Diplomarbeit

Diabetic foot infections: The surgical approach and the efficacy of fosfomycin as assessed by microdialysis

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Eidesstattliche Erklärung

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.

Graz, im Juni 2009 *Julia Metzler*

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ABSTRACT

Background: Severe bacterial infections of the diabetic foot might lead to limb loss in worst cases. Especially trauma, neuropathy and deformity are causing deep infections with bone and joint involvement. To enable healing of the infected ulcer, aggressive uncompromising surgery is essential as well as an adequate systemic antibiotic therapy.

Methods: The aim of our prospective study was to measure with the technique of in – vivo - microdialysis the concentrations of the anti MRSA (Methicillin - resistant Staphylococcus aureus) drug fosfomycin in the inflamed metatarsal bone compared to the concentrations of plasma and subcutaneous healthy tissue. Patients enrolled were suffering from diabetes mellitus with neuropathic ulcers and an absolute indication for resection of the metatarsal bone. Between 10/07 and 4/08 nine patients, six male, three female, mean age 66. 1 yrs (49 – 84), underwent surgery. At the end of surgery the microdialysis probes were inserted into the inflamed resected bone and into the contralateral subcutaneous leg. Patients were given fosfomycin Sandoz™ 100 mg /kg intravenously after surgery. Samples were collected over 6 hours from the bone as well as from plasma and subcutaneous tissue.

Results: Fosfomycin concentrations in plasma reached their peak with 377.3 ± 73.2 mg/L after half an hour. The concentration in the subcutis was 185.1 ± 34.2 mg/L after 1.0 ± 0.2 hours and 96.4 ± 14.5 mg/L after 3.9 ± 0.4 hours in the bone.
Measured concentrations in plasma and subcutaneous tissue are similar to previous data on healthy subjects. Fosfomycin penetrated well into bone, dispersion in bone took longer (T max. 3 – 4 hours) but equilibrated fully with plasma after 3 to 4 hours.

Conclusion: The prospective study using microdialysis demonstrated that effective bone concentrations of fosfomycin given intravenously, can be obtained in patients with severe diabetic foot infections.

ABSTRACT (German)

Grundlagen: Schwere bakterielle Infektionen am Fuß können bei Diabetikern im Extremfall bis zum Verlust einer Extremität führen. Vor allem durch Bagatelltraumata oder neuropathische Fußulcera kann es zu schwerwiegenden Infektionen mit Knochen- und Gelenksbeteiligung kommen. Eine Abheilung der infizierten Areale ist ausschließlich durch eine chirurgische Resektion der betroffenen Knochenanteile, sowie durch eine adäquate, systemisch verabreichte Antibiotikatherapie möglich.

Methodik: Das Ziel unserer prospektiven Studie war es, mit Hilfe der In - vivo - Mikrodialyse die Konzentrationen von Fosfomycin, einem gängigen Antibiotikum bei Infektionen am diabetischen Fuß, im infizierten Knochen zu messen und mit Werten des kontralateralen Fettgewebes und Serumwerten zu vergleichen. Die Studie wurde von der Ethikkommission der Medizinischen Universität Graz bewilligt. Die Studienteilnehmer waren Diabetiker mit radiologisch verifizierter Osteomyelitis und einer klaren Indikation zur Resektion des betroffenen Knochens. Zwischen 10/07 und 4/08 wurden nach Einholen des Informed Consent 9 Patienten, 6 Männer und 3 Frauen, im Durchschnittsalter von 66,1 (49 – 84) Jahren, operiert. Am Ende des Eingriffs wurden Mikrodialysesonden im Os Metatarsale und eine zweite Sonde im kontralateralen Bein subkutan platziert. Zum Zeitpunkt 0 wurde Fosfomycin Sandoz™ 100mg/kg intravenös verabreicht, und über 6 Stunden wurden vom Knochen und gesundem Fettgewebe als auch aus dem Serum Proben entnommen.

Ergebnisse: Die höchste Konzentration im Plasma erreichte Fosfomycin mit 377.3 ± 73.2 mg/L nach 30 Minuten. Die Konzentration in der Subcutis war 185.1 ± 34.2 mg/L nach einer ± 0.2 Stunden, während die maximale Konzentration im Knochen nach 3.9 ± 0.4 Stunden 96.4 ± 14.5 mg/L betrug.
Die gemessenen Konzentrationen im Plasma und der Subkutis sind mit den bisher publizierten Daten beim gesunden Probanden vergleichbar. Die Verteilung im Knochen dauert deutlich länger (Tmax. 3 – 4 Stunden), aber äquilibriert ab spätestens 4 Stunden mit Subkutis und Plasma.

Schlussfolgerungen: In unserer prospektiven Studie konnten wir unter Verwendung von In - vivo - Mikrodialysesonden beweisen, dass Fosfomycin, intravenös verabreicht, bei diabetischen Patienten mit schweren bakteriellen Fußinfektionen ausreichend hohe Konzentrationen im betroffenen Knochen erreicht.

ABBREVIATIONS

MRSA ..... Methicillin resistent Staphylococcus aureus
PAD ..... Peripheral arterial disease
DSN ..... Distal symmetric neuropathy
BMI..... Body Mass Index
PKC ..... Protein kinase C
HbA1c..... Glycosylated hemoglobin
HDL ..... High density lipoprotein
CRP..... C – reactive protein
NO ..... Nitric oxide
ABI ..... Ankle – brachial index
LDL ..... Low – density lipoprotein
MRA ..... Magnetic resonance angiography
CTA ..... Computed tomographic angiography
VRE ..... Vancomycin – resistent enterococci
G – CSF ..... Granulocyte colony stimulating factor
ISF ..... Interstitial space fluid
AUC..... Area under the curve
BW..... Body weight
PK ..... Pharmacokinetic
MIC..... Minimal inhibitory concentration

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1 INTRODUCTION

Approximately 246 million people suffer from diabetes mellitus - 5. 9% of the world's population.[1] Most of these people live in developing countries and it is becoming a greater problem. It is estimated that in 2025 about 380 million people will have diabetes - 7. 1% of the adult population.[1] It is increasing, particularly in developing countries, such as Africa, Asia and South America.[2] Diabetes mellitus is a serious, chronic illness, caused by both genetic and environmental factors. Complications such as heart disease, kidney failure, eye damage and complications on the foot may be severe.[1] A diabetic has a lifetime risk of 25 % to develop a foot ulcer.[3] We have to realize that every 30 seconds a lower limb is lost somewhere in the world as a result of diabetes.[4] The risk of a lower leg amputation is about 70% in people with diabetes.[1] Suffer from diabetes and its complications are a burden for each person. Moreover, it is also an extreme economic burden for the healthcare system of each country.[5]

2 THE DIABETIC FOOT

2.1 Pathophysiology of Foot Ulceration

The diabetic foot is a severe complication, which may affect people suffering from diabetes. It is often combined with infection, ulceration or destruction of the tissue [Fig.1]. The lifetime risk of developing a diabetic foot ulcer is relatively high: one of every seventh patient, who is suffering from diabetes, will develop a foot ulcer during his lifetime.[6] Some of these ulcers might need intensive treatment, are probably tied up with hospitalisation, and some of these patients might even loose their leg.

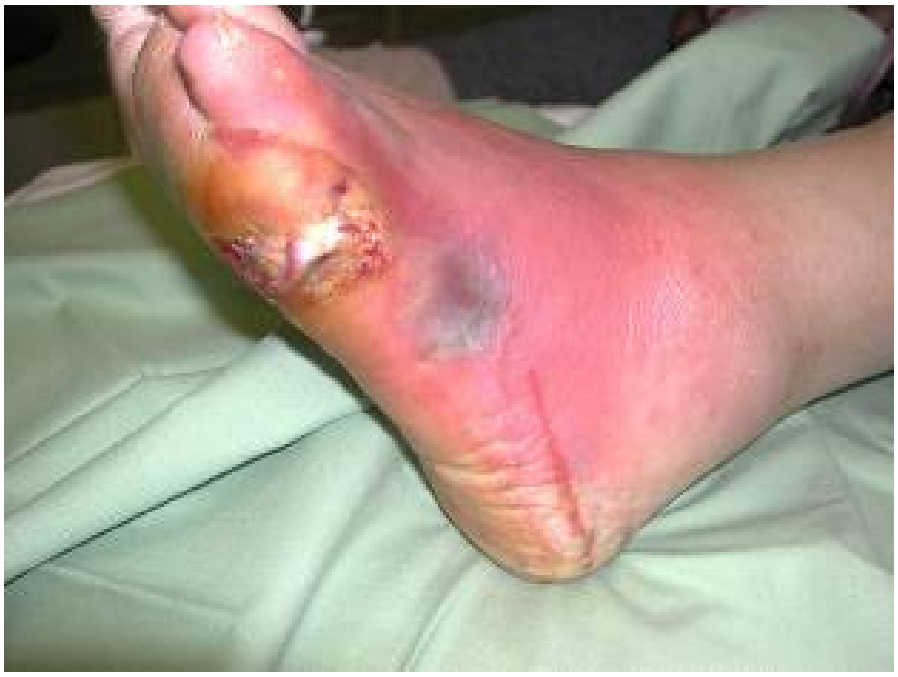


Fig. 1 Plantar ulcer affecting the fifth metatarsophalangeal joint as a severe limb - threatening infection

The two most important causes of diabetic foot ulcers are on the one hand neuropathy (sensory, motor and autonomic) and on the other hand peripheral arterial disease. But developing a foot ulcer usually involves several more risk factors occurring together, such as increased biomechanical stress, impaired skin perfusion, loss of sensation and external trauma, for instance a shoe related trauma.[1]

In a prospective study, data from 1229 diabetic patients with a new foot ulcer were analyzed.[7] Patients have been treated by diabetologists, vascular surgeons and orthopaedic surgeons. 1 of 14 diabetic foot centres in ten European countries were included. The

maximum follow up was 1 year, except that healing or amputation occurred before this period of time. The most important findings were that a huge number of patients had severe diseases. Furthermore infection and peripheral arterial disease (PAD) were present in one third of the patients. Prompers et al. also showed that non – plantar foot ulcers are more common than plantar ulcers, mainly in patients with severe disease. Severe comorbidity increases the severity of the diabetic foot. The prevalence of PAD was 49%, but ranged from 22 to 73% in the different centres. This difference is difficult to explain. On the one hand the reason could be related to the differences in the prevalence of PAD, on the other hand it is more plausible due to differences in healthcare systems. It is a fact that PAD and infections are important prognostic factors in diabetic foot disease.[6]

2.1.1 Polyneuropathy

Distal symmetric neuropathy (DSN) [Fig.2] is the most frequent neuropathy of diabetes but it might go along with carpal tunnel syndrome, ulnar neuropathy at the elbow and other focal neuropathies.[8] Studies have shown the association of DSN with risk factors that predict the progress of macrovascular disease. Hypertension is seen as a very important risk factor.[9] The United Kingdom Prospective Diabetes Study showed that control of hypertension not only markedly improved macrovascular outcomes but reduced microvascular progressions.[10] Dyslipidemia, microalbumineria, cigarette smoking and Body Mass Index (BMI) are other relevant risk factors.[11] Experimental studies suggest a multifactorial pathogenesis of diabetic polyneuropathy. Most aspects have been investigated in the diabetic rat model.[12] At the moment seven mechanisms are thought to act together to the pathogenesis.[12] [13] Compared to earlier years they are no longer regarded as being separated but playing together in multiple interactions, including metabolic and vascular factors:

(a) Increased flux through the polyol pathway that leads to accumulation of sorbitol and fructose, myo-inositol depletion and reduction in Na^+ - K^+ - ATPase activity.

(b) Disturbances in $n - 6$ essential fatty acid and prostaglandin metabolism, which results in alterations of nerve membrane structure and microvascular and hemorrheological abnormalities.

(c) Endoneurial microvascular deficits with subsequent ischemia and hypoxia as well as generation of reactive oxygen species (oxidative stress) and the so called hyperglycemic pseudohypoxia.
(d) Increased activity of protein kinase C β (PKC β). It is activated by hyperglycemia – driven de novo synthesis of diaglycerol and also by oxidative stress.[14] In vessels and vascular tissue, PKC, particularly the *β* isoform, is higher. In nerves, this is not usually observed.[13 15]

(e) Deficits in neurotrophism leading to reduced expression and depletion of neurotrophic factors such as nerve growth factor, neurotrophin – 3 and insuline – like growth factor, as well as alterations in axonal transport.

(f) Accumulation of non – enzymatic advanced glycation end – products on nerve and/or vessel proteins.

(g) Immunological processes with autoantibodies to vagal nerve, sympathetic ganglia and adrenal medulla as well as inflammatory changes.

