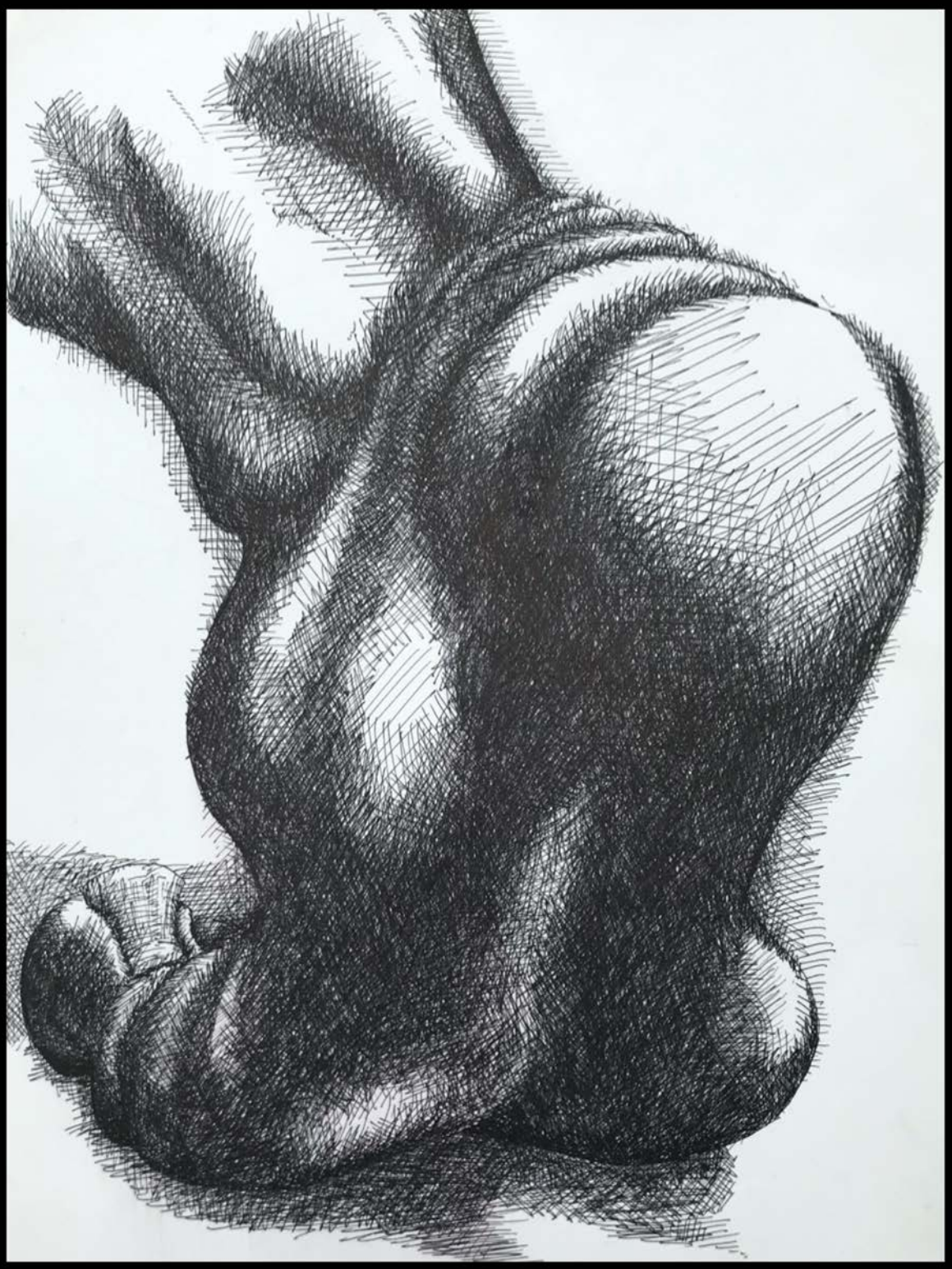
Universitat Autònoma de Barcelona

Clinical application of 2D perfusion angiography in critical limb ischemia revascularization

Efrem Gómez Jabalera

CLINICAL APPLICATION OF 2D PERFUSION ANGIOGRAPHY IN CRITICAL LIMB ISCHEMIA REVASCULARIZATION



EFREM GÓMEZ JABALERA

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Clinical application of 2D perfusion angiography in critical limb ischemia revascularization

Aplicació clínica de l'angiografia de perfusió 2D en el tractament de la isquèmia crítica de l'extremitat

Aplicación clínica de la angiografía de perfusión 2D en el tratamiento de la isquemia crítica de la extremidad

Efrem Gómez Jabalera

DOCTORAL THESIS – UAB 2020

Thesis directors:

Dr. Artigas Raventós, Vicenç

Dr. Bellmunt Montoya, Sergi

Dr. Dilmé Muñoz, Jaume

Thesis tutor:

Dr. Artigas Raventós, Vicenç

Doctorat en Cirurgia i Ciències Morfològiques
Facultat de Medicina
Departament de Cirurgia
Universitat Autònoma de Barcelona

A mi abuelo

Dr. Vicenç Artigas Raventós, MD, PhD, Surgical Department, Univesitat Autònoma de Barcelona, Barcelona; **Dr. Sergi Bellmunt Montoya, MD, FEBVS, PhD,** Hospital Universitari Vall d'Hebron, Univesitat Autònoma de Barcelona and **Dr. Jaume Dilmé Muñoz, MD, FEBVS, PhD,** Hospital de la Santa Creu i Sant Pau, Univesitat Autònoma de Barcelona, Spain.

They declare that **Efrem Gómez Jabalera** has performed under their supervision, the studies presented in the Dissertation Clinical application of 2D perfusion angiography in critical limb ischemia revascularization. This Dissertation has been structured following the normative for PhD Thesis to obtain the degree of **International Doctor Mention** and it is ready to be presented to a Tribunal.

Barcelona, June 2020

Director and Tutor	Director	Director
Vicenç Artigas Raventós	Sergi Bellmunt Montoya	Jaume Dilmé Muñoz

No sabía
si era un limón amarillo
lo que tu mano tenía,
o el hilo de un claro día,
Guiomar, en dorado ovillo.
Tu boca me sonreía.
Yo pregunté: ¿Qué me ofreces?
¿Tiempo en fruto, que tu mano
eligió entre madureces
de tu huerta?
¿Tiempo vano
de una bella tarde yerta?
¿Dorada esencia encantada?
¿Copla en el agua dormida?
¿De monte en monte encendida,
la alborada
verdadera?
¿Rompe en sus turbios espejos
amor la devanadera
de sus crepúsculos viejos?

Antonio Machado

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I. SUMMARY

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Summary

The endovascular treatment (EVT) of Peripheral Arterial Disease (PAD) is based on angiographic imaging and post-revascularization treatment success is based on the subjective interpretation of this visual assessment. 2D perfusion angiography (PA) is an image-processing software which may allow for the quantification of perfusion. In addition, a simpler anatomic classification system, able to describe the arterial disease burden below the groin, needs to be designed to determine the best therapy for any given patient.(1)

The aim of this thesis is to create an objective system to assess the success of EVT based on the quantification of tissue perfusion through PA, capable of accurately predicting the healing probability of ischemic ulcers. Secondly, we seek to describe a classification system of easy application during daily clinical practice that will also facilitate comparison of patients among clinical trials.

The Project was designed as a retrospective cohort study with consecutive patients undergoing EVT at a single specialized center for critical limb ischemia (CLI). The cases were analyzed with PA before and after treatment, and also ranked according to current classification systems (Rutherford, TASC and WIfI) and a new proposed classification: the Abano Terme Score (ATS). Demographic and clinical data were recorded and clinical follow-up was performed (at 1 and 6 months). The PA parameters were Arrival Time (AT), Peak Time (PT), Wash-in Rate, Width, Area Under Curve and Mean Transit Time (MTT). Two cohorts were defined based upon a time to heal of less or longer than 30 days.

From January 2015 to July 2016, PA analysis was performed on 580 consecutive patients that underwent EVT. Among them, 332 met the inclusion criteria to be studied, from which 123 were excluded for ulcer healing analysis (34 because of poor image quality, 50 patients had no ulcer, 20 died and 19 were lost at follow-up). Mean age was 72 years and 67.5% were men; 133 patients had Rutherford 5 and 76 had Rutherford 6 lesions, with similar distribution in both groups. The WIfI risk for amputation was also similar for both groups, and it was low in 24%, moderate in 14% and high in 62%. We found significant differences between groups in the healing time for the following cut-off values of PA parameters: AT>6 seconds and improvement of MTT>1.7 seconds or the MTT>4.1 seconds after the treatment. The ATS, while being a simpler classification than current used system, not only showed a better correlation with parameters such as the transcutaneous pressure of oxygen (TcPO2) and PA; but also demonstrated, in a subsequent analysis, a better correlation with ulcer healing and amputation free-survival in patients with Rutherford 5 lesions.

Resum

El tractament endovascular de la malaltia arterial perifèrica està basat en les imatges angiogràfiques. La interpretació subjectiva feta a partir d'elles és com més habitualment s'avalua l'èxit del tractament durant la revascularització. L'angiografia de perfusió 2D (AP) és un software de processat de la imatge que podria donar peu a la quantificació de la perfusió distal. Endemés, un sistema de classificació anatòmic més senzill, capaç de descriure la càrrega de malaltia arterial per sota de l'engonal, és necessari per poder triar el millor tractament per un pacient concret.

La intenció d'aquesta tesis és identificar una mesura objectiva per avaluar l'èxit del tractament endovascular basant-se en la quantificació de la perfusió del teixit amb l'AP; i per consegüent tenir la capacitat de predir amb major precisió la curació de les úlceres isquèmiques. Secundàriament, hem buscat adaptar un sistema de classificació que pugui ser aplicat amb facilitat a la pràctica clínica diària i que permeti la comparació entre pacients a assajos i estudis clínics.

La investigació del projecte present es va realitzar amb un estudi de cohorts retrospectiu amb pacients consecutius sotmesos a tractament endovascular en un únic centre especialitzat en tractament de la isquèmia crítica de l'extremitat. Als pacients se'ls va realitzar una AP abans i després del tractament, i també es van classificar d'acord amb els sistemes de classificació més utilitzats (Rutherford, TASC i WIfI) i amb una nova classificació proposta: l'score d'Abano Terme (ATS). Les dades demogràfiques i clíniques es van recollir i es va realitzar un seguiment clínic al mes i als 6 mesos. Els paràmetres de l'AP van ser el temps d'arribada (AT), el temps de pic (PT), la velocitat del rentat (WS), l'amplada (W), l'àrea sota la corba (AUC) i el temps de trànsit mig (MTT). Les dos cohorts es van definir en base a un temps de curació major o menor a 30 dies.

Des del gener de 2015 fins al juliol de 2016, 580 pacients consecutius van ser tractats endovascularment i van ser estudiats amb AP. D'entre ells, 332 complien els criteris d'inclusió, dels quals 123 van ser exclosos posteriorment de l'anàlisi per la curació de les úlceres (en 34 casos la imatge de l'AP tenia mala qualitat, 50 pacients no presentaven úlceres, 20 van finar i 19 no van completar el seguiment). L'edat mitja va ser de 72 anys i el 67,5% eren homes. 133 pacients presentaven lesions Rutherford 5 i en 76 les lesions eren Rutherford 6. El risc WIfI d'amputació va ser baix en un 24%, moderat en un 14% i alt en un 62%. Vam trobar taxa de curació als 30 dies, amb diferències estadísticament significatives entre els grups, per als següents punts de tall dels paràmetres de l'AP: AT > 6 segons i MTT > 4.1

segons o un increment del mateix MTT > 1,7 segons. L'ATS, sent un sistema de classificació més senzill que es existents actualment, no només es va correlacionar millor que amb la TcPO2 i amb l'AP; sinó que en un posterior anàlisis va demostrar millor correlació amb la curació de les úlceres i la supervivència lliure d'amputació en pacients amb lesions Rutherford 5.

Resumen (Castellano)

El tratamiento endovascular de la enfermedad arterial periférica está basado en las imágenes angiográficas. La interpretación subjetiva hecha a partir de ellas es el modo más habitual de evaluar el éxito del tratamiento durante la revascularización. La angiografía de perfusión 2D (AP) es un software de procesado de la imagen que podría permitir la cuantificación de la perfusión distal. Además, un sistema de clasificación anatómico más sencillo, capaz de describir la carga de enfermedad arterial por debajo de la ingle, es necesario para decidir el mejor tratamiento para un paciente dado.

La intención de esta tesis es identificar una medida objetiva para evaluar el éxito del tratamiento endovascular basado en la cuantificación de la perfusión del tejido con la angiografía de perfusión; y por ende ser capaz de predecir con mayor precisión la curación de las úlceras isquémicas. Secundariamente, hemos tratado de adaptar un sistema de clasificación que pueda ser aplicada fácilmente a la práctica clínica diaria y que permita comparaciones entre pacientes en ensayos clínicos y estudios.

La investigación de este proyecto se basó en un estudio de cohortes retrospectivo con pacientes consecutivos sometidos a tratamiento endovascular en un único centro especializado para el tratamiento de la isquemia crítica de la extremidad. Los pacientes fueron analizados con AP antes y después del tratamiento, y también se clasificaron de acuerdo con los sistemas más utilizados (Rutherford, TASC y WIfI) y con una nueva clasificación propuesta: el *score* de Abano Terme (ATS). Los datos demográficos y clínicos se recogieron y se realizó un seguimiento clínico a al primer mes y a los 6 meses. Los parámetros de la AP fueron tiempo de llegada (AT), el tiempo de pico (PT), la velocidad de lavado (WS), la amplitud (W), el área bajo la curva (AUC) y el tiempo de tránsito medio (MTT). Las dos cohortes se definieron en base a un tiempo de curación de menor o mayor a 30 días.

De enero de 2015 a julio de 2016, 580 pacientes consecutivos se sometieron a un tratamiento endovascular, realizándose en ellos un análisis con AP. Entre ellos, 332 cumplieron los criterios de inclusión, de los cuales 123 se excluyeron del análisis de curación de úlceras (34 debido a la mala calidad de la imagen de AP, 50 pacientes no presentaban úlceras, 20 fallecieron y 19 no completaron el seguimiento). La edad media fue de 72 años y el 67,5% eran hombres. 133 pacientes presentaban lesiones Rutherford 5 y en 76 las lesiones eran Rutherford 6. El riesgo WIfI de amputación fue bajo en 24%, moderado en 14% y alto en 62%. Encontramos una tasa curación a los 30 días , con diferencias estadísticamente significativas entre los grupos, para los siguientes valores de corte de los parámetros de la AP:

AT > 6 segundos y MTT> 4.1 segundos o un incremento del mismo MTT > 1.7 segundos. El ATS, siendo un sistema de clasificación más simple que los actualmente empleados, no sólo se correlacionó mejor con la TcPO2 y la angiografía de perfusión; sino que en un posterior análisis demostró una mejor correlación con la curación de las úlceras y la supervivencia libre de amputación en pacientes con lesiones Rutherford 5.

II. ABBREVIATIONS

Ankle Brachial Index	ABI
Atrial Fibrillation	AF
Amputation Free Survival	AFS
Arrival Time	AT
Abano Terme Score	ATS
Area Under the Curve	AUC
Body Mass Index	BMI
Below-The-Knee	BTK
Coronary Artery Bypass Graft	CABG
Coronary Artery Disease	CAD
Cardiovascular Disease	CD
Common Femoral Artery	CFA
Chronic Kidney Disease	CKD
Critical Limb Ischemia	CLI
Cerebro-Vascular Disease	CVD
Diabetes Mellitus	DM
Diabetic Foot Ulcer	DFU
Digital Subtraction Angiography	DSA
Duplex ultrasound	DUS
End-Stage Renal Disease	ESRD
Endovascular Treatment	EVT
Femoro-popliteal	FP
Global Limb Anatomic Staging System	GLASS
Great Saphenous Vein	GSV
Hyperspectral imaging	HI
High-Density Lipoprotein Cholesterol	HDL-C
Indocyanine Green Fluorescence Imaging	ICG-FI
Ischemic CardioMyopathy	ICM
Joint Vascular Societies Council	JVSC
Laser Doppler	LD
Medial Arterial Calcification	MAC
Major Adverse Cardiovascular Event	MACE
Major Adverse Limb Event	MALE
Multispectral optoacoustic tomography	MSOT
Micro OXYgen sensor	MOXY
Mean Transit Time	MTT
Objective Performance Goals	OPG
Perfusion Angiography	PA
Peripheral Arterial Disease	PAD
Percutaneous Coronary Intervention	PCI
Peak Time	PT
Relative Hemoglobin	rHb
Region Of Interest	ROI
Saturation of oxygen	StO2
Secondary HyperParaThyroidism	SHPT
Superficial Femoral Artery	SFA
TransCutaneous Pressure of Oxygen	TcPO2
Target Lesion Revascularization	TLR
Time to Peak	TP
TibioPeroneal Trunk	TPT
Time to heal	TTH
Texas Ulcer Classification	TUC
Toe Pressure	TP
Vascular Calcification	VC
Vascular Smooth Muscle Cells	VSMCs
Width	W
Wound Ischemia Foot Infection	WIfI
Wash Speed	WS

III. CONFLICTS OF INTEREST AND FINANCIAL SUPPORTS

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1. INTRODUCTION

1.1. Peripheral Arterial Disease

1.1.1. Definition and epidemiology of PAD

Peripheral artery disease (PAD) addresses chronic atherosclerotic disease that partially or completely obstructs ≥ 1 peripheral arteries.(2) It mainly affects the arteries of the lower limbs, but also the carotid and visceral arteries, and the arteries of the upper limbs.

The mechanism underlying PAD is an insufficient blood supply to the legs that provokes pain and muscular dysfunction, similar to that in coronary angina. Symptoms occur when walking and are relieved at rest in the case of intermittent claudication (IC); disease severity and the patient's degree of activity may modify clinical presentation.(3) Seventy-five percent of patients remain clinically stable due to the development of collaterals, metabolic muscular adaptation and/or the patients themselves altering the gait to favor non-ischemic muscle groups.

In the more severely affected cases, the pain may occur at rest, associating tissue loss and/or necrosis. As graded by Rutherford's and Leriche-Fotaine's classifications, this situation is known as critical limb ischemia (CLI): grade 4 in Rutherford's or grade III in Leriche-Fontaine's classification (pain at rest) and grades 5 and 6 in Rutherford's or grade IV in Leriche-Fontaine's classification (which meet the presence of an ischemic ulcer or gangrene). Critical limb ischemia (CLI) was first defined in published form in 1982,(4) and the threshold criteria have slightly changed.

It is estimated that >200 million people have PAD worldwide, with a spectrum from none to severe symptoms.(5) It is uncommon before the age of 50, and its prevalence rises sharply onwards. It is estimated that up to 23% of patients older than age 55 may suffer from PAD.(6) Data from Framingham Study show incidence increasing from <0.4 per 1000 in men 35-45 years to 6 per 1000 in men aged > 65 years.(7) Patients with CLI comprise approximately 2-3% of all cases of PAD, and the diagnosis portends a dismal prognosis.(8) From 2003 to 2008, the annual incidence of PAD and CLI were 2.35% and 0.35%, respectively; corresponding to a prevalence of 10.69% for PAD, and 1.33% for CLI.

Disease definition, that has evolved over time, is particularly important to precisely describe prevalence and incidence rates. Earlier definitions were focused on IC, using Rose criteria (an standardized questionnaire aimed at identifying IC symptoms); nowadays the Ankle Brachial Index (ABI) value of ≤ 0.90 is more widely used to define the disease.(3) Nevertheless, there are few epidemiological studies of PAD based on ABI, given the time and

resources required to periodically retest study subjects for incident disease. In the Limburg peripheral arterial occlusive disease Study, incidence rates for PAD were based on 2 ABI measurements of <0.95.(9)

Compared to other cardiovascular diseases (CVDs), rates are more similar for men and women. Among men, annual incidence of PAD was 1.7 per 1000 at the age of 40 to 54 years; 1.5 per 1000 at the age of 55 to 64; and 17.8 per 1000 at the age of ≥65. Annual incidence in women was higher: 5.9, 9.1, and 22.9 per 1000, respectively, for the same age groups.(9) In the Framingham Offspring Study, PAD based on ABI was found in 3.9% of men and 3.3% of women, for a ratio of 1.18.(10) On the other hand, in the Rotterdam Study, PAD was actually lower in men than in women, with prevalence of 16.9% and 20.5%, with a ratio of men:women of 0.82.(11) Still another population-based study from Southern Italy found prevalence of PAD to be similar in men and women, with male to female ratios from 0.89 to 0.99, depending on the age.(12) Finally, the Cardiovascular Health Study, an ABI of <0.90 was somewhat more prevalent in men (13.8%) than in women (11.4%; ratio, 1.21), but the association of disease with sex was not significant after adjustment for age and CVD status.(13)

1.1.2 Critical limb ischemia

The first description of CLI was made in 1982 by Earnshaw et al.(4) and intended to be applied only to non-diabetic patients. The threshold for CLI was set at an ankle pressure (AP) <40 mm Hg in the presence of rest pain and <60 mm Hg in the presence of tissue loss. Later on, at the European Consensus of 1992, CLI was defined as rest pain for more than 2 weeks, ulceration or gangrene, and an AP <50 mmHg or a toe pressure (TP) of <30 mmHg, including diabetic patients.(14) However, that definition is not universally supported, because the pressures required of healing in the presence ulceration may be higher. The Trans-Atlantic Inter-Society Consensus (TASC) II suggests that an ankle pressure of <70 mmHg or a TP <50 mmHg is more accurate for CLI condition.(15) Yet another definition is described by Vallabhaneni et al.(16), that found that CLI in diabetic patients relies mainly on the TP <30 mmHg, while a TP between 30 and 50 mmHg was associated with a low risk of limb loss if untreated. Despite all the efforts to attain a precise hemodynamic definition, up to one third of the patients with clinically determined CLI did not meet the usual hemodynamic criteria .(17)

In order to provide a descriptive and predictive stratification of patients, the Society for Vascular Surgery described the Wound, Ischemia and foot Infection (WIfI) classification system. Based on patients' lower extremity loss risk with respect to the natural history of the disease (if treated conservatively) and depending on the benefit of different treatment strategies, the WIfI classification refocuses the approach to the patient with a threatened limb and a component of chronic ischemia according to clinical disease severity.(1) According to the WIfI classification each of the three components (wound, ischemia and foot infection) are classified into grades according to specific criteria. Depending on the 3 components' grades, classes for each patient are assigned. The spectrum of symptoms is organized by a Delphi Consensus process into four clinical stages that grade both the risk of amputation and the benefit of revascularization, assuming the need for a prospective validation. Several studies have validated the WIfI classification for critical limb threatening ischemia as a predictor for limb survival and amputation free survival,(18–22) for time to wound healing(23) and subsequently for cost of care for diabetic foot ulcers.(24) However, the risk of major amputation at 1 year could be more dependent on the multidisciplinary approach of diabetic foot ulcers than the WIfI initial stage and the time for wound healing.(25)

1.1.3. Risk factors for PAD

The main risk factors are diabetes, smoking, chronic kidney disease, aging, hypertension, and dyslipidemia. In the following paragraphs all of them are exposed.

