. (1,12)

That leads to the conclusion that the sensitivity and specificity of conventional parameters like CRP and leukocyte count are not reliable enough. Hence the need of new inflammation markers, which respond fast to infection and have a high sensitivity and specificity, to optimise the diagnostic process.

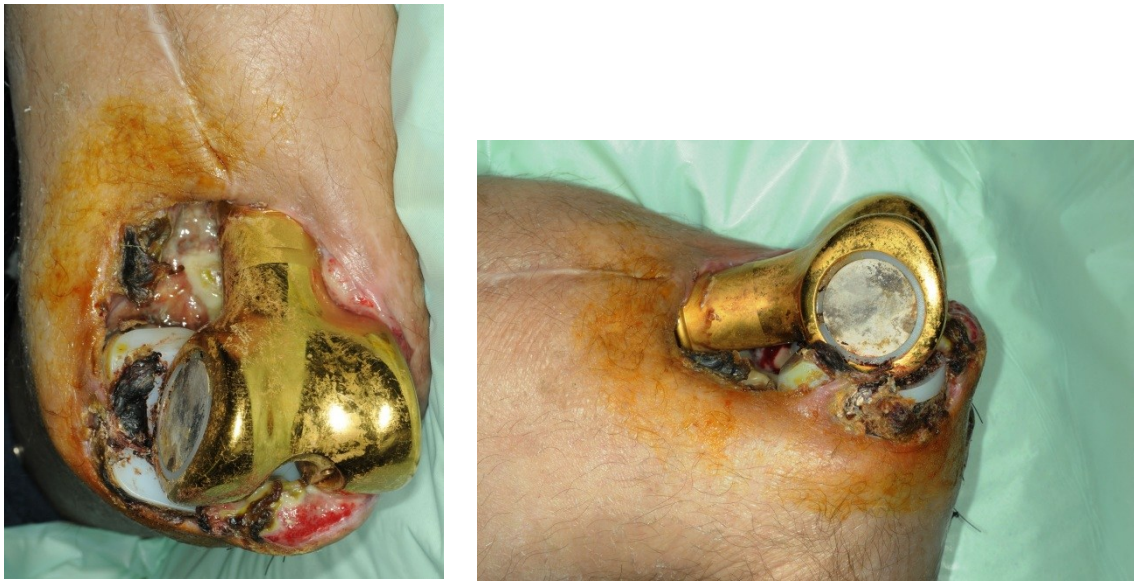


Fig 1: *Operative site of a 79 year old male patient. In the course of six months he developed a severe infection of the left knee with major skin defects after the implantation of a total femur prosthesis.*

4.1.2 Acute infection

An acute infection after implantation of an endoprosthesis begins up to 4 weeks after the intervention. The clinical picture consists of the typical signs of infection with pain, swelling, erythema, hyperthermia, impaired movement and supplementary fever. Also the conventional markers of infection (CRP, white blood cell count [WBC], ESR) remain increased after the operation. The sources often are infected haematomas deep to the fascia and superficial wound infections. Acute infections tend to turn into chronic infections, therefore an early diagnosis and proper treatment is pivotal for the patients' health. The pathway of infection is typically directly exogenous during the surgical intervention. Mostly caused by staphylococci and gram-negative pathogens. (11,13,14)

4.1.3 Chronic infection

According to the classification by Cierny, chronic infections include all infections, which begin at the earliest 4 weeks after surgery or start right after the operation, but last longer than 4 weeks. (13) The chronic infection is by some authors further classified in delayed and late infection.

4.1.3.1 Delayed infection

This kind of infection begins, as the name indicates, delayed, in average between 3 to 24 months after surgery. It is also caused by intraoperative acquired bacteria, but of lower virulence (e.g. coagulase-negative staphylococci, Propionibacterium acnes). The classical signs of infection can be very discrete or missing. Furthermore the body temperature reaches only subfebrile ranges. Often the inflammation is supported by host factors like illnesses (e.g. rheumatoid arthritis) and an immune deficiency. (11,14)

4.1.3.2 Late Infection

The late infection begins at the earliest two years after the implantation of an endoprosthesis. The patients had no pain after the intervention until the new onset of inflammation. The pathway is usually indirectly endogenous by haematogenous or lymphogenous spread of bacteria from another focus. In some cases it is an outbreak of a not healed acute or chronic infection. The isolated bacteria are again staphylococci, in some cases streptococci and Escherichia coli, or mixed infections. Signs of inflammation are often missing and CRP, ESR and leukocytes are in a normal or only slightly elevated range. (11,13,14)

4.2 Bacteria

Various bacteria have been identified to be the most common pathogens of orthopaedic infections. Between 60-70% of them are gram positive germs, while approximately a quarter of the infections are caused by gram negative rods. Moreover aerobic pathogens are predominant over the anaerobic. (11,13)

There are three different ways how pathogens get into the organism and can therefore cause implant infections. Firstly, directly exogenous, if the implant gets in contact with the skin or aerosols. Secondly, directly endogenous, if the implant gets in touch with the surrounding infected soft tissues. And thirdly, through a haematogenous or lymphogenous spreading of bacteria, which is the indirectly endogenous pathway. (13)

It depends on various factors, if it comes to a manifest infection after bacteria were brought into the joint. E.g. the immune defence of the host, the germinal load, the virulence and the antibiotic prophylaxis before, during and after the operation. Besides that, also some iatrogenic factors play a not so unimportant role, e.g. duration of the intervention and surgical technique. Additionally some bacteria are able to build a biofilm, which is a good protection from antibiotic treatment and other changes of the micro-environment. Moreover staphylococci and Escherichia coli have the ability to build so called small colony variants (SCV). These SCV

protect themselves by a very slow growth and demeaned metabolism. So they are hard or not at all detectable. This fact explains that they are usually found in delayed or late infections of deep implants. (13)

In microbiological testing mainly Staphylococcus aureus and coagulase-negative staphylococci (of this group mostly Staphylococcus epidermidis) could be proven. Other frequent pathogens were beta-haemolytic streptococci and streptococci of the viridans group. Besides in some few cases Propionibacterium acnes, Proteus mirabilis, Escherichia coli and enterobacter species were detected. (4,11,15,16)

4.3 Therapy

The PJI therapy consists of two equally important parts, namely surgical and conservative treatment. The figure below shows the different kinds of therapeutic possibilities of implant-associated infections.

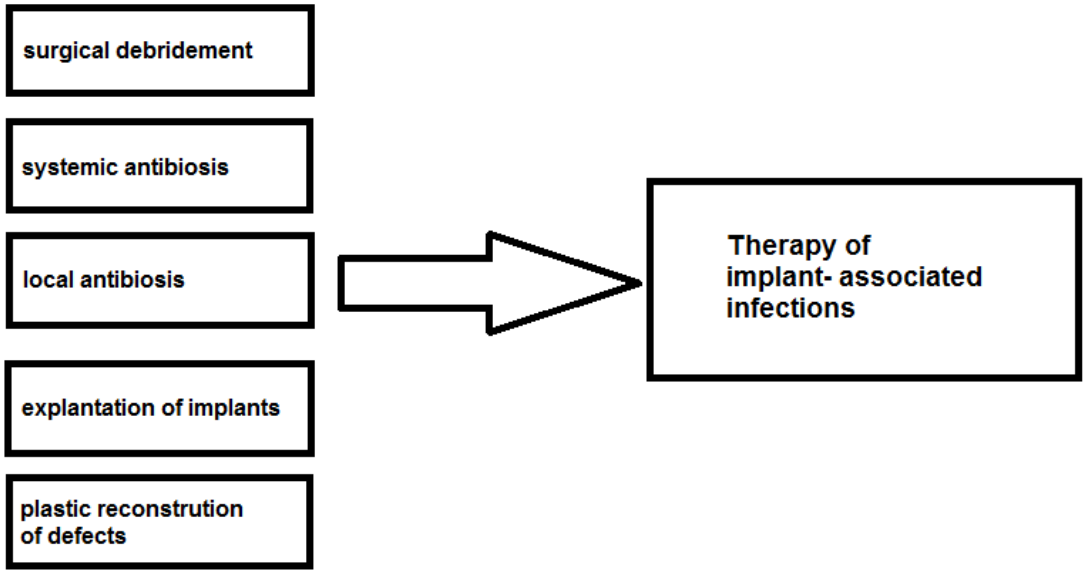


Fig 2: *Therapeutic possibilities of implant-associated infection, an adaption of* (13).

“Surgical treatments include debridement with retention of the prosthesis, one- or two-stage exchange, resection arthroplasty, arthrodesis, and amputation.” (10) In a one-stage exchange all parts of the prosthesis are removed and a new one is

implanted during the same procedure. In a two-stage exchange the prosthesis is explanted and replaced by an antibiotic-impregnated cement spacer. In a second operation after 6 weeks a new endoprosthesis can be implanted. (10)

The extent of the surgical debridement should be conforming to the extent of tumour surgery. Bone cement has to be erased completely and using a jet-lavage is beneficial. In case of an early infection, low virulence and few co-morbidities of the patient a one-stage exchange can be considered. Otherwise a two-stage exchange is indicated. During the surgical procedures of a two-stage exchange the edges of the wound can be adapted with strain less sutures. Major defects have to be covered with plastic-reconstructive methods. (13)

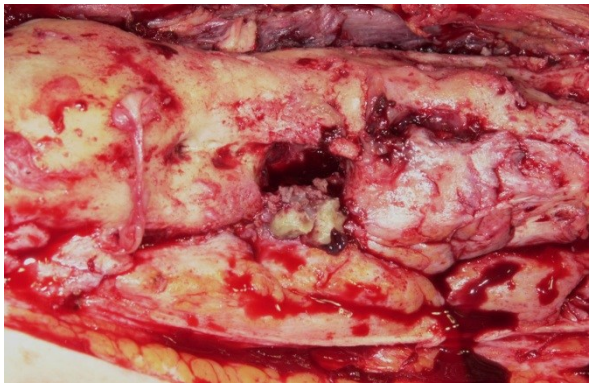


Fig 3a

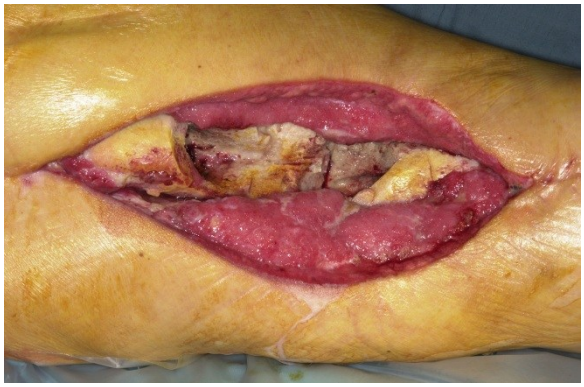


Fig 3b

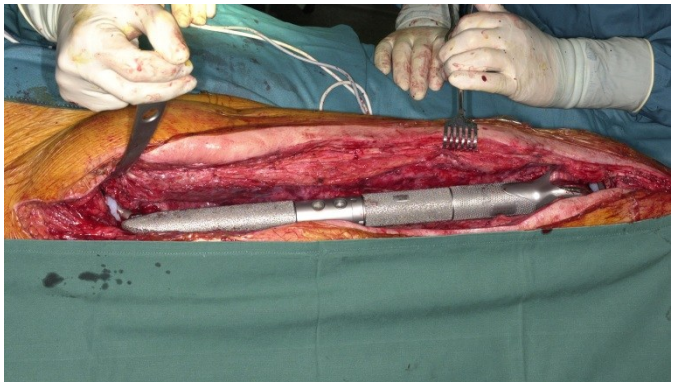


Fig 3c

Fig 3: *Therapeutic steps in a case of chronic osteomyelitis after a compound femur fracture (1975) in a 50 year old male patient. 3a) The operative site before debridement. The surgical intervention was performed in June 2002. 3b) After one month (July 2002) the infection is healing. The operative site shows granulation tissue. 3c) The same patient after implantation of a total femur prosthesis four month after the first intervention (October 2002).*

