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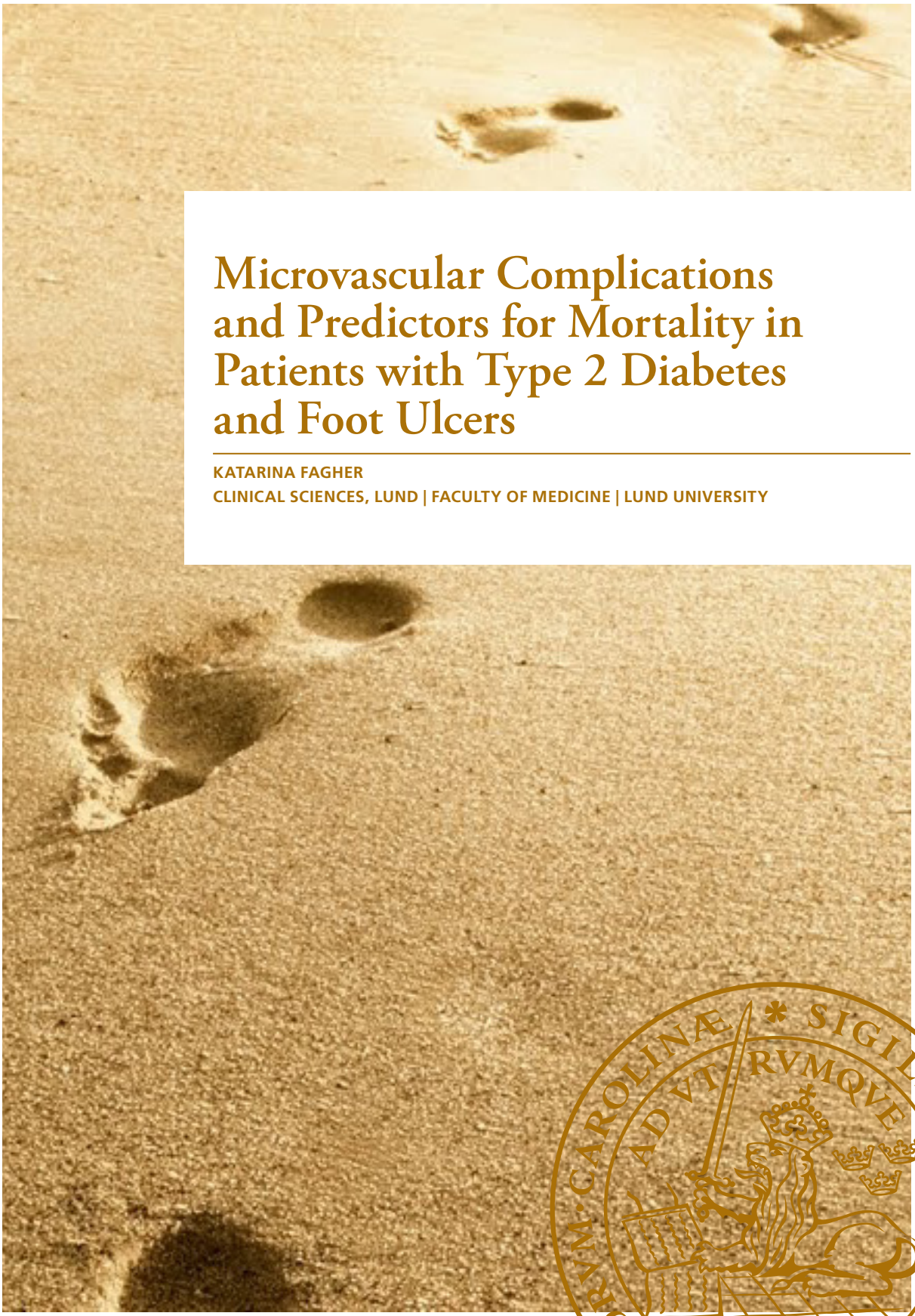
Fagher, Katarina

2018

Document Version:
Publisher's PDF, also known as Version of record



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Printed by Media-Tryck, Lund 2018 NORDIC SWAN ECOLABEL 3041 0903

FACULTY OF MEDICINE

Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2018:68
ISBN 978-91-7619-634-2
ISSN 1652-8220

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LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Segerfalksalen, Wallenberg Neurocentrum
Lund University.
June 1st 2018 at 13.00

Faculty opponent
Professor Thomas Nyström
Department of Clinical Sciences and Education, Södersjukhuset
Karolinska Institutet

Organization LUND UNIVERSITY	Document name Doctoral dissertation
	Date of issue June 1st 2018
Author(s) Katarina Fagher	Sponsoring organization

Title and subtitle: Microvascular complications and predictors for mortality in patients with type 2 diabetes and foot ulcers.

Abstract

A diabetic foot ulcer (DFU) is a common and serious complication of diabetes. It is today the leading cause of amputations and is associated with both increased morbidity and mortality. Individuals with DFU have 2.5 times higher mortality rates compared to patients with diabetes but without DFU, a risk that persists even after ulcer healing. Macrovascular complications, such as a higher burden of myocardial infarction, stroke and peripheral vascular disease can partly, but not alone explain this risk in mortality. The overall aim of this thesis is to evaluate risk factors for mortality to enable early risk stratification, in the high-risk population of individuals with type 2 diabetes and DFU.

(I). Long-term survival of 214 patients with type 2 diabetes and DFU was evaluated based on baseline HbA_{1c} level and QTc-time. After eight years 70.6% of the entire cohort was diseased. A low HbA_{1c} < 58 mmol/mol (< 7.5%) at baseline was associated with worse survival, particularly among those individuals with QTc interval > 440 ms, with 8-year mortality rates of 92.1%, compared to 48.8% in those with similar HbA_{1c}, but without QTc prolongation (p<0.0001).

(II). This study aimed to evaluate QTc interval as a risk factor for mortality among patients with type 2 diabetes undergoing an above-ankle amputation. In our cohort of 70 patients, we demonstrated that a prolonged QTc-time was independently associated with increased 3-year mortality (HR 2.2 (95% CI 1.11-4.38)), with the highest mortality seen among individuals within the highest quartile of QTc-time.

(III). Hyperbaric oxygen (HBO) therapy is a treatment that has been demonstrated to improve tissue oxygenation in hard-to-heal DFUs. The present study is a post-hoc analysis of the randomised, placebo-controlled Hyperbaric Oxygen Treatment in Diabetic patients with Chronic Foot Ulcers (HODFU) study, aiming to evaluate QTc time before and two years after HBO treatment. In this study, 73 patients were evaluated. After two years, median QTc time was significantly shorter in the HBO group compared to placebo group (438 ms vs 453 ms, p < 0.02) and fewer HBO-treated patients had a QTc time > 450 ms (22% vs 53% p < 0.02).

(IV). Transcutaneous oxygen pressure ($TcPO_2$) is often used together with ankle-brachial index (ABI) and toe blood pressure (TBP) for bedside vascular evaluation, to identify patients who might benefit from invasive revascularisation. The present study evaluated the predictive value of $TcPO_2$, ABI and TBP on 1-year mortality, in patients with type 2 diabetes and DFU. Of the 236 patients evaluated, 14.8% of them were diseased within one year. Patients with $TcPO_2$ < 25 mmHg had a higher 1-year mortality compared to those with $TcPO_2$ ≥ 25 mmHg (27.7% vs. 11.6%, p = 0.003), whereas TBP and ABI did not significantly predict 1-year mortality.

Conclusions: The results of the papers included in this thesis, indicate that screening with $TcPO_2$ as well as QTc-interval, might enhance identification of individuals with urgent need for medical assessment. Further, HBO therapy might have a protective effect against QT interval prolongation, however, future studies are needed to confirm this result.

Key words: Diabetic foot ulcers, microvascular complications, mortality, transcutaneous oxygen pressure, QT-interval.

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language: English
ISSN and key title: 1652-8220		ISBN: 978-91-7619-634-2
Recipient's notes	Number of pages 94	Price
	Security classification	

Signature  Date: 2018-04-25

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Coverphoto: Katarina Fagher

Lund University, Faculty of Medicine Doctoral Dissertation Series 2018:68
ISBN 978-91-7619-634-2
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University, Lund 2018

In memory of my Dad

Content

List of publications

This thesis is based on the following papers, referred to in the text by their roman numerals.

I. K Fagher, M Löndahl. The impact of metabolic control and QTc prolongation on all-cause mortality in patients with type 2 diabetes and foot ulcers. Diabetologia, 2013, 56(5): 1140–1147

II. K Fagher, A Nilsson, M Löndahl. Heart rate–corrected QT interval prolongation as a prognostic marker for 3-year survival in people with type 2 diabetes undergoing above-ankle amputation. Diabetic Medicine 2015: 32(5):679–685

III. K Fagher, P Katzman, M Löndahl. Hyperbaric oxygen therapy reduces the risk of QTc interval prolongation in patients with diabetes and hard-to heal foot ulcers. J Diabetes Complications 2015; 29(8): 1198–1202

IV. K Fagher, P Katzman, M Löndahl. Transcutaneous oxygen pressure as a predictor for short-term survival in patients with type 2 diabetes and foot ulcers: a comparison with ankle-brachial index and toe blood pressure. Accepted manuscript, April 11th, 2018, Acta Diabetologica

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Abbreviations

ACCORD study = Action to Control Cardiovascular Risk in Diabetes study

ACE-inhibitor = Angiotensin converting enzyme-inhibitor

ABI = ankle-brachial index

ADA = American Diabetes Association

ADVANCE study = Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation study

ARB = aldosterone receptor blocker

ATA = absolute atmosphere

CAN = cardiac autonomic neuropathy

CI = confidence interval

CVD = cardiovascular disease

DFU = diabetic foot ulcer

EASD = European Association for the Study of Diabetes

ECG = electrocardiogram

eGFR = estimated glomerular filtration rate

ESRD = end-stage renal disease

EURODIALE = European Study Group on Diabetes and the Lower Extremity

HBO therapy = hyperbaric oxygen therapy

HR= hazard ratio

IQR = interquartile range

IWGDF = International Working Group on the Diabetic Foot

MACE = major cardiovascular events

MDRD = Modification of Diet in Renal Disease

ms = milliseconds

NICE-SUGAR study = Normoglycaemia in Intensive Care Evaluation – Survival Using Glucose Algorithm Regulation study

OR = odds ratio

TBI = toe-brachial index

PVD = peripheral vascular disease

$TcPO_2$ = transcutaneous oxygen pressure

TBP = toe blood pressure

UACR = urinary albumin-to-creatinine ratio

UKPDS = United Kingdom Prospective Diabetes Study

WHO = World Health Organization

Abstract

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Introduction

Identifying risk factors for mortality and plausible mechanisms behind, in a predefined high-risk population of individuals with type 2 diabetes and foot ulcers is the unifying theme of this thesis.

Currently, 4% to 5% of the Swedish population is diagnosed with Diabetes Mellitus and the disease is growing in to epidemic proportions worldwide.[1] Diabetes can affect multiple organ systems, and consequently, the burden of diabetes-related complications is increasing and have become a major source of morbidity and mortality in urban countries. Patients with diabetes suffer from an approximate two-fold increase in cardiovascular and all-cause mortality compared to individuals without diabetes.[2,3]

A common complication to diabetes, with a lifetime risk estimated to more than 15%, is the development of a diabetic foot ulcer (DFU).[4-7] Once present, a DFU may cause disability, reduce quality of life and is today the leading cause of amputations in the lower limb.[8-10] Further, DFUs also have consequences on survival. A patient with a DFU has a 2.5 times higher mortality risk than a patient with diabetes, but without a foot ulceration.[11-13] This mortality risk is sustained even after ulcer healing, indicating that a DFU might serve as a marker for a general advanced disease.[14] After a major amputation the mortality risk is even higher, with five-year mortality rates exceeding 70%.[12,15] A higher burden of traditional cardiovascular risk factors could partly, but not alone, explain this risk increment and the role of other factors, including microvascular complications, needs further evaluation to improve strategies to identify high-risk DFU patients with urgent need for risk factor assessment.[13,14]

Classification and diagnosis of Diabetes Mellitus

Diabetes mellitus is a heterogeneous chronic disorder characterized by hyperglycaemia, requiring continuous medical care with a multifactorial approach to avoid long-term complications.

Diabetes can be classified into the following categories:[16]

- Type 1 diabetes (caused by an immune-mediated ß-cell destruction, typically leading to complete insulin deficiency)
- Type 2 diabetes (associated with progressive loss of insulin secretion and decreased insulin sensitivity)
- Gestational diabetes mellitus (diabetes during pregnancy; often resolved after delivery)
- Specific types of diabetes due to other causes. This category involves; monogenic diabetes syndromes (such as maturity-onset diabetes of the young (MODY)), diseases of the exocrine pancreas (such as cystic fibrosis and after pancreatitis), and drug- or chemical-induced diabetes (glucocorticoid use, Cushing syndrome and acromegaly)

Type 2 diabetes, is the most dominating type of diabetes contributing to almost 85% to 90% of all cases, whereas type 1 diabetes, the second most common type, only contributes to 5% to 10%.[1,17]

The WHO diagnostic criteria for diabetes mellitus are listed in table 1.[18,19]

Table 1.
WHO diagnostic criteria for the diagnosis of Diabetes Mellitus

A fasting[1] venous *or* capillary plasma glucose of ≥ 7 mmol/l**
Or
A venous plasma glucose of ≥ 11.1mmol/l or capillary of ≥ 12.2 mmol/l after OGTT**
Or
HbA_{1c} ≥ 48 mmol/mol (≥ 6.5 %)**
Or
Classic symptoms of hyperglycaemia and random plasma glucose ≥ 11.1 mmol/l (venous) or ≥ 12.2 mmol/l (capillary)

1. Fasting is defined as no caloric intake for at least 8 h.
2. OGGT Oral glucose tolerance test using a 75 g glucose solution.

** In the absence of symptoms of hyperglycaemia, a second test is required to confirm the diagnosis.

The diabetic foot ulcer

Background

The definition of a DFU, as a full-thickness wound below the ankle in a patient with diabetes, is based on consensus of the International Working Group on the Diabetic Foot (IWGDF).[20]

Already F G Banting, after achieving the Nobel prize 1923 for discovering insulin, stated in his Nobel lecture that "one of the most common complications of diabetes is gangrene". Today, 3% to 12% of all patients with diabetes have a history of foot ulcer, and the lifetime incidence of developing a DFU is estimated to more than 15%.[1,4,5] Further, a DFU is one of the most serious complications of diabetes, associated with both increased morbidity and mortality, and is today the leading cause of amputations.[9-11,13,14] In 2007, foot ulcers and their complications were linked to one third of costs associated with diabetes, and in 2012, the National Health Service in the United Kingdom, spent between £639 million and £662 million on foot ulcers and lower extremity amputation.[1,21] In Sweden, a health-economic study reported an average cost of a deep infected DFU, healed without amputation in 1997, to be 136 600 SEK per patient, whereas the average cost for a major amputation was estimated to 234 500 SEK.[22] When adjusting these costs for inflation (year 1997 to 2017 + 25,9% based on National Central Bureau of Statistics (SCB) Consumer Price Index), the approximate costs would amount to 171 979 and 295 236 SEK, respectively.

Pathophysiology of the diabetic foot ulcer

The development of a DFU is complex and involves several pathophysiological mechanisms. A minor trauma, or repetitive stress is often sufficient to trigger the development of a DFU in vulnerable feet, affected by either peripheral neuropathy, peripheral vascular disease (PVD) or, as in most of cases, both.[23,24] Once present, a DFU is often complicated by infection which, together with potential hyperglycaemia and oedema, further reduce the likelihood of healing.

Peripheral neuropathy

The association between diabetes and loss of peripheral nerve function has been known for long time. It was first recognized in 1864 by the French physician Marshal de Calvi, and in 1885, Frederick William Pavy described the condition called diabetic neuropathy, with the classical symptoms of hyperesthesia or anaesthesia, as well as loss of tendon reflexes in the lower extremities.[25]

Peripheral neuropathy affecting the lower limbs, is the most common form of diabetic neuropathy. The prevalence of peripheral neuropathy described in literature ranges from 16% to 66% in the general diabetic population.[1,6,26,27] The exact prevalence is however difficult to estimate since a significant amount of patients remain undiagnosed. Among individuals with DFU, presence of peripheral neuropathy is even more common. In the EURODIALE study, evaluating DFU patients, a prevalence of 86% was reported in this population.[28] Presence of peripheral neuropathy is not only a strong predictor for DFU, but also associated with an increased risk of falls, compared to age-matched controls.[29]

The pathological mechanism behind neuropathy is multifactorial. It is well known that poor glycaemic control with hyperglycaemia accelerates the process, and both older age and longer diabetes duration are well known risk factors for developing this complication.[26,27,30-33] The exact pathological mechanism is, however, not fully understood, although there is strong evidence that microvascular disturbance and hypoxia play a key role.[34] For instance, thickening of capillary basal membrane and hyalinization of small vessels supplying the nerves have been demonstrated in biopsy studies, and have been shown to correlate with degree of clinical symptoms.[35,36]

Peripheral neuropathy is divided into three groups; motor, sensory and autonomic neuropathy, each playing a vital role in the development of a DFU.

- *Motor neuropathy:* Damage to myelinated α-motor neurons cause loss of strength and function of the small muscles in the feet. One consequence of this is postural instability and unstable walking pattern, with increased risk of falling.[37] Further, imbalance between extensor and flexor muscles, due to different loss of strength between these muscles, results in claw deformities and subluxation of the toes. Consequently, parts of the toes and the metatarsal heads become exposed to abnormal pressure, with increased risk of ulceration.
- *Sensory neuropathy:* Loss of protective sensation is a key factor behind the DFU, associated with a vulnerability for repetitive stress and minor trauma.[37] This allows injuries to remain undetected and consequently, the risk of secondary infections and advanced ulcerations increases. Sensory nerves fibres have varying diameters, with all, but the thinnest C-fibre (associated with heat and pain sensation) being myelinated.[38]
- *Autonomic neuropathy:* Autonomic dysfunction in the lower limb has two major consequences. First, loss of sweating (sudomotor dysfunction) disturbs the natural moisture of the skin, resulting in dry skin with increased risk of cracking. Second, autonomic dysfunction affects the microvascular autoregulation of blood flow, which in normal conditions, redistributes blood supply to areas with increased need. When this autoregulation is disturbed, blood redistribution does not occur properly, which might aggravate tissue ischaemia.[37] Autonomic neuropathy involves small unmyelinated autonomic C-fibres in the sympathetic and parasympathetic system.

Damage to small myelinated (Aδ, associated with pain and cold sensation) or unmyelinated C-fibres (pain, heat and autonomic function) usually occur first.[39,40] Small fibre neuropathy is also frequently associated with neuropathic pain[37].

Peripheral vascular disease

Both macro- and microvascular impairment, when present, are major contributing factors in the development of a DFU, since oxygen delivery is crucial for ulcer healing and poor circulation is a strong risk factor for worse outcome and a non-healing ulcer.[41]

Traditionally, peripheral vascular disease, (or peripheral arterial disease), is characterized by an atherosclerotic disorder in the arteries of the lower limbs and the condition is markedly increased among individuals with diabetes, particularly in type 2 diabetes.[42] The risk of developing PVD increases by age, diabetes duration, smoking habits, and hypertension. Among patients with DFU approximate 50% have co-existing PVD.[28,42]

The most common symptom of PVD is intermittent claudication, with pain in the affected leg during activity, that typically resolves while resting. When the disease progresses into critical limb ischemia, rest pain might be present and the risk of developing gangrene increases.[42] However, although some patients develop symptoms, the majority remains asymptomatic due to neuropathy, making the true dimension of this complication difficult to determine.

PVD in patients with diabetes often differs to PVD in non-diabetic patients, as it more often involves distal arteries below the knee.[43] This sometimes complicates the possibilities for vascular intervention, and increase the risk of amputation.[43,44] Another difference seen in diabetic PVD, is the prevalently existing medial arterial calcification causing calcified and non-compressible vessels.[45] This consequently results in a diagnostic challenge with falsely elevated distal pressures, when evaluating peripheral circulation in patients with diabetes. The condition, was first described in 1903, by a German pathologist J. G. Mönckeberg, and is also termed Mönckeberg´s sclerosis.[46] Chronic kidney disease as well as neuropathy are factors that potentiate and increase the risk of medial arterial calcification in diabetes.[45,47,48]

The role of the microcirculation in the diabetic foot

Microvascular disturbance in the foot and skin was identified decades ago, although its impact on wound healing, and the relationship between micro- contra macrovascular circulation, is not fully clarified.[49,50] It has been previously shown, that the cutaneous microcirculation only partly depends on the macrovascular status, and that both components are independently important in the healing process.[50]

There are several structural and functional microvascular abnormalities seen in diabetes, such as thickening of basal membrane, reduced capillary size, pericyte degeneration, dysfunction involving nitric oxide pathways with reduced ability of vasodilation, and increased vascular permeability.[51,52] Further, small nerve fibre dysfunction, influencing the neurovascular control of local skin blood flow, is also involved in wound healing.[53] For instance, reduced sympathetic tone leads to increased blood flow in arteriovenous (AV) shunts.[54] This results in reduced oxygen and nutrient transport in the dermis and epidermis, and thus impaired ulcer healing.

Although microangiopathy contributes to the DFU, it should not be assumed to be the only cause of poor wound healing in a patient with DFU.[41]

Other factors contributing to the outcome of a diabetic foot ulcer

Infection

Infection in a DFU is a common and often devastating complication. In an ischaemic foot, presence of infection increases the oxygen demand. This might aggravate tissue necrosis and increase the risk of amputation.[55] A prompt and adequate treatment of an infected ulcer is therefore pivotal in the management of an DFU, to avoid amputations.

There is evidence in the literature that diabetes influences the immune system in several ways, making the patient more prone to infections.[56-59] Risk factors for diabetic foot infections are; ulcer deep, ulcer duration, a history of recurrent ulcers, a traumatic aetiology of the ulcer, presence of PVD, and renal impairment and transplantation.[60,61]

The predominant pathogens in infected DFUs are *staphylococcus aureus* and *β-haemolytic streptococci* (group B mostly, but also A and G). Other bacteria found, but to a less extend, are *coagulase negative staphylococcus*, *pseudomonas*, *Escherichia coli*, *proteus* and *klebsiella*. In deep wounds with abscesses and tissue necrosis, anaerobes are often present, and in osteomyelitis the microbiology often includes more than one bacteria.[62,63]

Osteomyelitis develops in approximate 15% of all DFU and is characterized by inflammatory process in the bone due to infection, and is associated with impaired healing rates and risk of amputations.[62] Deep wound extending to bone structure, wounds with area ≥ 2 cm, and non-healing wounds increases the likelihood of osteomyelitis.[62] Diagnostic tools involve probe-to-bone test, plain radiography, magnetic resonance imaging, and leukocyte scan. For microbiology, a bone culture is recommended, since the concordance of a superficial wound swab or a deep soft tissue culture is low.[64-66]

Hyperglycaemia

Hyperglycaemia is a common problem in patients with DFU, since inflammation increases insulin resistance due to increased levels of stress-hormones and pro-inflammatory cytokines, including tumour necrosis factor (TNF)-α, interleukin (IL) 1β, IL6, and IL8.[56] Hyperglycaemia is associated with impaired function in the cellular immune system, and this might be one contributing factor behind the higher risk of infections seen among individuals with diabetes.[57-59]

Oedema

Lower extremity oedema is commonly present in persons with DFU. Congestive heart failure, renal failure, hypoalbuminemia, venous insufficiency, and inflammation are factors that might contribute to the development. Oedema is a

prognostic factor to worse outcome among individuals with DFU, both with higher amputation rates as well as higher mortality.[67]

Management of the diabetic foot ulcer

Patients with DFU should be referred to a specialised DFU-unit including a diabetologist, podiatrist, diabetic nurse, orthopaedic technician, with rapid access to facilities for diagnosing PVD and, if indicated, vascular intervention and orthopaedic surgery.[66] Time has been demonstrated to be a crucial factor for outcome, and the National Diabetes Foot Care Unit in England found in an inventory that patients with DFUs assessed by an expert within two weeks, were more likely to heel their ulcers in 12 weeks and were also more likely to be alive.[68]

Ulcer characterization

Characterizing a DFU as neuropathic, ischaemic or neuro-ischaemic is important, to identify those individuals who would benefit from revascularisation procedures and to optimize wound care.[66]

Diagnosing neuropathy

Differential diagnosis to consider and exclude, before diagnosing a patient with diabetic neuropathy, is alcoholism, vitamin B deficiency, thyroid disorders, drug-induced, hereditary, infectious, and inflammatory causes. Laboratory and anamnestic screening for these disorders are therefore recommended.

Early detection of peripheral neuropathy is crucial to enable preventative management to reduce the risk of future foot complications. An annually screening with at least one of the following test is recommended in all patients with diabetes to identify patients at risk of developing foot complications:[66]

- *10-g Semmes-Weinstein Monofilament:* This is the most commonly used test to diagnose disturbance in pressure perception. The nylon monofilament is applied at the skin surface of the foot for two seconds, with sufficient force to cause the monofilament to bend. At least three sites on each foot is tested. Protective sensation is considered absent when two out of three answers are incorrect. A positive monofilament test has been highly predictive of foot ulcerations.[69]

- *Vibration testing:*
    - 128 Hz-tuning fork: This sensory test is carried out with a 128 Hz tuning fork applied with a constant pressure on the bony, dorsal side of the distal phalanx of the first toe. Three applications on each toe is recommended, with at least one application with the tuning fork not vibrating. Two or more incorrect answers suggests reduced protective sensation. If the patient does not feel vibration at the site of the toe, the test is repeated at the level of the malleoli.[70]
    - Biothesiometry: This is a test for vibration perception threshold and is carried out using an instrument consisting of a handheld part with a vibrating probe and a motor-part with a voltmeter. The probe is gently applied to the distal part of the great toe and vibration is increased using the voltmeter, until the threshold is reached where vibration is recognized. Two repetitive tests on each location is carried out and averaged, and values above 25 Volts are considered positive for neuropathy and has shown strong correlations with foot ulcerations.[70,71]
- *Ankle reflexes:* Absence of Achilles reflexes also correlates to increased risk of DFU.[4] The test is carried out with the Achilles tendon stretched, until the ankle is in neutral position, before striking it with the reflex hammer.[70]
- *Ipswich Touch Test:* This is a test were the examiner lightly touches the tips of the first, third and fifth toe of each foot with the index finger for 1-2 seconds, while patient having their eyes closed. The advantage of this test is its simplicity and availability where resources are limited. When compared to 10-g monofilament, the test had a sensitivity and specificity of 76% and 90% respectively.[72]

Diagnosing peripheral vascular disease

The following methods are often used for bedside vascular examination:

- *Palpation of the pedal pulse:* This test function as a first line screening, often used in primary bedside examination. It has a high inter- and intra-observer variability and thus, a low diagnostic value.[73]
- *Ankle blood pressure:* Measuring ankle blood pressure with a blood pressure cuff over the ankle and a Doppler placed over dorsal pedal and posterior tibial arteries, is the most widely used non-invasive test when diagnosing PVD. The value is used to calculate the ankle-brachial index (ABI), defined as the systolic ankle pressure divided by systolic arm blood

pressure. An ABI < 0.9 is often considered diagnostic for PVD and has in the general population been validated to be both sensitive and specific towards angiographic findings.[41,42,74] However, in patients with diabetes, and particularly among those with neuropathy, there are some important limitations with ABI as a diagnostic tool.[74] Due to the high prevalence of medial arterial calcifications and secondary arterial wall stiffness, there are risk of falsely elevated ABIs, which underestimates the severity of PVD.[45,75] Pathologically elevated ABI is often defined as ABI >1.3.[41] In patients with ABI < 0.5 and a non-healing DFU, urgent vascular imaging and revascularisation is recommended.[41]

- *Toe blood pressure (TBP)*: This test is performed with a small blood pressure cuff around the great toe. It could also be affected by digital calcification and consequently, be falsely elevated, although to a less extend compared to ABI.[74] A normal TBP is considered to be approximately 30 mmHg lower than the ankle pressure. Critical limb ischemia with impaired ulcer healing, is often present when TBP is < 30 mmHg, and urgent vascular imaging is recommended below this level.[41] Toe-brachial index (TBI), defined as TBP divided with systolic arm blood pressure, is sometimes calculated, and a TBI > 0.75 is usually defined as normal

- *Transcutaneous oxygen pressure ($TcPO_2$)*: $TcPO_2$ is the partial pressure of oxygen at the surface of the skin. It represents the oxygen diffusion through pre-heated skin minus the consumed oxygen in tissue and is considered to better reflect the microvascular status. Normal $TcPO_2$ is approximately 60 mmHg.[76] Impairment of microvascular function is accompanied by a decrease in $TcPO_2$.[77]

  In several studies, $TcPO_2$ has been a better predictor for ulcer healing compared to ABI and TBP. Ballard *et al* found a significant difference in healing outcome when $TcPO_2$ < 30 mmHg, Kalani *et al* found similar result using the cut-point of < 25 mmHg, and Pecoraro and Ladurner *et al* both suggested 20 mmHg as a cut-off level for supposing healing failure with a conservative treatment strategy.[78-81] Based on these studies, the IWGDF guidelines suggest urgent vascular imaging and vascular intervention, when $TcPO_2$ is < 25 mmHg.[41]

  There are several limitations with $TcPO_2$ measurements, and test results might be affected by presence of peripheral oedema, cellulitis and hypoxia due to other causes (heart failure, pulmonary diseases).