Diabetes Mellitus

The number of people with diabetes has risen from 108 million (prevalence of 4.7%) in 1980 to 422 million (prevalence of 8.5%) in 2014, as reported by the WHO.(26) This growth in prevalence has appeared more rapidly in middle- and low-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. In 2015 an estimated 1.6 million deaths were attributed to high fasting blood sugar levels, and almost half of them occurred before the age of 70 years. It is projected that by 2030, diabetes will be the 7th leading cause of death worldwide.

In patients with diabetes, for every 1% increase in hemoglobin A1c there is a corresponding 26% increased risk of PAD.(27) More severe or longstanding diabetes mellitus seems to be more strongly related to PAD,(3) but even insulin resistance without diabetes raises the risk of PAD approximately 40-50%, as it plays a key role in the clustering of cardiometabolic risk factors which include hyperglycemia, dyslipidemia, hypertension and

obesity. PAD in diabetic patients has an aggressive presentation, with early large vessel involvement coupled with distal microvascular disease and neuropathy.(15) Regardless of ischemia, the relative immunosuppression in diabetes increases the risk of amputation upon infection.(3) Amputation is 5 to 10 times more frequent in diabetics,(15) while death is 3 times higher.(28)

Smoking

Smoking, which is a modifiable risk factor, is one of the strongest risk factors for PAD. Indeed, active smoking has been shown to confer 2-4 times greater risk of PAD versus nonsmoking.(3) In addition, smoking cessation has been shown to improve the progression of PAD, as well as improving intermittent claudication or reducing mortality.(29) Regarding passive smoking and PAD, in a study conducted on women from Beijing who had never smoked, the hazard ratio (HR) for PAD was at 1.67, with a significant dose-response relationship.(30) To sum up, smoking is associated with a significantly higher relative risk for PAD compared to other atherosclerotic diseases.(31)

Chronic Kidney Disease

Chronic kidney disease (CKD) defined according to creatinine levels and particularly in the case of end-stage renal disease (ESRD) requiring dialysis has been associated with PAD in several studies.(3) Using β2-Microglobulin and cystatin C as markers of renal function, PAD could be predicted in men but not in women.(32) However, when measuring renal function with MDRD-4, CKD appeared to be a better predictor of mortality or myocardial infarction than other risk factors in patients with PAD.(33)

Aging

Supporting the data mentioned in the epidemiology section, the prevalence of PAD rises rapidly in population over 65 years of age.(15) As defined by an ABI of <0.9, the prevalence ranged from 2.5% in the age group 50–59 years to 14.5% in subjects > 70 years.(34)

Hypertension

The association of hypertension with PAD has been demonstrated in most studies in which blood pressure was studied. The odds ratio for hypertension ranged from 1.50 to 2.20, being the systolic blood pressure independently associated with PAD.(3) In the Framingham

Study, 30% of the risk of IC in the population was attributable to blood pressure >160/100 mm Hg.(35)

Dyslipidemia

In many studies, high lipid levels were significantly associated with PAD in multivariable analysis; in fact, 17% of PAD prevalence is attributable to hypercholesterolemia. Nevertheless, in some other studies, dyslipidemia was significant in the univariate analysis but dropped out of multivariable models in which other lipid measures were considered. High-density lipoprotein cholesterol (HDL-C) has been shown to be protective against PAD in most studies where it was evaluated.(3)

There is also some evidence suggesting that elevated triglycerides may play a role in disease progression or more severe PAD, and they could be independently associated with PAD since triglycerides frequently drop out as an independent risk factor.(3)

In recent studies, total cholesterol to high-density lipoprotein cholesterol (HDL-C) ratio is considered the best lipid measure of risk.(36) Despite all this, the dyslipidemia profile observed in insulin resistance and diabetes with low HDL-C and high triglycerides, is also associated with PAD.(3)

Other risk factors

Obesity as a risk factor fails to support a consistent, independent positive association with PAD. (3) Even in some studies a protective effect was found for claudication in men and seemed to have a U-shaped, nonlinear relationship with relative weight in women.(3,37) In any case, several studies found that central adiposity (by measuring waist/hip ratio rather than BMI), particularly in diabetic patients, was associated with significantly higher risk of PAD, as in coronary artery disease (CAD).(38,39)

Light-to-moderate alcohol consumption, as observed in CAD, is less consistent with PAD. [5] Due to the frequent association of alcohol consumption with other risk factors, the measure of its effects is uncertain. Depending on the population group it could differ. In Native Americans, a protective effect of alcohol was seen even in multivariable analysis,(40) but in elderly Japanese American men, alcohol intake was found to increase the risk of incident PAD.(41)

Several studies suggest a higher risk of PAD among Blacks and American Indians versus Non-Hispanic Whites, whereas there is a lower prevalence among Asians and Hispanics.(3)

The importance of homocysteine as a risk factor for PAD has been examined in many studies, with conflicting results. Other inflammatory markers, such as the C-reactive protein (CRP) and fibrinogen, have been shown, on the other hand, to be associated with PAD in many studies.(3) CRP, the neutrophil-to-lymphocyte ratio, homocysteine, and the urinary albumin-to-creatinine ratio appeared to be associated with higher mortality rates.(42) The neutrophil-to-lymphocyte ratio is found to be able to predict PAD and an elevated platelet-to-lymphocyte ratio can predict osteomyelitis in diabetic foot ulcers.(43)

1.1.4. Pathophysiology of PAD

Arterial wall composition

The arterial vascular wall is a dynamic tissue that is able to adapt and reorganize itself under both physiologic and pathologic stimuli. All arterial vessels except capillaries are composed of three concentric layers with distinct cell and interstitial composition (figure 1):(44)

- **Intima**, the innermost layer, is in contact with the blood flow. The intima layer is composed by a monolayer of endothelial cells, a very thin basal lamina and a subendothelial layer formed by collagen and elastic fibrils.
- **Media**, the middle layer of the vascular wall, is composed by vascular smooth muscle cells, collagen and a network of elastic fibrils. It is separated from the intima and the adventitia layers by the internal and external elastic lamina, respectively.
- **Adventitia**, the external layer of the vascular wall, consists of elastic fibers, fibroblast, collagen, nerves, and small blood vessels called vasa vasorum.

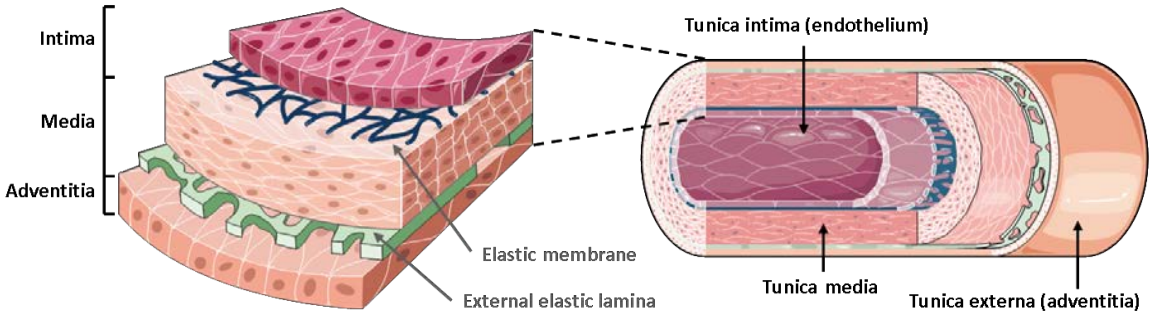

Figure 1. Arterial wall structure. The arterial wall consists of three concentric layers, called tunica intima, media and adventitia, separated by elastic membranes. (Courtesy of Prof. Badimon).

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the vascular wall, produced by lipid infiltration, foam macrophage accumulation on the inner wall, and subsequent focal thickening of the intimal layer. Lesion rupture leads to local thrombus formation, arterial occlusion, and tissue ischemia and necrosis, with disfunction of the affected organ. The atherosclerotic and thrombotic processes, with their clinical complications, appear interdependent and, therefore, are integrated under the term atherothrombosis (figure 2).(45)

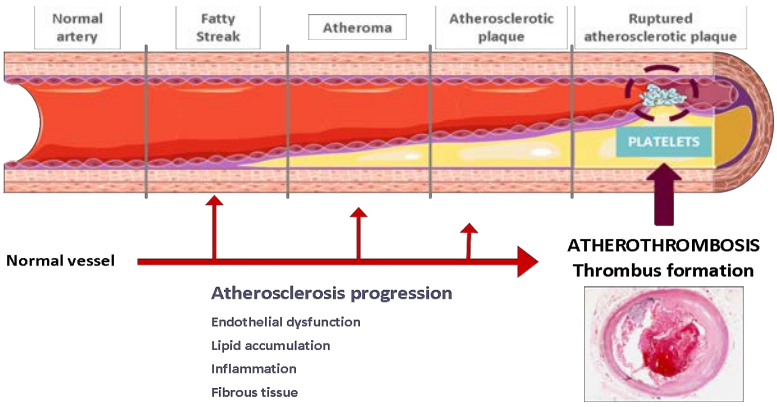

Figure 2. Atherothrombotic disease progression. Atherothrombosis chronically develop from early fatty streak to atheromatous plaque formation that by unpredictable disruption leads to platelet activation and thrombus formation. (Courtesy of Prof. Badimon).

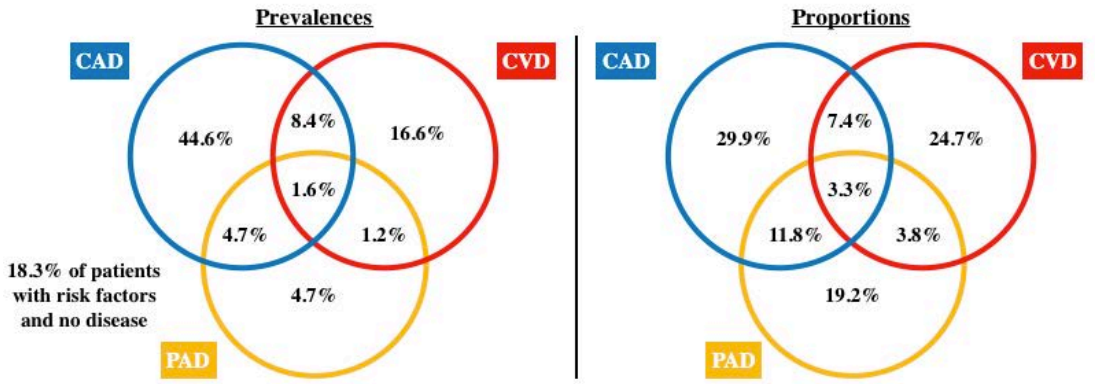

Figure 3. Cardiovascular disease prevalence and presentation. Prevalences of cardiovascular diseases observed in the REACH trial(46) and percentage of atherothrombotic events depending on the vascular territories affected. Data obtained from the CAPRIE trial.(47)

Atherothrombosis underlies the majority of cardiovascular events independently of the specific vascular bed in which they occur.(48) Indeed, a high percentage of atherothrombotic diseases occur in more than one area of the vasculature and, therefore, are classified as coronary, cerebrovascular or peripheral arterial disease. Data from the REACH(46) and CAPRIE(47) trials, represented in figure 3, explains the prevalence and polivascular disease presentation of atherothrombotic disease. The proportion of PAD patients with CAD and/or cerebrovascular disease (CVD) is 61%. CAD and CVD constitute the two leading causes of death worldwide,(49) followed by PAD, that is present in a 38.1% of the total amount of CVD patients.

Cardiovascular disease (CD) is the clinical manifestation of atherothrombosis, representing 17.9 million deaths globally (39%) and, consequently, a global health problem at present.(26) The number of deaths caused by CD is expected to reach 23.3 million by 2030 and, thus, CD is projected to remain the leading cause of death worldwide.(50)

Vascular calcification

Calcium is an essential ion in many metabolic pathway and is a widespread cellular signal effector. It is involved in the thrombosis cascade, the regulation of muscular contractility (including myocardium), neuronal activity, the endocrine system, and, paradoxically, in the genesis of vascular calcification (VC). Thus, calcium may accumulate in the spleen, liver, kidney, and the circulatory system, where it is deposited in the arterial intima and media layers and may eventually lead to obstruction.(51)

VC is a pathologic response to injuries or toxic stimuli involving inflammatory cells.(52) Historically, VC was considered to be a passive process, resulting of calcium (Ca^{2+})

and phosphate (P) ions exceeding solubility in tissue fluid, thereby inducing the precipitation and deposition of hydroxyapatite crystals. Far from being a passive process related to aging, it is an active and, maybe, potentially reversible process.(53,54) VC formation is now considered a complex, actively controlled intracellular molecular process. It involves the differentiation of macrophages and vascular smooth muscle cells (VSMCs) into osteoclast-like cells, similar to that which occurs in bone formation.(55–57) In fact, actual bone structure and bone related molecules and cell types have been found in calcified lesions.(58) The oxidative stress caused by locally generated hydrogen peroxide (H_2O_2) promotes the metaplasia of VSMCs in the vascular wall to an osteogenic phenotype. Further, this is associated with a significant loss of endogenous VSMC calcification inhibitors (matrix Gla protein, a calcium-binding protein involved in bone formation, pyrophosphate, and the inducible inhibitor osteopontin) and circulating inhibitors, such as fetuin-A.(55) To sum up, pathophysiological mechanisms resulting in VC can be broadly described as: (1) elevation in serum Ca^{2+} and P levels, (2) the induction of osteogenesis, (3) the inadequate inhibition of the mineralization process, and (4) the migration and differentiation of macrophages and VSMCs into osteoclast-like cells.(52,55,59)

In diabetic patients, advanced glycation end-products might promote mineralization of pericytes, and tight glycemic control might slow calcification in type 1 (but not type 2) diabetes. Moreover, the transcription factor proliferator-activated receptor gamma might deter calcification by inhibiting Wnt5a-dependent signaling in VSMCs.(60)

As previously mentioned, through the mechanisms of calcification there is an accumulation of Ca^{2+} and P in arteries with mineral deposits in the intima or medial layer,(51) leading to two different types of VC: intimal and medial (or Mönckeberg's disease).(55)

Intimal calcification is associated with atherosclerotic plaques and thought to result from modified lipid and low-density lipoprotein (LDL) particle accumulation, pro-inflammatory cytokines, and apoptosis within the plaque that induce osteogenic cell differentiation. Calcification in atherosclerosis alters plaque stability by direct mechanical stress within the fibrous cap and by increasing arterial stiffness and promoting hypertension.(53,60,61) Perhaps, intimal calcification is the organism's natural response to isolate the atherosclerotic plaque and interrupt the progression of abnormal cellular processes, thereby protecting the healthy adjacent intima.(55) As such, some studies have shown that calcification, specifically in the coronary bed, plays a major role in plaque stabilization.(62) In other territories, such as the carotid artery (where the main complications are caused by embolization rather than acute local occlusion), some studies associated the calcification

processes with plaque stability,(63) and other studies correlated a higher load of calcification with more hemorrhagic complications, suggesting that intraplaque calcification may not contribute directly to plaque stabilization.(64) Last but not least, it is worth mentioning that intimal calcification adopts a different composition depending on the territory affected. Specifically, femoral arteries are usually the most calcified vessels, presenting with advanced calcifications (sheet-like, nodule) and frequent osteoid metaplasia. In contrast, the plaques in carotid arteries display an increased lipid content.(53,65–67)

On the other hand, the medial arterial calcification (MAC) is considered to be more widespread in the lower abdominal region.(55) It is also known as Mönckeberg disease; since it was firstly described by Mönckeberg in 1903.(55) This process results from the osteogenic differentiation of VSMCs within the medial layer of the vessel wall.(68) Ca^{2+} accumulation begins as an amorphous mineral deposit and undergoes progressive remodeling, ultimately mineralizing into mature bone. Although medial calcification is generally not associated with luminal obstruction, the decrease in vessel wall elasticity and increase of its thickness ultimately lead to lumen loss and reduced perfusion, specially in the microvascular bed.(51)

Some conditions that promote VC are diabetes mellitus (type 1 and 2), ESRD, hyperphosphatemia and secondary hyperparathyroidism (SHPT). The latter is a condition associated with progressive renal failure, characterized by increased phosphate levels and diminished Ca^{2+}, which causes more Ca^{2+} to be taken from the bones and reabsorbed by the intestines and kidney. Subsequently, management of SHPT is central to the achieve a reduction of hyperphosphatemia and hypocalcemia without producing hypercalcemia. This could be achieved with oral phosphate binders, active vitamin D analogs and Ca^{2+} mimetics.(51) Phosphate binders (e.g., sevelamer; Renvela™) significantly decrease serum Ca^{2+} and are considered responsible for the lower rates of VC.(69) Concomitantly, a significant reduction in aortic calcification was observed in mice with CKD and SHPT under treatment with vitamin D receptor agonists.(70) In patients with CKD, vitamin D therapy decreases serum PTH levels, significantly reduces the incidence of cardiovascular events and improves survival.(71) In patients with ESRD and SHPT, treatment with cinacalcet (Sensipar™), which controls Ca^{2+} homeostasis by regulating the release of PTH, results in fewer hospitalizations for cardiovascular complications compared to placebo.(72) Cinacalcet, in combination with low-dose vitamin D, also attenuates coronary and aortic calcification in hemodialysis patients.(73) Figure 4 sums up the different mechanisms and factors implicated in VC.

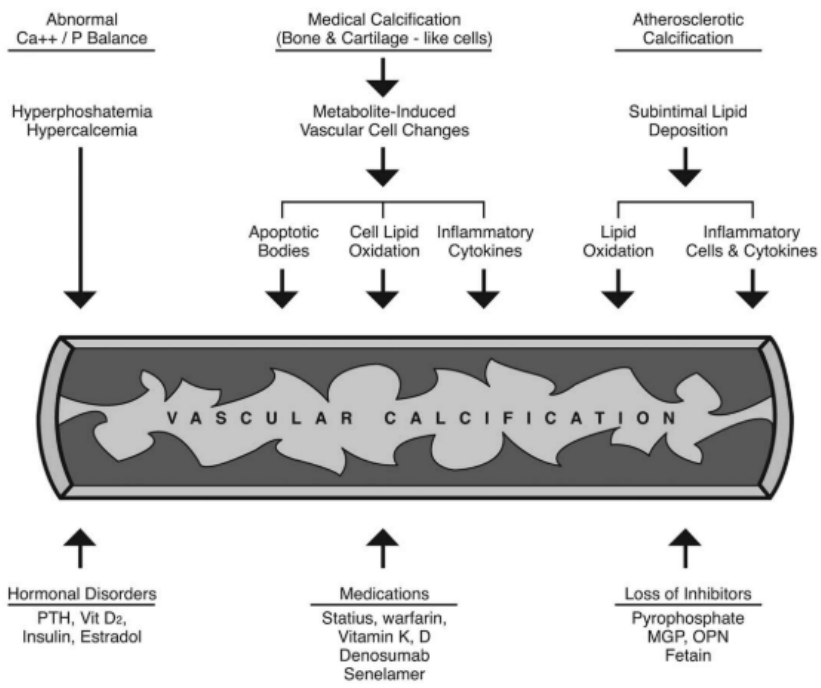

Figure 4. Diagram of mechanisms leading to vascular calcification.(51)

Arterial calcification is a strong risk factor and independent predictor of cardiovascular complications and mortality,(74,75) specifically affecting arterial stiffness in the case of MAC, as described by London et al.(76) Rennenberg et al. studied VCs among 218,080 subjects and found an increased risk (odds ratio, 95% CI) of 4.62 (2.24-9.53) for all-cause mortality, 3.94 (2.39-6.5) for cardiovascular mortality, 3.74 (2.56-5.45 for coronary events, 2.21 (1.81-2.69) for stroke and 3.41 (2.71-4.3) for any cardiovascular event.(77) VC is also associated with PAD, CKD and DM.(51)

From a radiological point of view, arterial intima atherosclerosis produces a patchy discontinuous appearance of large calcific deposits, while calcification in MAC is more diffuse and usually affecting the whole circumference of the vessel, adopting a “railroad track” pattern.(75) These findings can be seen on plain x-ray (figure 5) imaging and are well

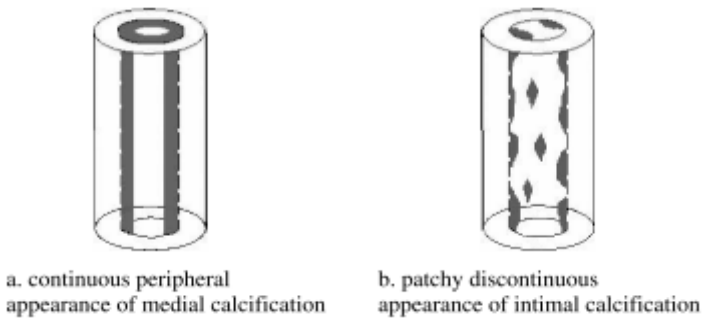

Figure 5. Diagram of X-ray appearance of MAC (a) and arterial intima calcification (b).(79)

correlated with microscopic findings.(78)

MAC is found in 11% of routine pelvic x-rays(80) and is commonly found in diabetic elder males with high cholesterol levels.(81) It is also associated with trophic foot ulcers and PAD,(82) more severely worsened in the peroneal and tibial arteries than in the thigh arteries. However, MAC seems to be related to diabetes and its microvascular manifestations rather than PAD itself.