The important parts of the conservative therapy are systemic as well as local antibiosis. Also it is pivotal that the antibiotics are able to infiltrate the bone and the soft tissue. It should be possible to continue the i. v. treatment with oral medication subsequently. (13) According to an article by Scheffer et al., in case of infection after total hip arthroplasty antibiosis is recommended for 3 months, after a total knee arthroplasty for 3-6 months. If antibiotic-impregnated cement for the spacer is used, the time of systemic antibiosis can be reduced to 4-6 weeks. (14) The release of local antibiotics out of the bone cement is limited to no more than 3 weeks. (13) Before the re-implantation of prosthesis the oral or systemic medication has to be stopped for two weeks. (13) At the Department of Orthopaedic Surgery/Medical University of Graz the antibiotic treatment is paused for a week preoperatively and continued afterwards for 4-6weeks.

4.4 Laboratory infection parameters

4.4.1 Procalcitonin

Procalcitonin (PCT) is the 116 amino acid prohormone of calcitonin, which is synthesized in the C-cells of the thyroid gland (17) and was “first described in the late 1970s”. (5)

Several studies show that it is significantly higher in cases of bacterial infection and sepsis, but not in viral infections. (5,9,18-20) It is still not known in which cells PCT is synthesized apart from those in the thyroid gland. (21) In a study with hamsters by Müller et al. the origin of the PCT enhancement could not be clearly detected. In hamsters and also in humans this marker is produced in several organs, e. g. liver, lung and kidney. While PCT increase in sepsis and bacterial infection, the level of the mature hormone is stable. (22)

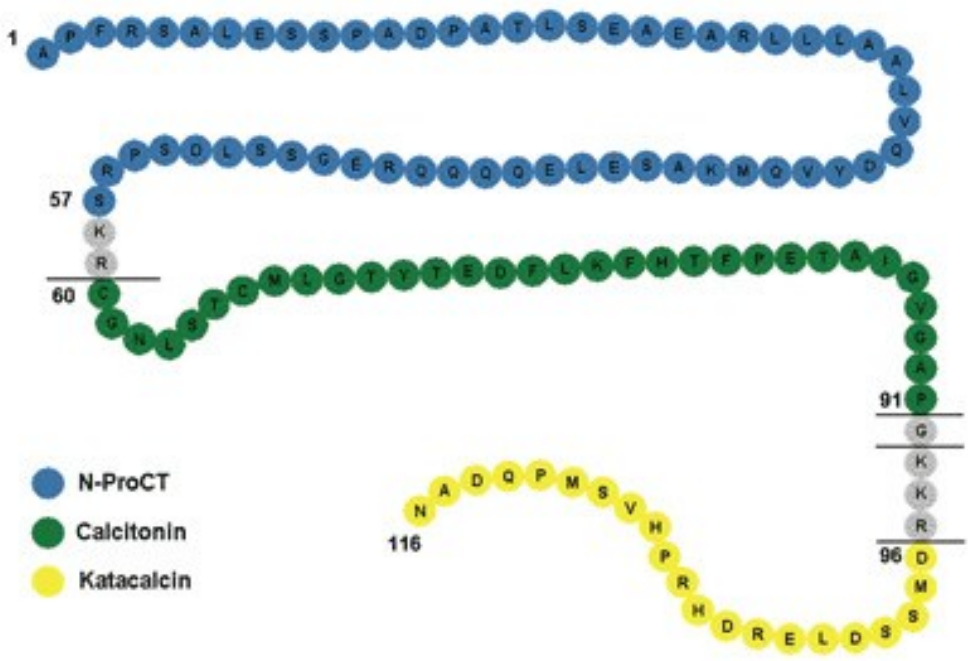


Fig 4: *Molecular structure of PCT; N-PCT and katacalcin have to be separated off to get the mature hormone calcitonin.*(23)

PCT increases 2-4 hours (h), in another publication 3-4 h after the onset of sepsis. (9,21,24) So PCT reacts not as fast as other proinflammatory cytokines, but clearly earlier than CRP. (9,25) It reaches its peak after 6 hours and remains elevated for at least 24 h. (21) In another study it peaks after 8h. (24) Some authors observed a mean half-life of 25-30 h (26) and 22-29h. (15) Several studies indicate that the history of PCT levels reflects the success or failure of the antimicrobial therapy efficiently, accurately and more reliably than CRP (9,18-20,25), as well as the success of antifungal therapy. (27) A persistently elevated PCT level or a delayed PCT peak could also suggest unfitting antimicrobials. (27)

4.4.2 Interleukin-6

Interleukin-6 (IL-6) has been reported to be a sensitive marker for bacterial infection, also after total joint replacement. (4,28,29) As IL-6 triggers the release of CRP in liver cells, it reacts much faster to infection then CRP. (28,30) Different cells, like monocytes, macrophages, fibroblasts and T2-lymphocytes produce IL-6 post traumatically. (28)

The IL-6 level increases rapidly after surgery, with a peak after 3-6h. (21,28) It reaches a baseline level after 8h and has a mean half-life of 15h; afterwards it decreases rapidly to normal concentrations. (20,21,28)

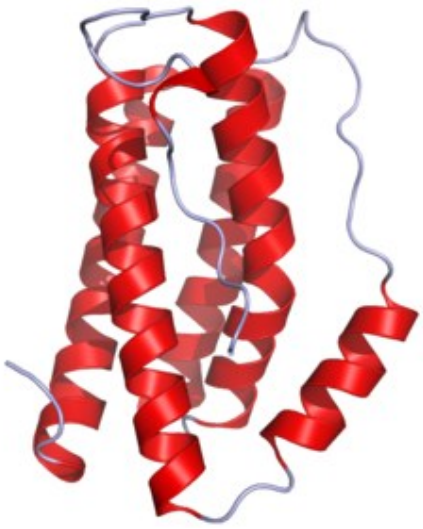


Fig 5: *Molecular structure of IL-6. (http://de.wikipedia.org/wiki/Interleukin-6)*

4.4.3 Fibrinogen

Fibrinogen (FBG) is an acute-phase protein (3) and also part of the coagulation system. (12) There is a close connection between inflammation and thrombosis. And after trauma or surgery the activation of prothrombotic elements and the systemic inflammatory response is remarkable. (31)

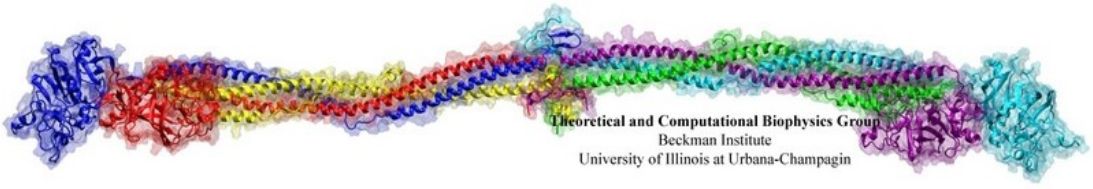


Fig 6: *Molecular structure of FBG.*
(http://www.ks.uiuc.edu/Gallery/Science/Structure/tn/fibrinogen-small_st2.jpg.html)

Activation of the coagulation cascade is part of the inflammatory reaction. Thrombin splits FBG into the insoluble fibrin, which forms the clot of thrombocytes. Proinflammatory cytokines have a pivotal role in the activation of coagulation through the expression of tissue factors and changes in the endothelium. (12) Considering this FBG could be helpful in the diagnosis of implant infections.

4.4.4 Interferon-alpha

Interferon cytokines, produced by leukocytes, are important parts of the innate and adaptive immune system. (32,33) Meanwhile three different types of Interferons (IFN´s) are discovered. Interferon-alpha (IFN-alpha) and Interferon-beta (IFN-beta) belong to the group of Type I IFN´s. They are pivotal for the immunity against most viruses. Type II IFN´s (Interferon-gamma [IFN-gamma]) are essential for anti-mycobacterial immunity, while the exact role of Type III IFN´s (Interferon-lambda [IFN-lambda]) is not quite clear yet. (33)

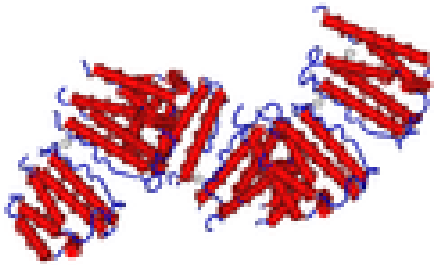


Fig 7: *Molecular structure of IFN-alpha.* (*http://commons.wikimedia.org/wiki/Cytokines)*

IFN-alpha has an important role in the modulation and regulation of different cytokines, e.g. it boosts the signalling effect of IL-6. By this it participates in the proinflammatory cascade after viral infections. (32) This suggests that it could be a useful laboratory marker for identifying viral, rather than bacterial infections. (34)

4.4.5 Leukocytes

Leukocytes are part of the unspecific innate immune system, like CRP and IFN´s. They are divided into three subsections, neutrophil, eosinophil and basophil granulocytes. Defence of bacterial infections is mostly part of the neutrophil granulocytes, which also distribute leukotrienes. By different chemotaxines (e.g. prostaglandines, complement factors, interleukin-8 [IL-8], tumour necrosis factor-alpha [TNF-alpha]) they are attracted to the location of an infection. (35)

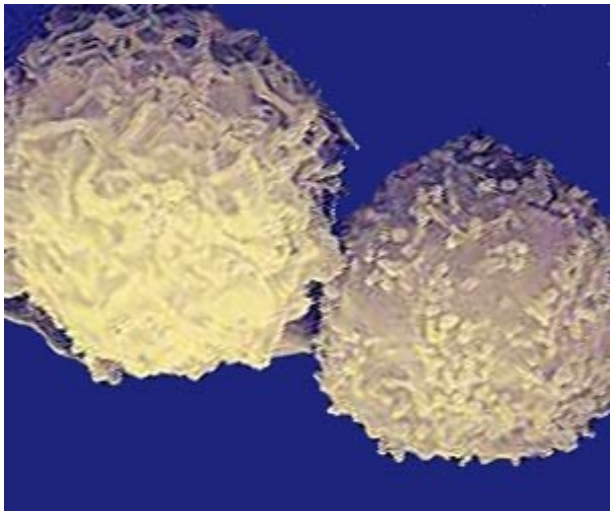


Fig 8: *Leukocytes under an electron microscope. (http://www.mg-i.ch/?page_id=373)*

Like all blood cells, leukocytes are built in the bone marrow. They have an important role in the activation and maintenance of the inflammatory reaction through the production of proinflammatory mediators. Moreover they secrete IFN-alpha and IFN-beta. (36)

Inflammation leads to a reactive leucocytosis. That explains why WBC is a good, but unspecific serum parameter of bacterial infections of all kind.