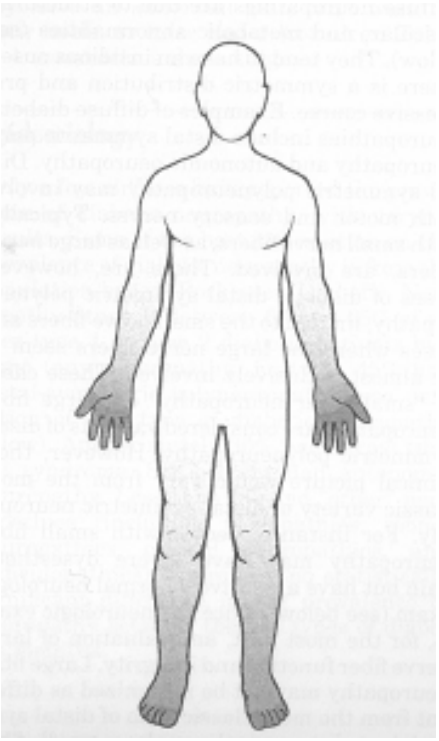
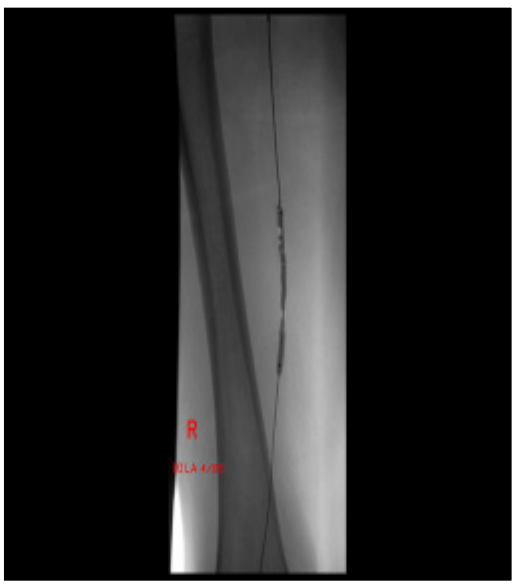


Fig. 2 Distal symmetric neuropathy (from Bowker JH and Pfeifer MA[99])

2.1.2 Peripheral Arterial Disease

Peripheral arterial disease leading to arterial insufficiency is an essential prognostic factor in a diabetic foot ulcer.[1] Arterosclerosis and medial sclerosis are the most frequent arterial diseases in people with diabetes. Arterosclerosis causes ischaemia because of narrowing and blocking the arterial lumen. Thus, medial sclerosis (Moenckeberg sclerosis) leads to calcification of the tunica media. As a consequence, arteries are getting stiff and inflexible – but without encroachment on the arterial lumen. So medial sclerosis, which is normally associated with neuropathy, does not cause ischemia, but the rigid arterial tube may interfere with indirect measurement of arterial blood pressure.[1] PAD is more than twice as common among diabetic patients matched with nondiabetic individuals.[16] [17] Atherosclerosis in people with diabetes is not only more common, it also affects younger individuals, there is no sex difference, it progresses faster, it is multi segmental [Fig.3, 4], more aggressive and last but not least more distal and commonly involves the tibial vessels.[1] [18]



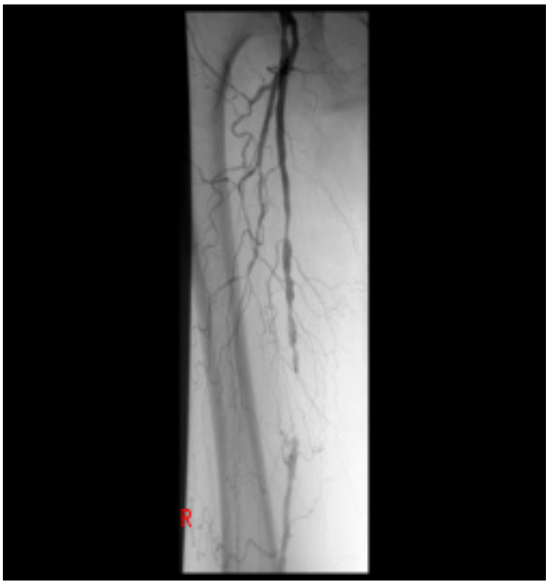


Fig. 3 Multi segmental peripheral arterial
Fig. 4 PTA; occlusive disease

Adler et al. investigated potential risk factors for the development of PAD.[19] They demonstrated that hyperglycemia, assessed by HbA1c, was associated with an increased

risk for incident PAD, independent of other risk factors, including age, elevated blood pressure, reduced high density lipoprotein (HDL), cholesterol, smoking, prior cardiovascular disease, peripheral sensory neuropathy and retinopathy. He demonstrated that each 1% increase in HbA1c was associated with a 28% increased risk of PAD and each 10 mmHg increase in systolic blood pressure with a 25% increase in risk.

The enormous importance of a good glycemic control was also shown by Selvin et al.[20] They showed that poor glycemic control (indicated by increased HbA1c levels) in patients with diabetes is combined with an increased risk of PAD, independently of known risk factors. This association was especially high for the symptomatic, more severe manifestations of the disease, including intermittent claudication and a PAD – related stay in hospital. Patients who had a HbA1c > 7.5% were more than five times as likely to develop intermittent claudication and also five times as likely to have to stay in hospital for PAD compared with comparable individuals with HbA1c < 6%.

The pathophysiology of PAD in a diabetic individual is similar to that in a non diabetic individual. Inflammation, more precisely increased levels of C – reactive protein (CRP), play a major role in the development of atherosclerosis. Individuals with an impaired glucose tolerance have elevated CRP levels.[21] Furthermore, CRP has procoagulant effects and is able to boost the expression of tissue factor.[22] It also inhibits endothelial cell nitric oxide (NO) synthase, which causes abnormal regulation of vascular tone and increases production of plasminogen activator inhibitor – 1, which avoids the formation of fibrinolytic plasmin from plasminogen.[23] [24]

Endothelial cell dysfunction is common in patients with diabetes and PAD. NO, a vasodilatator, is synthesized in healthy vessels. Poor glycemic control affects NO - mediated vasodilatation.[25] Not only NO, as a vasodilatator is decreased, but also the production of vasoconstrictors in diabetic individuals, for example endothelin - 1, is increased. Protein kinase C and nuclear factor kappa – B are activated and as a consequence, more reactive oxygen species are produced which increases the number of atherosclerotic lesions.[26]

Furthermore, also the major role of smooth muscle cells in diabetic patients has to be considered. It is proven, that advanced plaques in diabetic individuals have fewer muscle cells than healthy controls. Most likely, hyperglycemia – induced lipid modifications regulate the apoptosis of vascular smooth cells in advanced atherosclerotic lesions, causing plaque instability.[27]

The expression of glycoprotein Ib and IIb/IIIa receptors in platelets of diabetic individuals is elevated, increasing their thrombotic potential.[24] Hyperglycemia also increases blood coagulability by elevating the expression of tissue factor and antithrombin III, a potent anticoagulator.[28] The tendency towards coagulation and impaired fibrinolysis contributes to the increased thrombotic potential that characterizes diabetes.[25]

2.2 Symptoms

2.2.1 Different manifestations of neuropathy and PAD

	Neuropathy	PAD	combined
Symptoms	Hyperesthesia, Paresthesia, burning pain, stabbing pain, numbness and tingling in toes and legs, hot and cold sensations, muscle atrophy, sensory loss, the absence of sweating, warm and dry foot	Pale leg, leg coldness, numbness, atrophic skin, thickened nails, symptoms of claudicatio intermittens	Progressive damage and very poor wound healing
Pulse	palpable	not palpable	not palpable
Sensibility	reduced	normal	reduced
Typical complications	Malum perforans, Diabetic neuropathy [Fig. 5,6] Osteoarthropathy Charcot foot [Fig. 7, 8]	Gangrene	Combined complications with poor prognoses

Table 1 Manifestations of neuropathy and PAD

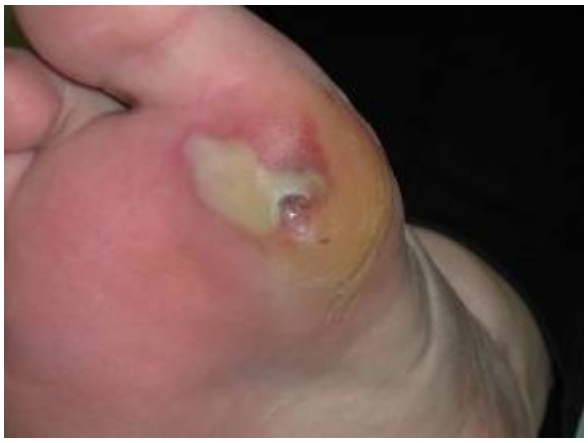
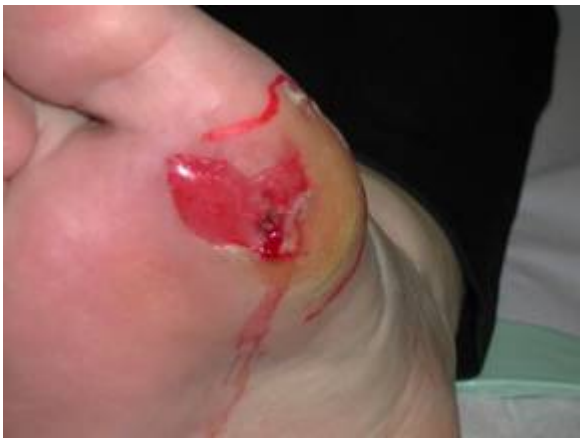



Fig. 5 Malum perforans, blister retaining pus
Fig. 6 After removal of the skin

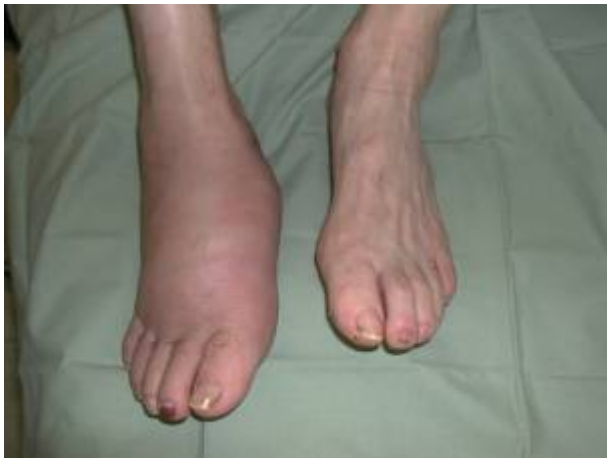


Fig. 7 Acute Charcot foot presenting with painless swelling, redness, difference of skin temperature (> 2 degrees higher than the contralateral foot)

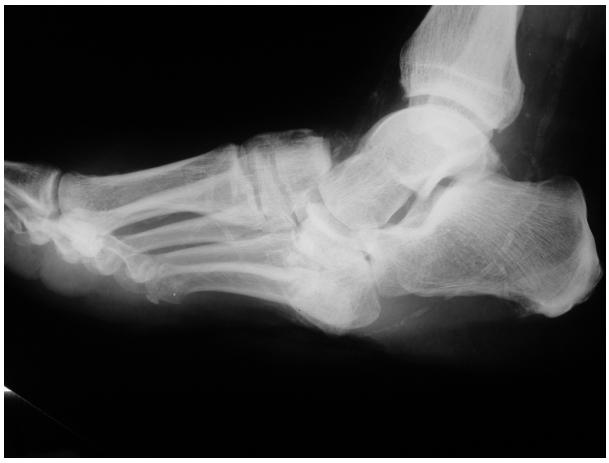


Fig. 8 X–ray: joint instability and talonavicular luxation

2.2.2 FONTAINE Classification of the 4 stages of PAD[29]

Peripheral artery occlusive disease, also known as „Claudicatio intermittens“ is commonly divided into four Fontaine stages, introduced by R. Fontaine in 1954.

Stage	Symptoms
I	asymptomatic
II	Claudicatio intermittens, pain free for a > 200 m walk (stage II a) Claudicatio intermittens, pain free for a < 200 m walk (stage II b)
III	Pain while resting
IV	Necrosis and gangrene [Fig.9]

Table 2 FONTAINE Classification of the 4 stages of PAD

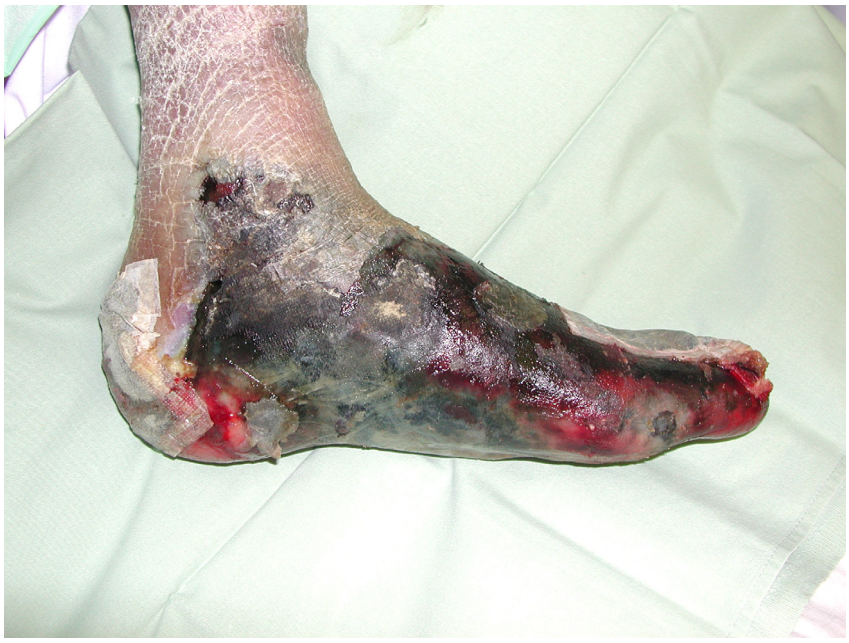


Fig. 9 Foot gangrene requiring major amputation

2.3 Diagnosis

2.3.1 Diagnosis of Polyneuropathy

Unqualified management of diabetic peripheral neuropathy leads to unnecessary morbidity and sizeable healthcare costs. Neuropathy is a major contributing factor in amputation and it is believed that half of the amputations in people with diabetes are preventable.
In the guidelines for the diagnosis and outpatient management of diabetic peripheral neuropathy Boulton et al. underlined the importance of a set of simple, practical aspects to be used by primary care physicians and by hospital physicians for the clinical assessment and management of neuropathy.[30] The aim was to create guidelines, which are applicable in daily practice.
Boulton et al. suggest that clinical assessment of neuropathy should form part of the annual review of the diabetic patient. The intention is to early detect the presence of clinical neuropathy.