Although a proper clinical examination in many cases can be diagnostic for PVD, its sensitivity for ruling out the disorder is low. The current IWGDF guideline

document recommends imaging and revascularisation if TBP < 30 mmHg or TcPO2 < 25 mmHg.[41] However, vascular evaluation should also be considered in patients with higher pressure levels if other predictors of poor prognosis are present, such as infection or large ulcer area. Further, IWGDF recommends re-evaluating patients for PVD and consider vascular imaging if an ulcer does not improve within six weeks.[41]

Vascular imaging:

- *Duplex Ultrasound imaging*: This is a non-invasive, well-tolerated technique that detects anatomical and hemodynamic abnormalities. The sensitivity of duplex scanning, compared to angiography ranges from 89% (iliac artery) to 68% (popliteal artery), and is highly operater-dependent.[82]
- *Computed tomography angiography (CTA)*; This is an imaging technique using iodine contrast. The original single CT system was limited to only assess large vessels, due to long exposure time and resolutions. However, the technique has enhanced with modern generations of CT scanners, and today, both the sensitivity and specificity to detect a > 50% stenosis are approximate 87% to 99%.[83,84] Heavy calcification can, however, mimic contrast and may under-diagnose the burden of disease, which limits the use in advanced PVD.
- *Magnetic resonance angiography (MRA)*; with gadolinium contrast allows imaging of the vessels, as well as the surrounding structures, with less difficulties to distinguish between calcification and contrast, since calcification has a different signal on MRA. The median sensitivity and specificity of a modern contrast enhanced MRA to detect a significant arterial stenosis, are approximate 95% and 97%, respectively.[85] An advantage with MRA compared to CTA is that there is no radiation exposure and gadolinium contrast is less nephrotoxic than iodine contrast, although the risk is still not insignificant. A contraindication to MRA is the use of pacemaker or certain implantable devices (for instance cochlea implants and aneurysm clips).
- *Digital Subtraction Angiography (DSA);* using iodine contrast is the golden standard to which other techniques are compared.[86] Today, it is not used as a diagnostic tool due to its invasive character with risk of complications, but is used when endovascular intervention is indicated, since this could be performed at the same time.

Ulcer classification systems

There are different DFU classification scales in use today, aiming to describe the lesion or ulcer as routine in clinical care, to follow progress or for research purposes. A few of the most commonly used systems, are stated below:

- *The Wagner classification scale;* is based on wound depth and vascular status. It is graded from 0, being a pre-ulcerative condition to 5, with advanced gangrene involving the whole foot.[87]
- *The University of Texas San Antonio wound classification system*; is based on both wound depth from grade 1, being a pre-ulcerative condition, to grade 4, a wound penetrating to bone, as well as presence of both ischaemia and infection, staged A-D (Stage A: no infection, Stage B: infection, Stage C: ischemia, Stage D: infection and ischemia).[88]
- *The Pedis system*; was developed by the IWGDF group in an effort to standardize wound classification and besides depth, vascular status and infection, it also includes wound size and neuropathy.[89]

Ulcer treatment

The following are to be considered routine management for DFU:

- *Off-loading:* One corner stone in the management of a DFU is off-loading, in order to relief pressure and biomechanical stress on the ulcer area. This can be managed in different ways; with total contact cast, removable cast-walkers, temporary therapeutic footwear, individually made insoles or customized shoes and limitations in walking and standing.[23] Non-removable off-loading devices has been superior to other removable devices, in order to heal neuropathic ulcers.[90] However, long immobilisation periods carry a risk of thrombosis, muscle wasting, as well as secondary ulcerations, that needs to be considered.
- *Restoration of perfusion:* Vascular intervention should always be considered in patients with DFU and critical limb ischemia, with primary goal of ulcer healing, pain resolving and avoidance of amputation. Techniques used are open reconstructive surgery (bypass surgery) or endovascular techniques, where use of the latter method has expanded rapidly over the last decade, and is now the dominating technique.[41] Endovascular techniques has several advantages regarding recovery rates, surgical complications, costs and hospitalization time. However, due to a considerably high rate of re-stenosis after endovascular treatments, there are still many situations were a distal bypass is the most durable method for

revascularisation, with superiority in long-term patency, freedom from re-intervention and limb salvage.[91]

- *Treatment of infection:* Infected ulcers should be treated with antibiotics after culturing. When initiating oral antibiotic therapy, before having the culture result, target *Staphylococcus aureus*. In deep infected, potentially limb-threatening wounds, parenteral broad-spectrum antibiotics should be considered, as well as the need for surgical intervention.

  The optimal duration of antibiotic administration is unknown. In conservatively treated osteomyelitis, prolonged treatment is often necessary and traditionally, twelve weeks has been recommended. Today, there are however trials demonstrating similar result with six weeks of treatment, suggesting that it is possible to reduce prescription of antibiotics, and this strategy is today supported in guidelines.[62,92,93] Using shorter antibiotic treatment must however, be accompanied with follow-up, to detect early signs of relapse. The effect and potential benefit of topical antibiotics into the wound, needs to be determined in randomised trials before recommended as routine praxis.[94]

- *Local wound treatment;* includes wound cleaning and debridement with a scalpel to remove slough, necrotic tissue and surrounding calluses, as well as choosing a proper dressing, based on exudate control and to maintain a moist environment. The number of dressings with different properties is increasing, but there is today either no, or insufficient evidence to support one specific dressing, in preference to any other.[95]

  In some cases, especially in post-surgical wounds, negative pressure wound therapy (NPWT) should be considered.[95] NPWT extracts wound exudate and seem to stimulate granulation tissue and there are randomised trials showing beneficial effects on post-surgical wounds.[96]

- *Optimize metabolic control and treat oedema:* Hyperglycaemia and elevated HbA_{1c} is considered a risk factor for worse wound healing, as well as complications after surgical procedures. Trials have demonstrated increased surgical morbidity after foot and ankle surgery indicating that optimized metabolic control should be considered before elective surgery.[97,98]

  Oedema management is often based on diuretics together with external compression using bandaging, compressions socks or intermittent pneumatic compression pumps. Presence of critical limb ischaemia must however be considered before initiating compression therapy, since constant external compression might aggravate tissue ischaemia. In those cases, intermittent pneumatic compression therapy is preferable, and has in

previous studies been demonstrated to improve tissue perfusion and enhance wound healing in patients with critical limb ischeamia.[99]

Obesity is considered a risk factor for both insulin resistance as well as oedema, and lifestyle intervention should be encouraged.

- *Pain control:* It is recommended to distinguish between neuropathic and ischemic pain, since appropriate treatment differ. Neuropathic pain is often more pronounced during resting, particularly at night time, and is often characterized as a burning or freezing, shock-like pain in the affected limb. Allodynia is also commonly present.[37,100] Ischaemic pain often resolves during resting, but in patients with critical limb ischaemia resting pain might be present. The pain intensity might, in these cases, depend on whether the patient lower, or raises the limb. Neuropathic pain is often more resistant to conventional pain treatment, and agents such as different types of antidepressants (tricyclic antidepressants, duloxetine, venlafaxine) and anticonvulsants (gabapentin, pregabalin) are routinely used, to reduce the use of opioids. Topical Capsaicin (as a cream or patch) is sometimes proposed as an alternative treatment, as it offers less systemic side-effects as well as drug-interactions.[100]

Treatments that are not included in routine management, but ought to be considered as adjunctive therapy in non-healing wounds, includes:

- *Systemic hyperbaric oxygen therapy (HBO):* This treatment modality developed primarily for decompression sickness, was in the early 1960s proposed as treatment for chronic wounds.[101] The treatment take place in either mono-place or multi-place hyperbaric chambers, where patients breath 100% oxygen at 2.0 to 2.5 absolute atmosphere (ATA), during a treatment session of 60 to 120 minutes (2.5 ATA is equivalent to being about 15 meters underwater). The treatment is repeated daily, for about 15 to 40 sessions. HBO therapy increases the partial pressure for oxygen in the blood and improves oxygen delivery in tissues. This has been shown to have multiple effects, both immediate and more long-term, involving angiogenesis, fibroblast activation, antibacterial effects, growth factor up-regulation and modulation of immune response with down-regulation of inflammatory cytokines.[102-106]

  There are randomised controlled trials evaluating HBO on wound healing in patients with chronic DFU, indicating that HBO enhances ulcer healing. However, conflicting evidence exists, and larger trials are required to determine the result, confirm cost-effectiveness, and identify those individuals most likely to benefit from this treatment.[95,107-113]

- *Biological therapy:* Local therapy with growth factors, platelet and leucocyte concentrates, are therapies that has been suggested to enhance wound healing, however, the clinical evidence today is sparse. There are ongoing randomised controlled trials, and whether these advanced wound products will be recommended treatments for hard-to-heal DFUs in the future, depends on these results.[95] Other products, sometimes used in non-infected chronic wounds, includes fibroblast-derived dermal substitutes composed of living, metabolically active, human fibroblasts, grown on tissue-engineered grafts or temporary mesh, as well as allografts derived from the submucosa of donated human placenta (amniotic membrane).[114] Few of these products have, however, been extensively evaluated in randomised controlled trials, and need higher level of evidence before they will be recommended in routine use.[95]

  Also, different types of autologous tissue-grafting (meshed skin graft, split-skin graft) are widely used, although the scientific evidence in the management of DFU is sparse.[95]

Healing and recurrence

> The strongest predictor of a diabetic foot ulcer is a previous diabetic foot ulcer.
>
> *David G Armstrong*

Approximate 70% of all DFUs heal within one year with appropriate therapy.[115] Worse healing rates are seen in patients with advanced PVD and multi-organ failure and in those unable to walk.[115]

Unfortunately, once healed many ulcers recur. In a review by Armstrong *et al*, on recurrence rates, they estimated a 40% risk of recurrence within one year after healing, and almost 60% after three years.[116] Consequently, they suggest clinicians to think in terms of remission rather than healing, once wound closure is achieved.[116] The reason behind the high recurrence rate is multifactorial. Initially, after ulcer closure, the skin is weaker and more vulnerable for stress and minor trauma. Further, the predictive factors behind ulcer development, such as neuropathy, foot deformities, sensory loss, and angiopathy, are often not resolved by healing.

The preventive work to reduce recurrence is an important and challenging issue for health care providers, and includes patient education, adherence to prescribed foot wear, podiatry care at a regular basis, and early detection of pre-ulcerative lesions, such as blisters and callus.[23,66]

Amputation

Of all lower extremity amputations performed, the majority occur in patients with diabetes, with the majority preceded by an ulcer.[8,9] In the EURODIALE study, approximately 5% of patients with a DFU required a major amputation, during the 12 month of follow-up.[115] Amputation rates are higher in ischemic ulcers, compared to pure neuropathic ulcers.[117] A study by Faglia *et al*, evaluated $TcPO_2$ as a predictor for above-the-ankle amputation, and demonstrated a prediction probability of 68% for amputation when $TcPO_2$ was < 10 mmHg. With increasing $TcPO_2$, the probability of amputation decreased, and $TcPO_2$ 10-19 mmHg, 20-29 mmHg, 30-39 mmHg, and 40-49 mmHg were associated with a probability of 44%, 22.5%, 6.1%, and 2.6%, respectively.[118] Amputations are divided into minor (when a toe or a part of the foot is removed) and major (above the ankle). There has been a reduction in major amputation over the past decades and this has been considered a marker for increased quality of care.[119] Nevertheless, sometimes in advanced wounds, not responding to conventional treatment, an amputation can actually be accompanied by improvement in quality of life and should not always be seen as a failure.

Comorbidities, their consequences and management of risk factors among individuals with diabetic foot ulcers

A DFU might be seen as a marker for advanced disease, as the majority of these individuals suffer from several diabetes complications.

Macrovascular complications

Presence of PVD increases the risk of other concomitant macrovascular complications. Long-standing hyperinsulinemia, elevated levels of glucose and free fatty acids, often found among individuals with type 2 diabetes, accelerate the atherosclerotic process. This process is characterized by endothelial dysfunction, inflammation, foam cell formation and development of fatty streaks, which proceed to atherosclerotic plaques.[120] Further, hyperglycaemia contributes to platelet dysfunction and thus increased athero-thrombotic risk.[121]

Cardiovascular disease

The prevalence of cardiovascular disease (CVD) is about two-fold higher among people with diabetes compared to non-diabetic patients, and approximate half of the

patients will die prematurely, due to the consequences of a CVD.[2,122] In gender-specific analysis, the cardiovascular mortality risk for women with diabetes is three-fold higher compared to women without diabetes, whereas a two-fold increase in cardiovascular mortality has been demonstrated in men.[2]

In the United Kingdom Prospective Study (UKPDS), evaluating patients with newly onset type 2 diabetes randomised to intensive versus conventional glycaemic control, the difference in myocardial infarction did not reach significance after the initial 10 years of follow up.[30] However, in the 10-year post-trial follow-up, a significant reduction of 15% was found in the intensively treated arm, demonstrating a positive effect in the long-term perspective, often mentioned as the "metabolic memory" or "legacy effect".[123]

Traditionally, CVD in diabetes has focused on the atherosclerotic coronary disease, but one of the earliest cardiac complications among individuals with diabetes is heart failure.[124] The mortality risk for patients with diabetes and left ventricular systolic dysfunction is doubled compared to patients with heart failure, but without diabetes.[125] Further, heart failure with preserved ejection fraction is a condition that has been associated with diabetes. Sandesara *et al*, found in a study of patients with heart failure with preserved ejection fraction (HFpEF) with and without diabetes, that diabetes was a risk factor for worse outcome (i.e. risk for hospitalisation, all-cause mortality, and cardiovascular mortality). The authors also concluded that the risk was highest among individuals with known microvascular complications, where neuropathy was the most common complication.[126]

Among individuals with DFU, the risk of CVD and cardiovascular mortality is even higher compared to the general diabetes population.[127] In a study evaluating the prevalence of cardiac disease in a DFU cohort of 80 patients, 24% of the patients had a history of myocardial infarction and 25% had known congestive heart failure. However, when examining patients with echocardiography, the number increased with 78% of the patients having signs of cardiac dysfunction.[128] This indicate that silent CVD is common in a DFU-population.

Presence of PVD and a low ABI have in several studies been shown to predict both fatal and non-fatal cardiovascular events, as well as all-cause mortality, in both the general and in the diabetes population.[9,129,130] In the Fremantle Diabetes Study of 1 294 type 2 diabetes patients, presence of ABI ≤ 0.90 was a strong predictor for cardiac death in a follow-up period of 10 years (HR 1.67 (1.13-2.47)), and in a meta-analysis of 16 cohort studies of both diabetes and non-diabetes patients, an ABI ≤ 0.90 was associated with a more than two-fold increase in all-cause mortality, cardiovascular mortality and major coronary events.[131,132] In a study by Moulik *et al*, evaluating mortality rates among patients with new-onset DFU stratified by ulcer aetiology, presence of an ischaemic ulcer was associated with worse outcome, with five-year mortality rates of 56%, compared to 45% in neuropathic, and 20% in

neuro-ischaemic ulcers. However, in that study, mean age was higher among patients with ischemic ulcer, and when adjusting for this, age turned out to be a confounding variable behind the higher mortality among these individuals.[117]

A low TBP or TBI and their association with CVD risk and mortality have been contradictory in literature, with some, but not all, studies indicating a predictive value.[130,133,134]

Cerebrovascular disease

The risk of cerebrovascular disease among patients with diabetes is increased approximate two-fold compared to individuals without diabetes.[122] Elevated HbA_{1c}, hypertension, and previous smoking, have in observational studies been identified as independent risk factors for stroke occurrence, among patients with diabetes without previous CVD disease.[135,136] In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, evaluating type 2 diabetes patients with either established CVD or with CVD risk factors, a reduction in stroke incidence was observed among patients with intensive blood pressure lowering therapy compared to a conventionally treated group (-14.2 mmHg difference), while no benefit was observed in patients who received intensive glucose-lowering therapy.[137,138]

In the DFU-population, cerebrovascular complications are more common, compared to individuals with diabetes without DFU.[139]

Microvascular complications

Diabetic microangiopathy is traditionally affecting the retina, nerves, and kidneys, and its relationship to long-term glycaemic control is well-known.[30,140,141]

In the foot, impairment of microvascular function is accompanied by a decrease in $TcPO_2$.[77] Presence of a low $TcPO_2$ has in previous studies been associated with a higher burden of other microvascular complications.[142,143] A low $TcPO_2$ has also been associated with major cardiovascular events (MACEs) in type 2 diabetes patients without a history of previous CVD or DFU.[144]

Retinopathy

Diabetic retinopathy correlates strongly with diabetes duration and is one of the most common causes of blindness among individuals with diabetes. Risk factors for developing retinopathy, besides diabetes duration, are hyperglycaemia, nephropathy, and hypertension.[145,146] A strict metabolic control has in large prospective studies been shown to reduce the risk of retinopathy progression.[30] Other diseases affecting the eye, such as glaucoma and cataract, are also more frequently seen among patients with diabetes.

Individuals with diabetes retinopathy have a higher incidence of other microvascular complications and also increased risk of developing DFU.[147,148]

Diabetic kidney disease

Diabetic kidney disease is defined as persistent elevated urine albumin excretion and/or impaired glomerular filtration rate due to diabetes, and occurs in approximately 20% to 40% of people with diabetes, where about half develop chronic kidney disease (CKD) stage 3 or higher (table 2).[149,150] The prevalence of end-stage renal disease (ESRD) is 10-fold higher in people with diabetes than without, and is accelerated by hypertension.[1,151]

Screening for albuminuria with urinary albumin-to-creatinine ratio (UACR) and estimation of glomerular filtration rate (eGFR) regularly is recommended to enable early diagnosis of diabetic kidney disease and to monitor progression.[150] Normal UACR is < 30 mg/g (< 3 mg/mmol), and two elevated tests within 3 to 6 months are recommended for diagnosis. Albuminuria is the earliest marker of diabetic kidney disease and is a strong predictor for CVD and mortality.[152,153]

Initiating angiotensin converting enzyme (ACE)-inhibitor therapy or angiotensin receptor blocker (ARB) therapy has been associated with reduction in albuminuria and improved renal outcome, and is recommended as first line antihypertensive therapy in patients with diabetic kidney disease.[154,155]

Recently, kidney effects of the SGLT-2 inhibitors empagliflozin and canagliflozin have been evaluated as secondary outcomes in large cardiovascular (CV) outcome trials, demonstrating beneficial effects versus placebo in reducing albuminuria and slowing progression of kidney disease.[156,157] Similarly, the CV outcome trials of the GLP-1 receptor agonists liraglutide and semaglutide, also demonstrated beneficial effects on the secondary renal parameters, compared to placebo.[158,159] These results need, however, to be further evaluated as primary outcomes in future trials before they can be definitely determined.

Diabetic kidney disease and DFU are often co-existing complications.[147] Patients with diabetic kidney disease have higher ulcer incidence, lower healing rates and increased risk of amputation and the association is almost linear with declining eGFR.[15,160,161] PVD in patients suffering from ESRD is more severe and often accompanied by diffuse arterial calcifications, involvement of more distal arteries, and by impaired microcirculatory perfusion.[47] A small study evaluating $TcPO_2$ in CKD-patients undergoing dialysis, indicates that there is an immediate decrease in $TcPO_2$ for up to four hours after dialysis.[162] On the other hand, there is also an opposite relationship, with DFU and PVD being independent risk factors for declining renal function over time. In a study evaluating the predictive value of ABI on renal function, participants with an ABI < 0.9 had a two-folded risk to double their creatinine level during three years of follow-up.[163]

Table 2.
Staging of chronic kidney disease (CKD)[164]

Stage	eGFR (ml/min/1.73 m^2)
No CKD	≥ 60 + no evidence of kidney damage*
1	≥ 90 + evidence of kidney damage*
2	60-89 + evidence of kidney damage*
3	30-59
4	15-29
5	<15

*Kidney damage is most often manifest albuminuria with UACR ≥30 mg/g, but can also include other abnormalities, such us pathological urinary sediment or radiographic abnormalities.

Cardiac autonomic neuropathy

Impairment of cardiovascular autonomic control, after excluding other reasons than diabetes, is called cardiac autonomic neuropathy (CAN).[37] It is a common and severe complication of diabetes with an initial prevalence of approximately 20%, increasing up to 65%, with increasing age and diabetes duration.[165-167] Symptoms and abnormalities associated with CAN involve resting tachycardia, orthostatic hypotension, silent myocardial ischemia, loss of heart rate variability, QT interval prolongation on ECG, disturbed circadian variation in blood pressure, and impaired baro-reflex sensitivity.[167] Presence of orthostatic hypotension and QT interval prolongation is associated with worse prognosis.[167,168] A meta-analysis of 15 studies, including 2 500 patients with diabetes, demonstrated that presence of CAN is a strong independent predictor for mortality with a relative risk of 3.45 (95% CI 2.66-4.47).[169] Also, in the ACCORD trial, presence of CAN was associated with increased mortality risk.[170]

The QT interval on the ECG reflects ventricular depolarization and repolarization in the heart, which is mediated by ion flow over the myocardial cell membrane. Loss of function in these ion channels cause an intracellular excess of positively charged ions and results in a prolonged repolarization, reflected by a prolonged QT interval on ECG.[171] A consequence of this is an increased risk of a specific type of ventricular tachycardia called Torsade de Pointes, which can precede to ventricular fibrillation and cardiac arrest.[172] Many conditions, as well as certain drugs, have besides CAN, been associated with QT interval prolongation, where some of the most common are stated in table 3.

When diagnosing CAN, several tests have been suggested for autonomic testing, but the golden standard today is heart rate response to deep breathing, standing, and Valsalva manoeuvre, together with blood pressure response to standing.[167] Orthostatic hypotension is defined as a reduction of at least 20 mmHg in systolic or 10 mmHg diastolic blood pressure.[173] A definite diagnose and staging of CAN

require more than one heart rate test, and the orthostatic hypotension test, where presence of orthostatic hypotension indicates a more advanced stage of CAN.

In clinical practice identifying patients with CAN is important when risk-stratifying and treating patients.[167] For instance, orthostatic hypotension has implications when adjusting anti-hypertensive treatments. Further, when targeting glycaemic control, patients with CAN might be more vulnerable to hypoglycaemia, since a pro-arrhythmical potential of hypoglycaemia mediated by a prolongation of the QT interval has been demonstrated.[174-176] Similarly, indications of drugs with a potential QT interval prolonging action might be re-evaluated. Also, presence of CAN may be pivotal when considering silent myocardial ischemia since this condition is two-fold as common in patients with CAN, compared to those without.[177,178]

Table 3.
Some of the most common conditions (A) and drugs (B) associated with QT interval prolongation[171,179,180]

A. Factors that predispose QT interval prolongation	B. Drugs associated with QT interval prolongation
Congenital Long QT syndromes	**Antiarrhythmic**: Amiodarone, Disopyramide, Procainamide, Sotalol, Ibutilide, Quinidine
Reduced left ventricular ejection fraction	**Antipsychotics:** Thioridazine, Pimozide, Ziprasidone, Chlorpromazine, Haloperidol, Olanzapine, Risperidone
Left ventricular hypertrophy	**Antibiotics:** Clarithromycin, Erythromycin, Levofloxacin, Trimethoprim-sulfamethoxazole, Pentamidine, Sparfloxacin
Myocardial ischemia	**Antidepressants:** Amitriptyline, Desipramine, Imipramine, Sertraline, Venlafaxine, Fluoxetine, Paroxetine
Bradycardia	**Other:** Droperidol. Fluconazole
Hypokalaemia	
Hypomagnesemia	
Hypocalcaemia	
Hypothyroidism	
Hypothermia	
Older age	
Female sex	
Cardiac autonomic neuropathy	
Hypoglycaemia	

Cardiovascular risk management

The benefits of CVD risk factor treatment strategies have been demonstrated previously. In the STENO-2 study, patients with diabetes type 2 and microalbuminuria, were randomised to either intensive or conventional risk factor modification, including metabolic control, blood pressure, and use of statin, aspirin and ACE-inhibitors. After intervention (7.8 years), a 20% absolute risk reduction in cardiovascular events was found, as well as a significant reduction in microvascular complications.[181] In a post-intervention follow-up, offering all patients intensive treatment, these beneficial effects of early intensive intervention, sustained over

time, with HR of 0.54 (95% CI 0.32-0.89) for all-cause mortality after 13.3 years.[182] This suggests that early intensive multifactorial approach has high impact on both macro- and microvascular complications, as well as on survival in patients with diabetes.

Among the DFU population, risk factor management has not been studied in detail. However, in a study by Young *et al*, introducing an aggressive CVD risk approach as standard practice at a DFU-unit in Edinburgh, five-year mortality was reduced from 48% to 27% ($p < 0.001$).[183] The improvement in survival was found both among patients with ischemic, neuro-ischaemic, as well as pure neuropathic ulcers.

CVD risk factor management according to ADA/EASD guidelines should be considered a priority among DFU-patients. These guidelines include:

- *Individualised lifestyle intervention and smoking cessation;* is fundamental for all patients with diabetes, including those with PVD.[184]
- *ECG-screening:* to detect signs of silent ischemic heart disease, as well as electrophysiological disturbance, such as atrial fibrillations and QT interval prolongation. A pathological finding should encourage further cardiac evaluation, and specific treatment.[185,186]
- *Lipids:* Patients with type 2 diabetes often differ in their dyslipidaemia compared to the general population, typically with lower HDL and higher triglycerides.[187,188] Effects of statins on CVD events has been confirmed by several studies and thus statin therapy is recommended as first-line therapy for all DFU-patients, except in cases of statin-intolerance. The beneficial effects of statins are not only associated with a reduction in CVD events and mortality, but also in a reduced incidence of, or worsening of intermittent claudication, as demonstrated in a subgroup of the Scandinavian Simvastatin Survival Study (4S).[189] The recommended goal for LDL in patients without CVD, or PVD is < 2.5 mmol/l and in patients with known CVD, or PVD a more stringent goal of < 1.8 mmol/l is recommended, and treatment should be optimized towards that goal.[185]
- *Blood pressure*: The prevalence of hypertension is higher among persons with diabetes compared to the general population and may be even more common among individuals with DFU.[11] Antihypertensive treatment primarily based on ACE-inhibitors or ARBs, is recommended for the general diabetic population, as well as the DFU-population.[190] Blood pressure goal due to ADA guidelines is < 140/90.[185] It is however important, that an orthostatic test is performed routinely before adjusting therapy, as autonomic neuropathy is a common co-existing complication in the DFU-population.