Smith et al.(79) reported a good predictive value of MAC for podiatric care requirements and for diabetes. MAC has a positive predictive value of 92.9% (95% CI: 69.2-98.7) for diabetes and a specificity of 99.9% (95%CI 99.4-100).

It has been reported that MAC has an OR (95% CI) for mortality of 1.5 (1-2.1), for amputations 5.5 (2.1-14.1), for proteinuria 2.4 (1.3-4.5), for retinopathy 1.7 (0.98-2.8) and for CAD 1.6 (0.48-5.4). Other studies found that MAC had an OR of 4.2 for cardiovascular death.(83) There's also correlation between MAC and other microvascular complications of diabetes, such as decreased vibration perception, serum creatinine and autonomic neuropathy.(84) The small vessels are the common factor among all previous clinical effects of MAC, suggesting a relationship between MAC and microvascular disease.

1.2. Present classifications and risk stratification systems of PAD

1.2.1. Leriche-Fontaine classification

Described in 1954, the Leriche-Fontaine classification is divided into four stages. The first one (stage I) is defined by demonstrable PAD by signs or explorations but with no symptoms. Stage II corresponds to intermittent claudication (explained below), subdivided in IIa as claudication of more than 150 m (non-disabling); and IIb, which affects the quality of life and supposes a limitation for the patient. CLI is confined to the last two stages: III and IV, both with the eventual presence of rest pain. In stage IV is also defined by the presence of an ulceration or gangrene in the foot or the limb.(85)

1.2.2. Rutherford classification

Firstly described in 1986(86) and afterward reviewed in 1997(87), the classification described by Rutherford et al. had the aim of ease description and to compare patients when reporting cases and studies of peripheral arterial occlusive disease in the literature. They described stages in acute and chronic limb ischemia but also the criteria to describe outcomes,

such as limb salvage, change in clinical status, patency or risk factors. The classification used to classify the stages of chronic limb ischemia is shown in Table 1.

Grade	*Category*	*Clinical description*	*Objective criteria*
0	0	Asymptomatic or no hemodynamically significant occlusive disease	Normal treadmill or reactive hyperemia test
	1	Mild claudication	Completes TE; AP after exercise >50 mmHg but at least 20 mmHg lower than resting value
I	2	Moderate claudication	Between categories 1 and 3
	3	Severe claudication	Cannot complete TE and AP after exercise < 50 mmHg
II*	4	Ischemic rest pain	Resting AP < 40 mmHg, flat or barely pulsatile ankle or metatarsal PVR; TP < 30 mmHg
III*	5	Minor tissue loss; nonhealing ulcer, focal gangrene with diffuse pedal ischemia	Resting AP < 60 mmHg, flat or barely pulsatile ankle or metatarsal PVR; TP < 40 mmHg
	6	Major tissue loss; above transmetatarsal level, functional foot no longer salvageable	

Table 1. Rutherford classification. AP: Ankle Pressure; PVR: Pulse Volume Recording; TE: Treadmill Exercise, consisting of 5 min at 2 mph on a 12% incline; TP: Toe Pressure. *Grades II and III correspond to critical limb ischemia. (87)

Symptomatic disease is stratified into six categories. Changes between categories correspond to clinical improvement after treatment. Gangrene is divided into two categories: affecting only the toes or with a larger tissue loss that could avoid the salvage of a functional foot remnant.

The zero grade is used to identify patients with no or little symptoms or sensations, valuable to allow a postoperative gauge at all levels.

Claudication is defined as an extremity pain or weakness produced by a constant amount of walking and that it is promptly relieved by stopping the muscular activity. Recommending patients to undergo intervention when disabled is enough for the clinical practice, but not enough precise for trials since disability is relative to age, occupation among other factors. The non-invasive vascular laboratory test criteria to define claudication higher than mild is to not being able to complete 5 minutes on the treadmill at 2 mph on a 12% incline; with a reduction in ankle pressure to ≤ 50 mmHg, thus corresponding to moderate or severe claudication. If only some of these criteria are accomplished, the stage corresponds to moderate claudication.

The grade II or category 4 in this classification corresponds to the ischemic rest pain and indicates diffuse foot ischemia. It cannot be controlled by analgesics and it is usually localized to the forefoot or near focal ischemic lesions, increasing at night or when lying down. The ankle pressure is commonly lower than 40 mmHg, and toe pressures ≤ 30 mmHg.

At the last grade, corresponds to cases with gangrene and depending on whether it is focal - usually to the toes, corresponding to category 5 - or more extended proximally for category 6. The upper limit of ankle pressure for this grade is 60 mmHg, a toe pressure of 40 mmHg or barely pulsatile tracing at the plethysmography recording.

The concept of critical limb ischemia corresponds to categories 4, 5 and 6 in this classification, thus defined by rest pain, nonhealing ulcer or gangrene besides the evidence of distal ischemia.

1.2.3. University of Texas ulcer classification

The goal of Lawrence et al.(88) from the Department of Orthopaedics, University of Texas Health Science Center, San Antonio defined this classification ultimately known as TUC classification. This system to improve communication, leading to a less complex, more predictable treatment course and, ultimately, improved results. The following classification uses a system of wound grade and stage to categorize wounds by severity. Wounds are graded by depth. Grade 0 represents a pre- or post-ulcerative site. Grade I ulcers are superficial wounds through the epidermis or epidermis and dermis but do not penetrate to tendon, capsule, or bone. Grade II wounds penetrate to tendon or capsule. Grade III wounds penetrate to bone or into a joint. Within each wound grade, there are four stages: non-ischemic clean wounds (A), non-ischemic infected wounds (B), ischemic wounds (C), and infected ischemic wounds (D). Table 2 is the original table for the classification.

	GRADE			
	0	I	II	III
A	Pre- or postulcerative lesion epithelized	Superficial wound, not involving deep tissues	Wound penetrating to tendon or capsula	Wound penetrating to bone or joint
B	As in A but with infection	As in A but with infection	As in A but with infection	As in A but with infection
C	As in A but with ischemia	As in A but with ischemia	As in A but with ischemia	As in A but with ischemia
D	As in A but with infection and infeccion	As in A but with infection and infeccion	As in A but with infection and infeccion	As in A but with infection and infeccion

Table 2. University of Texas Ulcer Classification (TUC), reported as the grade followed by the stage names.

1.2.4. TASC anatomical classifications

The Transatlantic Inter-Society Consensus (TASC II) for the Management of Peripheral Arterial Disease, has provided expert recommendations on the diagnosis and treatment of PAD. One of the main used aspects of these guidelines is the TASC artery lesion classification. It is based on anatomic criteria and provides a characterization of the patterns of disease and suggest treatment decisions. Based on the complexity and anatomical location of the lesions, recommendations tend to endovascular or open surgical repair.

TASC anatomical descriptions of the lesion patterns enable comparison between various grades of complexity throughout three territories: the aortoiliac, the FP and the BTK segment. The lesions are classified as A, B, C or D. Despite simpler lesions (A and B grades) are considered more suitable for endovascular repair and the more complex ones (C and D) for open surgery; TASC B and C lesions treatments could be considered with other factors such as the technical resources, patient status, and the surgeon's experience. they are not sufficient, anyway, to guide clinical decisions because of a lack of randomized clinical trials to generate particular recommendations.

Although the aortoiliac lesions are not concerned in this project, the aortoiliac classification worth a mention as a part of the TASC classification. As it is shown in Figure 6, the grades are: (15,89)

- TASC A: Common iliac artery stenosis or external iliac stenosis of ≤ 3 cm

- TASC B: Stenosis of infrarenal aorta of ≤ 3 cm, common iliac artery occlusion, external iliac stenosis of ≤ 10 cm or unilateral occlusion.

- TASC C: Bilateral common iliac artery occlusions, bilateral external iliac stenosis of ≤ 10 cm or unilateral stenosis extending to the common femoral artery, unilateral external iliac occlusion involving the internal iliac artery or unilateral heavily calcified occlusion of external iliac.

- TASC D: Infrarrenal aortoiliac occlusion, stenosis of the whole aortoiliac segment or diffuse pathology affecting the common femoral artery, bilateral external iliac occlusions or iliac stenosis in patients with aortic aneurysms.

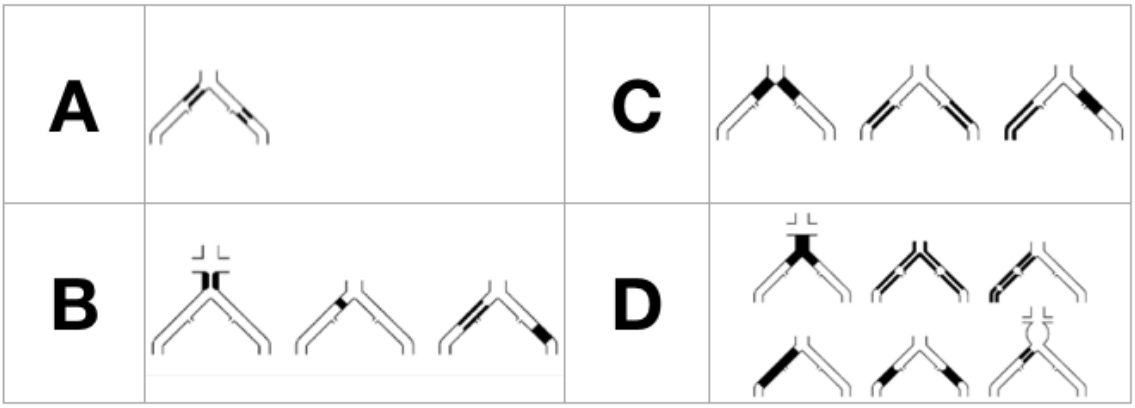

Figure 6. Aortoiliac TASC classification

In the FP sector, the criteria for each grade of the classification are (Figure 7):(15,89)

- TASC A: single stenosis ≤ 10 cm or occlusion ≤ 5 cm in length.

- TASC B: multiple lesions (stenosis or occlusions) ≤ 5 cm each, single lesion ≤ 15 cm (not involving infra-geniculate popliteal artery) or heavily calcified occlusion ≤ 5 cm.

- TASC C: multiple lesions totaling ≥ 15 cm or recurrent stenosis after failing treatment.

- TASC D: chronic total occlusions of SFA ≥ 20 cm or popliteal artery and proximal trifurcation vessels.

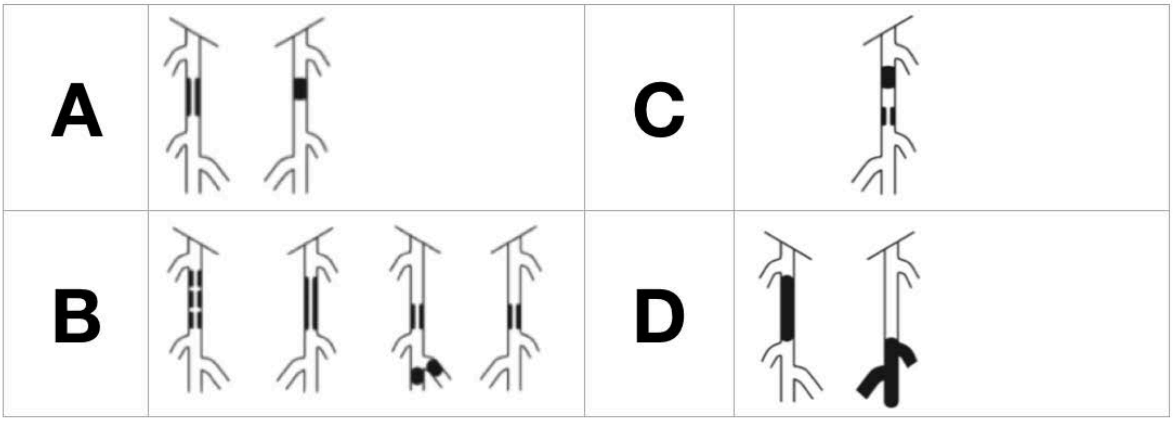

Figure 7. Femoropopliteal TASC classification

Finally, the 2015 update on TASC classification (Figure 8), mentioning the below-the-knee sector is defined by the lesions on the target vessel (assuming worse stenosis or occlusions in the other arteries):(89)

- TASC A: stenosis ≤ 5 cm in length

- TASC B: multiple stenosis of ≤ 5 cm each with a total length ≤ 10 cm or a single occlusion ≤ 3 cm in length

- TASC C: multiple stenosis or a single occlusion of > 10 cm in length

- TASC D: the same lesions of TASC C but with dense lesion calcification or no visualization of collaterals

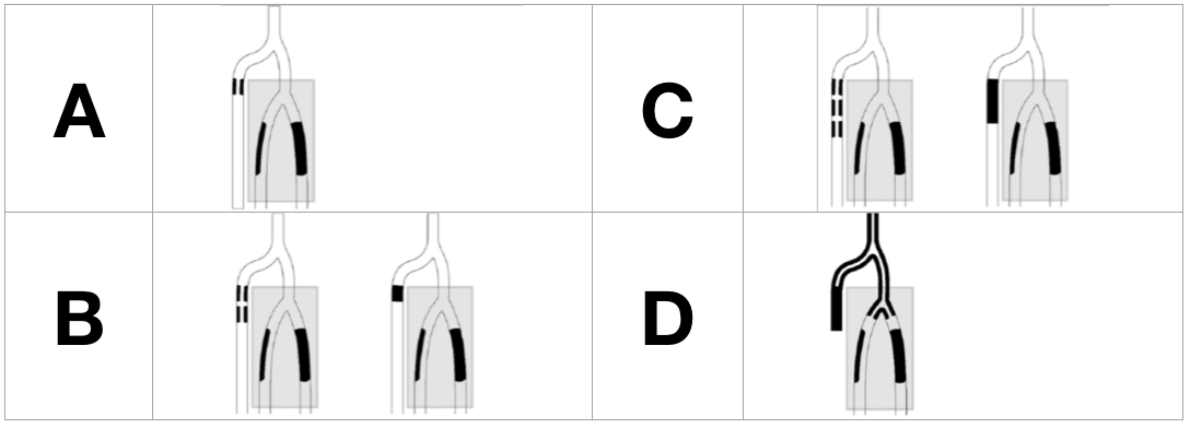

Figure 8. Below-the-knee TASC classification

Specific considerations on the application of the classification to the patient's population of the study are explained further in the discussion section.

1.2.5. SVS Wound Ischemia foot Infection (WIfI) stages(1)

First defined in 1982, CLI was intended to refer to patients with a threatened lower extremity because of chronic ischemia, but excluding diabetic patients whose should be analyzed separately.(4) Nevertheless, among patients with current accepted criteria for CLI, diabetes is highly prevalent. This liberal application of the term CLI could explain the issues in measuring and comparing outcomes of treatment options, especially as treatment options have rapidly expanded.

To provide a more precise description of the disease burden to allow accurate outcomes assessments and comparisons of various treatments in CLI patients, the Society for Vascular Surgery (SVS) Lower Extremity Guidelines Committee undertook the task of creating a classification taking into consideration the whole clinical scenario of CLI in diabetic patients. Also, it is suggested that an updated comorbidity index and a simpler anatomic classification system will need to be added to the classification. Not only to stratify the disease burden but to aid in the selection of the best therapy for any given patient.(1)

Thus, in addition to the perfusion, the extent of the ulcer and the degree of infection are weighted in this classification: Wound, Ischemia, and foot Infection (WIfI). Each of the three factors (or component) is graded on an increasing scale from 0 (none) to 3 (severe). The thresholds for the grades are the following:

- Wound:
    - 0: no ulcer
    - 1: shallow ulcer, without exposure of deep tissues
    - 2: bone, joint or tendon exposures
    - 3: extensive and deep ulcer
- Ischemia:
    - 0: ABI ≥ 0.8, Ankle pressure > 100 mmHg, TP or TcPO2 > 60 mmHg
    - 1: ABI 0.6 – 0.8, Ankle pressure 70 – 100 mmHg, TP or TcPO2 40 – 60 mmHg
    - 2: ABI 0.4 – 0.6, Ankle pressure 50 – 70 mmHg, TP or TcPO2 30 – 40 mmHg
    - 3: ABI < 0.4, Ankle pressure < 50 mmHg, TP or TcPO2 < 30 mmHg
- Foot Infection:
    - 0: No symptoms or signs of infection
    - 1: Local infection (after excluding an inflammatory response of the skin) involving only the skin and the subcutaneous tissue.
    - 2: Local deep infection or with erythema >2 cm, with no systemic inflammatory response.
    - 3: Local infection (as in grade 2) with the signs of SIRS, as manifested by two or more of the following: Temperature >38º or <36ºC, heart rate >90 beats/min, respiratory rate >20 breaths/min or PaCO2 <32 mm Hg, white blood cell count >12,000 or <4000 cu/mm or 10% immature forms.

The set of three grades on each component is used to define the class. The classes arranged in tables (Figure 9) and using a Delphi Consensus from the opinion of a group of experts, one of the 4 possible stages were assigned: very low (VL), low (L), moderate (M) or high (H). The stages were defined for both the estimate amputation risk and the likelihood of benefit of revascularization.(1) This system had been after validated several times by other independent studies.(18–20,22–25)

a, Estimate risk of amputation at 1 year for each combination

	Ischemia – 0				Ischemia – 1				Ischemia – 2				Ischemia – 3			
W-0	VL	VL	L	M	VL	L	M	H	L	L	M	H	L	M	M	H
W-1	VL	VL	L	M	VL	L	M	H	L	M	H	H	M	M	H	H
W-2	L	L	M	H	M	M	H	H	M	H	H	H	H	H	H	H
W-3	M	M	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	fI-0	fI-1	fI-2	fI-3	fI-0	fI-1	fI-2	fI-3	fI-0	fI-1	fI-2	fI-3	fI-0	fI-1	fI-2	fI-3

b, Estimate likelihood of benefit of/requirement for revascularization (assuming infection can be controlled first)

	Ischemia – 0				Ischemia – 1				Ischemia – 2				Ischemia – 3			
W-0	VL	VL	VL	VL	VL	L	L	M	L	L	M	M	M	H	H	H
W-1	VL	VL	VL	VL	L	M	M	M	M	H	H	H	H	H	H	H
W-2	VL	VL	VL	VL	M	M	H	H	H	H	H	H	H	H	H	H
W-3	VL	VL	VL	VL	M	M	M	H	H	H	H	H	H	H	H	H
	f-0	fI-1	fI-2	fI-3	fI-0	fI-1	fI-2	fI-3	fI-0	fI-1	fI-2	fI-3	fI-0	fI-1	fI-2	fI-3

Figure 9. WIfI consensus classification describing (a) the estimated risk of amputation at 1 year and (b) the estimated likelihood of benefit of revascularization.

1.3. Present methods for ischemic assessment

The initial assessment of PAD and CLI patients a direct inquiry on previous myocardial infarction, stroke, coronary artery bypass graft (CABG) and arterial surgery should be included. It is not exceptional that polivascular disease to be diagnosed for the first-time during PAD examination. An inquiry for cardiovascular risk factors is equally important. Upper extremity exertional pain, postprandial abdominal pain, and erectile dysfunction are other important potential clues in the medical interview.(90)

Physical examination should be systematic and include blood pressure in both arms, palpation of all peripheral and central pulses, notifying any thrill or murmur on them; pulse rate, rhythm and heart sounds; examination of the hands and abdominal aorta. On the feet, it is mandatory a careful examination, between the toes, assessing skin integrity, color, temperature and calf hair loss. The palpation of pulses is subjective and is influenced by the sensitivity of the fingers, the experience of the examiner, the obesity and edema of the patient and the warmth of the room. The femoral artery, whether pulsatile or occluded, should always be palpable unless the patient is very obese.(90)

In patients presenting with classic history of IC but with palpable foot pulses, there is usually proximal aortoiliac disease with collaterals through the pelvis, which produce adequate blood flow at rest but decreases suddenly with a small exercise like walking up and down the corridor or on a treadmill, or even by a repeated “tiptoe” while leaning on the couch.(90)

1.3.1. Ankle Brachial Index and ankle pressure.

Measuring the pressure in the ankle arteries has become a standard part of the initial evaluation and differential diagnosis of patients with suspected PAD. The ankle-brachial index (ABI) value raises from the ratio between the systolic pressure measured in the calf (for the peroneal, posterior and anterior tibial arteries) and the higher brachial pressure of either arm. The index leg is often defined as the leg with the lower ABI.(15) The ABI is cheap and non-invasive, which makes it potentially valuable in health care. A reduced ABI is a potent predictor of the risk of future cardiovascular events.(91)

The typical threshold for diagnosing PAD is ≤ 0.90 at rest, with an upper bound in normality from 1.2 to 1.4. An ABI <0.5 is often associated with critical limb ischemia. If we consider only the ankle pressure in patients with ischemic ulcers, it is typically 50–70 mmHg, and in patients with ischemic rest pain it stays around 30–50 mmHg.(15)

The ABI can represent a wide range of ankle, depending on the systolic actual pressure. For example, and ABI of 0.3 with a systolic blood pressure of 110 or 160 mmHg are 33 and 48 mmHg respectively, and near both opposite bounds in the ischemic pain threshold. However, ABI is better for comparing groups of patients or for monitoring a given patient before and after eventual treatments.(87)

However, the ABI is not a useful test for detecting PAD in patients with diabetes and/or renal insufficiency.(92) Due to vascular calcification, the tibial vessels at the ankle become non-compressible. This leads to a false elevation of the ankle pressure, in which ABI it typically >1.40. In these patients, additional non-invasive diagnostic testing should be performed.(15)

1.3.2. Toe pressure

Toe pressures (TP) have main importance in diabetic patients because it is the most distal pressure representing the big vessel circulation that can be measured, and the toe arteries are less prone to MAC.(93) The TASC II threshold for ischemia symptom appearance is below 50 mmHg.(15) In addition, recent European guidelines on diabetic foot management have

raised the level of probable wound healing from the previous 30–50 mmHg to 50–55 mmHg. In non-diabetics, the cut-off value for rest pain has been set on 30 mmHg.(93,94)

Few studies have shown the usefulness of TP compared with ABI in predicting outcomes of CLI patients.(95,96) This may be secondary to the predominance of small-vessel disease in the foot among patients with CLI. TPs are also less likely to give falsely elevated pressures than are APs.(16)

TP could be measured by three methods: the mercury strain-gauge, photoplethysmography (PPG) and laser Doppler (LD), the latter two being the ones still in use. As it is mentioned, PPG is based on detecting changes volume of the toe by emitting infrared light that penetrates the tissue under the probe. The LD emits infrared laser light that is reflected from moving particles (red blood cells). The PPG detectors need a pulsatile flow, whereas LD sensors detect minor flows; thus, LD allows lower values of toe pressure to be measured.(93) This test, as the TcPO2, is sensible to the vasodilatation due to the temperature of the ambient and probe, and it should be standardized. It is a quick test and thus suitable for everyday practice.(93)

1.3.3. Pulse volume recordings

Pulse volume recordings (PVR) provide a qualitative measurement by inflating cuffs at specified levels on each lower extremity. The cuff measures the minuscule change in the volume of the limb with each pulse, creating a volume/time trace. PVRs are useful in patients' extremely calcified vessels, as this modality relies on limb volume change rather than the compression of the vessels. Unfortunately, as it is mentioned, it is only a rough estimate of the level of disease, without correlation with the severity or prognosis of the disease. Nonetheless, PVRs can provide information about changes in arterial flow quality between segments of the main vessels of the limb, giving better anatomical information than other techniques.(97)

1.3.4. Photoplethysmography

Photoplethysmography (PPG) uses a transmitted infrared signal through each of the digits. The degree of transmitted signal varies depending on blood volume within the digit, blood vessel wall movement, and the orientation of red blood cells.(98) PPG is useful for the detection of disease below the knee as well as disease isolated to the forefoot and digits. The qualitative restoration of pulsed wave within previously "flat-lined" tracings can be assumed as an improvement of distal perfusion or suggesting restenosis when losing the pulsed

wave.(97) Anyway, PPG is only comparable with a previous reference on the same patient and used as subjective information. The role of PPGs in the surveillance of patients with distal ulcers is essential and may provide information about any restenosis of a distal pedal intervention.