4.4.6 C-reactive protein

CRP is the most frequently used marker of infection. It has a pivotal role in all sections of modern medicine, from outpatient care, over daily hospital routine, to general practitioner.

CRP is released from liver cells, which is triggered by different proinflammatory cytokines, e.g. IL-6 and TNF-alpha. (7,37) After surgical procedures CRP increases slowly and peaks two days afterwards. It has a mean half-life of 62h. (28) Another author observed the start of CRP-secretion after 4-8h and a peak after 36h. (24)

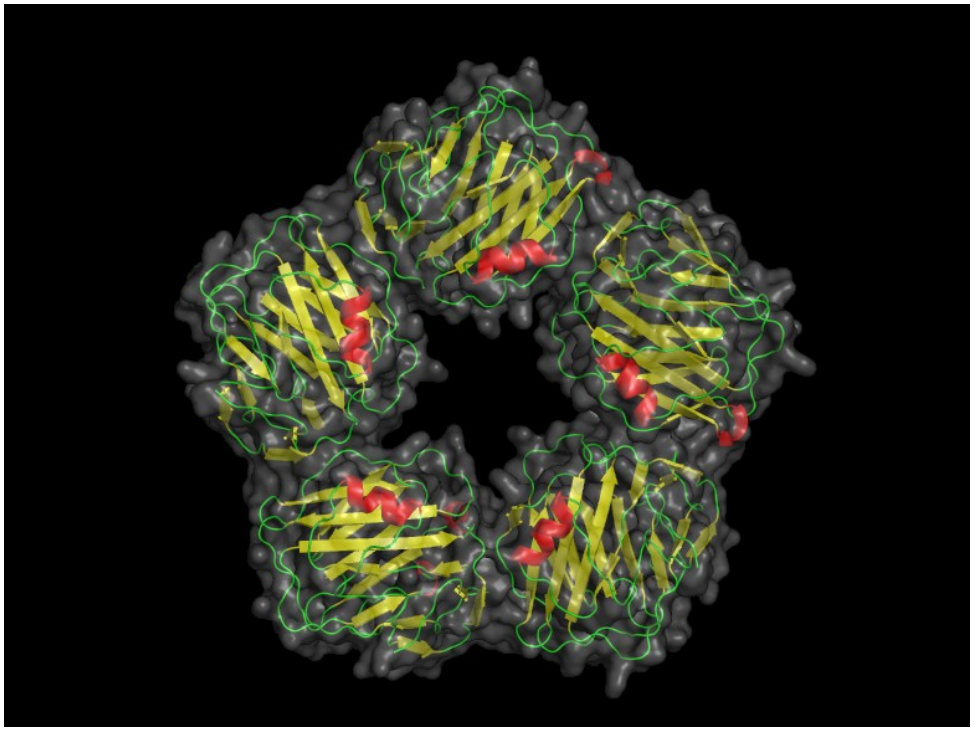


Fig 9: *Molecular structure of CRP. (http://en.wikipedia.org/wiki/File:CRP_pretty.png)*

It is a very sensitive marker for infection (4), but it is also elevated in people with lower socioeconomic status (8), patients with metabolic syndrome, diabetes and insulin-resistance and obese persons. (7) Besides surgical trauma, haematoma and the healing process can lead to increased CRP levels. (12) A study by Bottner et al. suggests that a cut-off level of 3.2 mg/dl is very sensitive for PJI. (4)

4.5 Aim of the study

In the current literature it has been suggested that PCT and IL-6 are markers for bacterial infections and that the sensitivity and specificity of PCT for sepsis and bacterial infections perform better than the sensitivity and specificity of CRP, IL-6 and IL-8. (4,9,38) The specific hypothesis was that there is a significant difference between PCT levels in patients with septic and non-septic arthritis. Because of the need of more accurate laboratory tests in the field of orthopaedic surgery, this study was undertaken to investigate the sensitivity and specificity of PCT, IL-6, FBG and IFN-alpha in comparison to the conventional markers of infection like leukocytes and CRP after total knee and hip endoprosthesis revisions and septic joint operations.

5. Materials and methods

5.1 Patients

The protocol for this prospective blinded study was approved by the Ethics Committee of the Medical University of Graz (internal number 19-279 ex 07/08).
Pre- and post-operative markers from 124 operations on 84 patients were analysed. Thirty-eight men (45.2%) and forty-six women (54.8%), with acute or chronic infection of knee and hip endoprosthesis or aseptic loosening of an implant were included after informed consent was obtained. The mean age of the enrolled patients was 64.8 (+/- 14.9) years, the youngest patient was 22 years old and the oldest was 88. The analysis included 54 patients with a single operation, 22 with two operations, six with three and two with four operations.

5.2 Classification

After the collection of the clinical and laboratory data the patients were divided into two groups: 1) patients with and 2) patients without bacterial infection of the orthopaedic implant. Two parameters as indicators of infection were defined: positive bacteriology or/and histologic signs of infection in intra-operatively obtained material. (16) In cases where one single bacteriological specimen was revealed to be positive but there was no clinical indication of bacterial infection and no evidence of infection at the six-month follow-up, the case was defined as not infected (suspected contamination).

One-hundred-thirty operative procedures (87 patients) were included. One patient was excluded as the preoperative measurements were missing. Of the remaining total of 129 operative procedures (87 patients), 124 (84 patients) had a clearly defined diagnosis in the histological analyses or in the bacterial cultures. In five cases (three patients) there was no clear histological diagnosis or a missing bacterial culture (drop-out of six operations or three patients).

Microbiological samples were collected before surgery by aspiration of the joint, during the operation and/or postoperatively out of drainage fluid. Parts of the synovial membrane were further collected in bovine-bouillon and synovial fluid samples were cultured for a minimum of 10 days.

Samples of the synovial membrane or pseudocapsule were taken during surgery and were examined histologically at the Department of Pathology/ Medical University of Graz. The specimens were always obtained from a defined localisation: in hip prosthesis “from the part of the membrane in contact with the neck” (16), and in knee prosthesis from the medial, parapatellar membrane. “The surfaces of pseudocapsules that face the joint cavity were identified and perpendicular to it sections were taken.” (16) The specimens for paraffin histology sections were fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin. “When more than five neutrophils per high-power field (40 times) in at least five separate microscopic fields were found”, the histology was considered positive for bacterial infection as described in the study by Bori et al. (16) Only paraffin sections and no frozen sections were used to avoid technical histological bias. (16)

5.3 Study protocol, laboratory testing

5.3.1 Testing procalcitonin

PCT was determined with a commercially available kit (Elecsys BRAHMS, Roche Diagnostics, Mannheim, Germany). It took 30 minutes to perform the test and required 30 µl of serum or EDTA-plasma. The detection limit was 0.2 ng/ml (normal<0.5 ng/ml).

5.3.2 Testing interleukin-6

IL-6 was determined with a commercially available kit (Elecsys BRAHMS, Roche Diagnostics). It took 30 minutes to perform the test and required 30 µl of serum or EDTA-plasma. The detection limit was 1.5 pg/ml (normal<10 pg/ml).

5.3.3 Testing fibrinogen

FBG was analysed by coagulometry (BCS, Siemens, Vienna, Austria) with sodium citrate blood. The normal range lies between 210 and 400 mg/dl. FBG was considered to indicate an infection, if the level was elevated over 400mg/dl.

5.3.4 Testing interferon-alpha

IFN-alpha was determined with a commercially available ELISA assay (Bender Med Systems, Vienna, Austria). The detection limit was 1 pg/ml (normal<260 pg/ml).

5.3.5 Testing leukocytes

Leukocytes were analysed by flow cytometry with EDTA-plasma (normal range 4.4-11.3 G/l). Leukocytes were considered to indicate an infection, if they were elevated over 11.3 G/l.

5.3.6 Testing C-reactive protein

CRP was analysed by immune-turbidimetry and required lithium-heparin blood. Up to a concentration of 0.5 mg/dl the CRP-level was considered normal.

5.4 Time and way of sampling

Blood was taken from the cubital vein on the day before surgery and on the first, third and seventh postoperative days to assess the parameters of interest (PCT, IL-6, IFN-alpha, leukocytes, CRP). Fibrinogen was collected only preoperatively.

5.5 Surgical procedure

The patients and operations were divided into 4 different cohorts, depending on the kind of surgical procedure.

The first cohort included the explantation of endoprosthesis and spacer-implantation, as well as lavage and insertion of a redon-drain with a change of inlay and also Girdlstone arthroplasty.

Cohort two had the purpose of comparison and enrolled aseptic loosening and change of inlay.

The procedures for cohort three were re-implantation of endoprosthesis and arthrodesis.

Cohort four enrolled all spacer-changes.