- *Assessment of albuminuria and eGFR:* At least once a year, screening with albumin-to-creatinine ratio in spot-urine, and calculation of eGFR using a validated formula, is recommended.[150] This is important to detect early progression of diabetic kidney disease, for proper dosing of medications and to minimize complications before considering iodinated contrast exposure.
- *Aspirin therapy:* In patients with present CVD or PVD, aspirin therapy should be considered unless intolerance is present and in those cases, another antiplatelet therapy is recommended.[185]
- *Beta-blockers*: should be considered to all patients with manifest CVD, if tolerated. The advantage of using beta-blockers actually seem to be more pronounced, particularly in heart failure patients with, compared to those without diabetes.[191] The use of beta-blockers in patients with PVD has been controversial since potential hemodynamic side-effects, leading to worsening of peripheral ischemia has been presumed. However, a Cochrane review evaluating the risk of beta-blockers in presence of PVD found no evidence for adverse effects, although they concluded that larger clinical trials were needed.[192]
- *Glycaemic control:* Glycaemic targets according to ADA/EASD is HbA_{1c} < 52 mmol/mol (< 7%) for most patients with diabetes.[193] If this goal is implemented early after diabetes diagnosis it has been associated with long-term reduction in future complications.[30] However, in patients with advanced disease, the optimal HbA_{1c} target is not fully clarified and the importance of individualized glycaemic targets based on comorbidities, expected life-time and risk of hypoglycaemia has been advocated.[138,193]

  The optimal metabolic target in a DFU population has previously not been evaluated.

Key messages of introduction

A diabetic foot ulcer is a common and severe complication of diabetes and should be considered a life-long condition with high risk of both ulcer recurrence, but also a high risk of future CVD-events and mortality. The mortality risk seems to be independent of the aetiology of the ulcer (i.e. ischemic, neuro-ischaemic or neuropathic) and traditional CVD risk factors can partly, but not alone, explain the increase in mortality. A multifactorial approach is fundamental, not only to manage foot problems, but also to recognize patients at increased risk of death. Additional tools to identify high-risk individuals who would benefit from urgent medical assessment are therefore warranted.

Aims

The overall aim of this thesis and PhD project was to identify potential risk factors for mortality, that might enable risk stratification in the in the high-risk population of patients with type 2 diabetes and foot ulcers.

- Evaluate QTc prolongation as a risk factor for mortality in DFU patients. (paper I, II)
- Evaluate the prognostic factor of HbA_{1c} and metabolic control for survival. (paper I)
- Evaluate if hyperbaric oxygen therapy has any impact on QTc prolongation. (paper III)
- Evaluate the prognostic value of $TcPO_2$ compared to ABI and TBP for short-term survival in patients with DFU. (paper IV)

Methods

Study site

All patients were recruited from diabetic foot units at Skåne University hospital in Lund (all papers), Skåne University hospital in Malmö (paper III), Ängelholm hospital (paper II), and Helsingborg hospital (paper III).

Skåne University Hospital in Lund is the primary hospital for approximate 350 000 inhabitants and Skåne University hospital in Malmö about 400 000 individuals.

Helsingborg and Ängelholm are primary hospitals for a population of about 200 000 and 70 000 individuals, respectively.

Study design

The different studies included in this thesis differ by design as follows.

Paper I is designed as a prospective cohort study evaluating QTc time on ECG and HbA_{1c} as predictors for long-term survival. Inclusion run-in period was two years (2002 to 2003) and patients were followed until they died or for a maximum of eight years.

In **Paper II**, data was retrospectively collected using medical and surgical records and all patients who underwent their first above-ankle amputation due to a DFU during year 2004-2010, were screened for participation. Patients were grouped based on QTc time ≤ / > 400 ms.

Paper III is a *post-hoc* analysis of ECGs recorded in the prospective, randomised, double-blinded, placebo-controlled HODFU-study (the Hyperbaric Oxygen in Diabetic patients with chronic Foot Ulcers). The primary endpoint of the HODFU-study, to evaluate HBO as adjunctive treatment on ulcer healing in patients with hard-to-heal DFUs, has been previously published.[107] Randomisation was done in blocks of ten using sealed, numbered opaque envelopes, stratified by TBP ≤ / > 35 mmHg. Patients fulfilling > 35 treatments out of 40 (per-protocol requirement) were

included in the analysis. [194] Inclusion period was between 2002-2007 and follow-up time was two years from the first treatment session.

Paper IV is a retrospective cohort analysis, including all type 2 diabetic patients who underwent vascular assessment measuring ABI, TBP and $TcPO_2$ at our DFU-unit in Lund during year 2013 to 2015.

Patients

All patients were treated according to international guidelines regarding the DFU and were evaluated for vascular intervention when indicated.

Inclusion criteria

- *Diabetes type:* In paper I, II and IV, patients with type 2 diabetes were included. In the ECG-analysis of the HODFU-study (paper III) ECGs of both type 1 and type 2 diabetes patients were evaluated.
- *Age:* All patients were older than 18 years. Maximum age for inclusion was < 80 years in paper I and II, and ≤ 90 years in paper IV.
- *Foot ulcer:* All patients included had at least one DFU below malleoli. Ulcer duration in paper I was > 4 weeks and in paper III ≥ 3 months. In paper II patients with DFU who underwent their first amputation above the ankle were included.
- *Vascular status:* In the HODFU-study (paper III) only patients with adequate peripheral circulation, or patients with non-reconstructable vascular disease were included.

Exclusion criteria

In all studies evaluating ECGs (paper I-III), patients without a baseline ECG recording and patients with ventricular stimulating pacemaker were excluded.

In paper I, patients with ongoing dialysis and lack of HbA_{1c} within four months from first visit were excluded.

In paper II patients who died within ten days after amputation, as a result of complications after surgery, were excluded from further analysis.

In paper III, patients with contraindications to HBO were excluded (i.e. severe pulmonary disease, untreated thyrotoxicosis, ongoing treatment with cisplatin, doxorubicin or disulfiram, women in fertile age without anticonception, patients with known malignancy, patients with a history of stroke or myocardial infarction within 30 days, misuse of drugs or alcohol, ongoing acute infection (CRP > 30 mg/l), a frail general condition, and participation in other ongoing studies.[194]

Baseline characteristics

Patients were risk stratified at baseline regarding the following traditional cardiovascular risk factors (in all papers, otherwise stated):

- *Hypertension*, defined as blood pressure ≥ 140/90 or the concomitant use of antihypertensive drugs.[185]
- *Hyperlipidaemia*, defined as total cholesterol > 5 mmol/l and/or LDL-cholesterol > 2.5 mmol/l, and/or ongoing treatment with cholesterol lowering medications.
- *Cardiovascular disease* was evaluated either as a composite risk factor including heart failure, known myocardial infarction, angina pectoris and cerebral vascular disease (paper IV), or as separate risk factors (paper I-III).
- *PVD* was defined as ABI ≤ 0.9, or ≥ 1.4, or previously performed vascular intervention (paper I). In paper II, the above stated, or TBP < 30 mmHg was used as a definition.
- *Renal function* was based on estimated glomerular filtration rate (eGFR) calculated from plasma creatinine level using MDRD-equation.[195] Reduced renal function was defined as eGFR < 60 ml/min/1.73m^2.
- *HbA_{1c}*, reported both in IFCC unit (mmol/mol) and DCCT standard (%).
- *Nephropathy* (paper III) was diagnosed when albuminuria > 30 mg/g (> 3 mg/mmol) on two separate occasions.
- *Smoking habits* (ever)
- *Diabetes duration* (years)
- *Concomitant medications*, both antidiabetic and cardiovascular medications as well as prescriptions of drugs with a plausible QTc prolonging action (paper I-III) (i.e. tricyclic antidepressants, selective serotonin re-uptake inhibitors, macrolides, quinolones, proton-pump inhibitors, and some beta-blockers, such as Sotalol) were evaluated.

Vascular assessment (all papers)

All vascular examinations were performed when patients were resting in a supine position.

In paper IV all measurements were performed with Periflux System 5000 diagnostic instrument (Perimed AB, Stockholm Sweden) at our DFU-unit in Lund, since this is routine praxis today in all new referrals to our unit.

When indicated patients were referred to our Vascular Department at Skåne University hospital in Malmö, for evaluation and consideration for vascular intervention.

Ankle-brachial index

Systolic ankle pressure was measured either with Periflux 5000 instrument (paper IV), which use a laser Doppler technique to measure systolic pressure in the extremity. In paper I-III, the ankle pressure was measured either with Periflux 5000 instrument, or with a pen Doppler over the dorsal pedal or tibial posterior arteries. The ABI was calculated as the ankle pressure divided by the systolic arm pressure. In paper I-III a threshold of ≤ 0.9 was used to define abnormal ABI.[42] In paper IV < 0.9 was used for definition.[41] Pathologically elevated ABI was defined as ≥ 1.4.[41]

Three separate measurements from each foot was performed and averaged. The lowest value of both feet was used in mortality analysis (in paper IV), otherwise the value of the affected leg with ulcer was used.

Toe blood pressure

Systolic TBP was measured at the great toe using either strain gauge technique or by laser Doppler technique (Periflux 5000). The difference in technique was due to a method change in the laboratory.

Three separate recordings were performed and averaged on each foot. In paper IV both TBP thresholds of < 30 and < 50 mmHg were evaluated.

Transcutaneous oxygen pressure

$TcPO_2$ measurements were performed at the dorsum of both feet at room temperature (21-24 °C), while the patient was breathing ambient air (basal $TcPO_2$).

Patients included in the HBO-trial were also evaluated with stimulated $TcPO_2$, after breathing 100% oxygen, but since this measurement strongly correlated to basal $TcPO_2$, only basal $TcPO_2$ was reported in paper III.[196]

The foot was carefully cleaned before applying the transducer using adhesive rings and contact liquid supplied by the manufacturer. After calibration and preheating the transducer to approximate 44°C, measurement was performed.

In paper IV, the cut-point of $TcPO_2 < / \geq 25$ mmHg as well as $TcPO_2$ quartiles were used in analysis. For mortality analyses, the lowest value of the two legs were used, while the value of the affected leg was evaluated for ulcer outcome.

ECG analysis and QTc interval calculation (paper I-III)

Standard 12-lead ECGs taken during resting conditions were used for QT interval measurements, using a Siemens ECG machine (Siemens Elema, Solna Sweden). The QT interval, defined as the time (milliseconds, ms) between the beginning of the Q wave and the end of the T wave, was calculated using the validated computer-based ECG program Siemens Sicard 440/740 (Siemens Elema, Solna Sweden). To correct for heart rate, Bazett´s formula ($QT/\sqrt{RR}$) was used to calculate the QTc interval.[197] All ECGs were manually checked by investigators to validate if a QTc prolongation was present. When the end of the T wave was not clearly defined (for instance in case of a T-U junction or a T-P junction) a tangent line extrapolated from the downslope of the T wave to the isoelectric point was used.[172]

Patients with ventricular paced rhythm were excluded since this make the QT interval calculation difficult, and no general consensus exists in how to manage these cases. In cases of atrial fibrillation an average of the two QTc intervals preceding the longest and the shortest RR-interval was used. Figure 1 demonstrate examples how to measure QT interval in two different clinical scenarios.

QTc prolongation was defined at the threshold of QTc > 440 ms.[198-200] Supplementary analysis was performed in paper II with QTc quartiles and in paper III the cut-point of QTc > 450 ms was also evaluated.[172]

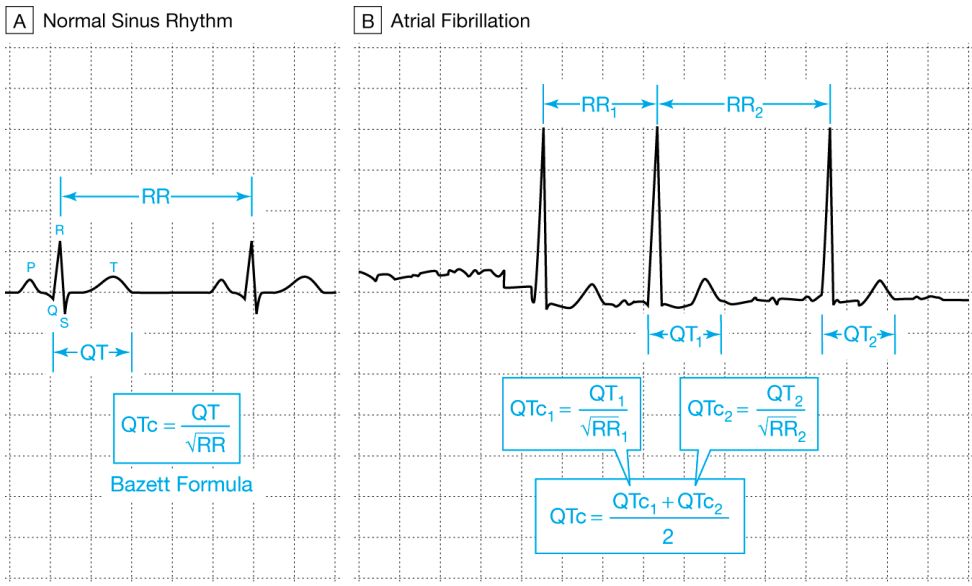

Figure 1.
Measuring QT interval during (A) normal sinus-rhythm and (B) atrial fibrillation and correction for heart rate using Bazett formula. Figure used with permission from: What Clinicians Should Know About the QT Interval. Al-Khatib et al. JAMA 2003.[201]

Hyperbaric oxygen therapy (HBO) (Paper III)

The treatments were performed in a multi-place hyperbaric chamber at the Department of Anaesthesiology, Helsingborg hospital. 40 treatment sessions were given, five days a week, during a period of eight weeks (with extension possible for a maximum of ten weeks). Patients were randomised to either breathing 100% oxygen or air, at 2.5 ATA in a double blinded way, through tightly fitted masks during the 90 minutes long treatment session, including 5 minutes of decompression.[194]

Patients who fulfilled > 35 treatment sessions were included in the final analysis, and follow-up time was two years.

Outcomes

The following parameters were evaluated in the studies

- Mortality data (paper I, II and IV) were obtained from the National Death Register in Sweden. Only all-cause mortality was evaluated.
- Ulcer healing and major amputation rates (paper IV): Ulcer healing was defined as complete epithelialization of the wound. Major amputation was defined as amputation above the ankle. Indication for amputation was a clinical decision due to progressive gangrene, refractory pain despite optimal treatment, or septic condition not responding to conservative strategies.
- Difference in QTc interval duration (paper III) was analysed as the difference in milliseconds between baseline and after two years.

Statistical analyses (all papers)

Statistical calculations were performed using Statistica software version 10 (Statsoft, OK, USA) (paper I) and SPSS (IBM, IL, USA) version 20-22 (paper II-IV), depending on paper. Non-parametric statistics were used in all papers. Continuous data are expressed as median and interquartile (IQ) ranges and categorical data as percentages (%).

Mann-Whitney U-test was used when assessing differences in continuous data and Chi-squared test or Fishers exact test for categorical data. For paired comparisons (paper III) when analysing effects of HBO treatment, Wilcoxon´s signed rank test (continuous data) and Mc Nemar´s test (categorical data) were used.

Survival analysis was performed with Kaplan-Meier estimates and to compare survival curves either Log rank test (paper II-IV) or Gehan Wilcoxon test (paper I) was performed. To quantify the difference in survival and to adjust for confounders Cox proportional regression analyses were performed to assess hazard ratios (HR) with confidence intervals (CI). Possible confounding variables either with independent impact on mortality, or with a significant or nearly significant ($p < 0.1$) difference between our groups at baseline, were entered stepwise in the Cox model, together with our principle variable. Those variables that either changed the coefficient of our principle variable, or had a potential independent impact on mortality, were kept in the final regression analysis.

In paper II, a multiple binary logistic regression analysis was performed to calculate odds ratios (OR) for each QTc quartile to estimate a dose-response relationship for QTc time and mortality.

A two-tailed p-value of < 0.05 was accepted as statistical significant.

Ethics (all papers)

For all papers, ethical approval was given by the Regional Ethics committee in Lund, Sweden, and the studies were carried out in accordance with the declaration of Helsinki, as revised in 2000.

All patients in the prospective studies (paper I and III) gave informed consent to participate in the studies. In the retrospective studies (paper II and IV) patients who were alive when recruited, were asked for informed consent according to decision in the ethical approval.

The HODFU-study is registered at clinicaltrials.gov, registration number NCT00953186.

Results

Paper I

The aim of this study was to evaluate the impact of HbA_{1c} and QTc time on long-term survival. We enrolled 224 patients with type 2 diabetes and at least one DFU with duration of at least four weeks, in the study. Due to lack of HbA_{1c} data, 10 patients were excluded from further analysis, and the remaining 214 patients (37.9% females) with a median age of 69.1 years, were grouped according to HbA_{1c} levels, as shown in Figure 2.

Figure 2
Schematic view of patients included in the study.

There was a significant difference between the HbA_{1c} groups in basal QTc time, with approximate 47% having a QTc interval > 440 ms in the lowest HbA_{1c} group, compared to approximate 30%, in the two other groups. Further, prevalence of PVD differed with more affected patients in the low HbA_{1c} group. Other traditional CVD risk factors such as age, smoking, gender, diabetes duration, renal function, previous myocardial infarction, heart failure, and hypertension, did not differ significantly between the groups. Antidiabetic treatment differed regarding insulin use, with the highest prescription rates within the highest HbA_{1c} group. Treatment with sulphonulurea in mono-therapy, differed by number, and was more frequently found in the lowest HbA_{1c} group (n.s). When evaluating all drugs, however, with a potentially hypoglycaemic action (insulin and sulphonulurea), there were no significant difference between the groups. Other diabetes medications, prescription of beta-blockers, or drugs with potential QTc action were also similar.

The all-cause eight-year mortality in the study was 70.6% (151 patients died), with the highest mortality found among patients with the lowest HbA_{1c} (figure 3).

In a subgroup analysis to evaluate the prognostic effect of a QTc time > 440 ms in different HbA_{1c} groups, the combination of a low HbA_{1c} and QTc time > 440 ms, was associated with the highest mortality (92% eight-year mortality), as demonstrated in figure 4.

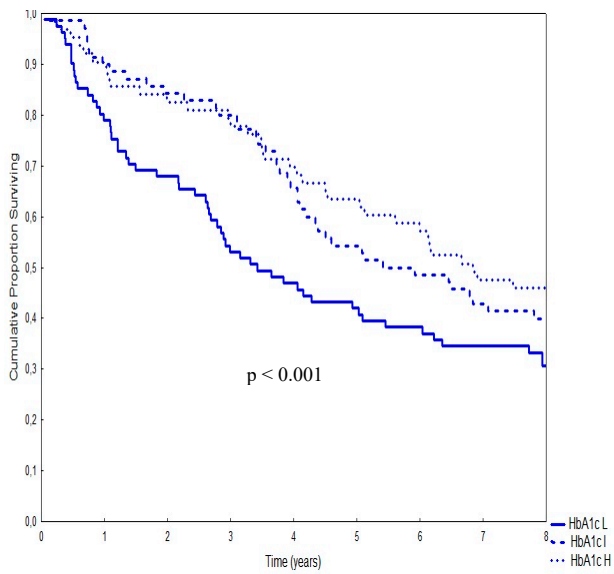

Figure 3.
Kaplan Meier curves estimating mortality in HbA_{1c} L (low < 58 mmol/mol), I (intermediate 58-74 mmol/mol) and H (high >74 mmol/mol)..

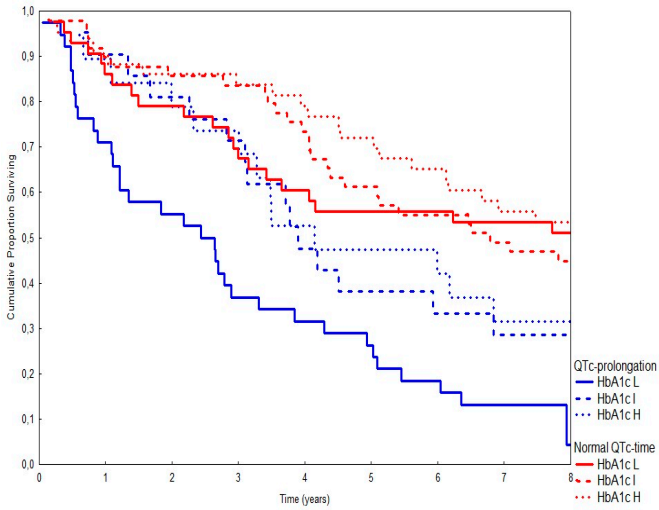

Figure 4.
Kaplan-Meier curves for survival based on QTc prolongation >440 ms (blue colour), QTc normal (red) in the different HbA_{1c} groups; L (Low), I (Intermediate) and H (High). p<0.0001 for pooled comparison.
For separate comparison;
QTc >440 ms/HbA_{1c} L vs QTc normal/HbA_{1c} L, I, H respectively: p<0.0001,
QTc >440 ms/HbA_{1c} L vs QTc >440 ms/HbA_{1c} I: p=0.023,
QTc >440 ms/HbA_{1c} L vs QTc >440 ms/HbA_{1c} H: p=0.015
QTc >440 ms/HbA_{1c} I vs QTc normal/HbA_{1c} H: p=0.025.
All other comparisons: NS.

Table 4.
Predictors for 4- and 8-year all-cause mortality in patients with type 2 diabetes and hard-to-heal ulcers based on a Cox regression model adjusted for confounding factors (age, sex, renal impairment, smoking, heart failure, previous myocardial infarction, PVD, usage of insulin or insulin-secretagogues, beta-blockers).

	4-year mortality HR (95% CI)	***p-value***	**8-year mortality HR (95% CI)**	***p-value***
HbA_{1c} < 7.5% (<58 mmol/mol)	1.78 (1.16-2.73)	0.009	1.41 (1.00-2.00)	0.052
Age (decade)	1.56 (1.12-2.17)	0.008	1.51 (1.17-1.95)	0.001
Female sex	0.61 (0.37-0.98)	0.042	0.63 (0.43-0.93)	0.019
QTc time >440 ms	2.08 (1.35-3.22)	0.001	2.00 (1.40-2.85)	<0.001
HbA_{1c} <7.5% (<58 mmol/mol) + QTc >440 ms	2.65 (1.66-4.24)	<0.001	2.76 (1.86-4.09)	<0.001

To adjust for possible confounding factors, a Cox regression analysis was performed and the HR for 4 and 8-year mortality, are demonstrated in table 4.

In summary, the results of paper I indicate that a low HbA_{1c}, particularly in the presence of QTc time > 440 ms, is an independent risk factor for increased mortality in the high-risk population of patients with type 2 diabetes and DFU.

Paper II

In paper II, we aimed to evaluate the predictive value of QTc prolongation on survival in patients with type 2 diabetes undergoing major amputations.

In this study, we enrolled 74 patients with type 2 diabetes who underwent a major amputation. Four patients were excluded, two with ventricular-stimulating pacemaker and two who died within ten days after surgery, due to acute complications (pneumonia and *staphylococcus* sepsis, respectively). The remaining 70 patients (> 80% male gender) with a median age of 72 (66-77) years, were included in the final analysis.

Approximate half of the patients had a baseline QTc time > 440 ms and patients were grouped accordingly. At baseline (day of surgery) patients with QTc time > 440 ms had a higher prevalence of coronary heart disease (20% vs 47%, p = 0.036), a higher prescription of beta-blockers (66.7% vs 36.1%, p = 0.015), a lower prescription of Metformin (6.1 vs 30.6%, p = 0.013), and a trend towards worse renal function (47% vs 25% with eGFR < 60 ml/min/1.73 m^2, p = 0.08). Other baseline characteristics or medications, did not differ significantly.

After three years of follow-up, 54% of our study population were diseased, and presence of a QTc interval > 440 ms was strongly associated with increased mortality (73% compared to 36% in patients with QTc time ≤ 440 ms, p < 0.001), as demonstrated in figure 5.

A multivariate Cox regression analysis was performed to adjust for confounders. Factors that differed, or nearly differed (p < 0.1), at baseline (i.e. coronary heart disease, eGFR < 60 ml/min/1.73 m^2, beta-blockers, and Metformin use) were entered as potential confounders in the Cox model. In this analysis prolonged QTc interval was associated with more than two-fold higher mortality risk, compared to QTc ≤ 440 ms (HR 2.2 (95% CI 1.11-4.38), p = 0.024). Also, treatment with Metformin seemed to have a protective effect (HR 0.22 (0.05-0.94), p =0.041), a result not confounded by eGFR.

Outcome differed in the different quartiles of QTc time, indicating a J-shaped association between QTc time and mortality, see table 5.

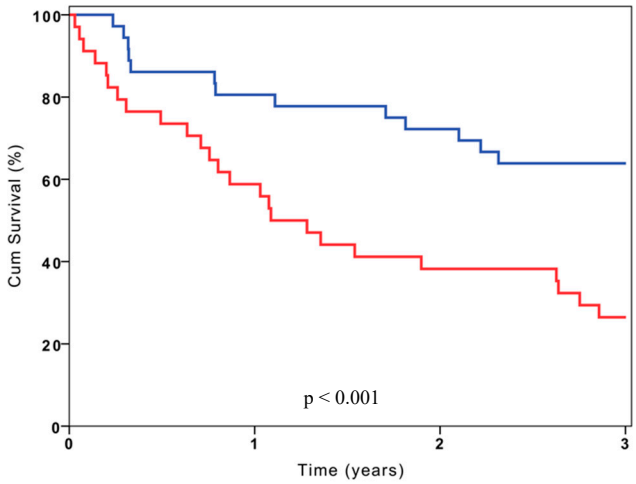

Figure 5.
Kaplan-Meier survival curves showing 3-year mortality in patients with QTc time > 440 ms (red line) and QTc time ≤ 400 ms (blue line).

Table 5.
Odds ratios (OR) for 3-year mortality in patients with type 2 diabetes undergoing major amputation grouped according to QTc quartiles with Q2 as reference group.

Quartile	*n*	Median (range) (ms)	3-year mortality (%)	OR (95% CI)	p-value
Q1	18	402 (376-414)	44.5	1.92 (0.63-5.89)	NS
Q2	17	421 (414-436)	29.4	1.0 (reference)	
Q3	17	450 (437-455)	64.7	4.40 (1.04-18.6)	0.04
Q4	18	469 (456-548)	72.2	6.24 (1.44-27.0)	0.01

In summary, the results from paper II indicate that QTc time > 440 ms is a risk factor for mortality among patients with type 2 diabetes undergoing above-ankle amputation, and that the risk increases with higher QTc intervals.

Paper III

The aim of this study was to evaluate the long-term effects of HBO therapy on the QTc interval. Of the 94 patients randomised in the study, 75 patients fulfilled the pre-defined criteria of more than 35 completed treatment sessions, and of them, two patients were excluded due to ventricular stimulating pacemaker. The remaining 73 patients, with a median age of 70 (61-77), years were included in the final ECG analysis. The flow chart of patients included in the study, are shown in figure 6.

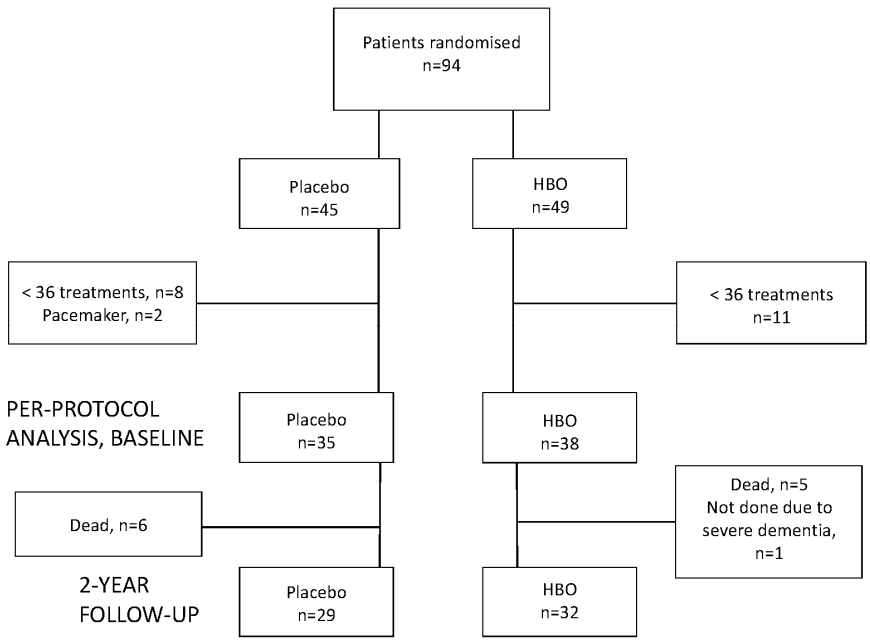

Figure 6.
Flow chart of patients included in the study.