1.3.5. Transcutaneous oxygen pressure (TcPO2)

Toe pressure and TcPO2 measurements are the most widely used noninvasive methods in assessing foot perfusion and wound healing potential.(93) The TcPO2 measurement allows the determination of the amount of oxygen that has diffused from the capillaries, through the epidermis, to an electrode sensor at the measuring site, usually placed at the dorsum of the foot.(99) The patient should be in supine position in a room maintained at 22ºC and the electrode at 45ºC. Finally, calibration time from 10 to 30 minutes is needed prior to the continuous measure. (99,100)

An improvement in the TcPO2 value postintervention compared with preintervention has been validated as an excellent marker of tissue reperfusion. TcPO2 values greater than 40 mm Hg in the area surrounding the ulcer or amputation site are considered predictive of successful healing. This test has the advantage of not being limited by non-compressible vessels, and in patients without pedal Doppler signals, it is one of the noninvasive tests that are helpful to assess perfusion.(100) Another study in diabetic patients made by Kalani et al.(101) found that the ulcers on the feet of patients with TcPO2 > 25 mmHg healed within 4–6 weeks; while when the TcPO2 was < 25 mmHg the ulcers did not heal (sensitivity: 85%; specificity: 92%). The TcPO2 values proposed by the TASC II(15) for a critical level is 30 mmHg. In figure 10(102) the probability of DFU healing is represented, according to the TcPO2, the TP and the AP. In all the three measures there is a range of medium probabilities for healing, being conflictive the choosing of a certain threshold.

Despite TASC II recommendations for the use of TcPO2 in all patients with CLI, the use of this diagnostic tool remains low related to its limited availability in the office setting, patient resistance to avoiding smoking and caffeine before the test, as well as the time and cost constraints associated with providing the temperature-controlled environment required to standardize the test.(99)

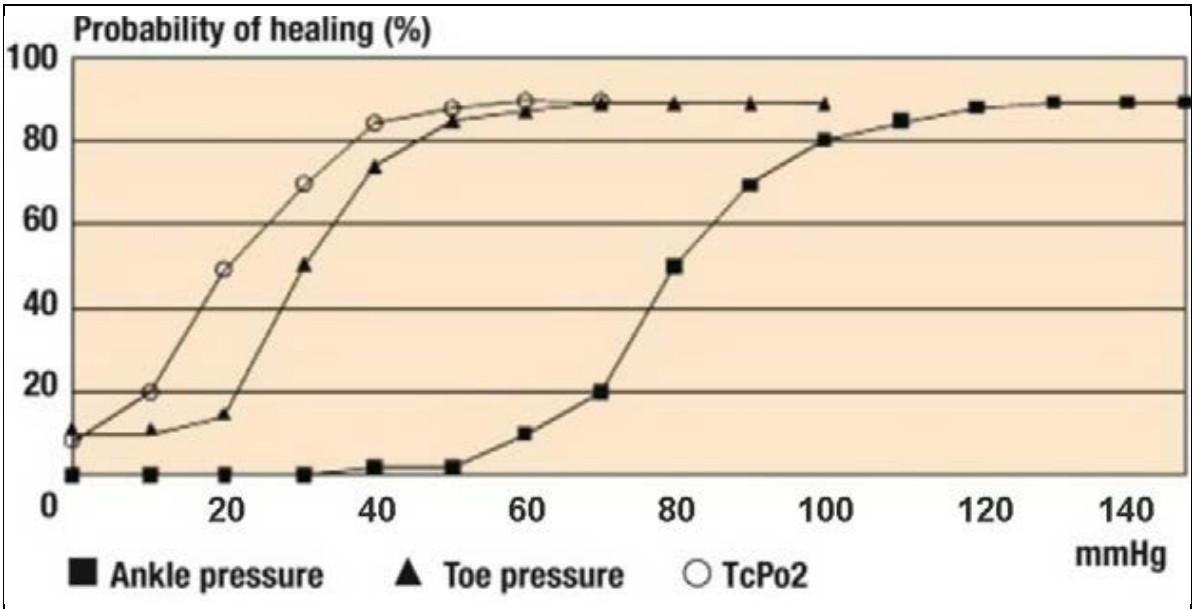

Figure 10. Probability of ulcer healing as related to different levels of systolic ankle pressure, toe pressure and TcPO2 (from the International Consensus of the Diabetic Foot 1999).

1.3.6. Tissue oxygen saturation mapping

On a similar basis than in the conventional TcPO2, a team of Kagaya et al.(103) used a near-infrared tissue oximeter monitor to detect the ischemic areas in the entire foot of a CLI patient. They measured the saturation of oxygen (StO2) of many points on the foot to make a map. The feasibility of the test relies on that the StO2 values can be measured within only10 seconds per point, by contrast to the conventional TcPO2. The contribution of this study is that the angiosomes are more variable and patient-dependent, likely due to collateralization and variations in microcirculation. This interesting fact could provide useful information to determine whether further interventions should be done and monitoring personalized treatment goals for each patient.

1.3.7. Hyperspectral imaging

Hyperspectral imaging (HI) consists of recording a series of images representing the intensity of diffusely reflected light from biological tissue at discrete wavelengths. The resulting set of images is called hypercube denoting spatial coordinates (x, y) and a spectral coordinate (λ). The analysis of the hypercube asses the distribution of chromophores, such as melanin or hemoglobin. That means that we can estimate the hemoglobin concentration and oxygen saturation from the tissue's diffuse reflectance.(104)

HI has been used to determine the spatial distribution of oxygen saturation in human skin(104,105) and to detect the changes in the diabetic foot.(106–108) Nouvong et al.(106) evaluated prospectively the predicting potential of HI for healing of ulcers in 66 diabetic patients (type 1 and 2). The sensitivity was 80% (43 of 54), the specificity was 74% (14 of 19), and the positive predictive value was 90% (43 of 48). A reason for false-positive was attributed to osteomyelitis and, on the other hand, callus could explain some of the false-negatives. For this study, the averaged oxyhemoglobin (T_{OXY}) and deoxyhemoglobin (T_{DEOXY}) concentrations, as well as oxygen saturation over an approximately 1-cm-thick band within the peri-wound area, were calculated around ulcers and compared between those healing and those non-healing. Figure 11 shows an example of the HI color coding in a peri-wound tissue.(106)

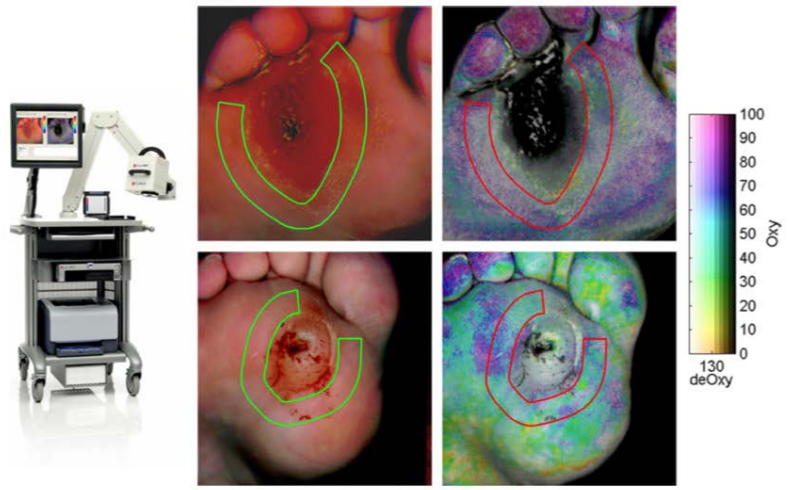

Figure 11. Hyperspectral imaging image sample. Visible (left) and hyperspectral (right) images of a healing DFU. The top panels show a healed DFU with oxy, deoxy and StO_2 values of 75, 34 and 69%, respectively. On the other hand, the bottom panels show a non-healed DFU, where the abovementioned values are 60, 53 and 53%.(106)

Using the same HI dataset, Yudovsky et al.(104) explored the risk of developing an ulcer in diabetic foot using HI images of diabetic feet collected before ulceration and dividing groups from the combined values for oxyhemoglobin (T_{OXY} = 18) and deoxyhemoglobin (T_{DEOXY} = 5.8). They found that diabetic foot ulcer formation could be predicted with a sensitivity and specificity of 95% and 80%, respectively. Those findings confirmed the results of a previous pilot study by Khaodhiar et al.(107)

To sum up, HI is noninvasive and can assess oxygen saturation with high spatial resolution in a relatively short time, with no special preparation.(109) However, HI has some limitations including the need to know the chromophores and structure of the tissue or even

the presence of wounds or scars prior to image analysis.(110,111) However, there are no large studies that could validate this technique as a reference in clinical guidelines.

1.3.8. Oxygen-to-see (O2C) method

The oxygen-to-see (O2C) method (LEA Medizintechnik GmbH, Gießen, Germany) is an optical measuring technique that uses both white light spectrometry and laser Doppler flowmetry. This combination allows measuring three parameters at a time: oxygen saturation (SatO2), relative hemoglobin (rHb) and blood flow.

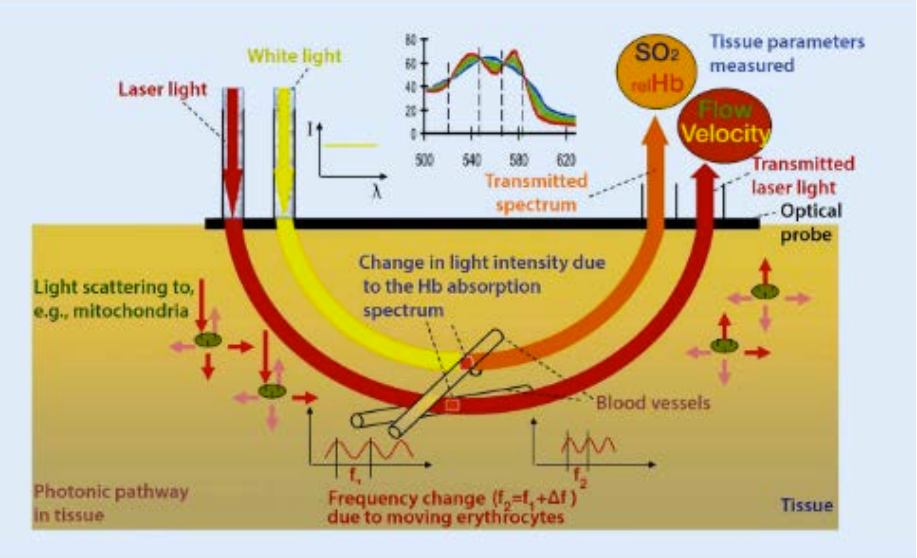

Figure 12. O2C measuring principle is a combination of white light spectrometry and laser Doppler flowmetry (courtesy of LEA Medizintechnik GmbH).

The method is based on the reflection from the skin of a broadband light signal (500–850nm) as well as light from a laser source (830nm). The white light source spectrum is used to determine SatO2 and rHb and the laser light determines blood flow (Figure 12). The penetration depth depends on the selected probe and it could be up to 8mm.(112)

The test showed high sensitivity and specificity for predicting the healing potential in above the ankle amputations.(113) This technique can also be used for periprocedural measurements in the foot. The probe can measure perfusion in a concrete angiosome (as shown in figure 13) after or during the revascularization. Indeed, several studies have demonstrated an increase of foot perfusion independently of the direct angiosome revascularization.(114,115) However, no large studies with clinical follow-up are available to validate this technique in clinical practice.

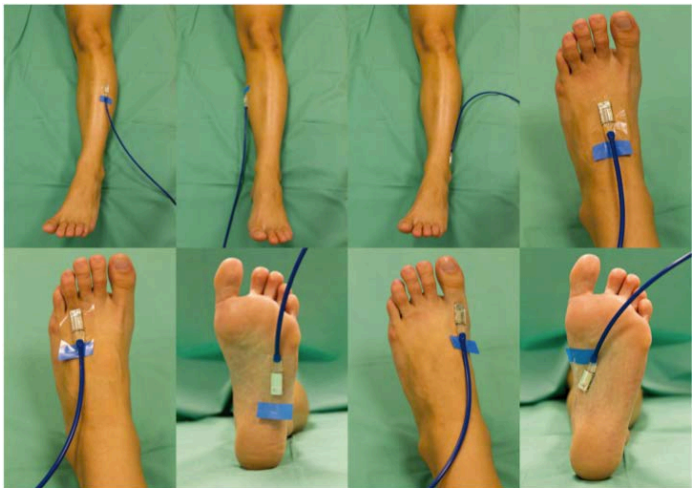

Figure 13. The O2C probe, as placed on several angiosomes.

1.3.9. Micro Oxygen sensors (MOXYs)

Performed by Montero-Baker et al.(116), the first-in-man evaluation of a potential tool that consists in micro-sensors placed in the subcutaneous tissue; from 2 to 4 mm below the skin surface, and were injected using an 18-gauge injector. They are settled in three locations of interest (with consideration of the ulcer or wound if present, angiosome anatomy or their likelihood of presenting a change in oxygenation after the revascularization). The micro-sensors (0.5*0.5*5 mm) were designed to remain in the body permanently, avoiding inflammatory reaction by physical and chemical properties. They were soft and tissue-like to minimize stress at the material-tissue interface caused by motion and pressure, which can damage or stimulate adjacent immune cells and prolong the inflammatory phase. From that study, 97.2% of sensors were successfully located and measured and the increase in median oxygen concentration measured in the treated feet was statistically significant (p=.0042), while arm sensors showed negligible or decreasing oxygen changes.

1.3.10. Indocyanine green fluorescence imaging

Fluorescence imaging consists in the injection, either intravenous (at the bedside or in the clinic) or intra-arterial (during an EVT, for instance), of indocyanine green (ICG) to quantify tissue perfusion (figure 14). The protocol for the intravenous technique consists in placing a camera at 20 cm over the foot while the patient is in supine position after at least 15 minutes of rest, with a room temperature between 20 and 25ºC removing dressings and extinguishing overhead lights. The injection dose of ICG is 0.1 mg/kg of 0.1% ICG intra-venously administrated solution, and pushed with 10 mL of normal saline to flush the intra-

venous route. After that injection, the capturing and recording go-ahead for 5 minutes.(117,118) After the injection, the detector emits low-level light at a wavelength of 760 nm over the skin, stimulating the fluorescence of the IGC molecule. This is captured by the camera and displayed on the monitor, after assigning a numerical value and therefore tracing a time/intensity curve. That can be measured in one specific point or within a freehand region of interest (ROI). The pre- and post-EVT curves can be plotted on the same graph, in the same way than in the PA.(97)

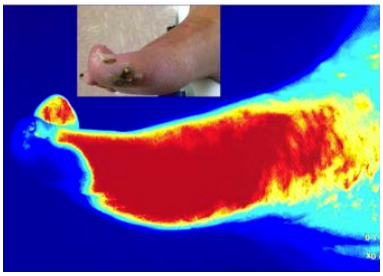

Figure 14. Fluorescence angiography image measuring surface foot perfusion using an intravenous injection of indocyanine green (inset: picture of the same foot).

This technique had been used initially to visualize retinal vascularity. It has a very strong safety record (1 adverse event per 40,000 administrations). IGC rapidly binds to albumin, so it remains intravascular for prolonged periods, making it an ideal marker to measure the perfusion. The hepatobiliary excretion makes it safe for CRD patients.(119)
A study made by Igari et al.(117) compared pre- and post-EVT ICG fluorescence imaging (ICG-FI) on Rutherford 2-4 patients. They found that a time from fluorescence onset to half the maximum intensity (T½) >20 seconds for predefined ROI (which included the distal region of the first metatarsal bone) was significantly correlated with a toe pressure of <50 mmHg (sensitivity: 0.77, specificity: 0.80).

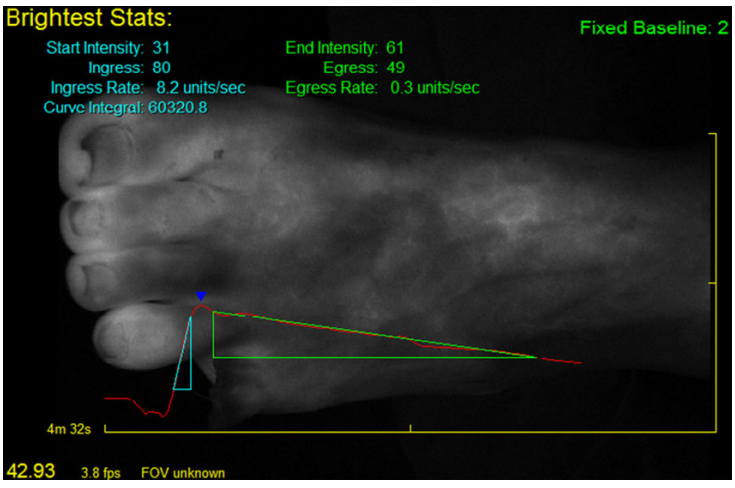

Figure 15. Time-intensity curves from ICG-FI. (courtesy of Rother et al.)

Another study made by Venermo et al.(118) was focused on CLI, with 66% of diabetic patients and 70% had an ischemic tissue lesion. They measured the ICG-FI in each leg.

They measured two parameters, the T½ and the PDE10 (defined as the increase of the intensity during the first 10 s). Time-intensity curves, with the aspect that is shown in figure 15, were repeatable and there was a strong correlation between TcPO2 and PDE10 was strong in diabetic patients (R=.70, p:.003). Other studies have confirmed the reliability and clinical correspondence with the information from the ICG-FI.(120) Anyway, the application of this technique become problematic in patients with ulcers and concomitant foot infection. In such cases, imaging could tend to overestimate perfusion.(121)

1.3.11. Peripheral duplex ultrasound imaging

Duplex ultrasound (DUS) is based on a gray-scale 2D imaging, color doppler and spectral waveform analysis. It has been a mainstay of vascular imaging for decades and it is still recommended as the first-line imaging study to the follow-up and even the intervention planning. It is non-invasive, yields true hemodynamic information, it does not affect the renal function and it is relatively inexpensive.(93)

There are several criteria to determine any stenosis as significant, besides of the anatomical and morphological information of the B-mode.(93) First, color doppler change from a smooth and uniform color associated with laminar flow to that of mixed color image associated with turbulence at and distal to the examined lesion. On the other hand, the form of the spectral doppler, from the normal triphasic or biphasic pattern with narrow spectral width to that of a monophasic tracing with spectral broadening distal to the lesion. Finally, the Peak Systolic Velocity (PSV) and the End Diastolic Velocity (EDV) are the more precise measures: a PSV of >200 cm/s and a ratio between two segments >2.0 translate a stenosis >50%, and PSV >300 cm/s, EDV 40-100 cm/s and a ratio >4.0 means a severe stenosis (>75%).(122)

The accuracy of DUS has been compared with the gold-standard, the Digital Subtraction angiography (DSA). Several meta-analyses(123) have shown high sensitivity (84%–91%) and specificity (93%–96%) DUS compared to DSA.

Moreover, there are some other advantages of this technique; like inquiring about the availability of saphenous vein for bypass grafts, the presence of a popliteal artery aneurysm or choosing the most useful outflow artery for distal bypass. DUS has a special interest due to its high sensitivity for the detection of patent tibial arteries; it is excellent for evaluating in-stent restenosis or occlusions(124) and the plantar artery or dorsalis pedis arteries when occluded could not be visible in DSA but can be seen in DUS.(93)

The main limitation of DUS exploration and the interpretation of its findings is that it is very operator dependent since DUS image alone is not informative without a summarizing cartography.(93) DUS is a more time-consuming examination than CTA and MRA. The visibility to the distal aorta, pelvic area, and the peroneal artery may be limited, added to the shadows caused by calcification, the reliability of the exploration could be diminished.(124) Moreover, DUS is less accurate when exploring arteries distal to occlusions or tight stenosis, where the velocity curve is not diagnostic due to slow velocity.(93)

1.3.12. Tomographic ultrasound imaging systems

Multispectral optoacoustic tomography (MSOT, iThera Medical GmbH, Munich, Germany) and other tomographic imaging systems based on ultrasounds suppose novel and promising techniques of noninvasive perfusion measurement. This technique combines conventional ultrasound with pulsed laser light in the near-infrared range. The laser pulses cause an extremely slight and transient rise in temperature in the tissue. This results in thermoelastic expansion, which generates an ultrasonic wave detected by highly sensitive detectors. Light excitation at specific wavelengths enables conclusions to be drawn on the composition of the tissue based on the absorption spectrum of several molecules (e. g., oxygenated Hb, deoxygenated Hb, melanin, lipids or contrast agents such as indocyanine green or methylene).(112,125)

Similarly, Plumb et al.(126) tested another method: photoacoustic imaging using a Fabry-Perot interferometer-based device which generates three-dimensional images of human vasculature at a depth of 14 mm. It was sensible to vasomotor microcirculatory changes induced by thermal stimuli, suggesting that it can provide valuable, objective and precise information about blood perfusion in the foot.