Patient's history

Questions should address the age of the patient, type of diabetes and symptoms. In general, the following areas should be asked:

- Age
- Diabetes: type, duration, therapy, level of glycemic control
- Physical factors: inability to see well or reach the feet
- Lifestyle: smoking, alcohol intake, nutrition, employment, sport/leisure activities, footwear
- Social circumstances: socio – economic status, mobility, home circumstances, social support, access to care
- Symptoms

Symptoms vary according to the stage and type of neuropathy. The following questions have to be answered:

- Presence/absence of symptoms
- Nature of symptoms
- Duration and progression of symptoms
- Nocturnal exacerbation
- Patients with chronic pain have to be asked if the pain is insidious, intermittent, bilateral, related/unrelated to treatment, if it occurs on walking or at rest, presence of foot ulcers in the past, presence of autonomic symptoms
- Patients with acute pain have to be asked about the presence of neuropathic pain or contact hyperesthesia

Finally, also other medical conditions/therapies which may be aetiological factors have to be remembered: vascular disease, HIV, vitamin B12 deficiency, hypothyroidism, weight loss, cancer, leprosy, syphilis, drug therapy, toxic exposure, paraproteinemia.

Examination of the Patient – Inspection of the feet

The patient should be asked to take off socks and shoes of both feet. The investigating physician has to pay attention to the following:

- Skin status: color, thickness, dryness, cracking, trophic changes
- Sweating
- Infection (interdigital fungal infection)
- Ulceration
- Calluses/blistering
- Deformity, e.g. Charcot joint or clowed toes
- Muscle atrophy
- Arches
- Palpation of the feet

Neurological Examination

These tests have to be done bilaterally and the result should be just yes/no or normal/abnormal.

- Pin prick test: a disposable dressmaker's pin for example should be used and the patient has to answer whether he can feel it and if yes, he also has to mention if it is painful
- Light touch: it is important to use a consistent method, perfectly a cotton wisp
- Vibration test: a 128 Hz fork should be used, initially on the big toe
- Ankle reflex: the ankle reflex has to be compared with the knee reflex

Additionally to these simple examinations, the following semiquantitative tests may be used:[1]

Semmes – Weinstein Monofilaments

It is known that the inability to sense the 10g monofilament at the toes or dorsum of the foot forecasts future occurrence of a diabetic foot ulcer, however at the moment there are no evidence – based data describing how often and where on the foot a monofilament should be applied. The benefits of this test are its simplicity and low costs.

Tests of Vibration Perception

Prospective studies have shown that a decrease in vibratory perception prefigures ulceration.[1] These studies used small, portable electronic instruments to semi – quantively diagnose the vibratory perception threshold. The disadvantage is that these instruments (for example biothesiometer or neuro – esthesiometer) are too expensive for most centers.

Vascular Examination - Documentation of systemic blood pressure and pulses.

Boulton et al. also mentioned in their report that for optimal care of people with neuropathy, a local muldisciplinary footcare team should be arranged. A diabetologist, a diabetes specialist nurse, a chiropodist, a podiatrist and a surgeon should be part of the team.[30]

2.3.2 Peripheral Arterial Disease

It is important to diagnose PAD in diabetic individuals early to start therapies that reduce the risk of atherothrombotic events, to handle foot problems and to improve quality of life and decrease disability. Generally PAD is underdiagnosed. A large - scale PAD screening study showed that only one third of patients with documented PAD had classical claudication symptoms.[31] The other patients either had atypical symptoms or were asymptomatic.

Assessment:

- Patient' s history.
- Physical examination, including blood pressure measurement, palpation of peripheral pulses as well as auscultation of pulses and bruits. Palpation of peripheral pulses means an examination of the femoral, popliteal and pedal vessels.[23] The absence of the dorsalis pedia pulse is less sensitive for PAD because up to 30% of these abnormalities may be due to an anatomic variation.[32]
- ABI (ankle – brachial index) screening, which represents the systolic blood pressure at the posterior tibial or dorsalis pedal level compared with brachial blood pressure. It is normally between 1.00 and 1.40.[33] The ABI is a reproducible and reasonably accurate measurement for the diagnosing of PAD. In PAD, the ankle systolic blood pressure is less than the brachial, so the ABI is decreased to <1.00; per definition, in PAD ABI is <0.90. Lower ABI indicates more severe PAD.[25] The ADA consensus statement suggests that a screening ABI should be performed in all diabetic individuals > 50 years of age. If normal (0.91 to 1.40), the test should be rerunned every five years. But a screening ABI should be done in any patient with symptoms of PAD.[23]
- If PAD is attested, an aggressive secondary prevention is warranted. The National Cholesterol Education Program/Adult Treatment Panel III guidelines approve a target low density lipopotein (LDL) cholesterol level of < 100mg/dl.[34] A recent update to these guidelines recommends a target LDL level of < 70 mg/dl for very high risk patients.[35]

In patients with an attested PAD in whom further investigation is requested (probably in the context of a planned revascularization procedure), a vascular laboratory evaluation for

segmental pressure and pulse volume recordings should be done. Both hemodynamic tests help in the localization of arterial occlusive lesion.[23] [32] If more detailed information of the morphological features of occlusion is required (for example for the decision about revascularization), other non – invasive imaging techniques, such as ultrasonic duplex scanning or magnetic resonance angiography (MRA) can be used.[25] Ultrasonic duplex scanning visualizes vessels, offers information on artery wall thickness, gives a measure of flow turbulence, and changes in blood flow velocity.[36] MRA and computed tomographic angiography (CTA), both non – invasive, open up a new era.[37] Contrast – enhanced MRA produces images that are comparable with conventional angiography.[38] CTA has definitely improved image quality – the CTA is replacing conventional angiography in many PAD imaging studies.[39]

2.4 Evaluation

Patients suffering from diabetes may develop several different types of foot wound, and any of these can become infected. Clinically, infection should be diagnosed on the basis of the presence of purulent secretion or at least two of the cardinal signs of inflammation (redness, warmth, swelling or induration and pain or tenderness).[1] However, not all ulcers are infected.[1] Lipsky et al.[40] showed that evaluation of the infection should be performed at 3 levels: the patient as a whole, the affected limb or foot and the infected wound. The aim is to assign the clinical extent and the microbial etiology of the infection, the biology or pathogenesis of the wound, any contribution of altered foot biomechanics to the cause of the wound, any contribution of vascular (mainly arterial) disease and the presence of any systemic consequences of infection.

2.5 Microbiology

Aerobic gram – positive Cocci are the dominant microorganism that colonize and acutely infect breaks in the skin. S. aureus and the hemolytic Streptococci (groups A, C and G, but mainly group B) are the most frequently isolated pathogens.[41] [42] [43] On the contrary, chronic wounds involve a complex colonizing flora, including Enterococci, various

Enterobacteriaceae, obligate anaerobes, Pseudomonas aeruginosa, and sometimes other nonfermentative gram – negative rods.[44] [45] [46] Hospitalization, surgery, and above all prolonged or broad – spectrum antibiotic therapy may predispose patients to colonization and/or infection with antibiotic – resistent organism (for example, MRSA or vancomycin – resistent enterococci [VRE]).[47] Even though MRSA strains have earlier been isolated mainly from hospitalized patients, community – associated cases are now becoming more common.[48] They are associated with worse outcome in patients with diabetic foot infections.[49] [50] [51] Vancomycin (or glycopeptide) intermediate S. aureus has been isolated in many countries. The first two reported cases of Vancomycin – resistent S. aureus affected diabetic patients with a foot infection.[52]

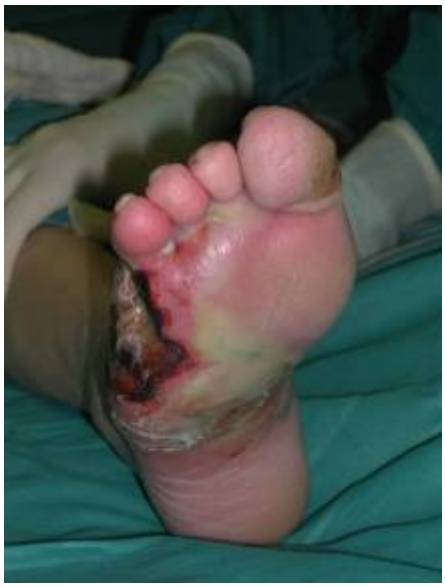
Because the impaired host defenses around necrotic soft tissue or bone allow low – virulence colonizers, such as coagulase – negative Staphylococci and Corynebacterium species ("diphteroids"), the pathogenetic role is not clear.[46] Patients who have not lately received antimicrobials often have monomicrobial acute infections (nearly always with an aerobic gram – positive Coccus). Chronic infections on the other hand are often polymicrobial.[41] [42] [46] [53] Cultures of specimens obtained from patients with mixed infections generally yield 35 isolates, as well as gram – positive and gram – negative aerobes and anaerobes.[43] [54] [55]

2.6 Determining the Severity of Infection

The International Consensus on the Diabetic Foot lately published a preliminary progress report on a diabetic foot ulcer classification system for research aims.[1] The most important statements are summarized by the acronym PEDIS (perfusion, extent/size, depth/tissue loss, infection and sensation).

Infection is categorized into four grades:

- Grade 1: No infection
- Grade 2: Involvement of skin and subcutaneous tissue only
- Grade 3: Extensive cellulitis or deeper infection [Fig. 10, 11]
- Grade 4: Presence of systemic inflammatory response syndrome



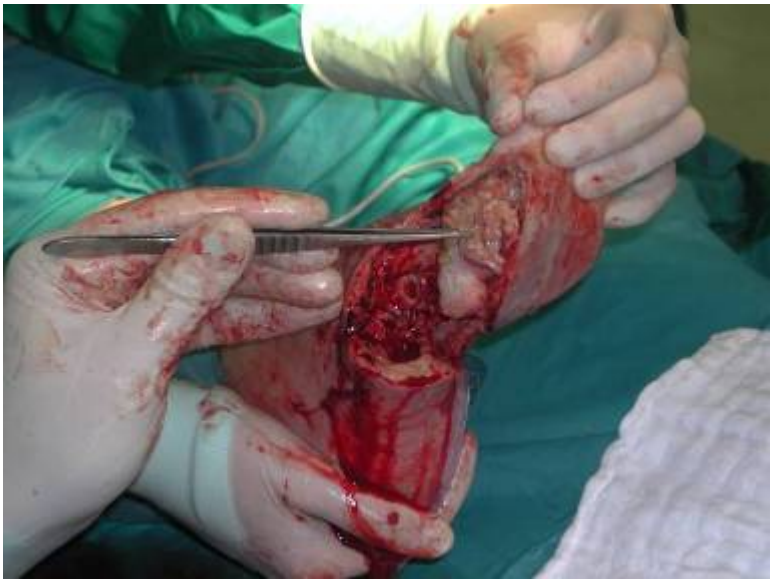


Fig. 10 Deep plantar phlegmon
Fig. 11 Surgical exploration finding extensive necrosis

For infected wounds the first and most important step is to find out the patients who require promptly hospitalization, parenteral and broad – spectrum empirical antibiotic therapy and an immediate assessment in therapeutic approach. Lipsky et al.[40] have defined these potentially life – threatening infections as "severe". Infections defined as "mild" have to be kept apart from clinically uninfected lesions, but are not so difficult to diagnose. The challenge is to define "moderate" infections, because the term covers a huge spectrum of wounds, some of them can be relatively complicated and even limb threatening.

2.7 Need for hospitalization

Lipsky et al. summarized the following items as a reason for hospitalization[40]:

- Systemic toxicity (for example fever and leukocytosis)
- Metabolic instability (for example severe hypoglycemia or acidosis)
- Rapidly progressive or deep – tissue infection
- Substantial necrosis or gangrene
- Presence of critical ischemia
- Requirement of urgent diagnostic or therapeutic interventions
- Inability to care for him/herself

Hospitalization is the most expensive part of treating a diabetic foot infection. However, patients with severe infections or patients with infections that are complicated by critical limb ischemia should stay in hospital.[56] [57] And, even some patients with mild and moderate infection may need hospitalization. The reason for this might be observation, urgent diagnostic testing or complicating factors that affect their wound care or adherence to antibiotic treatment. Again, most patients with mild or moderate infections can be treated as outpatients.