Median QTc time at baseline was 426 (410-440) ms and 426 (419-458) in patients randomised to HBO and placebo, respectively, and did not differ significantly. Neither did other baseline CVD risk factors or medications, both antidiabetic and drugs with QTc prolonging effect, differ between groups.

After two years of follow up, QTc time was re-evaluated in all remaining patients (n=61). There were a significantly longer QTc interval in the placebo group after two years, compared to the HBO group (456 (424-469) ms vs. 438 (425-453) ms, $p < 0.05$). When comparing QTc time after two years with baseline data, with each patient being their own control, a significant prolongation was found among patients in the placebo group, while HBO patients had a less pronounced, and not significant increase, in their QTc interval, as demonstrated in figure 7.

In summary, the results of paper III demonstrate a less pronounced QTc interval prolongation after two years, among patients receiving HBO-therapy, compared to those receiving placebo. This might indicate a plausible protective role of HBO therapy on QTc prolongation.

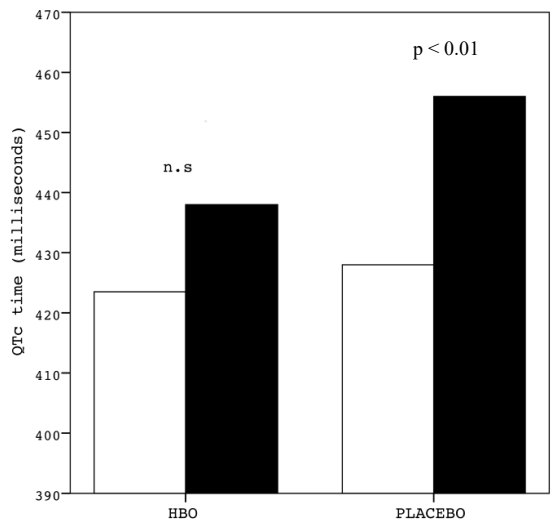

Figure 7.
Median QTc time at baseline (white bars) and after 2 years of follow-up (black bars) in patients treated with HBO and placebo respectively.

Paper IV

This paper aimed to evaluate the predictive value of $TcPO_2$, compared to ABI and TBP, on one-year all-cause mortality.

We included 236 type 2 diabetes patients (30% women), with a median age of 76 (69-82) years, with at least one DFU in the study. A $TcPO_2 < 25$ mmHg was detected in 47 patients, and this was associated with significantly lower three-months healing rates (8.8% vs. 24.9%, p= 0.045), as well as higher major amputation rates (23.4% vs. 4.2% in patients with $TcPO_2 \geq 25$ mmHg, $p < 0.001$). Presence of ABI < 0.9 or > 1.3 was also associated with worse ulcer outcome, whereas TBP did not predict wound healing in our study population.

Within one year, 14.8% of the study population died. A $TcPO_2 < 25$ mmHg was significantly associated with increased one-year mortality, as shown in figure 8 a. A non-significant trend towards worse outcome was seen among individuals with TBP < 30 mmHg (figure 8 b), while ABI did not predict mortality in this analysis (figure 8 c).

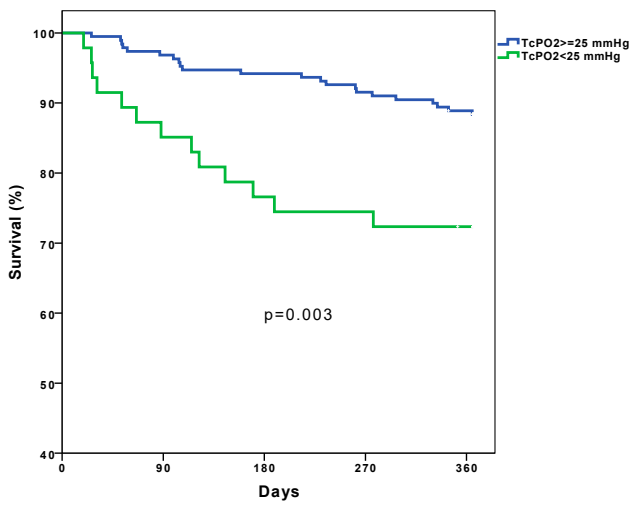

Figure 8a.
Kaplan-Meier survival curve with Log-rank test in DFU-patients grouped according to $TcPO_2$ < and ≥ 25 mmHg.

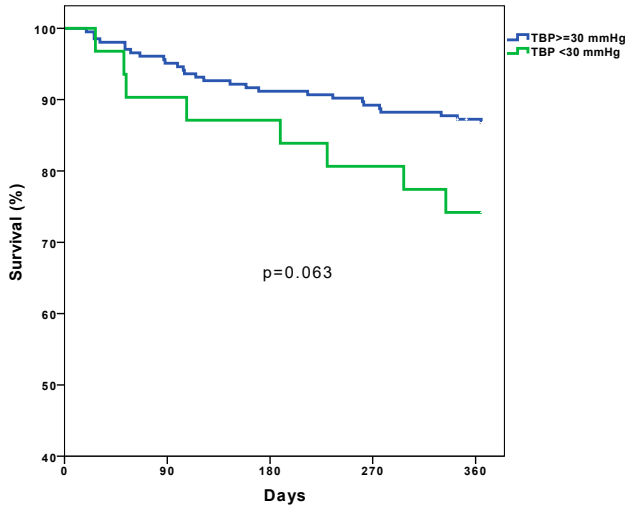

Figure 8b.
Kaplan-Meier survival curve with Log-rank test in DFU-patients grouped according to TBP < and ≥ 30 mmHg.

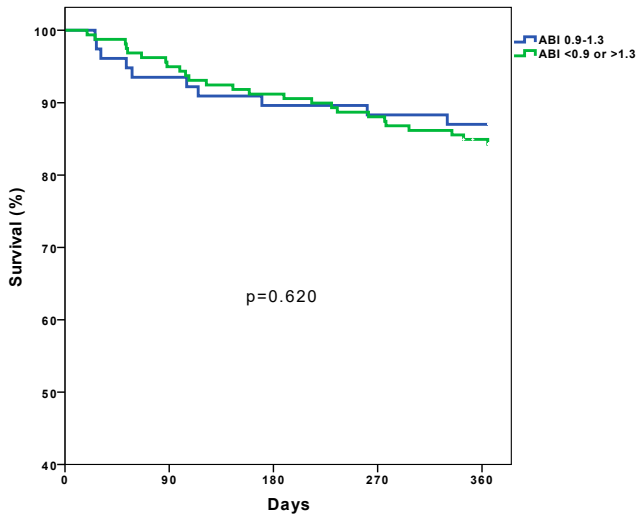

Figure 8c.
Kaplan-Meier survival curve with Log-rank test in DFU-patients grouped according to ABI 0.9-1.3 and < 0.9 or >1.3.

Patients with $TcPO_2 < 25$ mmHg had worse renal function, a factor also associated with increased mortality in the study. However, in a Cox regression analysis adjusted for renal impairment together with other plausible confounding factors, $TcPO_2 < 25$ mmHg was still an independent risk factor for one-year mortality with HR of 2.8 (95% CI 1.34-5.91, $p = 0.006$). Also, when evaluating $TcPO_2$ as a continuous variable, a significant association with survival was demonstrated. ABI or TBP did not predict one-year mortality in the Cox analysis.

In summary, the results of paper IV indicate that a screening with $TcPO_2$ not only predicts ulcer healing, but also might serve as an independent risk factor for one-year mortality in a high-risk population of type 2 diabetes patients with DFU. This finding might improve identification of DFU patients with urgent need for both vascular intervention and intensive cardiovascular risk factor evaluation.

Discussion

The overall aim of this thesis and PhD project was to identify risk factors and diagnostic tools, that might help us to identify those individuals with the highest mortality risk, among people with type 2 diabetes and foot ulcers.

There are some major findings in the studies included in this thesis. First, we concluded that mortality among our DFU-patients remains high, despite an improved health care over the last decade. We reported all-cause mortality rates of 15% after one year (paper IV), 50% after five years, and after eight years, more than 70% of our DFU-patients were deceased (paper I). Further, less than 50% of our patients were alive three years after a major amputation (paper II). These crude mortality rates are consistent with many other studies.[9,15,202,203] We demonstrated that presence of a QTc time > 440 ms, as well as a low $TcPO_2$ < 25 mmHg were strong independent risk factors for mortality, findings that might help us identifying patients with urgent need for medical evaluation. Another finding, was that a low HbA_{1c} seemed to be associated with worse outcome, particularly among individuals with prolonged QTc time, a finding plausibly mediated by hypoglycaemia.

Of course, one can speculate on causation and confounding. Our study designs do not allow us to establish causality and there are several methodological considerations in the studies that need to be highlighted.

HbA_{1c} and survival - implications and limitations

There is no doubt that prolonged hyperglycaemia and elevated HbA_{1c} are risk factors for developing diabetes-related complications, including CVD, but the optimal HbA_{1c} level and whether a low HbA_{1c} increases the risk of mortality, has been debated by many.

There are several limitations in the measurement of HbA_{1c}, such as assay interference, severe comorbidity, such as renal impairment, malignancy, cachexia, anaemia, and other conditions associated with increased red blood cell turnover.[204] These factors could potentially have impact on the HbA_{1c} value as well as survival, and might thus be confounders behind our result. In paper I, we did not, however, see any major differences in baseline characteristics, such as age, diabetes duration,

heart failure, and renal impairment between patients in the different HbA_{1c} groups. Further, when evaluating drugs with a potential hypoglycaemic action (insulin and sulfonylureas), the majority (> 80%) of the patients in the HbA_{1c} group < 58 mmol/mol (7.5%) were prescribed such drugs. These data do not exclude, but make severe frailty and advanced illness less likely, and the potential harm of hypoglycaemia must be addressed as a plausible explanation.

Previous studies have shown a linear relationship between HbA_{1c} and mortality among individuals with diabetes, whereas others demonstrate a more U- or J-shaped relationship. [205-209] In the observational study by Currie et al, evaluating a large cohort of type 2 diabetes patients, an U-formed association was found, with the lowest hazard ratios for mortality found at a HbA_{1c} level of 58 mmol/mol (7.5%).[207] However, in that study the authors did not adjust for potential comorbid conditions that might have confounded the result. Another study by Greenfield *et al*, evaluating both the impact of comorbidity and HbA_{1c} on survival in patients with type 2 diabetes, demonstrated that a low HbA_{1c} was associated with increased mortality in patients with high level of comorbidity, but not in those without.[204] In that study, diabetic foot disease was one of eight comorbid conditions that were included. In our study (paper I), we found that a low HbA_{1c} was particularly harmful among DFU patients with a QTc time above 440 ms, a condition that might be associated with CAN, as well as other cardiac diseases. Since the majority of our patients were treated with either insulin or insulin-secretagogues, with potential hypoglycaemic action, one hypothesis behind the increase in mortality in the group with low HbA_{1c} could be a vulnerability to malignant arrhythmias mediated by further prolongation of the QT interval during hypoglycaemia. There is convincing evidence in the literature about the harmful effects of hypoglycaemia; both during experimental settings and in clinical trials. In a study of 37 patients with type 1 diabetes undergoing blinded continuous glucose monitoring and ambulatory ECG, a large amount of asymptomatic hypoglycaemic events was found and hypoglycaemia was associated with increased risk of different arrhythmic and pro-arrhythmic states, such as bradycardia, ectopic heart beats, and QTc interval prolongation.[175] Similar results were found in a comparable study of 25 insulin-treated type 2 diabetes patients, with either a history of CVD or presence of at least two risk factors for CVD.[176] In experimental hypoglycaemic clamps in type 2 diabetes patients, QTc interval prolongation has been demonstrated during hypoglycaemia, with a mean increase of 76 ± 20 ms in the type 2 diabetes group in a study by Chow et al.[174,210] Further, a decline in potassium levels during hypoglycaemia has also been demonstrated, which might further aggravate the risk of arrhythmias.[174,210]

In clinical trials aiming to normalise, or lower HbA_{1c} in diabetes patients with high CVD risk, an intensive glucose-lowering therapy has not been shown to be beneficial. When the ACCORD study was published, demonstrating an increased mortality in the intensively treated arm, the harmful effects of hypoglycaemia in

vulnerable patients with manifest CVD complications, have been discussed frequently.[138] Also, in the ADVANCE trial, severe hypoglycaemia was linked to adverse outcome, and the in-hospital trial of intensive glucose-lowering therapy in critically ill patients (NICE-SUGAR trial), a significant association between hypoglycaemia and mortality was found, after adjusting for other risk factors.[211,212]

Very few of our patients were treated with newer antidiabetic drugs, without hypoglycaemic action, such as DPP4-inhibitors, GLP-1 inhibitors, or SGLT2-inhibitors. If a higher prescription rate of these drugs would have influenced mortality among our high-risk patients, we cannot tell from our data. Today, after the beneficial results of the cardiovascular outcome trials of empagliflozin, Canagliflozin, and liraglutide, on CVD events and mortality among type 2 diabetes patients with high CVD risk, these drugs are recommended as second-line treatment, in patient with manifest CVD.[157,213-215] Since individuals with DFU are considered high-risk patients for CVD, this shift in pharmacological approach might be of great importance to our patients with DFU. However, the need for individualized HbA_{1c} targets will persist, as renal impairment often limits usage of these new drugs. Consequently, the problem with hypoglycaemia will remain.

Paper I, with its limitations, was the first to demonstrate that a low HbA_{1c} seem to be associated an increased mortality risk among DFU patients; particularly in the presence of QTc prolongation. After our study was published, the result from a large epidemiological study from UK demonstrated the same result; with higher mortality among DFU-patients with low HbA_{1c} < 52 mmol/mol (7%).[13] Although our study design, as a prospective cohort study, might be afflicted with selection bias and random errors, our sample size was relatively large and representative of the DFU-population. Our statistical power might however, not have been sufficient to detect smaller differences between our groups. Further, as we cannot establish causality, our study might be considered hypothesis generating, indicating a potential vulnerability of hypoglycaemia among DFU-patients, with a prolonged QTc time. To determine the optimal HbA_{1c} targets in this population however, randomised controlled studies are needed and probably, the question *how* to reach the glycaemic target, will be of great importance, especially considering new drugs without hypoglycaemic action and with potential favourable CVD-effects.

The impact of QTc prolongation on survival among individuals with type 2 diabetes and foot ulcers

Prolonged QTc time is a clinical important risk factor for life-threatening ventricular arrhythmias and sudden death.[216,217] The impact of this condition has previously not been evaluated in the high-risk population of patients with type 2 diabetes and foot

ulcers. Our findings indicate that calculation of the QTc interval might be a simple diagnostic tool for risk stratification; both in the general DFU-population (paper I) and among DFU-patients who undergo major amputations (paper II).

Several limitations that might have influenced the result need to be highlighted. First, our study designs and the lack of data on neuropathy limits us to only speculate on underlying mechanisms, such as CAN, and the role of hypoglycaemia.

We found a high prevalence, 23%, 37%, and 51% (paper I, II, III respectively) of QT time > 440 ms in our studies, with the highest prevalence of QTc prolongation found among those patients undergoing amputation (paper II). One theory about the difference in prevalence, might be a higher burden of complications within the group of patients undergoing major amputation. Previous studies have shown higher prevalence of QTc prolongation among patients with diabetes, compared to those without, and multiple factors might explain this.[218] First, cardiac autonomic neuropathy (CAN) is suggested as one plausible underlying mechanism, but other important factors that might predispose QT prolongation are ischemic heart disease, heart failure, hypokalaemia, hypomagnesemia and hypoglycaemic events.[218-220] Further, age, female gender, congenital disorders, and use of QT-interval prolonging medications, are other known predisposing factors.[171] Whether the high prevalence of QTc prolongation in our studies is a marker for CAN, or simply a marker for a more advanced burden of cardiovascular complications, for instance silent myocardial ischemia, we can´t tell from our results. Although we adjusted for confounders in our analysis, we can´t exclude their interference. However, despite not knowing the exact underlying mechanisms, finding a prolonged QTc time seem to motivate an urgent cardiovascular risk evaluation.

In paper III, we demonstrated that DFU-patients receiving HBO therapy, to a less extent developed a prolonged QTc interval after two years, compared to those receiving placebo. This is an observation that has not been described before, and we can only speculate in plausible mechanisms behind this result. Previous studies have shown that HBO increases oxygenation in hypoxic tissue and improve microvascular function.[107,110,112] Further, few studies have indicated a direct effect of HBO on cardiac autonomic nerve function, and on the QT interval per see. For instance, in a study by Kardesoglu *et al*, a decrease in QTc dispersion was seen after 10 HBO sessions, and Sun et al found an increased heart rate variability after 4 weeks treatment.[221,222] These findings, together with our result in paper III, allow us to speculate on a hypothesis of improved microvascular function after HBO, resulting in a reduced progress in CAN, or in enhanced coronary microcirculation, that might be reflected by a lower risk of QTc prolongation. However, to establish these hypotheses, larger randomised studies aiming to primary answer these questions are needed.

There are some methodological limitations in the measurement of QT time that need to be underlined. First, the QT interval is strongly dependent on heart rate, and various methods for correcting for heart rate exists. These methods aim to determine the predicted QT interval at a reference heart rate of 60 beats/min (i.e. an R-R interval of 1.0 second).[172] The formula derived from Bazett, is one of the most commonly used in literature, and also the method we used in our studies.[172,197] This formula has been criticized, particularly in cases of slow or fast heart rates, where the formula might under- or overcorrect, respectively.[201] This could of course have influenced our results, but all ECGs were taken during resting condition, and severe symptomatic arrhythmias were not noticed when analysing the ECGs.

Another difficulty, when measuring the QT interval, is presence of atrial fibrillation or a wide QRS-complex; the latter either due to ventricular conduction defects or paced rhythm. We chose to exclude patients with a ventricular stimulating pacemaker, since there is no general consensus how to measure QT interval in standardized manner, in these individuals. Patients with ventricular conduction defects were not excluded and could thus have influenced our result. In cases of atrial fibrillation, the QT interval was calculated by taking the average of the QT intervals that follow the longest and the shortest RR-interval, a method recommended by several, but having limitations.[201]

Another factor, that might influence our result, is the definition a long QT interval. In the literature, QT times above 440 ms, or 450 ms, are cut-points often used when defining a prolonged interval.[172,198-200] In our studies (paper I-III), we chose the cut-off level of 440 ms, and this can partly explain the high prevalence of QTc prolongation in our studies. In paper II, we did therefore perform separate analysis of QTc time > 450 ms, as well as QTc quartiles, and confirmed that mortality increased with increasing QTc time, a result well-known from literature.[172]

Another limitation that might have influenced our results, is that we chose the same cut-off level for men and women. QT interval in women are suggested to be about 6 to 15 ms higher.[172] However, the gender differences become less important after 40 years of age, and practically disappears in older men and women.[172] Since our patient were in the older age span, we chose the same cut-of level for both women and men, but, adjusted for gender in our regression analysis.

Despite limitations in our design and methods, and uncertainty about the underlying cause behind QTc interval prolongation, we have demonstrated that a QTc interval > 440 ms seems to be a strong predictor for worse survival among DFU-patients, and that HBO-therapy might have beneficial effects on this condition. Our results suggest that screening with ECG might be an easy way to risk-stratify patients with DFU, to identify individuals that might favour an urgent cardiovascular risk evaluation.

$TcPO_2$ as a risk marker for mortality in patients with diabetic foot ulcers

$TcPO_2$ is a non-invasive method evaluating tissue perfusion and it is considered to better reflect the microvascular function in the skin. In paper IV, we demonstrated that a low $TcPO_2$ not only predicts ulcer healing, but also might serve as a strong independent risk factor for one-year mortality. $TcPO_2 < 25$ mmHg was associated with a 2.8-folded increase in one-year mortality (27.7% vs 11.6% in patients with $TcPO_2 \geq 25$ mmHg, $p = 0.003$). This suggests that screening with $TcPO_2$ might help us identifying DFU patients with both an urgent need for both revascularisation as well as cardiovascular risk evaluation.

There are several limitations in paper IV that need to be discussed. First, as a retrospective cohort study, conclusions must be drawn cautiously. There might be selection bias and confounders, as well as errors in data collection, influencing our results. For example, the parameter "ulcer healing" over time is hard to retrospectively review from patient's chart, and the result partly depends on the variation in the time span between patients visits, and when complete ulcer healing was reported. Secondary, ulcer duration at inclusion differed, consequently influencing healing time. One strength is, however, that our cohort is representative of our DFU-patients, as ABI, TBP, and $TcPO_2$ measurements are regularly performed at our department. However, although the vast majority of patients perform a vascular evaluation, there are exceptions. Patients with signs of prompt ulcer healing, as well as younger patients, might not have been vascular evaluated, and thus not included in the study. Further, there might have been some patients evaluated at other departments. Despite the limitations in study design, our study represents a relatively large cohort of patients with type 2 diabetes and DFU, with a primary endpoint not accomplished with biases, since mortality data, collected from the national death registry of Sweden, are accurate.

One finding that was not expected, was the relatively low utilization of vascular intervention. Among our patients with $TcPO_2 < 25$ mmH, only 25.5% performed vascular intervention, and additional 21.3% were assessed by a vascular surgeon, but intervention either failed or were impossible. These are considerably low rates, and in conflict with current recommendations from IWGDF.[41] However, not all individuals with significant limb ischaemia, will be suitable for invasive angiography or revascularisation, often due to severe comorbidity. The clinical decision is often based on risk-benefit assumptions, since an invasive procedure is associated with side effects and risks, and this reflects the complexity of the management of DFU patients with coexisting PVD.[41] We identified several different explanations to why revascularisation, or vascular imaging was not performed, such as severe comorbidity, renal failure, or in some cases, significant ulcer

improvement. However, in a small number of patients, no such reason was identified. This indicates a need to further improve the management of DFU patients with coexistent PVD. Our finding, although worrisome, is not unique. In a German study by Malyar *et al*, revascularisation rates of 18%, and angiography rates of 25%, were described.[9] In the EURODIALE study, a large multicentre study involving 14 European hospitals evaluating DFU patients in a "real world setting", they reported vascular imaging in 56% of patients with critical limb ischaemia, and of them, only 43% was revascularised.[223]

One could only speculate on whether the high frequency of amputation and mortality would have been different if a higher revascularisation rate was seen. There was a trend towards better one-year survival among those patients with low $TcPO_2$ having a vascular intervention performed. This might, however, be explained by the fact that these patients were healthier, as the borderline significance disappeared after adjusting for other risk factors in a Cox regression model. This was also the case in a study by Faglia *et al*, evaluating patients with critical limb ischaemia who did, or did not undergo revascularisation. In that study, 28.2% of the revascularised, compared to 81.6% of the non-revascularised patients died ($p = 0.009$). However, after the authors adjusted for other risk factors in a Cox regression model, only age turned out to predict mortality.[224] Nevertheless, all patients should undergo diagnostic screening to either rule-out, or to consider the possibility for vascular intervention, since this has profound impact on wound healing.[41,225] Due to the short follow-up time, and the limited number of patients in our study, it is not possible to draw any conclusions from our data, about the impact of successful vascular intervention on mortality.

Today, current guidelines do not recommend screening for coronary disease in asymptomatic patients with diabetes.[185] However, among DFU-patients asymptomatic CVD is common, and a more aggressive strategy might thus be warranted. A low $TcPO_2$ has previous been demonstrated to predict cardiovascular events (HR 1.78, (95% CI 1.44-2.23)) in a population of "relatively low-risk" patients with type 2 diabetes, without a history of previous CVD or DFU.[144] In a high-risk population of DFU-patients, this relationship has not been evaluated before. Our results, demonstrating a 2.8-fold increase in one-year mortality, indicate that a low $TcPO_2$ could be a marker of a more serious CVD, and might motivate urgent cardiovascular risk factor management, and perhaps also consideration of more advanced cardiovascular evaluation.[128,226,227]

Microvascular complications and mortality

Both macrovascular and microvascular complications are serious and contribute to increased morbidity and mortality among individuals with diabetes. Traditionally, this increase in mortality has primarily been associated with macrovascular atherosclerotic disease, such as myocardial infarction, stroke, and PVD as the dominating risk factors. However, the microvascular complications are often as important, and must be taken seriously when risk-stratifying patients.

The role of microvascular complications and mortality have been demonstrated by others. Sandesara *et al*, found that patients with heart failure with preserved ejection fraction had a marked increase in mortality, if microvascular complications were present.[126] When evaluating the results of the ADVANCE and ADVANCE-ON post trial, Mohammedi *et al* found that presence of microvascular complications (macro-albuminuria, requirement of retinal photocoagulation therapy, proliferative retinopathy or macular oedema), as well as macrovascular complications (myocardial infarction, angina pectoris, stroke and PVD) at baseline, were both independently associated with all-cause mortality, with comparative effects (HR 1.43 (1.20-1.71) vs 1.43 (1.30-1.57) respectively), and with the highest risk in patients with co-existing conditions (HR 2.01 (1.65-2.45)).[228] They also demonstrated that presence of microvascular disorders, predict other microvascular complications (HR 4.74 (3.86-5.82)), as well as major cardiovascular events (MACE) (HR 1.64 (1.37-1.97)). Conversely, macrovascular disorders at baseline were a strong predictor for new MACE (HR 2.04 (1.86-2.25)), whereas the association with microvascular events were weaker (HR 1.26 (1.06-1.51)). One limitation of the study by Mohammedi is that neuropathy was not evaluated, and thus, not included among the microvascular complications. This might have underestimated the role of microvascular disorders since neuropathy has been strongly associated with mortality.[117,167,229]

A low $TcPO_2$ has previously been associated with both microvascular complications, such as diabetic kidney disease and neuropathy, as well as with MACE.[142-144] We demonstrated in our study (paper IV), significant associations between a baseline $TcPO_2 < 25$ mmHg and one-year mortality, as well as with renal impairment, supporting the theory about a higher burden of both macro- and microvascular disorders among individuals with low $TcPO_2$. Renal impairment was also an independent risk factor for mortality in paper IV, similar with result shown by many.[153,230,231] Contrarily, in paper I, renal impairment did not predict mortality. One explanation for this discrepancy, might be the smaller cohort size, and the fact that patients on dialysis were excluded in paper I, and thus, a healthier population with a higher median eGFR was evaluated in this study.

We did not have quantitative data on peripheral neuropathy or CAN in our studies, which is a limitation. This allow us only to speculate on the role of neuropathy on both mortality and prolongation of the QT interval, among our high-risk population. From previous studies, neuropathy and CAN is well-known risk factors for mortality, and the latter has been associated with QTc prolongation.[170,217,232] Future prospective studies are needed, to sort out the true dimension of CAN among DFU patients, and its role for mortality. Nevertheless, we have demonstrated that routinely ECG screening with QT interval analysis in a DFU-population, provides important information regarding future risk, when considering further cardiovascular evaluation.

Conclusions and future perspective

The diabetic foot must be seen as a lifelong disease, not only affecting the foot per se, but also serving as a marker for an often advanced, general vascular disease, associated with poor prognosis. This thesis illustrates the complexity of the management of DFU-patients, often with co-existing macro- and microvascular complications, where a multifactorial approach is necessary to improve ulcer healing and to reduce life-threatening complications.

Our findings might help us identifying high-risk patients and to encourage considerations regarding risk factor management and further need for medical assessment, to further improve survival among individuals with type 2 diabetes and foot ulcers.