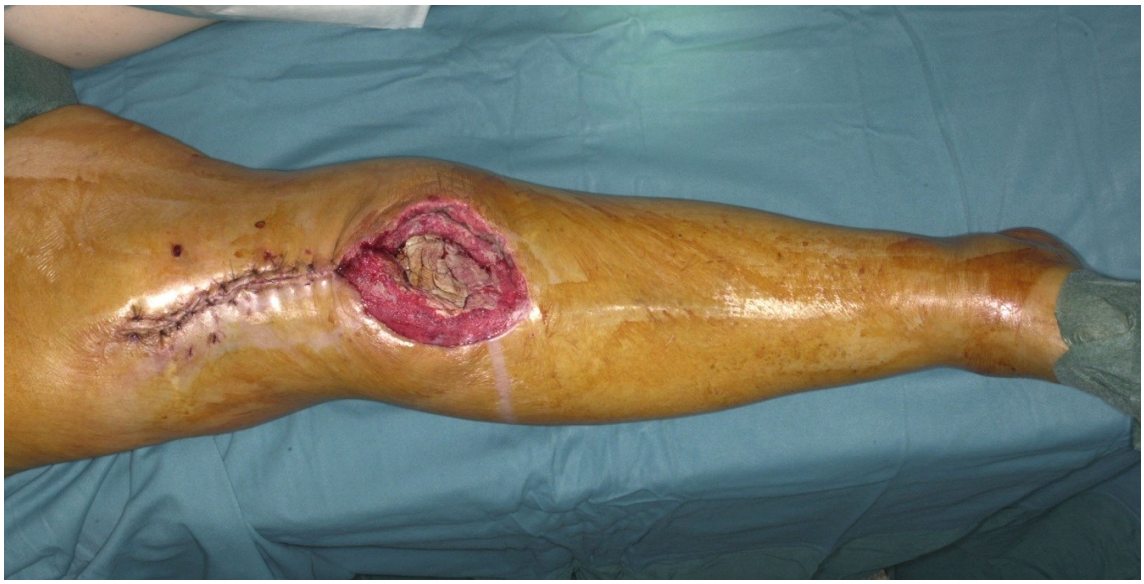


Fig 10: *Operative site of a 77 year old female patient viewing a cement-spacer, which was inserted after an acute infection of a primary total knee arthroplasty. The necrotic parts of the defect were excised and the wound covered with a vacuum assisted closure (VAC)-system.*

5.6 Exclusion criteria

To avoid interference with other inflammatory processes, patients with inflammation other than orthopaedic infection were excluded. Furthermore, patients with other possible preconditions for elevated inflammatory markers were excluded such as rheumatic diseases, adiposity (BMI>30), viral infections, malignancies or heavy smokers. Patients with renal or hepatic failure were also excluded.

5.7 Statistical methods

SPSS was used for all statistical analysis. The parameters of interest were PCT, IL-6, FBG, IFN-alpha, leukocyte count and CRP. Logistic regressions were performed to determine whether these data differed preoperatively regarding histology. To account for all measurements, non-linear mixed models were additionally calculated. For the significant variables, receiver operating characteristic (ROC) -curves were plotted. P-values of <0.05 were regarded as statistically significant.

6. Results

Seventy-eight (62.9%) operations were defined as septic (infection group), and forty-six (37.1%) operations had no signs of infection (non-infection group). The median age of patients was 65.7 (+/- 15.8) years in the infection group and 65.1 (+/- 14.6) years in the non-infection group. The knee was affected in 60.0% of the patients with infection and the hip in 40.0%. In the non-infection group 44.8% of the patients had knee surgery and 55.2% hip surgery.

6.1 Results of serum parameters

6.1.1 Results of procalcitonin

Based on the data for the first operation (one operation per patient), the preoperative PCT value was a significant predictor of infection (p=0.038). Based on the data for all operations, the preoperative PCT value was also significant (p=0.015). The PCT value of 0.055 had a sensitivity of 0.81 and a specificity of 0.54. The value of 0.35 had a sensitivity of 0.90 and a specificity of 0.67 (Fig. 11a).

Mean PCT did not change over time (p=0.816); furthermore there was no difference in progress for the two groups with and without infection (p=0.093). Changes from preoperative values to postoperative values in both groups, with and without infection, were significantly different from zero at any point except day one in the infection group (p=0.390). While differences from the preoperative value were significant in both groups, this change stabilized in the group without infection after the first day. In the infection group, however, there were more significant changes thereafter, namely between postoperative day three and day one, day seven and day one and day seven and day three (Fig. 12a).

6.1.2 Results of interleukin-6

Based on the data for the first operation only, preoperative IL-6 was not a significant predictor for infection (p=0.056). With the data for all operations, IL-6 was significant (p=0.012) for detecting infection. For IL-6 the value of 4.7 had a sensitivity of 0.81 and a specificity of 0.68. The value of 2.55 had a sensitivity of 0.92 and a specificity of 0.59 (Fig. 11b).

The progress of IL-6 between the groups with and without infection was statistically significantly different over time (p=0.001). Changes from preoperative values to postoperative day three and day seven in both groups were significant. In both groups the differences between day three and day one and day seven and day one were statistically significant (Fig. 12b).

6.1.3 Results of fibrinogen

When only the data of the first operation were evaluated, the preoperative FBG value was a significant predictor for infection (p<0.001). When the data of all operations were considered, the FBG value was also significant (p<0.001). For FBG, a value of 573.5 had a sensitivity of 0.81 and a specificity of 0.25. The value of 519 has a sensitivity of 0.90 and a specificity of 0.34 (Fig. 11c).

6.1.4 Results of interferon-alpha

Counting only the first operation, preoperative IFN-alpha was not a significant predictor for infection (p=0.402); this was similar when all operations were considered (p=0.432). In both histological groups the curves neither decreased nor increased (p=0.217). Changes from preoperative values in both groups were not significant at any time points and the differences between the time points were never significant (Fig 12c).

6.1.5 Results of leukocytes

Taking into account only the first operation the preoperative level of leukocytes was a statistically significant predictor for histology (p=0.001). The value of 6.27 had a sensitivity of 0.80 and a specificity of 0.68. The value of 5.48 had a sensitivity of 0.91 and a specificity of 0.34. Taking into account the entirety of operations, the level of leukocytes was also significant (p<0.001). The value of 6.58 has a sensitivity of 0.81 and a specificity of 0.59. The value of 5.68 has a sensitivity of 0.90 and a specificity of 0.39 (Fig. 11d).

The progress between the two curves was statistically significantly different over time (p=0.012). While differences from the baseline value were significant in both groups for day three and day seven, this change stabilized in the group without infection after the third day. In the infection group, however, there was also a significant change between day seven and day one (Fig. 12d).

6.1.6 Results of C-reactive protein

When only the first operation was evaluated, preoperative CRP was a significant predictor for the infection group (p<0.001). For CRP the cut-off point of 23.65 had a sensitivity of 0.80 and a specificity of 0.79. The cut-off point of 10.25 had a sensitivity of 0.91 and a specificity of 0.72. Counting all 124 operations, CRP was also significant (p<0.001). The value of 21.95 has a sensitivity of 0.81 and a specificity of 0.80. The value of 11.00 has a sensitivity of 0.90 and a specificity of 0.74 (Fig. 11e).

In the group with infection, values were higher pre-operatively and decreased post-operatively; in the group without infection the values increased post-operatively and decreased after day three. The progress between the two curves differs in a statistically significant manner over time (p<0.001) (Fig. 12e).

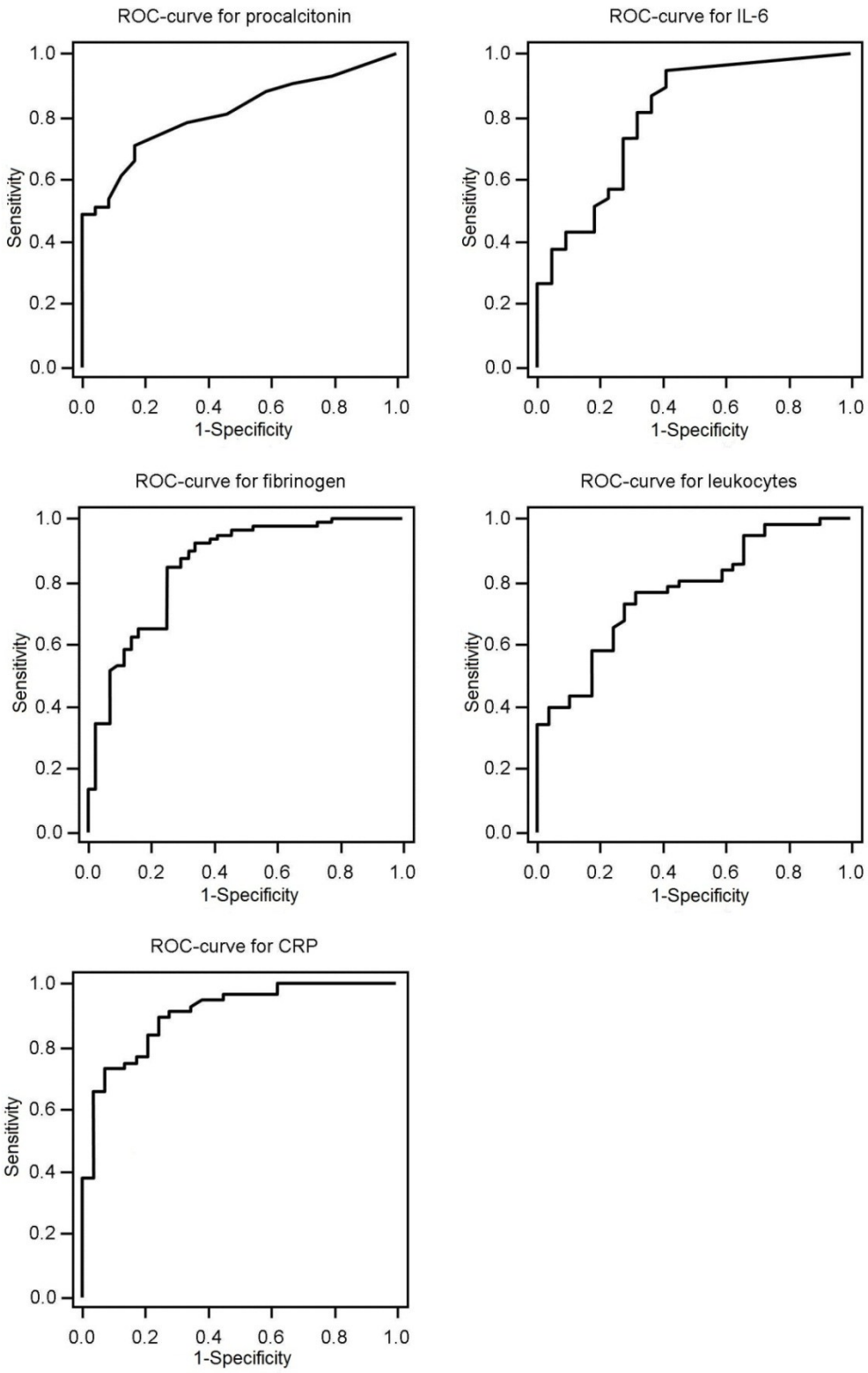
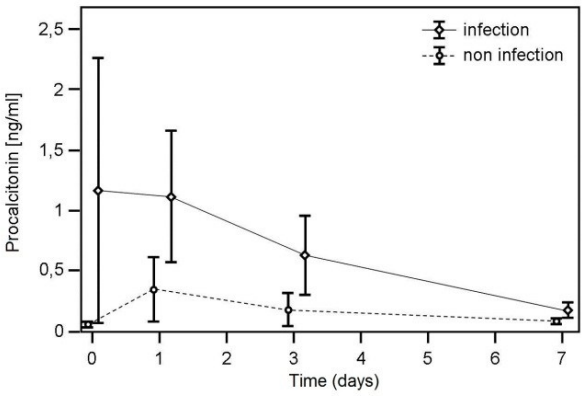
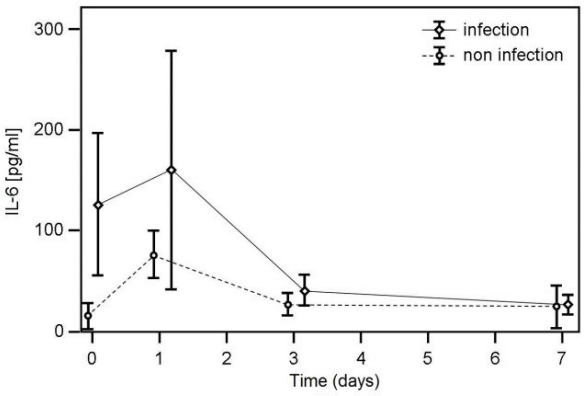