1.3.13. Computerized tomography angiography

Computerized tomography angiography (CTA) is comparable to the gold standard (catheter-based angiography) in detecting hemodynamically significant stenosis (>50%) with sensitivity, specificity, and accuracy of 99%, 98%, and 98%, respectively.(127) The main limitation relies on the below-the-knee (BTK) distribution, where the contrast in the small lumen of the tibial vessels can be difficult to make a distinction to the mural calcification. This often leads to lacking accuracy in diagnosis.

Dual-energy CTA is a technology able to subtract calcified plaque and soft tissues from contrast, creating CTA datasets and angiogram-like images. No detectable mean variation when analyzing vessel segments were found between dual-energy CTA compared with DSA, with less radiation than it used to be.(128)

1.3.14. Magnetic resonance imaging angiography

Magnetic resonance imaging (MRI) and CTA accuracy have become nearly equivalent in the below the groin segment., with a slight edge for MRI in the BTK distribution. Newer protocols, such as the time-resolved imaging of contrast kinetics (TRICKS) allows selecting the time point at which each segment of the vessel tree is optimally opacified. This technique increases sensitivity, specificity, and accuracy, particularly in CLI patients.(129) Time-of-flight (TOF) is also capable to avoid contrast use, but unfortunately, it is prone to artifact with nonlaminar flow typical of atherosclerotic plaque.(130)

More recently, magnetic resonance perfusion imaging using arterial spin labeling has been used to quantify arterial flow in the thigh and calf musculature, which has been shown to have equal or greater sensitivity for PAD compared with ABI.(131,132) Anyway, at present, both CTA and MRI roles are bounded in planning treatments. (97)

1.4. Natural history of critical limb ischemia

Patient outcomes in CLI are largely determined by morbidity and mortality caused by cardiovascular events and functional impairment caused by limb loss. Cardiovascular events such as myocardial infarction and stroke occur in 30-50% of patients with CLI, which face this risk over a 1-year period, and the same risk is faced over a 5-year period by the whole spectrum of PAD patients. Similarly, although the risk of major amputation is <5% in 5 years in patients with IC, it is of 30-50% in the first year in patients with CLI who are not revascularized.(133)

In the clinical scenario of CLI, revascularization is necessary and an attempt at treatment is reported in as many as 90% of CLI patients.(15) However, a subgroup of patients presents a favorable prognosis under conservative management. Marston et al.(134) reported 142 patients with limb ischemia (ABI < 0.7) treated at a comprehensive wound care center with amputation rates of 19% at 6 months and 23% at 12 months. Another study performed by Elgzyri et al.(135) evaluated 602 patients with diabetic foot ulcers who had TP < 45 mmHg or an AP < 80 mmHg. 50% of the cohort healed primarily without revascularization.

Nevertheless, besides the relieve of rest pain and/or faster healing rates, limb conservation with optimal revascularization, if feasible, would outweigh these performance goals.

Prognosis concerning limb salvage and survival in CLI patients has improved over the years.(136) As such, in large population-based studies performed between 1996 and 2006, a reduction of major amputation rates from 263 to 188 per 100,000 in Medicare beneficiaries with PAD (relative risk 0.71; 95% CI: 0.6–0.8) was observed.(137) Similarly, another study showed that among PAD population over 65 years of age, the adjusted odds ratio of lower extremity amputation per year between 2000 and 2008 was 0.95 (95% CI: 0.95–0.95, P<0.001).(138) Consistently, Egorova et al.(139) showed a reduction in surgical interventions in a US CLI population, where being major amputations decreased from 42% to 30% between 1998 through 2007. Furthermore, while the 1-year amputation-free survival (AFS) reported in trials performed during the period 1996-1999 was 28-40%, the same outcome from 2006 to 2010 was 48-81%.(140) This trend is supported by a decline in major amputation rates in CLI patients from 20-50% to 10-38% during the same period. This could be explained by either an improvement in revascularization techniques and endovascular options available, or a decrease in CLI incidence due to increased public awareness, better medical therapy and improved wound care.(136,139,141)

1.4.1 Treatment and management of critical limb ischemia

The clinical presentation of CLI depends on the degree of ischemia, the presence of infection and neuropathy. The primary goal is to preserve limb function and revascularization is a fundamental strategy for limb preservation, but in some patients, successful revascularization is not enough, such as in the case of cognitive impairment or non-ambulatory status.(130)

A revascularization planning should be made from arterial imaging, both functional and anatomical. Initial medical treatments include pain control, usually requiring narcotics, dressing for ulcers and tilting the bed downward to increase limb dependency and perfusion.(15)

In many centers, endovascular revascularization is the favored approach to CLI because of lower morbidity and mortality rates than those observed when performing open surgery. The optimal treatment strategy (endovascular versus open surgery) will depend on anatomic factors, comorbidities, patient preference, and operator experience and skill.(130)

The ESC 2017 guidelines of treatment of PAD(142) propose an algorithm (figure 16) to evaluate patient candidacy to revascularization.

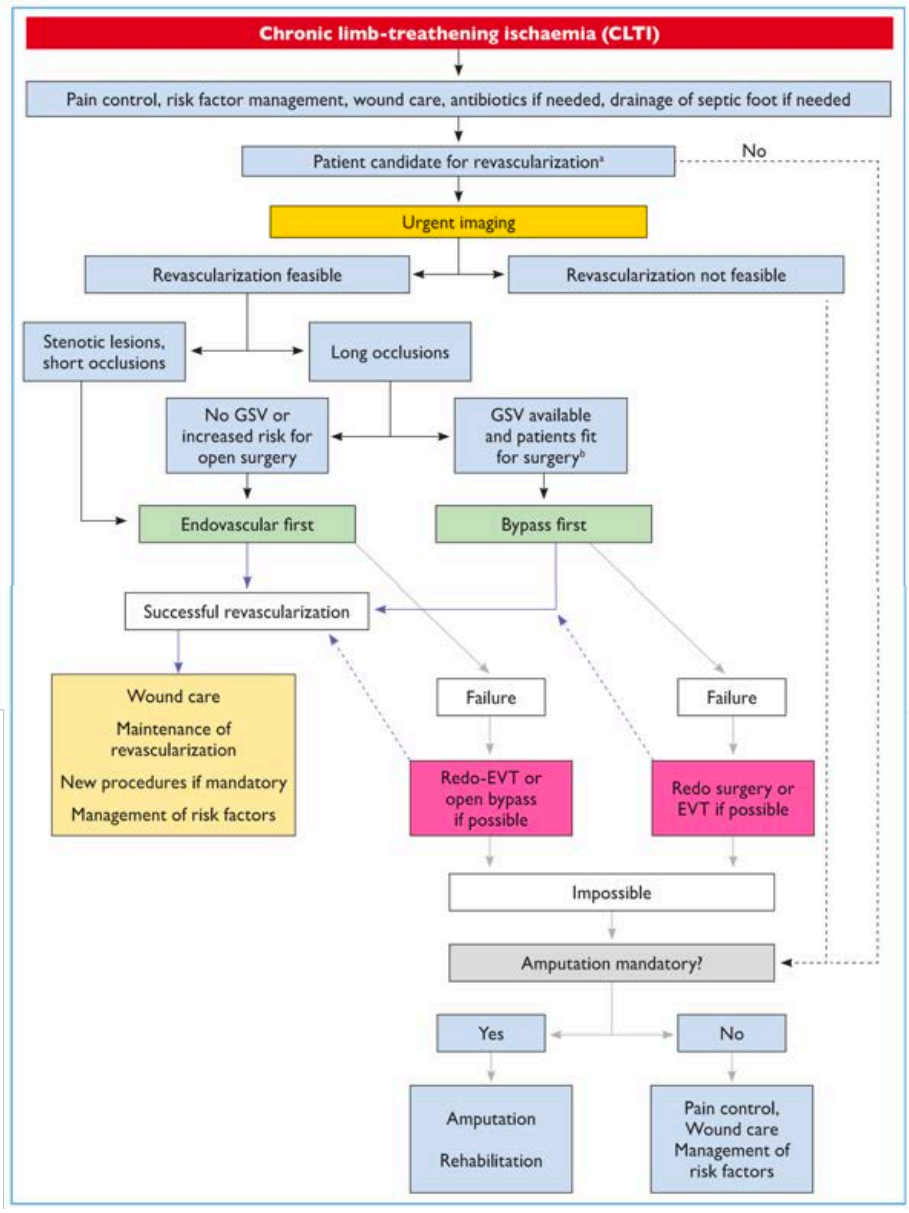

Figure 16. CLI treatment algorithm, as proposed in the 2017 European Society of Cardiology Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery.(142) GSV: great saphenous vein. [a)] In bedridden, demented and/or frail patients, primary amputation should be considered. [b)] In the absence of contra-indication for surgery and the presence of adequate target for runoff.

Open surgery has higher risks of perioperative myocardial infarction, death, and stroke than endovascular revascularization. However, in CLI without endovascular options or failed and a reasonable 2-year survival, the potential loss of limb and function may favor surgery.(143)

Common femoral disease often involves the profunda and superficial femoral artery (SFA) origins and in this segment. In such cases, surgical endarterectomy with patch angioplasty offers a more durable result than the endovascular recanalization. The procedure after the patch angioplasty can be followed with a hybrid endovascular-surgical approach, which is commonly used in multilevel disease.(143)

Bypass relies on good inflow and outflow, as well as on the conduit type, that could be autogenous or prosthetic. Autogenous saphenous veins provide better long-term patency than prosthetic grafts,(144,145) particularly when there is good quality, long, single-segment, autogenous vein with a diameter of at least 3.5 mm.(146–148) The 3 types of saphenous vein bypasses are reversed, non-reversed and in situ bypass.

The standard endovascular strategy is a step-by-step approach as shown in figure 17. An ultrasound-guided femoral antegrade ipsilateral access is often possible and it is more effective than contralateral retrograde or brachial approaches. The first-line approach, irrespective of the length of the lesion, is to cross in an endoluminal position. Nevertheless, in long calcified occlusions is frequent the need to go on the subintimal space. Despite of all, if the wire could not arrive at a distal lumen, a bailout strategy is the retrograde access techniques. Those are ideally performed the closer as possible to the lesion that is about to be treated.(149) In a femoropopliteal occlusion, the retrograde popliteal approaches could be made in P1 (from the adductor canal to the upper border of the patella) tract or P3 tract (from the joint line to the emergence of the anterior tibial artery) of the popliteal artery. When the occlusion is limited to the SFA, the P1 access is enough, but the occlusion or the subintimal dissection usually arrives at P2 or even P3. In that case could be helpful an anterolateral approach to the P3 tract of the popliteal artery or the tibioperoneal trunk (TPT), maintaining the patient in supine position.(150) In a CLI scenario, nonetheless, the more frequent affection is in the tibial vessels; thus, the need for a retrograde puncture will be in the anterior or posterior tibial arteries, dorsalis pedis, peroneal or extreme trans-metatarsal or trans-plantar arch access.(149,151)

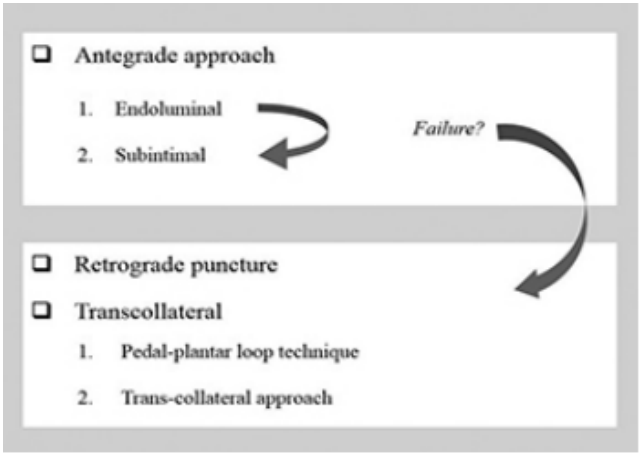

Figure 17. Step-by-step approach to chronic total occlusions.

After crossing the lesion, the usefulness of an atherectomy should be set.(152) Alternatively, primary balloon angioplasty is made, with or without predilatation. Finally, flow-limiting dissections or immediate recoiling to > 50% could justify the placement of a stent. Recently there's uprising evidence of the lower restenosis rates with the drug-eluting balloons and stents,(153–156) so we should consider the potential benefit of them on the patient we are treating. However, in late 2018, Katsanos et al. published a metanalysis reporting late all-cause mortality of patients in the DCB and DES arms of randomized clinical trials exploring the treatment of the superficial femoral artery of claudicants.(157) Despite the safety concern due to the finding, neither a plausible mechanism nor a specific cause of death were identified.

To take into account the big picture, the only randomized study comparing endovascular versus open surgical treatment of patients with CLI is the Bypass Versus Angioplasty in Severe Ischemia of the Leg (BASIL) study.(158) This trial published in 2005, demonstrated no difference in major amputation or death >5 years. Rates of myocardial infarction, wound infection, pulmonary complications were higher in the surgical group, and repeat revascularization was higher in the endovascular arm. However, after the time of the trial both techniques have improved as well as the perioperative mortality, particularly concerning the catheter-based techniques.

More recently, several proposals for important endpoints in CLI have converged on limb salvage (avoiding a major amputation) and the need for major reintervention (a new bypass graft or thrombolysis of treated segment), and assessments considering the quality-of-life and cost-effectiveness too.(159,160) As a result of these developments a new randomized trial of open surgery versus endovascular revascularization in CLI is being performed, the Best Endovascular Versus Best Surgical Therapy in Patients With Critical Limb Ischemia (BEST-CLI) study.(161) It has enrolled, by 2017, 775 patients out of 5000 aimed to be enrolled. It is a timely and critically needed trial that will significantly impact clinical practice by defining an evidence-based standard of care for patients with CLI.(162)

All efforts in revascularization and maintaining the walking ability usually go through a minor amputation. Major amputations (below or above the knee), on the other hand, limit functional self-sufficiency and require prosthesis to walk. Nonetheless, in certain cases, a primary amputation is the best option in patients with extensive tissue loss or infection, and in potentially non-ambulating patients.(163)

Complementary to all the aforementioned techniques, there are antibiotics and wound care which aim to improve perfusion, treat the infection, avoid pressure on a wound, debridement (by scalpel, collagenases, or even maggots(164)) and adequate nutrition. The perfusion can be increased by increasing local temperature of the limb with sheepskin boots or by negative pressure dressings (vacuum-assisted).(130)

1.4.2. Endpoints of treatment

The goals of treatment for CLI are to preserve life and limb function by healing the ulcer and relieving pain while minimizing the frequency and magnitude of interventions. Despite the scope of the problem, there's a lack of high-quality evidence to support current treatment paradigms in CLI. Conte et al.(165) set the Objective Performance Goals (OPG) for safety and efficacy for catheter-based treatments or endovascular treatments (EVT). However, the measured variables in the OPG do not say anything about the necessary results that revascularization should reach, but rather refer to the long-term clinical outcomes of a certain treatment.

In several reports, 10–15% of CLI patients not considered as suitable for revascularization heal with no amputation.(102) Thus, besides of risk scores and classifications, a part of the CLI population will heal whether we do or not the appropriate revascularization. However, an important tenet in restoring perfusion and blood flow is establishing in-line flow from the aorta to the affected foot or, more importantly, to the ulcer.(97) In CLI, where it is common the involvement of the tibial vessels, ideally, the normal

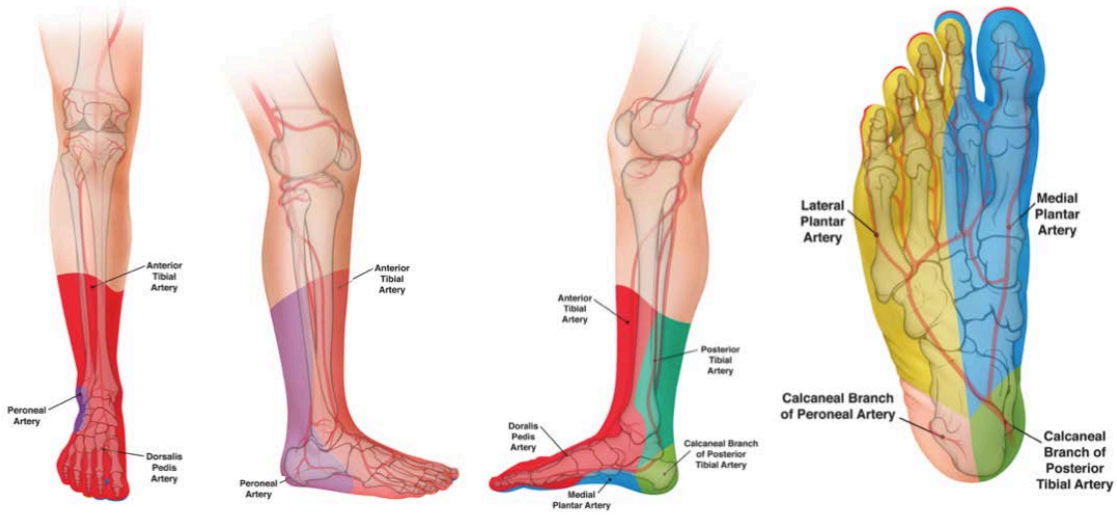

Figure 18. Angiosome skin-surface representation, courtesy of Desmond Bell in Podiatry Today.

vascular anatomy should be restored with a “complete” revascularization.(166) Nonetheless, it is not always feasible to achieve.

Thus, in case of being able to choose one vessel, the angiosome theory, first suggested by Taylor and Palmer,(167) could be helpful. This is particularly important in diabetic and ESRD patients with poor collateral communication between territories.(168,169) The anterior tibial and dorsalis pedis arteries supply the tissue comprising the anterodorsal surface of the calf, ankle, and foot; the posterior tibial artery supplies the plantar surface of the foot and posteromedial calf, ankle, and heel through its plantar and medial calcaneal branches; and the peroneal artery supplies the posterolateral calf and ankle as well as the lateral heel (figure 18).

Despite several studies have reported outstanding revascularization outcomes upon using the angiosome concept in treating diabetic feet,(168,170) there is much controversy regarding the necessity of angiosome-targeted angioplasty, with multiple studies falling on both sides of the debate. A meta-analysis by Chae et al.(171) identified four cohort studies reporting on 881 limbs, comparing the effect of angiosome-targeted angioplasty and indirect revascularization to treat CLI in diabetics. They found that angiosome-targeted angioplasty improved limb salvage rate (OR = 2.21, $p = 0.001$) and wound healing rate (OR = 3.3, $p < 0.001$). Consistently, several studies have favored the angiosome-targeted angioplasty with better limb salvage rates.(172–175) On the other hand, when adequate collateral vessels were present, the outcomes of indirect revascularization were similar to the angiosome-targeted ones,(176) although leaving the uncertainty of what is exactly the “adequate collateral vessel” presence.

Further, it worth mention that CLI in diabetic patients frequently develop concentric continuous vascular calcifications that could limit the effectiveness of endovascular angioplasty(174) and lead to revascularization of non-targeted vessels. In addition to that, when indirect revascularization is the only way to improve foot revascularization, it needs to be done.(171) Last but not least, since there is a reported 9%–12% of anatomical variations, we should consider them when we want to apply the angiosome theory.(177–179)

After choosing the target vessels, the revascularization could be deemed successful enough based on several sings. Angiographically we can take the patency of previously occluded vessels, improved vessel caliber and flow velocity, and decreased collateral flow as an intended result.(97) The presence of wound blush is associated with higher perfusion, which improves limb salvage rates.(180) In addition, palpable pulses can be considered an optimal result. Ultrasound imaging can also be performed after the procedure, with assessment

for distal runoff patency and blood flow improvement. Restoration of normal limb coloration, shortening of capillary refill time under 5 seconds can be seen after a successful intervention. (97)

1.5. 2D Perfusion angiography

The concept of perfusion is derived from the Latin *"perfundere"*, composed of *"per"* (around) and *"fundere"* (to pour). In physiology, perfusion refers to the circulation of blood through the vascular bed formed by biological tissue structures.

2D perfusion angiography (PA) is a modification of an original image processing software firstly developed to assess the penumbra pattern in acute stroke patients. However, its application in the CVD arena has been limited because in the brain a local area of ischemia is surrounded by normal brain, and 2D summation of damaged and normal perfused cerebral tissue renders precise quantification impossible.(181)

This thesis is focused on the foot dedicated PA software, developed by Philips medical (Best, The Netherlands) and is an image processing algorithm for DSA images based on the change of contrast density per pixel over time.(182) The purpose of this tool is to assist the physician in the diagnosis and quantification of the perfusion alterations in a given patient by providing a color-coded representation of a DSA run and depicting a curve from the average of time-density curves for each pixel into a selected Region Of Interest (ROI).

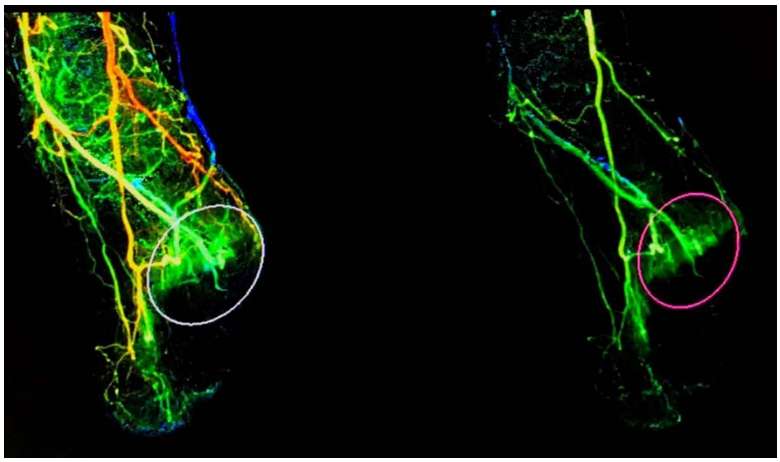

Figure 19. Color mapping of perfusion angiography. The image on the right side shows the PA before EVT, with the ROI placed over the ulcer-zone; while the image on the left corresponds to the perfusion angiography run after EVT.