In conclusions:

- Mortality is high among patients with type 2 diabetes and foot ulcers, despite advances in health care. Strategies for improving survival are thus strongly warranted.
- Prolongation of the QT interval is commonly present among individuals with type 2 diabetes and foot ulcers and is an independent risk factor for mortality. Whether the high prevalence is a marker for advanced concomitant coronary disease, or is associated with cardiac autonomic neuropathy, is not known but it indicates further need for medical evaluation.
- Low HbA_{1c} is associated with worse survival among patients with type 2 diabetes and foot ulcers, particularly among those with prolonged QTc interval. This might be mediated by increased vulnerability for hypoglycaemia, but we can only speculate in causality.
- Treatment with hyperbaric oxygen therapy seem to reduce the risk for QT interval prolongation over a period of two years. A hypothesis is that this is mediated by enhanced microcirculation.
- A low $TcPO_2$, reflecting a microvascular disturbance in the foot, is associated with worse ulcer healing and higher amputation rate. Further it is also a strong independent risk factor for one-year mortality among individuals with type 2 diabetes and foot ulcers.

Future perspective

> For one mistake made for not knowing, ten mistakes are made for not looking.
>
> *J A Lindsay*

A major barrier for optimal care for our DFU-patients is the fragmented health care system, in which health care providers often lack information from each other. A consequence of this, is that nobody takes the full responsibility for the patient. Traditionally, the DFU-unit treats the foot, while the patients over-all metabolic and CVD risk factor management is handled by primary health care, and communication in-between is often sparse. This thesis might hopefully inspire further improvement in the management and raise awareness of the diabetic foot patient, not only focusing on the foot per se, but on the whole patient. Our findings might propose screening with ECG and $TcPO_{2,}$ as valuable tools for risk stratification, to identify patients with urgent need for medical evaluation.

Questions remain though, inspiring to future studies. It would be of great interest to evaluate whether implementation of a basic risk factor strategy on our DFU-unit (targeting modifiable risk factors such as lipids, blood pressure control, aspirin therapy and metabolic control), in collaboration with primary health care, would affect survival. Also, as there has been a paradigm shift in antidiabetic treatment with novel therapies without hypoglycaemic action and with cardiovascular benefits, it would be of interest to evaluate these drugs in a DFU-population with and without QTc prolongation.

Furthermore, it would be of interest to evaluate patients with low $TcPO_2$ and QTc prolongation with echocardiography and myocardial imaging, to assess the dimension of co-existing silent coronary disease among these patients, to improve strategies in the future for the sub-group of DFU-patients with the highest risk.

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Acknowledgement

I would like to express my sincere gratitude to everyone who has supported me during my work with this thesis.

First, I would like to thank **Magnus Löndahl**, my supervisor, for believing in me and for being the one who introduced me into the field of diabetes. You are a brilliant scientist, an enthusiastic supervisor, and a true friend. I would not have accomplished this thesis without you, and I look forward continuing working together with you.

My co-supervisor **Mona Landin-Olsson**, for all your generous support during my PhD years. You are a true role model both as a clinician, scientist, and as a person.

My co-author **Per Katzman**, for sharing your great knowledge and for all valuable advises and fantastic conversations, not only scientific and clinical, but also about life in general.

My co-author **Anders Nilsson**, for contributing with good ideas and great wisdom in the field of diabetes.

Karin Filipsson, my dear friend and colleague, for making every day at work joyful. Teamwork with you is always a pleasure!

Karin Olsson, my dear friend and room-mate, for all shared moments (both good and more difficult ones) and for putting up with my messy desk all these years!

Sven Karlsson, for your excellent guidance and thoughtful advices as my clinical supervisor during my years as a resident at the department of Endocrinology.

Bodil Eckert, for sharing good clinical advices, and for being such a warm and inspiring role model at work.

Åsa, **Gunilla**, **Maria**, **Mirja**, **Anna-Karin** and **Iréne**, at the diabetic foot unit in Lund. Thank you for your support, and for your never-ending care of our patients.

Ulrika Moll, **Ola Lindgren**, **Henrik Borg**, and **Wathik al Salim**, for being great friends and colleagues. Look forward to a challenging competition at the tennis court, or a re-battle at Sherlocked soon.

All other colleagues at the department of endocrinology SUS, for providing an inspirational working environment, and for making me feel like I have the best work in the world.

Catharina Borna, **Johan Svahn**, **Johan Elf**, **Jadwiga Mroczkowska**, **Hans-Jörgen Nilsson**, and **Grunde Gjesdal**, at the former MAVA, for all shared laughter and excellent clinical discussions, both during work and after. A special thanks to **Hans-Jörgen**, for your great mentorship during my residence years. I would not have been the physician I am today without you.

Eva Degerman and **Sasha Koul**, for all your good advices and valuable comments during my half-time exam.

Anders Sellman, for good collaboration and interesting discussions in the field of the diabetic foot. I wish you the best luck with your thesis.

My mother **Ulla**, for endless love and support through life for me, and my family! You have been, and will always be, the best role model in life for me. I love you! **Rolf**, for all your support and for being the best "extra morfar" our family can wish for. Also, thank you for all valuable advices on "house-knowledge".

Klas and **Margaretha**, for your generous support and for being the dearest "parents-in-law" one could wish for, and the best "farmor and farfar" to Arvid and Harald. Without your help in daily life, this thesis wouldn´t have been possible.

My dear brothers, **Ola** and **Anders** for every laughter and tear we have shared during life. And **Tina** and **Abigail**, I´m so happy that you have become parts of our Fagher-family, and I look forward spending more time together with you!

My dear sister in-law **Maria**, for all good moments and long talks about everything in life, often while jogging or training. Together with you and **Jesper**, and your boys, **Isak** and **Jakob**, our family have spent so many happy moments and we look forward to spending many more!

Magnus Berglund for finding all grammar errors (hopefully!) and for all valuable comments on my text.

All dear friends, for enriching life outside work with so much fun; dinners, music, old memories, sports, tennis, long summer-nights (and winter-bath) in Köpegårda, and skiing-holidays. Me and my family look forward spending more time with you all!

All patients for participating in this thesis.

Above all, I would like to thank those closest to me; my husband **Andreas** for loving support, and for being a wonderful father to our children, **Arvid** and **Harald**. The three of you, always reminds me of what really matters in life! Jag älskar er!

Finally, my dad **Birger** (1942-2011), for believing in me, and encouraging me to start clinical research, and for sharing your sometimes controversial, but inspiring philosophy to always try your best to make a difference for your patients. I love you and miss you, always.

TACK

Populärvetenskaplig sammanfattning

Diabetesrelaterade fotsår är en vanlig och allvarlig komplikation till följd av diabetes. Det är idag inte bara den vanligaste orsaken till amputationer, utan innebär också en betydande risk för en förkortad livslängd. Denna risk kvarstår även efter att fotsåret är läkt och kan inte enbart förklaras av en högre förekomst av traditionella riskfaktorer, som till exempel hjärtkärlsjukdom. Syftet med denna avhandling har varit att försöka identifiera andra riskfaktorer med koppling till överlevnad hos individer med typ 2 diabetes och fotsår. Förhoppningen är att detta på sikt ska kunna leda till ett förbättrat omhändertagande och minska risken för livshotande komplikationer i denna patientgrupp.

Orsakerna till fotsår är mångfasetterade och omfattar bland annat diabetesrelaterade nervskador (neuropati) och försämrad funktion i stora och små kärl (makro- respektive mikrovaskulär sjukdom). Diabetesneuropati delas in i tre olika grupper; sensorisk neuropati (nedsatt känsel), motorisk neuropati (försämrad muskelfunktion) och autonom neuropati (det icke viljestyrda nervsystemet). Nedsatt känsel i fötterna kan till exempel medföra att individen inte känner av skoskav och mindre sår upptäcks därför inte i tid. Motorisk neuropati leder till obalans i fotens muskler och risk för felställda tår, vilket i sig ökar benägenheten för trycksår. Vid autonom neuropati i fötterna kan svettproduktionen minska vilket ökar risken för torrsprickor och sår. En annan allvarlig form av autonom neuropati är den som drabbar hjärta och kärl. Detta kan leda till instabilt blodtryck och i värsta fall rubbningar i hjärtats rytmreglering. Autonom neuropati i hjärtat är en av flera olika kända orsaker bakom förlängd QT-tid på EKG, vilket är starkt förknippat med en ökad risk för allvarliga rubbningar av hjärtrytmen och plötslig hjärtdöd.

Vi vet att åtgärder som sänker blodsockret på sikt skyddar mot diabeteskomplikationer. Behandlingseffekten brukar utvärderas med ett blodprov (HbA_{1c}), som mäter hur mycket socker som bundits till hemoglobinet. Ett högt HbA_{1c}-värde återspeglar ett högt medelblodsocker under de senaste två till tre månaderna och talar för att den blodsockersänkande behandlingen bör intensifieras. Ett lågt HbA_{1c}-värde anses vara optimalt för att skydda mot framtida komplikationer. Man måste dock beakta att vissa diabetesläkemedel (till exempel insulin) kan medföra en risk för allt för låga blodsockervärde (hypoglykemier). Detta måste i synnerhet uppmärksammas hos äldre och hos individer som redan är drabbade av komplikationer till sin diabetes.

I denna avhandling har vi visat att förlängd QT-tid är vanligt förekommande hos individer med typ 2 diabetes och fotsår (delarbete I), samt hos patienter som genomgått underbensamputation (delarbete II). Detta fynd verkar vara starkt förknippat med försämrad överlevnad. Vi fann även att kombinationen av förlängd QT-tid och ett lågt HbA_{1c} var särskilt ogynnsam (delarbete I), vilket eventuellt kan förklaras av en ökad sårbarhet för hypoglykemier hos individer med lång QT-tid. Slutsatsen av detta är att screening med EKG och analys av QT-tid hos individer med diabetesrelaterade fotsår kan ha betydelse för risk-klassificering, samt att dessa individer bör undvika hypoglykemier.

Sårläkning försämras vid syrebrist ute i vävnaden, vilket är vanligt hos personer med diabetes och svårläkta fotsår. Patienter med diabetesfotsår bör därför alltid utredas, och vid konstaterad kärlsjukdom erbjudas kärlkirurgisk behandling. Detta för att förbättra blodcirkulationen till foten och öka chanserna till sårläkning. Kärlkirurgisk behandling är emellertid inte alltid genomförbar av olika anledningar (t.e.x kan patienten vara allt för sjuk eller ha en allt för avancerad småkärlssjukdom). Vid dessa situationer kan tryckkammarbehandling (hyperbar syrgas) vara ett alternativ, då man genom ett ökat syrgastryck ökar koncentrationen av syre ute i vävnaden. I delarbete III undersökte vi om QT-tiden på EKG påverkades av tryckkammarbehandling. Vi fann att patienter som fick andas 100% syrgas i tryckkammare löpte mindre risk för QT-tidsförlängning jämfört med patienter som fick andas vanlig luft i tryckkammare.

För att utreda förekomst av åderförkalkning i benen undersöks patienterna traditionellt med blodtrycksmätning i benen (ankelblodtryck). Denna undersökning visar ofta felaktigt normala värden hos diabetespatienter på grund av att kärlen är stela och därmed svåra att trycka ihop med en blodtrycksmanschett. Ankelblodtryck kompletteras därför allt oftare med mätning av dels blodtryck i tårna och dels transkutant syrgastryck ($TcPO_2$), där det sistnämnda anses spegla den mikrovaskulära funktionen i större utsträckning. I delarbete IV fann vi ett starkt samband mellan låga $TcPO_2$-nivåer och en försämrad ettårs-överlevnad hos patienter med typ 2 diabetes och fotsår.

Sammanfattningsvis har vi kunnat visa att dödligheten hos patienter med typ 2 diabetes och fotsår är hög (femårsöverlevnad på endast 50 %). Vi har även visat att screening med EKG för att beräkna QT-tid, samt mätning av $TcPO_2$ skulle kunna hjälpa oss att identifiera högrisk-individer med ett skyndsamt behov av intensifierad medicinsk behandling och utredning.

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Paper I

Diabetologia
DOI 10.1007/s00125-013-2860-x

ARTICLE

The impact of metabolic control and QTc prolongation on all-cause mortality in patients with type 2 diabetes and foot ulcers

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Received: 20 November 2012 / Accepted: 24 January 2013

Abstract
Aims/hypothesis The increased all-cause mortality in patients with chronic diabetic foot ulcers cannot fully be explained by traditional cardiovascular risk factors. The significance of heart-rate-corrected QT (QTc) prolongation, a finding often seen in these patients, is unknown. Recently, the importance of metabolic control and hypoglycaemia has been discussed. The aim of this study was to evaluate the impact of different HbA_{1c} levels and QTc prolongation on all-cause mortality in the high-risk population of patients with type 2 diabetes mellitus and foot ulcers.
Methods All patients with type 2 diabetes, younger than 80 years, visiting our diabetes foot unit, with a foot ulcer duration >4 weeks, were screened for participation. Patients on dialysis were excluded. Patients were grouped according to HbA_{1c} level and QTc time ≤ or >440 ms.
Results Patients (n=214, median age 69.1 years) were grouped according to HbA_{1c} level (HbA_{1c}<7.5% [<58 mmol/mol] n=81, 7.5–8.9% [58–74 mmol/mol] n=70, >8.9% [>74 mmol/mol] n=63). Baseline characteristics, including use of potential hypoglycaemic drugs, were similar between groups. During the 8 years of follow-up 151 patients died (70.6%) and HbA_{1c} <7.5% (<58 mmol/mol) was strongly associated with increased mortality. The highest mortality was seen in patients with a combination of HbA_{1c}<7.5% (<58 mmol/mol) and QTc prolongation, with an 8 year mortality of 92.1% as compared with 48.8% in those with HbA_{1c}<7.5% (<58 mmol/mol) but without QTc prolongation.
Conclusion/interpretations HbA_{1c}<7.5% (<58 mmol/mol) in a high-risk population of patients with type 2 diabetes and foot ulcers is associated with a significantly higher mortality, particularly in patients with QTc prolongation.

Keywords Diabetes · Foot ulcers · HbA_{1c} · Metabolic control · Mortality · QTc prolongation

Abbreviations

ABI	Ankle brachial index
ACCORD	Action to Control Cardiovascular Risk in Diabetes
CAN	Cardiac autonomic neuropathy
CVD	Cardiovascular disease
DFU	Diabetic foot unit
eGFR	Estimated glomerular filtration rate
IQR	Interquartile range
MDRD	Modification of diet in renal disease
MI	Myocardial infarction
NICE-SUGAR	Normoglycemia in Intensive Care Evaluation – Survival Using Glucose Algorithm Regulation
PVD	Peripheral vascular disease
QTc interval	Heart-rate-corrected QT interval

Introduction

Patients with a history of diabetic foot ulcers are considered to be a high-risk population for cardiovascular disease (CVD) and increased all-cause mortality [1, 2]. This risk increment can not fully be explained by traditional CVD risk factors, such as peripheral arterial disease, history of myocardial

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Published online: 12 February 2013

infarction (MI) or renal dysfunction [2]. The importance of other diabetic complications, including microvascular disease and neuropathy, needs to be further evaluated.

In patients with type 2 diabetes, prospective studies have shown an association between the degree of hyperglycaemia, measured as HbA_{1c}, and incidence of cardiovascular events [3–6]. Based on these studies, EASD and ADA recommend a strict metabolic control with HbA_{1c} <7.0% (<52 mmol/mol) for most adults with diabetes [7, 8]. However, when the ACCORD (Action to Control Cardiovascular Risk in Diabetes) study was published, showing an increased mortality among patients randomised to receive intensive glycaemic control, a less stringent HbA_{1c} goal was suggested for patients with a history of severe hypoglycaemia, a limited life expectancy or advanced diabetic complications [9]. The importance of hypoglycaemia has frequently been discussed as a plausible factor or mediator of this increased mortality, although no study has been able to show causality [10–12]. Hypoglycaemia alters several risk factors for mortality, including vasoconstriction and cytokine responses, and increases the likelihood of lethal arrhythmias [13–17].

It has been demonstrated that patients with diabetes mellitus have a more frequent occurrence of heart-rate-corrected QT (QTc) interval prolongation than non-diabetic patients [18]. Prolonged QTc time is associated with an increased risk of both cardiovascular and all-cause mortality in the general population [19, 20]. Long QTc time is related to several factors–older age, coronary heart disease, intake of certain drugs and presence of cardiac autonomic neuropathy (CAN, a serious complication of diabetes), as well as hypoglycaemia [18, 21, 22].

The aim of our study was to evaluate the impact of different HbA_{1c} levels and QTc prolongation on all-cause mortality in a predefined high-risk population consisting of patients with type 2 diabetes mellitus and a history of hard-to-heal foot ulcers.

Methods

Patient characteristics and measurement of variables All patients with type 2 diabetes mellitus and age below 80 years, visiting our Diabetic Foot Unit (DFU; Skåne University Hospital, Lund, Sweden) during two consecutive years were screened for study participation. Those with an ulcer that did not heal within 4 weeks of treatment were included in this study. Patients with ongoing dialysis and those without an HbA_{1c} or ECG registration within a 4 month period following the first visit to the DFU were excluded.

Patients were grouped according to HbA_{1c} level: group 1, HbA_{1c} <7.5% (<58 mmol/mol); group 2, HbA_{1c} 7.5–8.9% (58–74 mmol/mol) and group 3, HbA_{1c} >8.9% (>74 mmol/mol).

HbA_{1c}, creatinine, cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerol were analysed in our local certified laboratory. Estimated glomerular filtration rate (eGFR) was derived from plasma creatinine level using the modification of diet in renal disease (MDRD) equation [23]. An eGFR≥60 $ml\,min^{-1}$ 1.73 m^{-2} was considered to indicate normal renal function. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or current use of blood-pressure-lowering drugs. Hyperlipidaemia was considered present if total cholesterol was >5.0 mmol/l, LDL-cholesterol was >2.5 mmol/l or the patient was prescribed a cholesterol-lowering drug. Peripheral vascular disease (PVD) was defined by the presence of an abnormal ankle brachial index (ABI) value (≤0.9 or ≥1.4) or a history of previously performed vascular surgery including percutaneous transluminal angioplasty [24]. The ABI was measured as the ratio of systolic ankle pressure divided by arm blood pressure. Systolic ankle pressure was analysed with a pen doppler over the dorsal pedal or posterior tibial arteries.

A standard 12-lead resting ECG was taken during a regular visit to the clinic, using a Siemens ECG machine (Siemens Elema, Solna, Sweden). The QT interval was calculated from the beginning of the QRS complex to the end of the T wave, using the validated Sicard 440/740 ECG computer-analysis programme (Megacart version 3 V4, 7/2.38/23; Siemens Elema) [25, 26]. The QT intervals were then corrected for heart rate to obtain the QTc value using Bazett's formula [27]. A QTc value greater than 440 ms was considered abnormal and patients in each HbA_{1c} group were divided according to QTc time ≤ or >440 ms [28]. Mortality data was obtained from the Swedish National Death Registry. This study was approved by the Local Ethics Committee in Lund, Sweden and was carried out in accordance with the Declaration of Helsinki, as revised in 2000. All patients have given their consent.

Statistical analysis Patient characteristics are summarised by descriptive statistics. Continuous variables are expressed as median and interquartile range (IQR; 25–75 percentile) and categorical variables are presented as percentages. Continuous data were compared using Mann–Whitney *U* test and categorical variables using Fisher's exact test. Univariate survival analysis was performed by Kaplan–Meier analysis and overall significance was calculated by the Gehan–Wilcoxon test.

Cox proportional hazard regression models were applied to study the independent association between HbA_{1c} level, QTc prolongation and mortality. Initial analyses included the following basal characteristics with plausible impact on mortality: sex, previous MI, heart failure, PVD, hyperlipidaemia,

renal impairment (eGFR ≥60, 45–59, 30–44, <30 ml min^{-1} min 1.73 m^{-2}), diabetes duration (decades), smoking, age (grouped according to decade of age) and usage of insulin, insulin-releasing agents or beta blockers. Potential confounding factors with a p value <0.10 were entered in the final analyses. The results of these Cox proportional hazards models are presented as HR with 95% CI.

All statistical analyses where performed using Statistica software version 10 (Statsoft, Tulsa, OK, USA) and statistical significance was assessed at the two-tailed 0.05 threshold.

Results

Baseline characteristics Of the initial sample of 224 patients with type 2 diabetes and foot ulcers, ten were excluded from the study due to lack of HbA_{1c} data. Thus, 214 patients (37.9% women) with type 2 diabetes mellitus were grouped according to their HbA_{1c} level (HbA_{1c}<7.5% [<58 mmol/mol] n=81, 7.5–8.9% [58–74 mmol/mol] n=70, >8.9% [>74 mmol/mol] n=63).

The median age in the whole study population was 69.1 years (range 63–76) and did not differ between groups. As shown in Table 1, QTc prolongation and PVD was most prevalent in patients with HbA_{1c}<7.5% (<58 mmol/mol). There were no differences between groups in the usage of drugs with potential hypoglycaemic effect, although insulin therapy was most frequently used in the group of patients with the highest HbA_{1c} levels. Prescription of beta blockers, as well as other drugs with potential effect on the QT interval (tricyclic antidepressants, certain antibiotics [e.g. macrolides and quinolones]) did not differ between groups and did not affect the outcome of this study.

Survival analysis During a follow-up period of 8 years, 151 patients died (70.6% of total). As shown in Fig. 1, an HbA_{1c} level <7.5% (<58 mmol/mol) was strongly associated with increased mortality.

In a Cox hazard model including the following possible confounding factors: HbA_{1c} level, age, sex, diabetes duration, hyperlipidaemia, renal impairment, smoking habits, presence of heart failure, previous MI, PVD, usage of insulin or insulin-releasing drugs, usage of beta blockers and QTc prolongation, only age, sex, HbA_{1c} level and QTc prolongation were associated (p<0.10) with mortality and thus included in the final analysis. The outcome of this analysis showed that both HbA_{1c}<7.5% (<58 mmol/mol) and presence of QTc prolongation were independently associated with a higher mortality (Table 2).

In a subgroup analysis evaluating the impact of QTc prolongation at different HbA_{1c} levels, highest mortality was found in patients with HbA_{1c}<7.5% (<58 mmol/mol) and QTc time >440 ms (Fig. 2). In these patients 8 year mortality was 92.1% as compared with 48.8% in those with HbA_{1c}<7.5% (<58 mmol/mol) but without QTc prolongation (p<0.00001). A statistically non-significant trend towards worse outcome in patients with QTc prolongation was seen in the other two HbA_{1c} groups (Fig. 2). In a Cox hazard model adjusted for the same plausible confounding factors as used in the previous analysis, HbA_{1c}/QTc (patients with both HbA_{1c} <7.5% [<58 mmol/mol] and QTc prolongation vs all other patients), age and sex was associated (p<0.10) with an increased 2, 4, 6 and 8 year mortality and thus included in the final analysis. Presence of hyperlipidaemia was associated with 2 year mortality in the initial but not in the final analysis (p=0.16). Patients with the combination of QTc time >440 ms and HbA_{1c}<7.5% (<58 mmol/mol) were at highest mortality risk in our study (Table 3).

A statistically significant correlation (r=0.23, p=0.034) was present between HbA_{1c} level and QTc time in the groups of patients not using any potentially QTc-prolonging drug (beta blockers, selective serotonin re-uptake inhibitors, macrolides, quinolones, proton-pump inhibitors or tricyclic antidepressants), whereas this was not the case in patients prescribed any of these drugs.

Discussion

It is well documented that improved metabolic control reduces microvascular complications in patients with type 2 diabetes mellitus, although it is not clear whether patients with type 2 diabetes mellitus and hard-to-heal foot ulcers benefit from strict glycaemic control. In our 8 year follow-up study evaluating survival in type 2 diabetes patients with hard-to-heal foot ulcers, short- as well as long-term all-cause mortality was higher in patients with HbA_{1c} levels below 7.5% (58 mmol/mol). Our finding is in accordance with the increased mortality in the intensively treated arm in the ACCORD trial and with the outcome of a large British cohort study including 47,970 patients with type 2 diabetes [9, 29]. The outcome of this latter study by Currie and coworkers showed a U-formed association between HbA_{1c} and all-cause mortality, with the lowest HR at an HbA_{1c} level of about 7.5% (58 mmol/mol). The adjusted HR of all-cause mortality for patients with the lowest HbA_{1c} decile was 1.59 [29]. Similar outcomes have been seen in studies evaluating the impact of HbA_{1c} levels in diabetic patients with severe renal complications [30, 31]. In a large cohort study including all diabetic patients with stage 3 and 4 chronic kidney disease in Alberta, Canada, the association between all-cause mortality and HbA_{1c} was J-shaped [30]. In a smaller study prospectively evaluating all-cause mortality in diabetic patients beginning dialysis treatment, a decreased mortality risk was observed with increasing

Table 1 Baseline characteristics of patients with different HbA_{1c} levels

Characteristic	HbA_{1c}<7.5% (<58 mmol/mol)	HbA_{1c} 7.5–8.9% (58–74 mmol/mol)	HbA_{1c}>8.9% (>75 mmol/mol)	*p* value
n	81	70	63	
Age (years)	72 (67–77)	71 (65–76)	67 (61–75)	NS
Diabetes duration (years)	11 (5–16)	11 (8–15)	12 (8–20)	NS
HbA_{1c} (%)	6.8 (6.3–7.2)	8.2 (7.8–8.5)	9.9 (9.2–10.8)	0.00000
HbA_{1c} (mmol/mol)	51 (45–55)	66 (62–69)	85 (77–95)	
QTc>440 ms (%)	46.9	30.0	30.7	0.019[a]
Creatinine (μmol/l)	82 (68–100)	85 (66–103)	73 (56–97)	NS
eGFR<60 mlmin^{-1} (1.73 $m)^{-2}$ (%)	28.4	34.8	27.0	NS
Sex, female/male (%)	32.1/67.9	38.6/61.4	44.4/55.6	NS
Previous MI (%)	33.3	28.9	22.2	NS
Heart failure (%)	24.7	28.6	19.1	NS
Hypertension (%)	75.3	68.6	68.3	NS
Hyperlipidaemia (%)	39.5	37.1	36.5	NS
Smoking (ever) (%)	56.8	51.4	54.0	NS
PVD (%)	58.0	42.9	25.4	0.07[b] <0.01[c] 0.04[d]
Previous vascular surgical intervention in a lower limb	34.6	20.0	12.7	0.07[b] <0.01[c] 0.35[d]
Glucose-lowering treatment				
Diet only (%)	11.1	1.4	1.6	NS
Metformin only (%)	6.2	11.4	4.8	NS
Sulfonylurea only (%)	19.8	14.3	11.1	NS
Metformin + sulfonylurea (%)	11.1	17.1	9.5	NS
Insulin alone or in combination (%)	51.9	55.7	73.0	NS[b] 0.01[c] <0.05[d]
Insulin or sulfonylurea alone or in combination (%)	82.7	87.1	93.7	NS
Beta blocker (%)	27.2	30.0	33.3	NS
Digoxin (%)	9.9	15.7	9.5	NS
Tricyclic antidepressant (%)	4.9	1.4	3.2	NS
Selective serotonin re-uptake inhibitor (%)	6.2	13.0	9.7	NS
Macrolide (%)	0	0	0	NS
Quinolone (%)	8.8	17.4	9.7	NS
Proton-pump inhibitor (%)	13.6	10.0	6.3	NS

Data are given as percentage or median (IQR)

[a] HbA_{1c}<7.5% (<58 mmol/mol) vs ≥7.5% (≥58 mmol/mol)

[b] HbA_{1c}<7.5% (<58 mmol/mol) vs 7.5–8.9% (58–74 mmol/mol)

[c] HbA_{1c}<7.5% (<58 mmol/mol) vs >8.9% (>75 mmol/mol)

[d] HbA_{1c}7.5–8.9% (58–74 mmol/mol) vs >8.9% (>75 mmol/mol)

HbA_{1c} [31]. The follow-up time in these studies was 46 and 32 months, respectively. Contrarily, in-hospital mortality following acute MI was not associated with HbA_{1c} levels in an American study using data from a nationwide voluntary register [32].

