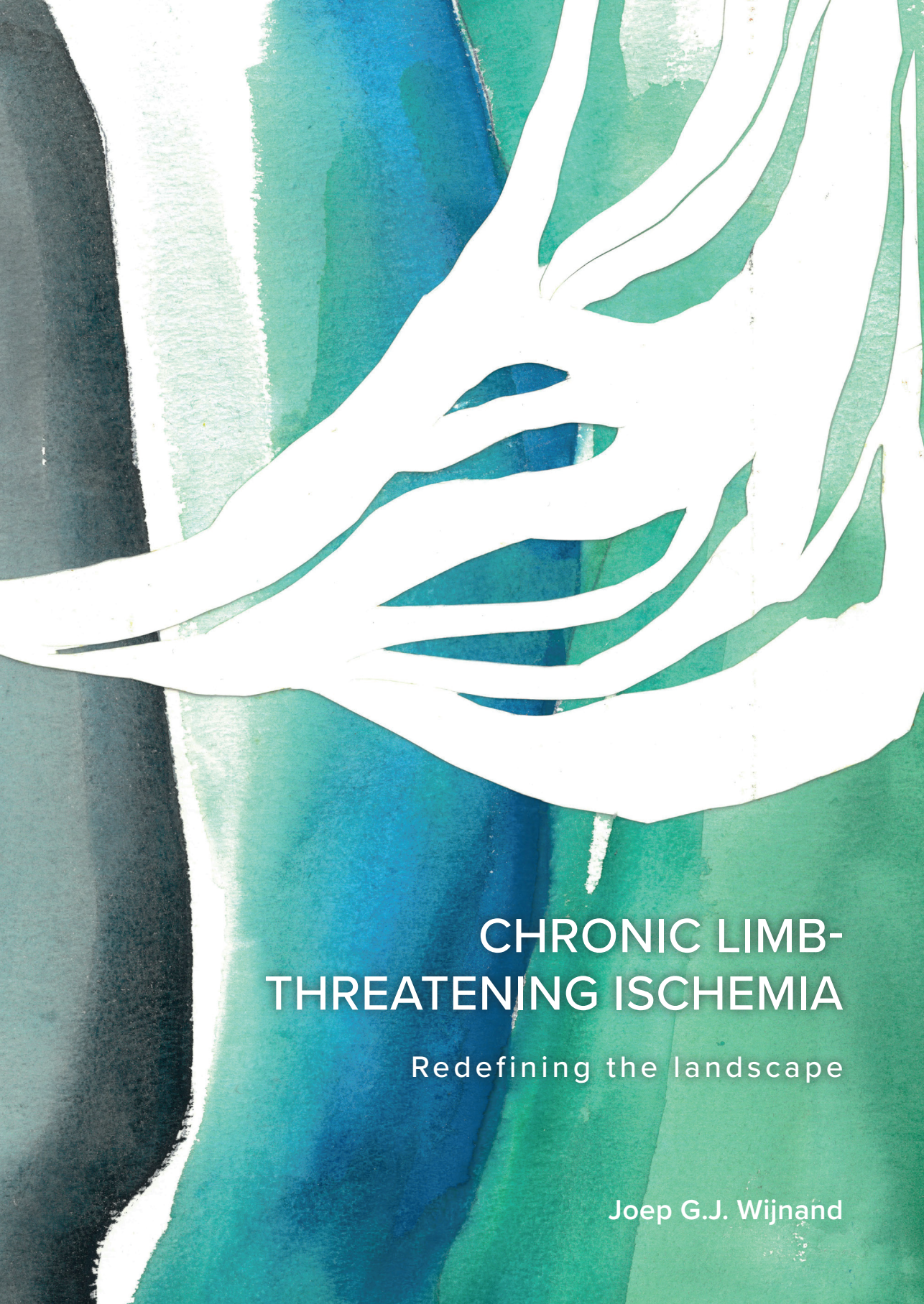

CHRONIC LIMB-THREATENING ISCHEMIA

Redefining the landscape

Joep G.J. Wijnand

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Cover Illustration: Cecile Wijnand (www.cecilewijnand.nl)
Cover: Ilse Modder (www.ilsemodder.nl)
Lay-out: Ilse Modder (www.ilsemodder.nl)
Printed by: Gildeprint Enschede (www.gildeprint.nl)

ISBN: 978-94-6496-058-7

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Chronic Limb-Threatening Ischemia - Redefining the landscape

Chronische ledemaat-bedreigende ischemie – Herdefiniëring van het landschap

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

maandag 25 maart 2024 des ochtends te 10.15 uur

door

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geboren op 8 mei 1988
te Assen

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Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged

Voor Opa Zwolle

"I can't change the direction of the wind, but I can adjust my sails to always reach my destination."

Jimmy Dean (1928-2010)

CONTENTS

GENERAL INTRODUCTION

Cardiovascular diseases are the leading cause of death worldwide. Damage to the smooth inner lining of the artery leads to stenosis or occlusion of the affected arteries. Many factors influence this process, including degeneration of tissues due to aging, inflammation, hypertension, hyperlipidemia, diabetes and smoking. Peripheral arterial disease (PAD) is one of the three major clinical manifestations of atherosclerosis, the other two being coronary artery and cerebrovascular disease.

In 2010, it was estimated that >200 million people worldwide were diagnosed with PAD. In the past 10 years, the prevalence increased by 28.7% in low-income countries and 13.1% in high-income countries. This increase appears to be partly due to population aging and the increase in other risk factors, particularly diabetes mellitus. In addition, it has been shown that overall increased affluence has contributed to overconsumption and other unhealthy lifestyles, resulting in increased incidence of cardiovascular disease at a younger age. Chronic limb-threatening ischemia (CLTI), defined as rest pain or tissue necrosis with ulceration or gangrene attributable to PAD, is at the end of the PAD spectrum and estimated to develop in 500-1,000 individuals per million persons per year in Western society. In the Netherlands, it is estimated that 85,000 individuals above the age of 55 have intermittent claudication. In 2009 it was estimated that there were 12,500 CLTI patients in the Netherlands, of whom 5,800 patients suffered from rest pain and 6,700 from gangrene. CLTI is associated with high risk for cardiovascular events and high mortality rates of 20% at six months, exceeding 50% within five years of initial hospitalization. To put this into perspective, of all patients who have a myocardial infarction, 28% are deceased within five years.

Despite advances in surgical and endovascular techniques, a substantial number of patients with CLTI (20-40%) is not eligible for revascularization procedures due to lack of a suitable bypass graft, comorbidity and anatomical factors such as location (tibial or pedal) or the extent of the disease. This may leave amputation as the only remaining option; amputation rates of 10-40% at six months are reported in no-option CLTI and this is associated with an even worse prognosis: perioperative mortality is 5-20%, a second amputation is required in 30% of the cases and full mobility is achieved in only 25-50% of patients. The median cost of managing a patient following amputation has been estimated almost twice that of successful limb salvage. With the expected increase in the incidence of CLTI and high cardiovascular risk, the disease poses a substantial burden on patients, healthcare providers, and public resources, underlining the urgent need for improved therapeutic solutions for these no-option CLTI patients.

CLTI is a multifactorial disease with mainly ischemic, neuropathic and microvascular determinants. Recognizing the multifactorial etiology has led to improved therapeutic

strategies for these patients. Nevertheless, a standardized care plan remains challenging. For instance, uniform staging and definitions are lacking. Consequently, it is hard to predict individual prognosis or treatment success rate. A framework is needed to standardize examination and treatment of this heterogeneous patient group. Furthermore, reliable CLTI risk prediction models could aid in clinical decision making. Tools have been developed to assist clinicians with predicting all-cause mortality, major amputation, amputation-free survival and perioperative events. Estimating the prognosis of a CLTI patient can guide therapeutic choices, whether revascularization is indicated and if so, which approach should be preferred. However, model validation and replication studies are limited. Models based on specific study populations often result in overfitting and produce over-optimistic estimates, therefore external validation is essential. Moreover, the large variation in disease severity in PAD, and even in CLTI-cohorts, contributes to differential results. Therefore, it is important to validate those models in several separate clinical cohorts and to directly compare the performance of these models to show superiority in a certain population.

A platform has been created for so-called evidence-based revascularization (EBR) in which definitions, staging and treatment are established. A “PLAN” should be made for each patient in which a classification is made in a quantifiable way based on three pillars: Patient risk, Limb severity and ANatomic pattern of disease (figure 1). Ultimately, PLAN will be the basis for identifying the best therapeutic option and providing adequate information to the patient regarding the expected success rate and durability of the treatment in question.

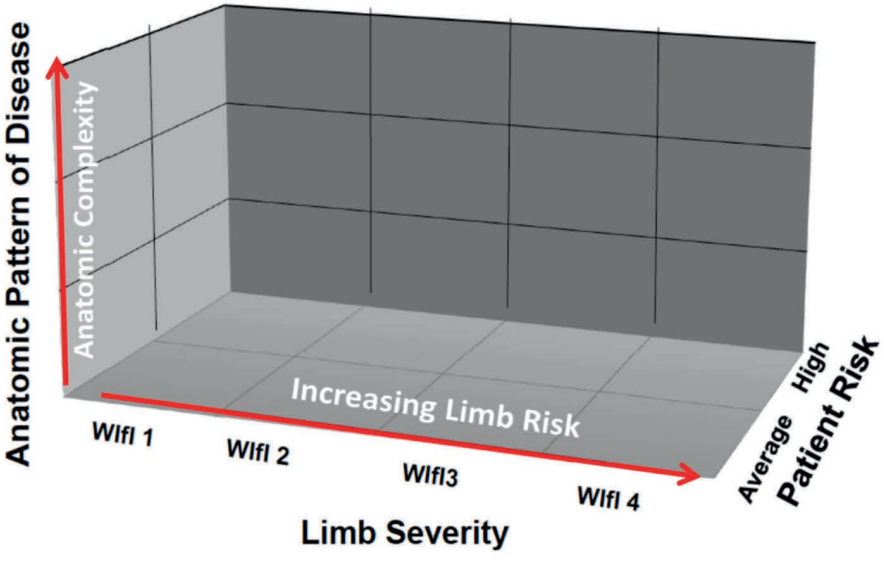


Figure 1 - Model for EBR in CLTI. WIfI: Wound, Ischemia, and foot Infection.

The current thesis provides an overview of the management of CLTI, focusing on validation of the PLAN pillars, exploratory feasibility studies regarding (the alternative use of) simple, low-cost diagnostic tools and the consideration of potential new therapies. The goal is to support future uniform and widely accepted CLTI definitions and classification, in order to improve the quality of future scientific research and thus the treatment and prognosis of patients.

THESIS OUTLINE

Part I - Predicting the course of CLTI

The first part will focus on the first pillar of "PLAN": Patient risk. Adequate prediction of the course of CLTI in terms of prognosis, treatment efficacy and patency in the individual patient is crucial, especially in a multifactorial disease with heterogenous manifestation like CLTI. For adequate prediction and improved clinical decision making, potential prediction models should be validated.

Several prediction models regarding post-procedural outcomes in CLTI patients have been developed based on Randomized Controlled Trials (RCT) to improve clinical decision-making. We aimed to determine model performance in predicting clinical outcomes in selected CLTI cohorts. In **chapter 1** we validated the BASIL, FINNVASC and PREVENT III models in data sets derived from a PAD registry study (Athero-Express) and two RCT's in CLTI in The Netherlands (JUVENTAS and PADI). Receiver-operating characteristics (ROC) curve analysis was used to calculate their predictive capacity. The primary outcome was amputation-free survival (AFS); secondary outcomes were all-cause mortality and amputation at 12 months after intervention. Patient-specific survival prediction is critical for informing treatment strategies, even for those without a clear option for revascularization. We validated a survival prediction model, developed in a revascularized Vascular Quality Initiative (VQI) cohort, in a Western European no-option CLTI cohort. The VQI survival prediction model was applied to the validation cohort to compare estimated mortality and observed mortality at two years after baseline; this is addressed in **chapter 2.**

In **chapter 3** our aim was to provide long-term survival and limb salvage rates for patients with non-revascularizable (NR) Chronic Limb-Threatening Ischemia (CLTI). Using prospectively collected data, derived from a RCT (JUVENTAS) investigating the use of a regenerative cell therapy, we retrospectively analyzed survival and limb salvage of the index limb in CLTI patients without viable options for revascularization at inclusion. The primary outcome was amputation free survival, a composite of survival and limb salvage, at five years after inclusion in the original trial.

Part II - A different perspective on CLTI

The second part of this thesis will address the third pillar of “PLAN”: Anatomic pattern of disease. **Chapter 4** discusses the Global Limb Anatomic Staging System (GLASS) for this purpose. The score is primarily used for endovascular interventions. Based on imaging (MRA/CTA/angiography), an optimal pathway is chosen that should lead to improved perfusion of the ankle and foot. This is the target artery path (TAP) and in this pathway two segments are scored based on the (length and degree of) stenosis and occlusion. Based on this, the sum of the distributed points allows estimation of technical success and the 1-year durability of an intervention.

One challenge with chronic ischemia of the leg, is the lack of methods to determine the degree of severity of ischemia. Minimal burdensome and practically easy to implement methods are needed that can also provide insight into the state of the blood vessels in the legs. Ideally this diagnostic tool is non-invasive and can accurately determine the tissue perfusion of the lower extremities.

In **chapter 5**, we explore the potential of the alternative application of a well-known measurement method. Nailfold capillaroscopy (NFC) is a non-invasive and fast method for imaging of capillaries of the proximal nailfold which is frequently used for diagnostics in rheumatic diseases. At the nailfold, capillaries run perpendicular to the skin and visualizing them using a microscope enables detection of morphologic abnormalities, including a decreased number of capillaries, dilated vessels, micro-hemorrhages and neovascularization which are a result of vascular inflammation and hypoxia. The presence of NFC abnormalities is one of the classification criteria for systemic sclerosis and can help to differentiate primary from secondary Raynaud’s. It is routinely used in the rheumatology outpatient office setting. NFC abnormalities are not only found in autoimmune disease, for instance, in patients with diabetes, NFC patterns are abnormal and correlate with the presence and development of diabetic neuropathy and other diabetic complications. NFC patterns and their related predictive value in patients with PAD have not been reported yet. Given the role of inflammation, presence of hypoxia, and the high prevalence of diabetes among PAD patients, NFC patterns in PAD patients are likely aberrant. Therefore, we hypothesized that NFC is feasible in PAD patients attending outpatient clinics and NFC patterns are abnormal. Secondly, we aimed to investigate the association between PAD and abnormalities in terms of quantitative and qualitative NFC measurements.

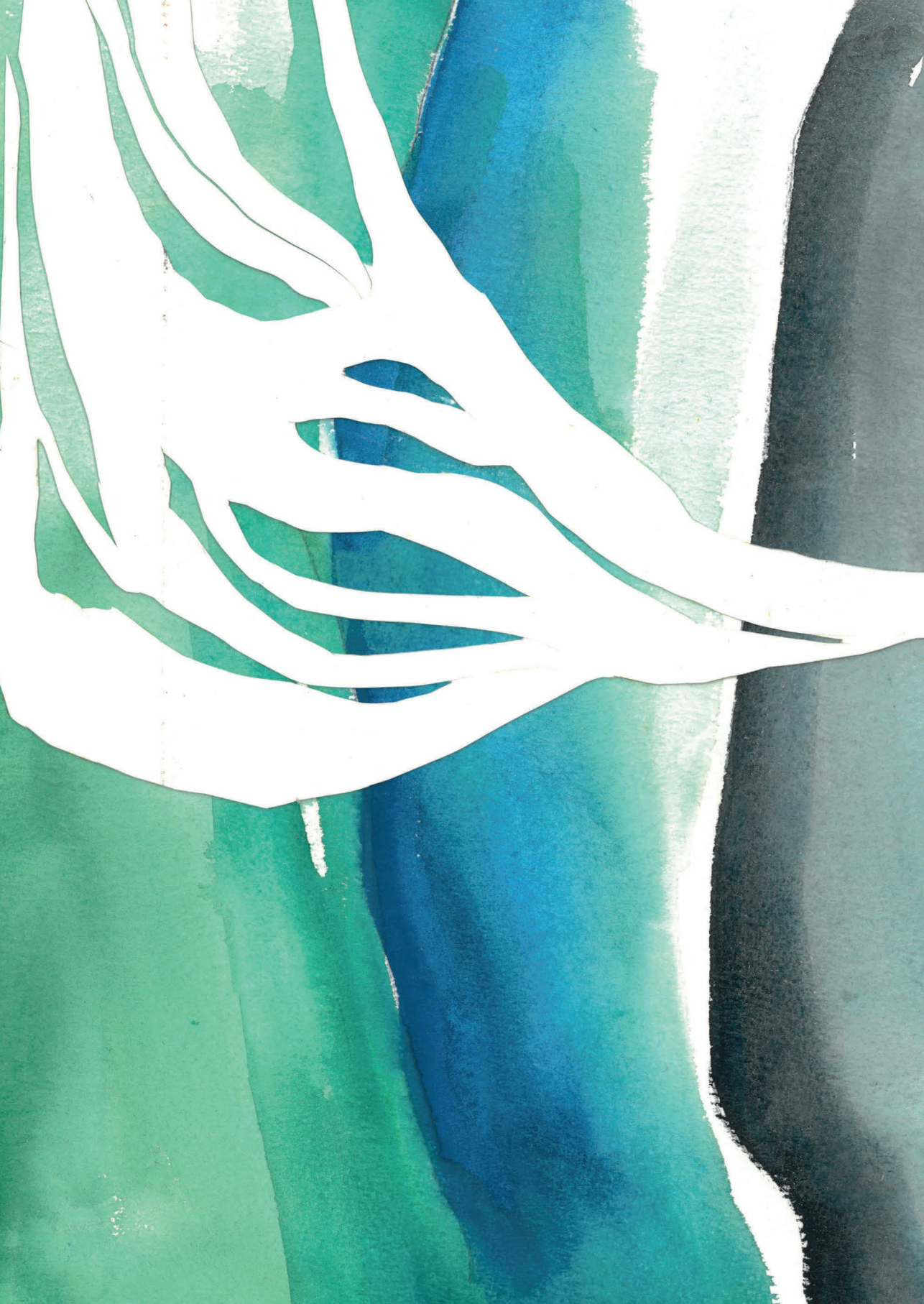
The last chapter of part II focuses on the evaluation of CLTI with transcutaneous oxygen tension measurement (TCpO2). The measurement of local oxygen pressure is believed to reflect the status of underlying vascularization of the skin. With the use of the so-called “Clark-electrode”, pO2 is measured by a platinum cathode and a silver anode covered with a thin membrane which is permeable for oxygen. The electronical reduction of oxygen allows

a current to flow which is proportional to the partial pressure of oxygen. To ensure dermal permeability for oxygen, the electrode is heated creating local hyperthermia liquefying the crystalline structure of the stratum corneum. Furthermore, hyperthermia creates underlying capillary vasodilatation allowing more oxygen diffusion. In **chapter 6,** the effect of different methods of TCpO2-use is described, and a general overview of TcPO2 methods within studies is demonstrated.

Part III - Cell therapy in CLTI

The third part of this thesis elaborates on potential novel therapies in PAD. In **chapter 7** we discuss current evidence and future directions with regard to cell therapy in CLTI. Subsequently in **chapter 8** we propose a design of a study on the effect of cell therapy in CLTI. In the last decade, cell-based therapies have been explored as a treatment option for patients with CLTI with no option for revascularization. Since 2002, several studies have suggested beneficial effects of cell-based therapies. However, the initial pioneering studies were heterogeneous, small, non-controlled, and non-blinded, precluding definite conclusions about treatment effects.

Mesenchymal stromal cells (MSC) are a subpopulation of bone marrow (BM) cells that can differentiate into various mesenchymal tissues (chondrocytes, osteocytes, adipocytes) under specific conditions. In vitro and vivo studies have demonstrated that MSC can home to injured tissue and secrete beneficial factors that suppress inflammation and improve angiogenesis. In addition, cell-to-cell interactions are also essential to immunomodulation and regenerative effects. Animal studies demonstrated that intramuscularly injected MSC improve neovascularization of mice in a 'hindlimb ischemia' model. Because of the immunomodulatory and vasculoregenerative properties, (Bone marrow-derived) mesenchymal stem cells (MSCs) may provide this novel therapy. Additionally, allogeneic cell therapy, which is possible for MSCs, is attractive as it may be used as 'off-the-shelf' available treatment and allows testing and selection of isolates before administration.



PART I

Predicting the course of CLTI

CHAPTER 1

Validation of Randomized Controlled Trial-derived Models for the Prediction of Post-intervention Outcomes in Chronic Limb-Threatening Ischemia

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Journal of Vascular Surgery 2020 Mar;71(3):869-879

ABSTRACT

Background

Chronic Limb-Threatening Ischemia (CLTI) represents the most severe form of peripheral artery disease (PAD) and has a large impact on quality of life, morbidity, and mortality. Interventions aim at improving tissue perfusion and averting amputation and secondary cardiovascular complications with an optimal risk-benefit ratio. Several prediction models regarding post-procedural outcomes in CLTI patients have been developed based on Randomized Controlled Trials (RCT) to improve clinical decision-making. We aimed to determine model performance in predicting clinical outcomes in selected CLTI cohorts.

Methods

This study validated the BASIL, FINNVASC and PREVENT III models in data sets from a PAD registry study (Athero-Express) and two RCT's in CLTI in The Netherlands (JUVENTAS and PADI). Receiver-operating characteristics (ROC) curve analysis was used to calculate their predictive capacity. The primary outcome was amputation-free survival (AFS); secondary outcomes were all-cause mortality and amputation at 12 months after intervention.

Results

The BASIL and PREVENT III models showed predictive values regarding post-intervention mortality in the JUVENTAS cohort with an area under the ROC-curve (AUC) of 81% and 70% respectively. Prediction of AFS was poor to fair (AUC ROC 0.60-0.71) for all models in each population, with the highest predictive value of 71% for the BASIL-model in the JUVENTAS population. The FINNVASC showed the highest predictive value regarding amputation risk in the PADI population with AUC of 78% at 12 months.

Conclusions

In general all models performed poor to fair on predicting mortality and amputation. Since the BASIL model performed best in predicting AFS we propose to use the BASIL model to aid the clinical-decision process in CLTI. However, improvements in performance have to be made in order for any of these models to be of real additional value in clinical practice.

INTRODUCTION

Chronic limb-threatening ischemia (CLTI), previously designated as critical limb ischemia (CLI), defined as rest pain or tissue necrosis with ulceration or gangrene attributable to peripheral artery disease (PAD), is estimated to develop in 500-1,000 individuals per million per year in Western society.(1) CLTI is associated with a high risk for cardiovascular events.(2–4) Six month amputation rates up to 40% and mortality rates of 20%, exceeding 50% at five years, from the incident diagnosis of CLTI have been reported.(5–8)

A recent nationwide registry study has shown consistent decreases in mortality and morbidity over time in CTLI-patients.(4) Proposed mechanisms for these improvements are advancements in preventive, medical and interventional treatment. However, despite these improvements, the relative mortality risk of CTLI-patients remains 2-5 times higher than in the general population. These poor prognostic figures underline the importance of further development and enhancement of tools to aid clinical-decision making.

Reliable CLTI risk prediction models could aid in clinical decision making. Over the last decade, several risk prediction models for CLTI have been developed, but validation and replication studies are limited. Models based on particular study populations often result in overfitting and produce over-optimistic estimates. Moreover, the large variation in disease severity in PAD, and even in CLTI-cohorts, contributes to mixed results. In that context it is important to validate those models in different clinical cohorts and to directly compare the performance of these models.

In order to assess validity and usefulness of these tools in daily clinical practice we tested three relatively commonly used CTLI prediction models; PREVENT, FINNVASC and BASIL in three different CLTI cohorts from the PADI-trial, JUVENTAS-trial and Athero-Express biobank.

METHODS

This study was conducted in accordance with the declaration of Helsinki. The medical ethics board in the participating hospitals approved the studies. All patients provided written consent. All patients who completed one-year follow-up or reached an endpoint in the first year were considered eligible for analysis. Resulting in the exclusion of eight (all from the AE cohort) subjects.

Study design

We assessed the performance of three commonly used CLTI prediction models,(9–11) which were derived from three eponymous cohorts (see Table 1); Bypass versus Angioplasty

in Severe Ischemia of the Leg [BASIL],(8) Prospective registry for surgical procedures performed for CLTI [FINNVASC](9) and the Prevention of Infrainguinal Vein Graft Failure [PREVENT III],(12) in three additional CLTI cohorts; JUVENTAS(13), PADI(14) and Athero-Express biobank(15) .

Initial model cohorts

All models were named after the initial cohort on which they were developed (Table 1). The BASIL trial consisted of 452 patients from the United Kingdom with CLTI. Patients were randomized for revascularization intervention using either balloon angioplasty or bypass surgery (BSX). AFS after three years was 38% and mortality rates were reported at 55% after this period. Vein-BSX was associated with significantly higher long term AFS and overall survival. Furthermore, through stratifying significant baseline factors, 2-year survival rates could be categorized ranging from 50% to 90%.(12) The PREVENT III study is a RCT (n=1404) conducted in the United States in which a new molecular therapy (edifoligide) was tested for the prevention of vein graft failure in patients undergoing infrainguinal revascularization for CLTI. 30-day mortality occurred in 2.7% of all patients. Major amputation occurred in 1.8% of all patients. Additionally, the molecular therapy did not offer protection for reintervention. (14) FINNVASC is a registry-study from Finland including data on 3,925 infrainguinal surgical revascularization procedures. 30-day major amputation rate was reported at 6.3% and the mortality rate after 30 days was 3.1%.(9)

Table 1. Specifics of initial study cohorts

Study	BASIL (N=452)	PREVENT III (N=1404)	FINNVASC (N=3925)
Design	Multicenter, randomized controlled trial comparing infrainguinal bypass versus angioplasty	Prospective, randomized, double-blinded, multicenter phase III trial of ex vivo vein graft treatment with edifoligide	Nationwide vascular registry for patients undergoing femoral endarterectomy, femoropopliteal or infrapopliteal bypass
Inclusion	Rutherford 4-6	Rutherford 4-6	Fontaine III + IV
Study results	No significant differences in amputation-free survival at 3 years	Ex vivo treatment of lower extremity vein grafts with edifoligide did not confer protection from reintervention for graft failure	-registry-
Model population	CLTI	CLTI	CLTI
Model endpoints	1 & 2 year survival 1 & 2 year major amputation	1 year AFS	30-day mortality 30-day major amputation
Model performance	AUC 1 year survival: 65.1%[11]	AUC 1 year AFS: 63.4%[18]	AUC 1 year AFS: 63.0%[18]

Validation Cohorts

PADI

The PADI trial is a randomized controlled trial (RCT) (2007-2013) in which percutaneous transluminal angioplasty (PTA) was compared to drug-eluting stents (DES) for treatment of infrapopliteal lesions in CLTI.(15) The primary outcome was therapy success, defined as 6-month patency of treated lesions (maximum of 50% restenosis on CTA). Secondary outcomes were Rutherford classification, minor and major amputation, and mortality. Six-month patency rates were 35.1% for PTA and 48.0% for DES.

Athero-Express biobank

The Athero-Express study (AE) is an ongoing tissue biobank collecting atherosclerotic plaques derived during surgical endarterectomy in two large Dutch tertiary referral centers. The AE started on March 24th, 2002 in the University Medical Center Utrecht (UMCU) and the St. Antonius Hospital Nieuwegein in The Netherlands.(16) Indication for surgery was made by an internal review board based on international guidelines and treatment was performed using standardized treatment protocols.

JUVENTAS

The JUVENTAS trial is a randomized, double-blind, placebo-controlled trial that included 160 patients with severe limb ischemia, who were no candidate for revascularization interventions (NO-CLTI), between 2006 and 2012.17 The aim was to investigate the effect of repetitive intra-arterial infusion of bone marrow mononuclear cells in NO-CLTI. Inclusion criteria were: age > 18 years, severe PAD (Fontaine class IIb, III, IV), ankle-brachial index (ABI) < 0.6 or unreliable ABI, no-option status and written informed consent. The primary outcomes were the incidence of major amputation and all-cause mortality at six months, with a median follow-up of 36 months. No significant differences in outcomes were observed between treatment arms.

Endpoints

The models differed in their choice of outcomes and chosen timeframe for prediction (see Table 1), which makes direct comparisons between the models challenging. In the present study we use amputation-free survival (AFS) as primary outcome for all analyses while amputation and all-cause mortality served as secondary outcomes. The total length of follow-up was chosen at 1-year for a cut-off in the primary analyses. Amputation was defined as any major amputation of the lower extremity, through or above the ankle joint.

Model specifics

In Table 2 an overview of the different risk prediction models is presented. The FINNVASC and PREVENT model both use a scoring method based on the presence of four to five risk factors present in the CLTI patient. All models require information on coronary artery

disease status and presence of tissue loss/gangrene. BASIL and PREVENT include renal function and age. FINNVASC is the only model that requires information on diabetic status. FINNVASC stratifies risk from 1-4 on the sum of points.18 PREVENT uses three risk categories for AFS; low (<4 points), medium (4-7 points) and high risk (>7). The BASIL prediction model calculates a continuous score and requires angiogram data for establishing below the knee Bollinger score. Prediction model data were calculated for each patient at the point of inclusion in the respective studies, which coincides with the pre-surgical screening in advance of the respective interventions.

Table 2. Critical limb ischemia prediction models

Models	PREVENT III	Score	FINNVASC	Score	BASIL
Risk factors	CAD	1	CAD	1	CAD
	Age>75 years Hematocrite <0.3	2 2	Diabetes mellitus	1	Age
	Tissue loss	3	Gangrene	1	Tissue loss
	Dialysis	4	Emergency procedure	1	Creatinine
					Smoking
					Bollinger score
					Ankle pressure
					BMI
					History of stroke/TIA
Model Specifics:	Risk categories cutoff at 3 and 8 points		Score indicates risk		Online risk calculator

Abbreviations: CAD; coronary arter disease, BMI; Body mass index, TIA; Transient ischemic attack.

Statistical analysis

To compare baseline characteristics between cohorts one-way ANOVA was used for continuous variables showing a normal distribution. Kruskal-Wallis test was used for continuous non-normally distributed variables. Chi-square test was used to compare categorical baseline characteristics between cohorts. Survival curves were constructed by use of the Kaplan Meier method. The accuracy of the FINNVASC, PREVENT and BASIL models in prediction of twelve-month AFS, mortality and amputation rate were determined using receiver-operating characteristics (ROC) curves. Confidence intervals around the ROC curves were created by bootstrapping (2000 iterations). The area under the curve (AUC) of all three prediction models were then compared within every cohort. Sensitivity analyses were performed to evaluate the choice of outcome and timepoint. Comparisons between related models were performed using the Net Reclassification Improvement (NRI). SPSS 21.0 (SPSS Inc, Chicago, Illinois, USA) and R 3.5.1 (R Core Team, Auckland, New Zealand) were used for all statistical analyses.