2.8 Treatment of infections

There is a discussion about the need for treatment. Some specialists discuss that a lot of apparently uninfected diabetic foot ulcers are subclinically infected and so they contain a high "bioburden" of bacteria (it is defined as > 105 organism per gram of tissue), causing "critical colonization" and impaired wound healing.[55] [58] [59] [60]

Other available data do not encourage the use of antibiotic therapy for the treatment of clinically uninfected wounds.[61] [62] Lipsky et al.[40] agree with these authors. They disadvise therapy for uninfected wounds because antibiotic therapies increase antimicrobial resistance, raise financial burden and may cause drug – related adverse effects. Sometimes it is a challenge to determine whether a chronic wound is infected, mainly when the foot is ischemic, has abnormal coloration or a fetid odor, has friable granulation tissue, is combined with unexpected pain or tenderness or last, when a properly treated ulcer does not heal over a long period of time.[63] In these rare cases, a brief culture – directed course of antibiotics may be helpful.

After determining the need for hospitalization, the patient has to be stabilized. The general metabolic status of the patient has to be observed, [64] including the assessment of fluid and electrolyte balances and the therapy of hyperglycemia, hyperosmolality, acidosis and azotemia. Critically ill patients who need surgery should be stabilized before intervention.

2.8.1 Antibiotic Regimen

Initial therapy is mostly empirical and should be based on the severity of the infection and on any available microbiological data, such as recent culture results or current gram – stained smear findings. For severe and more extensive, chronic moderate infections, it is the best to start a broad – spectrum antibiotic therapy. Antibiotics should have activity against gram – positive cocci (including MRSA in locations where this pathogen is common), as well as gram – negative and obligate anaerobic organisms. To guarantee adequate tissue concentrations, therapy should be given parenterally, at least initially.[40] Some authors believe to give broad – spectrum empirical therapy for most infected foot wounds[65] [66] but most of mild and many moderate infections can be treated with drugs with a relatively limited spectrum, such as those covering only aerobic gram – postitive cocci.[67] It is a fact that anaerobic organisms have been found in many severe infections,[45] but they are infrequent in mild to moderate infected wounds.[68] As a consequence there is no clear indication for anaerobic therapy in most infections.

For patients without gastrointestinal absorption problems and a mild – to – moderate infection, oral therapy often might be indicated. For mildly infected open wounds even the use of topical antimicrobial therapy is a possible alternative. So far, only a few data are available.[69]

Antibiotics diversify in how well they reach effective concentrations in infected diabetic foot wounds.[70] [71] [72] This is based on pharmacodynamic properties of the specific agent, and mainly on the arterial supply to the foot, rather than on diabetes.[73]

2.8.2 Indication for Surgery

Some infections need surgical interventions that range from drainage and excision [Fig.12, 13] of infected tissues to revascularization of the lower extremity and reconstruction of soft – tissue defects or mechanical misalignments.[74] [75] The surgeon has to decide the adequacy of the blood supply to the left viable tissues, has to pay attention to common surgical pitfalls (for example, infection spreading among foot compartments) and finally, has to

establish a strategy for eventual soft – tissue cover (for example primary close, delayed primary closure, secondary intention or even tissue transfer).[76]

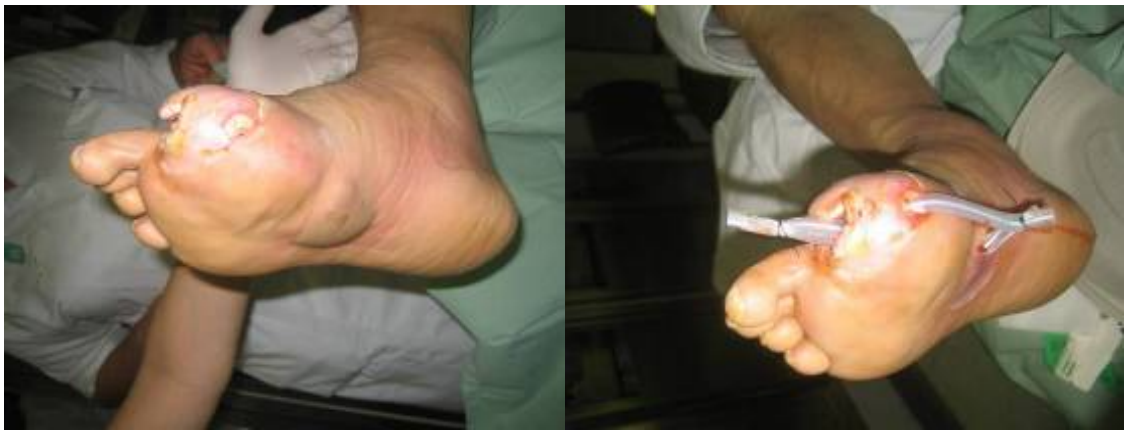
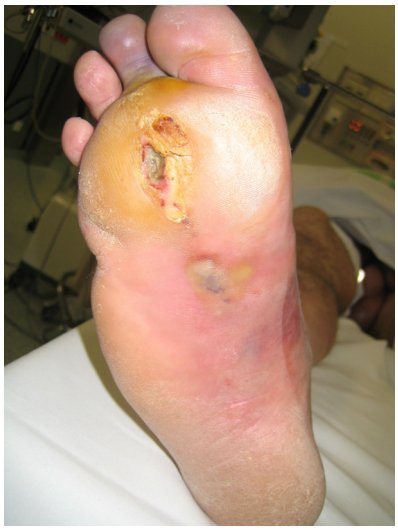


Fig. 12 Deep plantar phlegmon requiring surgery
Fig. 13 Plantar incision and drainage as an urgent procedure

Sometimes amputation is the best or only choice.[77] Urgent amputation is indicated when there is extensive necrosis or life – threatening infection [Fig.14 - 17].[78] Elective amputation may be indicated if a patient has recurrent ulceration (despite of maximal previous therapy).[79] Even though the surgeon tries to save as much of the limb as possible, it has to be noted, that a higher level of amputation that results in a more functional stump (even if a prothesis is necessary) might be a better alternative, than to save a foot that is mechanically useless or has no healing tendency. In patients with a dry gangrene, autoamputation may be a choice, particularly when surgery is a poor option. If the infected limb seems to be ischemic, the patient should be referred to a vascular surgeon.[80]



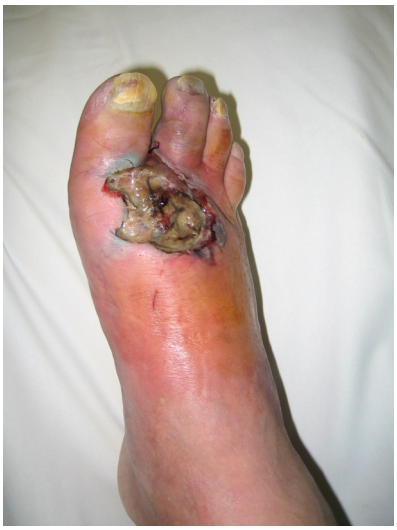
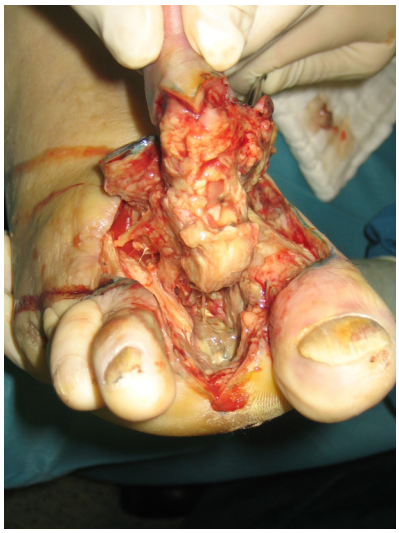


Fig. 14 A severe infection spreading
Fig. 15 Dorsal tissue necrosis from malum perforans



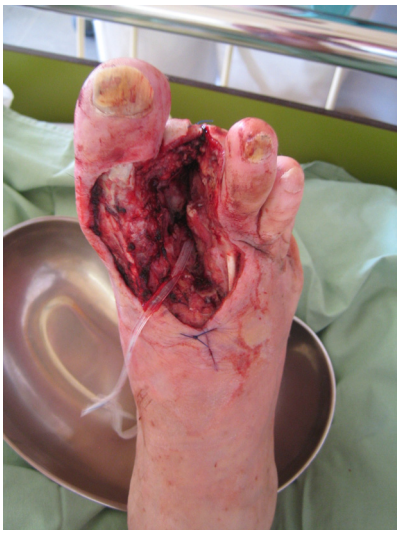


Fig. 16 Open ray amputation
Fig. 17 Open wound treatment

2.8.3 Planning wound care

The wound might need additional observation after the debridement performed during the initial treatment. The aim is to physically excise dead and unhealthy tissue, thus enabling wound healing.[81] [82] [83] Limited debridement can even be done as a bedside procedure, which does not need anaesthesia, mainly for a neuropathic foot. For sharp debridement a scissors or tissue nipper is mostly preferable to hydrotherapy or topical debriding agents.[83] [84] The wound should be dressed in a way that enables daily inspection.[85] [86]

Adjunctive possibilities of wound care treatment include:

- Wound vacuum – drainage systems[87] [88]
- Recombinant growth factors[89] [90]
- Skin substitutes[87] [91]
- Antimicrobial dressings[92]
- Maggot (sterile larvae therapy)[93]

Unfortunately, available evidence is insufficient to recommend routine use of any of these measures for treatment or prophylaxis.[40]
Two adjunctive treatments should be briefly commented. First, granulocyte colony stimulating factors (G – CSFs) have been investigated in five randomized studies. G – CSF

does not accelerate infection, but may significantly minimize the need for operative procedures.[94] Second, hyperbaric oxygen therapy might be a good alternative in treatment. A Cochrane report showed that hyperbaric oxygen therapy definitely reduced the risk of major amputation related to diabetic foot wounds.[95]

2.9 Follow up

Observation of the patient's response to therapy is relevant and should be performed daily for hospitalized patients and approximately every 25 days initially for outpatients. The first indicators of improvement are a decrease of local and systemic symptoms and clinical signs of inflammation. Blood test results, such as white blood cells counts[96] and inflammatory markers, like erythrocyte sedimentation rate[97] and the C - reactive protein level[98] are of limited significance, even though it may be calming to see elevated levels decreasing and on the other hand may be worrying if they do not.

In summary, there are four issues to remember, when a hospitalized patient is ready for discharge or an outpatient returns for follow up:

1. Select the definitive antibiotic regimen
2. Reevaluate the wound
3. Review the off – loading and wound care regimen
4. Evaluate glycemic control

3 SURGICAL CONSIDERATIONS

As mentioned in the introduction, it is important to realize that a patient suffering from diabetes has a lifetime risk of 25% to develop a foot ulcer[3] and as a consequence every 30 seconds a lower limb is lost somewhere in the world because of diabetes mellitus.[4] 85% of all amputations are caused by a foot ulcer.[1] Once a patient is suffering from an ulcus, the likelihood of reulceration is high. Likewise, once an individual has had an amputation, the risk of a subsequent amputation is up to 51% at 5 years.[99] The challenge for the physician is to regard amputations as reconstructive procedures and not as a failure of medical science or personal skills.[99]

Surgery of the diabetic foot can be classified into three broad categories:[100]

1. Elective surgical procedures: per definition procedures which bring an advantage for the patient but are not urgent.
2. Prophylactic surgical procedures: they are essential to prevent further damage to the foot.
3. Emergent surgical procedures: this stage requires immediate surgical intervention. Patients generally present themselves with a severe foot infection.

3.1 Important principles of surgical management[100]

Before surgery, patients need to be treated aggressively of their diabetes and comorbid conditions. The patient's nutritional status has to be assessed because wounds will not heal without adequate nutrition. Likewise, they will not heal with insufficient distal perfusion. Physicians should be able to recognize emergency diabetic foot problems and non – limb – threatening conditions. It is important that drainage of infection and debridement of necrotic, bacteria – laden tissues are in time. Inadequate antibiotic therapy and delay in surgery increase dramatically the risk of tissue loss and amputation. For most diabetic foot infections antibiotics alone are not sufficient.

3.2 Surgical Procedures

3.2.1 Incision and drainage[100]

Incision and drainage are the basics of treatment for nearly all infections of the diabetic foot, except streptococcal cellulitis. Initial drainage of an abscess can be done in the emergency department, as well as at the bedside, under local field block or regional ankle block anaesthesia. If a drainage is performed, all collections of pus have to be opened, with gentle probing of the superficial and deep tissues for sinus tracts. In some cases, sinus tracts need to be laid open. Bacteria – laden necrotic tissues have to be debrided and drainaged. In some cases amputation of toe(s) or ray(s) may be necessary to establish drainage, which is best performed in the operating room.

Gas in the soft tissue is a serious finding and requires immediate open drainage of all infected spaces in the operating room, followed by intravenous broad – spectrum antibiotics. This severe complication presents itself clinically by crepitus and crackling sensation noted on palpation of the affected soft tissue.