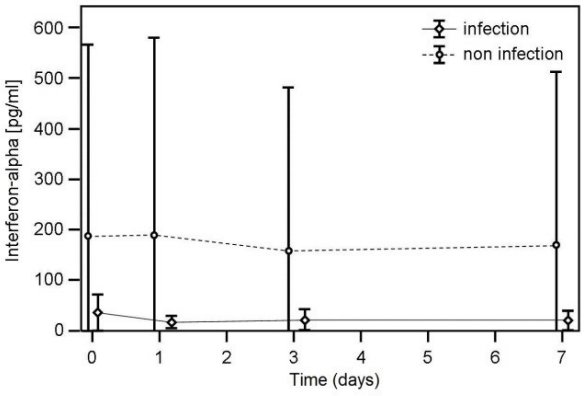
Fig 11: *The predictive performance of preoperative values of PCT, IL-6, FBG, leukocytes and CRP for infection. The ROC curve is a graphic plot of the positive rate (sensitivity) versus the false positive rate (specificity) for detection of infection. IFN-alpha was not significant, so ROC was not calculated.*



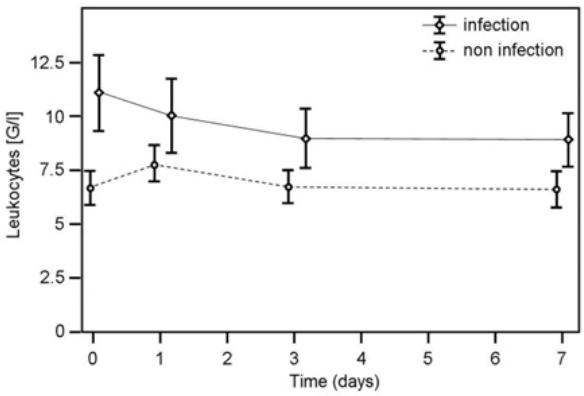
PCT is highly elevated in the infection group, but the variability among patients is very high. After day 1 it decreases rapidly until it reaches normal levels at day 7. In the non-infection group PCT is not detectable or very low. It increases slightly on day 1 with a fast decline afterwards.



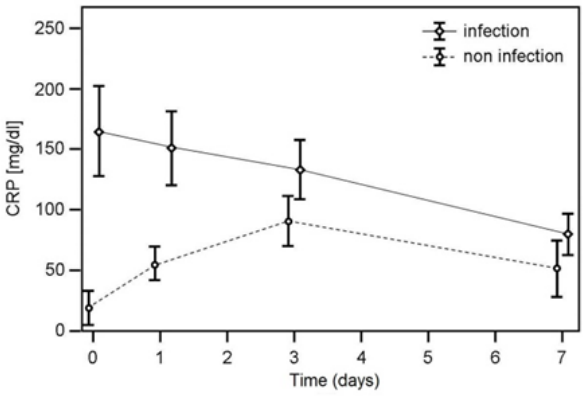
IL- 6 is elevated preoperatively in the infection group with a peak at day 1. There is also a very high variability among patients on this day. This is followed by a fast decrease on day 3, where it reaches baseline levels.



The curve of IFN-alpha does not change over time, either in the infection group, or in the non-infection group.



In the infection group leukocytes are slightly increased preoperatively, and already reach a normal level on day 1. The decline proceeds until day 3, after which a baseline level is reached. In the non-infection group leukocytes rise on day 1; after day 3 they again reach their baseline level.



CRP is highly elevated in the infection group and decreases continuously after surgery until day 7, while in the non-infection group the CRP levels increase after surgery, with a peak at day 3. This peak is lower than in the infection group. The decrease is similar to the infection group, but the CRP levels stay lower.

Fig 12: *Measurements of PCT, IL-6, IFN-alpha, leukocytes and CRP in time: day 0= day before operation; day 1= day of operation. The significant estimate of the baseline values indicated that variability among patients before operation is very high, which is an important factor to be considered when estimating the value of the variable at later times. FBG was only analysed preoperatively, so no time curve was possible.*

6.2 Outcome

From the total of 124 operations, in 68 cases (54.8%) the patient was healed. In 34 cases (27.3%) a revision was planned in advance and in 22 cases (17.7%) a second operation was not planned.

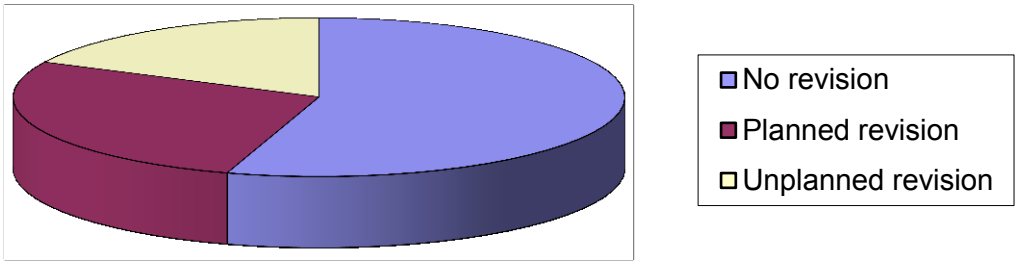


Fig 13: *Outcome of all 124 operations.*

In the infection group in 32 cases (41.0%) a revision was planned, in 19 cases (24.4%) a second intervention was not intended and in 27 cases (34.6%) only one operation was needed.

The results in the non-infection group are quite different. In 41 cases (89.1%) a single operation was performed and only in 2 cases (4.3%) a planned revision was needed. In 3 cases (6.5%) the second surgical intervention was not intended.

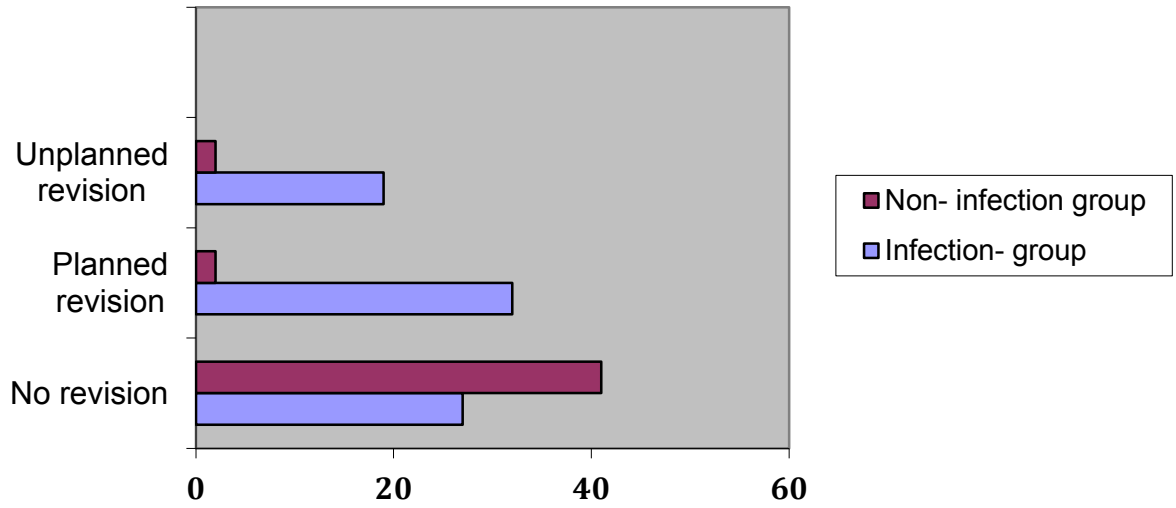


Fig 14: *Comparison of the outcome in the infection-group and the non-infection group.*

6.3 Identified bacteria

In 44 cases out of all operations bacteria could be identified. In the infection group staphylococci were found in 29 cases (52.7%), streptococci in 7 (12.7%) and enterococci, Propionibacterium acnes, as well as Aerococcus viridans were found in one case each (1.8%). In 16 cases (20.5%) in the infection group with positive histology for infection, no bacteria could be isolated after 14 days´ incubation.

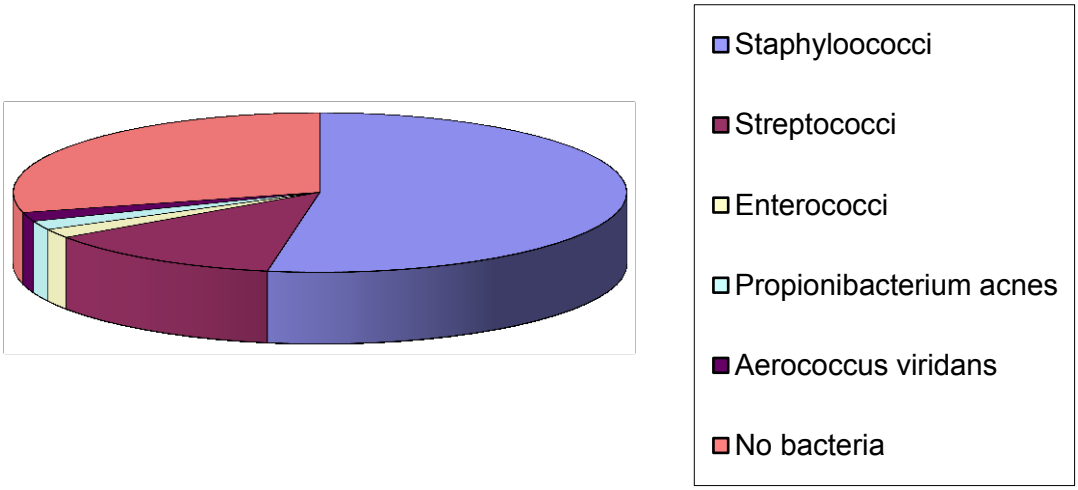


Fig 15: *Identified bacteria in the infection group.*

In the non-infection group, in three cases (10.3%) the isolated bacteria (all of them obtained postoperatively from the redon-drain) was a staphylococcus species. Enterobacter cloacae was found in one case (3.4%), as was Proteus mirabilis (3.4%). There were no clinical or laboratory signs of bacterial infection and the six-month follow-up gave no hint of infection; these five cases were defined as not infected and understood to reflect contamination.