RESULTS

Patient characteristics

Baseline characteristics of the three cohorts are presented in Table 3. Patients were oldest in PADI (73.6 ± 12.0) when compared to AE (68.5 ± 8.7) and JUVENTAS (67.0 ± 13.3). In all cohorts more males were treated than females with an overall male to female ratio of 2:1. Diabetes was present in 63% of all patients in PADI compared to approximately 37% in AE and JUVENTAS. Incidence of extra-peripheral vascular disease was high in all cohorts, with around 40% of all patients suffering from ischemic heart disease and 17% of all patients suffering from cerebrovascular disease. PAD severity based on Fontaine classification strongly differed across the three cohorts (<0.001). The number of Fontaine class 4 patients was highest in PADI with 86.3% compared to 63.1% in JUVENTAS and 43.7% in AE. However, JUVENTAS patients in contrary to PADI patients were ineligible for revascularization by definition. For this reason, limb survival amongst the latter cohort was better despite their higher Fontaine classification. Cardiovascular risk factors such as history of smoking, hypertension and hypercholesterolemia were highly prevalent in all cohorts. Not all data required for testing these models were available in all cohorts (see Table 2 and 3).

Table 3. Baseline characteristics CLTI-cohorts

	JUVENTAS (n=160)	PADI (n=131)	AE (n=398)	P-value
Age, years, mean (SD)	67.0(13.3)	73.6(12.0)	68.5(8.7)	<0.001
Men*	108(67.5)	91(69.5)	270(67.8)	0.940
BMI, kg/m2, mean (SD)	26.4(4.6)	Unknown	26.3(4.2)	-
Smoking status				
Ex-smoker	93(58.1)	29(22.1)	232(58.3)	<0.001
Current smokers	42(26.3)	31(23.7)	160(40.6)	<0.001
Diabetes	60(37.5)	82(62.6)	146(36.7)	<0.001
Hypertension(medication)	142(88.8)	Unknown	335(84.4)	<0.001
Hyperlipidemia	133(95.0)	Unknown	232(65.7)	-
Coronary disease	66(41.3)	49(37.4)	167(42.0)	0.644
Cerebrovascular disease**	23(14.4)	24(18.3)	63(16.8)	0.799
Impaired renal function***	36(22.5)	35(26.7)	46(11.6)	0.001
(prev.) Hemodialysis	5(3.1)	13(9.9)	Unknown	-
Fontaine classification				<0.001
3	59(36.9)	18(13.7)	224(56.3)	
4	101(63.1)	113(86.3)	174(43.7)	

* All variables in n(%), unless stated otherwise
** Previous stroke or transient ischemic attack
*** eGFR <45 mL/min per 1.73 m2

Follow-up

Outcomes after one-year follow-up are presented in Table 4. As anticipated the AE-population showed best outcomes when compared with PADI and JUVENTAS. This is particularly true for AFS and amputation. However, regarding all-cause mortality, the percentage is comparable to JUVENTAS. Patients in the PADI cohort had the poorest outcomes with only 66% reaching 12 months AFS, in particular due to high mortality rates. Amputation rate was highest in JUVENTAS with almost 22% compared to 14% and 6% in PADI and AE, respectively. In PADI 26% of all patients died within one-year follow-up.

Prediction models performance

In Table 5 an overview of the different AUC's stratified by prediction model and outcome are presented. In Figure 1 the ROC-curves of twelve-month AFS are shown. Overall, twelve-month AFS was best predicted by BASIL with AUC ranging from 66.3% to 70.9%. Both PREVENT and FINNVASC models showed comparable model performance ranging from an AUC of 60.2 to 68.7%. General performance of all models was moderate for prediction of twelve-month AFS.

Table 4. One-year clinical outcomes CLTI-cohorts

	JUVENTAS	PADI	Athero-Express
	(n=160)	(n=131)	(n=398)
Amputation free survival 12 months	105(65.6)	87(66.4)	339(85.2)
Amputation	35(21.9)	18(13.7)	25(6.3)
All-cause mortality	15(9.4)	34(26.0)	39(9.8)

One-year clinical outcomes of the three different prediction models in the three cohorts.
* All variables in n(%)

Table 5. ROC analysis for three different outcomes in CLTI cohorts

	AFS 12 months	Amputation 12 months	Mortality 12 months
PREVENT III	(95% CI)	(95% CI)	(95% CI)
JUVENTAS	62.2% (52.9-71.6)	54.5% (43.8-65.3)	70.1% (55.9-84.4)
PADI	63.4% (53.7-73.1)	58.9% (43.0-74.9)	60.9% (50.5-71.4)
AE	68.7% (60.8-76.7)	70.6% (59.2-82.1)	60.6% (50.6-70.7)
FINNVASC			
JUVENTAS	63.0% (53.4-72.5)	59.5% (48.5-70.5)	66.9% (53.8-79.9)
PADI	63.4% (53.9-72.8)	78.1% (67.2-89.1)	54.5% (44.2-64.7)
AE	60.0% (51.9-68.0)	67.9% (56.0-79.7)	52.4% (42.0-62.8)
BASIL			
JUVENTAS	70.9% (61.5-80.2)	64.5% (54.4-74.6)	80.9% (68.1-93.7)
PADI	68.2% (58.9-77.4)	60.0% (46.1-73.9)	66.5% (56.6-76.4)
AE	66.3% (58.5-74.2)	67.8% (57.7-77.9)	63.3% (53.6-72.9)

CI, confidence interval

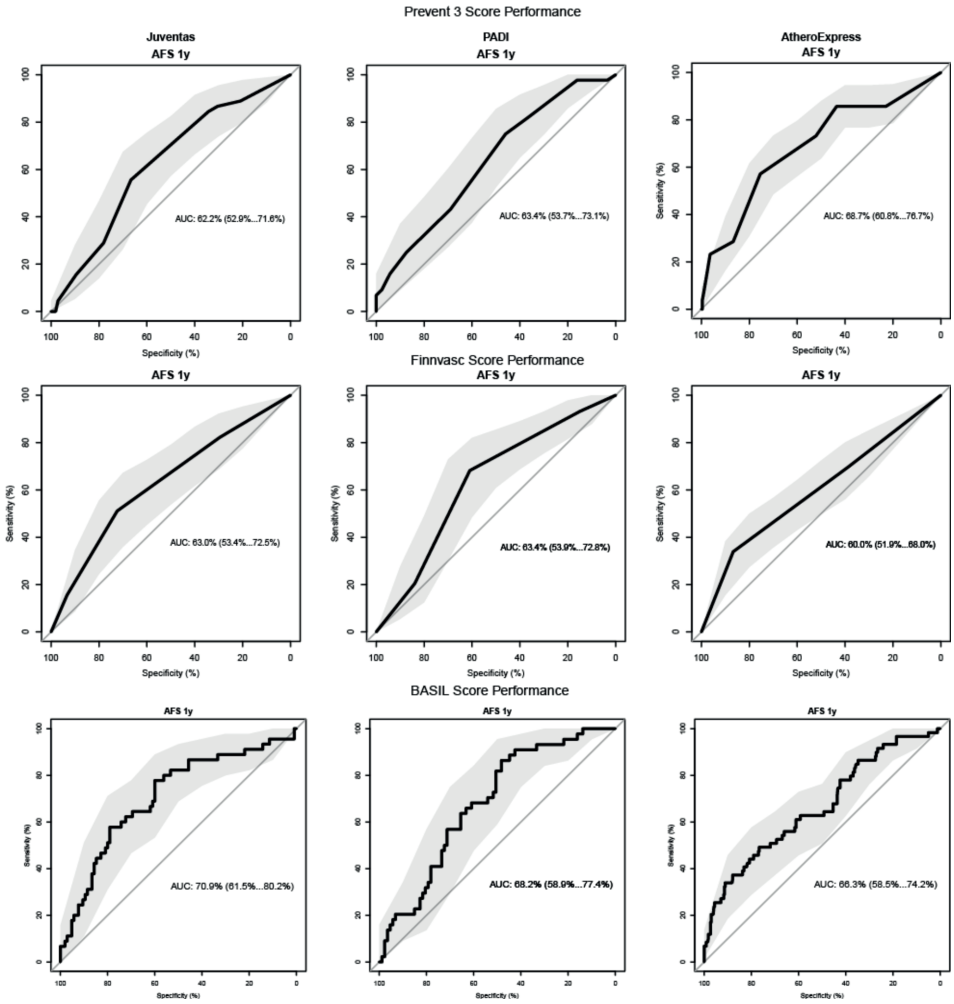

Figure 1 - ROC-curves for one year amputation free survival in the three different cohorts with the three different risk scores.

In Table 6 the group stratification and outcomes per model are shown. Additionally, in Figure 2 the logistic regression curves are presented for the risk classifications. In order to be able to compare the models we divided the BASIL in quartiles to create four groups. Sensitivity analysis showed that prediction model performance was relatively independent of the time-point chosen in all cohorts, with ROC curves stabilizing after 4 weeks (Figure 3). While models performed consistently over the different cohorts for the composite outcome of AFS, performance differed more notably between cohorts for the outcomes death and amputation. Which component outcome was better predicted, depended both on the cohort and the prediction model. In JUVENTAS all models performed poorly on amputation,

Table 6. Group stratification and outcomes

		JUVENTAS		PADI		Athero-Express		Group aggregate	
		Groupsize	AFS	Groupsize	AFS	Groupsize	AFS	Groupsize	AFS
FINNVASC	0	39	8 (20.5)	16	3 (18.8)	125	15 (12)	180	26 (14.4)
Score	1	59	14 (23.7)	51	11 (21.6)	156	19 (12.2)	266	44 (16.5)
	2	38	16 (42.1)	41	21 (51.2)	65	18 (27.7)	144	55 (38.2)
	3	14	7 (50.0)	23	9 (39.1)	1	1 (100)	38	17 (44.7)
PREVENT III	Low risk	90	20 (22.2)	40	8 (20.0)	251	24 (9.6)	381	52 (13.7)
	Moderate risk	55	23 (41.8)	79	29 (36.7)	101	29 (28.7)	235	81 (34.5)
	High risk	5	2 (40.0)	12	7 (58.3)	5	3 (60.0)	22	12 (54.6)
BASIL	1	37	5(13.5)	33	4(9.1)	100	7(7.0)	170	16 (9.4)
risk quartiles	2	38	7(18.4)	33	10(22.7)	99	15(15.2)	170	32 (18.9)
	3	37	13(35.1)	33	16(36.4)	99	10(10.1)	169	39 (23.1)
	4	38	20(52.6)	32	14(31.8)	100	27(27.0)	170	61 (35.9)

Outcomes of the different cohorts stratified by the FINNVASC, BASIL and PREVENT III risk score.
* All variables in n(%), AFS; amputation free survival

but well on mortality for instance. Interestingly, the FINNVASC model performed well on amputation in PADI and AE, and reasonable in JUVENTAS.

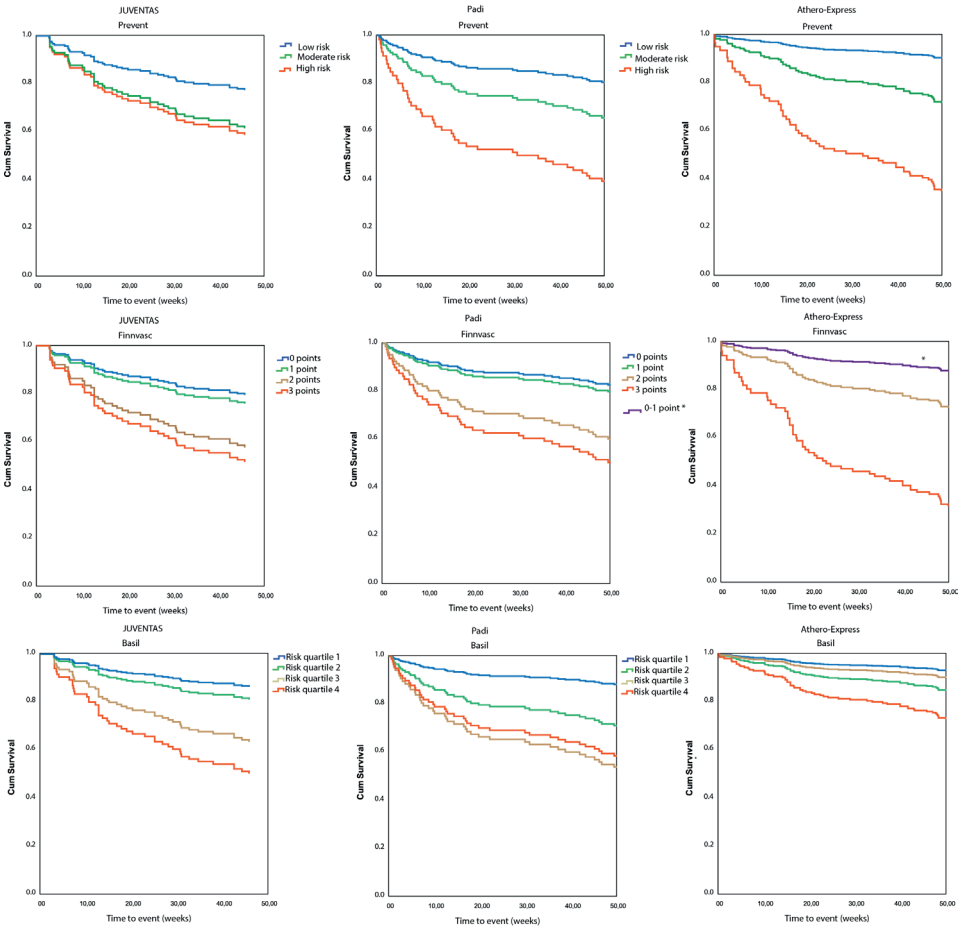

Figure 2 - Survival curves based on the FINNVASC, PREVENT III and BASIL risk prediction scores plotted per cohort.

While the risk factors that constitute the model scores largely overlap, FINNVASC is the only model that includes the presence of diabetes mellitus (DM) in the score. We therefore investigated whether addition of DM to the PREVENT model would improve classification for amputation and AFS. In order to maximize statistical power, we pooled the JUVENTAS, PADI and AE datasets for this purpose. The ORs for AFS at 12 months of the component variables in the Prevent 3 score were similar in rank to the original, with dialysis being the most important (OR=13.1) followed by tissue loss (OR=3.2) and Age (OR=2.1). History

of CAD (OR=1.4) and hypertension (OR=0.8) proved non-significant predictors for AFS. There were no differences between the cohorts in the association between model factors and outcomes. DM had a mutually adjusted OR of 2.0 (p=0.028) and therefore proved an independent predictor of AFS in the pooled cohorts. We next assigned DM 2 points on the Prevent 3 scale, on account of similarity in OR to Age. The thus expanded score indeed performed better than the original with an NRI of 0.28 (p=0.003) for AFS at 12 months and an NRI of 0.44 (p= 0.0014) for amputation at 12 months.

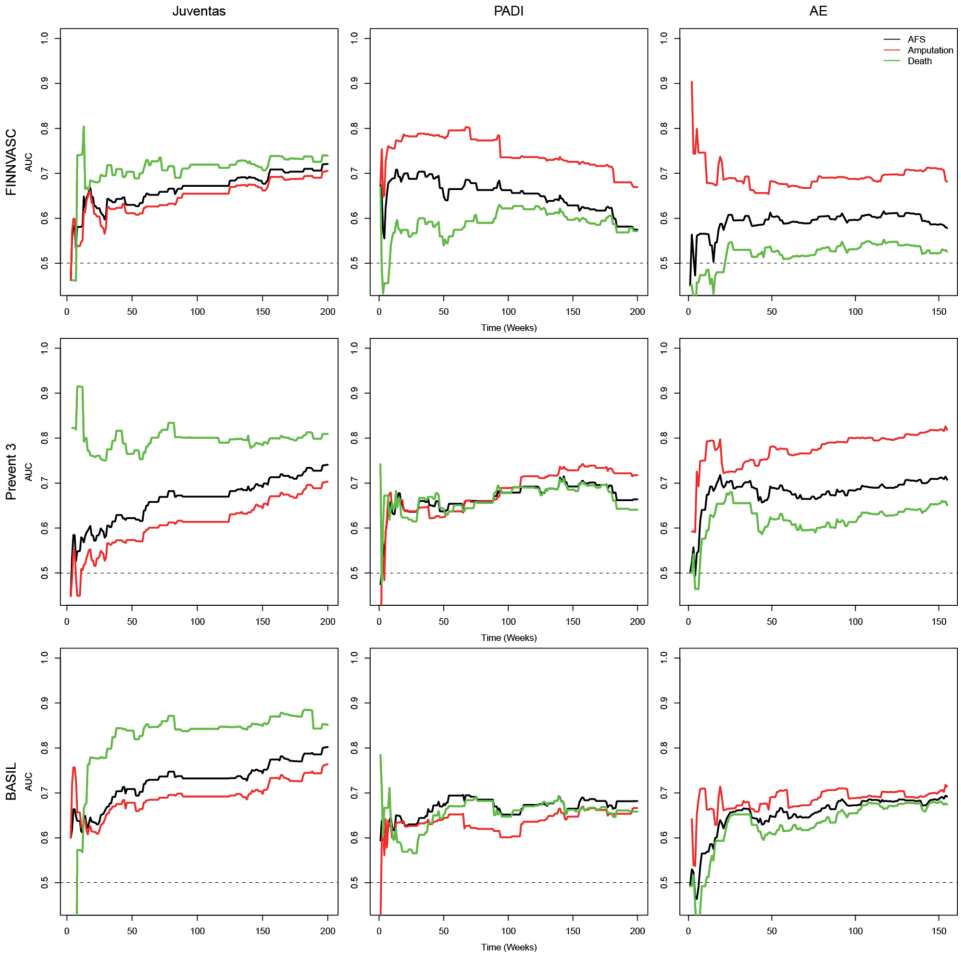

Figure 3 - AUC plotted per cohort for amputation free survival (black line), amputation (red line) and death (green line) at different time points during follow-up.

DISCUSSION

We have assessed three commonly used prediction models for CLTI in three different cohorts. Although our cohorts differ from the initial validation cohorts in terms of lesion localization, patient characteristics and type of intervention, performance was reproducible. Overall the BASIL model showed slightly better performance over the PREVENT and FINNVASC models for twelve months AFS. Interestingly BASIL performed best in the two most severe CLTI cohorts, namely JUVENTAS and PADI. The PREVENT model showed the best performance in the AE. Moreover, we studied the effects of the different components used in the risk prediction models. An important finding of this study is that by adding diabetes to the PREVENT model, predictive capacity significantly improved for both amputation and AFS 12 months after intervention.

Upon interpretation of these results it is essential to notice the differences in the chosen validation cohorts compared with the initial cohorts. The initial study cohorts are all comprised of patients undergoing surgical revascularization. Patients in the JUVENTAS study however, were not eligible for either surgical or endovascular revascularization often due to severe comorbidities. Interestingly, the AUCs of the different models ranged from 62.2% to 70.9% in the JUVENTAS-cohort and were comparable to the AUCs of 63.0% to 65.0% of the initial cohorts. Moreover, patients in PADI were oldest and often had a diagnosis of diabetes (62.6%) but models showed similar performance compared with the initial cohorts. Furthermore, the ineligibility for revascularization in JUVENTAS selects for a patient subset that has very generalized disease and is a non-classical type of CLTI. In this cohort it proved very difficult to predict amputation using classical risk factors, though mortality was predicted well. In AE there were many elderly patients with relatively little variance in age. For this reason, the age component in the models may have not predicted well. Additionally, FINNVASC might predict amputation better in PADI due to the fact that FINNVASC includes diabetes in its model and the fact that the incidence of diabetes is very different between PADI and the other two cohorts.

When we critically review the performances of the three different models we can conclude that overall model performance can be classified as poor to fair. When we compare the AUCs with historical cohorts we can confirm that these are in line with previous publications. (19) There were some differences, however, in model performance between the three intervention studies. When looking at 12-month AFS, the BASIL model performed better than PREVENT and FINNVASC. However, amputation at 12 months is best predicted by the FINNVASC model. Mortality after 12 months was best predicted by the BASIL model in the JUVENTAS cohort. The reason for variable model performance can partly be explained by the multifactorial complexity of CLTI. However, these models were initially not derived to predict the exact same endpoints. The factors that predict mortality and those that predict

limb loss are not concordant. Considering that diabetes is an important risk factor and is known to influence the pathophysiology of CLTI, addition of this risk factor seemed as a logical step. In our analysis we used the Net Reclassification Improvement(20) (NRI), to directly show the added value of adding diabetes mellitus as a model factor in the prediction model. This metric examines whether the additional model factor changes the predicted risk correctly, i.e. a subject that undergoes a major event has a higher a priori predicted risk and that subject that does not undergo an event has a lower predicted risk in the model. The highest possible value is 2, which occurs when the prediction model is 100% accurate with the addition of a new factor, whereas it performs at chance without the factor. Our results indicate that adding diabetes to the Prevent III model leads to a significant improvement in correctly identifying patients that will undergo a major event.

Starting point for further improvements of PAD risk prediction can come from different angles. Simple improvements could come from implementing information on exercise capacity or medical therapy prescription and compliance. While hemodynamic scores such as ABI are used only in BASIL, other scoring methods such as transcutaneous oxygen measurement and toe pressure measurements also merit consideration. Especially when considering that ABI is notoriously unreliable among diabetics, which were highly prevalent in these populations. Additionally, current trends point in the direction that one might consider the endpoints distinctly rather than combine them in a composite outcome such as AFS. For example WiFi may be better suited to predict limb loss whereas Vascular Quality Initiative (VQI) may be better to predict survival(19). In that situation, these models could be considered as a variant on the VQI model in representing the “patient risk” pillar complementary to “limb threat severity” and “anatomic pattern of disease” pillars in a more comprehensive evolution towards structured decision making in CLTI. Moreover, there is evidence emerging that hematological parameters can be used for improvement of secondary risk prediction. Recent studies have shown that by using specific features of full blood analyses such as counts and percentages from red blood cells, platelets and leukocytes, risk prediction significantly improved.(21,22) In a cohort of coronary angiography patients a panel of hematological predictors even outperformed high-sensitivity troponin I (hsTnI) and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) in secondary risk prediction.(22)
In a cross-sectional study of 6950 participants, the red cell distribution width, a marker of erythrocyte cell size heterogeneity, showed a strong graded increase in prevalence of PAD.(23) Moreover, this study showed that the predictive accuracy of the American College of Cardiology / American Heart Association (ACC/AHA)-defined PAD screening criteria improved from an AUC of 0.657 to 0.727 after addition of red blood cell distribution width. (24) These studies suggest that future risk prediction models likely comprise a wide range of clinical, functional, biochemical, and imaging variables.

The studies are in line with the argument that current risk prediction models simply do not cover all factors associated with CTLI disease progression and poor outcome. CLTI populations suffer from a form of end-stage atherosclerotic disease. This systemic atherosclerotic burden is reflected by the large prevalence of other affected vascular beds such as the coronary and the cerebral circulation. However, despite the fact that CLTI is a subgroup of advanced disease by definition, it still represents a heterogeneous population. This is reflected by differences in prognosis between the three cohorts studied in the current manuscript. This end-stage phenotype with often multi-segment vascular pathology can make risk prediction by use of four to five risk factors as used in PREVENT and FINNVASC an unjustified simplification for a complex problem. Considering the large heterogeneity in CLTI cohorts a possible approach for a new risk classification could come from novel statistical techniques such as phenomapping.(25) This unbiased clustering analysis may help identify distinct phenotypes in CLTI cohorts and has shown promising results in other heterogeneous clinical syndromes such as for instance heart failure with preserved ejection fraction.(25) This mapping strategy can help distinguish homogenous patient subcategories that have comparable success and outcome rates to the different treatment strategies. These phenomapping strategies can be used to combine the broad array of phenotypic data ranging from patient characteristics, blood biomarkers, imaging results to outcomes of functional vascular tests all in order to improve risk prediction.

Strengths and limitations

An important strength of this study is that we were able to study the performance of three different prediction models in three different CTLI-cohorts. First, we were able to study model performance in the surgical AE-cohort that is conceivably best comparable to the initial cohorts that are also comprised of patients undergoing surgical interventions. Second, we were able to show that prediction models initially built using data derived from interventional cohorts could be extrapolated to a no-option for revascularization population. Lastly, we were able to improve the risk PREVENT-model by adding diabetes into the risk score which could help improve clinical decision making. A limitation of this study is that since these cohorts were different in design some data on covariates were missing. Furthermore, we have to consider the fact that we validated the models for different outcomes then what they were originally designed for as a limitation.

CONCLUSION

In general, the three currently available CLTI risk prediction models perform poor to fair (AUC ROC 0.60-0.71) on predicting amputation and AFS. The best performing model in predicting AFS is the BASIL survival prediction model. We consider this model to be a useful addition

to the CLTI decision-making process in clinical practice. However, more research is needed in order to determine which factors should be included to evolve prediction models for specific PAD populations and how more advanced statistics, such as phenomapping could enhance risk prediction in CLTI. Aside from prediction of amputation risk, accurate survival prediction critically influences decision-making in patients with CLTI. Therefore, new models such as the VQI should be validated in order to differentiate patients into low-, medium-, and high-risk mortality groups. Eventually leading to evidence-based revascularization intervention recommendations that are in line with the newest treatment guidelines. Ideally, these prediction models should be validated prospectively.

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CHAPTER 2

External validation of the Vascular Quality Initiative prediction model for survival in no-option Chronic Limb-Threatening Ischemia patients

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Journal of Vascular Surgery 2020 Nov;72(5):1659-1666

ABSTRACT

Objective

Chronic Limb-Threatening Ischemia (CLTI) is associated with high morbidity and mortality rates. Over 50% of all CLTI patients die within five years after presentation. Patient-specific survival prediction is critical for informing treatment strategies, even for those without a clear option for revascularization. We validated a survival prediction model, developed in a revascularized Vascular Quality Initiative (VQI) cohort, in a Western European no-option CLTI cohort.

Methods

The VQI survival prediction model was applied to the validation cohort (n=150) to compare estimated mortality and observed mortality at two years after baseline. Performance of the VQI model was tested by evaluating discrimination using the ROC AUC and calibration using the Hosmer-Lemeshow goodness-of-fit test.

Results

The two-year survival rate was 79% in the validation compared to 81% in the VQI cohort. Baseline characteristics were significantly different for 13 out of 17 variables. The c-statistic was 0.86 (95% CI 0.78-0.95), which indicates good discrimination. The Hosmer-Lemeshow goodness-of-fit test had a p-value of 0.30, which indicates good fit.

Conclusions

This is the first external validation of the VQI survival prediction model. The good model performance suggests that this model can be used in different CLTI populations, including no-option CLTI, and underlines its contributory role in this challenging population.

INTRODUCTION

Globally, over 200 million people are living with Peripheral Arterial Disease (PAD) and this prevalence has increased over the last decade.(1) While Chronic Limb-Threatening Ischemia (CLTI) represents less than 10% of all PAD patients, it comes along with a huge burden in terms of morbidity, mortality and socio-economic costs. At 12 months, both the mortality and amputation rate are approximately 20%.(2) Estimates indicate that over 50% of all CLTI patients die within 5 years after presentation.(3) A more recent Dutch study supports this five year estimate and shows a slight decrease of all-cause mortality between 1998 and 2010.(4)

CLTI is a multifactorial disease with mainly ischemic, neuropathic and microvascular determinants. Recognizing the multifactorial etiology has led to improved therapeutic strategies for these patients. Yet, choices for revascularization are mostly based on expert opinion or personal preference of the physician treating the patient. There is no standardized therapeutic approach to the CLTI patient and therefore the recently published Global Vascular Guidelines attempts to provide a framework for evidence-based revascularization (EBR).(5) This framework is composed of three dimensions; 1. Patient risk; 2. Limb status; and 3. ANatomical pattern (PLAN). Staging of the limb with WIfI and the anatomical pattern of disease using GLASS are the fundaments of the second and third dimension of PLAN. A reliable tool to assess patient risk, the first element of the PLAN framework, has yet to appear.

Multiple tools have been developed to assist clinicians with predicting all-cause mortality, major amputation, amputation-free survival and perioperative events. The only randomized controlled trial comparing open lower extremity bypass (LEB) with endovascular intervention (PVI) (Bypass versus Angioplasty in Severe Limb Ischemia, BASIL) showed that open bypass is the preferred treatment in patients with survival longer than 2 years.(6) Estimating the prognosis of a CLTI patient is therefore not only useful to inform the patient. It can also affect therapeutic choices, whether revascularization is indicated and if so, which approach should be preferred. A prognostic model derived from BASIL-data was externally validated and showed modest performance in this heterogeneous population. Moreover, variables in BASIL are less convenient and not available in routine clinical practice.(7) Other models show similar limited predictive value.(8-10)

Recently a prediction model was developed using the Vascular Quality Initiative (VQI) database, in which CLTI patients underwent either an endovascular intervention or infrainguinal bypass. The chosen covariates were easily obtainable and internal validation showed acceptable discrimination.(11) However, external validation of the VQI-derived model has not been performed yet. The model was developed in patients who had undergone

revascularization; its performance in patients without a revascularization option is unknown. If the VQI model proves to have good performance in a no-option CLTI population this would underline that the model could be useful in a broader population to substantiate treatment decisions on amputation and palliative strategies and aid optimal selection of patients in future regenerative therapeutic trials (e.g. cell therapy) in no-option CLTI patients.Therefore, our goal is to investigate the applicability and performance of the VQI-model in predicting survival at two years in a well-defined Western European no-option CLTI population.

METHODS

Study design

Our goal was to apply the VQI survival predictive model for two-year mortality on the baseline characteristics in the JUVENTAS cohort, and compare them with the observed mortality at two years. We chose to analyze 2-year mortality and disregard the 30-day time point because of the clinical relevance and limited sample size available. We analyzed the performance of the VQI survival prediction model by evaluating discrimination and calibration.

The study was approved by the institutional review board of the University Medical Center Utrecht. The study was conducted according to the Declaration of Helsinki, and all patients provided written informed consent.

Initial model cohort

The specifics of the VQI model and cohort can be found in the original article.(11) In summary, the model was derived from a large eponymous cohort of CLTI patients in the United States of America, who underwent open or endovascular revascularization of the infrainguinal segment of the lower extremity between 2003 and 2017. The primary end point was survival at two years, defined as freedom from all-cause mortality. A backward stepwise selection was performed after univariate analysis which resulted in a Cox proportional hazards model with 12 covariates; age; race; rest pain or tissue loss; smoking status; coronary artery disease (CAD); congestive heart failure (CHF); chronic obstructive pulmonary disease (COPD); chronic kidney disease stage; ambulation status; pre-operative use of beta blocker, antiplatelet and statin (see table I for corresponding coefficients).

The equation for the Cox survival model is

$$h(t,X)=h_0(t)\exp(\sum[\beta_i x_i])$$

where X =(x1, x2,..., xp) are the covariates and h_0 (t) is the hazard function, with an empirical

baseline hazard of 0.952 at 2-years in the VQI cohort.

Internal validation demonstrated acceptable discrimination with a c-statistic of 0.72 at 2 years. Hosmer–Lemeshow goodness-of-fit (GOF) test was $P < 0.05$.

Validation Cohort

The JUVENTAS trial was a single-center, double-blind placebo-controlled randomized controlled trial that was performed between 2006 and 2012 in The Netherlands.(12) It showed no difference in major amputation or all-cause mortality 6 months after repetitive infusion (3 times at 3-week intervals) of Bone Marrow Mononuclear Cells (BMMNC) in 160 patients with severe, non-revascularizable (no-option) PAD, compared to placebo. No-option status, either due to poor health status or technical impossibility for revascularization, was determined by a multidisciplinary team of experts.