A plausible explanation for this increased mortality might be a higher frequency of hypoglycaemic episodes in patients with a lower HbA_{1c}. It is well proven that the use of insulin or insulin secretagogues can cause severe and fatal hypoglycaemia. In type 1 diabetes mellitus, 6–10% of all

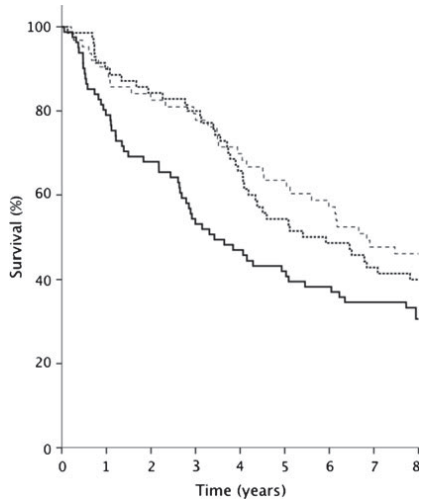

Fig. 1 Kaplan–Meier survival curve showing 8 year mortality in patients grouped according to HbA_{1c} levels, $p<0.01$ for pooled comparison. For separate comparisons: HbA_{1c}<7.5% (<58 mmol/mol) vs HbA_{1c} 7.5–8.9% (58–74 mmol/mol), $p=0.06$. HbA_{1c}<7.5% (<58 mmol/mol vs HbA_{1c}>8.9% [>74 mmol/mol]), $p=0.02$. HbA_{1c} 7.5–8.9% (58–74 mmol/mol) vs HbA_{1c}>8.9% (>74 mmol/mol), NS Solid line, HbA_{1c}<7.5% (<58 mmol/mol); dotted line, HbA_{1c} 7.5–8.9% (58–74 mmol/mol); dashed line, HbA_{1c}>8.9% (>74 mmol/mol)

mortality is estimated to be associated with hypoglycaemia [33–35]. In addition, in the Normoglycemia in Intensive Care Evaluation – Survival Using Glucose Algorithm Regulation (NICE-SUGAR) study a strong association between hypoglycaemia and all-cause mortality was seen [11]. The follow-up period in NICE-SUGAR was 90 days and the median time from the first episode of hypoglycaemia to death was 7 days. A causal relationship between hypoglycaemia and mortality is likely for several reasons. Cardiac arrhythmias occurring during hypoglycaemia were first described in psychiatric patients treated with insulin shock in the 1930s [36]. Several case reports have also described an association between arrhythmia and spontaneous hypoglycaemia [37, 38]. Further, hypoglycaemia triggers an array of counter-regulatory responses that would normally return blood glucose to non-pathological levels. Glucagon, adrenaline (epinephrine), noradrenaline (norepinephrine), cortisol, growth hormone, corticotropin, pancreatic polypeptide and the autonomic nervous system are all activated, leading to alterations in blood flow and blood composition, vasoconstriction, white-cell activation and a release of inflammatory mediators and cytokines[13–15, 17]. Several studies have also shown an association between hypoglycaemia and QTc prolongation in type 1 as well as type 2 diabetes mellitus [39, 40]. In our study QTc prolongation might be linked to hypoglycaemia, as it was more frequently present in the group of patients with the lowest HbA_{1c} levels (47 vs 30% in the other two groups), wherein 83% were prescribed drugs with hypoglycaemic effects (insulin or sulfonylurea). Further, a negative correlation between QTc time and HbA_{1c} was present in our patients without QTc-prolonging drugs, suggesting a plausible association between these two risk factors.

QTc prolongation, a condition known to be associated with CAN, has been shown to increase the risk of arrhythmia and sudden death in diabetic patients [41, 42]. In the ACCORD trial, the presence of CAN, defined as a combination of pathological QT index and abnormal heart-rate variability, was associated with increased mortality risk [43]. The highest mortality rate in our study was among patients with both QTc prolongation and HbA_{1c}<7.5% (<58 mmol/mol). In this group 8 year mortality was 92% as compared with 49% in those patients with similar HbA_{1c} but normal QTc time.

Although a causal relationship between these two factors and mortality is plausible, it will be difficult (and perhaps impossible) to sort out the true determinants of our outcome. However, the increased mortality in the group of patients with prolonged QTc time and the lowest HbA_{1c} level could not be explained by differences in traditional cardiovascular risk factors, such as MI, heart failure, hypertension or smoking habits, and our findings were sustained after being

Table 2 Predictors of all-cause mortality in patients with type 2 diabetes mellitus and hard-to-heal ulcers based on Cox proportional analyses adjusted for plausible confounders

All-cause mortality	HbA_{1c}<7.5% vs ≥7.5%[a]	*p* value	Age, risk per decade	*p* value	Female sex	*p* value	QTc prolongation	*p* value
2 year mortality	1.88 (1.05, 3.36)	0.033	1.72 (1.07, 2.75)	0.025	0.61 (0.32, 1.16)	NS	1.74 (0.98, 3.12)	0.060
4 year mortality	1.78 (1.16, 2.73)	0.009	1.56 (1.12, 2.17)	0.008	0.61 (0.37, 0.98)	0.042	2.08 (1.35, 3.22)	0.001
6 year mortality	1.47 (1.01, 2.14)	0.045	1.63 (1.22, 2.17)	0.001	0.64 (0.42, 0.97)	0.035	1.96 (1.34, 2.86)	0.001
8 year mortality	1.41 (1.00, 2.00)	0.052	1.51 (1.17, 1.95)	0.001	0.63 (0.43, 0.93)	0.019	2.00 (1.40, 2.85)	0.000

Data are given as HR (95% CI)

[a] <58 mmol/mol vs ≥58 mmol/mol

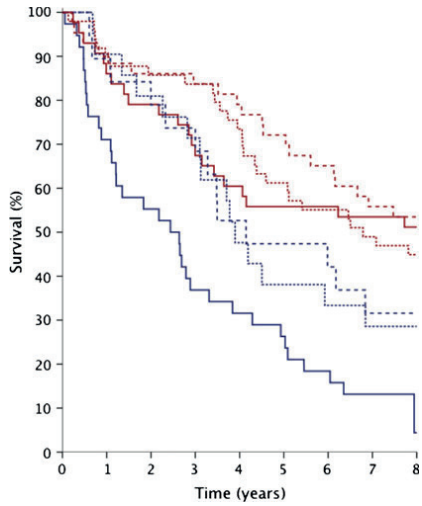

Fig. 2 Kaplan–Meier survival curve showing 8 year mortality in patients grouped according to HbA_{1c} levels and presence of QTc prolongation, defined as QTc time >440 ms. $p<0.0001$ for pooled comparison. For separate comparisons: QTc prolongation and HbA_{1c}<7.5% (<58 mmol/mol) vs all three groups with normal QTc-time separately $p<0.0001$; vs group with QTc prolongation and HbA_{1c} 7.5–8.9% (58–74 mmol/mol) $p=0.023$; and vs group with QTc prolongation and HbA_{1c} >8.9% (>74 mmol/mol) $p=0.015$. QTc prolongation and HbA_{1c} 7.5–8.9% (58–74 mmol/mol) vs normal QTc time and HbA_{1c}>8.9% (>74 mmol/mol), $p=0.025$. All other comparisons NS. Blue lines, patients with QTc prolongation; red lines, patients without QTc prolongation; solid line, HbA_{1c}<7.5% (<58 mmol/mol); dotted line, HbA_{1c} 7.5–8.9% (58–74 mmol/mol); dashed line, HbA_{1c}>8.9% (>74 mmol/mol)

adjusted for possible confounders. PVD, as well as a history of previous vascular surgical intervention in the lower limb, was more frequently present in the group of patients with the lowest HbA_{1c}. The 8 year mortality rate was higher in patients with a history of vascular intervention in a lower limb than in those without such intervention (74.1 vs 58.6%, $p=0.032$); however, in the Cox proportional hazard model neither this intervention nor presence of PVD were independently associated with mortality. This outcome is in accordance with the result of a UK study evaluating factors associated with mortality in diabetic patients with novel foot ulcers [44]. In this study presence of PVD was associated with a higher mortality rate but after adjustment for confounding factors only older age turned out to be a significant predictor of mortality.

Autonomic neuropathy, which might not only lead to decreased adrenergic awareness of hypoglycaemia but also to increased risk of cardiac arrhythmia, may have been more frequently present in those patients with prolonged QTc time[45]. An alternative explanation is that QTc prolongation in combination with a low HbA_{1c} level, occurs as a result of disease processes that confer a predisposition to death, and that this cluster of risk factors represents a marker, rather than a cause, of an increased risk of death.

Possible limitations of this study include selection bias, although this is not a critical issue as all patients with diabetic foot problems in our catchment area are, with few exceptions, referred to our clinic. Our registry, including all patients visiting our clinic, as well as all charts from the years of inclusion, was carefully monitored. Mortality data are robust as the Swedish National Death Registry capture data on 100% of all deaths. The fact that we only included baseline clinical variables and did not account for time-varying covariates, might affect outcome. Further, QTc time might be influenced by several factors, including drugs and acute heart disease. All ECGs at our clinic were taken during non-acute, resting conditions, which limits (although does not exclude) biases such as myocardial ischaemia or acute heart failure. Neither could we identify any significant differences in prescription patterns of the most commonly used drugs with plausible QTc-prolonging actions (including beta blockers, tricyclic antidepressants, macrolides and quinolones) between groups. We did not collect data on the frequency of hypoglycaemia, so we can therefore only

Table 3 Importance of the combination of QTc prolongation and HbA_{1c}<7.5% (58 mmol/mol) on all-cause mortality in patients with type 2 diabetes mellitus and hard-to-heal ulcers based on Cox proportional analyses adjusted for plausible confounders

All-cause mortality	HbA_{1c}<7.5%[a]+long QTc time vs HbA_{1c}<7.5%[a]+QTc ≤440 ms and HbA_{1c}≥7.5%[a]	*p* value	Age, risk per decade	*p* value	Female sex	*p* value
2 year mortality	2.80 (1.54, 5.09)	<0.001	1.76 (1.10, 2.82)	0.019	0.56 (0.29, 1.07)	0.079
4 year mortality	2.65 (1.66, 4.24)	<0.000	1.63 (1.18, 2.27)	0.003	0.54 (0.34, 0.87)	0.012
6 year mortality	2.55 (1.67, 3.87)	<0.000	1.69 (1.27, 2.25)	<0.000	0.58 (0.39, 0.87)	0.09
8 year mortality	2.76 (1.86, 4.09)	<0.0000	1.57 (1.21, 2.02)	0.001	0.57 (0.39, 0.84)	0.004

Data are given as HRs (95% CI)

All statistically significant risk factors are presented in the table

[a] <58 mmol/mol

speculate on its importance as a possible cause or mediator of the increased mortality seen in patients with low HbA_{1c} level and prolonged QTc interval. Still, a causal relationship is plausible, and in the EURODIAB insulin dependent diabetes mellitus (IDDM) complications trial an annual occurrence of at least three severe hypoglycaemic events was, independently of confounders, associated with presence of QTc prolongation [46]. Another confounder might be that HbA_{1c} may decrease with older age, as well as being associated with terminal disease, but in our study no differences in age, medications or comorbidities were seen between HbA_{1c} groups. In this study we have neither evaluated cause of death nor autonomic nervous function, which limits our opportunity to identify any relationship between QTc time, autonomic function and cause of death. Despite these limitations we identified a group of patients with clinically and statistically significant increased mortality. However, to fully evaluate the importance of metabolic control and QTc time on mortality in this high-risk population a larger prospective multicentre study needs to be initiated.

In conclusion, in this high-risk population of patients with type 2 diabetes mellitus and hard-to-heal foot ulcers, an HbA_{1c} level below 7.5% (58 mmol/mol) was associated with higher mortality, particularly in the presence of QTc prolongation. This finding indicates the clinical importance of ECG screening and suggests that, in patients with prolonged QT intervals, drugs which are known to affect QT interval and hypoglycaemia should be avoided.

Funding This study was supported by the Swedish Diabetes Foundation, Sydvästra Skånes Diabetesförening, Krapperup Foundation, Crafoord Foundation, Skane county council's research and development foundation and Faculty of Medicine (ALF), Lund University, Sweden.

Duality of interest Both authors declare there is no duality of interest associated with this manuscript.

Contribution statement Both authors have substantially contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafted the article and given final approval of the version to be published.

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Paper II

DIABETICMedicine

DOI: 10.1111/dme.12632

Research: Complications

Heart rate-corrected QT interval prolongation as a prognostic marker for 3-year survival in people with Type 2 diabetes undergoing above-ankle amputation

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Accepted 7 November 2014

Abstract

Aim To evaluate whether heart rate-corrected QT interval is a plausible prognostic factor for survival after major amputation in people with Type 2 diabetes.

Methods All people with Type 2 diabetes aged < 80 years who underwent a major amputation at two hospitals with multidisciplinary diabetic foot teams were evaluated and grouped according to whether their heart rate-corrected QT interval was ≤ or > 440 ms.

Results A total of 70 patients with a median age of 72 years were included in the study. During the 3 years of follow-up, 38 patients (54%) died. Heart rate-corrected QT interval prolongation was present in 51.4% of the patients and was strongly associated with 3-year mortality (73 vs 36%; $P < 0.001$). In a Cox proportional hazard model, heart rate-corrected QT interval prolongation was the strongest independent risk factor for 3-year mortality [hazard ratio 2.20 (95% CI 1.11–4.38)]. Treatment with metformin seemed to have a protective effect [hazard ratio 0.22 (95% CI 0.05–0.94)].

Conclusions The findings of the present study indicate that heart rate-corrected QT interval prolongation is associated with increased mortality in people with Type 2 diabetes undergoing above-ankle amputation.

Diabet. Med. 32, 679–685 (2015)

Introduction

Foot ulcers and their complications are a major cause of morbidity and mortality in people with diabetes [1–3]. The lifetime risk of amputation is 10–30 times higher in people with diabetes compared with the general population [4–6]. Despite a high standard of clinical care for these people in recent years, the all-cause mortality risk after amputation has remained high, with a 3-year mortality rate of ~37–52% [7–11]. This excess mortality after amputation cannot fully be explained by traditional cardiovascular risk factors, such as myocardial infarction, hypertension, renal dysfunction and peripheral vascular disease, and there are still no clinically useful markers to identify people with the highest mortality risk after major amputation [2].

In a recently published study evaluating the prognostic impact of prolonged heart rate-corrected QT (QTc) interval and metabolic control in people with chronic diabetic foot ulcers, those with a QTc interval > 440 ms, especially in combination with an HbA_{1c} level < 58 mmol/mol (< 7.5%), had a significantly higher mortality rate [12]. QTc interval prolongation is a plausible consequence of cardiac autonomic neuropathy in diabetes and is a well-known predictor of lethal arrhythmias [13,14]. Studies also indicate that QTc interval increases during hypoglycaemia [15,16]. Whether QTc prolongation and metabolic control have any prognostic impact on the high mortality risk after major amputation, however, has not previously been studied. The aim of the present study, therefore, was to evaluate the significance of QTc time and HbA_{1c} as risk factors for mortality after above-ankle amputation in people with Type 2 diabetes.

Patients and methods

We retrospectively reviewed all people with diabetes who underwent their first above-ankle amputation at Skåne University Hospital, Lund and Ängelholm Hospital,

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What's new?

- Patients with Type 2 diabetes undergoing major amputations still have a very high mortality risk despite improved cardiovascular risk management during the last decades.
- This risk increment cannot fully be explained by traditional risk factors and none of these risk factors can be used as good predictors for survival.
- In the present paper we show that corrected QT interval prolongation, a plausible marker for autonomic cardiac neuropathy, may be used as such a risk marker. In a consecutive group of patients, the 3-year mortality rate was doubled in patients with a corrected QT interval > 440 ms than in those with a normal corrected QT interval.
- This novel finding might be clinically useful for future risk stratification in this high-risk population.

Ängelholm, Sweden between 1 January 2004 and 30 June 2010. All patients with Type 2 diabetes who were aged < 80 years were included for further analysis of their medical records in the study. Individuals without an electrocardiogram (ECG) taken within a 4-month period before amputation and those with a ventricular-stimulating pacemaker (making it impossible to calculate a correct QTc interval) were excluded from the study, together with people who died within 10 days after amputation as a result of complications after surgery.

A standard 12-lead resting ECG was taken before surgery using a Siemens ECG machine (Siemens Elema, Solna, Sweden). In the few cases lacking a preoperative ECG, a standard non-acute 12-lead resting ECG, taken no longer than 4 months before amputation, was analysed. The QT interval, defined as the time from the beginning of the QRS complex to the end of the T-wave on the ECG, was calculated using the validated Sicard 440/740 ECG computer-analysis programme (MEGACART version 3 V4, 7/2.38/23; Siemens Elema) [17]. The value was then corrected for heart rate using Bazett's formula, to obtain the QTc time [18]. QTc intervals > 440 ms were considered prolonged and patients were grouped accordingly [19].

Baseline characteristics at the time of amputation, including medical history regarding coronary heart disease (i.e. myocardial infarction, angina pectoris, history of coronary revascularization procedure), diabetes duration, heart failure, hypertension, peripheral arterial disease, hyperlipidaemia, smoking habits, pharmacological treatment and laboratory data (HbA_{1c}, cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol and creatinine) were assessed from patients' charts. Estimated glomerular filtration rate (eGFR) was calculated from plasma creatinine using the modification of diet in renal disease equation [20].

Hyperlipidaemia was defined as total cholesterol > 5.0 mmol/l, LDL cholesterol > 2.5 mmol/l or on-going treatment with a cholesterol-lowering drug. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or use of blood pressure-lowering drugs. Peripheral vascular disease was defined as either presence of a pathological ankle brachial index (defined as ≤ 0.9 or ≥ 1.4 mmHg), an arterial toe-blood pressure ≤ 30 mm Hg or previously performed vascular intervention in the lower extremity [21].

We obtained the 3-year mortality data from the National Death Registry of Sweden. The study was approved by the Regional Ethics Committee in Lund, Sweden and was carried out in accordance with the declaration of Helsinki, as revised in 2000. All patients gave their consent according to the decision of the Regional Ethics Committee.

Statistical analysis

Statistical analyses were performed using SPSS (IBM Corp., Chicago, IL, USA) version 20. Continuous data are expressed as median and interquartile ranges and categorical data as percentages. To assess differences, the Mann–Whitney *U*-test was used for continuous variables and Fisher's exact test for categorical data. Survival analyses were performed using Kaplan–Meier estimates and significances were calculated using log-rank tests. To assess the independent prognostic impact of prolonged vs normal QTc interval on all-cause mortality, Cox regression analysis was performed. Possible confounding variables with a significant or nearly significant ($P < 0.1$) difference between the groups at baseline [myocardial infarction, renal impairment (eGFR < 60 ml/min/1.73 m^2), treatment with β-blockers and metformin), were manually entered into the model, together with our variable of interest (QTc prolongation). Those variables found to have a significant ($P < 0.05$) or potential ($P < 0.15$) association with mortality or those found to change the coefficient of our principal independent variable (QTc prolongation) markedly were retained in the final regression analysis. The results of the Cox regression model are presented as hazard ratios with 95% CIs. To analyse the dose–response relationship between QTc time and 3-year mortality, we performed a logistic regression analysis to estimate the odds ratios for each QTc quartile. A two-sided *P* value of < 0.05 was taken to indicate statistical significance.

Results

During the study period, 74 consecutive people with Type 2 diabetes and a maximum age of 80 years underwent an above-ankle amputation. Four people were excluded from further analysis according to our exclusion criteria, two with a ventricular-stimulating pacemaker making QTc interval calculation impossible, and two who did not survive the first

10 days after amputation (one because of staphylococcus sepsis and one because of pneumonia). The remaining 70 patients, with a median (interquartile range) age of 72 (66–77) years, were grouped according to QTc and HbA_{1c} level < or ≥ 58 mmol/mol (7.5%).

Baseline characteristics

A total of 51.4% of the study population had QTc prolongation. A history of coronary heart disease was more common among those patients with QTc prolongation, as shown in Table 1, but no other differences were seen between groups regarding traditional risk factors such as age, sex, diabetes duration, hypertension, renal dysfunction and peripheral vascular disease. Antidiabetic treatment, the use of drugs with a potential QTc-prolonging action and β-blockers were analysed and the results are shown in Table 2. The prescription of β-blockers was higher among those patients with QTc prolongation (66.7 vs 36.1%; $P = 0.015$), while metformin treatment was more common in the group with a normal QTc time (30.6 vs 6.1%; $P = 0.013$). Treatment with insulin or other drugs with a potential hypoglycaemic effect did not differ between the groups, neither did prescription of drugs with a known QTc-prolonging action.

Survival analysis

During the 3 years of follow-up, 54% of our study population died. As shown in Figure 1, QTc time > 440 ms was strongly associated with 3-year mortality (73%, compared with 36% in those with normal QTc time; $P < 0.001$).

In Cox regression analysis, a prolonged QTc interval (> 440 ms) was associated with more than double the 3-year mortality risk [hazard ratio (95% CI 1.11–4.38; $P = 0.024$)], after adjusting for the possible confounding factors mentioned above. Treatment with metformin seemed to have a protective effect in the present study, with a hazard ratio of 0.22 (95% CI 0.05–0.94; $P = 0.041$), but renal impairment (eGFR < 60 ml/min/1.73 m^2), myocardial infarction or use of β-blockers did not affect the outcome. The results of the Cox regression analyses are shown in Table 3. When analysing the outcome in the different QTc quartiles (Q1 = 376–414 ms, Q2 = 415–436 ms, Q3 = 437–455 ms, Q4 = 456–548 ms), we found the highest 3-year survival among patients in quartile 2, in which > 70% of the patients were alive after 3 years compared with < 30% of those in the highest quartile. The 3-year mortality and the odds ratios for the different QTc quartiles are shown in Table 4.

When analysing the impact of HbA_{1c} levels < or ≥ 58 mmol/mol (7.5%) among patients with and without QTc prolongation, there was a trend towards worse outcome among patients with the combination of prolonged QTc time and HbA_{1c} < 58 mmol/mol (7.5%), although it did not reach statistical significance (Fig. 2).

Discussion

The mortality rate in patients with diabetes who have undergone amputation has remained high despite improvements in cardiovascular risk factor intervention during the last decades. In the present study, 54% of our patients died within 3 years of amputation, a finding consistent with those of other published studies. A German study from 2011 also reported a 3-year mortality rate of 54% [11], an Italian study from 2001 reported a 3-year mortality of 52% in patients undergoing major amputation [8], and a US study from 2010, reviewing patients with diabetes undergoing both major and minor amputations, reported an overall 3-year

Table 1 Baseline characteristics in patients with and without heart-rate corrected QT interval prolongation (> 440 ms)

	QTc ≤ 440 ms, $n = 34$	QTc > 440 ms, $n = 36$	P
Median (IQR) age, years	73.5 (66–77)	71.5 (66–77)	n.s
Median (IQR) HbA_{1c}			
mmol/mol	60 (48–73)	63 (49–79)	n.s
%	7.6 (6.5–8.8)	7.9 (6.6–9.4)	n.s
Median (IQR) diabetes duration, years	18 (9–28)	18 (13–26)	n.s
Median (IQR) QTc time, ms)	415 (402–423)	457 (451–470)	
Median (IQR) creatinine level, μmol/l	81.5 (65–117)	99.0 (74-230)	0.087
Median (IQR) eGFR, ml/min/1.73 m^2	76 (55–112)	68 (23–95)	n.s
eGFR < 60 ml/min/1.73 m^2, %	25.0	47.1	0.08
Sex: female /male, %	19.4/80.6	14.7/85.3	n.s
Preious myocardial infarction, %	20.0	46.9	0.036
Heart failure, %	33.3	22.2	n.s
Hypertension, %	80.6	97.0	n.s
Hyperlipidaemia, %	73.5	78.1	n.s
Smoking (ever), %	78.8	68.6	n.s
Peripheral arterial disease, %	88.9	86.2	n.s

QTc, heart rate-corrected QT; IQR, interquartile range; n.s., nonsignificant; eGFR, estimated glomerular filtration rate.
P values < 0.1 are shown, otherwise n.s. is stated.

Table 2 Use of medications at time of amputation in patients with and without QTc prolongation (> 440 ms)

	QTc ≤ 440 ms	QTc > 440 ms	
Antidiabetic treatment, %			
Diet only	2.8	0	n.s
Metformin	30.6	6.1	0.013
Sulphonylurea	11.1	3.0	n.s
Insulin alone or in combination	88.9	100.0	n.s
Insulin or sulphonylurea alone or in combination	94.4	100.0	n.s
Other drugs, %			
β-blocker	36.1	66.7	0.015
Sotalol	0	0	n.s
Digoxin	22.2	6.1	n.s
Tricyclic antidepressive drugs	8.3	8.8	n.s
Selective serotonin reuptake inhibitors	19.4	30.3	n.s
Macrolides	0	0	n.s
Quinolones	11.1	15.2	n.s
Protone-pump inhibitors	25.0	39.4	n.s

n.s., nonsignificant.
P values < 0.1 are shown, otherwise n.s. is stated.

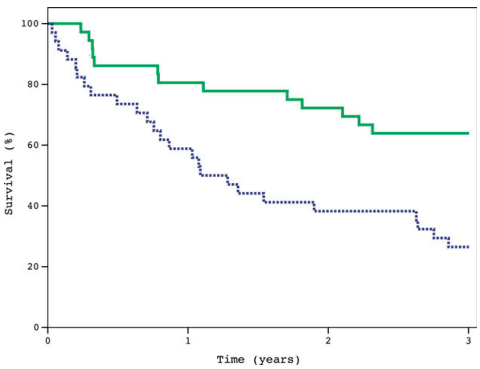

FIGURE 1 The 3-year survival rates in patients with Type 2 diabetes with (dotted blue line) and without (solid green line) heart-rate corrected QT (QTc) interval prolongation, defined as QTc time > 440 ms. $P < 0.001$.

mortality of 39.5%, and when comparing foot, above-ankle and above-knee amputations, the two higher levels were associated with higher mortality rates, similar to the present results [9].

Peripheral vascular disease and neuropathy are the two leading causes of amputation among people with diabetes. Peripheral vascular disease is considered to be associated

Table 3 Predictors, based on Cox regression analyses, of all-cause 3-year mortality in people with Type 2 diabetes mellitus undergoing an above-ankle amputation

	Hazard ratio	95% CI	*P*
QTc interval > 440 ms vs. normal	2.20	1.11–4.38	0.024
Metformin	0.22	0.05–0.94	0.041

QTc, heart rate-corrected QT.

Table 4 Odd ratios for 3-year mortality in people with Type 2 diabetes mellitus undergoing above-ankle amputation in the different heart rate-corrected QT interval quartiles with quartile 2 as the reference group

Quartile	*n*	3-year mortality, %	Odds ratio (95% CI)	*P*
Q1	18	44.4	1.92 (0.63-5.89)	0.36
Q2	17	29.4	1.0 (reference)	
Q3	17	64.7	4.40 (1.04–18.6)	0.04
Q4	18	72.2	6.24 (1.44–27.0)	0.01

Quartile (Q) definitions: Q1, QTc time 376–414 ms, median 402 ms; Q2, QTc time 414–436 ms, median 421 ms; Q3,QTc time 437–455 ms, median 450 ms; Q4, QTc time 456–548 ms, median 469 ms.

with a higher incidence of cardiovascular complications and mortality. In a UK study evaluating 5-year mortality after minor or major amputation, a history of a vascular surgical intervention in the lower limb was found at a higher rate in deceased as compared with surviving patients with diabetes, but in a Cox regression analysis revascularization procedures were not independently associated with mortality [7]. Furthermore, in a study by Moulik *et al.* [22] evaluating mortality stratified by ulcer aetiology, there was a higher 5-year mortality among patients with ischaemic ulcers than among those with neuropathic ulcers (56 vs 45%; $P = 0.01$). When adjusting for older age at presentation of ischaemic ulcers in a multinominal regression analysis, however, only age was found to predict mortality and was considered to be a confounding factor. In the present study, the prevalence of peripheral vascular disease at the time of amputation was high, did not differ between groups and was not associated with the difference seen in survival rate.