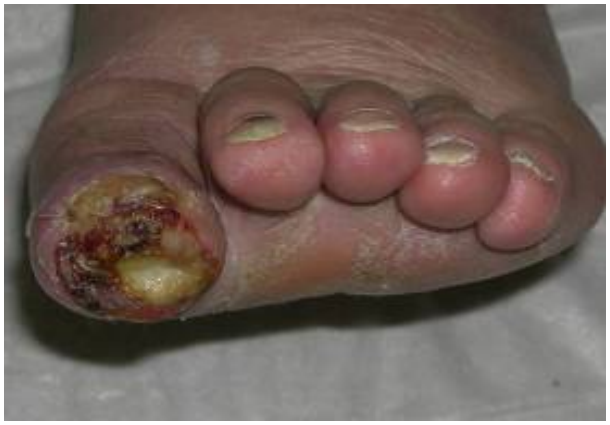
3.2.2 Partial Foot Amputations and Disarticulations[99 101]

The following will give a short summary of the most important levels of amputation, starting with the great toe.

Toe Disarticulations

In the case of osteomyelitis of the distal phalanx of the great toe, sufficient skin might be salvaged to enable closure of an interphalangeal disarticulation [Fig.18 - 21]. Probably it might be necessary to trim the prominent condylar portions of the proximal phalanx, as well as to shorten it by removing the articular cartilage to permit a closure without tension. By shortening the tendon, the sesamoid bone within the flexor hallucis longus tendon at the level of the interphalangeal joint is removed. The proximal phalanx will help to stand balanced due to preservation of the windlass mechanism, in comparison to disarticulation

at the metatarsophalangeal joint where it is lost because of the removal of the flexor hallucis brevis/sesamoid complex.



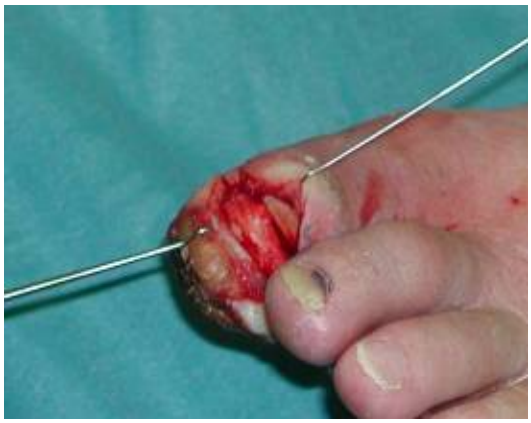
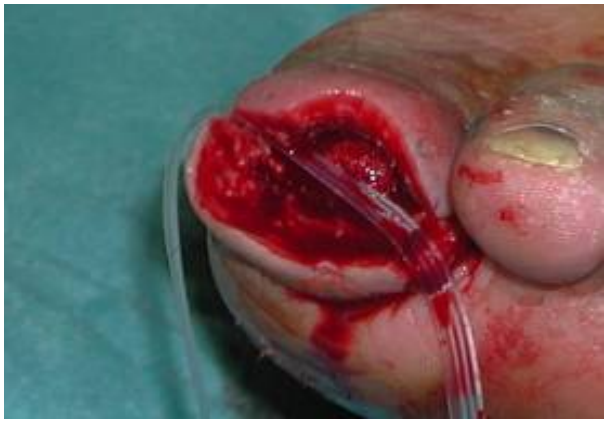


Fig. 18 A distal phalangeal osteomyelitis of the great
Fig. 19 Amputation preserving the proximal toe phalanx



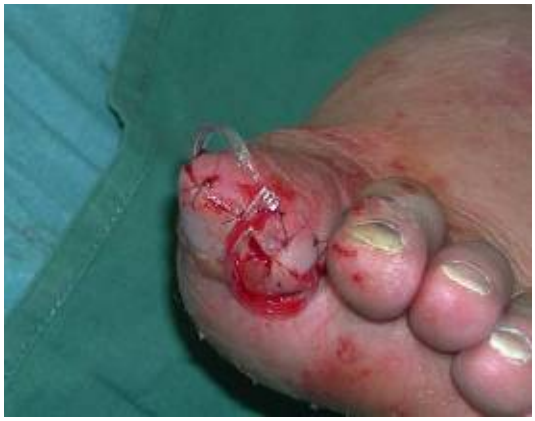


Fig. 20 Easyflow - drainage
Fig. 21 Wound closure without skin tension

The metatarsophalangeal joint is the next common site in the great toe. The flexor hallucis brevis tendon insertion is released and the sesamoid bone will displace proximally. Thus, the prominent crista on the plantar surface of the first metatarsal head is exposed. Sometimes the medial sesamoid might produce a bony prominence. Hence, the sesamoids and their fibrocartilaginous plate should be excised, as well as the crista removed with a rongeur. Furthermore, the articular cartilage has to be removed and the metatarsal head smoothly rounded with a file. Disarticulation of the second toe at the metatarsophalangeal joint, by removing the lateral support from the great toe, may create a hallux valgus (bunion) deformity. This problem can be avoided by removing the second metatarsal

through its proximal metaphysic along with the toe. The first and third metatarsal can approximate each other, resulting in a good cosmetic and functional outcome.
Dry gangrene may affect all five toes because of occlusion of their end arteries but without significant change in proximal perfusion. Then, disarticulation of all five toes with primary coverage of the metatarsal heads is possible, provided that the plantar and dorsal incisions are made as distally as possible.

After great toe disarticulation at the MTP joint, walking function may be altered because the first ray fails in the final transfer of weight during late stance phase and a decrease in foot stability medially due to loss of the windlass mechanism.

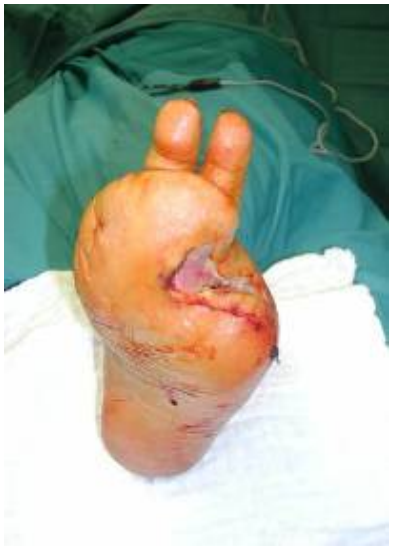
Ray Amputations

A ray amputation means that a toe and a variable portion of its metatarsal are excised. The metatarsal shaft of the first ray should be left as long as possible to allow an effective elevation of the medial arch by a custom – molded insert. The most common indication of a first ray amputation is an ulcer beneath the first metatarsal head that has already penetrated the MTP joint capsule.
Single amputation of ray two, three or four will sparely affect the width of the forefoot. Resection should be performed through the proximal metaphysis. The tarsometatarsal joints should be left intact. If the fifth metatarsal has to be amputated, it should be transected obliquely with an inferolateral - facing facet. If multiple rays have to be removed, it is both functionally and cosmetically a poor choice. If all except the first rays are involved, it can be left as the only one. However, with a good pedorthic fitting it is preferable to a transmetatarsal amputation.[102] [103]

Transmetatarsal Amputation

This form of amputation should be considered when two or more medial rays must be amputated [Fig. 22, 23]. Regarding the metatarsal shaft length, it is important to save as much as can be covered with good plantar skin. The use of fragile split – skin graft distally and plantarly should be avoided. Because of that, both plantar and dorsal transverse skin incisions should be made at the base of the toes.

To prevent postoperative equinus deformity, a well – padded short leg cast in slight dorsiflexion should be applicated.



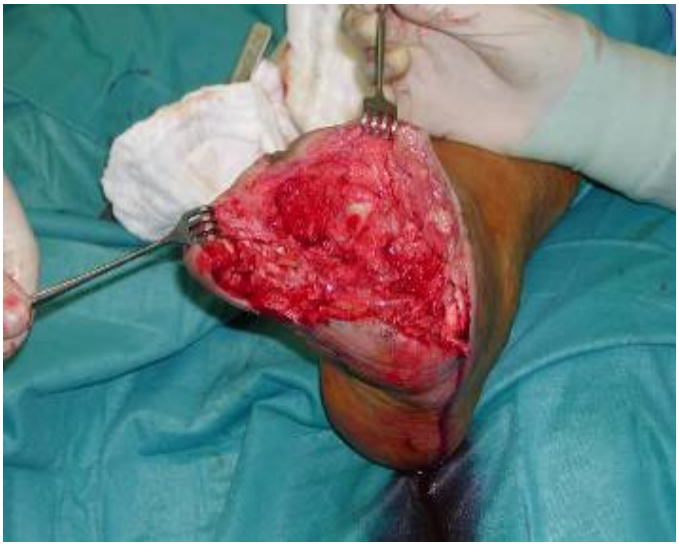


Fig. 22 Osteomyelitis requiring resection
Fig. 23 Transmetatarsal amputation choice of therapy of another ray

Tarsometatarsal (Lisfranc) Disarticulation

This type of surgery has first been described by Lisfranc. It might be used in cases of foot infections in diabetics. Patients should be selected carefully, since failure to control the infection at this level will risk the failure of a Syme ankle disarticulation. This surgery results in a major loss of forefoot lever length; thus the tendon insertions of the peroneus brevis, peroneus longus and tibialis anterior muscles have to be preserved, to maintain a muscle balanced residual foot. These muscles will aid to counteract the triceps surae complex and prevent equinus contracture as well as provide inversion and eversion of the residual foot. Careful dissection will spare the proximal insertions of the peroneus longus and tibialis anterior tendons on the medial cuneiforme bone. The distal insertions of these tendons on the first metatarsal can also be dissected and reattached for reinforcement. The first, third and fourth metatarsals might be disarticulated, but the “keystone” base of the second metatarsal should be left in place to preserve the proximal transverse arch. A portion of the base of the fifth metatarsal can preserve the insertion of the peroneus brevis tendon. Because of a dramatically loss of forefoot lever length, the massive triceps surae complex can easily overpower the weaker dorsiflexors, which might lead to equinus contracture. This complication might be avoided by doing a primary percutaneous

fractional Achilles tendon lengthening. Then a cast with the foot in a plantigrade or slightly dorsiflexed position should be applicated.

Midtarsal (Chopart) Disarticulation

This disarticulation is through the talonavicular and calcaneocuboid joints, only leaving the talus and calcaneus. It is most useful in trauma and for selected cases of foot tumor but only occasionally in diabetic foot infections because of its proximity to its heel pad. At the time of disarticulation the function of all ankle dorsiflexors is lost. Active dorsiflexion can be restored by attachment of the anterior tibial tendon to the talus, either through a drill hole in the talar head or with sutures or staples to a groove in the distal aspect of the head.[104] Letts et al. advise in their study to remove two to three cm of the Achilles tendon, rather than to lengthen it, to restore relative balance. To prevent equinus contracture, a cast with the hindfoot in slight dorsiflexion should be applicated.

Higher amputation levels will not be described here because they are not so frequent in diabetic patients.

4 MICRODIALYSIS

4.1 Introduction

Concentration of an antiinfectivum should be maximized on target location, but concentration in other different compartments should be kept low. This is an ideal antimicrobial therapy because a therapeutic profit is given by minimization of adverse effects. In opposition to this optimum we have to remember that systemically given agents accumulate differently in different compartments after a time of equilibration.[105]

Some diagnostic and therapeutic decisions in everyday life are focused on measuring blood concentrations of endogenous molecules. A lot of biochemical and pharmacological processes, however, take place in the tissue. Estimating tissue chemistry should theoretically offer more correct data, and this can now be achieved relatively cheaply and minimally invasively with microdialysis.[106]

The following pages will give a short review on the technique of microdialysis and its application in clinical research, drug monitoring and drug developing.