For our analysis, two patients with missing information on outcome were excluded from the validation analyses. Another eight patients with Rutherford classification stage 3 were excluded because they did not meet the CLTI criteria; per definition they had no rest pain or ischemic wounds.

Endpoints

The primary outcome was all-cause mortality after two years after inclusion.

Statistical analysis

Chi-squared analysis was performed to compare categorical baseline characteristics of the JUVENTAS and VQI group. A logistic regression model for mortality at 2 years was constructed using the coefficients for the corresponding Cox model in the original article. Three survival curves were constructed by the use of the Kaplan Meier method. With each curve representing one tertile of the VQI Cox survival score.

Calibration refers to the agreement between observed outcomes and predictions.(13) A Hosmer-Lemeshow GOF model was used to assess calibration. We used $g = 10$. A p-value of <0.05 means the model is not a good fit; that is, a nonsignificant p-value indicates good calibration.

We constructed Receiver Operating Characteristics (ROC) curves, with calculation of the concordance (c-) statistic, which is equal to the Area Under the Curve (AUC) in binary outcomes. A c-statistic is a unitless index interpreted as the probability of a random pair of patients in which the patient who meets the endpoint has a higher predicted probability compared with the subject who does not. (14)

A c-statistic of <0.6 was considered fail, 0.6 to 0.7 was considered poor, 0.7 to 0.8 was considered fair, 0.8 to 0.9 was considered good, and 0.9 to 1 was considered excellent in the ability to predict the all-cause mortality of the patient.

SPSS 21.0 (SPSS Inc, Chicago, Illinois, USA) and R 3.5.1 (R Core Team, Auckland, New Zealand) were used for the execution of all statistical analyses.

RESULTS

Patient characteristics

In the JUVENTAS cohort, for a total of 150 patients the two year follow-up was completed. Baseline characteristics of the VQI and JUVENTAS cohorts are presented in Table I. Distribution of the age-groups was significantly different between JUVENTAS and VQI (P=0.036). The majority of the patients was male (68% vs. 61%, P = 0.10), white (94 % vs. 76%, P = <0.001) and had tissue loss (66% vs. 70%, P = 0.38) in both cohorts. 14% of the patients never smoked, 59% had only a history of smoking and 27% were current smokers. This was significantly different in the VQI cohort with 30%, 41% and 30%, respectively (P = < 0.001). Hypertension was less common in JUVENTAS (63% vs. 90%, P = < 0.001). CHF was present in only 6% compared to 23% (P < 0.001), diabetes in 37% vs. 61% (P < 0.001) and most patients had no history of COPD (86% vs. 77%, P = 0.017). A large proportion of the patients had previous ipsilateral treatment in the JUVENTAS trial (81% vs. 39%, P < .001). Use of beta blockers (44% vs. 61%, P < 0.001) and statins (83% vs. 66%, P < 0.001) was statistically different in the cohorts.

Table I. Baseline characteristics of JUVENTAS and VQI cohort.

Characteristic	Beta-Coefficient	JUVENTAS (N=150)	VQI (N=38470)	P-value
Age, years				0.036
< 60	Referent	45 (30)	8400 (22)	
60 - 70	0.32	45 (30)	12105 (31)	
71 - 80	0.68	40 (26)	10030 (26)	
> 80	1.17	20 (13)	7935 (21)	
Sex, male		102 (68)	23533 (61)	0.10
Race, non white	- 0.25	9 (6.0)	9376 (24)	< 0.001
Indication				0.38
Rest pain	Referent	51 (34)	11674 (30)	
Tissue Loss	0.43	99 (66)	26796 (70)	
Smoking status				<0.001
Never	Referent	21 (14)	11368 (30)	
Prior History	0.09	89 (59)	15621 (41)	
Current	0.11	40 (27)	11412 (30)	

Table I. Continued.

Characteristic		Beta-Coefficient	JUVENTAS (N=150)	VQI (N=38470)	P-value
Hypertension			95 (63)	34577 (90)	< 0.001
CAD					0.005
	None	Referent	90 (60)	26715 (70)	
	History of MI, asymptomatic or stable angina	0.18	59 (39)	10799 (28)	
	Unstable angina / MI within 6 months	0.31	1 (0.7)	916 (2.4)	
CHF		0.49	9 (6.0)	8888 (23)	< 0.001
Diabetes			56 (37)	24328 (63)	< 0.001
COPD					0.017
	None	Referent	129 (86)	29766 (77)	
	Not treated or on medication	0.24	21 (14)	7670 (20)	
	Home oxygen	0.52	0 (0)	1034 (2.7)	
Chronic kidney disease stage					0.004
	GFR > 90	Referent	31 (21)	7295 (22)	
	GFR > 60-89	0.02	69 (46)	12597 (37)	
	GFR 30-59	0.22	34 (23)	11506 (34)	
	GFR 15-29	0.64	12 (8.0)	1949 (5.8)	
	GFR <15	1.09	4 (2.7)	303 (0.9)	
Ipsilateral Treatment			122 (81)	15066 (39)	< 0.001
Major Amputation			10 (6.7)	2799 (7.3)	0.90
Ambulation status					0.015
	Independent	Referent	88 (59)	24576 (64)	
	With assistance	0.33	39 (26)	10066 (26)	
	Wheelchair bound	0.52	23 (15)	3266 (8.5)	
	Bedbound	0.91	0	428 (1.1)	
Baseline medication					
	Betablocker	0.12	66 (44)	23353 (61)	<0.001
	Antiplatelet	- 0.13	105 (70)	29029 (75)	0.15
	Statins	-0.19	124 (83)	25264 (66)	<0.001
Endpoints					
	Survival		119 (79)	31880 (83)	0.29

Beta-coefficients are derived from the VQI model. Characteristics without a beta-coefficient are not implemented in the VQI model.
Numbers of patients are given with percentages within the brackets.
Chi-squared analysis was used for all categorical characteristics.
For baseline medication, numbers correspondent with the number of patients that use medication at baseline.
CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; MI, myocardial infarction; GFR, glomerular filtration rate.

Follow-up

All-cause mortality within two years after inclusion in the JUVENTAS was 21% (n=31) compared to 17% (n= 6590) in the VQI population (P = 0.29). In figure 1, the Kaplan-Meier

curves are shown for tertiles of the VQI Cox survival score to give an indication at what time a patient dies, depending on his survival probability. The highest tertile (the highest risk scores derived from the VQI model) mortality is higher compared to the lower two tertiles. At two years, 50% of the patients in the highest tertile have died, compared to 8.1% and 3.6% in the middle and lowest tertile, respectively.

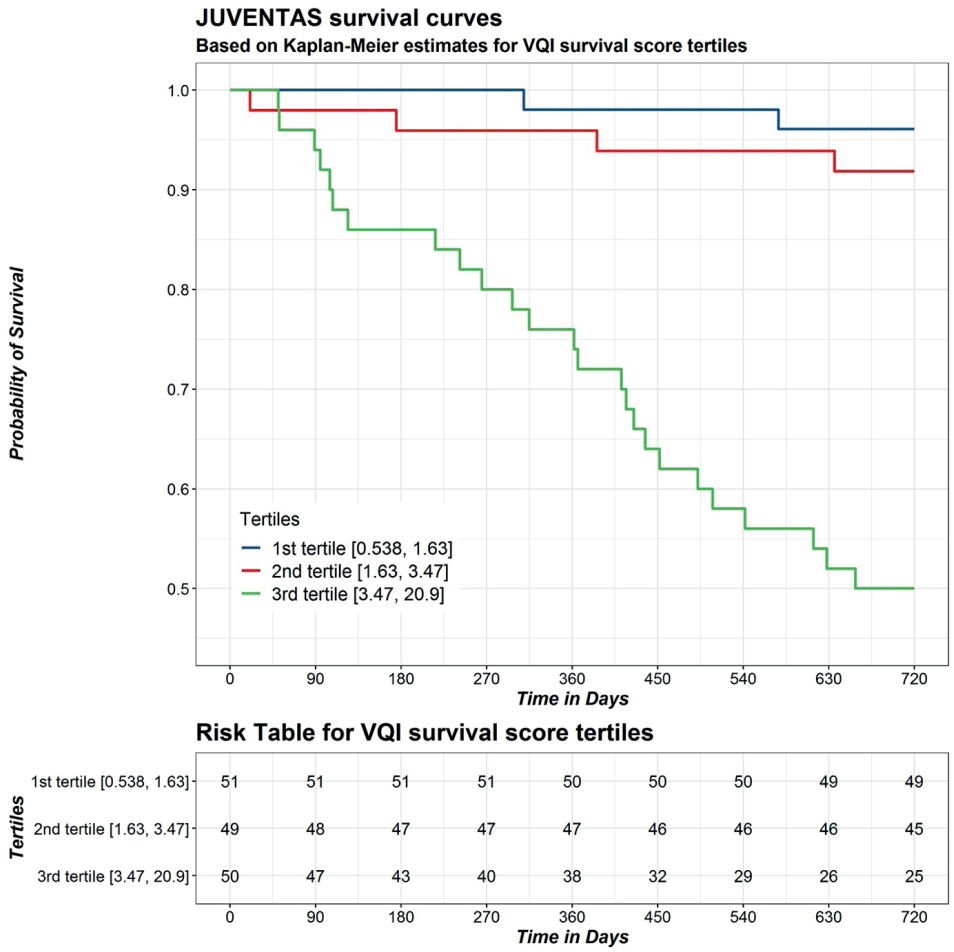

Figure 1 - Kaplan-Meier curves for VQI survival model score tertiles.
A survival curve is produced with the Kaplan-Meier method. This survival curve is made to show a clear survival difference when stratifying for survival scores obtained with use of the VQI survival model. Tertiles of VQI survival scores were made ranging from [0.538 – 1.63], [1.63 – 3.47] and [3.47 – 20.9]. The first (and lowest) tertile indicates lower risk of mortality, the last (and highest) tertile indicates higher risk of mortality.

Prediction model performance

In figure 2 the ROC-curve of the two year all-cause mortality is shown. The AUC was 0.86 (95% CI = 0.78-0.95) which is generally regarded as providing good discrimination. The p-value of the Hosmer-Lemeshow GOF was 0.30, indicating an agreement between observed outcomes and prediction, and therefore good model calibration.

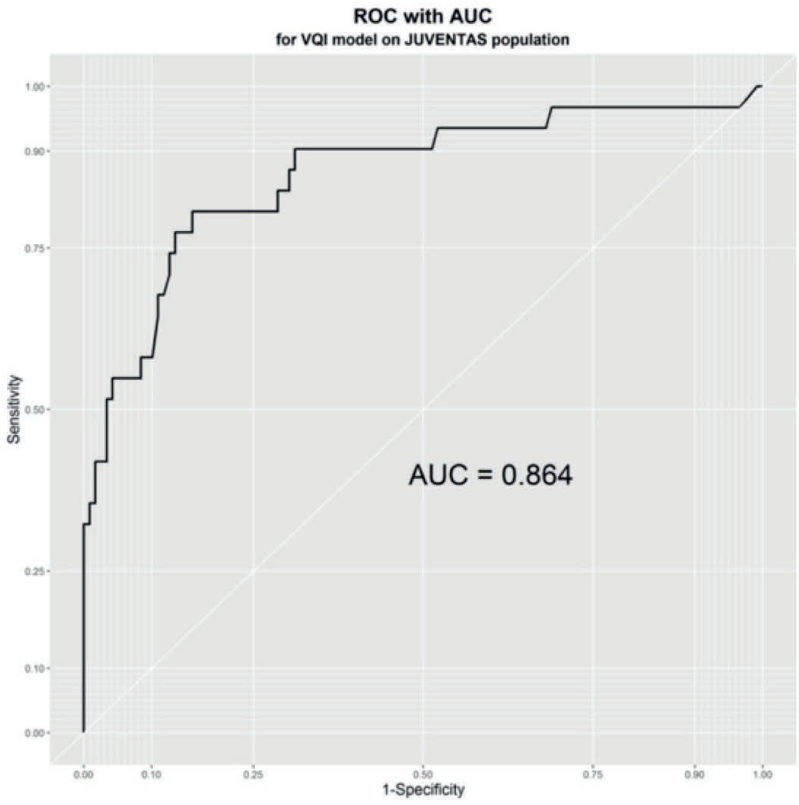

Figure 2 - Discrimination of the model (ROC with AUC) for overall mortality at two years.
Receiver operating characteristics (ROC) curve for predicted and observed mortality at two years are obtained from all JUVENTAS patients. The area under the curve (AUC) is 0.864 (95% CI: 0.781 -0.948).

Figure 3 shows the calibration plot of the predicted probabilities and actual probabilities of mortality. There is an under-estimation of low predicted probability (0.1 – 0.3), indicating that patients with a predicted probability in this range have an actual mortality that is slightly higher. There is good estimation when predicted probability is higher (0.5 – 1.0).

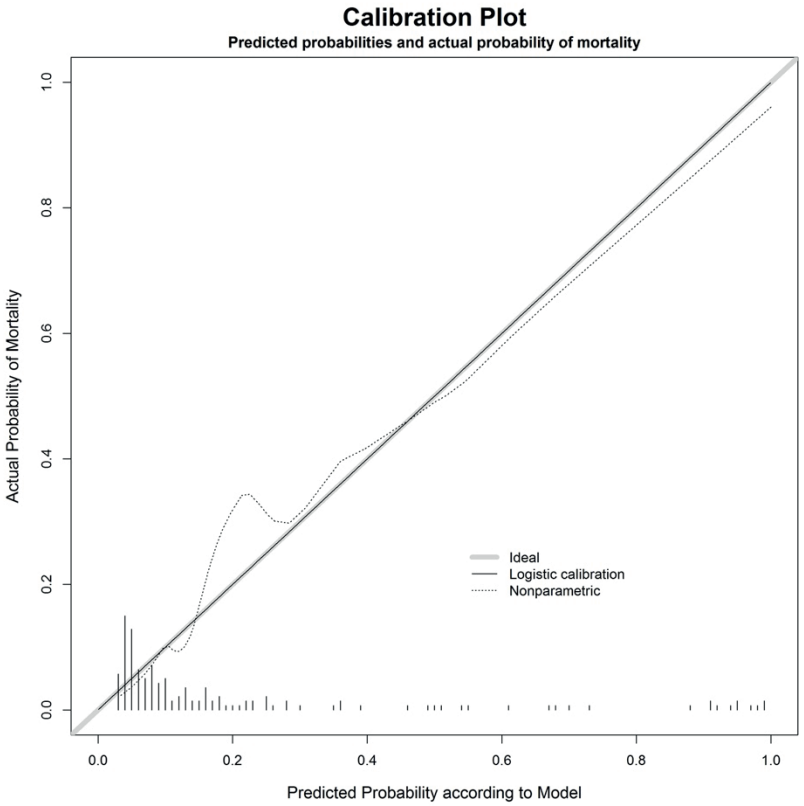

Figure 3 - Calibration plot for VQI survival model at two years.
Standard calibration curve for the predicted probability of mortality and actual probability of mortality. The diagonal line indicates the ideal line (where predicted probability matches the actual probability of mortality). On top of the x-axis, an indication of the amount of patients is given in a histogram to show the distribution of patients along the predicted probability according to the VQI survival model (on the x-axis). For a lower probability of mortality (left side of the x-axis), there are relatively more patients compared to a higher probability of mortality (right side of the x-axis).

Supplemental Figure 1 shows a regression curve of survival based on the VQI Cox survival score, 30 is not the highest possible score but no patient exceeded this risk score in the JUVENTAS cohort.

To test whether the predictive performance was coincidentally good only at two years, we calculated the ROC AUC for 180 and 365 days as well. Likewise, this shows good discrimination with an ROC AUC of 0.83 (9 events) and 0.86 (16 events), respectively.

DISCUSSION

The initial VQI survival prediction model showed good performance in an internal validation study of patients who underwent revascularization. Here we present the first external validation study of the VQI model in an independent well-defined no-option CLTI cohort from Western Europe. Our study showed good discrimination (c-statistic = 0.86) and a non-significant Hosmer-Lemeshow GOF test (P=0.30) indicating good calibration. Our findings support the VQI model as a good tool to evaluate survival estimates of no-option patients as part of the first dimension of the PLAN framework.

Survival predictions in no-option patients may seem less relevant as surgical revascularization-strategies are not expected to be successful or patients are deemed to be too frail to be exposed to the risks of an intervention. However, a reliable estimation of the prognosis of no-option CLTI patients could be very relevant, especially with the perspective of future therapeutic options. The model is useful in providing an optimal selection of no-option patients who might benefit most from novel therapeutic strategies (e.g. biologics). These trials are costly and results are affected when there is high loss to follow-up due to earlier (unexpected) death. Patients within the third tertile of survival scores perform much worse compared to patients in the first two tertiles. Therefore, in this high risk group the least advantage from other treatments is expected and palliative care should be considered. Furthermore, patients within the lower risk groups have better life-expectancies and should be the focus of therapeutic research in the future.

When reviewing the JUVENTAS and the VQI cohort, the majority of baseline characteristics differ significantly despite both cohorts included CLTI patients, which underlines the heterogeneity of the CLTI population. For age > 80, race, hypertension, diabetes, CHF, COPD and use of beta blockers the incidences and therefore risk indicators were much higher in the VQI cohort. While for history of smoking, MI, and ipsilateral treatment and use of statins the covariates in the JUVENTAS were more prevalent. The difference in baseline characteristics and hence predictor values between the cohorts may be explained by demographic and genetic differences, different structure of the healthcare system and most importantly, patient selection. The VQI cohort is derived from patients who were able to receive either surgical or endovascular intervention and were prospectively followed from this time point. The JUVENTAS trial, in contrast, was a study performed in patients without revascularization options. The specific reason to be considered no-option varied between patients, the majority was deemed (technically) ineligible for revascularization and in other patients the poor overall condition precluded an intervention.

Despite these differences, survival was not significantly different between both groups and in line with previous literature.(2)

The latter could partially be explained by the exclusion criteria in the JUVENTAS trial: life expectancy less than one year and no history of malignancy in the past ten years. This may have reduced the number of patients at risk for mortality in the trial. Furthermore, it could be hypothesized that inclusion in a clinical trial per se is related to better outcomes and patients who are motivated and consent to participate in a clinical trial have characteristics that put them in a prognostic better no-option subgroup, for example: their compliance could be higher and risk factors are addressed better due to a prevalent follow-up within the trial. This selection bias complicates a direct baseline comparison between the cohorts.

When looking at the covariates independently; age, CKD stage and ambulation were most contributory for discrimination in our population. This is no surprise as beta coefficients were highest in these groups, although very few patients in JUVENTAS were in the highest risk categories (i.e. >80 years old, GFR <15 and bedbound). In this respect, a limitation of the model might be the dichotomization of age and CKD stage. Part of the information is lost this way.

In the VQI model, coronary artery disease and preoperative ambulation violated the proportional hazard assumption. When checking the Schoenfeld residuals of the covariates independently, we found no interaction with time in the JUVENTAS data. This is probably due to a combination of the very small deviance in coefficient (ranging -0.011 to -0.005, as reported in the original article) and the small sample size in JUVENTAS.

The majority of the covariates used in the VQI survival model could be regarded as general health-indicators. In this model, only the indication for intervention can be considered a direct CLTI dependent risk factor for mortality. By expanding the predictive model with more PAD specific variables, performance could perhaps be improved. It is unfortunate that ABI-measurements were missing in >40% in the VQI cohort, as it would be interesting to see whether including ABI would lead to increased model performance. Other studies have shown ABI to be a good predictor for long-term survival in PAD.(15-17) However, ankle pressure was not incorporated in the BASIL predictive score due to the lack of statistical significance and other prediction models have not incorporated ABI either.(7, 18) In the future, it could be assessed whether other factors like perfusion (e.g. toe pressure or toe-brachial-index) and blood-derived parameters or biomarkers further improve the VQI model.(19)

However, the main advantage of this model is the overall easy availability of its covariates, and even without CLTI-specific covariates the VQI survival model performs well. Moreover, in comparison with the BASIL and PREVENT III survival models which were validated within the JUVENTAS cohort, the VQI model has better performance.(20) Therefore, the VQI model seems to serve as a good indicator for no-option patients in the first dimension of the PLAN framework. Although the JUVENTAS cohort comprises a very specific and high risk patient

selection, we were able to show that the VQI model performs well in this subgroup. In absence of alternative prediction models, we therefore suggest that based on our analysis, the VQI model can be applied on no-option CLTI patients for the prediction of survival. To extend its validity and utility, external validation on all-comers should be performed.

Strengths and limitations

As JUVENTAS is a clinical trial, cohort covariates were gathered prospectively and missing data were scarce. There was a low loss of follow-up within the JUVENTAS trial, even though original follow-up stopped at 6 months. Only two patients were lost at the two year interval and were excluded.

A limitation of this study is its small absolute number of events. The small event size in our study could lead to an overestimation of the model's performance. The relatively low number of events could be due to selection bias in the JUVENTAS trial. In order to have lower loss to follow-up in the original trial and reduce loss of patients due to other factors than the one studied, patients with concomitant disease with an estimated life expectancy of less than one year were excluded from the trial. This could be considered as another limitation of this study, because the population actually included in the trial could be considered a relatively healthier selection than the general no-option population. However, estimation of frailty was purely subjective and when looking at the Kaplan-Meier curves a linear decrease in probability of survival across the whole two-year interval is seen. If the initial estimation was better we would expect a more convex slope if high risk patients were excluded, with fewer deaths in the first year and increased mortality in the following year. Consequently, this underlines the difficulty of accurately predicting mortality, which shows the importance of a prediction model, such as VQI, for this CLTI-subgroup.

In conclusion, in this first external validation study, the VQI survival model showed good performance. Our study indicates that the model is not only applicable on surgically or endovascular treated patients in the United States of America, but can also be used in non-revascularizable Western European CLTI patients. Therefore, the VQI survival prediction model seems a reasonable tool to be used as part of the three dimensional PLAN strategy as proposed in the Global Vascular Guidelines for the treatment of CLTI. Whether the model can be further improved with the addition of blood-derived or CLTI-specific parameters, such as ABI, should be elucidated in future studies. Moreover, the VQI model should be validated in a large prospective all-comer CLTI population to show its usefulness in the general CLTI population.

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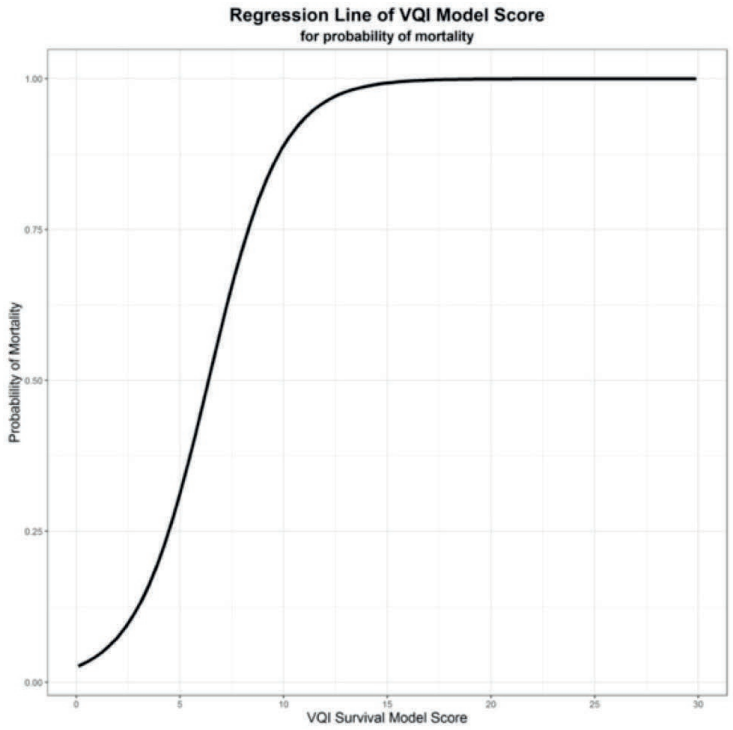
SUPPLEMENATERY MATERIAL



Supplemental Figure 1. Regression curve of mortality probability.
A regression curve shows the probability of mortality dependent on the VQI survival model score. Patients with a score of 5 have approximately 31% chance of mortality within two years. Patients with a score of 10 have approximately 88% chance of mortality

CHAPTER 3

Long-term survival and limb salvage in patients with non-revascularizable Chronic Limb-Threatening Ischemia

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European Journal of Vascular and Endovascular Surgery 2021 Aug;62(2):225-232

ABSTRACT

Objectives

Our aim was to provide long-term survival and limb salvage rates for patients with non-revascularizable (NR) Chronic Limb-Threatening Ischemia (CLTI).

Design

Retrospective review of prospectively collected data

Methods

Using prospectively collected data, derived from a randomized controlled trial (JUVENTAS) investigating the use of a regenerative cell therapy, we retrospectively analyzed survival and limb salvage of the index limb in CLTI patients without viable options for revascularization at inclusion. The primary outcome was amputation free survival, a composite of survival and limb salvage, at five years after inclusion in the original trial.

Results

In 150 patients with NR-CLTI, amputation free survival was 43% at 5 years after inclusion. This outcome was driven by an equal rate of all-cause mortality (35%) and amputation (33%). Amputation occurred predominantly in the first year. Furthermore, 33% of those with amputation subsequently died within the investigated period, with a median interval of 291 days.

Conclusions

Five years after the initial need for revascularization, about half of the CLTI patients who were deemed non-revascularizable survived with salvage of the index limb. Although the prospect of these high-risk patients is still poor, under optimal medical care, amputation-free survival seems comparable to that of revascularizable CLTI patients, while major amputation rate within one year, especially among NR-CLTI patients with ischemic tissue loss, is very high.

INTRODUCTION

Despite medical and technological treatment advances, patients with peripheral artery disease (PAD) still have a high morbidity and mortality risk as compared to the general population.(1) This is in particular true for patients with Chronic Limb-Threatening Ischemia (CLTI) as reported 5-year all-cause and cardiovascular mortality rates twice as high (57% and 29%) compared with patients with intermittent claudication (IC) (31% and 15%), respectively, according to a Dutch national registry study.(1) Furthermore, the amputation rate in CLTI patients of 15-20% at one year reflects a large impact on quality of life and healthcare costs.(2)

Alarmingly, the prevalence of PAD will likely grow as populations are ageing and prevalence of risk factors for PAD, such as diabetes mellitus (DM), increase. Between 2017 and 2045 the prevalence of DM is expected to rise from 451 to 693 million people worldwide.(3) Already, up to 30% of all patients with IC and 50% of all patients with CLTI are diagnosed with DM, which co-prevalence is associated with lower revascularization success rates, decreased wound healing and higher amputation and mortality rates as compared to non-diabetics. (4–8) As a consequence, the increasing prevalence of patients with DM will expectedly lead to a parallel increase in the number of patients with non-revascularizable or so-called “no-option” PAD, and specifically no-option or non-revascularizable CLTI (NR-CLTI).

Although the clinical prognosis of NR-CLTI patients has been reported, the evidence is limited to one-year mortality and amputation rates in non-consecutive case series and randomized controlled trials that report these outcomes as an ancillary result. Available data combined in a meta-analysis investigating the natural history of NR-CLTI, reported a one year mortality and amputation rate of 22%.(9) Within this analysis, consisting of 11 studies, only two studies reported a follow-up exceeding two years but both are dated more than 30 years ago (study periods were 1979-1986 and 1971-1983, respectively).(10,11) Hence, the current long-term prognosis of CLTI patients without revascularization options remains unclear, while knowledge about the contemporary prognosis in this specific population is valuable for numerous of reasons: e.g. counselling patients and family, substantiating treatment decisions (not limited to PAD alone, as these patients often have multiple morbidities), the timing of palliative care and optimal selection of patients for future (regenerative therapy) trials.

The aim of this study was to provide long-term survival and limb salvage rates for NR-CLTI patients. Therefore, we investigated the five-year survival and amputation-free survival in ‘no-option CLTI patients’ who participated in a randomized controlled trial (RCT).

METHODS

The details of the JUVENTAS trial design were previously published.(12) In short, in this single-center, double-blind, placebo-controlled RCT, the clinical effects of repetitive infusion of bone marrow mononuclear cells into the common femoral artery were investigated in 160 patients. Notable inclusion criteria were the ineligibility for surgical or endovascular revascularization (thus deemed non-revascularizable ((NR)), as defined by a multidisciplinary team of vascular surgeons and radiologists in the University Medical Centre of Utrecht, and severe PAD consisting of severe intermittent claudication, persistent recurring rest pain or non-healing ulcers present for more than four weeks. Noteworthy exclusion criteria were a history of malignancy within the ten years prior to inclusion and a life expectancy of less than one year.

The primary outcome of the initial study was major amputation of the index limb within six months after randomization. All-cause mortality was a secondary outcome. Inclusion was conducted between 2006 and 2012. No effect of the trial intervention was observed.(13)
For the current study, only the NR-CLTI population included in the JUVENTAS trial was analyzed. For our baseline we used the original information, without any new retrospectively reconstructed data (such as the Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification).(14) But in addition to the original protocol, information about major amputation and all-cause mortality was successfully requested from the general practitioners, more than five years after inclusion (N = 158). The patient and the referring hospital were contacted when the follow-up was unknown by the general practitioner (N = 2). The leg on which a patient was included in the original trial was defined as the index limb. Major amputation was defined as amputation through or above the ankle joint. The primary outcome of this study was ipsilateral amputation-free survival (AFS), the inverse composite of ipsilateral major amputation and all-cause mortality. The study was conducted according to the Declaration of Helsinki, the medical ethics board in the participating hospital approved the study, and all patients provided written informed consent.

Statistical analyses

Baseline characteristics, such as risk factors, medication use, wound-characteristics and the ankle-brachial index (ABI), stratified for AFS, are provided. Categorical variables were reported as numbers with percentages, non-normally distributed data were reported as median with interquartile ranges (IQR) and normally distributed results were given as mean with standard deviation (SD). Normality of data was analyzed using the Shapiro-Wilk test. Continuous variables were analyzed using Students t-test or Mann Whitney U-test as appropriate. Categorical variables were analyzed using Fisher's exact test.

Additional analyses were performed to evaluate contributing factors for lower limb

amputation and all-cause mortality. Scaling (z-transformation) was performed after log10 transformation of non-normally distributed continuous variables. Univariate Cox proportional hazard regression was performed on a selection of risk factors with a plausible relation to the outcome. Multivariate analysis was performed including predictors with a P value < .10 in univariate analyses using a forward stepwise approach. The proportional hazard assumption was verified by examining the Schoenfeld residuals.

The statistical analyses were performed by using SPSS for Windows version 25.0 (SPSS Inc., Chicago, IL) and R version 4.0.0 (R Core Team, Auckland, New Zealand).

RESULTS

Patient Characteristics

Of the original 160 included patients, eight patients had severe IC (Rutherford stage 3), and were excluded from analyses (none underwent amputation or died within five years). Of the remaining 152 CLTI patients, two were lost to follow-up in an early phase. Hence five-year follow-up data were available for 150 patients, including 102 males (68%), with a median age of 67 (IQR 56–76) years, and of whom 56 (37%) patients had DM. At time of inclusion, 51 patients had rest pain (Rutherford stage 4), 90 patients had ischemic ulceration not exceeding the digits of the foot (Rutherford stage 5) while nine patients had severe ischemic ulcers or gangrene (Rutherford stage 6).