Thus, the PA analysis will be visualized by the tool either as a pixel-by-pixel color map (figure 19), or a time-density graph (figure 20). The tool, therefore, enables an evaluation of the flow in the imaged tissue but also a comparison between subsequent runs during the intervention.

From that PA analysis we can obtain 6 parameters, which are numeric measures of the average time-density curve, and are defined as follows:

1. **Arrival Time (AT):** AT is the time elapsed between the first frame selected for processing and the frame where contrast is detected. This is the time between main arterial inflow and inflow into the small vessels of interest.

2. **Peak Time (PT):** PT is the time between the contrast uptake point (AT) and the time at which the contrast has maximum density.

3. **Wash-in Rate (WR):** The WR is defined by the slope of the uptake curve. The WR gives an indication of the flow rate or speed in the big foot vessels.

4. **Width (W):** The W is measured between the inflection points on the wash-in curve and wash-out curve. The W parameter is an alternative to the Mean Transit Time (MTT) parameter, but it does not consider any asymmetry in the time-density curve.

5. **Area Under the Curve (AUC):** The AUC is the area under the curve between the AT and the point that the contrast has left the vessel.

6. **MTT:** MTT is the time between AT and the point of the center of gravity in the time-density curve. This functional parameter is an indication of the average time it takes for contrast to pass through the tissue.

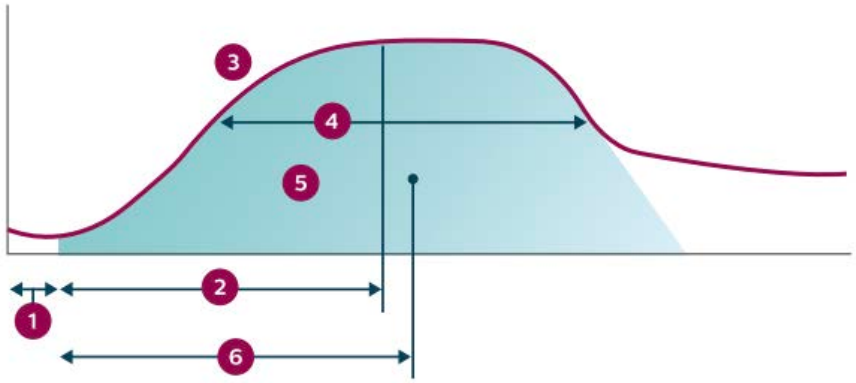

Figure 20. Representation of the PA parameters over the curve: (1) Arrival Time, (2) Peak Time, (3) Wash-in Speed, (4) Width, (5) Area Under the Curve and (6) Mean Transit Time.

The foot should ideally be immobilized before beginning the procedure to avoid extensive movement that will cause the run to be unquantifiable. Methods to ensure complete immobilization include the use of a dedicated footrest, general anesthesia, or various creative alternatives with tape, X-ray compatible rest devices, and non-abrasive wraps. Toe movement can also be excluded when defining the ROI.(181)

Techniques of PA measuring are already used, but we don't understand the physiological meaning of the parameters analyzed and we cannot link them up to the type or degree of stenotic and occlusive lesions and neither to the clinical prognosis. Therefore, PA cannot be used in decision-making.(183)

2. JUSTIFICATION

Nowadays there is no objective measure to assess the success of EVT in CLI patients. PA is an image-processing software which may help in quantifying the degree of post-EVT perfusion. Thus, our aim is to define an objective goal based on PA to ensure successful EVT of CLI patients.

Furthermore, a simpler anatomic classification system, able to describe the arterial disease burden below the groin, needs to be created to assess optimal therapy for any given patient.

3. HYPOTHESIS AND OBJECTIVES

HYPOTHESIS

Perfusion angiography can give us objective information on the degree of distal blood irrigation to the lower limbs in patients with peripheral arterial disease, which is pivotal in clinical outcomes. This degree of irrigation could be deduced from the perfusion parameters, which might be also related to patterns of stenotic and occlusive lesions.

It is the hypothesis of this thesis that the information generated in perfusion angiography could have a higher prognostic value on the healing of ischemic ulcers and patency of revascularized limbs.

OBJECTIVES

Given the previously described state of the art, the main objectives of this thesis are:

a) to give a clinical meaning to imaging parameters of the perfusion angiography; and,

b) to set an objective measure to determine success in endovascular therapies; and,

Following, the secondary objectives of this thesis are:

c) to describe a novel classification with perfusion, clinical and anatomical information of the arterial steno-occlusions burden in the whole limb; and,

d) to correlate the perfusion angiography parameters with non-invasive diagnostic methods (mainly the TCPO2); and,

e) to explore the effects of an incomplete plantar arch on perfusion angiography.

3. METHODS

4.1. Study design

4.1.1. Setting

A prospective cohort study was developed in the Interventional Radiology Department as a part of a diabetic foot clinic in the Policlinico Abano Terme (Abano Terme, Veneto, Italy). It was anticipated a study population with high prevalence of diabetic patients with CLI and an estimated volume of 800 patients per year. The clinical follow-up was at least 6 months after intervention.

4.1.2. Patient selection

The criteria to select a patient to perform the Perfusion Angiography (PA) analysis was chosen randomly as only 1 case every 2 consecutive patients (independently from clinical stage or expected technical complexity), due to daily time schedules limitations for the clinical practice.

4.1.3. Inclusion criteria

Every patient who received EVT for CLI (with Rutherford classification ranging from 4 to 6) and for very short distance claudication (considered in Rutherford 3) in which the PA before and after treatment had been performed; with the technical requirements described above and with comparable initial and final projections.

4.1.4. Exclusion criteria

Firstly, PA studies with artifact because of movements of the foot were firstly excluded from analysis either because this affects the curve of the PA graph or because the PA protocol could not be properly done. Further considerations to this point are developed below in “Perfusion angiography measurement”.

Patients with borderline end-stage renal disease or allergy to iodinated contrast media were studied with CO2 angiography. Subsequently, those ones were not considered to be selected.

Those patients without a PA after treatment (not done because of lack of collaboration from the patient or because a non-standardized PA technique in the after-treatment PA) were only considered for the hemodynamic analysis, and not for clinical follow-up endpoints.

To end with, patients whose pre-treatment PA analysis could not be analyzed were analyzed for post-treatment PA variables.

4.1.5. Interventions

Interventions were performed under local anesthesia. A percutaneous antegrade approach of the common femoral artery was taken to place an 11 cm 6F sheath (St. Jude Medical®, Saint Paul, Minnesota). Standard DSA projections (Philips Allura Xper FD20, Philips Healthcare®, Best, the Netherlands) will be done pre- and post-EVT. The protocol for contrast injection consisted in 9 mL of Visipaque 270 mg/ml (iodixanol, GE Healthcare Inc.®, Princeton, NJ) administered at a speed of 3 ml/s using a coupled injector. The projection was lateral or anterior-posterior views, with a maximum magnification while minimizing the distance between the foot and the detector and ensuring the foot remains completely visible. The projection setting was retained for all PA runs in the same patient.

The injection for the PA analysis was done from the short sheath in the proximal superficial femoral artery (SFA) to avoid losing the hemodynamic information of the femoropopliteal (FP) lesions. Ideally, we might have to measure up any eventual aorto-iliac stenosis and cardiac ejection fraction; but the information obtained was at least as representative as possible of the hemodynamic of the whole limb. This way, the information obtained from the study was applicable to decision making on the clinical practice since it's the normal scenario in the EVT with intention to treat CLI patients.

The foot of the patient had not been immobilized if there was no need and no movement artifacts were detected in the PA run. In case of foot movement during de PA, it will be tied up to the operating table with a tape over a protection to avoid abrasive lesions on the delicate skin of a CLI foot.

The PA was measured prior to the revascularization and afterwards. PA images were obtained at a frame rate of 3/s until the operator extends the acquisition. Time of acquisition depended on a subjective impression that the contrast had adequately arrived to the foot. The maximum time of acquisition supported for analysis is 30 seconds (90 frames). The image was analyzed by the Philips software Interventional Workspot 1.3.1 / 2D Perfusion 1.1.6©.

4.1.6. Variables

The collected variables were:

- Demographic: Age (years), gender, smoking habits (no/former/active), weight (Kg), height (cm), Body Mass Index (BMI).

- Comorbidities: diabetes mellitus, hypertension, atrial fibrillation (without pacemaker rhythm), ischemic cardiomyopathy, chronic kidney disease, end-stage renal disease, cerebrovascular disease, autoimmune disease.

- Medications: Antiplatelet treatment (no/simple/double), anticoagulation and corticoid therapies.

- Vascular history: ipsilateral or contralateral endovascular treatment, ipsilateral or contralateral surgical treatment, contralateral major amputation, CABG and PCI. Also the baseline Rutherford classification(87) and the WIfI clinical stage(1) for benefit of revascularization, TCPO2 (mmHg) and Texas Ulcer Classification (TUC)(88).

- Laboratory: Creatinine before and after EVT (mg/dL), Reactive C Protein before EVT (mg/dL), Hb before and after EVT (g/L)), leucocytes before and after EVT ($x10^9$/L) and neutrophils before and after EVT ($x10^9$/L).

- Intervention variables: Plain angioplasty, drug coated balloon, bare metal stent, drug eluting stent or atherectomy, both in above or below the knee.

- Conventional angiography classifications: TASC classification(15,89) femoral popliteal (FP) and Below-The-Knee (BTK), pre and post, and the "Abano Terme Score" (ATS) for each artery of the limb.

- Perfusion Angiography (PA) measurement: Each parameter pre-treatment and post-treatment, measured both for the whole foot and for the ROI of the ulcer. Quality of the PA will be also collected.

4.1.7. Clinical follow-up

There were 2 follow-up checkpoints: at 1 and 6 months: Date of visit, TCPO2 (mmHg), target lesion revascularization (TLR), ulcer healing and date of event, WIfI stage(1) and TUC classifications(88), amputation free survival, Major Adverse Limb Event (MALE), Major Adverse Cardiovascular Event (MACE) as a stroke, clinical significant infarction or arterial thrombosis in any vascular region, Death.

Patients who missed the clinical follow-up of the diabetic foot clinic had been phone called twice during the data collection in order to find out if they presented eventually a MALE or they died (not being reported on clinical follow-up). Patients who died during

follow-up and those who missed a follow-up control before their ulcers healed, were also excluded from analysis. If the date in which the ulcer healed could not be inquired but we know instead that it healed and the limb was salvaged, the case was excluded from analysis of the time to heal, but included in the general limb salvage rates.

4.1.8. Data collection

Data were collected by the main investigator using a form database software (Ninox Software GmbH®, Berlin Germany), and several control variables were collected only to ensure the precision and truthfulness of the collected data. Those variables were data collection check, marks to review cases or data and marks on the image quality. Data from one case could not be accidentally merged with another case because of the form entry data process.

4.1.9. Ethics

Data was collected at that single center and the study was conducted in full compliance with the Declaration of Helsinki, after being approved by the local Institutional Review Board. All patients provided informed consent before the procedure.

4.2. Perfusion angiography measurement

4.2.1. Projection

The projection of the PA was mainly done in an antero-posterior view of the foot, but it could had been also done in a standard lateral projection. In both ways the area had been the same, including from peroneal bifurcation (at the level of the malleolus) until the proximal part of the toes. There was no advantage in measuring above the malleolar area because it would had given information about the flow in a concrete tibial artery, but not about the foot.

Anyway, the antero-posterior projection was preferable to evaluate the terminal blood flow of the food and to discriminate between precise zones of the ulcers. An exception for this would have been the case of heel ulcers or in a prior Chopard or Lisfranc amputation, in which a lateral view for the PA may be better. However, in both AP and lateral projections the area of the ROI had been theoretically the same, including from peroneal bifurcation (at the level of the malleolus) until the proximal part of the toes; in order to avoid confounding information about the flow in a concrete tibial artery, and not in the ulcer or the entire foot.

4.2.2. ROI tracing

In case that our aim was to analyze the perfusion to the whole foot, the ROI included the whole foot arch, from the malleolar level to the toes (excluding outline borders and areas with movement artifacts). For the perfusion of the ulcer zone, instead, we traced the ROI over the tissue affected by the ulcer. Subsequently, the angiosome concept had been neglected because we were measuring the perfusion in the concrete ulcer tissue, regardless of the anatomical variations and the angiosome targeted revascularization.

The outlining of the ROI had been traced respecting anatomical symmetry of the arteries between both projections (before and after EVT). If we excluded a concrete zone of the foot from the ROI to avoid movement artifact, the ROI in the comparative PA image also excluded the same zone, even if there is no artifact in the latter. This way both images were comparable from the hemodynamic point of view.

4.2.3. Time of exposure

Regarding the time of the exposure and analysis of the PA, it's important to consider that this time was decided from the arbitrary duration of the Digital Subtraction Angiography (DSA) run and it depended on the contrast arrival. This duration was a decision took during the intervention and cannot had been equalized for all patients. Thus, the more adjusted measurement to the limb's real state was to analyze the whole duration of the PA run.

The color bar scale had been the same in both PA images and fully ranged in order to improve the resolution. Nevertheless, it did not modify the value of the parameters that are the object of study. It does not seem to be useful to set trim the exposure time too much because the behavior of the PA graph was not coherent and might be a consequence of some gap into the software algorithm.

To avoid artifacts, time of exposure was cut-off from the ending tail, but not from the start because in the latter fashion time dependent parameters would have been wrongly modified.

In the cases with runs longer than 90 frames, only the last 30 seconds were analyzed and the delay time before analysis should was added to the arrival time value. The other affected parameter by this hypothetical issue was the Area Under the Curve (AUC), but it could had not been corrected because we can add the time, but the AUC in the deleted frames of long series were not recorded. The rest of the parameters, since they only depend

theoretically on the morphology of the curve they would remain invariable after a time delay before analysis.

4.2.4. Artifact management

The PA image will be considered optimal when we can only see the arteries in the PA image. That implies that the bone or the skin could not be seen and the curve follows a physiological morphology.

It was considered as an artifact any mark of the bone's or skin's outlines in the PA image, also the speckled color tracing in the zone of the bone marrow. Anyway, the magnitude of those artifacts was dependent on how much is the time-density curve modified. Here, the artifact was seen as a peak appearing through the curve. Usually they could be seen isolated but if there were too many, the peaks could not be identified, thus, the curve was useless and should be excluded. That is, the artifact that revealed a unique movement was reflected on the curve as a unique peak; on the other hand, the artifact that depicted a speckled color tracing in the PA image was represented as multiple small peaks that could have increased the slope of the curve and might have modified the values of the parameters.

If the maximum density in the curve did not match in time with the corresponding color (codified by the time-color bar) with which the foot arteries were depicted, it would have mean that the curve was not representative of the arterial flow but modified by an artifact. Subsequently, the run was not valid and had been excluded. The other face of the coin of this fact was used as a checkpoint, ensuring that the color corresponding to the time of the maximum point in the curve was matching the main color of the PA image. Another strategy used to test the truthfulness of the time-density curve was to scroll the time of exposition (from second 0.3 till the end of the run) and observing that the arteries appeared in the PA image synchronized with the graph curve, denoting that the curve reflected the contrast in the arteries and it had no relation with any other artifact.

If the movement artifact was clearly limited to a zone in the foot, the ROI had been drawn excluding that zone and the PA took to analysis. Symmetrical ROIs were always taken from the PA image before and after treatment. To ensure this symmetry, we took anatomical references (arterial bifurcations, shape, etc.) and not with the provided chained tool, in order to increase precision.

4.3. Conventional angiography: measurement and classification

The standard DSA projections of the limb were those described by Manzi et al.(184) The angiographies were recorded as a maximum opacity pictures and stored for later classification according to TASC and the below described score.

4.3.1. TASC classification

The angiographies before EVT and thereafter were classified according the TASC classification(89) for both FP and BTK sectors. The TASC classifications does not have any grade to describe a patient with no stenosis; therefore, it was classified as a "no lesions angiography", besides the TASC categories A, B, C or D. The criteria for each grade of the classification in the FP sector were:

- TASC A: single stenosis ≤ 10 cm or occlusion ≤ 5 cm in length

- TASC B: multiple lesions (stenosis or occlusions) ≤ 5 cm each, single lesion ≤ 15 cm (not involving infra-geniculate popliteal artery) or heavily calcified occlusion ≤ 5 cm.

- TASC C: multiple lesions totaling ≥ 15 cm

- TASC D: chronic total occlusions of SFA ≥ 20 cm or popliteal artery and proximal trifurcation vessels.

A specific consideration on femoral popliteal TASC B classification was that a short stenosis in the popliteal artery will be considered as TASC A, in order to give it a more hemodynamic sense, rather than a technical one. In TASC D, the possibility of an occlusion of the common femoral artery (CFA) is not considered because the EVT is not suitable on the CFA occlusions.

The TASC classification for the below-the-knee sector obeyed the following criteria over the target vessel (assuming worse stenosis or CTO in the other arteries):

- TASC A: stenosis ≤ 5 cm in length

- TASC B: multiple stenosis of ≤ 5 cm each with a total length ≤ 10 cm or a single occlusion ≤ 3 cm in length

- TASC C: multiple stenosis or a single occlusion of > 10 cm in length

- TASC D: the same lesions of TASC C but with dense lesion calcification or no visualization of collaterals

To study the differences between pre and post TASC classifications, categorical values of 0, 1, 2, 3 and 4 were given to “No lesions”, “A”, “B”, “C” and “D”, respectively.

4.3.2. Hemodynamic angiography based “Abano Terme Score”

We described a score to ease the classification and to better describe the lesions of the whole limb, as well as to include hemodynamic parameters.

It was inspired on the Joint Vascular Societies Council (JVSC) classification described by Toursarkissian et al.(185) referring to the lesions on the out-flow arteries in the foot. The JVSC classification gave a score from 0 to 3 to each artery on the foot (dorsalis pedis from the ankle joint level to the level at which it gives off its arcuate branch, lateral plantar, and medial plantar) and the calf (anterior tibial, posterior tibial and peroneal including the tibioperoneal trunk) on the basis of the most severe stenosis depending on the following grading:

- 0 points in a stenosis of 0-20%

- 1 points in a stenosis of 20-50% or in tandem stenosis of <20%. This value will be also given to no flow-limiting dissections or mild recoiling after angioplasty.

- 2 points in a stenosis of >50% or in tandem stenosis of <50%

- 2.5 points in an occlusion of <50% of the vessel length

- 3 points in an occlusion of >50% of the vessel length

Afterwards, a “foot score” (sum of the scores obtained for the three foot vessels plus 1) and a “calf score” (sum of the scores obtained for the three calf vessels plus 1) were finally obtained in the JVSC system. Our attempt was to adapt this score to the whole limb. Based on the same grading criteria, we provided a punctuation to each artery from the groin to the ankle (superficial femoral artery, popliteal, anterior tibial, posterior tibial and peroneal arteries). For convenience in this manuscript, we named it as “Abano Terme Score (ATS)”.

The total scoring for the whole limb was obtained by adding up the score given to the FP and the BTK region. To obtain the score of the FP region we added the squares of the scores of the SFA and the popliteal artery. In the BTK area instead, we multiplied the scores of the anterior and posterior tibial arteries, and then the score for the peroneal artery was added. The rationale behind this strategy is that the presence of lesions in the FP area is hemodynamically more deleterious, while the lesions in one tibial artery could be properly compensated by the other tibial artery if the latter has no stenosis. The peroneal artery alone,

otherwise, could not replace both tibial arteries. The formula to calculate the global limb ATS score is explained in figure 21.

$$limb\ ATS = SFA^2 + Pop^2 + (ATA \times ATP$$

Figure 21. Formula for the global limb ATS. ATS: Abano Terme Score; ATA: anterior tibial artery ATS; ATP: posterior tibial artery ATS; Per: peroneal artery ATS; Pop: popliteal artery ATS; SFA: superficial femoral artery ATS.

According to this scoring, in the case of a long occlusion of all the anterior tibial (deserving a score punctuation of 3), a posterior tibial without any stenosis and good outflow (corresponding to a punctuation of 0) and an isolated stenosis of >50% in the peroneal artery (punctuation of 2), the score for BTK in this case would be 2 (since 3×0+2 = 2). Some examples are shown in figure 22.

In case of anatomical variations, typically the hypoplasia of the anterior or the posterior tibial arteries, the score value is given to the artery that procures blood supply to the theoretical angiosome of the hypoplastic artery. The latter is usually the peroneal artery. The tibio-peroneal trunk will be scored as a part of the posterior tibial artery.

Prior FP bypasses had been scored as if they were the native FP, with eventual stenosis or occlusions, provided that they are equivalent from the hemodynamic point of view.

The advantages to evaluating limbs using this type of score are several. First, an index of the global hemodynamic significance of the lesions will be obtained, aiding in the correlation with the degree of perfusion. Indeed, it is mandatory to correlate hemodynamics and perfusion to accurately quantify the severity of the lesions.

Second, the score provides a simplified report of the steno-occlusion pattern in a limb, which is very useful in daily clinical practice and in the standardization of descriptive analysis of the disease burden of a limb. For instance, if we want to describe an IC patient we can understand that a compatible score would be a 2.5+1+0+0+0; or, on the other side of the coin, a typical CLI pattern in a diabetic patient would be 2+0+3+3+0.