6.4 Result for the cohorts

Considering all 124 operations, cohort one includes 73 operations, 34 (27.4%) explantations of endoprosthesis with spacer-implantation, 35 (28.2%) cases of lavage and insertion of a redon-drain with change of the inlay and 4 (3.2%) Girdlestone-arthorplasties.

Cohort two contains a total of 22 operations, 20 (16.1%) cases of aseptic loosening and 2 (1.6%) inlay changes.

In cohort three are also 22 surgical procedures, 19 (15.3%) re-implantations of endoprosthesis and 3 (2.4%) cases of arthrodesis.

Cohort four encases 7 (5.6%) changes of spacers.

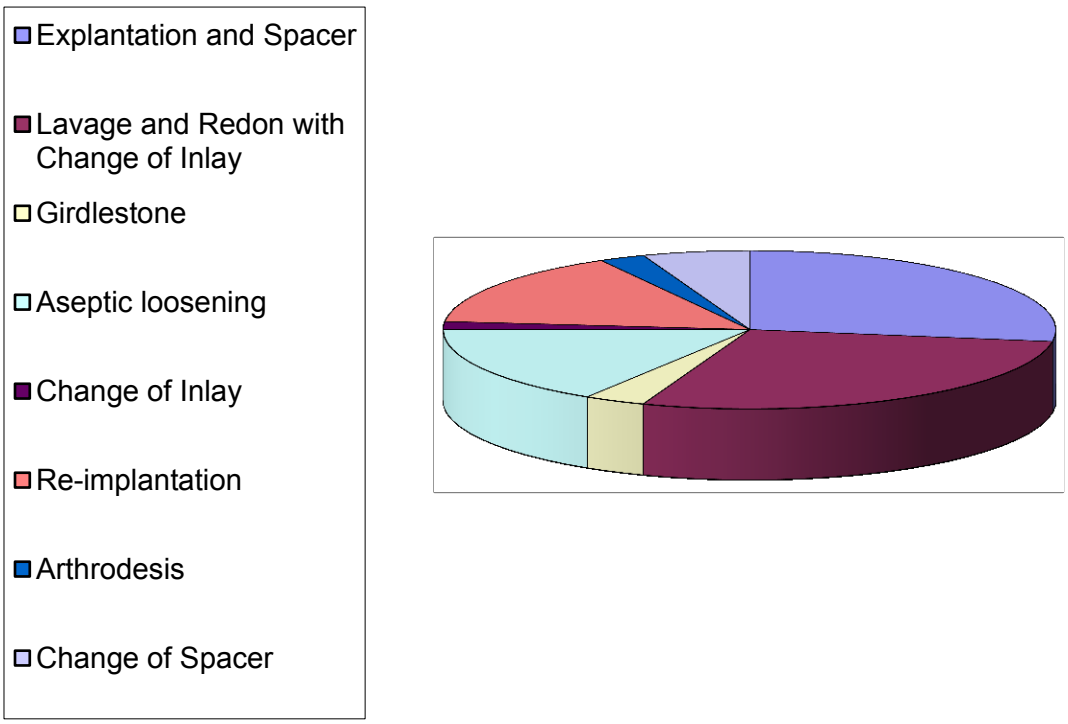


Fig 16: *Surgical procedures of all 124 operations.*

In the infection-group cohort one included 68 operations, 33 (42.3%) explantations of endoprosthesis with spacer-implantation, 32 (41.0%) cases of lavage and redon-insertion with change of the inlay and 3 (3.8%) Girdlestone-arthroplasties.

In cohort two is 1 (1.3%) case of before considered aseptic loosening and 1 (1.3%) change of inlay.

Cohort three consists of 3 (3.8%) re-implantations of an endoprosthesis.

In cohort four are 5 (6.4%) cases of spacer-changes.

The results in the non-infection group are contrary to the infection-group.

In cohort one are 5 cases, 1 (2.2%) explantation, 3 (6.5%) cases of lavage with redon-insertion and change of the inlay and 1 (2.2%) Girdlestone-arthroplasty.

Cohort two consists of 20 surgical procedures, 19 (41.3%) cases of aseptic loosening and 1 (2.2%) change of the inlay.

Cohort three includes 19 operations, 16 (34.8%) re-implantations and 3 (6.5%) cases of arthrodesis.

In cohort four are 2 (4.3%) changes of spacer.

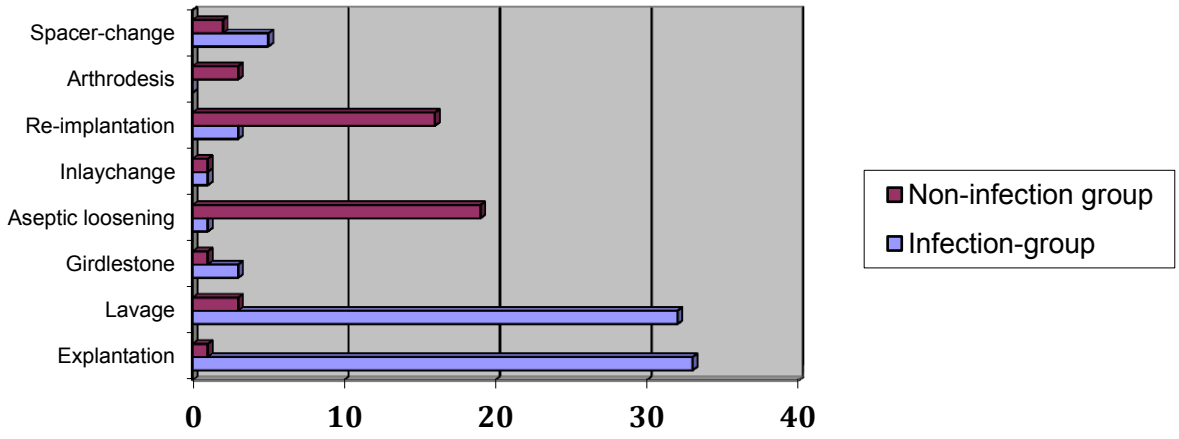


Fig 17: *Comparison of the surgical procedures in the infection-group and the non-infection group.*

7. Discussion

In this study the serum parameters PCT, IL-6, FBG, IFN-alpha, leukocyte count and CRP were analysed with respect to their sensitivity and specificity in detecting bacterial joint infection in the field of orthopaedic surgery. This is of major interest, because bacterial infections of joints and implants are common complications in orthopaedic surgery, leading to longer hospital stays and a higher morbidity when not diagnosed in time; but in case of aseptic loosening, the reason for unnecessary and inadequate therapy.

Clinical signs of infection (swelling and erythema of the joint, positive scintigraphy, pus, fever and elevated serum markers like CRP and WBC), can be false negative or positive. The usefulness of microbiological samples taken prior to or during surgery is debateable, since infections of endoprosthesis are usually associated with a lower number of pathogens. And microbial contamination during sample taking is common. (29,39)

In this study a system to determine the diagnosis of bacterial infection was defined, based mainly on histologic analyses of the species obtained intraoperatively and bacterial culture. Only in unclear cases blood parameters were used for a definitive diagnosis. Compared with all other diagnostic tools, a combination of results from bacteriology and histological analysis of the interface membrane has the highest diagnostic value. In this study the protocol following Bottner et al. (4) was used - the definition for septic endoprosthesis was a positive culture and histological evidence of deep infection.

Worthington et al., however, established the diagnosis of septic or aseptic loosening using criteria including pre-surgical imaging data, elevated pre-surgical serum CRP or positive microbiology cultures yielding indistinguishable micro-organisms from two or more clinical samples. (29)

In contrast to this, Uckay et al. based “the diagnosis of infection upon the presence of pus and of pathogens obtained from intra-operative microbiological cultures.” (40)

Fottner et al. based their diagnosis of septic or aseptic loosening on the presence of a positive bacterial culture obtained from pre-operative joint taps (5) and report 100% positive results of cultures for septic arthritis (41), which seems high.

Elsewhere in the current literature, positive cultures of septic arthritis have not been reported to be as sensitive. (41)

Hügle et al. defined septic arthritis by detecting “bacteria in the synovial fluid by Gram stain or culture, or detection of bacteria in blood cultures in the presence of arthritis”. (42)

In Savarino et al., PJI was diagnosed when two out of four criteria were fulfilled. These criteria included microbiological growth in at least two cultures, the presence of pus in aspirate or preoperative tissue, “tissue polymorphonuclear neutrophils” or elevated serum parameters (ESR, CRP and FBG). (3)

Schindler et al. defined PJI by purulence of the joint and/or three or more cultures of the same microorganism. (39)

Piper et al. considered PJI, if one out of four criteria (pus around the implant, histologic proven infection, fistulation or the same bacteria in “periprosthetic tissue culture and sonicate fluid culture”) was fulfilled. (43)

The fundamental premise for evaluating sensitivity and specificity of a laboratory marker for joint infection is a clear definition of an ongoing septic process inside a joint. Because it is sometimes not possible to detect bacteria in joint or blood culture and the specificity of histological signs for infections is low. (16) This seems to be a major bias in most of the above-mentioned studies.

7.1 Sensitivity and specificity of procalcitonin

In this study the preoperative PCT level is significant; when all operations are evaluated the value of 0.055 ng/ml has a sensitivity of 81% and a specificity of 54%. The value of 0.35ng/ml has a sensitivity of 90% and a specificity of 67%. This study also shows that the variability of PCT among patients is very high before surgery. That leads to the conclusion that a single PCT value is of poor informative value. Therefore it is inevitable to determine the baseline PCT value for each patient at admission and to make a follow up.

With further development, PCT may be useful to distinguish between bacterial infections of the joint and other causes of postoperative inflammation.