Outcomes

After five years, 64 out of 150 patients (43%) survived without major amputation of the index limb. Of the other 86 patients, 53 (35% of total) died and 49 (33% of total) underwent a major amputation. In 16 patients, amputation was performed prior to their death within the five-year interval. The median time between amputation and death was 291 days (IQR 35-583). The Kaplan-Meier curve for AFS, amputation, and mortality, are demonstrated in figure 1. As seen, all-cause mortality is evenly distributed along the 5-year interval while amputation occurs predominantly within the first year. The one-year AFS was 70% (95% CI 63–78), attributed to 24% (95% CI 17–31) major amputation and 11% (95% CI 6-16) mortality.

Determinants of outcomes

Table I summarizes the baseline characteristics of the 150 included patients, stratified by the five-year composite outcome. Male gender (P = .033), higher age (P < .001), higher Rutherford stage (P = .004), history of a cerebrovascular event (P < .001) and cardiogenic chest pain (P = .001), use of diuretics (P = .031), lower glomerular filtration rate (P = .013), HDL-cholesterol (P = .005) and hemoglobin (P = .004) were significantly more common in the group with the composite of amputation and mortality.

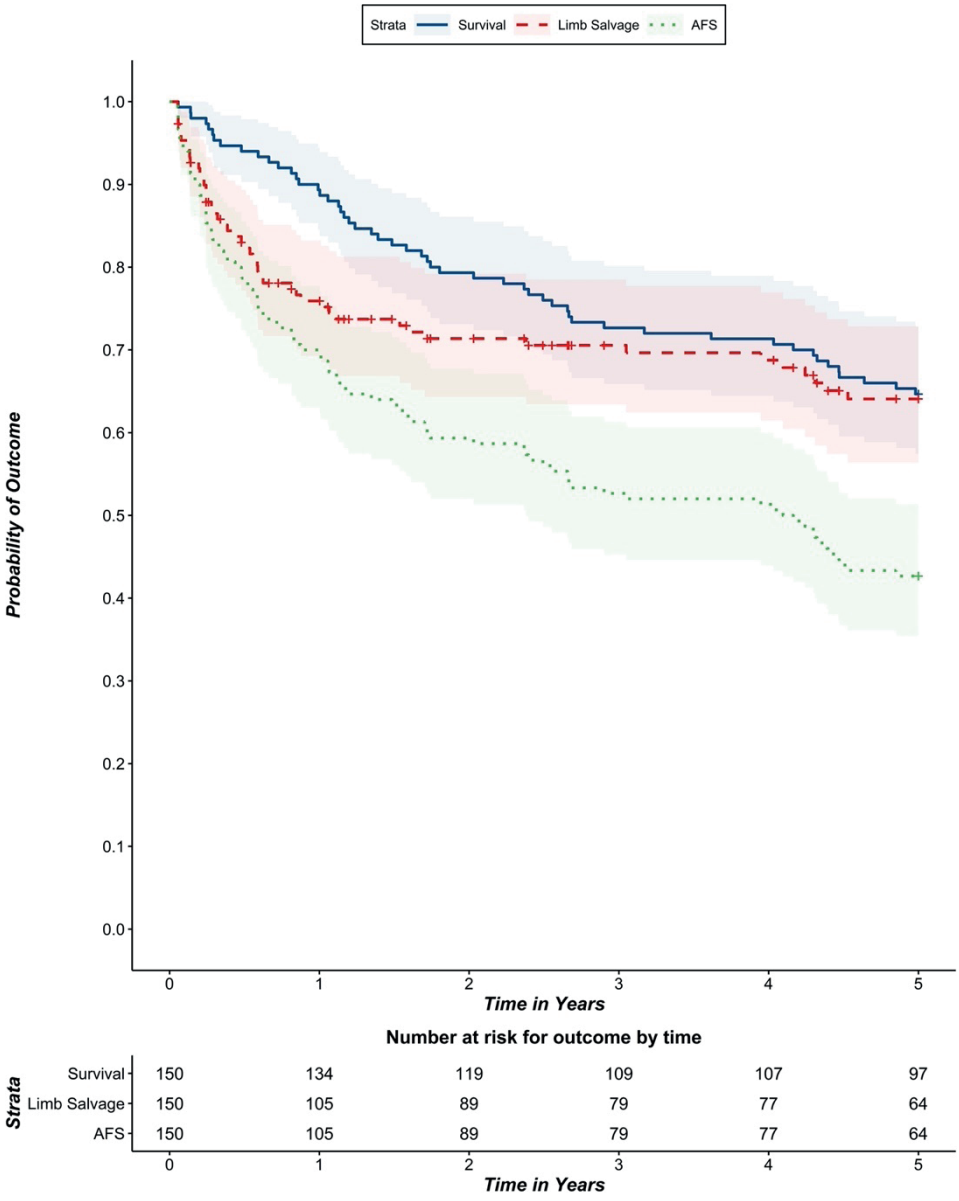

Figure 1 - Kaplan Meier Curve for amputation free-survival, survival and limb salvage
Kaplan-Meier curves for amputation free-survival, survival and limb salvage during a 5-year period, with confidence intervals. Censoring in limb salvage is applied when a patient dies, there is no loss to follow-up.

Table I. Patient characteristics stratified by endpoint

	Amputation Free Survival N = 64	*Amputation or Mortality N = 86*	*P value*
Gender = Female (%)	27 (42.2)	21 (24.4)	.033
Age (median [IQR])	60.50 [51.50, 70.00]	71.00 [62.25, 79.00]	<.001
BMI (median [IQR])	26.56 [24.53, 29.17]	25.15 [22.72, 27.77]	.055
Peripheral Artery Disease			
Rutherford Classification (%)			.004
Rutherford 4	31 (48.4)	20 (23.3)	
Rutherford 5	31 (48.4)	59 (68.6)	
Rutherford 6	2 (3.1)	7 (8.1)	
History of (%)			
Cerebrovascular Event	2 (3.1)	20 (23.3)	.001
Cardiogenic chest pain	15 (23.4)	44 (51.2)	.001
Coronary Intervention	13 (20.3)	32 (37.2)	.040
Contralateral Major Amputation	2 (3.1)	8 (9.3)	.24
Contralateral Minor Amputation	3 (4.7)	5 (5.8)	1.0
Ipsilateral Minor Amputation	5 (7.8)	10 (11.6)	.62
Contralateral Bypass	9 (14.1)	16 (18.6)	.61
Contralateral PTA or Stent	13 (20.3)	21 (24.4)	.69
Ipsilateral Bypass	34 (53.1)	41 (47.7)	.62
Ipsilateral PTA or Stent	37 (57.8)	53 (61.6)	.76
Dialysis	2 (3.1)	3 (3.5)	1.0
Hypertension	37 (59.7)	53 (63.1)	.80
Diabetes Mellitus	19 (29.7)	37 (43.0)	.13
Smoking			.12
Never	6 (9.4)	15 (17.9)	
History of Smoking	36 (56.2)	51 (60.7)	
Currently	22 (34.4)	18 (21.4)	
Medication, use of (%)			
Antiplatelets			.008
None	20 (31.2)	25 (29.1)	
Aspirin	41 (64.1)	39 (45.3)	
Clopidogrel	1 (1.6)	6 (7.0)	
Aspirin + Clopidogrel	1 (1.6)	13 (15.1)	
Persantin	1 (1.6)	3 (3.5)	
Anticoagulants			.74
None	42 (65.6)	51 (59.3)	
Acenocoumarol	19 (29.7)	29 (33.7)	
Fenprocoumon	3 (4.7)	6 (7.0)	
Lipid Lowering Drugs			.97
None	10 (15.6)	15 (17.4)	
Statin	51 (79.7)	66 (76.7)	
Ezetimibe	0 (0.0)	1 (1.2)	
Statin + Ezetimib	3 (4.7)	4 (4.7)	

Table I. Continued.

	Amputation Free Survival N = 64	*Amputation or Mortality N = 86*	*P value*
ACE inhibitors	20 (31.2)	38 (44.2)	.15
Angiotensin-2 receptor blockers	13 (20.3)	18 (20.9)	1.0
Diuretics	22 (34.4)	46 (53.5)	.031
Beta-blockers	24 (37.5)	42 (48.8)	.22
Laboratory Results			
Glomerular Filtration Rate (median [IQR])	78.36 [64.12, 86.76]	62.04 [44.34, 86.83]	.013
Total cholesterol (mmol/l) (median [IQR])	4.40 [3.50, 5.17]	4.20 [3.32, 4.80]	.15
Triglycerides (mmol/l) (median [IQR])	1.40 [0.90, 1.92]	1.45 [1.00, 2.05]	.44
HDL cholesterol (mmol/l) (median [IQR])	1.32 [0.96, 1.55]	1.06 [0.84, 1.30]	.005
Hemoglobin (mmol/l) (median [IQR])	8.40 [7.88, 8.95]	7.80 [7.12, 8.50]	.004
Thrombocytes ($\times 10^3/mm^3$) (median [IQR])	283 [223, 330]	279.50 [234, 343]	.92
Leucocytes ($\times 10^3/mm^3$) (median [IQR])	7.90 [6.83, 9.72]	8.55 [7.03, 10.15]	.17
Outcomes			
Mortality	0	53	
Amputation	0	49	

IQR, interquartile range; *PTA,* percutaneous transluminal angiography; *ACE,* Angiotensin-converting-enzyme; *HDL,* high-density lipoprotein; parametric continuous data was tested with the Students t-test, non-parametric continuous data was tested with the Mann-Whitney U test, categorical test were performed with Fisher's exact test.

The results of univariate and multivariate Cox proportional hazard regression analyses are detailed in Table II for the composite outcome and Table III for individual outcomes. Age (HR 1.77 (95% CI 1.35-2.32); P < .001), Rutherford 5 (HR 1.79 (95% CI 1.07-2.99); P = .027), Rutherford 6 (HR 3.48 (95% CI 1.46-8.27); P = .005) and HDL-cholesterol (HR 0.68 (95% CI 0.53-0.88); P = .003) were independent predictors for the composite of amputation and mortality. Figure 2 presents AFS for these predictors, in which the continuous variables age and HDL-cholesterol are categorized based on their median value. Similarly; history of a cerebrovascular event (HR 2.49 (95% CI 1.19-5.20); P = .015), history of contralateral amputation (HR 3.3 (95% CI 1.44-7.60); P = .005), higher leukocytes (HR 1.48 (95% CI 1.12-1.95); P = .006) and lower hemoglobin (HR 0.72 (95% CI 0.56-0.92); P = .010) were predictors for amputation, whereas age (HR 2.26 (95% CI 1.49-3.44); P < .001), lower glomerular filtration rate (HR 0.64 (95% CI 95% CI 0.47-0.87); P = .004) and HDL cholesterol (HR 0.54 (95% CI 0.39-0.76); P < .001) were independent predictors of mortality. For all of these, the proportional hazard assumption holds and thus these predictors were not time-dependent.

Table II. Results of Cox proportional hazard regression analyzing the predictors for the composite of amputation and death

	Composite endpoint N = 86			
	Univariate analysis		Multivariate analysis [c]	
	HR (95% CI for HR)	P value	HR (95% CI for HR)	P value
Gender (Female)	0.63 (0.38-1)	.062		
Age [b]	1.73 (1.3-2.3)	<.001	1.77 (1.35-2.32)	<.001
BMI [b]	0.97 (0.92-1)	.21		
Rutherford 5 [a]	2.08 (1.25-3.46)	.005	1.79 (1.07-2.99)	.027
Rutherford 6 [a]	4.05 (1.71-9.61)	.001	3.48 (1.46-8.27)	.005
Cerebrovascular Event	2.93 (1.8-4.9)	< .001		
Cardiogenic chest pain	2.07 (1.4-3.2)	< .001		
Coronary Intervention	1.65 (1.1-2.6)	.026		
Contralateral Major Amputation	1.99 (0.96-4.1)	.063		
Contralateral Minor Amputation	1.19 (0.48-2.9)	.70		
Ipsilateral Minor Amputation	1.15 (0.59-2.2)	.68		
Contralateral Bypass	1.29 (0.75-2.2)	.35		
Contralateral PTA or Stent	1.13 (0.69-1.8)	.63		
Ipsilateral Bypass	0.88 (0.58-1.3)	.56		
Ipsilateral PTA or Stent	1.19 (0.77-1.8)	.44		
Dialysis	0.87 (0.27-2.7)	.81		
Diabetes Mellitus	1.45 (0.94-2.2)	.090		
ACE inhibitors	1.39 (0.91-2.1)	.13		
Angiotensin-2 receptor blockers	1.05 (0.62-1.8)	.87		
Diuretics	1.64 (1.1-2.5)	.023		
Beta-blockers	1.37 (0.9-2.1)	.14		
Glomerular Filtration Rate [b]	0.81 (0.65-1)	.064		
Total cholesterol [b]	0.85 (0.69-1)	.12		
Triglycerides [b]	1.06 (0.86-1.3)	.57		
HDL cholesterol [b]	0.74 (0.59-0.92)	.008	0.68 (0.53-0.88)	.003
Hemoglobin [b]	0.73 (0.61-0.88)	< .001		
Thrombocytes [b]	1.07 (0.86-1.3)	.56		
Leucocytes [b]	1.15 (0.94-1.4)	.17		

Empty fields are not entered into the final model; [a] Rutherford 5 and 6 are compared with Rutherford 4 stage [b] Non-parametric continuous data was log transformed and scaled to provide an HR per standard deviation increase. [c] Multivariate HRs were calculated with the Cox proportional hazard analysis using a forward stepwise approach (derived from factors with P < .10 in univariate analysis).

Table III. Results of Cox proportional hazard regression analyzing the predictors for amputation and mortality

	Amputation N = 49				Mortality N = 53			
	Univariate analysis		Multivariate analysis[c]		Univariate analysis		Multivariate analysis[c]	
	HR (95% CI for HR)	P value	HR (95% CI for HR)	P value	HR (95% CI for HR)	P value	HR (95% CI for HR)	P value
Gender (Female)	0.8 (0.43-1.5)	.48			0.51 (0.26-1)	.049		
Age [b]	1.27 (0.93-1.7)	.14			2.27 (1.5-3.5)	<.001	2.26 (1.49-3.44)	<.001
BMI [b]	0.93 (0.87-1)	.061			0.97 (0.91-1)	.40		
Rutherford 5[a]	2.24 (1.11-4.55)	.025			2.26 (1.15-4.42)	.018		
Rutherford 6[a]	5.20 (1.77-15.3)	.003			2.69 (0.86-8.46)	.09		
Cerebrovascular event	2.06 (1.02-4.2)	.044	2.49 (1.19-5.20)	.015	2.28 (1.2-4.3)	.012		
Cardiogenic chest pain	1.47 (0.84-2.6)	.18			2.54 (1.5-4.4)	<0.001		
Coronary Intervention	1.49 (0.83-2.7)	.18			1.58 (0.91-2.8)	.11		
Contralateral Major Amputation	3.16 (1.4-7)	.010	3.3 (1.44-7.60)	.005	0.75 (0.23-2.4)	.62		
Contralateral Minor Amputation	1.26 (0.39-4.1)	.70			1.61 (0.58-4.5)	.36		
Ipsilateral Minor Amputation	0.76 (0.27-2.1)	.60			1.63 (0.77-3.5)	.20		
Contralateral Bypass	1.56 (0.79-3)	.20			0.89 (0.42-1.9)	.75		
Contralateral PTA or Stent	1.27 (0.67-2.4)	.46			0.85 (0.44-1.7)	.64		
Ipsilateral Bypass	1.02 (0.58-1.8)	.95			0.66 (0.38-1.1)	.13		
Ipsilateral PTA or Stent	1.37 (0.76-2.5)	.30			0.85 (0.5-1.5)	.57		
Dialysis	1.03 (0.25-4.2)	.97			1.04 (0.25-4.3)	.96		
Diabetes Mellitus	1.78 (1-3.1)	.043			0.94 (0.54-1.7)	.84		
ACE inhibitors	1.41 (0.8-2.5)	.24			1.3 (0.76-2.2)	.34		
Angiotensin-2 receptor blockers	0.89 (0.43-1.8)	.76			0.78 (0.38-1.6)	.49		
Diuretics	1.3 (0.74-2.3)	.36			1.94 (1.1-3.3)	.017		
Beta-blockers	0.95 (0.53-1.7)	.85			1.96 (1.1-3.4)	.016		
Glomerular Filtration Rate [b]	1.08 (0.81-1.5)	.59			0.56 (0.43-0.73)	<.001	0.64 (0.47-0.87)	.004

Table III. Continued.

	Amputation N = 49				Mortality N = 53			
	Univariate analysis		Multivariate analysis[c]		Univariate analysis		Multivariate analysis[c]	
	HR (95% CI for HR)	P value	HR (95% CI for HR)	P value	HR (95% CI for HR)	P value	HR (95% CI for HR)	P value
Total cholesterol [b]	0.72 (0.55-0.95)	.020			0.87 (0.67-1.1)	.29		
Triglycerides [b]	0.93 (0.7-1.2)	.59			1.12 (0.85-1.5)	.41		
HDL cholesterol [b]	0.76 (0.57-1)	.057			0.62 (0.47-0.83)	.001	0.54 (0.39-0.76)	<.001
Hemoglobin [b]	0.69 (0.54-0.88)	.002	0.72 (0.56-0.92)	.010	0.71 (0.55-0.9)	.005		
Thrombocytes [b]	1.39 (1.1-1.8)	.019			0.83 (0.63-1.1)	.20		
Leucocytes [b]	1.3 (1-1.7)	.052	1.48 (1.12-1.95)	.006	1.05 (0.81-1.4)	.72		

Empty fields are not entered into the final model; [a] Rutherford 5 and 6 are compared with Rutherford 4 stage [b] Non-parametric continuous data was log transformed and scaled to provide an HR per standard deviation increase. [c] Multivariate HRs were calculated with the Cox proportional hazard analysis using a forward stepwise approach (derived from factors with P < .10 in univariate analysis).

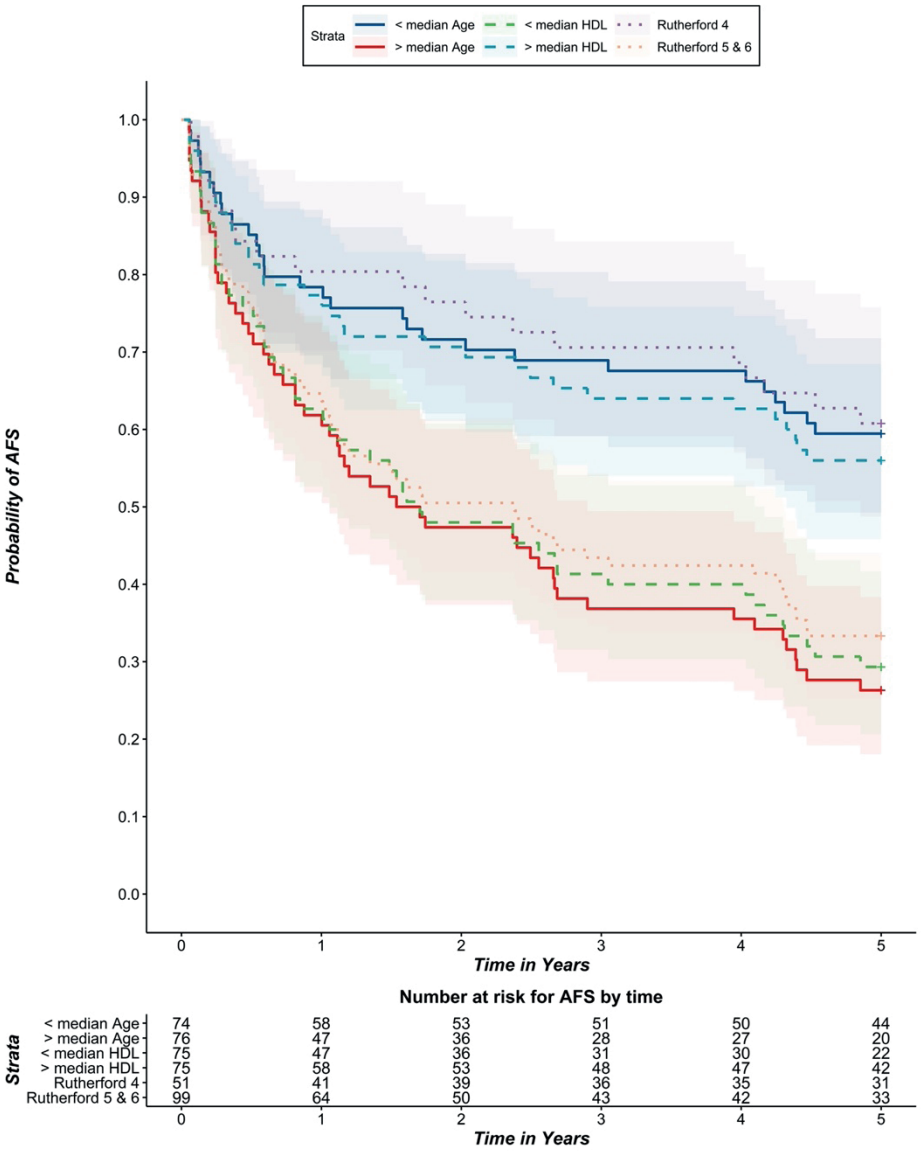

Figure 2 - Kaplan Meier Curves for amputation-free survival stratified by (categorized) predictors
Kaplan-Meier amputation-free survival curves during a 5-year period, with confidence intervals, stratified by independent risk factors (Rutherford classification, median age and median HDL). For a more simplified presentation, Rutherford 5 and Rutherford 6 are combined.

DISCUSSION

The long-term prognosis in our well-defined and granulated CLTI population without options for revascularization (NR-CLTI) revealed to be poor with 43% of the patients completing five years survival without limb loss. This result was driven by an equal rate of all-cause mortality and amputation: one third of the patients died (35%) and one third underwent amputation of the index limb (33%). Furthermore, a third of those with limb loss after inclusion died within the five-year time-interval (33%).

Our data correspond to the findings of a small long-term retrospective observational study (N = 30), the only published equivalent, reporting a five year mortality of 30% for this NR-CLTI subgroup.(15) No registry studies have been performed and thus prognostic information for NR-CLTI patients is very limited. As such, our data provide the best available insight in nowadays perspective for these patients in terms of mortality and limb salvage.

Two registry studies concerning the 'real-world' CLTI-population reported a higher all-cause mortality rate of 57% and 54% for five and four years respectively. This may relate to the fact that our study-population was younger and had lower prevalences of history of coronary artery disease and DM.(1,16) In trial-selected patients treated for severe limb ischemia, BASIL reported an AFS of 38% within the completed follow-up (3 – 7 years), which was mainly driven by mortality (56%), possibly due to an older study-population.(17) Although the overall amputation rate was not given, only 7% of the patients that were alive at the final follow-up underwent amputation, compared to 22% in our study. This seems particularly high, but four year amputation rates of CLI patients in a retrospective cohort and according to Rutherford stages 4,5 and 6 (12%, 35%, and 67%, respectively) were more comparable with ours (20%, 38% and 56%, respectively).(18) In our cohort, 33% of those who underwent amputation subsequently died within the investigated period. This rate is relatively low as amputation is an established risk factor for death and five year mortality rates have been reported up to 85% in elderly CLI amputees, and seven year rates after below and above the knee amputations in a Veteran cohort (published in 2003) were 72% and 80%, respectively.(19–21) However, subjects were much older in both studies which troubles comparison.

More published evidence is available on the short-term outcomes of this subgroup. At one year, NR-CLTI patients in JUVENTAS were at an especially high risk of amputation (24% of total), but mortality was lower (11%). In comparison, two meta-analyses reported one-year amputation rates of 22% and 34%, and mortality rates of 22% and 20%.(9,22) This is perhaps due to a similar design of some of the included studies in these meta-analyses: most recent short-term prognostic data are derived from small RCTs investigating gene or cell therapy in no-option patients.(23–25) Other (older) case series included in these meta-analyses do not provide up-to-date information for the current CLTI population, especially since recent

studies show gradual reduction of amputation and mortality rates.(1,9–11,26,27)

Short- and long-term results considered, our results indicate that an NR status is associated with an increased early risk of major amputation, although this risk is weaning off in the subsequent years. In contrast, mortality is fairly evenly distributed along the follow-up. This is important for both patients and physicians, and might imply that an NR status is not the primary cause of mortality, but rather a gradation of a common denominator: progressive systemic atherosclerotic disease. Direct comparison between CLTI and NR-CLTI is difficult, but outcomes are generally in the same order of magnitude. In contrast, a more benign (PAD) population with means of intervention recently revealed considerably better outcomes, as all-cause mortality and amputation rates of just 9.1% and 3.5% at 3 years in the placebo-arm of the recent VOYAGER-trial demonstrate.(28) Since the difference in outcomes for CLTI and NR-CLTI patients is less pronounced than that of CLTI- and IC-patients, an NR status is perhaps not a major risk factor. With regard to this concept, although long-term prognosis is poor, we believe that an NR status in CLTI does not drive towards immediate amputation per se if best medical/wound treatment can be applied, contrary to what perhaps seems the general belief of vascular specialists, and neither does this amputation always lead to premature death (as compared to CLTI patients with revascularization options). The emphasis for management of these high-risk patients should therefore lie on strategies to decrease the amputation risk in the short term, and enable optimal management of comorbidities in the long term. This approach could facilitate vascular specialists in medical management and patient counseling and is otherwise crucial in the design of future regenerative trials, and their selection of patients.

Putting our outcomes into perspective is troublesome due to a paucity of prognostic data for NR-CLTI, and heterogeneity of study design and populations. The disparity of our results with some of the current literature could be attributed to a trial effect, selection bias, definition and time. The so-called 'trial-effect', has been suggested to influence outcome, although little evidence is available on this topic.(29,30) However, extensive care and strict surveillance, as implemented in these trials, are thought to reduce adverse outcomes in cardiovascular disease and thus hypothetically support this claim.(31–33) If these assumptions are valid, our relatively benign results compared to the CLTI registry studies suggest that extensive care could improve the prognosis of the no-option patient significantly, even for a relatively short amount of time (as in this study), and thus more effort is warranted to enable optimal management.

On the other hand, differences of our outcomes, caused by a discrepancy of real-world and trial patients, are possibly the result of selection bias. The participation in a time-consuming study with potential adverse events could potentially favor a compliant patient with ultimately a lower a-priori risk for mortality due to better adherence and disease awareness.

Undoubtedly, the exclusion criteria in JUVENTAS in combination with an average-to-good ambulatory state (a non-ambulatory state disincentives to participation due to the frequency of follow-ups) influences both short- and long-term outcomes.(34–36) Furthermore, there is a lack of a standard definition of 'no-option', which could comprise of patients without feasible intervention, and patients whose medical condition is too frail to justify the exposure to additional intra- and post-operative risks. Patients included in JUVENTAS match the first category, as established by a multidisciplinary team of vascular surgeons and radiologists in an academic hospital. However, other mentioned studies combined these categories, which subsequently influences these outcomes.(25–27)

Whether the no-option patient of today is comparable to no-option patients of 10 or 20 years ago in terms of AFS is arguable as secondary and tertiary prevention have improved and innovations have led to improved revascularization alternatives.(1,37) Furthermore, a time-depending shift in etiology (macro- to microvascular) could lead to different patient characteristics. However, the main principle of our no-option definition remains the same: all patients are subject to inadequate perfusion, resulting in high grade ischemia, without any means of treatment in the foreseeable future. A uniform description should be considered for general use and research, in which we propose an emphasis on the 'no-option anatomy' category, as mentioned in the Global Vascular Guidelines on the management of CLTI.(38)
A limitation of our analysis is the extension of original follow-up without additional contacts or visits within this interval. However, the endpoints remained the same and almost no loss to follow-up occurred. The two patients lost to follow-up were removed from analysis because there was a significant gap between their last confirmed medical status and five-year follow-up. We included both treatment and placebo arms in our analyses. The JUVENTAS trial did not find a treatment related effect on AFS. At five years we reaffirm no difference in AFS (46 vs 40, P = .53), amputation (27 vs 22, P = .56) or mortality (29 vs 24, P = .58) for treatment versus placebo, respectively.(13) Thus, including patients from both trial arms is justified.
In conclusion, the current study provides the necessary contemporary long-term follow-up data for NR-CLTI patients. The poor amputation free survival and general survival underscores the poor prospect of these patients. In comparison with other studies, our analysis suggests that AFS and survival in NR-CLTI are no worse than in CLTI patients with revascularization options.

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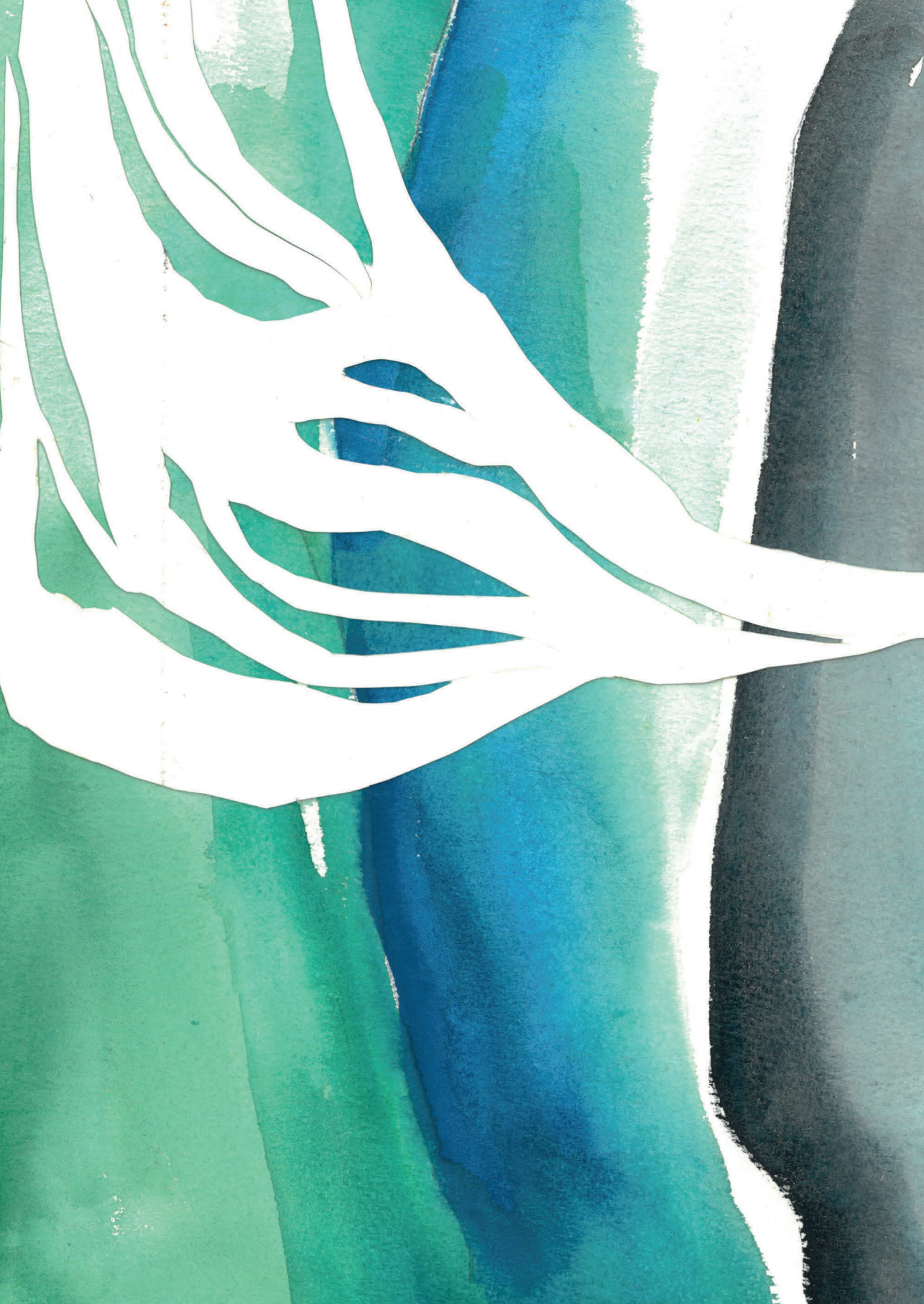

PART II

A different perspective on CLTI

CHAPTER 4

The Global Limb Anatomic Staging System (GLASS) for CLTI: Improving Inter-observer Agreement

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Journal of Clinical Medicine 2021 Aug 4;10(16):3454

ABSTRACT

Objective

The 2020 Global Vascular Guidelines aim at improving decision making in Chronic Limb-Threatening Ischemia (CLTI) by providing a framework for evidence-based revascularization. Herein, the Global Limb Anatomic Staging System (GLASS) serves to estimate the chance of success and patency of arterial pathway revascularization based on the extent and distribution of the atherosclerotic lesions. We report the preliminary feasibility results and observer variability of the GLASS.