4.2 History

The idea of microdialysis goes back to the 1960s, when push – pull cannulas, dialysis sacs and dialytrodes were inserted into animal tissues to learn about tissue chemistry directly.[107]

The technique of microdialysis has been established in preclinical tests in 1972. Up to 1978 microdialysis studies were only performed on animals, looking on a description of a relative concentration difference. After some period of time the description of an absolute concentration difference seemed to be necessary. Methods of calibration have been developed.[105]

4.3 Principles of microdialysis

In - vivo - microdialysis measures the chemical composition of the interstitial tissue fluid - it is the fluid to which cells and other target structures are directly exposed. Compared to imaging data collection or biosensors, which serve as detecting tools, microdialysis is a sampling tool and has to be combined to an analytical device. Depending on having an appropriate analytical assay, virtually every soluble molecule in the interstitial space fluid can be measured by microdialysis.[106]

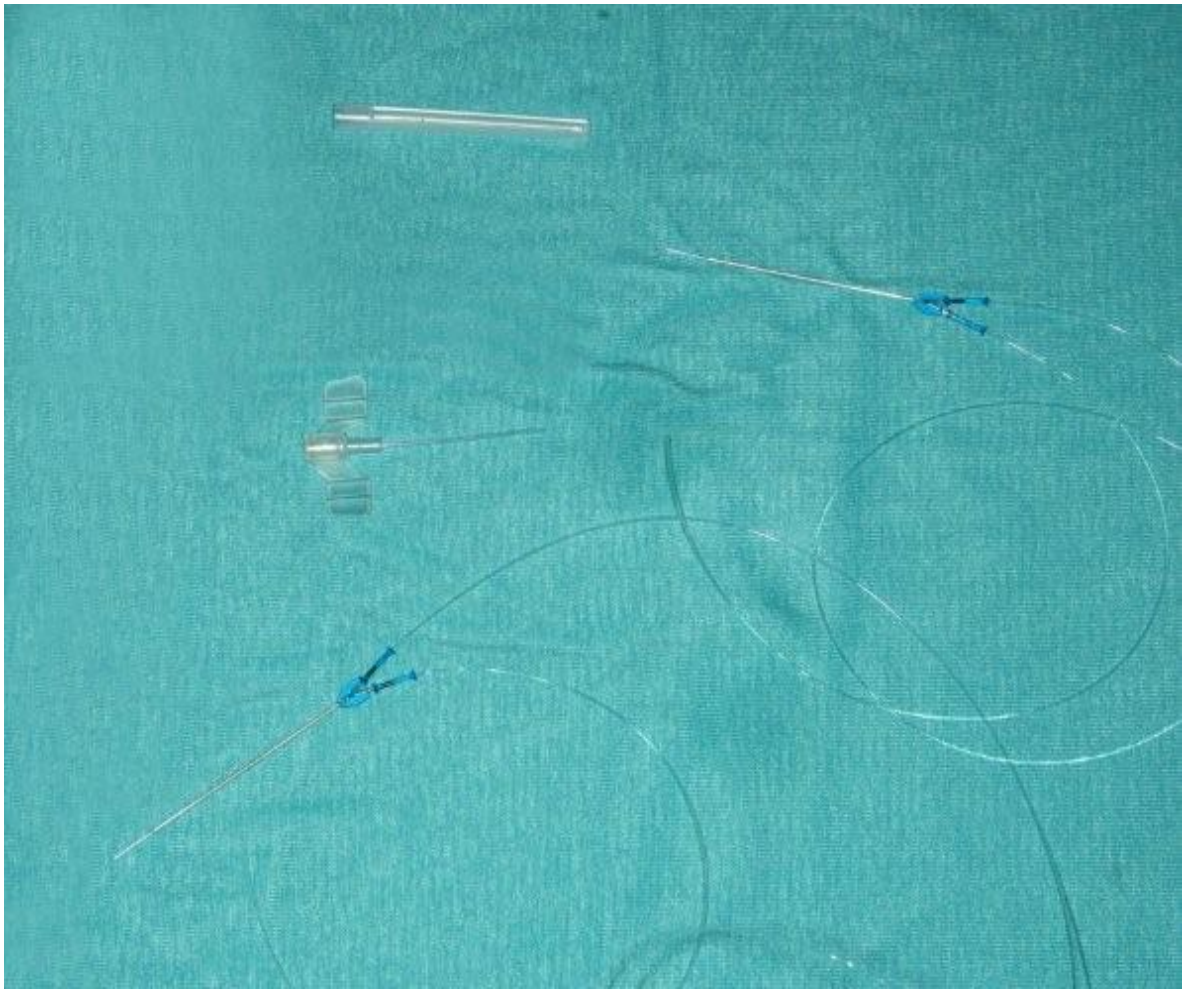


Fig. 24 A microdialysis probe with two interleaved and stucked together cannulas, the thinner and internal probe is a little bit longer. At the tip of the probe is a semipermeable membrane, which is fused to the external probe. The probe's inflow tubing is connected to a microperfusion pump and so the probe is constantly perfused with a physiological solution. Samples are continuously collected from the outflow tubing.[105] [106]

Microdialysis is based on sampling of dissoluble molecules from the interstitial space fluid through a semipermeable membrane at the tip of the probe.[106] The probe is constantly perfused with a physiological solution rate of 1 – 10 µl/min.
When the probe is implanted into the tissue, molecules present in the extracellular fluid at concentration (C tissue) are filtered by diffusion out of the extracellular space into the probe, resulting in a concentration (C dialysate) in the perfusion medium.[108] Samples can be either analyzed on line or might be collected for future analysis.[106]

It is essential that for most analytes, equilibrium between interstitial space fluid (ISF) and the perfusion medium is incomplete. (C tissue > C dialysate) The factor by which the concentrations are cohesive is defined as recovery. Microdialysis probes need to be calibrated to obtain ISF concentrations from dialysate concentrations. There are some techniques which might be used. The most comfortable technique for human pharmacokinetic studies is the "retrodialysis", or "delivery" method proposed by Ståhle.[109] The underlying idea is that the diffusion process is completely equal in both directions through the semipermeable membrane.[110] So the study drugs are added to the perfusion medium, and the disappearance rate through the membrane and in - vivo recovery are equal. The in - vivo recovery formula defines:

Recovery (%)
= 100 - (100 × analytes concentration dialysate × analytes concentrationperfusate-1).[108]

It has been demonstrated that intraindividual variation in microdialysis experiments ranges between 10% and 20% for different analytes.[105]

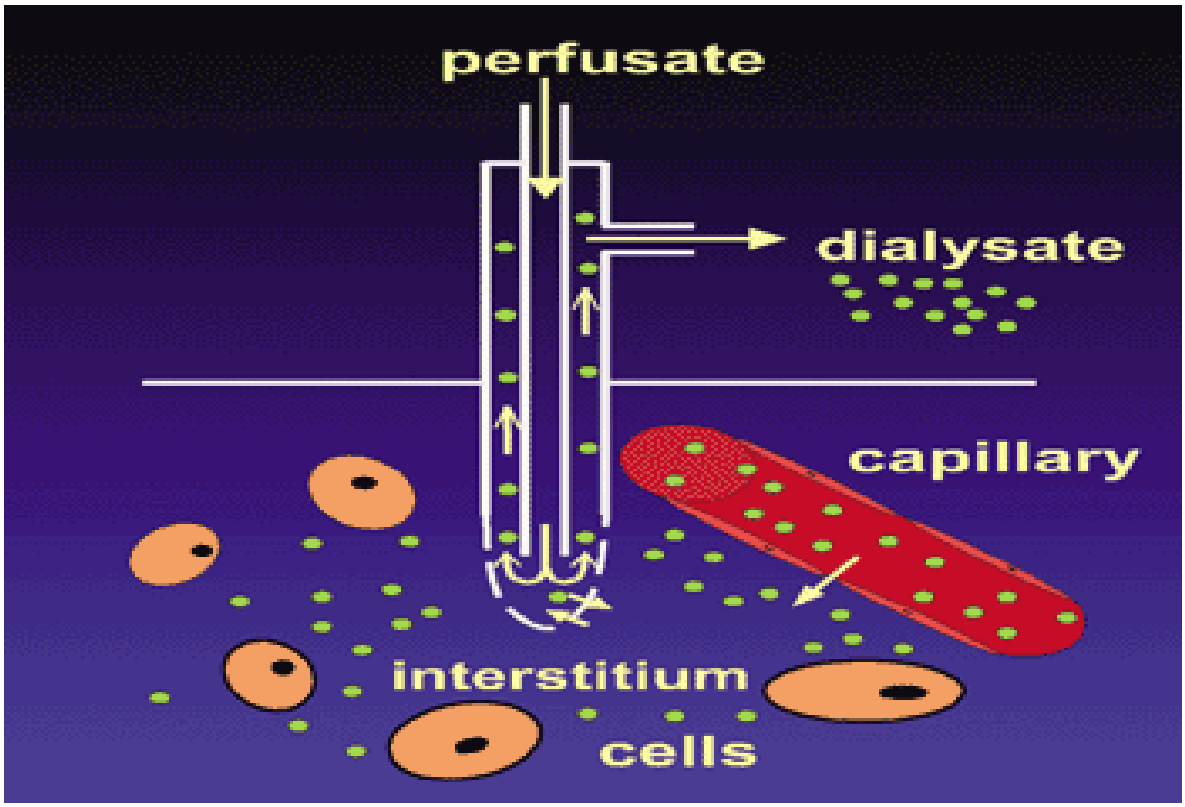


Fig. 25 The inflow tubing is connected to a microperfusion pump and so constantly perfused with a carrier medium, a physiological solution (perfusate). When the probe is inserted into tissue, molecules diffuse out of the interstitial space into the perfusion medium. The fluid, enriched with the substances which have to be analyzed, flows out through the outflow tubing (dialysate). Samples are collected and analyzed.[106] [105]
(Reproduced with the permission of the authors)

4.4 Practical features

There is no special need of practical skills to insert a microdialysis probe. In soft tissues and the skin it can be managed by any health care professional and is not more painful than placing an intravenous cannula. After 10 minutes most patients do not feel any pain. The probes might be left in situ for many hours, but in special cases they can be left for several weeks.[106]

4.5 Limitations

Although microdialysis offers many benefits compared to other techniques, unfortunately it still is limitated by the availability of sufficiently sensitive chemical assays. Microdialysis with conventional dialysis membranes might only dialysate molecules with a

maximum weight of 50 kD, even though the development of ultrafiltration membranes and metal meshes might improve the limitations.[106] Another problem is the fact that the used solution is a hydrophilic fluid, thus only water – soluble substances can be transported. The use of lipid or lipophil substances, like estrogen or nicotine, is limited.[105]

4.6 Application of microdialysis in clinical research

In 1987 the first report on microdialysis in humans was published. It was a study about interstitial glucose[111] and was confined to adipose tissue.[111] [112] Since then numerous studies have been published on microdialysis in other human tissues such as brain[113], heart[114], lung[115] and solid tumors.[116]

At the moment, microdialysis is very popular in neurointensive care for monitoring secondary ischemia, a serious complication after brain trauma.[117] Microdialysis detects characteristic changes in the concentrations of energy related metabolites which might be earlier and more exact markers of ischemic events than brain pressure.[106] Because of many other encouraging results on microdialysis in neurosurgery, some departments of neurosurgery consider to implement microdialysis in routine care.[106] Microdialysis has also been used in cardiac surgery to monitor concentrations of troponin T and aspartate transaminase in patients, who underwent cardiac surgery.[114] Furthermore microdialysis is used in plastic surgery to early detect imminent ischemia in myocutaneous flaps.[118]

4.7 The importance of microdialysis in clinical pharmacology

Because of the fact that microdialysis evaluates concentrations of unbound, i.e. pharmacologically active drugs in the interstitium (where most bacterial infections are localized), it is a well known technique for "tissue – penetration" by antimicrobial drugs.[119] [108] With microdialysis it has been shown that in healthy subjects interstitial concentrations of ß – lactams are in the range of free serum concentrations compared to chinolones and macrolides, where interstitial levels are lower than those from biopsies. In case of septicaemia or septic shock, concentrations of antiinfective drugs are probably different in different spaces from the predicted data.[108] [120]

5 HIGH FOSFOMYCIN CONCENTRATIONS IN BONE AND PERIPHERAL SOFT TISSUE IN DIABETIC PATIENTS PRESENTING WITH BACTERIAL FOOT INFECTION

5.1 Introduction

Severe soft tissue infections of the foot in patients suffering from diabetes may lead to serious or even life threatening complications. The combination of PAD, neuropathy, minor trauma or neuropathic plantar pressure, which often is not noticed by patients because of their neuropathy, might result in deep infections. In worst cases, bones and joints are involved. It is extremely important to avoid delayed diagnosis and a prolonged conservative treatment. A multidisciplinary team has to take part in treating diabetic patients to enable the healing of an ulcer. A surgeon has to be part of the team, because aggressive surgical therapy can be extremely relevant in some patients. The aim always is to prevent limb loss. Therefore, extended incisions of anatomical spaces, uncompromising surgical debridement, open toe, ray amputations or the opening of plantar or dorsal compartments, excision of necrotic tendons, plantar fascia and resection of infected bone are mainly important.

Another essential part of therapy is immediate empirical antibiotic therapy, before information about the causal bacteria is available. Apart from choosing an adequate antiinfectivum, sufficient levels of the antimicrobial agent in the involved tissue or bone have to be reached.

Frossard et al.[121] demonstrated in a recent study that the AUC (Area under the curve) 0 - 8 of fosfomycin in the interstitial fluid of unaltered human soft tissues reached 50% to 70% of the correspondending AUC 0 - 8 for serum, which shows a high degree of penetration into the target side. The concentration of fosfomycin was high enough to eradicate bacterial isolates, like Staphylococcus aureus, in vitro. So it was clearly shown that the concentration of fosfomycin in noninflamed tissue was high enough.

Legat et al.[122] investigated in their study the penetration of fosfomycin in inflamed tissue of elderly patients with severe uncomplicated cellulitis of lower extremities or diabetic foot infection, analyzed by microdialysis. This study showed that the AUC values used for inflamed subcutaneous tissues were not significantly different from those for noninflamed tissue. Fosfomycin concentrations were high enough to inhibit the growth of relevant bacteria, such as Staphylococcus aureus.

Joukhadar et al.[123] described fosfomycin concentrations in muscle interstitium and plasma for a range of clinically relevant pathogens in critically ill patients. He demonstrated that fosfomycin concentrations in muscle interstitium completely equilibrated with plasma within 80 minutes in critically ill patients demonstrating that concentrations were satisfying. In addition, fosfomycin may be used as an alternative antiinfectivum in severe soft tissue infections in critically ill patients.