Infections after orthopaedic surgery are responsible for longer hospital stays, more complications and higher costs for the health care system. Timely and suitable

antibiotic, and when necessary surgical treatment should be undertaken to reduce patient morbidity and mortality. (1) PCT can help to reveal if an antimicrobial therapy is effective or not, and so could reduce the duration of medication and the length of hospitalization. (19) But according to a study by Ruiz-Alvarez PCT levels are not directly correlated with mortality. (44)

In a study by Bottner et al. PCT has at a cut-off level of 0.3 ng/ml a sensitivity of 0.33 and a specificity of 0.98, so it is very specific for bacterial infections, but not very sensitive. (4) This is oppositional to the findings of this study, where the sensitivity is higher than the specificity. Also Bottner suggests that a determination of CRP and IL-6 can identify all patients with a deep implant infection. The evaluation of PCT he recommends to avoid false positive results. (4)

Fottner demonstrates in his study that the sensitivity (53.3%) of PCT at a cut-off level of 0.5 ng/ml is not high enough to discriminate between infectious and non-infectious cases of arthritis. So he suggests the reduction of the cut-off level of PCT to raise the diagnostic value. (5)

The study by Hügle et al. shows that serum PCT is an effective marker to distinguish between septic and non-septic arthritis by using a highly sensitive PCT test kit. He found significantly higher PCT levels in patients with septic arthritis than patients with non-septic arthritis. “At a cut-off level of 0.1 (0.25) ng/ml, sensitivity for septic arthritis was 100 (93) % and specificity was 46 (75) %.” (42)

Hunziker finds that after orthopaedic surgery single PCT values are of moderate use to diagnose infection. But that the development of PCT levels differs between infection and non-infection. Also commonly used parameters like CRP and WBC are similar for all patients and are not at all helpful in discriminating between true postoperative infection and the non-specific systemic inflammatory response due to surgical stress or underlying trauma. (1)

Martinot et al. concluded that “serum PCT is a poorly sensitive but specific marker of bacterial arthritis. A low serum PCT level does not rule out bacterial arthritis and only positive values of PCT have a diagnostic value in bacterial arthritis.” (15) In the study by Martinot et al. serum PCT (>0.5 ng/ml) revealed 54.5% sensitivity and 93.5% specificity for bacterial arthritis. (15)

Uckay comes to the conclusion that serum PCT has no additional value in the follow up after septic orthopaedic surgery. (40) But with a cost of approximately 50-70$ the evaluation of PCT is a quite expensive laboratory marker. Therefore PCT does not have an advantage in clinical use in comparison to CRP. (5,40)

In a study by Worthington et al., serum PCT was not helpful to distinguish between infection and non-infection in loosening after total-hip-arthroplasty. (29)

Considering the quite different results of all studies it is debateable, if the expensive parameter PCT has enough additional value in the follow-up of infected patients and therefore is justified in daily clinical practice. Maybe further studies with a bigger study population can clarify the situation.

7.2 Sensitivity and specificity of interleukin-6

Based on the data for the first operation, preoperative IL-6 was not a significant predictor for infection. But evaluating the data of all operations, IL-6 was significant for detecting infection. The value of 4.7 pg/ml had a sensitivity of 81% and a specificity of 68%. The value of 2.55 pg/ml had a sensitivity of 92% and a specificity of 59%.

In a study by Bottner et al., IL-6 has with a level of 12 pg/ml a sensitivity of 0.95 and a specificity of 0.87. In his opinion a combination of CRP and IL-6 could identify all cases of PJI. (4)

Worthington finds in his study a sensitivity of 81% and a specificity of 77% at a cut-off level of 9 pg/ml. He concludes that IL-6 could be used in diagnosing periprosthetic inflammation. (29)

In the study by Wirtz et al. IL-6 levels are highly correlated to the extent of infection. Because of its biological characteristics it increases and decreases more quickly than CRP. Therefore it could be a more useful inflammation marker than CRP. (28)

In this study the parameter IL-6 was significant for bacterial infection but CRP was superior in sensitivity and specificity. Keeping in mind that IL-6 increases earlier

than CRP with infection, IL-6 seems useful for early detection of a septic process and for selecting antibiotic therapy.

7.3 Sensitivity and specificity of fibrinogen

FBG is a significant marker of infection. When evaluating the data of all operations, the value of 573.5 mg/dl had a sensitivity of 81% and a specificity of 25%. The value of 519 mg/dl had a sensitivity of 90% and a specificity of 34%.

In Savarino et al. FBG had at a cut-off level of 432 mg/dl a sensitivity of 0.93 and a specificity of 0.86. (3)

Sedlar found that FBG was decreased after surgery and stayed at low levels up to 48h afterwards. (31)

Beloosesky came to the conclusion that FBG is not helpful in detecting complications after hip-fracture surgery. (45)

Increased fibrinogen levels in plasma indicate a tendency toward hypercoagulation and in consequence entail a higher risk of thrombosis. The preoperative value of serum FBG was significantly higher in the infection group.

As it is part of the acute-phase reaction, it could be a useful marker in the diagnostic process of joint infection. Further studies will be needed to evaluate the development of FBG in the follow up after septic joint surgery.

7.4 Sensitivity and specificity of interferon-alpha

IFN-alpha stayed at a baseline level in both groups and was not useful at detecting a bacterial infection of a joint or endoprosthesis. This can be explained by the fact that IFN-alpha has an important role in antiviral immunity, but not in antimicrobial immunity. (32)
There are no other studies to which the results of this study could be compared.

7.5 Sensitivity and specificity of leukocytes

In this study the leukocyte count of 6.58 G/l has a sensitivity of 81% and a specificity of 59%. The value of 5.68 G/l has a sensitivity of 90% and a specificity of 39%.

In a study by Fottner et al. WBC had a sensitivity of 20% and a specificity of 100% for the correct diagnosis of septic arthritis. (5)

According to a study by Hügle et al. the median WBC has no statistically significant correlation with septic arthritis. So he concluded that WBC is not useful for the detection of septic arthritis. (42)

In Savarino et al. the leukocyte count revealed no statistically significant difference between infection of a total knee arthroplasty and the comparison group. (3)

As every kind of inflammation leads to an elevation of the WBC, it is a sensitive but very unspecific marker of infection.

7.6 Sensitivity and specificity of C-reactive protein

In this study CRP level of 21.95 mg/dl has a sensitivity of 81% and a specificity of 80%, while the value of 11.00 mg/dl has a sensitivity of 90% and a specificity of 74%.

In a study by Bottner et al. CRP had at a cut-off level of 3.2 mg/dl a sensitivity of 0.95 and a specificity of 0.96. He suggests that a combination of CRP and IL-6 could identify all patients with infection of endoprosthesis. (4,4)

Fottner finds in his study a sensitivity of 100% and a specificity of 0% for CRP in detecting septic arthritis with a cut-off level of 0.5 mg/dl. Thus it is not possible to make a diagnosis using CRP alone. (5)

In a study by Hügle et al. CRP has at a cut-off level of 118 mg/l a sensitivity of 79% and a specificity of 68%. That is lower than in this study, although the cut-off level is higher. Hügle comes to the conclusion that PCT is more accurate in the diagnosis of bacterial arthritis. (42)

Piper et al. (43) revealed in their study for CRP at a cut-off level of 14.5 mg/l a sensitivity of 79% and a specificity of 88% to detect PJI of the knee and at a cut-off level of 10.3 mg/l a sensitivity of 74% and specificity of 79% for detection of hip PJI.

After all CRP is not a very sensitive marker for endoprosthetic infection, because it can also be increased without underlying infection. (1) But with a cost of approximately 10-20$ it is a rather cheap laboratory test. (39,40)

7.7 Time progression of the parameters

PCT is highly elevated in the infection group, but the variability among patients is very high. After day 1 it decreases rapidly until it reaches normal levels at day 7. In the non-infection group PCT is not detectable or very low. It increases slightly on day 1 with a fast decline afterwards.

IL-6 is elevated preoperatively in the infection group with a peak at day 1. There is also a very high variability among patients on this day. This is followed by a fast decrease on day 3, where it reaches baseline levels.

FBG was only analysed preoperatively, therefor no time curve was possible.

The curve of IFN-alpha does not change over time, either in the infection group, or in the non-infection group.

In the infection group leukocytes are slightly increased preoperatively, and already reach a normal level on day 1. The decline proceeds until day 3, after which a baseline level is reached. In the non-infection group leukocytes rise on day 1; after day 3 they again reach their baseline level.

CRP is highly elevated in the infection group and decreases continuously after surgery until day 7, while in the non-infection group the CRP levels increase after surgery, with a peak at day 3. This peak is lower than in the infection group. The decrease is similar to the infection group, but the CRP levels stay lower.

7.8 Discussion of identified bacteria

In this study in the infection-group in 52.7% staphylococci-species could be identified. In 12.7% streptococci caused the PJI. Enterococci, Propionibacterium acnes and Aerococcus viridans were proven each in 1.8%. Also in 20.5% of all cases no bacteria could be isolated after 14 days of incubation. These findings are similar to the results of other studies.

In a study by Fitzgerald staphylococci-species could be isolated in 25 cases of 49. Besides 9 cases of streptococci were identified. Also Propionibacterium acnes were proven in 1 case and in 2 cases enterobacter species were found. (11)

In the study by Schindler in recurrent PJI the causing pathogen was in 33% Staphylococcus aureus, in 17% coagulase-negative staphylococci and in 25% streptococcus species. (39)

Also in a study by Bottner et al. the main pathogens were Staphylococcus and Streptococcus species. (4)

Fottner found in his study also 7 cases of Staphylococcus aureus, 3 cases of coagulase-negative staphylococci and 2 cases of streptococci. (5)

7.9 Limitations of the study

The relatively small sample size and heterogeneity of the study population and pathogens are a limitation to the study. Furthermore, the extent of surgical procedure differed largely. When more than one operation is counted for each patient, the different baseline values of the laboratory markers between the patients could be a bias. Another limitation is that fibrinogen could not be evaluated postoperatively.

8. Conclusion

This study showed that - besides conventional parameters like leukocyte count and CRP - PCT, IL-6 and FBG showed significance for detecting joint infection. Interferon-alpha was not elevated in PJI.

However, these serum parameters alone are not reliable enough to reach the correct diagnosis in joint infections. The therapeutic decisions are still difficult to make and depend on the experience of the attending physician. Various factors are influential in the decision-making process such as fever, pain, swelling and erythema of the joint. It is still not possible to give an exact cut-off point for conventional inflammation markers that could be used in the decision-making process.

In my opinion – considering the low costs and high significance of CRP – PCT and IL-6 have low additional informative value in the decision as to whether an implant is infected or not. The low impact of this additional information may not justify higher costs. FBG is usually analysed for preoperative coagulation control anyway and gives a hint if there is an ongoing bacterial infection.

An additional analysis of the parameters PCT and IL-6 is nevertheless worthwhile in special cases, when it is totally unclear whether there is a bacterial implant infection or an aseptic inflammation and when it is important to analyse the impact of an antibiotic therapy. In such cases these new parameters may give a supplementary hint for deciding whether or not a bacterial infection is present.

9. References

(1) Hunziker S, Hugle T, Schuchardt K, Groeschl I, Schuetz P, Mueller B, et al. The value of serum procalcitonin level for differentiation of infectious from noninfectious causes of fever after orthopaedic surgery. J Bone Joint Surg Am 2010 January;92(1):138-148.