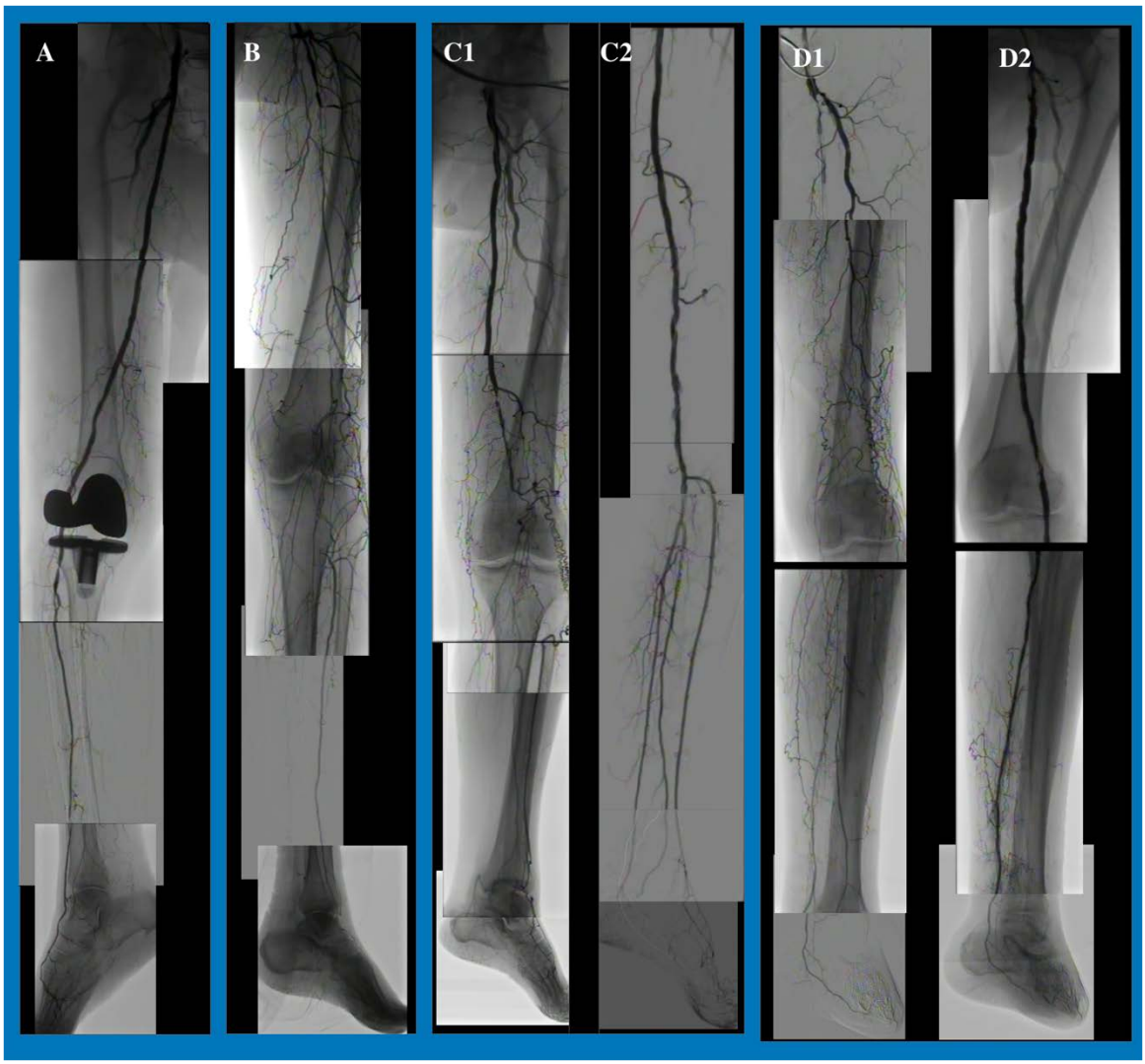

Figure 22. Examples of the ATS system. Composition of images from DSA conventional angiographies. The projections vary from the above the knee and the ones below the knee. The ATS is calculated in the two cases on the left to show its descriptive ability regarding the disease burden in a limb, being (A) 0+0+0+3+3 (ATS for the whole limb=3) and (B) 3+3+0+3+3 (ATS for the whole limb=21). The two cases on the right demonstrate the ATS' capacity to describe the global post-procedural improvement of the limb. In the case (C), the ATS score improved from 13 (2+3+0+3+0) to 0 (0+0+0+0+0). The case (D) improved its ATS of 28 (3+3+3+2.5+2.5) to 3 (0+0+3+0+3). ATS: Abano Terme Scores; DSA: Digital Subtraction Angiography.

4.4. Endpoints and groups for analysis

The independent variables of the study were the PA parameters after the EVT. They were measured on the whole foot and in the ROI corresponding to the ulcer. Thus, the angiosome concept was not applied since the area of tissue measured was straightly the ulcer, independently to the tibial vessel to be treated. To avoid confounding factors, such as central hemodynamic variables, we also annotated the delta (Δ) changes (increases or decreases) of the values of a certain PA parameter.

TASC classification and ATS values were annotated separately and with the changes through the EVT (Δ).

The dependent variable for the main endpoint was the presence of an ulcer or not. In cases without an ulcer, as in Rutherford 3 and 4 patients, the groups were divided if they needed a target lesion revascularization (TLR) or not. Herein, the endpoint is not referred to the concrete lesion but a reintervention on the same limb.

On the other hand, in patients with and ulcer, the dependent variable was the time to heal (TTH), and patients were classified according to whether the TTH is lower (group A) or higher (group B) than a specific amount of days. The optimal TTH interval was explored from several time cut-off points: 30 days, 40 days and 120 days. The reasons for choosing several cut-off points was the uncertainty on the prediction ability of PA, besides from other variables that contribute to the healing process (cures, nutrition status, etc). The 30- and 40-days cut-off points were chosen due to their easiness to determine precisely the healing process because of more frequent controls in the first weeks. The 120 days cut-off was chosen as suggested by Reed et al.(186) who found a higher probability of MALE in patients who did not heal by 4 months. As a last consideration, if a patient has required a TLR or a major amputation, the case was be considered as non-healing, thus, included in group B.

4.5 Statistical analysis

Descriptive and frequency statistical analysis were obtained and comparisons were made by use of the software IBM SPSS Statistics 22.0®. Categorical variables were reported as frequencies (percentages) and continuous variables as mean ± SD or median (interquartile range), as appropriate. Distributions of the PA parameters were checked for normality using Q-Q plots. Box-Plots were used to represent values of quantitative variables among the study population.

Changes in TASC classification were assessed with the McNemar-Bowker´s test. Pre-post changes of PA parameters (before and after EVT) were assessed by the paired samples t-test. Intergroup differences between dependent variables (TTH) and PA parameters were performed using the Student's t test. Receiver characteristic operator curves (ROC) were configured in order to calculate cut-off points for PA parameters with best sensitivity and specificity to predict a best outcome. These variables were then categorized and compared to TTH with the Pearson's chi-square or the Fisher's exact test, as appropriate. Kaplan-Meier curves were performed to evaluate the time of ulcer healing depending on the different values of PA parameters, determining the log-rank test to assess statistical significance between groups.

Logistic regression models adjusted by potential confounding factors were performed in order to identify variables which could be independent predictors of TTH. Predicted probabilities of the model were obtained and a ROC curve was configured in order to measure its predictive ability.

Associations between TCPO2 and values of PA and ATS parameters were assessed with Spearman's correlation coefficients.

Pre-post changes in Classification Scores (ATS) were evaluated with the Wilcoxon test. ATS were compared with TTH using the Mann-Whitney U test. Spearman's correlation coefficients were used to assess correlations between classification Scores (ATS) and PA parameters; for this purpose, a specific database with both pre and post values evaluated as different cases was created.

A p-value<0.05 was considered as statistically significant.

5. RESULTS

5.1. Description of the population

From January 2015 to July 2016, out of 1189 patients treated for CLI, 580 patients were selected for a PA study protocol. The complete PA protocol described for this project was achieved in 332 patients; but not in the rest due to several reasons: popliteal selective injection, hand injection (without using the coupled injector), non-standardized projections, lack of preoperative or postoperative runs or significant differences between them on the projection or excessive movement artifact detectable before performing the PA analysis.

Among cases analyzed with PA, 39 patients were excluded in a second step: 21 patients died with unhealed ulcers and 18 were lost during follow-up. Among the missing cases we suspect three possible reasons: the patient had been managed in another hospital or city (with clinical success or requiring an amputation) or had died, although the latter could not be confirmed through the final data review phone call).

Figure 23 shows an algorithm of the aforementioned exclusions before acquiring the baseline study population (N=293) for specific analysis. Among this population we found 250 cases with ulcers (Rutherford 5 and 6 classifications), where the main endpoint used was the TTH; and 44 other cases without ulcers (Rutherford 3 and 4), compared to the TLRs as the main endpoint.

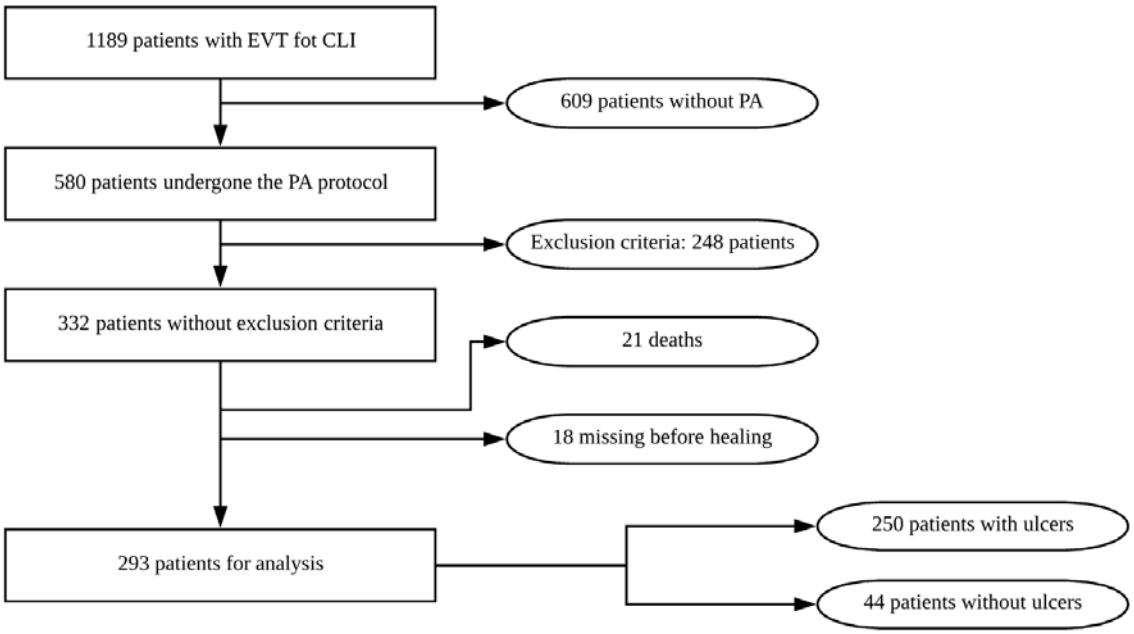

Figure 23. Algorithm of exclusions, from every patient with a PA run and undergoing an EVT to cases fit to be analyzed. CLI: Critical Limb Ischemia; EVT: Endovascular Treatment; PA: Perfusion Angiography.

In the cases where only the post-treatment PA parameters were used for analysis, only one correct post-treatment image was required, obtaining an N=246. On the other hand, when the analysis was to be over the Δ PA parameters, a correct pre-treatment image was also required, thus, the final population was N=222. In each case, as it is shown in figure 24, cases were divided again whether they had ulcers or not.

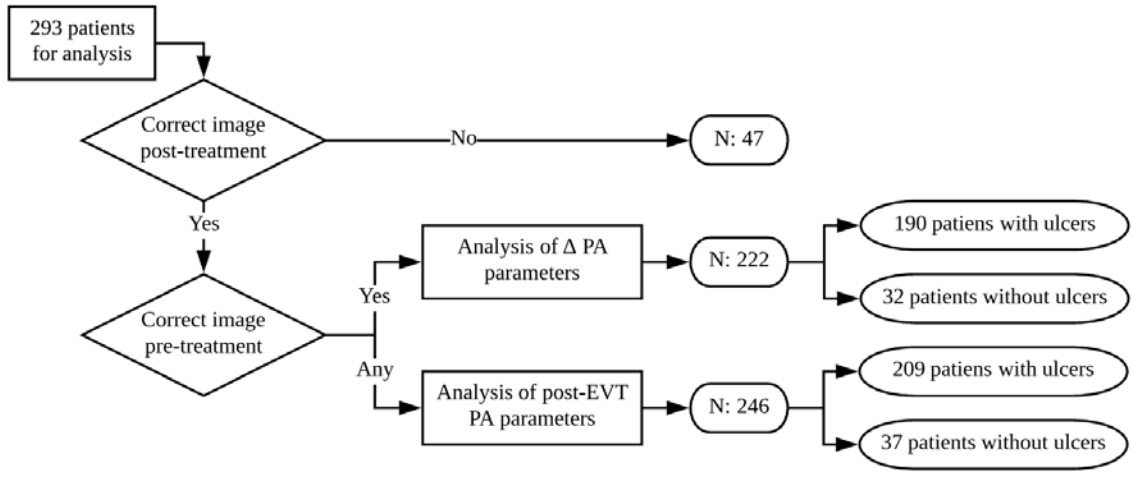

Figure 24. Subgroups for analysis of the perfusion angiography. PA: Perfusion Angiography.

In the baseline study population (N=293), the mean age was 72 years and 67.5% were men. Common comorbidities were diabetes mellitus (DM), hypertension, chronic kidney disease (CKD), end-stage renal disease (ESRD), atrial fibrillation (AF), ischemic cardiomyopathy (ICM), cerebrovascular disease (CVD) and autoimmune diseases (AD). Missing data regarding smoking habits was lacking (information was retrieved in only 20.2% of the cases) and thus the calculated prevalence was assumed not representative. Data on any previous revascularizations (of the limb or the coronary arteries) and minor ipsilateral amputations or major contralateral amputations was also collected. The frequencies of all of them, as well as the antiplatelet, anticoagulation and corticoid therapies, are described in Table 1.

	N=293
Age (years)	72.2 ± 10.4
Male	201 (68.6%)
BMI (Kg/m²)	27.2 ± 4.5
DM	272 (92.8%)
Hypertension	280 (95.6%)
AF	59 (20.1%)
CKD	148 (50.2%)
ESRD	27 (9.2%)
ICM	117 (39.6%)
CVD	81 (27.6%)
Autoimmune disease	18 (6.1%)
Previous ipsilateral EVT	118 (40.3%)
Previous ipsilateral bypass	20 (6.8%)
Minor ipsilateral amputation	82 (28%)
Previous contralateral EVT	96 (32.8%)
Previous contralateral bypass	13 (4.4%)
Major contralateral amputation	13 (4.4%)
Previous PCI	38 (13%)
Previous CABG	48 (16%)
Single antiaggregation	220 (75.1%)
Double antiaggregation	44 (15%)
Anticoagulation	53 (18.1%)
Corticoid therapy	10 (3.4%)

Table 3. Baseline characteristics of cases for analysis. Data are shown as N(%) or mean±SD. AF: Atrial Fibrillation; BMI: body mass index; CABG: coronary artery bypass graft; CKD: Chronic Kidney Disease; CLI: Critical Limb Ischemia; CVD: Cerebro-Vascular Disease; DM: Diabetes Mellitus; ESRD: End-Stage Renal Disease; ICM: Ischemic CardioMyopathy; PCI: percutaneous coronary intervention.

	preEVT	postEVT
Creatininte (g/dL)	1.7 ± 1.5	1.6 ± 1.6
C-reactive Protein (mg/dL)	2.97 ± 5.6	-
Hemoglobine (g/L)	119.4 ± 18.7	119.6 ± 60.8
Leucocytes (*10⁹/L)	8.9 ± 3.1	12.4 ± 6.4
Neutrophiles (*10⁹/L)	5.99 ± 2.8	5.78 ± 2.3

Table 4. Laboratory tests did not reveal any clinically relevant difference due to the EVT. Data are shown as mean ±SD.

	N=293
Intervention time (minutes)	58.2 ± 28
POBA	294 (100%)
Femoropopliteal DCB	21 (7.1%)
BTK DCB	8 (2.7%)
Femoropopliteal BMS	13 (4.4%)
Femoropopliteal DES	12 (4.1%)
BTK DES	2 (0.7%)
Atherectomy	5 (1.7%)

Table 5. EVT variables. Data are shown as N(%) or mean±SD. BMS: bare metal stent; BTK: Below-the-knee; DCB: drug coated balloon; DES: drug eluting stent; POBA: plain old balloon angioplasty.

		N=293
$TCPO_2$		22 ±15.8
TUC	0C	45 (15.3%)
	IC	26 (8.8%)
	ID	2 (0.7%)
	IIC	40 (13.6%)
	IID	16 (5.4%)
	IIIC	27 (9.2%)
	IIID	138 (46.9%)
Rutherford	3	6 (2%)
	4	37 (12.6%)
	5	163 (55.4%)
	6	88 (29.9%)
WIfI stage	Low	71 (24.1%)
	Moderate	42 (14.3%)
	High	181 (61.6%)

Table 6. Baseline clinical stages frequencies. Data are shown as N(%). TCPO2: TransCutaneous Pressure of Oxygen; TUC: Texas Ulcer Classification; WIfI: Wound Ischemia foot Infection.

Laboratory tests and the kind of EVT performed are shown in Tables 2 and 3, respectively. At the baseline clinical stage, Rutherford classification(87), the Texas Ulcer Classification (TUC) (88) and the WIfI clinical stage(1) were annotated and described in Table 4. The plantar arch had been cropped in 11 cases (3.8%) and we found 52 cases (17.7%) with anatomical variations. Proximal arterial occlusive disease was detected in 14 cases (4.8%) and a there was a pre-planned amputation in 170 cases (58%).

5.2. Distribution of the endpoints and PA parameters

As the main endpoint, for the cases with ulcers, we have chosen the TTH and its mean was 75.9 ± 85.9 days and the process of healing of ulcers is described in figure 25. The equivalent endpoint in Rutherford 3 and 4 cases was freedom from TLR, which was observed in 23 out of 37 (62.2%) patients during follow-up months.

The distribution of TASC classifications, ATS scores and WIfI clinical stages(1) are represented in figures 26, 27 and 28, respectively. The subgroup used here was that without exclusion criteria and with a good quality image of PA run after the EVT (N=246).

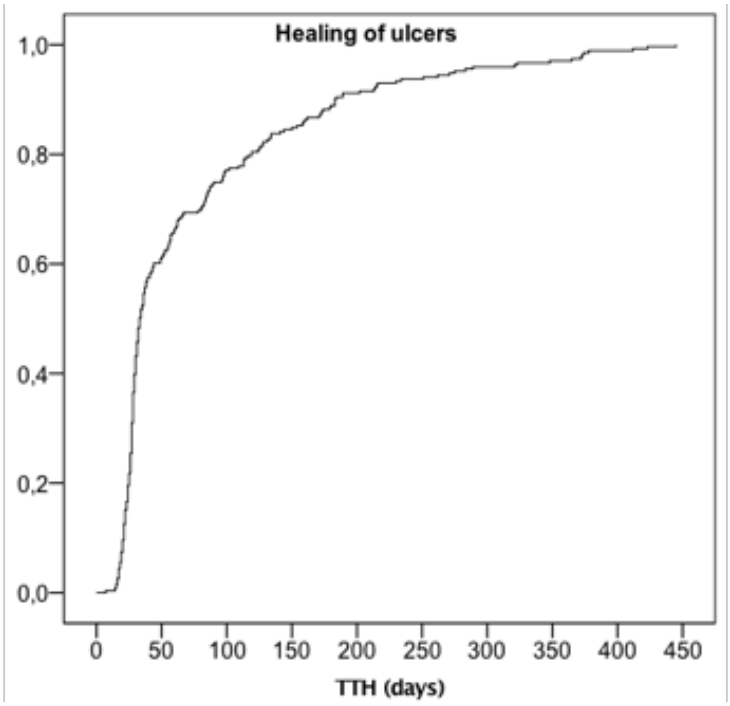

Figure 25. Ulcer healing rate. One minus survival curve for the healing of the ulcers during follow-up. The analyzed data is over the ulcer healing rate at 6 months, which was the 70.6%. TTH: Time to Heal.

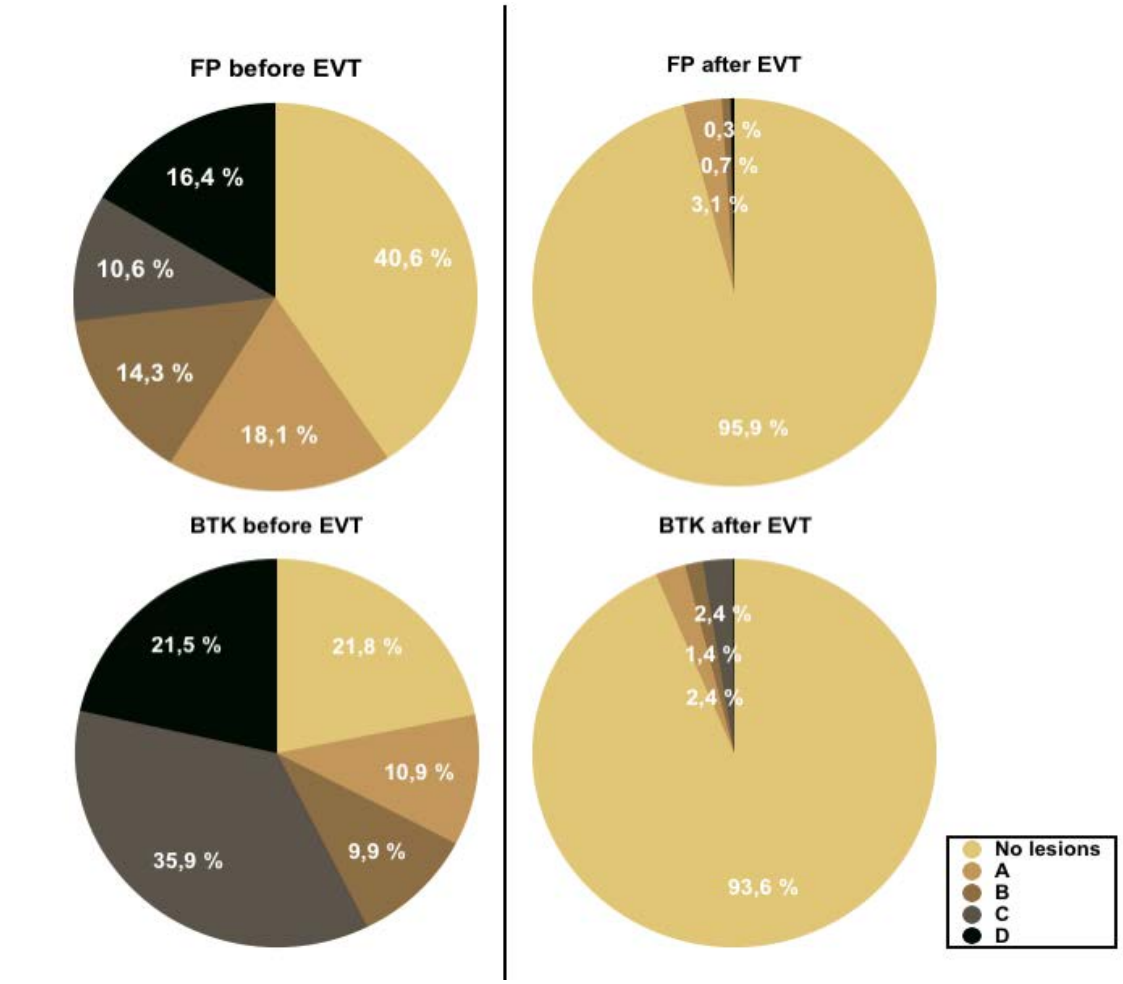

Figure 26. TASC classifications of the before and after treatment angiographies, in the femoropopliteal (FP) and in the below the knee (BTK) regions. EVT: Endovascular Treatment.