The main question is whether adequate antibiotic concentrations can be reached in bone tissue of diabetic patients suffering from ulcers. So far, fosfomycin concentrations in bone were only assessed by single – point measurements from biopsies collected after application of doses, which are therapeutically not meaningful.[124] [125]

The aim of this study was to demonstrate details of concentration – *versus* – time profiles of fosfomycin in plasma and metatarsal bone in a diabetic foot infection after application of a single dose of 100 mg per kg body weight (BW), measured by microdialysis.

Some of the following data will be published in J Antimicrobial Chemotherapy under the title: High fosfomycin concentrations in bone and peripheral soft tissue in diabetic patients presenting with bacterial foot infection.

5.2 Subjects and Methods

This study was performed as a single – center, non placebo – controlled open study. It was approved by the Ethics Committee of the Medical University of Graz, Austria and performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines of the European Commission at the Division of Plastic Surgery, Department of

Surgery, Graz, Austria. Analytical work was performed at the laboratory of J&P MEDICAL RESEARCH Ltd, Vienna, Austria.

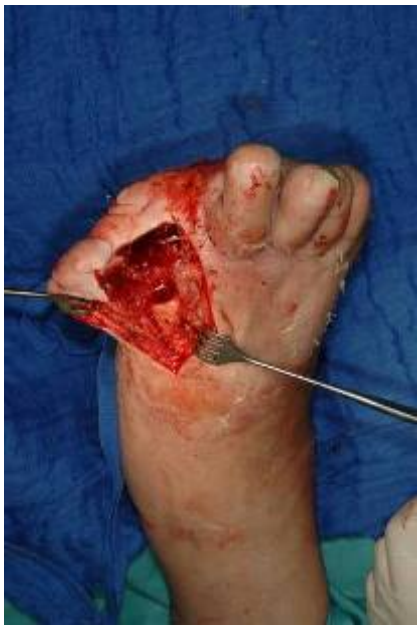
All patients were given a detailed description of the study and an informed consent was obtained from all patients.

The study population included 9 patients with diabetes mellitus (3 female, 6 male, aged 48 – 83, BMI 22.0 – 37.2 kg/m²). The patients were suffering from metatarsal osteomyelitis and/or diabetic foot infections, staged 3B and 4B, according to the University of Texas Classification System.[126] The study subjects required surgical debridement with partial metatarsal bone resection and adjuvant systemic antimicrobial therapy. Exclusion criteria included allergy to fosfomycin, pre-treatment with the study drug within one week before start of the study, pregnancy and nursing women, serum creatinine > 1.6 mg/dl (141µmol/L), severely impaired liver function, severe heart insufficiency and lung edema. Before patients were included into the study, all of them obtained conservative treatment for diabetic foot infection. Six patients had a percutaneous transluminal angioplasty in their medical history. All study subjects were suffering from type II diabetes.

5.3 Microdialysis and sampling procedures

At the end of surgical debridement, after necrotic bone has been removed, one microdialysis probe (shaft length 50 mm, membrane length 10 mm, molecular cut – off 20,000; CMA Microdialysis AB Stockholm, Sweden) was inserted into healthy bone, very close to the resected metatarsal bone [Fig.26]. For this purpose a channel of about 2 mm in caliber and 2 cm in length was drilled into healthy bone [Fig.27]. The tip of the probe was inserted into the channel by the guidance of an adapted plastic cannula. [Fig.28]. (Venflon™, Becton Dickinson, Heidelberg, Germany). The plastic cannula made it possible to place the tip of the probe exactly and safely into the target tissue. A second microdialysis probe was implanted into healthy subcutaneous tissue of the contralateral leg, also placed with the help of a venflon. [Fig.29]. Both probes were connected via tubing to a precision pump (SP101i syringe pump, WPI Inc., Sarasota, FL, USA) and constantly perfused with 0.9% sterile saline solution at a flow rate of 1.5µL/min. After one hour equilibration time, a single dose 100 mg/kg of fosfomycin (Fosfomycin Sandoz™,

Kundl, Austria) was applied as intravenous infusion over 30 minutes. Afterwards, microdialysate samples were collected every 20 minutes from 0 – 3 hours and every 30 minutes from 3 – 6 hours. Venous blood samples were collected from a venous cannula, placed on one arm, also at a 20 minutes intervall from 0 – 3 hours, and from 3 – 6 hours once in every 60 minutes. Microdialysis probe calibration was assessed by the retrodialysis method. Collected microdialysates and plasma aliquots were centrifugated at 1.600 × g for 10 minutes and after this stored frozen at minus 70 ºC until analysis.



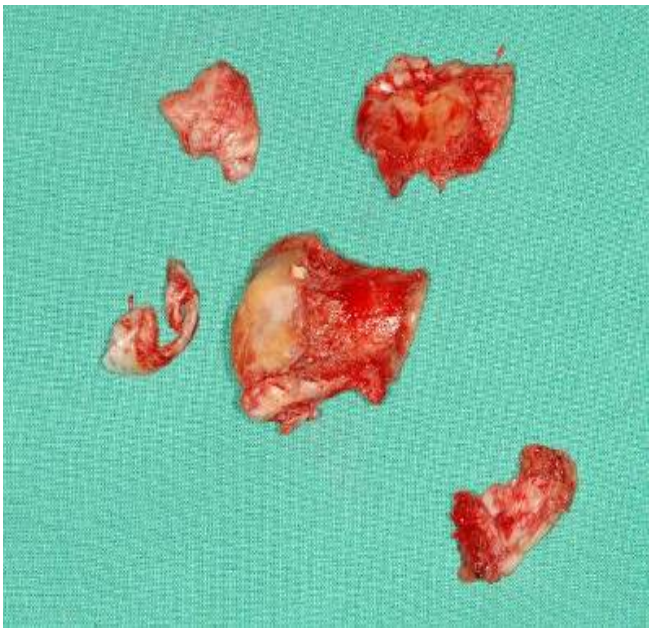


Fig. 26 Resection of the distal MT I
Fig. 27 Resected sequestrating bone

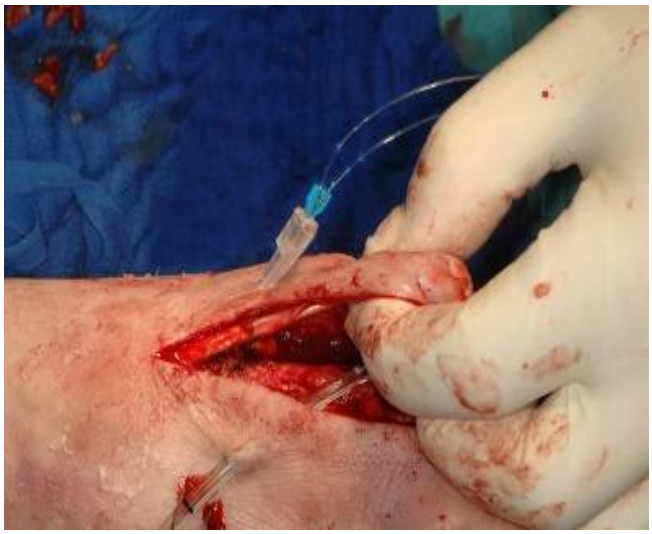


Fig. 28 Adapted plastic cannula as guidance into osseous channel
Fig. 29 Microdialyses probe postioned

5.4 Analysis

5.4.1 Chemical Analysis

Fosfomycin concentrations in plasma and in microdialysates were measured by high performance liquid chromatography.[127] [128] The samples were diluted with drug – free human plasma or 0.9% saline. Plasma samples had to be prepared by ultrafiltration. The lower limit of quantification was 5 mg/L. The coefficients of inaccuracy (relative error) and imprecision (relative standard deviation) ranged between 0.8% and 12.0%.

5.4.2 Pharmacokinetic (PK) analysis and statistics

Data were recorded using a commercially available computer program (Kinetica, version 3.0; Innaphase, Philadelphia, USA). The concentrations at 12 hours were calculated by using the formula: $C_{12h} = C_{6h} \times {}^{e-6k}$. More precisely C_{6h} is the concentration at the time – point 6 hours after start of infusion and k is the elimination rate constant at the ß – phase of elimination. The linear trapezoidal rule was used to calculate the areas under the concentration – time curves from 0 - 6 hours (AUC_{0-6}) and 0 - 12 hours (AUC_{0-12}) in plasma and interstitial fluid. The time fosfomycin concentration remained above the minimal inhibitory concentration (MIC) of selected bacteria (T > MIC) was calculated for plasma and subcutis by the formula $T > MIC = \ln (C_{1h}/MIC)/k + 1$. The calculation of T>MIC for bone was corrected afterwards. Wilcoxon's tests were used for the comparison of the AUCs in plasma, subcutaneous adipose tissue and bone within subjects. A two – sided ρ – value of < 0.05 was considered significant. Multiple testing for significance was adjusted by use of the Bonferroni method.

5.5 Results

The study demonstrated that penetration of fosfomycin into bone is adequate. Concentrations of fosfomycin in bone are completely equal to bone about 3 hours after the

start of the infusion. The concentration of fosfomycin in noninfected subcutaneous adipose tissue was very similar the concentration – versus – time profiles in plasma [Fig.30].

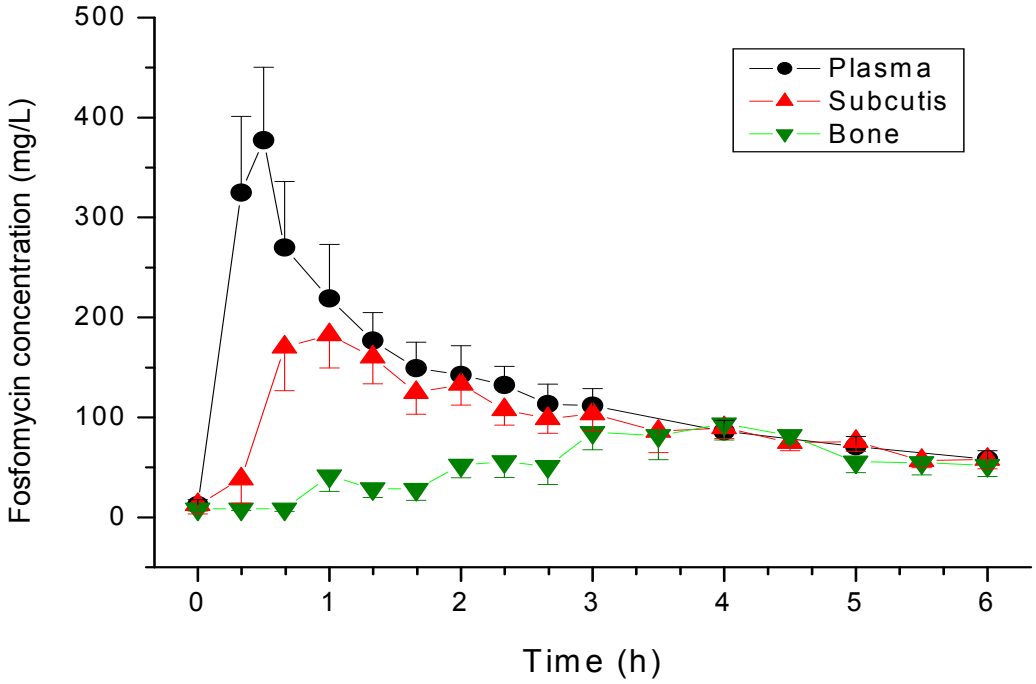


Fig. 30 Concentration – versus – time profiles of fosfomycin in plasma, subcutaneous adipose tissue and metatarsal bone in severe diabetic foot infection after a single dose of 100 mg/kg fosfomycin (*n*=9).

Our study showed that half – lives of fosfomycin in bone, plasma and subcutaneous adipose tissue were approximately 3 hours.

In - vivo recovery of fosfomycin in microdialysates was 81.2 ± 5.6% and 67.7 ± 8.6% for subcutaneous tissue and bone. Fig. 30 shows the concentration – versus – time profiles of fosfomycin in plasma, subcutaneous adipose tissue and metatarsal bone in patients (n = 9). The concentration – versus – time profiles for plasma and subcutaneous adipose tissue were relatively similar.

The main PK parameters are presented in table 3. Fosfomycin concentrations in plasma reached their peak with 377.3 ± 73.2 mg/L after half an hour. In the subcutis the concentration was 185.1 ± 34.2 mg/L after 1.0 ± 0.2 hours, in the bone with 96.4 ± 14.5 mg/L after 3.9 ± 0.4 hours.