(2) Parvizi J, Jacovides C, Zmistowski B, Jung KA. Definition of periprosthetic joint infection: Is there a consensus?. Clin Orthop Relat Res 2011 July;13:e-pub, ahead of print.

(3) Savarino L, Tigani D, Baldini N, Bochicchio V, Giunti A. Pre-operative diagnosis of infection in total knee arthroplasty: An algorithm. Knee Surg Sports Traumatol Arthrosc 2009 June;17(6):667-675.

(4) Bottner F, Wegner A, Winkelmann W, Becker K, Erren M, Gotze C. Interleukin-6, procalcitonin and TNF-alpha: markers of peri-prosthetic infection following total joint replacement. J Bone Joint Surg Br 2007 January;89-B(1):94-99.

(5) Fottner A, Birkenmaier C, von Schulze Pellengahr C, Wegener B, Jansson V. Can serum procalcitonin help to differentiate between septic and nonseptic arthritis?. Arthroscopy 2008 February;24(2):229-233.

(6) Bauer TW, Parvizi J, Kobayashi N, Krebs V. Diagnosis of periprosthetic infection. J Bone Joint Surg Am 2006 April;88-A(4):869-882.

(7) Bo M, Raspo S, Morra F, Isaia G, Cassader M, Fabris F, et al. Body fat and C-reactive protein levels in healthy non-obese men. Nutr Metab Cardiovasc Dis 2004 April;14(2):66-72.

(8) Owen N, Poulton T, Hay FC, Mohamed-Ali V, Steptoe A. Socioeconomic status, C-reactive protein, immune factors, and responses to acute mental stress. Brain Behav Immun 2003 Aug 2003;17(4):286-295.

(9) Pramod J, Singh A. Sepsis Biomarkers. Am J Med 2008 June;121(6):e11.

(10) Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Eng J Med 2004 October;351(16):1645-1654.

(11) Fitzgerald RH, Nolan DR, Ilstrup DM, Van Scoy RE, Washington JA, Coventry MB. Deep wound sepsis following total hip arthroplasty. J Bone Joint Surg Am 1977 October;59(7):847-855.

(12) Laffey JG, Boylan JF, Cheng DCH. The systemic inflammatory response to cardiac surgery. Anesthesiology 2002 July;97(1):215-252.
(13) Seifert J, Apostel S, Frank M, Stengel D, Ekkernkamp A. Limits of clinical pathways. Implant-associated infections. Orthopade 2010 August;39(8):777-784.
(14) Scheffer D, Hofmann S, Pietsch M, Wenisch C. Infections in orthopedics and traumatology. Pathogenesis and therapy. Orthopade 2008 July;37(7):709-718.
(15) Martinot M, Sordet C, Soubrier M, Puechal X, Saraux A, Liote F, et al. Diagnostic value of serum and synovial procalcitonin in acute arthritis: A prospective study of 42 patients. Clin Exp Rheumatol 2005 May;23(3):303-310.
(16) Bori G, Munoz-Mahamud E, Garcia S, Mallofre C, Gallart X, Bosch J, et al. Interface membrane is the best sample for histological study to diagnose prosthetic joint infection. Mod Pathol 2011 April;24(4):579-584.
(17) Becker KL, Nylen ES, White JC, Muller B, Snider RH. Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: A journey from calcitonin back to its precursors. J Clin Endocrinol Metab 2004 April;89(4):1512-1525.
(18) Schuetz P, Batschwaroff M, Dusemund F, Albrich W, Burgi U, Maurer M, et al. Effectiveness of a procalcitonin algorithm to guide antibiotic therapy in respiratory tract infections outside of study conditions: A post-study survey. Eur J Clin Microbiol Infect Dis 2010 March;29(3):269-277.
(19) Hatzistilianou M. Diagnostic and prognostic role of procalcitonin in infections. ScientificWorldJournal 2010 October;10:1941-1946.
(20) Gendrel D, Raymond J, Assicot M, Moulin F, Iniguez JL, Lebon P, et al. Measurement of procalcitonin levels in children with bacterial or viral meningitis. Clin Infect Dis 1997 June;24(6):1240-1242.
(21) Gendrel D, Bohuon C. Procalcitonin, a marker of bacterial infection. Infection 1997 May/June;25(3):133-134.
(22) Müller B. White JC. Nylén ES. Snider RH. Becker KL. Habener JF. Ubiquitous expression of the calcitonin-I gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab 2001 January;86(1):396-404.
(23) McGee KA., Baumann NA. Procalcitonin: Clinical utility in diagnosing sepsis. CLN 2009 July;35(7):10-12.

(24) Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: A systematic review and meta-analysis. Clin Infect Dis 2004 July;39(2):206-217.
(25) Rau B, Steinbach G, Gansauge F, Mayer JM, Grunert A, Beger HG. The potential role of procalcitonin and interleukin 8 in the prediction of infected necrosis in acute pancreatitis. Gut 1997 December;41(6):832-840.
(26) Viallon A, Zeni F, Lambert C, Pozzetto B, Tardy B, Venet C, et al. High sensitivity and specificity of serum procalcitonin levels in adults with bacterial meningitis. Clin Infect Dis 1999 June;28(6):1313-1316.
(27) Robinson JO, Lamoth F, Bally F, Knaup M, Calandra T, Marchetti O. Monitoring procalcitonin in febrile neutropenia: What is its utility for initial diagnosis of infection and reassessment in persistent fever? PloS one 2011 April;6(4):e18886.
(28) Wirtz DC, Heller KD, Miltner O, Zilkens KW, Wolff JM. Interleukin-6: A potential inflammatory marker after total joint replacement. Int Orthop 2000;24(4):194-196.
(29) Worthington T, Dunlop D, Casey A, Lambert P, Luscombe J, Elliott T. 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 2010;67(2):71-76.
(30) Selberg O , Hecker H, Martin M, Klos A, Bautsch W, et al. 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 2000 August;28(8):2793-2798.
(31) Sedlar M, Kudrnova Z, Trca S, Mazoch J, Malikova I, Kvasnicka J, et al. Inflammatory response in patients undergoing hip surgery due to osteoarthrosis or different types of hip fractures. Osteoarthritis Cartilage 2008 January;16(1):26-33.
(32) Anders H, Lichtnekert J, Allam R. Interferon-alpha and -beta in kidney inflammation. Kidney Int 2010 May;77(10):848-854.
(33) Zhang S, Boisson-Dupuis S, Chapgier A, Yang K, Bustamante J, Puel A, et al. Inborn errors of interferon (IFN)-mediated immunity in humans: Insights into the respective roles of IFN-alpha/beta, IFN-gamma, and IFN-lambda in host defense. Immunol Rev 2008 December;226(1):29-40.

(34) Moulin F, Raymond J, Iniguez J, Ravilly S, Lebon P, Gendrel D. Serum alpha-interferon in lower respiratory tract infections of children. Pediatr Infect Dis J 1996 October;15(10):883-886.
(35) Bauer C. Immunabwehr. In: Klinke R, Silbernagl S, editors. Lehrbuch der Physiologie. 4., korrigierte Auflage. Stuttgart New York : Thieme; 2003. p. 197-208.
(36) Silbernagl S. Entzündung. In: Silbernagl S, Lang F, editors. Taschenatlas der Pathophysiologie. 2., korrigierte Auflage. Stuttgart New York : Thieme; 1998. p. 48-51.
(37) Brunkhorst FM, Eberhard OK, Brunkhorst R. Discrimination of infectious and noninfectious causes of early acute respiratory distress syndrome by procalcitonin. Crit Care Med 1999 October;27(10):2172-2176.
(38) Prat C, Dominguez J, Rodrigo C, Gimenez M, Azuara M, Jimenez O, et al. Procalcitonin, C-reactive protein and leukocyte count in children with lower respiratory tract infection. Pediatr Infect Dis J 2003 November;22(11):963-967.
(39) Schindler M, Christofilopoulos P, Wyssa B, Belaieff W, Garzoni C, Bernard L, et al. Poor performance of microbiological sampling in the prediction of recurrent arthroplasty infection. Int Orthop 2011 May;35(5):647-654.
(40) Uckay I, Garzoni C, Ferry T, Harbarth S, Stern R, Assal M, et al. Postoperative serum pro-calcitonin and C-reactive protein levels in patients with orthopaedic infections. Swiss Med Wkly 2010 December;140:w13124.
(41) Kesemenli CC, Memisoglu K. Differentiation between septic and nonseptic arthritis. Arthroscopy 2008 July;24(7):852.
(42) Hugle T, Schuetz P, Mueller B, Laifer G, Tyndall A, Regenass S, et al. Serum procalcitonin for discrimination between septic and non-septic arthritis. Clin Exp Rheumatol 2008 May/June;26(3):453-456.
(43) Piper KE, Fernandez-Sampedro M, Steckelberg KE, Mandrekar JN, Karau MJ, Steckelberg JM, et al. C-reactive protein, erythrocyte sedimentation rate and orthopedic implant infection. PloS one 2010 February;5(2):e9358.
(44) Ruiz-Alvarez MJ, Garcia-Valdecasas S, dePablo R, Sanchez-Garcia M, Coca C, et al. Diagnostic efficacy and prognostic value of serum procalcitonin concentration in patients with suspected sepsis. J Intensive Care Med 2009 January/February;24(1):63-71.

(45) Beloosesky Y, Grinblat J, Pirotsky A, Weiss A, Hendel D. Different C-reactive protein kinetics in post-operative hip-fractured geriatric patients with and without complications. Gerontology 2004 July/August;50(4):216-222.

10. References of figures

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Fig 4: (23)

Fig 5: http://de.wikipedia.org/wiki/Interleukin-6 (29.8.2011)

Fig 6: http://www.ks.uiuc.edu/Gallery/Science/Structure/tn/fibrinogen-small_st2.jpg.html (29.8.2011)

Fig 7: http://commons.wikimedia.org/wiki/Cytokines (29.8.2011)

Fig 8: http://www.mg-i.ch/?page_id=373 (29.8.2011)

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11. Curriculum vitae

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Professional Experience

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10/2010- 11/2010	Medical University of Graz, Department of Otorhinolaryngology
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8/2007 Department of Orthopaedics, LKH Amstetten

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Courses and lectures next to my studies:

10/2005- 1/2006	Philosophy courses at Karl-Franzens-University of Graz
1/2005	Elective subject: Sports traumatology
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1/2005	Elective subject: Traffic medicine: Whiplash-injury of the cervical spine
1/2007	Special study module: Clinical topographic anatomy of the extremities
1/2008	Special study module: Clinical topographic anatomy of the visceral organs
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<u>Additional skills:</u>

Language: German (mother tongue), English, Spanish, Latin

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