Methods

GLASS separately scores the femoropopliteal (FP) and infrapopliteal (IP) segment based on stenosis severity, lesion length and the extent of calcification within the target artery pathway (TAP). In our step-wise approach we used two angiographic datasets. Each following step was based on the lessons learned from the previous step. The primary outcome was inter-observer agreement measured as Cohen's Kappa, scored by two (step 1+2) and four (step 3) blinded and experienced observers respectively. Step 1 (n=139) and 2 (n=50) were executed within a dataset of a Dutch interventional RCT in CLTI. Step 3 (n=100) was performed in randomly selected all-comer CLTI patients from two vascular centers in the United States.

Results

In step 1 kappa values were 0.346 (FP) and 0.180 (IP). In step 2, applied in the same dataset, the use of other experienced observers and a provided TAP, resulted in similar low kappa values 0.406 (FP) and 0.089 (IP). Subsequently, in step 3, the formation of an altered step-wise approach using component scoring, such as separate scoring of calcification and adding a ruler to the images resulted in kappa values increasing to 0.796 (FP) and 0.730 (IP).

Conclusion

This retrospective GLASS validation study revealed low inter-observer agreement for unconditioned scoring. A step wise component scoring provides acceptable agreement and a solid base for further prospective validation studies to investigate how GLASS relates to treatment outcomes.

INTRODUCTION

While Chronic Limb-Threatening Ischemia (CLTI) represents less than 10% of all PAD patients, it comes with a considerable burden in terms of morbidity, mortality and socio-economic costs. Despite improvement of the therapeutic armament, the amputation rate is up to 20% at twelve months(1,2) while over 50% of all CLTI patients die within 5 years after presentation.(3,4)

Choices for revascularization are still not standardized and largely based on expert opinion and personal preference of the physician treating the patient(5). Existing anatomic classifications in PAD are based on the location and severity of individual arterial lesions (e.g. TASC)(6) or quantify the overall burden of atherosclerotic disease.(7) These individual lesion-based classification systems correlate poorly with clinically effective revascularization in patients with CLTI, and leave vascular specialists trying to integrate data for arterial segments into a management strategy for the whole limb.

The 2020 Global Vascular Guidelines aims at improving structured decision making in CLTI by providing a framework for evidence-based revascularization (EBR).(8) This framework is composed of three dimensions; 1. Patient risk; 2. Limb status; and 3. ANatomical pattern (PLAN). Components of each of those three dimensions are respectively: The Vascular Quality Initiative prediction model (VQI) for determining overall patient risk,(9) WIfI for limb staging, and the Global Limb Anatomic Staging System (GLASS) for identifying different anatomical patterns of disease and related chance of success of revascularization. The writing group of the Global Vascular Guideline defined three GLASS stages based on the likelihood of immediate technical failure and one-year limb-based patency (LBP) following endovascular intervention of the selected TAP.

Although already incorporated in the guideline and suggested as a promising additional tool for EBR strategies to predict the success of lower extremity arterial revascularization, GLASS needs proper prospective validation. Our goal was to examine the consistency of GLASS scoring and to maximize inter-observer agreement to facilitate its application.

METHODS & RESULTS

GLASS scoring principles

The GLASS scoring principles have been reported previously in detail.8 In short GLASS staging requires separate scoring of the femoropopliteal (FP) and infrapopliteal (IP) segments (Figure 1A and 1B). Before doing so, the observer must identify the target artery pathway (TAP), which is the preferred IP artery for revascularization in the case (Figure 2).

Femoro-popliteal (FP) Grading	
0	Mild or no significant (<50%) disease
1	Total length SFA disease <1/3 (<10 cm); may include single focal CTO (< 5 cm) as long as not flush occlusion; popliteal artery with mild or no significant disease
2	Total length SFA disease 1/3-2/3 (10-20 cm); may include CTO totaling < 1/3 (10 cm) but not flush occlusion; focal popliteal artery stenosis <2 cm, not involving trifurcation
3	Total length SFA disease >2/3 (>20 cm) length; may include any flush occlusion <20 cm or non-flush CTO 10-20 cm long; short popliteal stenosis 2-5 cm, not involving trifurcation
4	Total length SFA occlusion > 20 cm; popliteal disease >5 cm or extending into trifurcation; any popliteal CTO

Figure 1A - Femoropopliteal (FP) disease grading in GLASS
*involvement of trifurcation means disease includes the origin of either the anterior tibial or tibioperoneal trunk
*severe calcification (e.g. >50% of circumference, diffuse, bulky, or "coral reef" plaques) within the TAP increases the within-segment grade by +1

Infra-popliteal (IP) Grading	
0	Mild or no significant (<50%) disease
1	Focal stenosis <3 cm not including TP trunk
2	Total length of target artery disease < 1/3 (<10 cm); single focal CTO (< 3 cm not including TP trunk or target artery origin)
3	Total length of target artery disease 1/3- 2/3 (10-20 cm); CTO 3-10 cm (may include target artery origin, but not TP trunk)
4	Total length of target artery disease >2/3 length; CTO > 1/3 (>10 cm) of length (may include target artery origin); any CTO of TP trunk

Figure 1B - Infrapopliteal (IP) disease grading in GLASS
*IP grading is applied only to the primary selected vessel in the TAP
*severe calcification (e.g. >50% of circumference, diffuse, bulky, or "coral reef" plaques) within the TAP increases the within-segment grade by +1
*TP trunk disease is only included if the TAP is the posterior tibial or peroneal artery

The TAP is defined by the proceduralist and thus identified either prospectively during a case, from operative notes, or based on imaging evidence of the IP artery that was primarily targeted for intervention. In the absence of such information the least diseased IP artery on imaging is selected as the TAP. Furthermore, it is important to realize that GLASS was originally designed to be used for angiographic imaging. Hence, all the imaging and scoring that has been done in this study, pertains to angiograms.

Combinations of grade scores for the FP and IP segments are used to define three GLASS stages (table 1) based on estimating the likelihood of immediate technical success and 12-month LBP, defined as maintained patency of the TAP following endovascular intervention. GLASS stages for the limb thus reflect a gradient of TAP complexity:

- Stage I: Average Complexity Disease: technical failure < 10% AND >70% 12-month LBP
- Stage II: Intermediate Complexity Disease: technical failure < 20% AND

12-month LBP 50-70%

- Stage III: High Complexity Disease: technical failure >20%; OR <50% 12-month LBP

For the present retrospective study we used existing data sets to validate the GLASS. We report on the three stages that we run through in order to improve inter-observer agreement levels for GLASS scoring. The three steps were not predetermined. Each following step was the resulting effect of the outcome of the previous one.

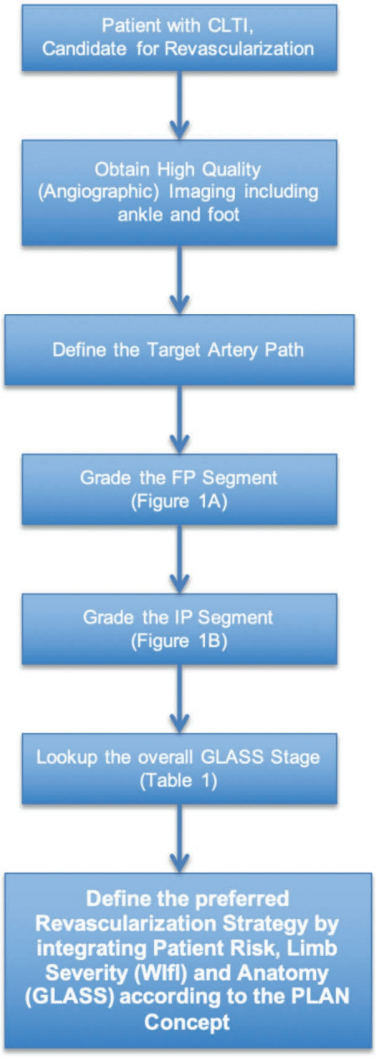

Figure 2 - Flowchart illustrating use of GLASS for staging infrainguinal arterial disease

Table 1. GLASS stages

	Infrainguinal GLASS Stage					
FP	4	III	III	III	III	III
Grade	3	II	II	II	III	III
	2	I	II	II	II	III
	1	I	I	II	II	III
	0	NA	I	I	II	III
		0	1	2	3	4
	IP Grade					

FP = femoropopliteal, IP = infra-popliteal

Statistics

Cohen's kappa values for variability were obtained using SPSS for Windows version 25.0 (SPSS Inc., Chicago, IL). Kappa values of < 0 reflects 'poor', 0 to 0.20 'slight', 0.21 to 0.4 'fair', 0.41 to 0.60 'moderate', 0.61 to 0.8 'substantial', and above 0.81 'almost perfect' agreement. In addition to ordinal percentage agreement calculation, Kappa considers the possibility of the agreement occurring by chance. These statistics were applied in this and all subsequent steps.

Step I – Initial inter-observer analysis within RCT data

The inter-observer variability of the original GLASS was examined retrospectively in the Dutch multicenter PADI trial cohort(10) (Percutaneous Transluminal Angioplasty Versus Drug-Eluting Stents for Infrapopliteal Lesions in Critical Limb Ischemia), consisting of 139 patients eligible for predominantly infrapopliteal revascularization enrolled between 2007 and 2013. The study protocol, detailed patient characteristics and study results were previously published.9 GLASS stage was determined based on the scoring system provided in figure 1A and 1B. Scoring was done by two experienced and independent radiologists. All 139 cases were used during step I.

Results step I

Kappa for FP was 0.346 [95% CI 0.126-0.566] and kappa for 0.180 [95% CI 0.078-0.282] (IP); fair and slight interobserver agreement respectively. Evaluating the scoring process together with the observers, resulted in a strong belief that specific components in the scoring system, such as 'severe calcification', are more responsible for causing low agreement rates than others. Therefore, we decided that calcification kappa should be determined independently; resulting in a kappa value of 0.208 [95% CI -0.116-0.532] for FP calcification and 0.071 [95% CI -0.080-0.231] for IP calcification. It was also necessary to rule out certain potential factors contributing to variability such as differences in the observer-selected target artery pathway. Investigating the role of these factors lead to the second step in our validation process.

Step II – Calibration series within Dutch RCT

Step 2 consisted of a second scoring series by two vascular surgery fellows in the UCSF Medical Center (San Francisco, CA), where diagnostic angiographies are incorporated in standard patient work-up and daily clinical practice. 50 random cases from the same PADI cohort were scored using the same method as in phase 1, however prior to scoring each limb, the TAP was predefined and provided to both independent observers.

Results step II

Kappa for FP was 0.406 [95% CI 0.102-0.710] and kappa for IP was 0.089 [95% CI -0.007-0.171]; again, fair and slight agreement respectively. These findings confirmed that the high variability between observers was not caused by the background nor the TAP selection. Therefore, we aimed to identify potential contributing factors by splitting the original GLASS into a non-composite component scoring system. Making it possible to assess each separate component within the GLASS scoring system.

Step III - Adjusted GLASS inter-observer variability in US CLTI cohort

In step 3 we adjusted the scoring routine (table 2), based on the findings in step 1 & 2, using component scoring to stimulate a more systematic approach. A total of 100 all-comer CLTI patients were randomly and retrospectively selected from two vascular centers in the United States; the Baylor College of Medicine (Houston, TX) and the UCSF Medical Center (San Francisco, CA). Each center provided 50 cases and two observers. So, two separate 2x2 comparisons were done. To be able to trace back consistent discrepancies we designed a roadmap for each segment by using only non-composite components scoring (table 2). This approach enabled us to determine which component scores performed relatively better and worse in terms of observer agreement levels.

Results step III-A

Almost all kappa's for component scores increased drastically compared to the initial FP and IP combination scores. For instance, the total occlusion kappa value for the superficial femoral artery (SFA) and infrapopliteal (IP) trajectory reached almost 0.5. Contrarily, calcification alone showed much lower agreement levels (table 3). In particular determining "severe calcification" in the IP trajectory caused high variability rates.

Results step III-B

After consulting the observers in a "consensus session," we found out that estimating length was another common factor amongst component scores causing high variability rates. This was confirmed in a sample of 20 randomly selected cases within the US cohort by adding a ruler to the images (for the purpose of measuring lesion length adequately), Kappa values increased to 0.796 [95% CI 0.656-0.936] (FP) and 0.730 [95% CI 0.61-0.85] (IP), representing substantial interrater agreement.

Table 2. improved step-wise method

SFA-segment
Total stenosis length
0 = mild or no significant (<50%) disease
1 = <1/3 (<10 cm)
2 = 1/3-2/3 (10-20 cm)
3 = >2/3 (>20 cm)

CTO
0 = no CTO
1 = <5 cm
2 = <1/3 (10 cm)
3 = flush occlusion <20 cm
4 = non-flush CTO 10-20 cm long
5 = SFA occlusion > 20 cm
Popliteal-segment

Total stenosis length
0 = mild or no significant disease
1 = <2 cm
2 = 2-5 cm
3 = >5 cm
4 = extending into trifurcation
5 = popliteal CTO
Infra-popliteal segment

Total stenosis length
0 = mild or no significant (<50%) disease
1 = <3 cm
2 = < 1/3 (<10 cm)
3 = 1/3- 2/3 (10-20 cm)
4 = >2/3
CTO
0 = no CTO
1 = single focal CTO (< 3 cm not including TP trunk or target artery origin)
2 = 3-10 cm (may include target artery origin, but not TP trunk)
3 = > 1/3 (>10 cm) of length (may include target artery origin)
4 = any CTO of TP trunk

SFA = superficial femoral artery, CTO = chronic total occlusion

Table 3. Kappa scores divided by component and center

Component score	UCSF 50	Baylor 50
SFA TSL	0.399 [0.195-0.603]	0.557 [0.398-0.736]
SFA CTO	0.498 [0.242-0.754]	0.486 [0.246-0.726]
FP Calc++	0.143 [-0.097-0.383]	0.483 [0.243-0.723]
Pop TSL	0.535 [0.354-0.716]	0.390 [0.236-0.544]
IP TSL	0.240 [0.062-0.418]	0.387 [0.217-0.557]
IP CTO	0.488 [0.324-0.652]	0.470 [0.289-0.651]
IP Calc++	-0.120 [-0.220- -0.020]	0.291 [-0.089-0.671]

SFA = superficial femoral artery, TSL = total stenosis length, CTO = chronic total occlusion, FP = femoropopliteal, IP = infra-popliteal, [95% confidence interval]

DISCUSSION

Through sequential optimization we were able to obtain moderate interrater agreement for most non-composite (component) scores in GLASS. For the most part the observer variability was attributable to the subjective aspect of estimating lesion length and severity of calcification. Although all of our observers were highly trained and experienced in reviewing angiographic imaging, high interobserver variability occurred for these components throughout the whole scoring process. Even though absolute accuracy in centimeter is not critical because the length-categories are estimates and described in proportions (e.g. 1/3 or 2/3 of length); when adding a ruler to the image interpretation, estimating lesion length became more reliable and reproducible, ultimately leading to 'almost perfect' agreement levels (kappa 0.8).

In comparison, existing classifications such as TASC show poor to moderate inter-observer agreement with kappa values ranging from 0.11 to 0.54.(11) Moreover, individual lesion- or segment-based grading systems are less useful in daily practice where complex disease patterns are commonly encountered, especially in CLTI.(11) The clinical success of revascularization, particularly in patients with tissue loss, nearly always requires the restoration of direct arterial flow to the foot. In this regard GLASS poses an important improvement over the lesion-based systems.

GLASS is a promising tool that can represent the "anatomical pattern" pillar in PLAN. When combined with tools for stratification of patient-risk and severity of limb threat, GLASS can facilitate the development of specific EBR strategies in CLTI. Furthermore, GLASS may enhance research quality in the future as it allows improved patient stratification and thus more homogeneous study populations can be formed and better comparative studies can be executed to study the effect of specific endovascular interventions(12).

Future research should evolve around two main points; prospective validation and correlation with clinical presentation, treatment success and (limb-based) patency. Furthermore, the effects of pedal arch disease and patient factors on revascularization outcomes deserve attention in future research. Moreover, based on our results it is important to search for a reproducible method to grade severity of calcification before its predictive value for infrainguinal interventions can be incorporated as factor within the GLASS scoring system. However, the system is meant for real world clinical use and the current descriptor of "severe" is meant to be used whenever the treating specialist believes that the degree of calcification would significantly diminish the outcomes of an endovascular intervention in that segment. By design, this is subjective. There is no existing data to support any approach at present, except it is well established that severe calcification is indeed an important risk factor for both technical success and patency. Additionally, it would be interesting to see

if and how artificial intelligence could play a role in an alternative approach in order to overcome described obstacles by implementing a computerized algorithm.

Our results show that accurate assessment of lesion length is essential to improve inter-observer agreement. Furthermore, severe calcification, particularly in the tibial arteries, is established as a negative predictor of technical success for interventions and portends higher amputation risk.(13,14) Considering the central role of CTA (and MRA) in diagnostic work-up in many countries, the ease of measuring lesion length with this imaging techniques and potential improved estimation and quantification of calcification underlines the need to also validate GLASS in CTA (and MRA) in future studies.

GLASS was designed for prospective use by clinicians who would define the preferred TAP based on the case at hand. In this regard it is likely that the assessment of lesion length, complete versus subtotal occlusion, and even severity of calcification will be simpler to perform by the proceduralist in real-time. A mobile app has been developed for the Society for Vascular Surgery (SVS interactive practice guideline; see provided weblink(15)) that will be released imminently for clinical use. In contrast, our study applies directly to the use of GLASS for retrospective or registry-based research studies to better compare outcomes across stages of disease complexity. In these cases, use of a specified workflow as described herein and a core lab team of readers will help to insure the most consistent grading of disease patterns.

The main limitations of this study are the limited number of observers and the limited study size, especially in the second step. While fixing the TAP is a simplification of the GLASS and could be considered a limitation of our study, we experienced that in order to be able to record interobserver agreement, a fixed TAP was an essential requirement to obtain some level of standardization. Our preliminary validation data and optimization of the GLASS scoring system should be prospectively validated in a larger study population. Furthermore, given the new aspect of the scoring system, there might be a learning curve for the observers in the process. Part of the improvement may be contributed to this phenomenon. However, the observers from Houston showed similar agreement levels in step 3 during their first run compared to their UCSF counterparts who also served in the second step. It would be interesting to see and objectivate how intra-observer agreement evolves once one becomes more familiar with the scoring system. Not including intra-observer agreement could also be considered as a limitation of this study.

CONCLUSION

We developed a step-wise approach for retrospective review of lower extremity angiograms using GLASS. Our systematic method improved the inter-observer agreement rates from an unacceptable low to an acceptable Kappa value. Further prospective validation studies using these workflows will determine the relationship between GLASS stage and treatment results in CLTI.

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CHAPTER 5

Capillaroscopy of the Nailfold in patients with Peripheral Artery Disease of the Lower Limb (CAPAD study)

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European Journal of Vascular and Endovascular Surgery 2022 Jun;63(6):900-901

Nailfold capillaroscopy (NFC) is a non-invasive and fast method for imaging of capillaries of the proximal nailfold which is frequently used for diagnostics in rheumatic diseases. At the nailfold, capillaries run perpendicular to the skin and visualizing them using a microscope enables detection of morphologic abnormalities, including a decreased number of capillaries, dilated vessels, micro-haemorrhages and neovascularization which are a result of vascular inflammation and hypoxia.

The presence of NFC abnormalities is one of the classification criteria for systemic sclerosis and can help to differentiate primary from secondary Raynaud's. It is routinely used in the rheumatology outpatient office setting. NFC abnormalities are not only found in autoimmune disease, for instance, in patients with diabetes, NFC patterns are abnormal and correlate with the presence and development of diabetic neuropathy and other diabetic complications. NFC patterns and their related predictive value in patients with peripheral artery disease (PAD) have not been reported yet. Given the role of inflammation, presence of hypoxia, and the high prevalence of diabetes among PAD patients, NFC patterns in PAD patients are likely aberrant. Therefore, we hypothesized that NFC is feasible in PAD patients attending outpatient clinics and NFC patterns are abnormal. Secondly, we aimed to investigate the association between PAD and abnormalities in terms of quantitative and qualitative NFC measurements.

Data were collected prospectively on three randomly selected days from patients visiting the outpatient clinic of a single vascular surgery unit between April and June 2018. All patients with a history of PAD (Intermittent Claudication (CI; n=17) or Chronic Limb-Threatening Ischemia (CLTI; n=9)) who consented to the procedure were eligible. Controls without a history of PAD or CLTI(n=10) were visitors accompanying the patients. The study was approved by the institutional review board and conducted in accordance with the declaration of Helsinki. All participants provided written informed consent.

NFC was performed on the digits of the upper limb by an experienced physician (FR). NFC was performed according to Cutolo et al(1). Additionally, in order to minimize noise from the impact of microtrauma, due to occupation and hobbies, only images from the fourth and fifth finger were used for analyses. After application of one drop of hypo-allergenic mineral oil, one overview image (at 100x magnification) and 4 single segment images (at 200x magnification) were obtained with the DinoLite CapillaryScope (DLC).

Image quality and qualitative parameters were assessed by an experienced rheumatologist (JS). Quantitative scoring was performed by two different observers (JW, FR) as described and validated by Cutolo et al(1) and Barchetta(2). All observers were blinded throughout the whole process.

The capillaroscopic images of 24 patients and nine controls were of sufficient quality for analysis. In only three cases image quality was poor and insufficient for analyses. Age, gender, diabetes mellitus (DM) and smoking status did not differ significantly between healthy controls (n=9), CI (n=15) and CLTI (n=9) patients.

There were no significant abnormalities in quantitative measures. Mean capillary diameter was within range of normal reference values(3). Mean capillary count per millimetre was similar in healthy controls, CI or CLTI with 6,7 (SD 0,86); 6,2 (SD 1,1); 6,5 (SD 1,5) respectively. Among the qualitative measures, both haemorrhages and non-specific qualitative abnormalities (NSQA; ranging from disturbed capillary architecture, lower density, abnormal morphology, dilatation, areas of decreased vascularity and atypical branching) were most prevalent in the CI-group. Two observations, both prominent venous plexus (PVP) and mega capillaries (MC), occurred exclusively in CLTI.

The abovementioned data shows that NFC is clinically feasible in PAD patients. While the study design and sample size did not allow for formal robust statistical analyses, patients with CLTI had abnormalities that were absent in controls and patients with CI. Strengths of this study are the fact that it was performed in a real-world situation. Furthermore, image analysis was performed by experienced, independent and blinded observers.

PVP and MC are associated with endothelial dysfunction. Biomarkers of endothelial dysfunction are present in CLTI(4). In Systemic Sclerosis the extent of abnormalities on NFC has been linked to circulating biomarkers of inflammation and endothelial dysfunction,(5) which have also been shown to be altered in CLTI. Our findings suggest that NFC abnormalities may also be used as markers for inflammation and endothelial dysfunction in PAD, although this needs to be confirmed in larger studies. This feasibility study provides pilot data for larger (prospective) follow-up studies to assess the added value of NFC in the evaluation of the poor cardiovascular prognosis in PAD and CLTI patients in particular(6). In analogy with rheumatologic diseases(7), it would be of interest to investigate whether cardiovascular risk management strategies could lead to reduction of PVP and MC. NFC may hold promise as a non-invasive, inexpensive and relatively easy diagnostic and prognostic tool in patients with PAD.

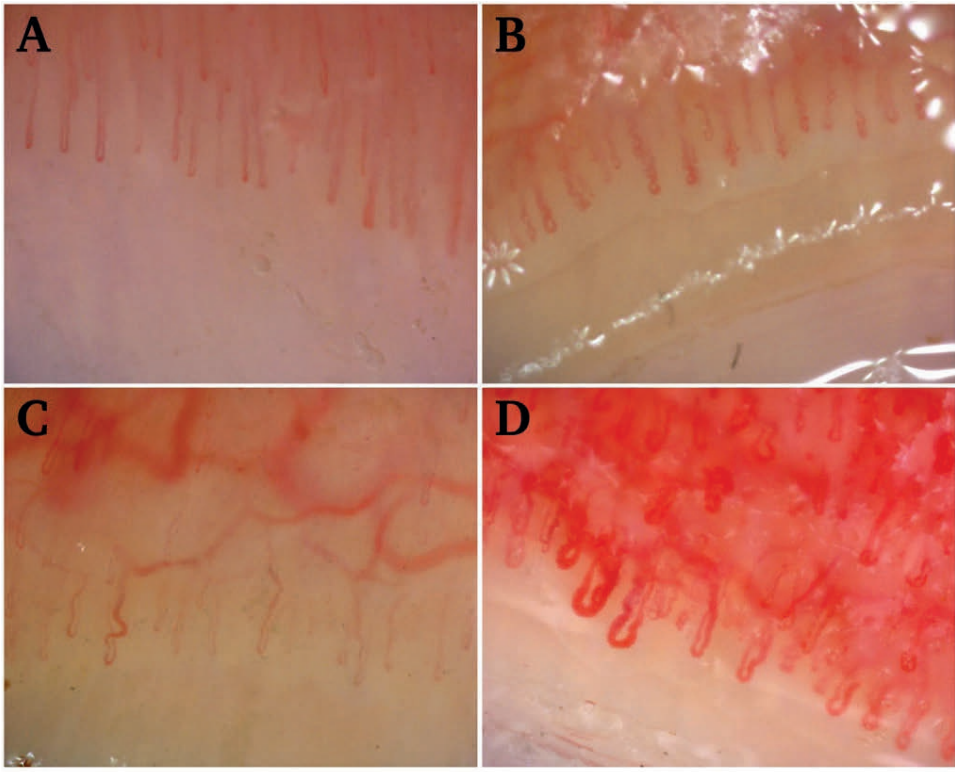

Figure 1 - Examples of nailfold abnormalities found in PAD patients with Rutherford >4
Panel A: 200x magnification. No apparent abnormalities. Panel B: 200x magnification. Dilated capillary loops and a lower capillary density. Panel C: 200x magnification. Mega capillaries. Panel D. 50x magnification. Prominent venous plexus. PAD: peripheral artery disease.

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CHAPTER 6

Applicability of transcutaneous oxygen tension measurement in the assessment of Chronic Limb-Threatening Ischemia

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Angiology 2020 Mar;71(3):208-216

ABSTRACT

Transcutaneous oxygen tension measurement (TcPO2) is widely applied for the evaluation of Chronic Limb-Threatening Ischemia (CLTI). Nevertheless, studies that focused on the clinical value of TcPO2 have shown varying results. We identified factors that potentially play a role in TcPO2 measurement variation such as probe placement, probe temperature, and the use of a reference probe. In this review of the current literature we assessed the application of these factors. A systematic search was conducted. Parameters that were assessed were probe placement, probe temperature, and mentioning and/or use of a reference probe. In total, 36 articles were eligible for analysis. In 24 (67%) studies probes were placed on specific anatomical locations. Seven (19%) studies placed probes, regardless to the location of the ulcer, adjacent to an ischemic lesion or ulcer (peri-lesion). Selected temperature setting of the probe differed; in 18 (50%) a default probe temperature of 440C was selected and in 13 (36%) a different temperature was selected. In 31 (84%) studies the use of a reference probe was not reported. TcPO2 is applied diversely in patients with CLTI. Homogeneity in TcPO2 protocols is warranted for reliable clinical application and to compare future TcPO2 research.

INTRODUCTION

Chronic limb-threatening ischemia (CLTI) is an increasing major health care problem worldwide with a large impact on quality of life, morbidity and mortality, and health care expenses.(1-3) The main treatment goals of CLTI management are wound healing, prevention of amputation and preservation of ambulation.(1) Transcutaneous oxygen tension measurement (TcPO2) has been proposed as a promising non-invasive tool for the diagnosis and evaluation of CLTI, especially in diabetes.(4,5)

The measurement of local oxygen pressure is believed to reflect the status of underlying vascularization of the skin. With the use of the so-called "Clark-electrode", pO2 is measured by a platinum cathode and a silver anode covered with a thin membrane which is permeable for oxygen.(6) The electronical reduction of oxygen allows a current to flow which is proportional to the partial pressure of oxygen.(6) To ensure dermal permeability for oxygen, the electrode is heated creating local hyperthermia liquefying the crystalline structure of the stratum corneum. Furthermore, hyperthermia creates underlying capillary vasodilatation allowing more oxygen diffusion.(7,8)

Despite the use of TcPO2 in clinical practice, its added value for the diagnosis and evaluation of therapy in CLTI patients is strongly debated as studies have shown poor to moderate reliability and reproducibility.(9-13) A recent review on TcPO2 reported a sensitivity ranging from 0.61 to 0.82 for the prognosis of diabetic foot ulcer (DFU) healing.(12) Moreover, reported TcPO2 threshold values for the detection of ischemia or potential non-healing DFU's differ significantly.10, (14-24)

Recently, the Society of Vascular Surgery proposed the "Wound, Ischemia, and foot Infection, (WIfI)" classification system to assess the risk of limb amputation and chance of successful revascularization in patients with CLTI.(25) Moreover, the European Society for Vascular Surgery (ESVS) has adopted and implemented the WIfI in their most recent guideline on management of peripheral artery disease (PAD).(26) Based on the aggregate of grades determined by wound presence, level of ischemia and severity of infection a treatment strategy can be proposed as well as an estimation of the prognosis can be made. The WIfI recommends that if arterial calcification precludes reliable ankle-brachial index or toe-pressure measurement, ischemic grade should be classified with TcPO2.

Since WIfI scores should ideally translate into treatment decisions, it is essential that TcPO2 results are reliable and reproducible. This underlines that TcPO2 measurement should be conducted homogenously and factors that may interfere with the test should be kept to a minimum. We assume that the discrepancies between reported threshold values in studies on TcPO2 are, at least partially, due to differences in protocols for measurement.

Our objective is to review methods of TcPO2 measurement and explore potential factors that influence these values in CLTI patients.