Compartment	C_{max} (mg/L)	T_{max} (h)	$T_{½\beta}$ (h)	AUC_{0-6} (mg·h/L)	AUC_{0-12} (mg·h/L)	AUC_{0-6} tissue/ AUC_{0-6} plasma
Plasma	377.3 ± 73.2	0.5 ± 0.0	3.6 ± 1.2	785.1 ± 107.2	1013.8 ± 108.4	–
Subcutis	185.1 ± 34.2	1.0 ± 0.2	3.7 ± 1.4	592.7 ± 77.5	821.3 ± 91.3	0.76 ± 0.05
Bone	96.4 ± 14.5	3.9 ± 0.4	2.2 ± 0.6	330.0 ± 55.3	511.0 ± 100.7	0.43 ± 0.04

Table 3 Main PK parameters Pharmacokinetic indices of fosfomycin following intravenous administration of 100 mg/kg (*n*=9) in plasma and tissues

5.6 Discussion

Most of the published data on fosfomycin address the pharmacokinetic profile of fosfomycin in serum. Less information is available on the ability of the drug to penetrate into the fluid of the interstitial space of human soft tissue. Recent studies have shown that fosfomycin attains high concentrations in noninflamed tissue[121] and also penetrates well into the infected soft tissue of patients with uncomplicated cellulitis of the lower extremity or diabetic foot infections.[122] Our results of fosfomycin are in accordance with these previous data, confirming that the intravenous dose of 100 mg/kg was adequate. Concentrations of fosfomycin peaked in bone between 79 and 124 mg/L and fully equilibrated with plasma after 3 - 4 hours after the start of the infusion. [Fig.30]

In comparison to a healthy study population[129] [121] a moderate prolongation of half - life was noticed in our patients, which is probably a result of the reduced glomerular filtration rate. The ratios of the AUC 0 - 6 for bone to plasma and for subcutaneous adipose tissue to plasma ranged between 0.37 - 0.48 and 0.70 - 0.68. In our study the microdialysis probes were inserted into noninflamed bone, thus very close to the surgically resected infected bone tissue. Theoretically, the PK profile of fosfomycin in inflamed and noninflamed bone may be different, however based on several previous studies we have some cumulative evidence that inflammation does not significantly influence the PK profile of fosfomycin.[123] [122] [130] [131] As a result our study fosfomycin's ability of penetration in

diabetics and a healthy study population seems to be similar. [121] [122] In conclusion, fosfomycin in a dose of 100 mg/kg BW, 2 – 3 times daily seems to be high enough in patients with severe foot infection and osteomyelitis to obtain concentrations in the human bone tissue, which sufficiently eradicate MRSA and other relevant pathogens.

6 INFORMED CONSENT

Patienteninformation und Einwilligungserklärung
zur Teilnahme an der klinischen Studie

Pharmakokinetik von Fosfomycin und Clindamycin gemessen durch In- vivo-Mikrodialyse in Metatarsalia bei Osteomyelitiden und diabetischen Fußinfektionen
(Vollständiger Titel der klinischen Studie)

Sehr geehrte Teilnehmerin, sehr geehrter Teilnehmer!

Wir laden Sie ein an der oben genannten klinischen Studie teilzunehmen. Die Aufklärung darüber erfolgt in einem ausführlichen ärztlichen Gespräch.

Die Teilnahme an einer klinischen Studie ist freiwillig und kann jederzeit ohne Angabe von Gründen durch Sie beendet werden, ohne dass Ihnen hierdurch Nachteile in Ihrer medizinischen Betreuung entstehen.

Klinische Studien sind notwendig, um verlässliche neue medizinische Forschungsergebnisse zu gewinnen. Unverzichtbare Voraussetzung für die Durchführung einer klinischen Studie ist jedoch, dass Sie Ihr Einverständnis zur Teilnahme an dieser klinischen Studie schriftlich erklären. Bitte lesen Sie den folgenden Text als Ergänzung zum Informationsgespräch mit Ihrem Arzt sorgfältig durch und zögern Sie nicht Fragen zu stellen.

Bitte unterschreiben Sie die Einwilligungserklärung nur

- wenn Sie Art und Ablauf der klinischen Studie vollständig verstanden haben,
- wenn Sie bereit sind, der Teilnahme zuzustimmen und
- wenn Sie sich über Ihre Rechte als Teilnehmer an dieser klinischen Studie im klaren sind.

Zu dieser klinischen Studie, sowie zur Patienteninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

1. Was ist der Zweck der klinischen Studie?

Der Zweck dieser klinischen Studie ist der Nachweis eines wirksamen Gewebespiegels der verabreichten Antibiotika im Knochen. Dieser Gewebespiegel gibt Aufschluss über notwendige Dosierungen der verabreichten Medikamente und könnte für zukünftige Patienten mit ähnlicher Erkrankung bessere Ergebnisse erbringen.

Die Behandlung ihrer Erkrankung erfolgt unabhängig von ihrer Teilnahme an der Studie in gleicher Weise.

Die tiefreichende Infektion, ausgehend von einem Fußgeschwür mit Knochenbeteiligung macht einen chirurgischen Eingriff notwendig. Der geschädigte und von Bakterien befallene und z. T zerstörte Knochen muss entfernt werden, damit die Infektion ausheilen und das Fußgeschwür abheilen kann. Die über eine Vene verabreichten Antibiotika müssen den Knochen in ausreichender Konzentration erreichen um den angrenzenden gesunden Knochen vor einer neuen Infektion zu schützen.

2. Wie läuft die klinische Studie ab?

Diese klinische Studie wird an Chirurgischen Universitätsklinik in Graz durchgeführt, und es werden insgesamt ungefähr 10 Personen daran teilnehmen.

Ihre Teilnahme an dieser klinischen Studie wird voraussichtlich 1-4 Tage dauern.

<u>Folgende Maßnahmen werden ausschließlich aus Studiengründen durchgeführt:</u>

Während dieser klinischen Studie werden im Abstand von 20 min-60 min die folgenden Untersuchungen durchgeführt: Entnahme von Gewebsflüssigkeit aus den zuvor gelegten Sonden, Blutabnahme aus einer zuvor gelegter Kanüle am Unterarm. Sie werden kurz nach der durchgeführten Operation im Bett liegend betreut werden. Die Dauer der Entnahmen von Gewebsflüssigkeit und Blut wird über 8 Stunden erfolgen.

3. Worin liegt der Nutzen einer Teilnahme an der Klinischen Studie?

Es ist nicht zu erwarten, daß Sie aus Ihrer Teilnahme an dieser klinischen Studie gesundheitlichen Nutzen ziehen werden.

Jedoch können die erzielten Ergebnisse für zukünftige Patienten wertvolle Erkenntnisse zur Dosisanpassung und Wirksamkeit antibiotischer Substanzen bedeuten und somit zum Extremitätenerhalt beitragen.

4. Gibt es Risiken, Beschwerden und Begleiterscheinungen?

Das Legen der Mess-Sonden erfolgt während der notwendig gewordenen Operation in Narkose und wird daher keinerlei Beschwerden verursachen. Die Entnahme der Gewebsflüssigkeit erfolgt ebenfalls schmerzlos. Zur Ruhigstellung des operierten Fußes

werden sie in den ersten Stunden danach eine Gipshülse angelegt bekommen. Die parallel durchgeführten Blutabnahmen aus einer gelegten Leitung am Unterarm wird ebenfalls schmerzfrei erfolgen, bzw. nicht mehr Schmerzen verursachen als eine übliche postoperative Blutabnahme. Lokale Schwellungen und Blutergüsse sind möglich, aber auch im Rahmen des postoperativen Verlaufes zu erwarten. Eine postoperative Infektion und Wundheilungsstörung kann vorkommen, hängt aber in erster Linie mit ihrer Grunderkrankung zusammen und nicht mit der durchgeführten Messung.

Teilnehmer an der klinischen Studie sind in etwaigen Schadensfällen versichert und können sich bei Bedarf an folgende Adresse wenden.

Wiener Städtische

Allgemeine Versicherungs- AG

HF2 Haftpflicht Fachabteilung

Argentinierstrasse 22

1040 Wien

Tel. 050 350

Polizzenummer : 08-N811.95.7

Bei Unklarheiten im Schadensfall können sich Teilnehmer an der Studie direkt an die Versicherung oder zur Unterstützung auch an die Patientenanwaltschaft oder Patientenvertretung wenden.

5. Zusätzliche Einnahme von Arzneimitteln?

   Keine

6. Hat die Teilnahme an der klinischen Studie sonstige Auswirkungen auf die Lebensführung und welche Verpflichtungen ergeben sich daraus?

   Keine

7. Was ist zu tun beim Auftreten von Symptomen, Begleiterscheinungen und/oder Verletzungen?

Sollten im Verlauf der klinischen Studie irgendwelche Symptome, Begleiterscheinungen oder Verletzungen auftreten, müssen Sie diese Ihrem Arzt mitteilen, bei schwerwiegenden Begleiterscheinungen umgehend, ggf. telefonisch (Telefonnummern, etc. siehe unten).

8. Wann wird die klinische Studie vorzeitig beendet ?

Sie können jederzeit auch ohne Angabe von Gründen, Ihre Teilnahmebereitschaft widerrufen und aus der klinischen Studie ausscheiden ohne dass Ihnen dadurch irgendwelche Nachteile für Ihre weitere medizinische Betreuung entstehen.

Ihr Prüfarzt wird Sie über alle neuen Erkenntnisse, die in bezug auf diese klinische Studie bekannt werden, und für Sie wesentlich werden könnten, umgehend informieren. Auf dieser Basis können Sie dann Ihre Entscheidung zur weiteren Teilnahme an dieser klinischen Studie neu überdenken.

Es ist aber auch möglich, dass Ihr Prüfarzt entscheidet, Ihre Teilnahme an der klinischen Studie vorzeitig zu beenden, ohne vorher Ihr Einverständnis einzuholen. Die Gründe hierfür können sein:

- Sie können den Erfordernissen der Klinischen Studie nicht entsprechen;
- Ihr behandelnder Arzt hat den Eindruck, dass eine weitere Teilnahme an der klinischen Studie nicht in Ihrem Interesse ist;

9. In welcher Weise werden die im Rahmen dieser klinischen Studie gesammelten Daten verwendet?

Sofern gesetzlich nicht etwas anderes vorgesehen ist, haben nur die Prüfer und deren Mitarbeiter Zugang zu den vertraulichen Daten, in denen Sie namentlich genannt werden. Diese Personen unterliegen der Schweigepflicht.

Die Weitergabe der Daten erfolgt ausschließlich zu statistischen Zwecken und Sie werden ausnahmslos darin nicht namentlich genannt. Auch in etwaigen Veröffentlichungen der Daten dieser klinischen Studie werden Sie nicht namentlich genannt.

10. Entstehen für die Teilnehmer Kosten? Gibt es einen Kostenersatz oder eine Vergütung?

Durch Ihre Teilnahme an dieser klinischen Studie entstehen für Sie keine zusätzlichen Kosten.

11. Möglichkeit zur Diskussion weiterer Fragen

Für weitere Fragen im Zusammenhang mit dieser klinischen Studie stehen Ihnen Ihr Prüfarzt und seine Mitarbeiter gern zur Verfügung. Auch Fragen, die Ihre Rechte als Patient und Teilnehmer an dieser klinischen Studie betreffen, werden Ihnen gerne beantwortet.

Name der Kontaktperson: .Dr. Michael Schintler

Ständig erreichbar unter: 0316 385 81904 /0699 19377598

Name der Kontaktperson: Dr. Stephan Spendel

Ständig erreichbar unter: 0316 385 81215 / 0664 2212161

Name der Kontaktperson: .....

Ständig erreichbar unter: .....

12. Sollten andere behandelnde Ärzte von der Teilnahme an der klinischen Studie informiert werden?

Nein

13. Einwilligungserklärung

Name des Patienten in Druckbuchstaben: .....

Geb. Datum: ..... Code: .....

Ich erkläre mich bereit, an der klinischen Studie Pharmakokinetik von Fosfomycin und Clindamycin gemessen durch In- vivo- Mikrodialyse in Metatarsalia bei Osteomyelitiden und diabetischen Fußinfektionen teilzunehmen.

Ich bin von Herrn/Frau Dr. Michael Schintler ausführlich und verständlich über Durchführung, mögliche Belastungen und Risiken, sowie über Wesen, Bedeutung und Tragweite der klinischen Studie, sich für mich daraus ergebenden Anforderungen aufgeklärt worden. Ich habe darüber hinaus den Text dieser Patientenaufklärung und Einwilligungserklärung, die insgesamt fünf Seiten umfasst gelesen. Aufgetretene Fragen wurden mir vom Prüfarzt verständlich und genügend beantwortet. Ich hatte ausreichend Zeit, mich zu entscheiden. Ich habe zur Zeit keine weiteren Fragen mehr.

Ich werde den ärztlichen Anordnungen, die für die Durchführung der klinischen Studie erforderlich sind, Folge leisten, behalte mir jedoch das Recht vor, meine freiwillige Mitwirkung jederzeit zu beenden, ohne dass mir daraus Nachteile für meine weitere medizinische Betreuung entstehen.

Ich bin zugleich damit einverstanden, dass meine im Rahmen dieser klinischen Studie ermittelten Daten aufgezeichnet werden. Um die Richtigkeit der Datenaufzeichnung zu

überprüfen, dürfen Beauftragte der zuständigen Behörden beim Prüfarzt Einblick in meine personenbezogenen Krankheitsdaten nehmen.

Beim Umgang mit den Daten werden die Bestimmungen des Datenschutzgesetzes beachtet.

Eine Kopie dieser Patienteninformation und Einwilligungserklärung habe ich erhalten. Das

Original verbleibt beim Prüfarzt.

.....

(Datum und Unterschrift des Patienten)

.....

(Datum, Name und Unterschrift des verantwortlichen Arztes)

(Der Patient erhält eine unterschriebene Kopie der Patienteninformation und Einwilligungserklärung, das Original verbleibt im Studienordner des Prüfarztes.)

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