In a large meta-analysis comparing mortality between people with diabetes either with or without a history of foot ulceration, the presence of the latter was associated with an increased risk of all-cause mortality (RR 1.89, 95% CI 1.60–2.23) [3] and the number of cardiovascular deaths was greater among patients with foot ulcers; however, the proportions of people with cardiovascular mortality were similar between the groups, implying that the excess cardiovascular risk observed in patients with diabetes and foot

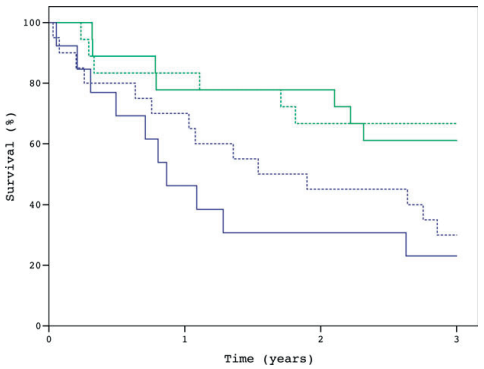

FIGURE 2 Kaplan–Meier survival curve showing 3-year mortality in patients grouped according to HbA_{1c} levels and presence of heart-rate corrected QT (QTc) interval prolongation (defined as QTc time > 440 ms). $P = 0.017$ for pooled comparison. For separate comparisons: patients with normal QTc time and HbA_{1c} < 58 mmol/mol (7.5%; green solid line) compared with those with normal QTc time and HbA_{1c} ≥ 58 mmol/mol (7.5%; green dotted line), $P = 0.839$. Patients with QTc time > 440 ms and HbA_{1c} < 58 mmol/mol (7.5%; blue solid line) compared with those with prolonged QTc time and HbA_{1c} ≥ 58 mmol/mol (7.5%; blue dotted line), $P = 0.420$.

ulcers only partly accounts for the higher mortality rate and that other factors play a role in the high mortality seen in these patients. Such a factor might be the presence/absence of neuropathy. Carrington *et al.* [23] have shown increased mortality rates in people with diabetes, microvascular disease and neuropathy and suggested that lower motor nerve conduction velocity, lower transcutaneous oxygen pressure ($TcPO_2$) levels and higher mortality rates are caused by similar aetiological mechanisms. Further, a pooled analysis of 15 studies including 2900 people with diabetes has associated the presence of cardiac autonomic neuropathy, an often over-looked complication that is frequently present in people with foot ulcers, with increased mortality risk [24]. One plausible manifestation of cardiac autonomic neuropathy is prolonged QTc time and, despite several limitations as a diagnostic marker for autonomic failure [25], QTc prolongation is an established risk factor for mortality in people with and without diabetes [13,14,26,27]. In a recently published study of 214 patients with Type 2 diabetes and chronic foot ulcers, QTc prolongation was an important prognostic marker for mortality [12]. Whether QTc prolongation has any impact as a prognostic marker in the very high-risk group of people with diabetes facing an above-ankle amputation has to our knowledge previously not been reported. The novel results of the present study indicate that prolonged QTc time is associated with higher all-cause mortality also in this high-risk population. In the present study population, consisting of patients with Type 2 diabetes with a median age of 72 years, 73% of the patients with prolonged QTc time at baseline died within a 3-year follow-up period after major amputation, as compared with 36% of those with normal QTc time. Patients with prolonged QTc time had a higher prevalence of myocardial infarction, but this factor was not found to be a confounding factor. Furthermore, an eGFR < 60 ml/min/1.73 m^2 did not seem to influence mortality in the present population, a result that contrasts with previously reported outcomes, but that might be explained by a more preserved renal function in the present study population as compared with those in the studies by Tentolouris *et al.* [7] and Lavery *et al.* [9] evaluating survival after minor and major amputation. A total of 34.7% of the present overall study population had an eGFR < 60 ml/min corresponding to 49.6% in the Lavery *et al.* population. In addition, above-ankle amputations were more commonly present in patients with chronic kidney disease in the Lavery *et al.* study, which might have influenced the outcome. QTc time was not included as a variable in the aforementioned two studies.

In the present study, the presence of a QTc time > 440 ms was associated with a 2.20-fold higher 3-year mortality risk after major amputation, a result similar to our previously reported study of people with chronic diabetic foot ulcers [12]. The prevalence of prolonged QTc interval was higher in the present study population of patients undergoing major amputation (51.4%), compared with the previous study of people with chronic diabetic foot ulcers (36.4%), which might reflect a worse autonomic function among patients undergoing amputation.

The highest 3-year mortality rate was found in patients within the highest QTc quartile, indicating a progressively increasing relationship between QTc time and mortality. The lowest mortality was found in the second quartile and not in the first, indicating that this relationship might be J-shaped; however, the difference between quartile one and two did not reach statistical significance in our small study population. Previous studies evaluating the relationship between QT interval and mortality in the general population have had inconsistent results, with some studies showing a progressively increasing association [28,29], and others, a J- or U-shaped association [19,30]. One reason for these inconsistent results could be that the intervals used for evaluating the association between QT time and mortality are often wide and differ between studies, making it difficult to compare findings and to evaluate the exact dose–response relationship; however, despite these inconsistent results within the lower QTc range, there is substantial evidence supporting higher mortality among those with higher QTc durations [19,28–30].

In the previous study of people with chronic diabetic foot ulcers, the combination of prolonged QTc time and HbA_{1c} level < 58 mmol/mol (7.5%) was associated with a worse all-cause mortality [12]. In the present study, there was only a trend towards worse outcome among patients with a combination of a low HbA_{1c} level [< 58 mmol/mol (7.5%)] and QTc prolongation, but the

limited number of patients in each subgroup might have influenced this result and larger studies are needed to confirm this finding.

Two unexpected results were found; one was the difference in prescription of metformin between patients with and without prolonged QTc time, despite similar baseline characteristics including renal function and diabetes duration, another was that metformin treatment was independently associated with a higher survival rate. Caution should be taken when drawing conclusions, however, as the numbers are very low. Several explanations for these findings are possible. Metformin might have been prescribed to healthier patients, although we could not identify any differences between users and non-users, or patients on metformin might have had fewer hypoglycaemic episodes, thereby preventing prolongation of the QT interval. Whether metformin treatment has any direct impact on myocardial de- and repolarization and thereby influences the QT interval is not known.

The present study has some strengths and limitations that should be noted. Although the number of people in the study was limited, all patients who underwent a major amputation at our two hospitals during the inclusion period were identified and screened. Mortality data are correct, as all deaths are registered in the national death registry. Nevertheless, as autopsies are rarely performed in Sweden and a significant proportion of our patients died outside a hospital, data on cause of death are less robust. Consequently, we have chosen to evaluate all-cause mortality only. Furthermore, important explanatory variables such as detailed information on autonomic nervous function and frequencies of hypoglycaemia were often not identified. The lack of data on autonomic nervous function and cause of death limits us to speculation only about any relationship between these factors and QTc prolongation.

The present study was powered to identify QTc prolongation as a plausible risk factor for mortality but underpowered to allow further subgroup analyses, including the impact of different HbA_{1c} levels on mortality in the high-risk group of patients with long QTc time. Several drugs may have a possible QTc-prolonging action (e.g. sotalol, tricyclic antidepressants, selective serotonin reuptake inhibitors, macrolides and quinolones), but we could not identify any significant difference in prescription patterns between groups.

This is the first study evaluating the impact of QTc time on survival in people with Type 2 diabetes undergoing major amputation. Within the limitations of the present study design, our novel finding suggests that QTc prolongation is also associated with a considerably higher risk of all-cause mortality in this high-risk population. Whether this higher mortality rate is a manifestation of cardiac autonomic neuropathy, or whether prolonged QTc time is a marker of another known or unknown condition that increases the mortality rate, remains to be established.

Funding sources

This study was supported by the Swedish Diabetes Foundation, Sydvästra Skånes Diabetesförening, the Krapperup Foundation, the Crafoord Foundation, Skåne county council's Research and Development Foundation and the Faculty of Medicine, Lund University, Sweden.

Competing interests

None declared.

Acknowledgements

We are grateful to statistician Helene Jacobsson, Department of Biomedical Statistics and Epidemiology, Centre of R&D Skåne, Region Skåne, Sweden, for valuable statistical advice.

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Paper III

Journal of Diabetes and Its Complications 29 (2015) 1198–1202

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Hyperbaric oxygen therapy reduces the risk of QTc interval prolongation in patients with diabetes and hard-to-heal foot ulcers

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ARTICLE INFO

Article history:
Received 28 April 2015
Received in revised form 23 July 2015
Accepted 24 July 2015
Available online 29 July 2015

Keywords:
Diabetes mellitus
Foot ulcer
Diabetic autonomic neuropathy
Hyperbaric oxygen therapy
Diabetes complications

ABSTRACT

Aims: Heart rate corrected QT (QTc) interval prolongation is a risk factor associated with increased mortality. Hyperbaric oxygen therapy (HBO) has previously been shown to have acute beneficial effects on QTc dispersion. The aim of this study was to evaluate long-term effects of HBO on QTc time in diabetic patients with hard-to-heal foot ulcers.
Methods: In a prospective, double-blinded placebo-controlled study, patients were randomized to 40 treatment sessions with either HBO or air (placebo), at 2.5 ATA. Patients fulfilling >35 completed treatment sessions were included in the evaluation.
Results: Of the initial 75 patients (38 HBO/37 placebo), two were excluded due to pacemaker use. Baseline characteristics were similar between groups. At the 2-year follow-up, QTc time was significantly shorter in the HBO compared to the placebo group (438 vs. 453 ms, $p < 0.05$). Further, fewer HBO treated patients had a QTc time >450 ms (22 vs. 53 %, $p < 0.02$). This difference seemed to be caused by a significant prolongation of the QTc interval in the placebo group (427 (419–459) at baseline vs. 456 ms (424–469) after 2 years), whereas no significant change was seen in HBO treated patients.
Conclusions: HBO treatment might protect against QTc prolongation in this high-risk diabetic population.

Introduction

Diabetic foot ulcers (DFU) are a major health problem and an important risk factor for morbidity and mortality among people with diabetes mellitus (Brownrigg et al., 2012). This excess mortality has in part, been explained by an increased burden of traditional cardiovascular risk factors, such as hypertension, myocardial infarction, heart failure, renal dysfunction and smoking. However, these risk factors cannot fully explain this excess mortality risk, and lately studies have demonstrated an association between heart rate corrected QT interval (QTc) and all-cause and cardiovascular mortality in the general population (Goldberg et al., 1991; Montanez, Ruskin, Hebert, Lamas, & Hennekens, 2004) as well as in people with diabetes (Christensen et al., 2000; Cox et al., 2014; Fagher & Londahl, 2013). Several factors, i.e. hereditary disorders, coronary heart disease, cardiac autonomic neuropathy and microvascular disease, may all contribute to QTc prolongation (Dekker, Schouten, Klootwijk, Pool, & Kromhout, 1994; Festa, D'Agostino, Rautaharju, Mykkanen, & Haffner, 2000; Macfarlane, McLaughlin, Devine, & Yang, 1994).

Systemic hyperbaric oxygen therapy (HBO) is a medical treatment for hard-to-heal diabetic foot ulcers that has been demonstrated to increase oxygenation in hypoxic tissue and enhance microvascular function (Faglia et al., 1996; Game et al., 2012; Kalani, Jorneskog, Naderi, Lind, & Brismar, 2002; Londahl, Katzman, Nilsson, & Hammarlund, 2010). Further, HBO may also have acute beneficial effects on QT dispersion, as demonstrated in a study by Kardesoglu et al. (2008), and this might have a protective effect on the risk for ventricular arrhythmias and sudden death. Whether HBO therapy has any long-term effect on QTc time has to our knowledge previously not been studied. The aim of this study was to evaluate long-term effects of HBO on QTc time in diabetic patients with multiple complications.

Materials and methods

Design and procedures of the randomized, double-blinded, placebo-controlled Hyperbaric Oxygen Treatment in Diabetic Patients with Chronic Foot Ulcers (HODFU) study evaluating effects of HBO in patients with chronic diabetic foot ulcers, have been reported in detail previously (Londahl et al., 2006; Londahl et al., 2010). The outcomes for the group receiving HBO were compared with those of the group receiving treatment with hyperbaric air (placebo). The study was performed in an

Conflict of interest statement: All authors declare there is no duality of interest associated with this manuscript.

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http://dx.doi.org/10.1016/j.jdiacomp.2015.07.023
1056-8727/

ambulatory setting and study treatment was given as an adjunct treatment at a multi disciplinary foot clinic.

Patients provided signed informed consent before any study related procedure was performed. The study was approved by the Ethics Committee, Lund University, and was conducted according the Declaration of Helsinki. It is registered on clinicaltrials.gov, registration number NCT00953186.

All participants in the HODFU study were diabetes patients with at least one foot-ulcer below the ankle, with duration of at least 3 months, treated at a multidisciplinary foot clinic.

Before inclusion patients were evaluated regarding the possibility for vascular surgical intervention and only patients with adequate peripheral circulation or patients without reconstructable peripheral arterial disease were included. Exclusion criteria were presence of contraindication for HBO treatment (Londahl et al., 2006; Londahl et al., 2010), (i.e., severe pulmonary disease, untreated thyrotoxicosis, pneumothorax, ongoing treatment with cisplatin, doxorubicin or disulfiram, women in fertile age not receiving adequate anti-conception therapy) or patients with known malignancy, a history of stroke or myocardial infarction within 30 days, misuse of alcohol or other drugs, acute infection (C-reactive protein >30 mg/l), declined general condition and patients participating in other clinical trials.

Patients were randomized to 40 treatment sessions (90 minutes long, five days a week for eight weeks) with either oxygen or air, at 2.5 absolute atmospheres (ATA) in a hyperbaric chamber. Randomization was stratified according to arterial toe blood pressure ≤ 35 mmHg/>35 mmHg and done in blocks of ten using sealed and numbered opaque envelopes.

All HODFU study participants fulfilling the per-protocol requirement of at least 36 completed study treatment sessions were included for analysis. Patients with a ventricular stimulating pacemaker, making it impossible to calculate a correct QTc interval, were excluded from the present ECG evaluation study.

All patients were evaluated with standard 12-lead resting ECG, using a Siemens ECG machine (Siemens Elema, Solna, Sweden) before randomization and at two-year follow-up visit. The QT interval, defined as the time between the beginning of the Q wave and the end of the T-wave represents the duration of the electrical depolarization and repolarization of the ventricular walls of the myocardium (Rautaharju et al., 2009). Depending on the ECG configuration the end of the T-wave was defined as either the point where the downslope of the T wave cross the isoelectric line or the onset of the U-wave in presence of a T-U junction or the onset of the P-wave in presence of a T-P junction. It was calculated using the validated Sicard 440/740 ECG computer-analysis program (Megacart version 3 V4, 7/2.38/23; Siemens Elema) (Macfarlane et al., 1990; Willems et al., 1991), and was then corrected for heart rate using Bazett's formula to get the QTc interval. In this study QTc prolongation was defined as a QTc value >440 milliseconds (ms) (Algra, Tijssen, Roelandt, Pool, & Lubsen, 1993; Tentolouris et al., 1997). We also evaluated the cut-point of 450 ms, since this alternative level also is used in the literature (Cox et al., 2014; Rautaharju et al., 2009). Two researchers independently evaluated all ECGs in a blinded manner.

Laboratory data were analyzed at the local certified laboratory at Helsingborg hospital. Hyperlipidemia was defined as total cholesterol >5.0 mmol/l, LDL-cholesterol >2.5 mmol/l or on-going prescription of a cholesterol-lowering drug. Estimated glomerular filtration rate (eGFR) was calculated from plasma creatinine level using the modification of diet in renal disease (MDRD) equation (Levey et al., 2006). eGFR <60 ml min^{-1} 1.73 m^{-2} was considered to indicate renal impairment. Nephropathy was considered present in case of a Urine to Albumine Ratio (UACR) >30 mg/g (or 3,5 mg/mmol) on 2 separate occasions. Hypertension was defined as systolic blood pressure ≥140, a diastolic blood pressure ≥90 or prescription of a blood pressure-lowering drug.

Vital statuses of all participants were determined from the Swedish National Death Registry.

Statistical analysis

Statistical analyses were performed using SPSS (IBM, IL, USA) version 20. Continuous data are given as median and interquartile range (IQR) and categorical variables as percentages. To assess differences between groups Mann-Whitney U-test were used for continuous variables and Chi-squared test were used for categorical data. For paired comparisons before and after treatment Wilcoxon's signed rank test (continuous variables) and Mc Nemar's test (categorical variables) were performed. A two-tailed p-value <0.05 was considered as statistical significant.

Results

A total number of 75 patients fulfilled at least 36 completed treatment sessions and were included in the study (Fig. 1); 38 were randomized to HBO and 37 to placebo. Two patients were excluded due to pacemaker use. Baseline characteristics of the remaining 73 patients (38 HBO, 35 placebo), with a median age of 70 (61–77) years, are given in Table 1. There were no differences in traditionally cardiovascular risk factors between groups at baseline and QTc time was similar in patients randomized to HBO (426 (410–440) ms) and placebo (426 (419–458)). Further, no difference was found in prescription pattern of drugs with hypoglycemic action or drugs with known QTc prolonging effect between groups. After two years, follow-up data from the 61 remaining patients were evaluated (Fig. 1). By then, QTc time was significantly shorter in the HBO group as compared to the placebo group, 438 (425–453) vs. 456 (424–469) ms, $p < 0.05$. This difference seemed to be caused by a significant prolongation of the QTc interval in the placebo group (427 ms (419–459) at baseline vs. 456 ms (424–469) after 2 years, whereas no significant change was seen in HBO treated patients (426 ms (412–439) vs. 438 ms (425–453) (Fig. 2).

The proportion of patients with prolonged QTc interval >440 ms and >450 ms were lower two years after HBO (Table 2A) compared to placebo (Table 2B).

Discussion

People with diabetes are at a higher risk for cardiovascular disease and all-cause mortality compared to a general population and this risk is even higher among patients with a history of a DFU (Brownrigg et al., 2012). However, this excess risk cannot fully be explained by a higher prevalence of cardiovascular disease (CVD) or known CVD risk factors (Regidor et al., 2012). Lately, several studies have indicated the importance of long QTc time, a well-known predictor for malignant arrhythmias and death, as a predictor of all-cause mortality in patients with diabetes (Cox et al., 2014; Fagher & Londahl, 2013). QTc prolongation is more frequently found in patients with diabetes than in non-diabetic patients (Festa et al., 2000). The mechanism behind the higher prevalence of prolonged QTc time among people with diabetes is not fully understood, but possible contributors could be a higher incidence of myocardial infarctions as well as presence of cardiac autonomic neuropathy, factors known to be associated with QTc prolongation (Festa et al., 2000; Tentolouris et al., 1997). Further, it is also known that QT interval is prolonged during hypoglycemia (Landstedt-Hallin, Englund, Adamson, & Lins, 1999).

Early intervention with improved metabolic control reduces the risk of developing microvascular complications, including neuropathy (American, 2014; UKPDS, 1998), but in patients with manifest neuronal dysfunction there is to our knowledge no effective method or treatment to enhance nervous function.

HBO is a therapy used in the management of chronic diabetic foot ulcer management to increase tissue oxygenation and improve wound healing (Faglia et al., 1996; Kalani et al., 2002; Londahl et al.,

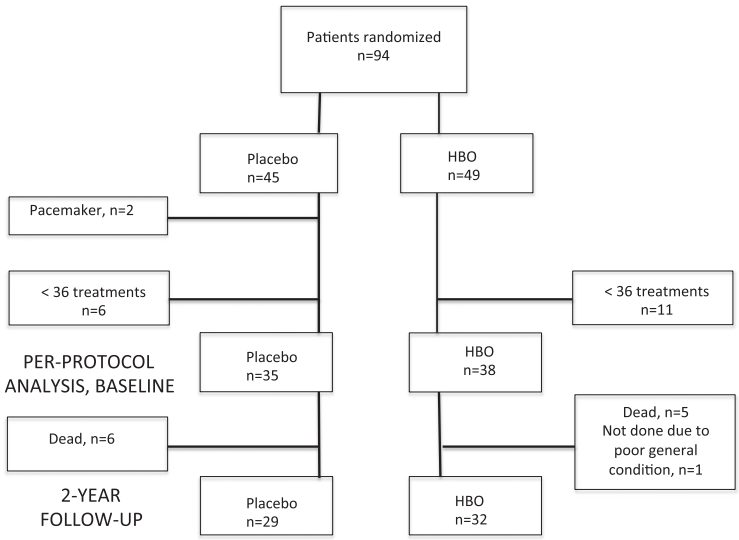

Fig. 1. Flow chart of patients included in the HODFU study at start and number of patients included in the per-protocol analysis at baseline and at 2-year follow-up.

2010). Our results indicate that HBO therapy might have a protective long-term effect on QTc prolongation.

Table 1
Baseline characteristics and medical treatment of patients fullfilling at least 36 out of 40 HBO/placebo treatment sessions. Categorical data are given as percentages and continuous data as median and interquartil range. p Values <0.1 are given as figures, otherwise n.s. is stated.

Baseline characteristics	HBO	Placebo	p Value
	n = 38	n = 35	
Age (years)	67 (55–75)	71 (64–79)	n.s
Sex male/female (%)	76/24	89/11	n.s
Diabetes type 1/2 (%)	26/74	29/71	n.s
Creatinine (μmol/l)	87(67–140)	102 (77–141)	n.s
eGFR (ml min^{-1} 1.73 m^{-2})	66 (41–99)	63 (40–82)	n.s
eGFR <60 ml min^{-1} 1.73 m^{-2} (%)	39	40	n.s
QTc time (milliseconds)	426 (410–440)	426 (419–458)	n.s
Diabetes duration (years)	23 (10–33)	20 (16–36)	n.s
HbA1c (%)	7.9 (6.9–8.9)	8.1 (7.0–9.1)	n.s
HbA1c (mmol/mol)	63 (52–74)	65 (53–76)	
Coronary/ congestive heart disease (%)	40	51	n.s
Stroke (%)	16	14	n.s
Atrial fibrillation (%)	21	34	n.s
Hypertension (%)	76	74	n.s
Hyperlipidemia (%)	87	89	n.s
Nephropathy (%)	89	83	n.s
Dialysis (%)	5	3	n.s
Smoking (ever) (%)	68	69	n.s
Ankle brachial index	0.76 (0.57–1.13)	0.79 (0.62–1.01)	n.s
Toe blood pressure (mmHg)	50 (30–85)	52 (30–75)	n.s
Toe blood pressure <35 mmHg (%)	30	33	n.s
TCPO2 (mmHg)	45 (32–55)	53 (39–70)	n.s
Medical Treatment (%)			
Insulin	90	91	n.s
Metformin	13	14	n.s
Sulfonylurea	16	20	n.s
Quinolones	8	9	n.s
Statins	66	66	n.s
Beta-blockers	34	43	n.s

We know from earlier studies that neuronal regulation of the heart progressively decreases with age, a process that seems to be aggravated in patients with diabetes, plausibly due to autonomic neuropathy (Kuo et al., 1999; Macfarlane et al., 1994; Mangoni, Kinirons, Swift, & Jackson, 2003; Masaoka, Lev-Ran, Hill, Vakil, & Hon, 1985). A few previous studies have indicated that HBO could have acute beneficial effects on autonomic nerve function. Kardesoglu et al. demonstrated in a small study of 30 patients with diabetes undergoing HBO therapy for non-healing foot ulcers a significant

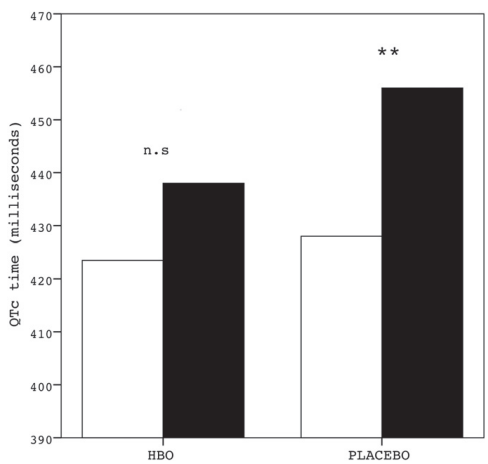

Fig. 2. Median QTc-time at baseline (white bars) and at two-year follow-up (black bars) in patients treated with hyperbaric oxygen therapy and placebo, respectively. ** $p < 0.01$.

Table 2A
ECG evaluation before and 2 years after treatment with HBO. Categorical data are given as percentages and continuous data as median and interquartile range. p Values <0.1 are given as figures, otherwise n.s. is stated.

HBO group, n = 32	Baseline	2 year follow-up	p-value
QTc time (milliseconds)	424 (411–438)	438 (425–453)	n.s
Heart rate (beats/min)	78 (67–88)	75 (66–85)	n.s
QTc >440 ms (%)	16	38	0.065
QTc >450 ms (%)	16	25	n.s

decrease in QTc dispersion after 10 HBO sessions (2 weeks), although mean QTc time did not change significantly (Kardesoglu et al., 2008). Further, in a prospective study by Sun et al., evaluating heart rate variability in patients with diabetic foot ulcers undergoing HBO therapy, there was an increase in heart rate variability after 4 weeks of HBO therapy, compared to an age-, sex- and disease-matched control group not receiving HBO therapy, and they suggested that HBO might enhance the sympathovagal balance of the heart (Sun, Yang, & Kuo, 2006). However, Sun et al. did not evaluate QTc time. Further, Lund et al. suggested in a study of 10 healthy volunteers, that HBO treatment immediately influences and increases parasympathetic tone (Lund et al., 1999), with a significant increase in respiratory arrhythmia during HBO. They could however not differentiate whether the effect was caused by oxygen therapy or pressure in this small study, nor did they analyze QTc time. In a Cochrane review from 2011 evaluating effects of adjunctive HBOT in the treatment of acute coronary syndrome, there was some evidence found from small trials to suggest that HBO is associated with a reduction in the risk of death, the volume of damaged muscle, the risk of major adverse coronary events and time to relief from ischaemic pain. However, the number of studies and patients included were small and the author's conclusion was that routine application of HBOT to these patients cannot be justified from this review (Bennett, Lehm, & Jepson, 2011). Our prospective randomized double-blinded placebo-controlled study, evaluating long-term effects of HBO therapy compared to hyperbaric air in patients with diabetes and hard-to-heal foot ulcers indicates that HBO treatment might attenuate QTc interval prolongation. Two years after treatment fewer patients in the HBO, compared to the placebo group, had a prolonged QTc interval and this difference seemed to be related to a progress in QTc time in the placebo group. This prolongation was not seen in patients receiving HBO.

We can only speculate on the mechanisms behind our findings. The etiology of neuropathy in diabetes is not fully understood, but there is strong evidence that impaired blood flow and tissue hypoxia plays a major role in developing neuronal dysfunction (Cameron, Eaton, Cotter, & Tesfaye, 2001). The fact that microvascular changes are involved in diabetic neuropathy was first recognized in a human biopsy study by Fagerberg in 1959, demonstrating structural changes such as thickening and hyalinization of the small interneuronal vessels walls from people with neuropathy (Fagerberg, 1959). These changes, caused by capillary basal membrane thickening, pericyte degeneration and endothelial cell hyperplasia, were in a study by Malik et al. markedly increased in nerve capillaries compared to skin capillaries and did strongly correlate with the degree of clinical signs (Malik et al., 1989).

Table 2B
Electrogardiographic data before and after treatment with placebo. Categorical data are given as percentages and continuous data as median and interquartil range. p Values <0.1 are given as figures, otherwise n.s. is stated.

Placebo group, n = 29	Baseline	2 year follow-up	p Value
QTc time (milliseconds)	426 (419–458)	456 (424–469)	0.010
Heart rate (beats/min)	74 (62–80)	73 (65–85)	n.s
QTc >440 ms (%)	31	59	0.008
QTc >450 ms (%)	28	52	0.016

The plausible importance of hypoxia as a major contributor to neuropathy can be implicated as non-diabetic patients with chronic hypoxia developing neuropathy with neuropathological changes similar to those seen in diabetes (Malik et al., 1990). In a study by Carrington et al., reduced motor nerve conduction velocity was both associated with decreased transcutaneous oxygen levels ($TcPO_2$) and increased mortality, suggesting a similar etiological mechanism between neuropathy and hypoxia (Carrington et al., 2002). Thus, improved microcirculation might preserve neural function, including cardiac autonomic function, thereby avoiding further detoriation of the QTc-time. Also, improved microcirculation in cardiac tissue might per se be involved.