METHODS

For this review, we conducted a systematic search on PubMed, EMBASE and the Cochrane Library for peer-reviewed publications on TcPO2 in CLTI patients using synonyms for "transcutaneous –oximetry, -oxygen or TcPO2" and "critical limb or peripheral arterial disease and ischemia" and reviewed references of reviews. All observational studies and clinical trials on CLTI and TcPO2 were included. Exclusion criteria were: lack of TcPO2 measurement, absence of CLTI e.g. diabetic foot ulcers without ischemia, non-human studies, language other than English, or if no full text was available. All eligible articles were analyzed for the protocol used to measure TcPO2 and – if available - the relation of TcPO2 values and outcomes. The following potential parameters were assessed: probe placement, probe temperature, and mentioning and/or use of a reference probe. All studies were reviewed by two researchers (BL and JW) independently.

RESULTS

A flowchart of the systematic search is shown in Figure 1. A total of 437 publications were screened. After exclusion, 36 articles remained eligible for analysis. An overview of the study characteristics and results is demonstrated in table 1. A total of 13 studies involved an experimental intervention, 8 studies on success of percutaneous transluminal angioplasty (PTA), 7 observational studies, 6 studies on experimental diagnostics and 2 studies on wound healing after amputation.

Probe placement

In 24 (67%) studies probes were placed on specific anatomical locations. These locations were on the dorsum of the foot (n=20, 54%), ankle (n=3, 8%) and calf (n=1, 3%) (table 1). A total of 7 (19%) studies placed probes adjacent to an ischemic lesion or ulcer (peri-lesion) irrespective of the location of the lesion. In 5 (14%) studies the specific location was not reported (table 1).

Probe temperature

Among the selected temperature settings of the probe the following was noted; in 18 (50%) studies a default probe temperature of 440C was selected, in 8 (22%) studies 450C, in 2 (5%) studies 43.50C, in 2 (5%) studies 44.50C and in 1 (3%) study 420C. In 5 studies (14%) probe temperature was not reported.

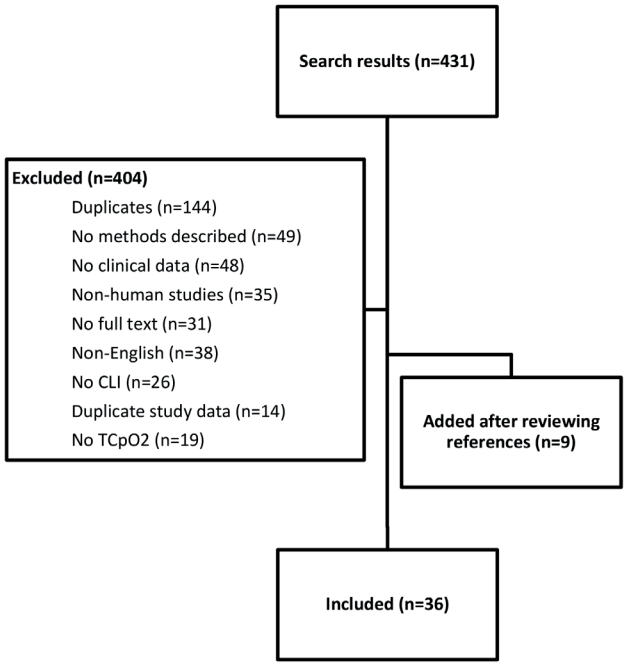

Figure 1 - Study selection

Reference probe

In 31 (84%) studies, the use of a reference probe was not reported. In 4 studies (11%) the reference probe was applied at the chest (e.g. subclavicular) region and in one (3%) study on the arm and in one (3%) on the contralateral limb. Only one study used reference probe values for the interpretation of systemic oxygenation.

Relation of TCpO2 and outcome

As apparent from the studies shown in Table 1 ulcer healing and limb prognosis were in general poor if TcPO2 is <20 mmHg and were in general good if >40 mmHg, however even these values varied between studies.

Table 1. Study characteristics and results

	Subjects	Probe position(s)	Reference probe	Probe temperature (°)	Conclusion
Wagner [59] (2003)	34	Yes, subclavicular	Dorsum	45	No deterioration with tcpO2 >45 mmHg (±20)
Benhamou [60] (2012)	48	NR	1st MT (metatarsal)	44	A TcPO2 less than 40mmHg at the onset of thehaemodialysis could identify patients at high risk of death and patients requiring vascular treatment.
Kalani [13] (1999)	50	Yes, subclavicular	1st MT	44	probability of ulcer healing is low when TcPO2 is <25 mmHg.
Petrakis [23] (2000)	60	NR	Foot dorsum	44	Limb salvage in trophic lesions <3 cm2 24.2 (± 6.2) mmHg, Trophic lesions >3 cm2 20.7 (± 5.3) mmHg
Khodabandehlou [14] (2004)	33	NR	1st MT	44	94% of patients with TcPO2 < 10 mmHg deteriorated; while only 53% of those with 10 < TcPO2 < 30 mmHg, improved
Caselli [16] (2005)	43	NR	2nd MT	44	TCpO2 <20 mmHg no limb salvage, TCpO2 >35 mmHg limb salvage
Jacqueminet [49] (2005)	32	NR	1st MT	44	Partial or total healing with TCpO2 > 27 ± 9 mmHg, clinical deterioration with TCpO2 < 20 ± 9 mmHg
De Graaff [61] (2003)	96	NR	1st MT	44	Use of tcPO2 and TP measurements in management of suspected CLI does not have advantage over the clinical judgement of an experienced vascular surgeon.
Faglia [50] (2007)	564	NR	Peri-lesionair (dorsum)	NR	In patients in whom PTA is effective in only the iliac–femoral–popliteal, or only in the peroneal axis, the change in TcPO2 can help to determine the probability of avoiding major amputation.
Gersbach [62] (2007)	87	NR	1st MT	45	Static tcpO2 determinations were also insufficiently reliable: 10/12 limbs (83%) with supine tcpO2 >15 mmHg were salvaged, yet also in 5/12 limbs with tcpO2 < 15 mmHg (42% = false negative rate)
Nouvong [15] (2009)	54	NR	Ankle	44	Non-healing ulcer (46 ± 16 mmHg) and healed ulcer (48 ±15 mmHg)
Ferraresi [52] (2009)	101	NR	Peri-lesionair (dorsum)	NR	n/a
De Marchi [63] (2012)	48	NR	NR	44	n/a

Table 1. Continued.

	Subjects	Probe position(s)	Reference probe	Probe temperature (°)	Conclusion
Ladurner [21] (2010)	141	NR	Foot dorsum	NR	The overall amputation rate increased with decreasing tcpO2 readings (group < 20 mmHg: 26%, group 20-40 mmHg: 10%, group > 40 mmHg: 5%, p=0.014),
Uccioli [9] (2010)	551	NR	2nd MT	44	Healing 46.8 ±1.4 mmHg, non-healing 41.8 ±3.2 mmHg
Prochazka [18] (2010)	96	NR	Peri-lesionair	NR	n/a
Ruangsetakit [19] (2010)	50	Yes, subclavicular	Dorsum	45	None of patients with a TcPO2 of <20mmHg (group 1) showed signs of ulcer healing, whereas all of the patients with a TcPO2 of >40mmHg (group 3) showed a progression towards healing during the study period
Löndahl [20] (2011)	75	NR	3rd MT	42	No ulcer healed when basal TcPO2 was <25 mm and all ulcers healed when TcPO2 was >75 mmHg. In patients with TcPO2 26–50 and 51–75 mmHg, healing rates were 50% and 73%, respectively.
Kim [54] (2011)	23	NR	Peri-lesionair (plantar)	44	In the 28 limbs with ulcers, 25 limbs revealed marked improvements in TcPO2 values (> 30 mmHg).
Redlich [22] (2011)	28	NR	1st MT	NR	In the nonamputation group, at 3 months after PTA, TcPO2 values 41.0 ± 4.5 mmHg, from 22.8 ± 4.3 at baseline. Amputation group was 18.9 ±6.1 mmHg at day nine post PTA.
Andrews [55] (2013)	307	NR	Dorsum	45	10 (29%) of 34 patients with supine TcPO2 values lower than 20 mm Hg and uncontrolled DM healed within 3 months. The optimal cut point (healing or failure of healing) in the data of this study was 38 mm Hg.
Humeau-Heurtier [64] (2017)	84	Yes, chest	Ankle and toe	44.5	n/a
Katsui [65] (2018)	16	NR	Ankle and 1st MT	44	n/a
Pardo [58] (2015)	40	NR	Dorsum	44	n/a
Kavros [66] (2008)	48	NR	Peri-lesionair	45	
Klingel [67] (2005)	12	NR	Dorsum	44	n/a

Table 1. Continued.

	Subjects	Probe position(s)	Reference probe	Probe temperature (°)	Conclusion
Kram [68] (1989)	40	Yes, arm	Calf	44	In patients with calf tcPo2 values greater than 20 mmHg, 96% (27/28) had successful healing after BKA.
Kumagai [69] (2016)	10	NR	NR	43.5	n/a
Lenk [70] (2005)	7	NR	NR	43.5	n/a
Madaric [71] (2016)	62	NR	Forefoot	44	Surviving patients with limb salvage at the 12-month follow-up (39/62 patients) were characterized by higher tcpO2 levels (16 ± 10 vs 10 ± 9 mmHg, p = 0.01),
Malyar [72] (2014)	16	NR	Peri-lesionair	44.5	n/a
Melillo [73] (2016)	26	Yes, subclavicular	NR	45	Ilioprost treatment success was almost certain when tcPO2 was >23 mm Hg
Nilsson [74] (1998)	10	NR	NR	45	n/a
Paraskevas [75] (2006)	74	NR	Peri-lesionair	45	n/a
Scheffler [76] (1992)	64	NR	Forefoot	44	In conclusion, tcPo 2 limits of 10 and 45 mmHg for room air breathing readings in supine and sitting position, respectively, should be applied as discriminatory values. Measurements localized outside this two-dimensional range most probably are not associated with critical limb ischemia.
Ubbink [77] (1992)	49	NR	Dorsum	44	The optimal TCpO2 cut-off value for presence of CLI is 35 mmHg.

DISCUSSION

We found substantial differences in probe placement, probe temperature and reference probe application in studies on TcPO2 use in CLTI. Moreover, cut-off values for wound healing and limb prognosis varied between studies.

It is known that the abovementioned variations in TcPO2 protocols affect the obtained values. For example, in general the lower extremity has different oxygen tension levels influenced by local factors, such as skin thickness.(27) Hence, the selected probe site affects TcPO2 value. Although ischemic lesions may occur on different anatomical locations on the lower extremity,(28) standardization of TcPO2 probe location is crucial to reduce the intra- and inter-patient variability.

Furthermore, TcPO2 measurement using the Clark-electrode is influenced by the selected probe temperature.(29-32) Cutaneous warming of, for example, the diabetic foot has shown a profound effect on TcPO2 values showing a 40.8 ± 23.8 mmHg difference between a probe temperature of 37°C and 44°C.29 TcPO2 manuals suggest a probe temperature between 43-45°C,(33) 44-45°C,(34) or 45°C,(35) hence the majority of studies used 44°C as default temperature. However, a consensus statement of an expert panel proposed a default temperature of 45°C.(36) Since, studies on the effect of TcPO2 probe temperature on TcPO2 values in CLTI and its relation with prognosis are lacking, the optimal probe temperature in CLTI remains unknown.

Finally, it is suggested that TcPO2 values are influenced by the systemic oxygen level. Therefore, the placement of a reference probe on the thorax is recommended to correct for systemic hypoxia. We found that 6 studies mentioned the use of a reference probe. However, in 2 of these studies, values or interpretation of the reference probe remained unclear. An indicator which takes into account the systemic oxygen influence is the Regional Perfusion Index (RPI),(37,38) which is calculated by dividing the TcPO2 values of the limb by TcPO2 thorax values. RPI has been successfully applied to evaluate vascularization of the extremities,(39-41) which is also recommended by TcPO2 measurement device manufacturers. However, thorax TcPO2 values are subject to the same variability as for the limb, hence the ratio of the two could mathematically result in a greater variability than that of each factor separately. Therefore, more comparative research is required to validate and determine accurate thresholds for RPI and their added value in comparison to absolute cut-off TcPO2 values in CLTI for usage in clinical practice. Another proposed strategy is to add pulse oximetry (SO2) to rule out arterial hypoxemia.(33-35) However, studies on SO2 and its correlation to TcPO2 values of the lower limb are lacking.

A major limitation in this review is the impossibility to address the specific impact of probe location, probe temperature and use of reference probe on the measured TcPO2 values due to the fundamental differences in study aim and therefore patient population in the individual studies. Moreover, the study design of the included studies was very heterogeneous: 13 studies involved an experimental intervention, 8 studies on success of PTA, 7 observational studies, 6 studies on experimental diagnostics and 2 studies on wound healing after amputation. Still, we demonstrate that the method of TcPO2 use differs undoubtedly and point out the sensitivity of TcPO2 measurement in general. Hence, it might be suggested that heterogeneity in method of use alters TcPO2 values.

With the recently introduced WIfI classification system in the current ESC guidelines,(26) TcPO2 plays a role in grading ischemia and the determination of the treatment strategy in patients with CLTI. For example, if a patient has a shallow ulcer on the foot without gangrene and no signs of infection and TcPO2 measurement is 40 mmHg, the patient is categorized as having a very low risk of limb amputation and low requirement for revascularization. However, if the same patient has a TcPO2 of 29 mmHg there is a moderate risk of limb amputation and a high requirement for revascularization.(26) This indicates a prominent role for TcPO2 to determine treatment strategy. Moreover, the usefulness of the ankle systolic pressure, imbedded in the WIfI classification, to predict the risk of major amputation in CLTI is currently under debate.(42) Especially in CLTI patients with concomitant diabetes mellitus or chronic renal failure, TcPO2 seems more reliable in comparison to current macrovascular diagnostics.(43) Furthermore, other factors in method of TcPO2 use should be addressed to provide an adequate risk stratification scheme for TcPO2, such as, positioning of the patient (supine vs. sitting) and additional oxygen inhalation, both methods have proven to increase the predictive value of wound healing prognosis.(44,45) All these factors underline the importance of a standardized method of TcPO2 measurement and careful interpretation of these values. The effect of altering specific variables during TcPO2 assessment remain unclear. Therefore, we are currently conducting an observational study to investigate the influence of probe location and the added value of the RPI on wound healing prognosis. Also, in our vascular laboratory we are conducting experimental TcPO2 studies to investigate a new photo-optical form of TcPO2. It has been suggested that photo-optical TcpO2 is not affected by probe temperature compared with the standard electro-chemical TcPO2.

CONCLUSIONS

TcPO2 for the evaluation of limb perfusion in CLTI patients is applied in standard clinical practice and is included in WIfI and international guidelines on PAD management, therefore affecting treatment decisions. However, there is a strong heterogeneity in methods used to assess TcPO2. A substantial diversity in probe temperature, probe location and the

use of a reference probe was found among studies regarding TcPO2 in CLTI. The varying values and different conclusions of the reviewed studies underline the importance of a homogeneous protocol in order to interpret and possibly compare measurement values and use these values to guide treatment decisions. Prior to the implementation of TcPO2 as part of treatment guidelines it is mandatory that (international) TcPO2 measurement protocols become available and are validated to guarantee reliable and reproducible TcPO2 results.

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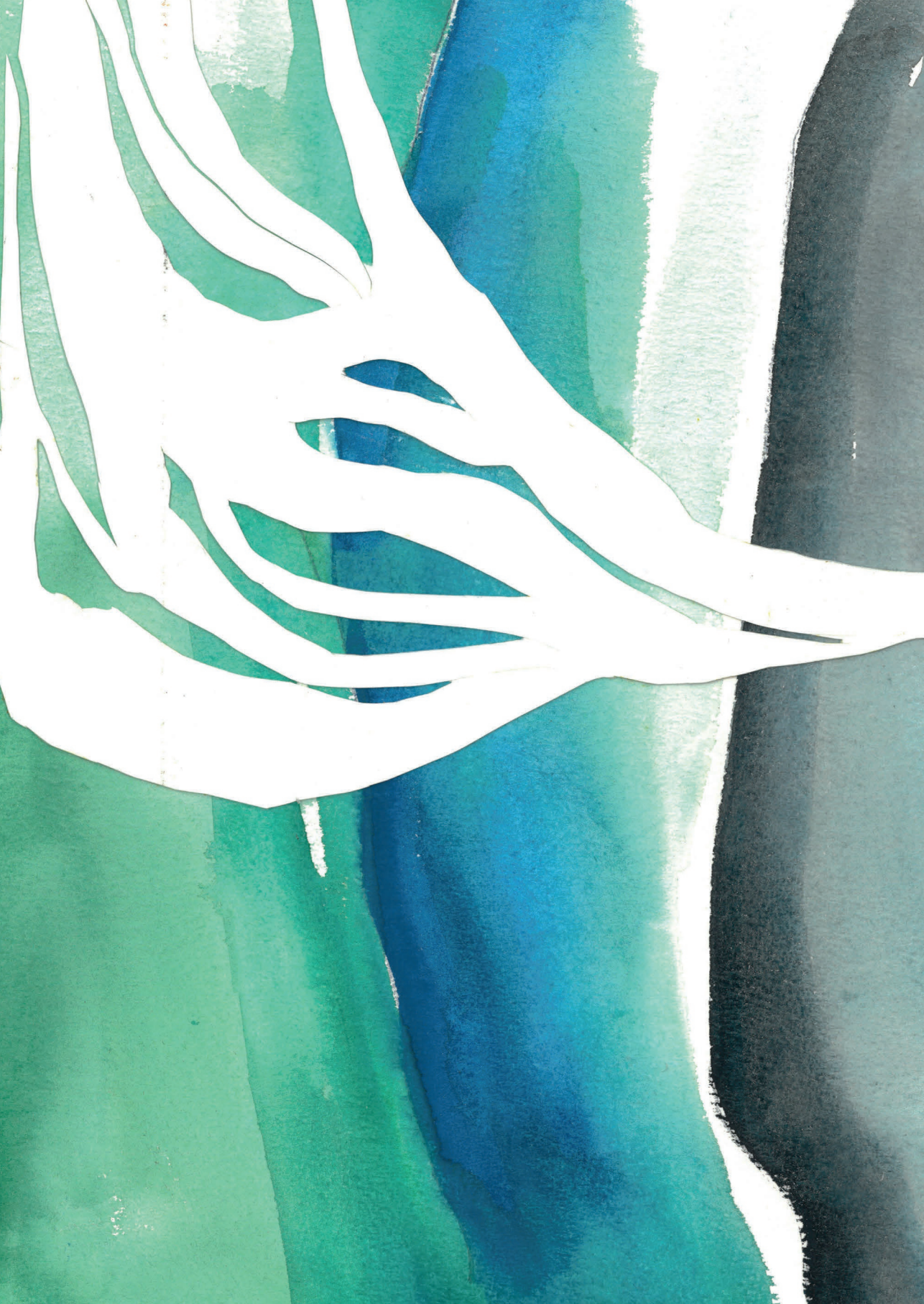

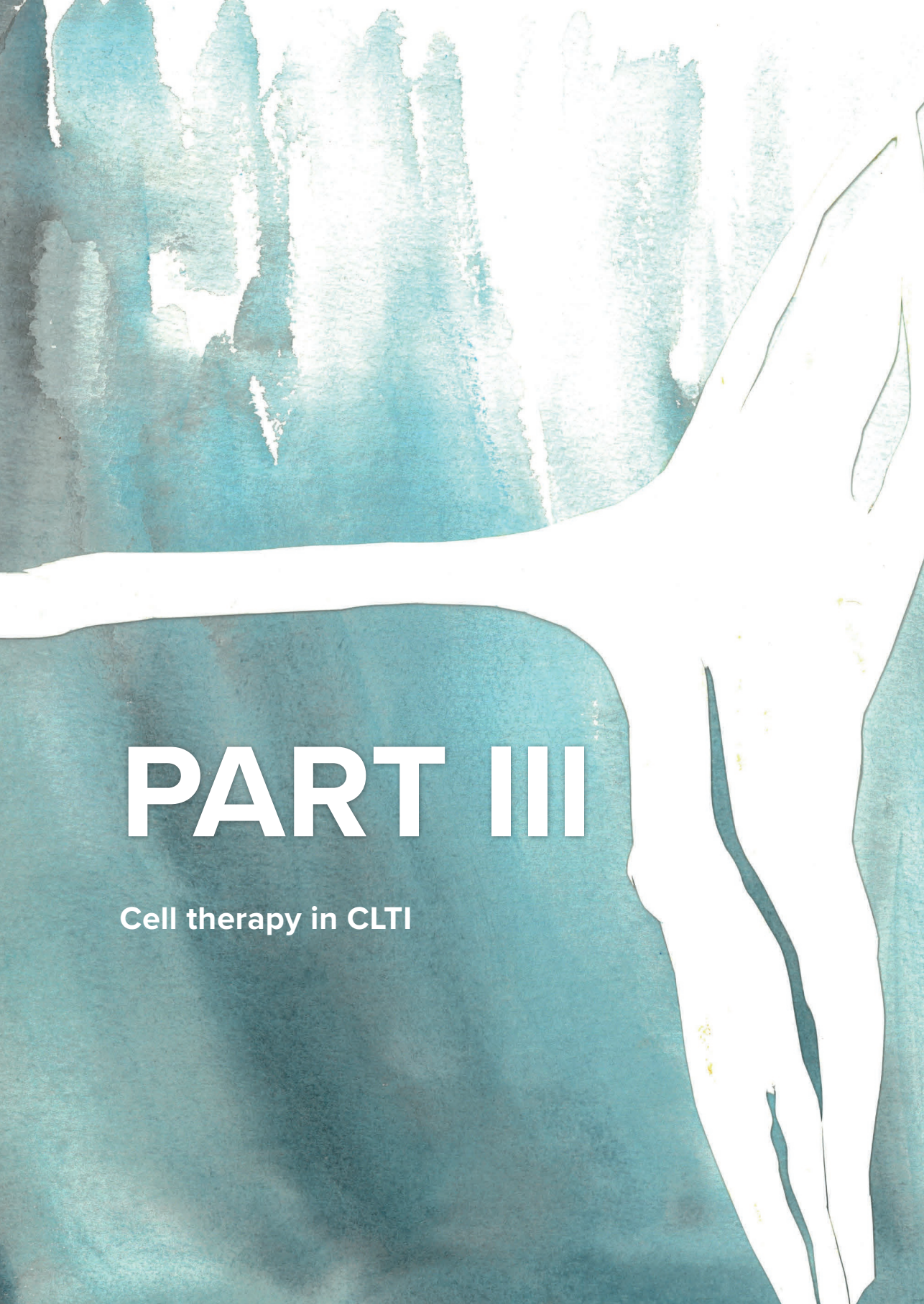

PART III

Cell therapy in CLTI

CHAPTER 7

Cell Therapy for Chronic Limb-Threatening Ischemia: Current Evidence and Future Directions

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Stem Cells Translational Medicine 2018 Dec;7(12):842-846

INTRODUCTION

Peripheral Artery Disease (PAD) arises from atherosclerosis of major arteries, with a predilection for the lower limbs. In its most severe manifestation, occlusion of limb arteries reaches a point where metabolic demands of the tissue can no longer be met; this stage is termed Chronic Limb-Threatening Ischemia (CLTI) or Critical Limb Ischemia (CLI). CLTI poses a great unmet need for novel treatments, as 20-40% of the CLTI patients are not eligible for conventional revascularization, ultimately leading to amputation, associated with an immense medical and socio-economic burden (1-3). No-option status in these patients is due to extensive and diffuse, often infrapopliteal, atherosclerotic lesions, comorbidity and/or lack of a suitable bypass graft (4, 5). Novel approaches that target neovascularization provide a potential solution for these no-option patients. Cell-based therapies seem the most promising (6), although initial enthusiasm has abated after negative results in the first generation of progenitor cell trials. Here we will provide a concise review on the available evidence on and future directions for cell therapy in CLTI. In that context we will also briefly address literature regarding cell therapy in myocardial infarction (MI) because objectives in both MI and CLTI trials are to enhance revascularization through cell therapy.

CELL THERAPY FOR CLTI – THE PRESENT STATE OF AFFAIRS

The rationale behind using progenitor cell therapy as a treatment for ischemic cardiovascular disease was motivated by the discovery that human blood contains progenitor cells that home to ischemic tissues (7) and augment angiogenesis (8). Relatively soon thereafter, a first generation of progenitor cell trials have been conducted using bone marrow mononuclear cells (BM-MNCs), a direct BM isolate which contains a variety of different cell-types, mostly from the hematopoietic line. The primary hypothesis was that BM, as the reservoir of hematopoietic stem cells, also contains endothelial progenitor cells (EPCs) (9). These putative EPCs were initially thought to promote angiogenesis through the formation of new vessels (7) as they actively homed to ischemic areas after injection. Early, uncontrolled clinical studies using BM-MNCs were promising, but placebo-controlled trials gave conflicting results. A large, double-blind, placebo-controlled randomized trial by our group, the JUVENTAS trial, showed no treatment effects of BM-MNC administration over placebo (10), which was corroborated by a meta-analysis (11). Similar results were obtained with BM-MNC therapy for other indications such as MI, where an aggregated study comprising over a thousand patients that were treated with BM-MNCs for MI failed to find a consistent positive effect (12).

Advancing insight into the biology of progenitor cells has in parallel, revealed that the mechanisms of effect involved in progenitor cell-therapy are different and more complex than initially thought. The use of cell surface markers to identify EPCs has been shown to be prone to isolation artifacts (13, 14), and several ontologically distinct cell populations display EPC markers. Furthermore it has been shown that BM-derived cells do not stably incorporate into newly formed vessels and only play an auxiliary role in neovascularization (15). True vasculogenic ability has only demonstrated for a single cell-type, designated the endothelial colony forming cell (ECFC) (16), which cannot be obtained from BM. While the auxiliary angiogenic effects of BM-MNCs have been demonstrated very consistently in animal models, it is likely that they only occur with BM isolates from comparatively young subjects without comorbidities (17, 18). This restricts successful application of BM-MNCs in MI to specific subsets (19) of patients, and likely severely limits it in CLTI.

Collectively, these observations have precipitated a switch away from undefined raw cell isolates such as BM-MNCs, towards better-defined cell therapy products (20). Whereas in the therapy for MI, the focus has shifted away from angiogenic cell therapy to cardiomyocyte regeneration (21), in CLTI the primary objective remains to augment angiogenesis.

Angiogenesis can be induced via different cell-based strategies (Figure 1): by supplying endothelial-like cells, such as endothelial colony forming cells (ECFCs) directly, which will spontaneously organize into new vessels that integrate with the existing vasculature. Alternatively angiogenesis can be promoted indirectly by cells that secrete factors that remodel the extracellular matrix and recruit resident endothelial cells. In this category are circulating endothelial cells (CACs), which are of monocyte/myeloid origin, and may potentially act as bridging monocytes in angiogenesis (22). Alternatively there are Mesenchymal Stromal Cells (MSCs), which are of pericyte orgin (23) and secrete a host of paracrine angiogenic and immunomodulatory factors (24). It is likely that there are synergistic effects of combining these approaches, using a combination of ECFCs and a supportive cell-type (25, 26). At present however, translation to a clinical product has proven difficult as ex vivo expansion of the above-mentioned cells requires specialty cell culture additives that are difficult and costly to obtain for clinical grade production (27). For this reason only Mesenchymal Stromal Cells (MSCs), have advanced to a second generation of clinical trials, as they can be relatively easily obtained from different tissues such as BM, placenta and adipose tissue and reproducibly expanded ex vivo (see table 1 for overview).

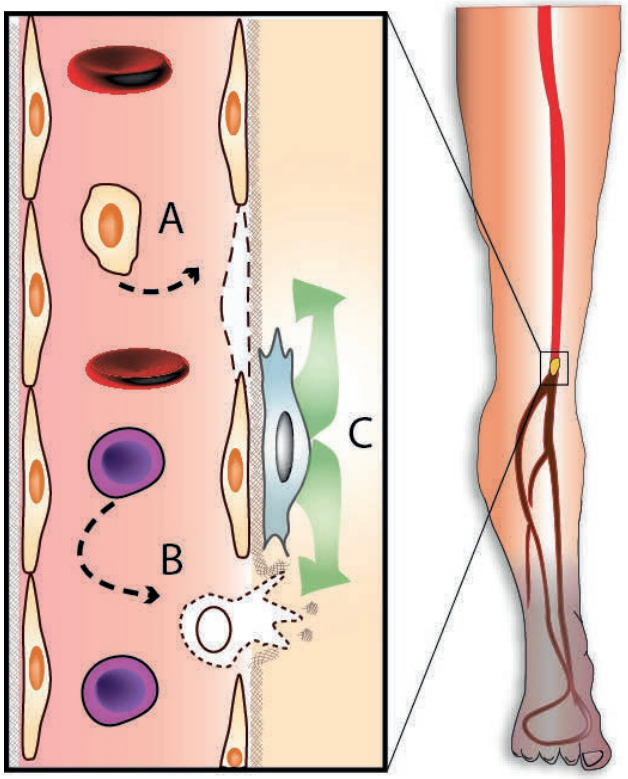

Figure 1 - Different potential modes of action of cell therapy.

A. Direct angiogenesis through introduction of endothelial-like cells that will form new capillaries through vasculogenesis and fill endothelial defects.

B. Indirect angiogenesis through introduction of monocyte-like cells, that will remodel the extracellular matrix and will recruit and guide new endothelial sprouts.

C. Indirect angiogenesis through paracrine effects, including modulation of monocyte differentiation and recruitment of endothelial cells.

Table 1. Overview of MSC trials in CLTI

Author	Year	N	Design	Injection sites	Total dose & source
Kim[28]	2006	4	No control group	NM	$1*10^6$ allogeneic HLA matched UCB-MSCs
Dash[29]	2009	24	Open label; control group (1:1 randomization)	NM	$45\text{-}60*10^6$ autologous BM-MSCs
Lu[30]	2011	41	Double blind study; randomly assigned treatment per leg; 1 leg treated with normal saline, the other treated with MNC or MSC	20	$9.3*10^8$ autologous BM-MSCs
Gupta[31]	2013	20	Placebo controlled; double blind	40-60	$2*10^6$ allogeneic BM-MSCs / kg body weight
Gupta[32]	2016	90	Nonrandomized; low dose, high dose or standard care	40-60	$(1 \text{ or } 2)*10^6$ allogeneic BM-MSCs / kg body weight

MSC THERAPY FOR CLTI

While MNC have proven to effectively enhance angiogenesis and neovascularization in preclinical studies, it has been suggested that the pro-angiogenic effect of MSCs is superior compared to MNCs in preclinical studies (33, 34). In vitro and vivo studies have demonstrated that MSC can home to injured tissue and secrete beneficial factors that suppress inflammation and improve angiogenesis via paracrine pathways (35). Several small exploratory clinical trials showed positive effects of MSCs in the treatment of CLTI compared to standard of care or placebo (29, 31, 36). Clinical studies that directly compare BM-MSCs with -MNCs for the treatment of CLTI are scarce. Only one study directly compared the two strategies in 41 diabetic CLTI patients, suggesting that MSC might be better tolerated and more effectively enhance perfusion and ulcer healing compared to MNC (30). The disappointing results of clinical trials on MNC in CLTI, the promising effects of MSCs and several practical benefits of MSCs, in particular the potential for allogeneic application, have led to increased interest of MSCs as potential option for cell therapy in CLTI A similar switch is also observed for studies in cardiac disease (37).

MSCs, rather uniquely among transplanted cell grafts, are only minimally immunogenic (37) and display strong immunomodulatory properties (38), which makes allogeneic application possible, at least in a single administration, as it is still unclear whether rejection occurs upon repeated administration (37). At the present state of knowledge, allogeneic administration of MSCs is the most promising route to clinical application with the advantage of providing an off-the-shelf available product. An allogeneic product significantly reduces the burden on the patient, as patients will not have to undergo a (BM) harvesting procedure. Whereas in BM-MNCs it has been shown that patient-derived cell isolates show decreased pro-angiogenic effects (18), this does not necessarily apply to MSCs (39). In a previous study we did not observe reduced angiogenic potency in CLTI MSC isolates in a murine hind limb ischemia model (39, 40). In a clinical trial comparing efficacy of autologous versus allogeneic MSCs in non-ischemic dilated cardiomyopathy however, allogeneic MSCs had a more favorable side-effect profile and a trend towards greater improvement in ejection fraction. Additionally the occurrence of SAEs was substantially lower in allogeneic then autologous MSCs; 28% vs. 64% respectively(37). Furthermore, an advantage of allogeneic MSC therapy is that the (angiogenic) potency of the cell isolate can be tested in advance. Demonstrable potency will likely be of importance in the quality control of cell therapy products for clinical regulation (41). As a single MSC isolate generally is sufficient to treat dozens of patients, a priori batch testing can conceivable improve clinical outcomes, provided that validated assays are developed (41, 42). Lastly, allogeneic MSC administration is significantly less costly. Costs for expansion and quality testing of a BM isolate are high, which in autologous application will be the per-patient cost, but which can be split over multiple patients in allogeneic application (43).

CURRENT CHALLENGES AND FUTURE DIRECTIONS

Allogeneic application makes MSC-therapy interesting for commercial parties, as a defined cell product can be comparatively easily patented and produced by in-house companies, without the complications of harvesting donor material for each patient. 15% of all clinical trials worldwide involving cell therapy are industry-sponsored and the vast majority of the remainder by leading academic centers. Additionally facilities and logistics involved in the development of cell therapy products are becoming more available and less expensive due to increased administration as standard of care or as investigational novel treatment in other diseases (44). However, development of evidence-based accepted approaches remains challenging, due to high developmental costs, regulatory hurdles, and batch-per-batch product variation. Some of these factors may be less relevant for non-cellular cell-based therapies, such as exosome-based therapies, which could make commercialization less difficult (45). Another important CLTI-specific limiting factor is that, historically, the design of high-quality studies for the treatment of CLTI has proven notoriously difficult (46). Improved clinical management and technical advances in revascularization approaches, endovascular interventions in particular, have led to a near doubling of 1-year amputation-free survival for CLTI patients since the first trials with BM-MNCs. Therefore larger and better-designed trials are required to determine the potential added value of novel therapies in CLTI (47, 48). In the light of these considerations, small phase I/II (30, 31) or pragmatically designed studies (32) have provided valuable first indications about potential efficacy of MSCs in CLTI. However, at no point can they substitute evidence from placebo-controlled double-blind randomized trials. Public demand for cell-based therapies has been such, that smaller commercial parties are offering cell treatments in the absence of evidence – positive or negative - potentially putting patients at risk (49), leading to public discussions with respect to ethical issues regarding regenerative medicine approaches (50).

We therefore would encourage increased openness and standardization, both in the use of the investigational cell product and trial design. Convincing evidence for efficacy of MSC therapy will only come from well-designed Randomized Controlled Trials (RCTs) using hard and clinically relevant outcomes, which would be ideally related with future imaging methods to evaluate collateralization and neovascularization. It seems increasingly unlikely that single investigative centers will achieve sufficient statistical power to show efficacy. International collaborative efforts and data sharing are necessary to push the field forward and maintain scientific integrity.

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CHAPTER 8

Rationale and design of the SAIL trial for intramuscular injection of allogeneic mesenchymal stromal cells in no-option critical limb ischemia

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Journal of Vascular Surgery 2018 Feb;67(2):656-661

ABSTRACT

Background

Critical limb ischemia (CLI) represents the most severe form of peripheral artery disease (PAD) and has an immense impact on quality of life, morbidity and mortality. A considerable proportion of CLI patients is ineligible for revascularization, leaving amputation as only option. Mesenchymal stromal cells (MSCs), because of their vasculoregenerative and immunomodulatory characteristics, have emerged as a potential new treatment.

Methods

The primary objective of this trial is to investigate whether intramuscular administration of allogeneic bone marrow (BM)-derived MSCs is safe and potentially effective. The SAIL (allogeneic mesenchymal Stromal cells for Angiogenesis and neovascularization in no-option Ischemic Limbs) trial is a double-blind, placebo-controlled randomized clinical trial to investigate the effect of allogeneic BM-MSCs in patients with CLI, who are not eligible for conventional revascularization. A total of 66 patients will be included and randomized (1:1) to undergo 30 intramuscular injections with either BM-MSCs (5*10^6 MSCs per injection) or placebo in the ischemic lower extremity. Primary outcome i.e. therapy success, a composite outcome considering mortality, limb status, clinical status, and changes in pain score, will be assessed at six months. All study-related procedures will take place in the University Medical Center Utrecht in The Netherlands.

Conclusions

If our results indicate that intramuscular allogeneic BM-MSC therapy for CLI is safe and potentially effective, this will have important consequences for treatment of patients with CLI. A large multicenter clinical trial with longer follow-up focusing on hard endpoints should then be initiated to confirm these findings.

RATIONALE AND BACKGROUND

Critical limb ischemia (CLI) is a major health care problem that is estimated to develop in 500-1,000 individuals per million persons per year in Western society(1). Five-year mortality rates exceeding 50% from the incident diagnosis of CLI have been reported(2, 3). Additionally, CLI is associated with important functional impairment, a major impact on quality of life, and high treatment costs, especially when amputation is inevitable(4, 5). Despite the technical evolution in especially endovascular interventions in the past decade, 20-40% of the CLI patients are not eligible for revascularization procedures due to the anatomical location of the lesions, the extent of the disease or co-morbidity(2). High amputation rates of 10-40% at six months are reported in this "no-option" population(3, 6). Efficacious new interventions aimed at limb salvage and improvement of peripheral circulation will have major impact on quality of life and may avert amputation and associated burden and costs. In the last decade, cell-based therapies have been explored as a treatment option. Especially mesenchymal stromal cells (MSCs), because of their immunomodulatory and vasculoregenerative properties, have emerged as a potential new treatment. MSCs are a subpopulation of BM cells that can differentiate into different mesenchymal tissues, depending on the conditions. (clinicaltrials.gov identifier NCT03042572).

MATERIALS AND METHODS

Study Design and Patient Population

The SAIL trial is a randomized, double-blind, placebo-controlled trial with 6 months follow-up for the primary outcome and five years for secondary outcomes. All study-related procedures including screening, inclusion, treatment and follow-up will take place in the University Medical Center Utrecht in The Netherlands. A total number of 66 patients with proven chronic CLI, defined as rest pain and/or non-healing ulcers due to peripheral artery disease (PAD), who are not eligible for surgical or endovascular revascularization, will be included in this trial. No-option status is assessed based on conventional vascular imaging, which is discussed at a weekly consensus meeting including vascular surgeons and endovascular specialists, who are not involved in the trial. Potential participants are screened according to the criteria stated in Table 1. After obtaining written informed consent, subjects will be randomized by the Cell Therapy Facility of the University Medical Center Utrecht (UMCU) by means of a computerized table. The clinical trial team remains blinded to the treatment allocation throughout the duration of the trial. The trial is estimated to take two years until analysis of the primary outcome starts.

The study will be conducted according to the Declaration of Helsinki and is approved by the Central Committee on Research Involving Human Subjects (CCMO, The Netherlands),

trial number: NL59038.000.16.

Table 1. Overview of studies on intramuscular MSC administration in CLI. NM: not mentioned.

Author	Year	N	Design	Injection sites	Total dose
Kim(31)	2006	4	No control group	NM	$1*10^6$ allogeneic HLA matched UCB-MSCs
Dash(32)	2009	24	Open label; control group (1:1 randomization)	NM	$45\text{-}60*10^6$ autologous BM-MSCs
Lu(12)	2011	41	Double blind study; randomly assigned treatment per leg; 1 leg treated with normal saline, the other treated with MNC or MSC	20	$9.3*10^8$ autologous BM-MSCs
Lasala(33)	2011	10	No control group	40	$30*10^8$ MNCs and $30*10^6$ MSCs (both autologous)
Lasala(26)	2012	25	Patient blinded to treatment; most affected leg treated with randomly assigned low or high dose product, less affected leg treated with placebo	40	Low dose: $9*10^8$ BM-MNCs and $9*10^6$ BM-MSCs, high dose: $18*10^8$ BM-MNCs and $18*10^6$ BM-MSCs
Gupta(11)	2013	20	Placebo controlled; double blind	40-60	$2*10^6$ allogeneic BM-MSCs / kg body weight
Gupta(14)	2016	90	Nonrandomized; low dose, high dose or standard care	40-60	$(1 \text{ or } 2)*10^6$ allogeneic BM-MSCs / kg body weight

Intervention

Subjects will be 1:1 randomized to receive intramuscular injections with either allogeneic BM-MSCs or placebo (see Figure 1 for a flow chart of the study design), which will be administered in the most affected leg.

Allogeneic BM-MSCs will be obtained from BM of healthy volunteers. The crude BM extract will be purified by density gradient centrifugation and the resulting mononuclear cell fraction will be seeded in culture medium (α-MEM, 5% human Platelet Lysate and 2 IU/ml Heparin) in CellStack flasks. Cells will be expanded to passage 3 and cryopreserved until injection. Prior to release, all donor isolates will be tested for bacterial/fungal contamination, endotoxins, mycoplasma and the absence of transmissible infectious diseases.

After the induction of analgesia using intravenous fentanyl, patients will either receive 30 intramuscular injections with either BM-MSCs (5*10^6 MSCs per injection; a total of 150*10^6 MSCs) or placebo (50% Human Serum Albumin 20% and 50% sodium chloride 0.9%). Blinded syringes are provided and an experienced clinician will inject cell suspensions in the ischemic lower extremity.

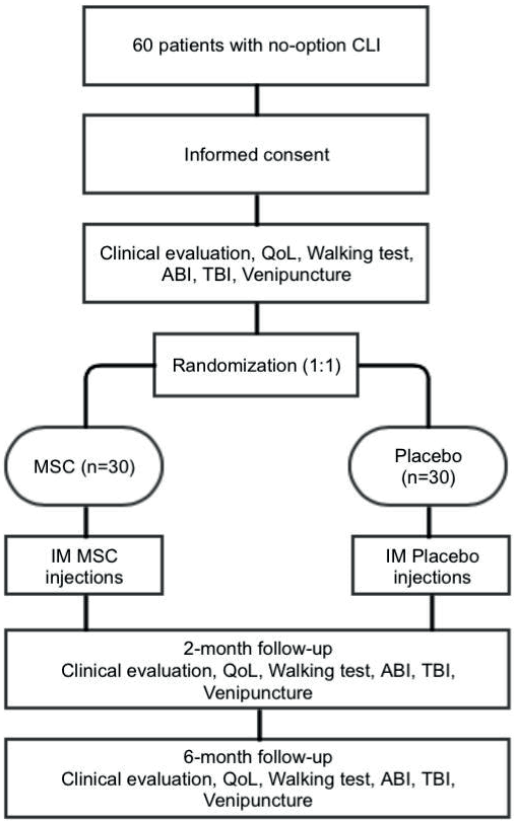

Figure 1 - Flow chart of the study design of the SAIL trial. CLI: critical limb ischemia, ABI: ankle-brachial pressure index, TBI: toe-brachial pressure index, MSC: mesenchymal stromal cells, QoL: quality of life.

Outcomes and safety data

Follow-up visits will be scheduled at 1 week and 2 & 6 months post-treatment. Primary outcome is therapy success at 6 months, assessed as composite outcome: to be a "success," a subject must: 1. Be alive, 2. Be without a major amputation of the index limb, 3. Have not worsened in Rutherford classification or visual analogue pain scale, and 4. Have improved in either Rutherford classification or visual analogue pain scale (18). Subjects not meeting all of the criteria are classified as treatment failures. Secondary outcomes are the incidence of major (through or above the ankle joint) and minor (distal from the ankle joint) amputations, mortality, improvement of pain free walking distance, changes in the number and extent of leg ulcers, ulcer healing, clinical classification (Fontaine and Rutherford classification), ankle-brachial index (ABI) and toe-brachial index (TBI), and quality of life (EuroQoL 5-D [EQ5D] and Short Form 36 [SF-36]).

An independent Data and Safety Monitoring Committee (DSMC) will review the status and conduct of the clinical trial, evaluate all causes of death and cardiovascular events and make recommendations to the clinical research group concerning the trial's continuation and modification, based on prespecified stopping rules and evolving evidence. All patients will be carefully monitored for side effects due to intramuscular injections, changes in clinical parameters (temperature, blood pressure, heart rate) and changes in renal, hepatic and metabolic parameters. Sequential safety monitoring will be performed on major side effects.

Power calculation

The sample size calculation for the SAIL trial is based on the primary outcome. Based on previously reported therapy failure rates of approximately 62.5% (50-75%) in the placebo group in studies reporting the same composite outcome and considering a 50% reduction of the therapy failure rate as clinically relevant(18, 19), the following sample size calculation is obtained. With a one-tail β of 0.30, an α of 0.05, and a 1:1 randomization ratio, 60 patients should be included in the present trial. With an anticipated loss to follow-up of 10% 66 patients should be ultimately included.

Table 2. Inclusion and exclusion criteria of the SAIL trial.

Inclusion criteria	Exclusion criteria
• Age > 18 years • Severe PAD (Fontaine class III-IV/ Rutherford class 4-5) • Ankle brachial index < 0.6 or unreliable (non-compressible or not in proportion to the Fontaine classification) • Not eligible for surgical or endovascular revascularization • Written informed consent	• History of malignancy or neoplasm (within the past 10 years) • Serious known concomitant disease with life expectancy of less than one year • Rutherford 6 in which amputation on the short term (within 1-2 weeks) is inevitable • Pregnancy or unwillingness to use birth control measures • Uncontrolled acute or chronic infection with systemic symptoms • Follow-up impossible

DISCUSSION

Since 2002, several small and often uncontrolled clinical studies have suggested beneficial effects of cell-based therapies in patients with CLI. However, thus far, no large placebo-controlled RCTs have reported on efficacy of MSCs for the treatment of CLI.

Preclinical studies have demonstrated that MSCs can home to injured tissue and secrete factors that suppress inflammation and improve angiogenesis(7). In animal models with hind limb ischemia intramuscularly injected MSCs greatly improve neovascularization(8). A preclinical study comparing efficacy of BM mononuclear cells (MNCs) and BM-MSCs

suggested that MSCs are superior to MNCs in promoting neovascularization(2).

Studies published until 2012 were previously reviewed and summarized(9), with the conclusion that BM-derived cell therapy might be promising in CLI. We performed a second meta-analysis in 2015, after the results of five additional randomized placebo-controlled trials had been published(10). This meta-analysis of 10 randomized placebo-controlled trials of BM-derived cell therapy in 499 patients with CLI showed no advantage of cell therapy on the primary outcome measures of amputation, survival, and amputation-free survival (AFS). Importantly, most of these RCTs used BM-MNCs and only 2 RCTs involved BM-MSCs. While the MSC studies suggested a benefit, they were not adequately designed and very small, with only 30 patients receiving MSCs (11, 12). Lu et al. investigated MSC therapy in patients with diabetic ulcers of mixed etiology and showed positive results on pain scores, ankle/brachial-index (ABI) and walking distance (see Table 2). They also suggested that autologous BM-MSC therapy may be better tolerated and more effective than BM-MNCs in diabetic CLI. The potential superiority of BM-MSC compared to BM-MNCs was corroborated by a meta-regression analysis (13). Gupta et al. showed in a small placebo-controlled RCT (10 vs. 10) that intramuscular allogeneic MSC injection in CLI patients was safe and promising, with improvement of ABI and ankle pressure without serious adverse events (SAEs) related to the intervention(11). Recently, a larger (n=90) phase II, prospective, non-randomized, open-label, multicenter, dose-ranging study in patients with CLI due to Buerger's disease was published. This study showed clinical benefit (reduction in rest pain, healing of ulcers, improvement in ABI and total walking distance) of intramuscular injection of adult human cultured, pooled, allogeneic BM-MSCs at a dose of 2 million cells/kg (14).

Administration route and dosage

For this trial, we chose the intramuscular route. So far 3 small RCTs showed potential benefit of intramuscular administration of MSCs(11, 12, 14). An animal study indicates that muscle tissue improves secretory functions and supports the injected MSCs(20). Furthermore, significant entrapping and embolus formation in the lungs has been shown in animal studies after systemic intravenous administration(21, 22). Lung entrapping has also been observed in humans(23). Intra-arterial administration also poses a risk of iatrogenic damage to the artery, dissection of the vessel wall, dislodgement of atherosclerotic lesions and surrounding nerves. Based on current literature we opted for a total dose of 150*106 MSCs distributed over 30 injections sites (5*106 MSCs per injection-site; see Table 2). Previous clinical studies did not show a clear dose-response relationship(24-26) or even an inverse dose-response relationship (15, 27).

Allogeneic MSCs

The administration of allogeneic MSCs has several advantages in comparison to autologous MSCs. First, the burden on the patient is significantly less, as patients will

not have to undergo a BM harvesting procedure. Second, in allogeneic MSC therapy the pro-angiogenic capacity of the cell isolate can be tested in advance. We observed a considerable heterogeneity between donor MSC isolates, with about 25% of isolates not inducing increased neovascularization compared to placebo(28). In autologous application of MSCs, this heterogeneity will likely affect trial results and therapeutic potential. In allogeneic application, a selection of the best donor isolates can be made to reduce variability in treatment response. Lastly, there is considerable difference in treatment cost. In allogeneic MSC application, MSCs from altruistic donors can be expanded prior to the study and thus be used as off-the-shelf available therapy. This makes the therapy instantly available and enables per-batch instead of per-patient (pre-treatment) testing, which saves costs. Additionally, allogeneic application of MSC might be more efficacious, as the POSEIDON-trial showed that in ischemic cardiomyopathy injection of allogeneic BM-MSCs was safe and more effective than autologous MSCs(17).

Safety

Several clinical studies using allogeneic MSCs have been performed in patients with myocardial infarction and allogeneic cells did not cause an acute rejection or detectable allo-antibodies(15, 16). Allogeneic BM-MSCs have also been studied in a variety of non-cardiovascular diseases. No local reaction such as inflammation or any other signs of rejection were observed after injection of allogeneic MSCs around fistula tracts of patients with Crohn's disease (n=21) (27). A meta-analysis including 216 patients receiving allogeneic BM-MSCs reports no infusion-related toxicity other than transient fever (29). In four years, no treatment-related adverse events occurred in patients with systemic lupus erythematosus treated with allogeneic MSCs (n=15)(30).

CONCLUSION

The SAIL Trial will assess safety and efficacy of intramuscular administration of allogeneic BM-MSCs in patients with no-option CLI. If this trial shows safety and efficacy of allogeneic BM-MSCs in no-option CLI, this is the basis for a future large multi-center trial to definitely prove efficacy of allogeneic BM-MSCs in CLI. If BM-MSCs ultimately prove to be effective in no-option CLI, it fulfils an urgent unmet medical need for CLI patients who are not eligible for conventional revascularization strategies.

Acknowledgments, Funding sources and Disclosures

Acknowledgments/ Funding sources: we gratefully acknowledge the support by ZonMw grant 40-41400-98-9026 (ZonMw is an independent self-governing organisation and is responsible for health care research funded by the Dutch government).

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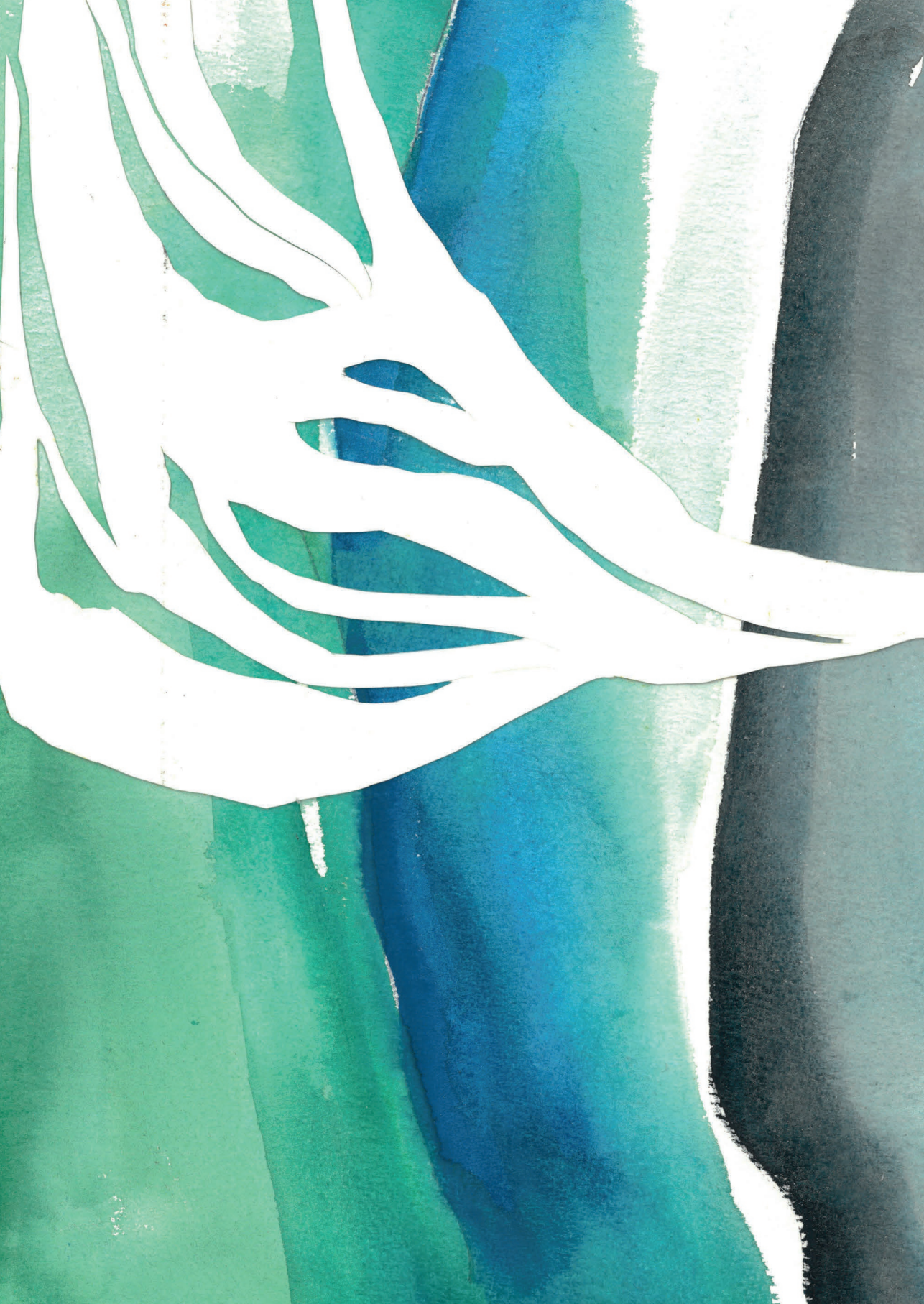


PART IV

CHAPTER 9

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

Peripheral arterial disease (PAD) is one of the three major clinical manifestations of atherosclerosis, the other two being coronary artery (CAD) and cerebrovascular disease. This thesis provides an overview of the management and prognosis of PAD and more specifically chronic limb-threatening ischemia (CLTI). This thesis aimed to contribute to improved definition, classification, prognostic modeling, and clinical decision making in CLTI patients and is divided in three main topics. Part 1 and 2 focus on the validation and improvement within two pillars of the "PLAN" concept of evidence-based revascularization (EBR)[1]. PLAN stresses a structured management approach based on Patient risk, Limb severity, and ANatomic pattern of disease, in that order of priority. In this thesis we validated different prognostic models for CLTI (part I) and tested and improved the "Global Limb Anatomic Staging System" (GLASS). Furthermore, we provide exploratory feasibility studies regarding (the alternative use of) simple, low-cost and non-invasive diagnostic tools, such as transcutaneous oxygen tension (TCpO2) measurement and nailfold capillaroscopy (NFC) in PAD (part II). We also discuss potential regenerative therapies in CLTI, especially in patients who are not eligible for conventional revascularization intervention (part III).

Here, we provide a summary and general discussion of the presented studies as well as future perspectives.

OPTIMIZATION OF PREDICTION IN CLTI

All three models (BASIL[2], PREVENT III[3] and FINNVASC[4]) tested in **chapter 1** showed poor to fair predictive capability for predicting amputation, amputation-free survival (AFS) and mortality when retrospectively validated in three different study cohorts that were prospectively obtained (JUVENTAS[5], PADI[6] and Athero-Express[7]). The BASIL model could provide a solid basis for a more optimized prognostic model to be developed in future studies towards a pragmatic and accurate tool to support shared-decision making and therapeutic choices in CLTI. For instance, one might consider targeting distinct endpoints rather than combine them in a composite outcome such as AFS. One model may be better suited to predict limb loss, whereas the other may be better to predict survival. Furthermore, the addition of important prognostic factors such as inflammatory parameters such as IL-6 may be desirable. This approach may allow for more precise and accurate results, as well as greater flexibility in terms of how the model can be applied.

In **chapter 2** we conducted the first external validation of the Vascular Quality Initiative (VQI) survival prediction model, which is designed to predict two-year mortality risk in patients with CLTI. The model was derived from a large eponymous cohort of CLTI patients in the United States of America, who underwent either an open or endovascular infrainguinal revascularization between 2003 and 2017. The VQI survival prediction model was applied

to the JUVENTAS validation cohort (n=150) to compare estimated mortality and observed mortality at two years. This retrospective (prospectively collected data) single-center validation study of the VQI model showed satisfactory performance when used in 150 non-revascularizable Western European patients with CLTI. Our findings suggest that the VQI model is useful to predict all-cause mortality in different CLTI populations, including non-revascularizable CLTI patients.

In **chapter 3** we present the long-term results of the JUVENTAS population (N = 150). In its original publication the JUVENTAS trial showed no difference in major amputation or all-cause mortality 6 months after repetitive infusion (3 times at 3-week intervals) of Bone Marrow Mononuclear Cells (BMMNC) in 160 patients with severe, non-revascularizable (no-option) PAD, compared to placebo. Five years after the initial indication for revascularization (i.e. inclusion in the RCT), about half of the CLTI patients who were deemed non-revascularizable survived with salvage of the index limb. Although the prognosis of these high-risk patients is still poor, with the intensive trial-related medical care, their chances of surviving without amputation appear to be similar to those of patients with revascularizable CLTI.

DIAGNOSTIC TOOLS IN CLTI

GLASS (Global Lower Extremity Arterial Revascularization Success Score) is a relatively new angiography-based predictive tool that has been developed to evaluate the success of lower extremity arterial revascularization strategies and is part of the 2020 Global Vascular Guideline[1] (GVG). In **chapter 4**, we retrospectively studied GLASS inter-observer agreement in 289 angiograms. A stepwise component scoring system provides an acceptable level of agreement and a strong foundation for further prospective validation studies to assess the correlation between GLASS and treatment outcomes. Since our report several studies have used our improved stepwise scoring system[8].

The use of NFC is not only limited to assessment of the microcirculation in autoimmune disease, but can also be used to diagnose and monitor the progression of various other conditions, such as the development of diabetic vascular complications. In **chapter 5** we show that NFC is clinically feasible in PAD patients. While the study design and sample size did not allow for formal robust statistical analyses, patients with CLTI had abnormalities that were absent in controls. This feasibility study provides pilot data for larger (prospective) follow-up studies to assess the potential added value of NFC in PAD and CLTI diagnosis, prediction and prognosis.

Transcutaneous oxygen tension measurement (TcPO2) is a widely accepted and utilized method for assessing limb perfusion in patients with CLTI. It is included in the Wound, Ischemia, and foot Infection (WIfI) model and international PAD guidelines, making it one of the factors to guide therapeutic decisions. In **chapter 6**, we demonstrated that there is

a wide range of specific techniques used to measure TcPO2 in CLTI. Variations in probe temperature, probe location, and the use of a reference probe were observed among the studies. The importance of a consistent protocol is highlighted by the diverse results and conclusions of the reviewed studies.

STEM CELL THERAPY IN CLTI

In **chapter 7**, we discuss how future research in cell therapy should progress. To provide convincing evidence of the efficacy of mesenchymal stem cell (MSC) therapy, well-designed Randomized Controlled Trials (RCTs) should be conducted, using well-defined and clinically relevant outcomes, which would be ideally integrated with future imaging methods to evaluate collateralization and neovascularization. Individual research centers will not achieve sufficient statistical power to demonstrate efficacy. Collaboration between international researchers and data sharing are essential for advancing the field of science and upholding its standards of integrity. Therefore, we strongly suggest that more transparency and uniformity should be adopted in both the use of the experimental cell products and trial design in order to be able to adequately compare results or even merge data on an individual patient level. In line with the abovementioned ambitions we, the SAIL (Allogeneic mesenchymal Stromal cells for Angiogenesis and neovascularization in no-option Ischemic Limbs) trial group, proposed a new double-blind, randomized, placebo-controlled trial in 60 patients. The aim of this study would be to assess the feasibility, safety, and potential clinical benefits of intramuscular injection of allogeneic BM-MSCs. The methodology is outlined in **chapter 8.** In the following chapter we will discuss which treatment modalities should be prioritized and why.

CHAPTER 10

Future perspectives

FUTURE PERSPECTIVES

In the last decade the number of patients with PAD has increased, a trend that will continue the next decades. Within ten years after diagnosis, 2% of patients will undergo major amputation as the disease progresses. With ageing of the population associated with an increase of cardiovascular risk factors, such as obesity, sedentary lifestyle and DM in particular, it is very likely that the prevalence of CLTI will rise even further[9, 10]. Therefore, limiting this burden and minimalizing its consequences is an important challenge to contemporary health care.

Individual treatment decisions in CLTI are based on a multifactorial process guided by co-morbidities, patient preferences, anatomical characteristics and previous interventions. To adequately determine which treatment strategy is preferred for an individual patient, it is of utmost importance that physicians around the globe speak the same language when it comes to definitions, classifications and use the same outcomes in study context in CLTI. This is a must in order to be able to develop robust RCTs for new treatment modalities. Currently, PAD or CLTI trials typically involve a relatively small number of patients resulting in low statistical power and limited level of evidence. Therefore, treatment regimens are often adopted from extrapolated data from cardiology trials. Although PAD and CAD are both caused by atherosclerosis, there are also substantial differences which may hamper such an extrapolation. For instance, the risk of major adverse cardiovascular events (MACE) and major adverse limb events (MALE) differ between these two entities. Furthermore, pharmacotherapy does not necessarily work nor has it the same effect in PAD and CAD. This strategy needs to change and high quality PAD-specific studies should be initiated in order to stimulate the evolvement of novel treatments and improve prognosis with regards to this important global health-issue.