Strengths and limitations

There are both strengths and limitations in our study. Despite the limited number of patients, our data are reasonably robust as the study is designed as a randomized double-blinded placebo controlled study. We can conclude that the effect is not related to the hyperbaric pressure per se, since there was a significant difference in progress of the QTc interval between the study groups. The low number of participants might enhance the influence of other potential causes of QTc prolongation on our results. However, baseline characteristics were similar and we did not find any difference in prescription patterns of common drugs with a known QTs prolonging action (for instance beta-blockers and quinolones) between groups. We cannot answer the question whether HBO has any beneficial effects on peripheral neuropathy, since we didn't collect quantitative data on this, but our study might provide a clinical basis for larger studies in the future to answer this question and to confirm our present findings.

Despite these limitations, our study is to our knowledge the first to demonstrate that HBO therapy might have a protective effect on long-term progression of the QTc interval among patients with diabetes.

Conclusions

In conclusion, HBO seems to have a protective effect on QTc prolongation in patients with diabetes and chronic foot ulcers.

Acknowledgement

The study was supported by the Thelma Zoega's Foundation, Swedish Diabetes Foundation, Sydvästra Skånes Diabetesförening, Krapperup Foundation, Skåne County Council's Research And Development Foundation and Faculty of Medicine (ALF), Lund University, Sweden.

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Paper IV

Transcutaneous oxygen pressure as a predictor for short-term survival in patients with type 2 diabetes and foot ulcers: a comparison with ankle-brachial index and toe blood pressure

Manuscript accepted for publication in Acta Diabetologica April 11th, 2018

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Abstract:

Aims: Ankle-brachial index (ABI) is the most commonly used test when diagnosing peripheral vascular disease and is considered a marker for cardiovascular risk. Transcutaneous oxygen pressure ($TcPO_2$), a test associated with microvascular function, has in several studies shown better correlation with diabetic foot ulcer (DFU) healing. Whether a low $TcPO_2$ could be a marker for mortality in the high-risk population of DFU patients has not been evaluated before. The aim of this study was to evaluate the predictive value of $TcPO_2$ in comparison with ABI and toe blood pressure (TBP) on 1-year mortality in type 2 diabetes patients with DFU.

Methods: Type 2 diabetes patients aged ≤ 90 years with one DFU who attended our multidisciplinary DFU-unit during year 2013-2015 and were screened with $TcPO_2$, ABI and TBP, were retrospectively evaluated. 1-year mortality was assessed from the national death register in Sweden.

Results: A total of 236 patients (30% women) with a median age of 76 (69-82) years were evaluated in this study. 14.8% of the patients died within 1 year. $TcPO_2 < 25$ mmHg was associated with a higher 1-year mortality compared with $TcPO_2 \geq 25$ mmHg (27.7% vs. 11.6%, p = 0.003). TBP and ABI did not significantly influence 1-year mortality. In a Cox regression analysis adjusted for confounders, $TcPO_2$ were independently predicting 1-year mortality with a hazard ratio (HR) for$TcPO_2 < 25$ mmHg of 2.8 (95% CI 1.34-5.91, p=0.006).

Conclusions: This study indicates that a low $TcPO_2$ is an independent prognostic marker for 1-year mortality among patients with type 2 diabetes and DFU.

Abbreviations

ABI Ankle-brachial Index

CVD Cardiovascular disease

DFU Diabetic foot ulcer

eGFR Estimated glomerular filtration rate

HR Hazard ratio

IWGDF The International Working Group on the Diabetic Foot

IQR Interquartile range

MACEs Major cardiovascular events

MDRD Modification of diet in renal disease

PVD Peripheral vascular disease

$TcPO_2$ Transcutaneous oxygen pressure

TBP Toe blood pressure

Introduction

Diabetic foot ulcers (DFU) are a common complication of diabetes mellitus, associated with both an increased risk of amputations in the lower limb and a higher risk of cardiovascular disease and death[1-3]. Peripheral vascular disease (PVD) is frequently contributing, together with peripheral neuropathy in the development of a DFU, and the presence of PVD is considered a predictor of worse outcome both for ulcer healing and survival[4, 5]. Ankle-brachial index (ABI) is the most commonly used test for the diagnosis of PVD and has also been associated with increased cardiovascular risk in the general population[6]. However, in patients with diabetes, both the diagnostic and the predictive value of ABI may be limited due to a high prevalence of false-negative values as a result of medial artery calcification[7-9]. Further, ABI does not reflect microvascular dysfunction, a condition often seen in patients with DFU. Transcutaneous oxygen pressure ($TcPO_2$) is a non-invasive method measuring tissue perfusion and is considered to better reflect the micro-vascular status in the skin. The International Working Group on the Diabetic Foot (IWGDF) recommend in their guideline document, urgent vascular imaging and if feasible revascularization when TBP < 30 mmHg or $TcPO_2$ < 25 mmHg, as patients with higher levels are more likely to heal their ulcers[10]. It has previously been demonstrated a plausible association between $TcPO_2$ and mortality in patients with diabetes, but without significant PVD, or ongoing

DFUs[11]. Also, in a study by Gazzaruso *et al*, a low $TcPO_2$ has been considered a risk factor for major cardiovascular events (MACEs) in patients with type 2 diabetes, but without a history of foot ulcer, or previous cardiovascular disease[12]. Whether a low $TcPO_2$ could be a marker for mortality in the high-risk population of DFU patients, has not been evaluated before. The aim of this study, was to evaluate the predictive value of $TcPO_2$, in comparison with ABI and TBP, on 1-year all-cause mortality in patients with type 2 diabetes and DFU.

Methods

For this study, we retrospectively enrolled patients with type 2 diabetes, aged ≤ 90 years with at least one DFU who visited our DFU-unit between year 2013-2015. All patients were examined with $TcPO_2$, ABI, and TBP, measured with Periflux System 5000, diagnostic instrument (Perimed AB, Stockholm Sweden). These non-invasive assessments are routine in all new referrals at our DFU-unit.

$TcPO_2$-measurements were performed at the dorsum of both feet, while patient was breathing ambient air, in a resting supine position at room temperature, between 21°C and 24° C. The site on the foot was carefully cleaned before the transducer was applied to the skin, using adhesive rings and contact liquid, supplied by the manufacturer. The measurement was performed after calibration and preheating of the transducer to approximate 44° C. Patients were stratified according to $TcPO_2$ < and ≥ 25 mmHg, and to evaluate mortality related to different $TcPO_2$ levels, patients were also grouped according to $TcPO_2$ quartiles.

The systolic ankle pressure and TBP were also evaluated during resting, in a supine position. Three measurements were performed on each foot and averaged. ABI was calculated by dividing the systolic ankle pressure with the systolic arm pressure, and ABI 0.9-1.3 was considered normal[13]. TBP was measured at the great toe, and TBP < 30, as well as < 50 mmHg, was used in the analyses. The lowest value (of ABI, TBP and $TcPO_2$) of the two legs was used in mortality analysis, while the value of the affected foot was used when analysing ulcer healing.

Baseline characteristics were assessed from patient medical records, and included age, gender, diabetes duration, as well as co-morbidities, such as history of hypertension, hyperlipidaemia, cardiovascular disease (CVD), as well as concomitant medication, and laboratory data (HbA_{1c}, LDL-cholesterol and creatinine). CVD was defined as verified myocardial infarction, angina pectoris, heart failure, or cerebral vascular disease. Hypertension was defined as blood pressure ≥ 140/90, or ongoing treatment with antihypertensive drugs. Hyperlipidaemia was defined as LDL >2.5 mmol/l, or the ongoing treatment with cholesterol-lowering drugs. Glomerular filtration rate (eGFR) was estimated from

plasma creatinine, using the modification of diet in renal disease (MDRD) equation[14].

All patients were treated according to international guidelines, at our multi-disciplinary diabetic foot clinic, and were evaluated for vascular intervention when indicated. Ulcer healing, defined as complete epithelialisation within 12 weeks, and above-ankle amputation during follow-up were assessed from patient charts. Mortality data were obtained from the National Death Registry of Sweden. Approval of the study was given by the Ethics Committee in Lund, Sweden.

Statistical analysis:

Continuous data are expressed as median and interquartile ranges (IQR; 25-75 percentile), and to assess differences Mann-Whitney U tests were performed. Categorical data are expressed as percentages, and Fisher´s exact test was used to compare differences. Survival analyses were performed with Kaplan-Meier estimates, and significances calculated with log-rank tests. A Cox regression analysis was performed to adjust for confounding factors. Those factors with a significant ($p < 0.05$), or nearly significant ($p < 0.1$) difference between groups at baseline, as well as those with a significant association with mortality in the univariate analysis, were stepwise entered in a multivariate Cox model. Only those variables that changed the p-value of our variables of interest, or significantly predicted mortality, were kept in the final model. The results of the Cox analysis are given as Hazard ratios (HRs), with 95% confidence interval (CI). All statistical analyses were performed using SPSS program (IBM, version 22). A two-sided p-value < 0.05 was taken as statistical significant.

Results

We enrolled 236 type 2 diabetes patients with DFU, with a median age of 76 years (69-82), visiting our multidisciplinary diabetic foot clinic between year 2013 and 2015. A total number of 47 patients had a baseline $TcPO_2 < 25$ mmHg, and among them, the probability for healing was low and only 8.8% successfully healed their ulcers after 12 weeks. Further, these patients suffered an increased risk of above-ankle amputations (23.4% vs. 4.2% in $TcPO_2 \geq 25$ mmHg, $p = 0.001$). Minor amputation, or auto-amputation was performed in 17 (9.0 %) patients during follow-up in the group with TcPO2 ≥ 25 mmHg group, compared to 6 (12.8%) in the TcPO2 < 25 mmHg group (n.s). Baseline characteristics, as well as healing, and major amputation rates of the study population, and of patients stratified by $TcPO_2 <$ and ≥ 25 mmHg, are given in table 1.

Table 1.
Baseline characteristics of all patients and patients stratified according to $TcPO_2$<25 mmHg.

	All patients	**$TcPO_2$ < 25 mmHg**	**$TcPO_2$ ≥ 25 mmHg**	***p*-value**
n	236	47	189	
Age (years)	76 (69-82)	74 (68-81)	77 (69-82)	n.s
Sex (male/female %)	69.9/30.1	57.4/42.6	73.0/27.0	0.050
Diabetes duration (years)	15 (8-23)	18 (8-26)	15 (8-22)	n.s
HbA1c (mmol/mol)	59 (50-70)	60 (48-75)	59 (49-68)	n.s
HbA1c (%) (DCCT)	7.5 (6.7-8.6)	7.6 (6.5-9.0)	7.5 (6.6-8.4)	n.s
eGFR (ml min^{-1} $1.73m^2$)	60 (42-81)	54 (38-68)	63 (43-87)	0.009
Previous CVD	65.3	70.2	64.0	n.s
Antidiabetic treatment -Insulin (%) -Sulphonylurea (%) -Incretins (%) -SGLT2 inhibitors (%) -Metformin (%)	 69.8 10.6 7.2 0.9 38.0	 78.3 6.4 10.6 0.0 34.0	 67.7 11.7 6.4 1.1 39.0	 n.s n.s n.s n.s n.s
ACEI/ARBs (%)	66.1	70.2	65.1	n.s
Hypertension (%)	94.5	93.6	94.7	n.s
Hyperlipidemia (%)	87.1	86.4	87.3	n.s
Smoking (ever) (%)	26.2	35.0	24.1	n.s
ABI	0.9 (0.6-1.1)	0.7 (0.5-1.0)	0.9 (0.6-1.1)	0.016
TBP (mmHg)	65 (45-93)	45 (26-79)	70 (49-100)	<0.001
TcPO2 (mmHg)	41 (28-52)	11 (5-22)	44 (37-54)	
Ulcer healed within 3 months (%)	23.1	8.8	25.5	0.045
Revascularisation during follow up (%) Vascular intervention not possible (%)	16.1 9.3	25.5 21.3	13.8 6.3	0.073 <0.001
Above-ankle amputation during follow-up (%)	8.1	23.4	4.2	<0.001

Data are expressed as percentages (%) or median ±IQR.
p-value > 0.1 is expressed as n.s

ABI < 0.9 or > 1.3 was significantly associated with worse ulcer outcome (12-week healing rate of 15.7% compared to 32.7%, in patients with normal ABI, p = 0.004), but no significant association between ulcer healing and TBP was found (both < 30 mmHg and < 50 mmHg analysed). Within the population of patients with $TcPO_2$ < 25 mmHg, the revascularisation rate during follow up was 25.5%. Additional 21.3% were vascular assessed, but intervention was not manageable, a decision made by a vascular surgeon. The remaining 53.2% (n = 25) of patients in $TcPO_2$ < 25 mmHg was separately evaluated concerning plausible reasons why vascular diagnostics, or revascularisation were not performed (Table 2).

Table 2.

Identified possible causes why revascularisation, angiography or vascular assessment by a vascular surgeon was not performed within patients with $TcPO_2$ <25 mmHg (n=25).

Identified possible causes	Patients (n)
Performed vascular imaging, but were not evaluated further.	5
Healed ulcer	8
Severe renal impairment	4
Severe comorbidity	1
Patients died before evaluation	1
Unknown reasons	6

After 1-year of follow-up, 35 patients were deceased (14.8%). Patients who died within 1 year were significantly older (81 (75-84) vs. 75 (68-82) years, p = 0.005), and had worse renal function, compared to survivors (eGFR 50 (26-66) vs. 63 (46-83) ml min^{-1} $1.73m^2$, p = 0.002). $TcPO_2$ < 25 mmHg was significantly associated with a higher 1-year mortality rate (27.7% vs. 11.6%, p = 0.003) as demonstrated in figure 1a. The impact of different $TcPO_2$ levels on mortality is illustrated in figure 2. There was a not significant trend (p = 0.061) in the Kaplan-Meier analysis of worse survival rates in patients with TBP < 30 mmHg (figure 1b). ABI < 0.9 or > 1.3 was not linked to a higher mortality rate (figure 1c). There was a trend towards better 1-year survival among patients with $TcPO_2$ < 25 mmHg who underwent a revascularisation procedure (91.7% vs. 65.7% in patients not re-vascularised, p = 0.094).

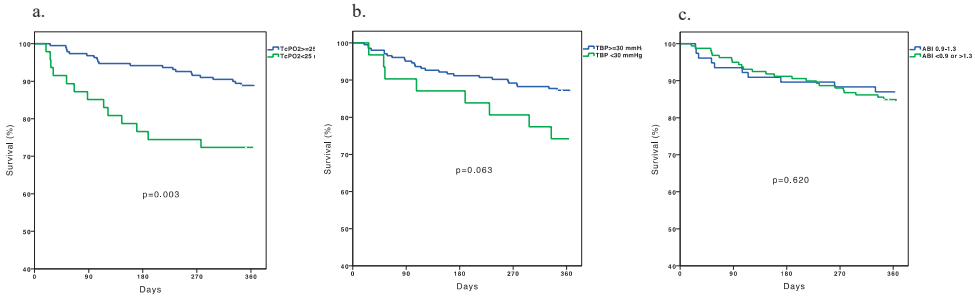

Figure 1 a-c
Kaplan-Meier survival curve with Log rank test in DFU patients grouped according to:

A. $TcPO_2$ < 25 mmHg (green) and ≥ 25 mmHg (blue) p=0.003
B. TBP < 30 mmHg (green) and ≥ 30 mmHg (blue), p=0.063
C. ABI 0.9-1.3 (green) and <0.9 or >1.3 (blue), p=0.620

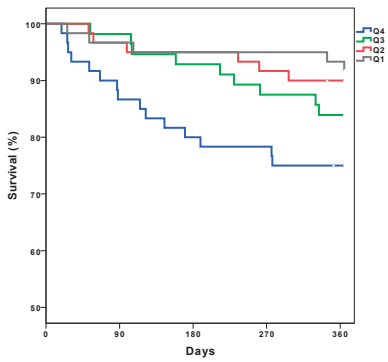

Figure 2.
One-year mortality (%) in patients divided into different quartiles of TcPO2 and HR calculated for each quartile.
Q1 = reference (grey): ≥52 mmHg, Q2 (red): 41-51 mmHg, Q3 (green): 29-40 mmHg, Q4: (blue) ≤28 mmHg
Pooled comparison (log rank) p=0.032.
For separate comparisons:
Q1 vs Q4: HR 3.4 (1.2-9.4), p=0.018. Q1 vs Q3: HR 2.0 (0.67-5.9), ns. Q1 vs Q2: HR 1.2 (0.4-4.0), ns.

To adjust for confounders, a Cox proportional analysis was performed. The following factors (age, gender, eGFR, three months ulcer healing, revascularisation, and above-ankle amputation) that either differed, or nearly differed between groups at baseline, or were significantly associated with mortality in a univariate analysis were entered stepwise into a multivariate Cox model, together with our variables of interest. Of these plausible confounding factors, only age and renal function (eGFR)

were significantly predicting mortality, and thus kept in the final model together with $TcPO_2$ and TBP. ABI, revascularisation, or ulcer healing at 3 months, did not predict one-year mortality or were significantly confounders, and consequently not entered in the final Cox model. There were a few cases of missing data and these were consequently censored in the Cox analysis (n = 2 in ulcer healing at 3 months and n = 2 in eGFR). The result of the final multivariate Cox analysis is given in table 3, and as shown, a $TcPO_2$ < 25 mmHg was an independent predictor for 1-year mortality with a HR of 2.8 (95% CI 1.34-5.91, p = 0.006). Also, when analysing $TcPO_2$ as a continuous variable, a significant association between increased survival with each mmHg increasing $TcPO_2$ level was found. TBP was not an independent predictor for mortality.

Table 3.
Results of the Cox regression models with TcPO2 and TBP analyzed first as continuous and then as dichotomized variables.

	HR (95%CI)	95% CI	p-value
TcPO2 (continous)*	0.979	0.959-0.999	0.039
TcPO2 < 25 mmHg*	2.814	1.341-5.905	0.006
Age *	1.061	1.010-1.115	0.018
eGFR *	0.984	0.969-0.999	0.034
TBP (continous)*	1.001	0.989-1.012	n.s
TBP < 30 mmHg*	1.240	0.526-2.923	n.s
ABI (continous)	0.623	0.248-1.568	n.s
ABI <0.9 or >1.3	0.831	0.399-1.730	n.s

* = variables entered in the final multivariate model.

Discussion

$TcPO_2$ has in several studies been associated with ulcer healing, and has in previously published studies been associated with cardiovascular events and mortality in patients with type 2 diabetes, but without DFU and/or a history of previous CVD[11, 12, 15-17]. Our study, is the first to demonstrate that $TcPO_2$ also may be a predictor for mortality in the high-risk population of patients with DFUs, with a 2.8-folded increase in 1-year mortality among patients with $TcPO_2$ < 25 mmHg. We demonstrated a continuous relation between $TcPO_2$ levels and survival, with increasing survival for each mmHg higher $TcPO_2$. Today, the current consensus among experts is that ulcer healing more likely occurs when $TcPO_2 \geq 25$ mmHg and TBP ≥ 45 mmHg, and that urgent vascular imaging should be considered

when $TcPO_2$ < 25 mmHg and TBP < 30 mmHg[10]. In our study, 91.8 % of our patients with baseline $TcPO_2$ < 25 mm Hg failed to heal their ulcers in 3 months, a result similar with others. In the study by Pecoraro *et al* there was a 39-fold increase in healing failure if $TcPO_2$ was < 20 mmHg[18]. Kalani *et al* evaluated the threshold of $TcPO_2$ < 25 mmHg, and found impaired healing in 31 out of 34 ulcers (91.2%), compared to improvement in ulcer area in 34 of 37 patients with $TcPO_2$ ≥ 25 mmHg[15]. In the Kalani study, ulcer improvement seemed to be more prevalent, compared with our results, but as they evaluated improvement (defined as a decrease in ulcer area of 25%) after 12 months, instead of complete epithelization after 3 months, as we did, it is difficult to compare these results with ours[15]. In another study, evaluating hyperbaric oxygen therapy and wound healing, no ulcer healed when $TcPO_2$ was < 25 mmHg[16].

We observed a high rate of above-ankle amputations (23.4%) in the group of people with $TcPO_2$ < 25 mmHg (median $TcPO_2$ 11 (5-22) mmHg). Faglia *et al* reported major amputation rates of 9.8% in their entire cohort, consisting of patients with PVD (defined as ankle-pressure < 70 mmHg, TcPO2 < 50 mmHg, or obstruction identified at duplex scanning). In their study, the prediction probability of above-ankle amputation correlated to the level of $TcPO_2$ (after revascularisation), with a probability of 2.6, 6.1, 22.5, 44.0 and 68.0% when $TcPO_2$ was 40-49, 30-39, 20-29, 10-19, and < 10 mmHg respectively[17]. We did only report baseline $TcPO_2$, which is a limitation, and reported a total amputation rate of 8.1% in our entire cohort, with a median $TcPO_2$ of 41 (28-52). In a separate analysis of our patients with $TcPO_2$ < 50 mmHg (n=174), the rate increased to 9.8 %, similar to the result by Faglia *et al*. Patients who undergo major amputations are considered to have even higher mortality rates, but neither above-ankle amputations, nor healing failure was associated with 1-year mortality or was confounding the predictive value of $TcPO_2$ on mortality, in our study[19-21]. TBP or ABI were not significantly associated with mortality in our study, which is in contrast to previous results. In a study by Zobel *et al*, a significant association between toe-brachial index (TBI), ABI and mortality was found[22]. Our cohorts differed, however, in baseline criteria as they included patients with type 2 diabetes and microalbuminuria, but no clinical signs of CVD, compared to our unselected high-risk population of DFU-patients. Further, follow-up time was 6 years in the study by Zobel, compared to 1 year in our study. Therefore, one can speculate on whether the trend towards worse outcome seen in among our patients with TBP < 30 mmHg, would have significantly affect mortality in a longer perspective. Another study, including 81 type 2 diabetes patients with a history of myocardial infarctions, evaluated the risk of recurrent MACE when TBP < 50 mmHg and reported a HR of 3.83 (1.45-10.1)[23]. Further, in the Hoorn study, a population-based cohort study, including 155 patients with, and 469 patients without type 2 diabetes, Hanssen *et al* demonstrated a significant association between ABI < 0.9 and all-cause mortality in both patients with and without

diabetes, after a median follow-up time of 17.2 years[24]. Our present study, evaluating 1-year mortality in the high-risk population of patients with type 2 diabetes and DFU, indicates that TcPO2 might be a useful tool for risk assessment, with a prognostic value in the short-term perspective superior compared to ABI and TBP.

Concerning revascularisation, the observed rate of 25.5% among patients with $TcPO_2 < 25$ mmHg is considerably low. Additional 21.3% performed diagnostic imaging and were assessed by a vascular surgeon but were not amenable for revascularization. More than half of the patients with $TcPO_2 < 25$ mmHg did not, however, perform a complete diagnostic assessment, which is inconsistent with the recommendation by IWGDF. When evaluating those cases separately, we found cases with prompt ulcer improvement, as well as patients with severe comorbidity, including renal failure, among these individuals, but also a few cases with unknown reasons were limb ischemia with possibility for revascularisation might have been under-diagnosed. Patients with TcPO2 < 25 mmHg who underwent re-vascularisation procedures, had a trend towards better survival, but in the 1-year perspective this finding did not reach significance, or was confounding our results. In a study by Faglia *et al*, patients with identified critical limb ischaemia had higher survival rates after successful intervention, during 6 years of follow-up[21]. However, when the authors adjusted for confounders in a Cox model, only age turned out to predict mortality in that study[21]. Our low revascularisation rate is, however, an important observation, but not unique in our setting. In a German study by Malyar *et al*, only 18% of the patients were re-vascularised, and another 25% were evaluated with angiography[25]. Also, in the EURODIALE study, vascular imaging was only performed in 56% of the patients with severe limb ischaemia, and of them, only 43% were re-vascularised[26]. This might reflect the complexity of patients with DFUs, where several factors and often severe comorbidity need to be considered before intervention. Nevertheless, further optimising the multidisciplinary approach, when managing DFU-patients is desirable.

We can only speculate about the mechanism behind the increase in all-cause mortality among our patients with low $TcPO_2$. Traditionally cardiovascular risk factors such as smoking, hypertension, and previous CVD, did not significantly relate to either $TcPO_2$ or mortality, in our study. One possible explanation could be that a low $TcPO_2$ on the foot, might serve as a marker for impaired microcirculation in general, with a higher burden of microvascular complications. It has previously been shown by Huang *et al*, a significant negative association between $TcPO_2$ and microvascular events (albuminuria and distal polyneuropathy) in type 2 diabetes patients, with a 10-fold risk of microvascular complications when $TcPO_2$ was < 50 mmHg[27]. Similar, our study demonstrated a significant association between a low $TcPO_2$ and renal impairment, and a low eGFR was also independently associated with mortality, results previously shown by many[28-30]. eGFR did not, however,

confound the impact of $TcPO_2$ on mortality in the Cox analysis. Another possible mechanism contributing to the increased mortality rate among patients with low $TcPO_2$, could be a higher prevalence of neuropathy[31]. In a study by Arora *et al*, a lower $TcPO_2$ in the forearm and foot, was found in patients with diabetic neuropathy, compared to non-neuropathic patients, as well as healthy subjects[32]. Another study by Pfutzner *et al*, indicated a relation between a microvascular disturbance and small fibre neuropathy[33]. As small fibre neurons are involved both in peripheral neuropathy, and in the serious diabetes complication cardiac autonomic neuropathy, the latter could be a plausible mechanism contributing to increased mortality in patients with microvascular impairment[34]. In our study, we lack quantitative data on neuropathy, and thus, we can only speculate on a causality.

Our study has some strengths and limitations that need to be stated. The study design, as a retrospective cohort study might be afflicted with bias, and the findings might only serve as a hypothesis generating for future prospective studies. However, one strength is our relatively large cohort size, which is representative of the DFU-population, as most patients with DFUs in our region are transferred to our multidisciplinary DFU-unit. Further, the vast majority of our patients are routinely screened with $TcPO_2$, TBP and ABI and are seen at a regularly basis at our department, until ulcer healing. Mortality data are accurate since all deaths in Sweden are registered in our National Death Register. We only report all-cause mortality, due to the low frequency of autopsies in Sweden, and thus, we can only speculate on underlying cause of death. The lack of quantitative data on neuropathy and albuminuria is a limitation, and the association between $TcPO_2$ and cardiac autonomic neuropathy needs further studies. Several factors, besides microvascular function, might contribute to a low $TcPO_2$ that we did not correct for, such as advanced pulmonary diseases or heart failure with chronic hypoxia, as well as local factors such as tissue oedema and inflammation. Only one patient in the $TcPO_2$ < 25 mmHg group were suffering from chronic obstructive pulmonary disease, but since we didn´t measure oxygen saturation, undiagnosed hypoxia could have affected our result.

Besides limitations, our novel findings indicate an independent association between a low $TcPO_2$ and increased 1-year mortality, in patients with type 2 diabetes and DFU. This suggests that screening with $TcPO_2$ might improve identification of DFU-patients with urgent need for both vascular intervention and intensive cardiovascular risk factor assessment.

Author´s contribution

KF, PK and ML contributed to the design of the study. KF and ML performed data analysis. KF wrote, and all authors reviewed the article. ML is the garantor of this work and is responsible for accuracy of data.

Funding

This study was supported by the Swedish Diabetes Foundation, Sydvästra Skånes Diabetesförening, Krapperup Foundation, Skåne county council´s research and development foundation and Faculty of Medicine (ALF), Lund University, Sweden.This study was supported by the Swedish Diabetes Foundation, Sydvästra Skånes Diabetesförening, Krapperup Foundation, Skåne county council´s research and development foundation and Faculty of Medicine (ALF), Lund University, Sweden.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical standards: Approval of the study was given by the Ethics Committee in Lund, Sweden and patients were asked for informed consent according to decision in the ethical approval.

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