EVIDENCE-BASED REVASCULARIZATION

The recently published GVG focus on the definition, evaluation, and management of CLTI with the goals of improving EBR and highlighting critical research needs[1]. The term CLTI is preferred over the previously used term critical limb ischemia (CLI), as the latter implies a specific level of impaired perfusion, while CLTI encompasses a broader range of impaired perfusion and stresses the chronic state of the disease. By definition CLTI is a medical condition characterized by the presence of PAD in conjunction with rest pain, gangrene, or a non-healing lower limb ulcer. The guideline provides a framework to standardize treatment and research in this specific group of patients with a heterogenous profile with respect to co-morbidities, anatomic pattern of disease, and treatment options. This framework aims to help reduce the extremely high risk of MACE and MALE in these patients by selecting the best suitable approach.

Effective revascularization is the cornerstone of limb salvage in CLTI. Although multiple techniques are available, there are limited high-quality data to support EBR. Two recent studies[11,12] emphasized the need for a systematic and unambiguous approach in order to improve decision-making, clinical outcomes, and cost-effectiveness. Ideally, "PLAN" should be used as a blueprint; PLAN is an acronym for the three pillars of this framework; Patient risk, Limb severity and ANatomic pattern of disease.

Patient risk

The PLAN-algorithm evaluates the patient's eligibility for limb-saving surgery based on their peri-procedural risk, cardiovascular morbidity and estimated life expectancy. If the risk is deemed acceptable and the patient is expected to live long enough to benefit from a potential invasive procedure, then limb-sparing surgery may be recommended. If the risks do not outweigh the improvement in quality of life, then a wait-and-see approach, amputation, or a palliative course of treatment may be the best option. Based on life expectancy, a distinction is also made between bypass or endovascular intervention. For example, the BASIL trial showed that bypass surgery should be preferred beyond survival expectancy of two years. However, several prognostic models created to date (VQI, BASIL and PREVENT-III) have demonstrated moderate quality, as shown in this thesis.

Limb severity

The second step consists of staging of the affected leg based on the presence of a wound, level of ischemia and presence or severity of infection. For this purpose, the Society of Vascular Surgery (SVS) Lower Extremity Threatened Limb Classification System may be utilized. It is a descriptive and predictive classification that bundles ischemic and diabetic classifications. This is done through three pillars: Wound Size, Degree of Ischemia and the Presence of Foot Infection (WIfI). This approach is comparable to the TNM classification system used in oncology. By combining these scores, it is possible to divide the risk into four categories: very low, low, moderate, and high for both the 1-year risk of amputation without revascularization and the necessity of revascularization. The WIfI classification system, which was developed based on expert consensus, has been validated in multiple studies and has been shown to have predictive value for duration of hospitalization and occurrence of myocardial infarction.

Anatomical classification

Finally, the anatomic pattern of the disease is important, identifying specific therapeutic options and providing a model to estimate probability of success. The Global Limb Anatomic Staging System (GLASS), which is similar to the SYNTAX score[13] in CAD, can be used for this purpose. And like SYNTAX, the score is used primarily for endovascular interventions. Using imaging data, an optimal pathway is selected that is expected to restore the blood flow to the ankle and foot. This is called the target artery path (TAP) and in this pathway the

femoropopliteal segment and the infrapopliteal segment are scored based on the (length and degree of) stenosis and occlusion. The GLASS score is intended to be used to assess the technical success and 1-year patency of an intervention[1,8]. It is of importance that future research focuses on primary validation of GLASS in different imaging techniques such as CTA and MRA, so no baseline angiography is necessary. Hence, the latter is no standard of care in most countries in Europe.

Artificial intelligence

There have been various reports on the use of artificial intelligence (AI) such as machine learning (ML) in cardiovascular diseases, but its potential applications for patients with PAD have not been extensively documented. Most studies have concentrated on developing automated detection and characterization of arterial lesions based on imaging, such as CTA[14]. AI holds numerous possibilities for assisting and enhancing the management of patients suffering from PAD and potentially be a supportive tool in clinical decision-making. Specific applications could evolve around combining clinical risk factors and other (bio) markers to increase the accuracy of prediction, identification of factors associated with hospital readmission after vascular procedures using big (international) data or automatic and early identification of patients with PAD or CLTI in medical records.

NOVEL TREATMENTS AND MANAGEMENT STRATEGIES

In PAD, therapy and management have two main aims; 1. Reduction of cardiovascular risk and 2. Limb preservation. Conventional treatment choices in PAD consist of exercise therapy and revascularization. Furthermore, Cardiovascular Risk Management (CVRM) is essential for CLTI patients, who are at a very high risk for cardiovascular events. It involves lifestyle modifications, including more physical activity and smoking cessation, and cardiovascular risk(factor) management. To lower the risk of MACE and MALE, lipid lowering, antithrombotic and antihypertensive drugs and therapies for diabetes mellitus have proven to be effective.[15] Additionally, emerging therapies such as cell therapy, organic nitrates, and antioxidants are currently under investigation.[15]

Inflammation and pharmacological prevention

Although there have been advancements in surgery, antithrombotic therapy, and interventions that modify risk factors, such as lipid-, blood pressure-, and glucose-lowering interventions, patients suffering from PAD still face a high residual cardiovascular risk. Hence, strategies to address this residual risk are urgently needed. Inflammation has a significant role in the development and progression of atherosclerosis. Recent studies have shown that anti-inflammatory therapy can reduce the risk of MACE in patients with CAD[16, 17]. As a result, there have been efforts to examine anti-inflammatory agents as a supplementary therapeutic option for the prevention and treatment of atherothrombosis.[18] A significant proportion of cases involving blood vessel reconstruction, such as stenting

or balloon angioplasty, and venous bypass grafting result in intimal hyperplasia (IH) of the vessel wall. This is caused by endothelial injury during the vascular intervention, which can cause vessel restenosis and potentially life-threatening consequences for patients.[19] Drugs used for preventing IH have primarily targeted the vascular smooth muscle cell proliferation pathway involved in IH development. However, limitations such as endothelial toxicity and incorrect drug administration timing have prompted the exploration of alternative pharmacological approaches that are more effective in controlling IH.

Revascularization

To improve limb perfusion and minimize the risk of amputation revascularization is an essential part of the therapeutic approach in CLTI. However, to date it is largely unclear whether surgical or endovascular revascularization is a more effective initial strategy for improving limb outcomes. Two recently published RCT's demonstrated largely opposing results in this regard[11,12]. One consisted of 345 CLTI patients and showed that an endovascular first strategy was associated with a better AFS, which was largely driven by fewer deaths in the endovascular treatment group.[12] The other RCT included 1830 CLTI patients, and demonstrated that of CLTI patients with a suitable great saphenous vein for surgical revascularization, the occurrence of MALE or death was notably lower in the surgical group compared to the endovascular group.[11] In the group where no saphenous vein was available, there was no difference in outcomes between surgical and endovascular revascularization. However, a limitation of this study and such studies in general is that they are subject to selection bias. In this particular study only patients with infrainguinal PAD, eligible for both open and endovascular revascularization, were included.

Cell Therapy

According to preclinical studies conducted on animal hind limb ischemia models, stem cells injected intramuscularly into the hind limb can enhance blood flow through an angiogenic mechanism. Similarly, initial studies conducted in humans have demonstrated improved vascularity in the treated extremity, as measured by ABI. The specific mechanism behind this phenomenon is largely unknown. Despite early promising results, to date there are no phase 3 trials that have shown cell therapy to be effective. A meta-analysis[20] of randomized placebo-controlled trials of stem cell therapy demonstrated no improvement in AFS or major amputation rates. However, secondary outcomes such as ABI were significantly improved in the treatment group. A more recently completed phase 1 trial with allogeneic placental stem cells (PLX-PAD cells) has shown promising safety and potential efficacy[21]. These cells are believed to be immune privileged and would potentially offer an "off-the-shelf" treatment option.

The fact that present and recent trials face slow recruitment rates of no option patients, might suggest that fewer patients require alternative treatments. However, it is more

likely that this effect is caused by shifting definition or delayed acknowledgement of ”no-option” status. Increasing number of patients are offered revascularization therapy, due to improved endovascular techniques and ongoing absence of alternatives. Additionally, improved pharmacological therapy contributes to this phenomenon as well. However, revascularization may fail or only partly reduce symptoms. Therefore, the need for novel treatments for no-option patients remains relevant.

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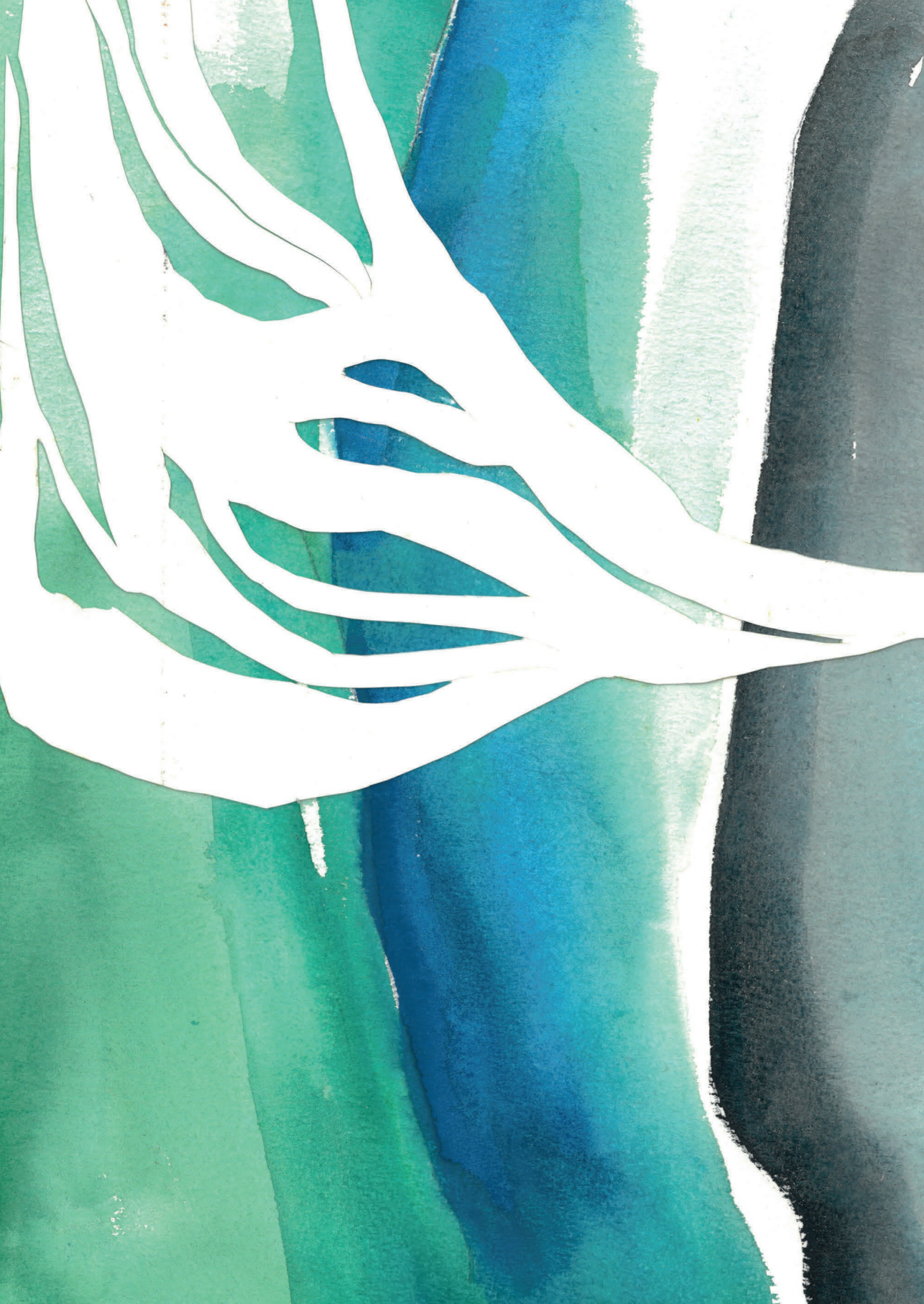


PART V

APPENDIX

Samenvatting
List of publications
Acknowledgements
Curriculum vitae

SAMENVATTING

Hart- en vaatziekten vormen doodsoorzaak nummer één wereldwijd. Het betreft een systemische ziekte van de slagaders waarbij zowel door verwijding als vernauwing van het bloedvat gezondheidsproblemen ontstaan. Perifeer (buiten het hart of hersenen gelegen) arterieel (slagaderlijk) vaatlijden (PAV) is een vernauwing van de slagaders die doorgaans wordt veroorzaakt door atherosclerose (slagaderverkalking). Beschadiging van de gladde binnenbekleding van de slagader leidt tot accumulatie van immuun cellen en vettige eiwitten, met als gevolg een afname van de diameter van het bloedvat. Op dit proces van beschadiging en vernauwing van bloedvaten hebben vele factoren invloed, zoals degeneratie van weefsels door veroudering, hoge bloeddruk, cholesterol, diabetes (suikerziekte) en roken.

Vernauwing van slagaders in de benen leidt ertoe dat de spieren in de benen onvoldoende bloed en uiteindelijk onvoldoende zuurstof krijgen aangeleverd. Dit tekort resulteert in een versneld optreden van verzuring of spierpijn. Het ziektebeeld is progressief en kan er uiteindelijk voor zorgen dat patiënten met PAV niet of nauwelijks nog kunnen lopen zonder krampende pijn in de spieren te ervaren. Pijn die pas wegtrekt na even stil te hebben gestaan voor bijvoorbeeld de etalage van een winkel; claudicatio intermittens ofwel ‘etalagebenen’ genoemd.

In 2010 werd geschat dat er wereldwijd >200 miljoen mensen lijden aan PAV. In 10 jaar steeg de prevalentie met 28.7% in landen met een laag inkomen en 13.1% in landen met een hoog inkomen. Deze stijging lijkt mede te berusten op vergrijzing en de toename van overige risicofactoren, in het bijzonder diabetes mellitus. Daarnaast is aangetoond dat de algehele toegenomen welvaart heeft bijgedragen aan overconsumptie en andere ongezonde leefgewoonten, met als gevolg dat hart- en vaatziekten ook steeds vaker op jongere leeftijd ontstaan. In latere stadia van PAV kunnen er zelfs rustpijn of niet-genezende wonden optreden. Deze eindstadia van de ziekte noemen we chronische ledemaat-bedreigende ischemie (CLBI). In Nederland gaat het naar schatting om 85.000 55-plussers met claudicatio intermittens. CLBI kwam in 2009 voor bij 12.500 Nederlanders. Van de patiënten die leiden aan dit vergevorderde stadium overlijdt 51 tot 58% binnen vijf jaar na de eerste ziekenhuisopname. Om dit in perspectief te plaatsen: van alle patiënten die een hartinfarct krijgen, overlijdt 28% binnen vijf jaar na dato. Voor borstkanker is dit 11%.

De eerste stap in de behandeling van PAV is het behandelen van de risicofactoren zoals roken en hoge bloeddruk. De andere belangrijke pijler is het weer doorgankelijk maken van de slagader met behulp van een chirurgische interventie. Dit kan op verschillende manieren geschieden, bijvoorbeeld door met behulp van een dotter (een opblaasbare ballon) de vernauwingen op te heffen, het plaatsen van een stent (buisje) om de opgeheven

vernauwing te behouden of het omleiden (bypass) van een slecht of niet doorgankelijk bloedvat met behulp van een implantaat of eigen weefsel. Een van de grootste hedendaagse uitdagingen binnen het diagnostisch en therapeutisch proces van PAV is dat in meer vergevorderde stadia van de ziekte patiënten vaker niet in aanmerking komen voor een van de genoemde therapieën. Voor deze patiënten wordt gezocht naar nieuwe behandelmogelijkheden zoals stamceltherapie. Dit onderwerp wordt in **'part 3'** van dit proefschrift beschreven.

Stamceltherapie zou door een complex samenspel van cellen en andere factoren in een milieu waar een chronisch relatief zuurstoftekort heerst, aanzetten tot angiogenese, ofwel de vorming van (nieuwe) bloedvaten door het lichaam zelf. Hiervoor zouden cellen van de patiënt zelf of van een donor gebruikt kunnen worden. In diermodellen en bij andere ziektebeelden bij mensen is dit fenomeen reeds aangetoond. Tot concrete conceptualisatie van een efficiënte en effectieve therapie voor patiënten die niet in aanmerking komen voor conventionele behandeling heeft dit nog niet geleid. In dit proefschrift hebben we vastgesteld dat de heterogeniteit in gebruikte celproducten, studie-populaties, design en andere factoren hier mede toe hebben geleid. Allogene (cellen van donor) toepassing van MSC-therapie (mesenchymale stromale cellen) zijn voor commerciële partijnen het meest interessant, omdat een gedefinieerd celproduct relatief eenvoudig kan worden gepatenteerd en geproduceerd door in-house bedrijven, zonder de complicaties van het oogsten van donormateriaal bij elke patiënt in het geval van autologe therapie. We zouden daarom meer openheid en standaardisatie aanmoedigen, zowel bij het gebruik van het onderzoekscelproduct als bij het studie-ontwerp. Overtuigend bewijs voor de werkzaamheid van MSC-therapie zal alleen komen van goed ontworpen gerandomiseerde gecontroleerde onderzoeken (RCT's) met harde en klinisch relevante resultaten, die idealiter verband zouden houden met toekomstige beeldvormingsmethoden om neovascularisatie te evalueren. Het lijkt steeds onwaarschijnlijker dat afzonderlijke onderzoekscentra voldoende statistische power zullen bereiken om de werkzaamheid aan te tonen. Internationale samenwerking en het delen van gegevens zijn nodig om het veld vooruit te helpen en de wetenschappelijke integriteit te behouden.

Een andere belangrijke uitdaging wordt gevormd door het feit dat progressie van PAV naar CLBI multifactorieel en onvoorspelbaar is. Hierdoor blijkt het opstellen van een gestandaardiseerd zorgplan lastig. Het ontbreekt mede aan een uniforme stadiëring van ziekte-ernst. Derhalve is er een platform gemaakt voor zogenaamde *evidence-based revascularization (EBR)* waarin definities, stadiëring en behandeling worden vastgelegd. Er dient bij elke patiënt een 'PLAN' te worden gemaakt: waarbij op kwantificeerbare wijze een indeling wordt gemaakt op basis van drie pijlers: *Patient risk, Limb severity en ANatomic pattern of disease.* Uiteindelijk zal PLAN de basis vormen voor het identificeren van de beste therapeutische optie en het verschaffen van adequate informatie voor de patiënt

met betrekking tot de te verwachten slagingskans en duurzaamheid van de betreffende behandeling.

'Part 1' van dit proefschrift besteden we aandacht aan de eerste pijler 'patient risk.' In PLAN wordt gekeken of de patiënt een geschikte kandidaat is voor een ledemaat sparende ingreep, afhankelijk van het peri-procedurele risico en de levensverwachting. Indien de risico's niet opwegen tegen de verbetering van kwaliteit van leven kan er voor afwachtend beleid, een amputatie of een palliatief traject worden gekozen. Op basis van de levensverwachting wordt ook een onderscheid gemaakt tussen bypass of endovasculaire interventie. In **chapter 1** onderzoeken we van drie bekende en gangbare predictiemodellen het vermogen om te voorspellen wat de kans is dat een patiënt binnen bepaalde tijd komt te overlijden of een amputatie zal ondergaan op basis van verschillende patiënt-karakteristieken. We concluderen dat alle drie de modellen slecht tot matig in staat zijn om een redelijk accurate voorspelling te doen voor de individuele patiënt. In **chapter 2** wordt voor het eerst een relatief nieuw predictiemodel (VQI) voor overleving gevalideerd in een extern cohort. De resultaten laten zien dat het model goed presteert als het gaat om het voorspellen van mortaliteit in patiënten met eindstadium PAV. Dit geldt ook voor de subgroep van patiënten die niet meer in aanmerking komt voor welke operatieve interventie dan ook. In **chapter 3** stellen we vast dat in de no-option CLBI-populatie van de JUVENTAS-studie, vijfjarige amputatievrije overleving vergelijkbaar is met de CLBI-populatie die nog wel in aanmerking komt voor revascularisatie.

'Part 2' van dit proefschrift gaat mede over de derde pijler van PLAN; 'anatomic pattern of disease.' **Chapter 4** gaat hiervoor in op het *Global Limb Anatomic Staging System (GLASS).* De score wordt primair gebruikt voor endovasculaire interventies. Op basis van beeldvorming (MRA/CTA/angiografie) wordt een optimale route gekozen die moet leiden tot verbeterde perfusie van de enkel en voet. Dit is de *target artery path (TAP)* en in dit traject worden twee segmenten gescoord op basis van de (lengte en mate van) stenosering en occlusie. Aan de hand hiervan kan er door de som van de verdeelde punten een schatting worden gedaan over technisch slagen en de 1 jaar-duurzaamheid van een interventie. Een uitdaging met chronische ischemie van het been, is het bepalen van de mate van ernst van ischemie. De meeste nauwkeurige methode die nu gebruikt wordt betreft een scan. Echter deze scan is kostbaar, niet overal beschikbaar en in sommige gevallen zelfs schadelijk voor de patiënt. Daarom zijn minder belastende en praktisch eenvoudiger uitvoerbare methoden nodig, die ook inzicht kunnen verschaffen over de staat van de bloedvaten in de benen. In **chapter 5** onderzoeken we de potentie van de alternatieve toepassing van een bekende meetmethode. Capillaroscopie is een beproefde methode binnen de reumatologie, waarbij met behulp van een speciaal vergrootgals wordt gekeken naar kwalitatieve en kwantitatieve variabelen met betrekking tot de kleine haarvaatjes in de nagelriem van de vingers. Er werden specifieke afwijkingen gevonden bij CLBI-patiënten, die bij de

mildere vormen van PAV (nog) niet aanwezig zijn. Deze bevinding in combinatie met het gegeven dat PAV onderdeel is van een systemisch vaatlijden, opent de deuren voor meer onderzoek naar nieuwe methoden of de alternatieve toepassing van bestaande. Zoals het meten van zuurstofspanning onder de huid. Deze meetmethode wordt de “transcutane zuurstofspanningsmeting (TCp02)” genoemd. In **chapter 6** onderzoeken we of TCp02 in studies op gelijke wijze wordt toegepast. Aangezien TCp02 een gevoelig meetinstrument betreft, is een gestandaardiseerde toepassing wenselijk om uitkomsten goed te kunnen vergelijken. Onze conclusie is dat de TCp02 meting door verschillende ziekenhuizen erg verschillend wordt toegepast. Dit tast op zijn minst de betrouwbaarheid van de bevindingen aan, echter onduidelijk is in hoeverre dit van invloed is op de individuele metingen.

Dit proefschrift biedt een overzicht van management van CLBI, waarbij de nadruk ligt op validatie van PLAN-pijlers, oriënterende haalbaarheidsstudies met betrekking tot (de alternatieve toepassing van) eenvoudige, voordelige diagnostische hulpmiddelen en de beschouwing van potentiële nieuwe therapieën. Het doel is om bij te dragen aan het verkrijgen van eenduidige definities, uniforme classificering en optimalisatie van indicatiestelling binnen CLBI, teneinde de kwaliteit van toekomstig wetenschappelijk onderzoek en daarmee de behandeling en prognose van patiënten te verbeteren.

LIST OF PUBLICATIONS

1. **Validation of Randomized Controlled Trial-derived Models for the Prediction of Post-intervention Outcomes in Chronic Limb-Threatening Ischemia**
   J.G.J. Wijnand, I.D. van Koeverden, M. Teraa, M.I. Spreen, W.P.T.M. Mali, H. van Overhagen, Gerard Pasterkamp, GJ. de Borst, M.S. Conte, H. Gremmels, M.C. Verhaar
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2. **External validation of the Vascular Quality Initiative prediction model for survival in no-option Chronic Limb-Threatening Ischemia patients**
   M.C. Verwer, J.G.J. Wijnand, M. Teraa, H. Gremmels, J.P. Simons, M.S. Conte, M.C. Verhaar, GJ. de Borst
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7. **Cell Therapy for Chronic Limb-Threatening Ischemia: Current Evidence and Future Directions**
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ACKNOWLEDGEMENTS | DANKWOORD

Beste **Gert Jan**, voor je begrip, steun, geduld en natuurlijke gave om mij als onderzoeker te motiveren. And **prof. Conte**, for inviting me to do research at the University of California in San Francisco for a couple of months. It was a great honor and pleasure.

Beste **Martin**, voor je inspanningen en hulp als co-promotor, ik had mij geen betere kunnen wensen.

Beste **Marianne**, voor het bieden van de kans, ondanks dat alles anders liep dan gepland.

Beste **Hendrik**, **Ian**, **Maarten**, **Femke** en **Steven**, voor jullie onmisbare bijdrage als coauteurs.

Beste hooggeleerde **leden van de beoordelingscommissie**, voor het beoordelen van dit proefschrift.

Beste **Joost** en **Mathijs**, paranimfen. Jullie hebben geen idee waar dit boekje over gaat en dat is prima. Wat wij allemaal hebben meegemaakt en uitgespookt zou niet passen in een boek dat twee keer zo dik is.

Beste **Aarent**, **Bernard** en **Robert**. Door jullie kon ik het bestaan als arts-onderzoeker volhouden. Elke dag op de 7e verdieping van de toren met jullie was een feestje. Van acceptatie en (met name) rejection-pils om 10 uur 's ochtends, heerlijke setjes en haardvuren van Arie, slappe typetjes van Bernard, voetbalpraat, gezelligheid en kookkunsten van Baggio, tot eindeloos ondernemen, "even iets bellen," steppen door Hamburg, volledig all-in reizen als BHV en BLS-instructeurs op een decadente skitrip met een groep tandartsen en een congres met roadtrip langs de east coast van de VS. Wetenschap was wat ons bond als belangrijkste bijzaak. Ik vind het prachtig om te zien dat we allemaal onze dromen aan het verwezenlijken zijn, als vaders, partners en dokters.

Beste **Janine**, **Marjolein**, **Constance**, **Armelle**, **Djurre**, **Robin**, **Marieke** en **Leonie**, voor het meerijden tijdens deze tweede studententijd. Jullie hebben een belangrijke rol gespeeld in het vormgeven van alle mooie herinneringen.

Beste **Maurits**, **Niels**, **Folkert**, **Gijs**, **Paul**, **Laurens**, **Lucas** en **Hilde**, voor de onvergetelijke tijd in San Francisco. Het surfen, de feesten. Wat hebben wij veel gave dingen meegemaakt. Zonder twijfel een van de mooiste perioden uit mijn leven.

Beste **Andor** en **Ornis**, mijn beste maatjes uit Utrecht, voor het planten van het promotiezaadje in het Academiegebouw van Utrecht, in de herfst van onze studententijd.

Beste **Jonathan**, mijn trouwe vriend aan de andere kant van de oceaan, voor je humor, je bijna dagelijkse belletjes en onze gemeenschappelijke interesses. Ik koester onze roadtrip langs de west coast van de VS samen met Keppel.

Beste Robberts; **Puijk** en **Keppel**, voor het samen oplopen tijdens een enerverende fase in ons leven, die door eenzelfde ritme, eindeloos ouwehoeren en veel snode plannen tot een dierbare vriendschap heeft geleid.

Beste **Jeroen** en **Mo**, mijn maatjes uit het MCL, voor het relativeren, het sparren en het lachen op de werkvloer en daarbuiten.

Beste **stafleden** en **assistenten** van de plastische chirurgie en chirurgie in het MCL, met name mijn opleiders **Chantal**, **Irene** en **Marloes**, voor de prettige samenwerking en de geboden ruimte en flexibiliteit die mij in de gelegenheid stelden dit proefschrift af te ronden.

Lieve **vrienden** en **familie**, voor het vragen wanneer mijn boekje af is, het antwoord is nu.

Lieve **Opa**, voor de motivatie. “Goed je best doen.” Voor u heb ik het afgemaakt.

Lieve **papa**, **mama**, **Julie** en **Mytza**, voor jullie warmte, liefde, lichtend voorbeeld en stabiele basis in mijn leven. Jullie hebben mij gemaakt tot wie ik ben. Zonder te pushen, werden kosten noch moeite gespaard om mij de kansen te bieden om te floreren in velerlei facetten van het leven. Doorzettingsvermogen zit in ons bloed. Ik hou van jullie.

Lieve **Vanessa**, wat een intense en prachtige jaren hebben wij al doorgemaakt samen. Zo veel avonturen en mijlpalen. Ik prijs mezelf gelukkig met zo’n lieve, vrolijke, geestige, positieve, zelfverzekerde, stabiele en relaxte vrouw aan mijn zijde. Je hebt me geïnspireerd met jouw doorzettingsvermogen. Ik zou willen dat ik je tijdens jaar 1 van geneeskunde al mee uit had durven vragen. “Tot straks.” Of toen we allebei ANIOS en later collega arts-onderzoekers waren bij de chirurgie in het UMCU. Dan had ik veel eerder geweten wat voor prachtpersoon jij bent. Sometimes, the thing you’ve been looking for is right there beside you all along. Bo ta e amor di mi bida. Mi soñonan a bira realidat. Op naar boek nummer vier; het trouwboek van onze aanstaande bruiloft op Curaçao! Las aventuras continuan...

Lieve **Ole**, mijn kleine grote liefde, mijn eigenwijze, intelligente, zachtaardige en temparamentvolle strijder. Je kunt nog niet lezen, maar je zal niet de enige zijn die dit boek niet leest. Je maakt me een trotse en gelukkige vader en je helpt me om stil te staan in het sneltreinleven dat we soms leiden.

CURRICULUM VITAE

Joep Gijsbert Jan Wijnand was born on the 8th of May 1988 in Assen, the Netherlands, as the eldest of three children. He was raised on the Island of Texel. After primary school he went to the OSG de Hogeberg in Den Burg, where he obtained his VWO-level high school certificate in 2006. Directly hereafter Joep started his medical training at the University Medical Center in Utrecht. During medical school, he worked as a student tutor in anatomy and clinical education at the Medical faculty. In 2012 he performed a clinical internship at the University of Buenos Aires, Argentina. Besides medical school he developed a career in top sailing and participated in various board functions. Starting from 2013 he gained clinical experience as a surgery and dermatology resident-not-in-training, at the Diakonessenhuis hospital and UMCU in Utrecht. Everything with the aim of ultimately realizing his long-cherished dream of becoming a plastic surgeon.

In 2016, Joep started his PhD trajectory at the dept. of Vascular Surgery and the dept. of Nephrology and hypertension at the University Medical Center Utrecht. During this period, he was coordinating investigator of the 'SAIL' trail, a randomized controlled trial with Allogeneic mesenchymal Stromal cells for Angiogenesis and neovascularization in no-option Ischemic Limbs. He also set up a biobank for amputated lower limbs. To date he is (co-)author of 9 papers in the field of (vascular) surgery, especially regarding the management of chronic limb-threatening ischemia. In that context he worked for several months at the Dept. of Vascular Surgery at the University of California in San Francisco, US. Besides his work as a researcher he co-founded the successful company HAP-arts, that trained and seconded MD PhD students with clinical experience to assist general practitioners in the overflowing emergency primary care.

In December 2019, Joep started working as a resident at the dept. of Plastic, Hand, Reconstructive and Esthetic surgery at the Medical Center Leeuwarden in Friesland. In July 2021 he joined the training program to become a Plastic Surgeon, which he expects to complete in early 2027.

“Bij Sparta jasje uit.”

Michiel Romeyn - 1997