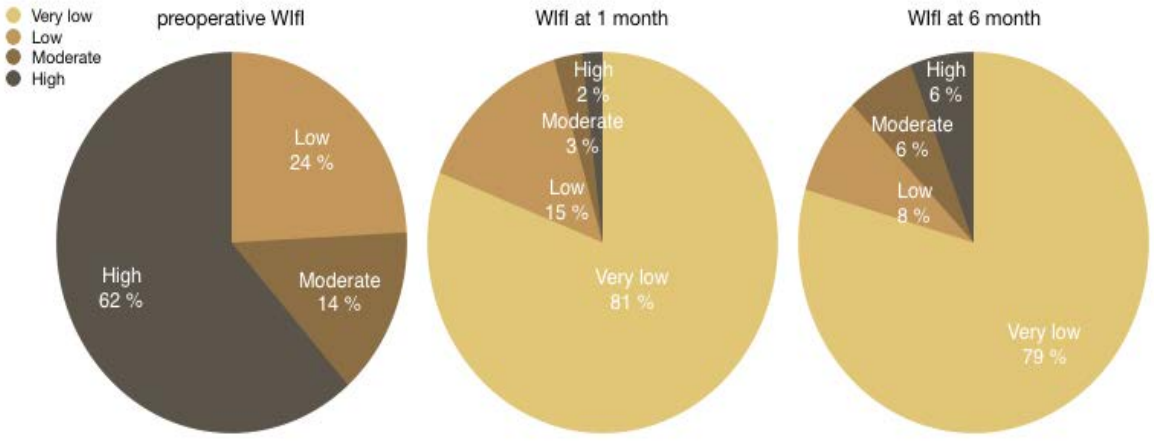

Figure 27. Evolution of WIfI clinical stage preoperatively and during follow-up. WIfI: Wound Ischemia Foot Infection.

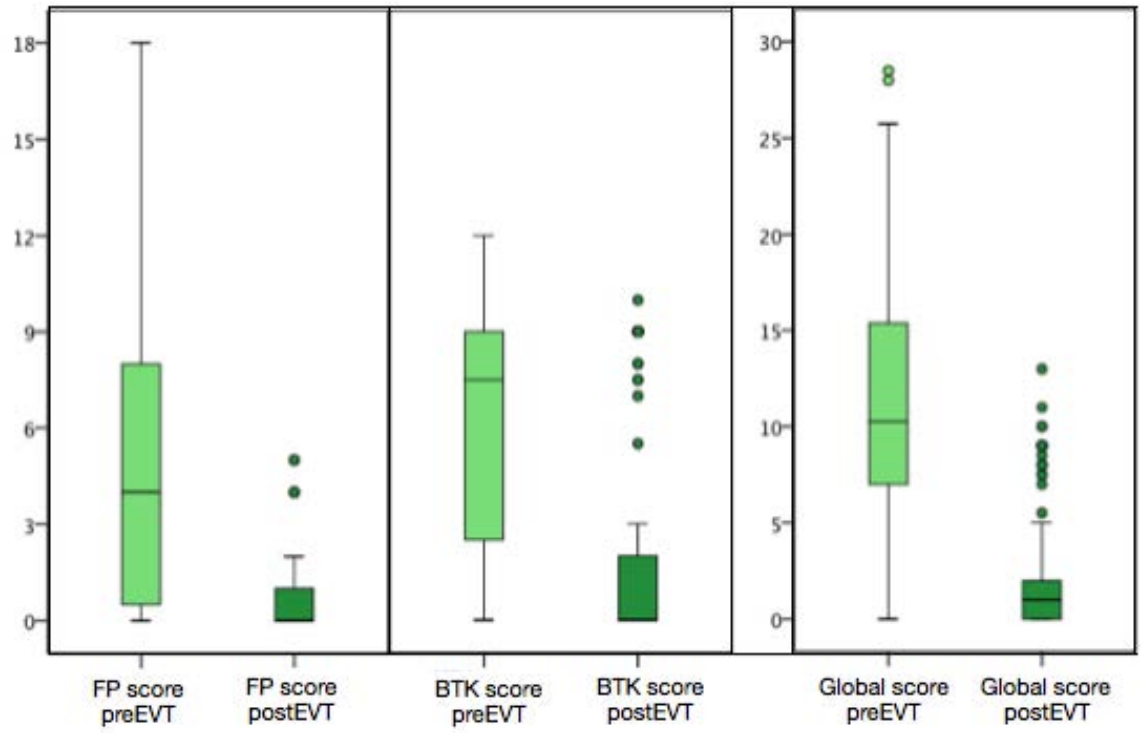

Figure 28. Box-plots of ATSs, either for pre- and post-EVT. ATS: Abano Terme Score; BTK: Below-The-Knee; EVT: Endovascular Treatment; FP: Femoro-popliteal.

		preEVT	postEVT	*p value*
TASC of FP		1.4 ± 1.5	0.1 ± 0.3	<0.001
TASC of BTK		2.2 ± 1.5	0.2 ± 0.7	<0.001
ATS of FP		4.7 ± 4.6	0.3 ± 0.7	<0.001
ATS of BTK		6 ± 3.9	1.09 ± 2.1	<0.001
ATS of the limb		10.6 ± 5.8	1.43 ± 2.3	<0.001
PA parameters (foot)	AT	8.8 ± 3	6.3 ± 2.7	<0.001
	PT	3.7 ± 1.2	4.3 ± 1.3	<0.001
	WS	41 ± 21.9	48 ± 25	<0.001
	W	3.1 ± 0.8	3.5 ± 1	<0.001
	AUC	3622 ± 2398	6455 ± 4948	<0.001
	MTT	3.7 ± 1.1	4.6 ± 1.3	<0.001
	AUC/s	0.3 ± 0.2	0.5 ± 0.4	<0.001
PA parameters (ulcer)	AT	9.1 ± 3.1	6.4 ± 2.8	<0.001
	PT	4.3 ± 3.6	4.8 ± 1.6	0.065
	WS	27.7 ± 24.4	35.7 ± 30.4	<0.001
	W	3.2 ± 0.9	3.8 ± 1.2	<0.001
	AUC	3212 ± 2523	6207 ± 6128	<0.001
	MTT	4.2 ± 1.2	5.1 ± 1.7	<0.001
	AUC/s	0.2 ± 0.2	0.5 ± 0.5	<0.001

Table 7. Changes in TASC, ATS and PA parameters from before to after EVT. Data are shown as mean ± SD. AT: Arrival Time; ATS: Abano Terme Score; AUC: Area Under the Curve; BTK: Below-The-Knee; FP: Femoro-popliteal; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; W: Width; WS: Wash Speed.

The means ± SD of TASC, ATS and PA parameters measured on the foot and at the ulcer are all exposed in Table 7. All the parameters presented statistically meaningful differences through the EVT. The frequencies of PA parameters after EVT in the study population are represented in figure 29a for those parameters measured in seconds, and in figure 29b for those other parameters which have no units. For this picture the group used was also the N=246; as in the previously mentioned classifications. All values measured on the ulcer, except the PT, showed significant differences, meaning that they are measures affected by the EVT revascularization. In Table 6, instead, we can see the changes in ATS and PA parameters through the EVT.

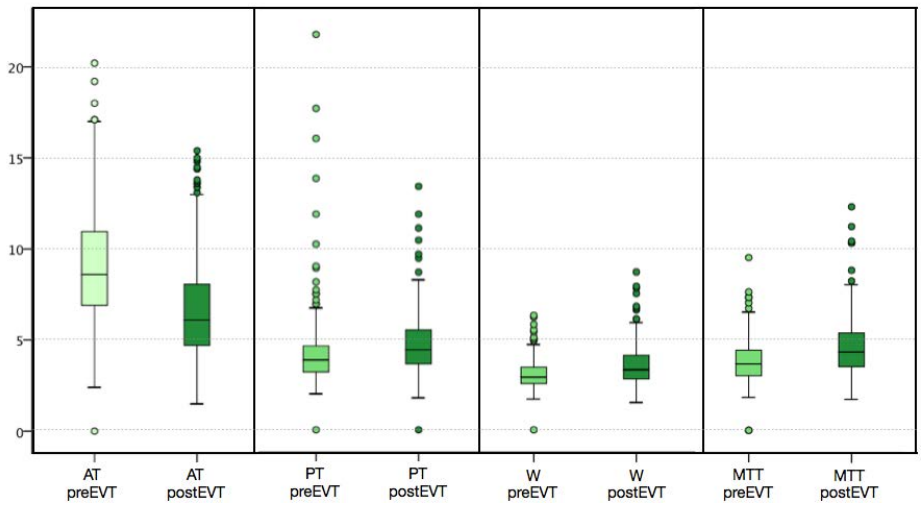

Figure 29a. Box-plots of PA parameters with seconds as units of measure, either pre- and post-EVT runs. AT: Arrival Time; EVT: Endovascular Treatment; MTT: Mean Transit Time; PT: Peak Time; W: Width.

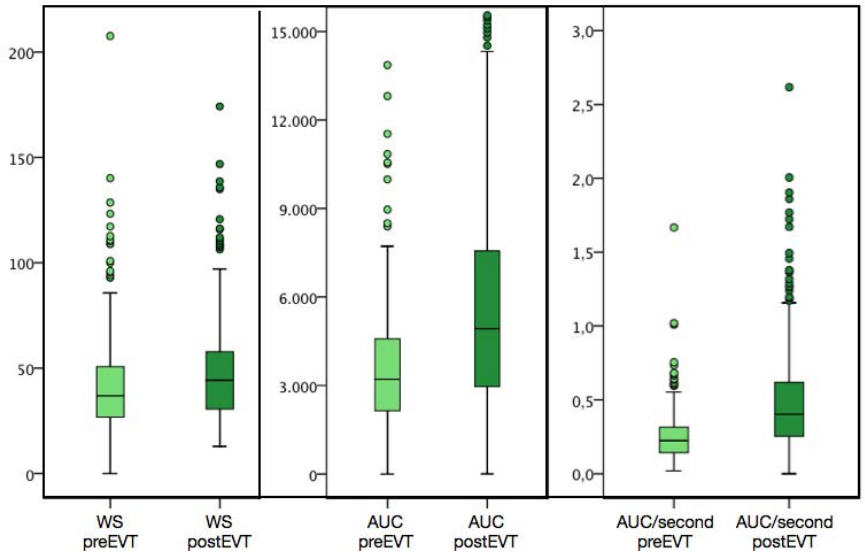

Figure 29b. Box-plots of PA parameters witout units, either pre- and post-EVT runs. AUC: Area Under the Curve; EVT: Endovascular Treatment; WS: Wash Speed.

		N=222
Δ ATS of FP		-4.3 ± 4.5
Δ ATS of BTK		-4.9 ± 3.8
Δ ATS of the limb		-9.2 ± 5.2
PA parameters (foot)	ΔAT	-2.5 ± 3
	ΔPT	0.6 ± 1.5
	ΔWS	7 ± 23.2
	ΔW	0.5 ± 1.1
	ΔAUC	2832 ± 5053
	ΔMTT	0.8 ± 1.5
	ΔAUC/s	0.3 ± 0.4
PA parameters (ulcer)	ΔAT	-2.7 ± 3
	ΔPT	0.5 ± 3.8
	ΔWS	8.1 ± 22
	ΔW	0.6 ± 1.3
	ΔAUC	3011 ± 5607
	ΔMTT	0.9 ± 1.8
	ΔAUC/s	0.3 ± 0.5

Table 8. Distribution of ΔATS and ΔPA parameters. Data are shown as mean±SD. AT: Arrival Time; ATS: Abano Terme Score; AUC: Area Under the Curve; BTK: Below-The-Knee; FP: Femoro-popliteal; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; W: Width; WS: Wash Speed.

	Follow-up at 1 month (N=293)	Follow-up at 6 months (N=293)
Time	31 ± 17	217 ± 69
Missing	14 (4.2%)	4 (1.2%)
$TCPO_2$	49.5 ± 13.3	32.3 ±17.3
Ulcer healing rate	160 (54.6%)	197 (70.6%)
TLR	23 (7.9%)	57 (20.6%)
AFS	293 (100%)	283 (96.6%)
MALE	-	86 (33.6%)
MACE	20 (6.8%)	16 (5.5%)
Death	7 (2.1%)	14 (4.2%)

Table 9. Follow-up events frequencies. The N=332 was used for missings and deaths. Data are shown as N(%) or mean±SD. AFS: Amputation Free Survival; MACE: Major Adverse Cardiovascular Event; MALE: Major Adverse Limb Event; TCPO2: TransCutaneous Pressure of Oxygen; TLR: Target Lesion Revascularization.

5.3. Follow-up

The follow-up events frequencies and means are explained in Table 9. Only the final study MALE rate was collected. A total of 188 (64%) of minor amputations were performed. Due to the low rate of major amputations (3.4%), a Kaplan-Meier curve for the amputation free survival (AFS) is not represented. Among the 21 cases that died, the mean days to the event were 177 ± 167 days.

5.4. Predictive ability of clinical success in patients with rest pain

There were 37 patients with Rutherford 3 and 4 classifications with a good image in the PA post-EVT, the baseline characteristics for this group are exposed in Table 8. Among them we found 11 cases (29.7%) of anatomical variations, while the proportion of anatomical variations in cases with ulcers (17.7%) was lower, although there were no statistical differences (p=0.146). Proximal arterial occlusive disease was present in 2 cases (5.4%). No cases had a previously surgical cropped plantar arch.

	N=37
Age	70.5 ± 10.6
Male	23 (62.2)
BMI (Kg/m²)	27.7 ± 4.8
DM	30 (81.1%)
Hypertension	35 (94.6%)
AF	4 (10.8%)
CKD	14 (37.8%)
ESRD	1 (2.7%)
ICM	12 (33.3%)
CVD	11 (30.6%)
Autoimmune disease	1 (2.7%)
Previous ipsilateral EVT	13 (35.1%)
Previous ipsilateral bypass	3 (8.1%)
Minor ipsilateral amputation	3 (8.1%)
Previous contralateral EVT	13 (35.1%)
Previous contralateral bypass	3 (8.1%)
Major contralateral amputation	4 (10.8%)
Previous PCI	7 (18.9%)
Previous CABG	3 (8.1%)
Single antiaggregation	31 (83.8%)
Double antiaggregation	6 (16.2%)
Anticoagulation	3 (8.1%)
Corticoid therapy	1 (2.7%)

Table 10. Baseline characteristics of the subgroup of patients without ulcers (Rutherford 3 and 4). Data are shown as N(%) or mean±SD. AF: Atrial Fibrillation; BMI: body mass index; CABG: coronary artery bypass graft; CKD: Chronic Kidney Disease; CLI: Critical Limb Ischemia; CVD: Cerebro-Vascular Disease; DM: Diabetes Mellitus; ESRD: End-Stage Renal Disease; ICM: Ischemic CardioMyopathy; PCI: percutaneous coronary intervention.

	TLR	no TLR	*p value*
AT	6.5 ± 2.9	5.7 ± 2.9	0.379
PT	3.8 ± 1	4 ± 1	0.669
WS	45.7 ± 21.2	52.5 ± 26.4	0.420
W	3.3 ± 1.1	3.3 ± 0.8	0.985
AUC	5259 ± 3316	5671 ± 3188	0.709
MTT	4.1 ± 1.1	4.1 ± 1	0.775
AUC/s	0.5 ± 0.2	0.5 ± 0.3	0.751
ΔAT	-2.8 ± 3.4	-2.4 ± 3.5	0.728
ΔPT	0.9 ± 1.4	0.92 ± 1.3	0.976
ΔWS	6.3 ± 23.7	5.5 ± 24.5	0.933
ΔW	0.7 ± 1.2	0.7 ± 1	0.860
ΔAUC	3520 ± 3155	1978 ± 6025	0.420
ΔMTT	1.1 ± 1.5	1 ± 1.3	0.834
ΔAUC/s	0.4 ± 0.2	0.3 ± 0.4	0.485

Table 11. Differences in post-EVT and ΔPA parameters for TLR during follow-up. Data are shown as N(%) or mean±SD. Patients with good post-EVT image were N=37 and patients with good both pre- and post-EVT were N=32. AT, PT, W and MTT are expressed in seconds. AT: Arrival Time; AUC: Area Under the Curve; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; TLR: Target Lesion Revascularization; W: Width; WS: Wash Speed.

	TLR (N=14)	no TLR (N=23)	*p-value*
DM	12 (85.7%)	18 (78.3%)	0.687
Hypertension	13 (92.6%)	22 (95.7%)	1
AF	1 (7.1%)	3 (13%)	1
CKD	5 (35.7%)	9 (39.1%)	0.835
ESRD	0	1 (3%)	1
ICM	5 (35.7%)	7 (30.4%)	1
CVD	6 (42.9%)	5 (21.7%)	0.273
Autoimmune disease	0	1 (3%)	1

Table 12. Comorbidities and possible confounding factors among cases without ulcers and suitable for PA analysis (N=37). The percentages ar in relation with the column N. AF: Atrial Fibrillation; CKD: Chronic Kidney Disease; CVD: Cerebro-Vascular Disease; DM: Diabetes Mellitus; ESRD: End-Stage Renal Disease; ICM: Ischemic CardioMyopathy.

Comorbidities were equally distributed between cases which required a TLR and those that did not, as it is shown in Table 9. Anyway, similar results (exposed in Table 10) came out from exploring the predictive power of PA parameters for requiring a TLR.

5.5 Predictive ability of clinical success in patients with ulcers

The TTH used to divide groups A and B was first calculated at 30 days. Then, when using the post-EVT PA parameters, only the cases with an adequate post EVT image of PA were used (N=209). The baseline characteristics, Rutherford(87) classifications and WIfI clinical stages(1) showed no significant differences between groups (table 11). In a subsequent analysis we took cases with adequate images both before and after EVT (N=190) to use then the increments in the parameters (Δ).

The results of the comparisons between TTH and post-EVT ΔPA parameters are shown in Table 12, where we found significant differences in AT, PT, MTT, ΔPT, ΔW and ΔMTT.

	TTH>30 days (N=136)	TTH<30 days (N=73)	*p-value*
DM	126 (97.3%)	71 (92.6%)	0.172
Hypertension	133 (97.8%)	70 (95.9%)	0.432
AF	27 (19.9%)	15 (20.5%)	0.905
CKD	73 (53.7%)	36 (49.3%)	0.547
ESRD	18 (13.2%)	4 (5.5%)	0.082
ICM	55 (40.4%)	22 (30.1%)	0.203
CVD	40 (29.4%)	15 (20.5%)	0.165
Autoimmune disease	9 (6.6%)	6 (8.2%)	0.680
Surgically cropped plantar arch	9 (6.6%)	2 (2.7%)	0.231
Pre-planned amputation	97 (71.3%)	45 (61.6%)	0.153
Rutherford 5	81 (59.6%)	52 (71.2%)	0.094
Rutherford 6	55 (40.4%)	21 (28.8%)	0.094
WIfI Low	12 (8.8%)	10 (13.7%)	0.547
WIfi Moderate	21 (15.4%)	11 (15.1%)	0.547
WIfI High	103 (75.7%)	52 (71.2%)	0.478

Table 13. Comorbidities and possible confounding factors. Among cases with ulcers and suitable for PA analysis (N=209). WIfI High was compared to non-High and the Low and Moderate ones with other stages particularly. AF: Atrial Fibrillation; CKD: Chronic Kidney Disease; CVD: Cerebro-Vascular Disease; DM: Diabetes Mellitus; ESRD: End-Stage Renal Disease; ICM: Ischemic CardioMyopathy; TTH: Time To Heal; WIfI: Wound Ischemia Foot Infection.

	TTH > 30 days	TTH<30 days	*p value*
AT	6.1 ± 2.6	7.3 ± 3.1	0.004
PT	4.6 ± 1.5	5.1 ± 1.7	0.014
WS	36.9 ± 31.2	31.3 ± 36.2	0.197
W	3.7 ± 1.1	3.9 ± 1.1	0.128
AUC	6210 ± 6549	5724 ± 4815	0.582
MTT	4.9 ± 1.5	5.4 ± 1.7	0.022
AUC/s	0.5 ± 0.6	0.4 ± 0.4	0.113
ΔAT	-2.8 ± 2.7	-2.7 ± 3.5	0.714
ΔPT	0.13 ± 4.5	1.14 ± 1.9	0.009
ΔWS	8.7 ± 22.2	6.6 ± 22.1	0.112
ΔW	0.5 ± 1.2	0.8 ± 1.4	0.037
ΔAUC	3059 ± 6222	2973 ± 4517	0.862
ΔMTT	0.6 ± 1.7	1.2 ± 1.9	0.01
ΔAUC/s	0.3 ± 0.5	0.3 ± 0.3	0.756

Table 14. Differences in post-EVT and ΔPA parameters for TTH at 30 days. Data are shown as mean ± SD. Patients with good post-EVT image (N=209) for post-EVT and good both pre- and post-EVT (N=190). Units of AT, PT, E and MTT are expressed in seconds. Mann-Whitney was used for WS, W and for Δ parameters. AT: Arrival Time; AUC: Area Under the Curve; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; TTH: Time To Heal; W: Width; WS: Wash Speed.

Using ROC curves, represented in figure 30, we retrieved the best sensitivity and specificity cut-off points for post-EVT PA parameters, which are described in Table 13. The ones that showed statistical differences were analyzed with a chi-square test to calculate the sensitivity and specificity of each one, also described in Table 13.

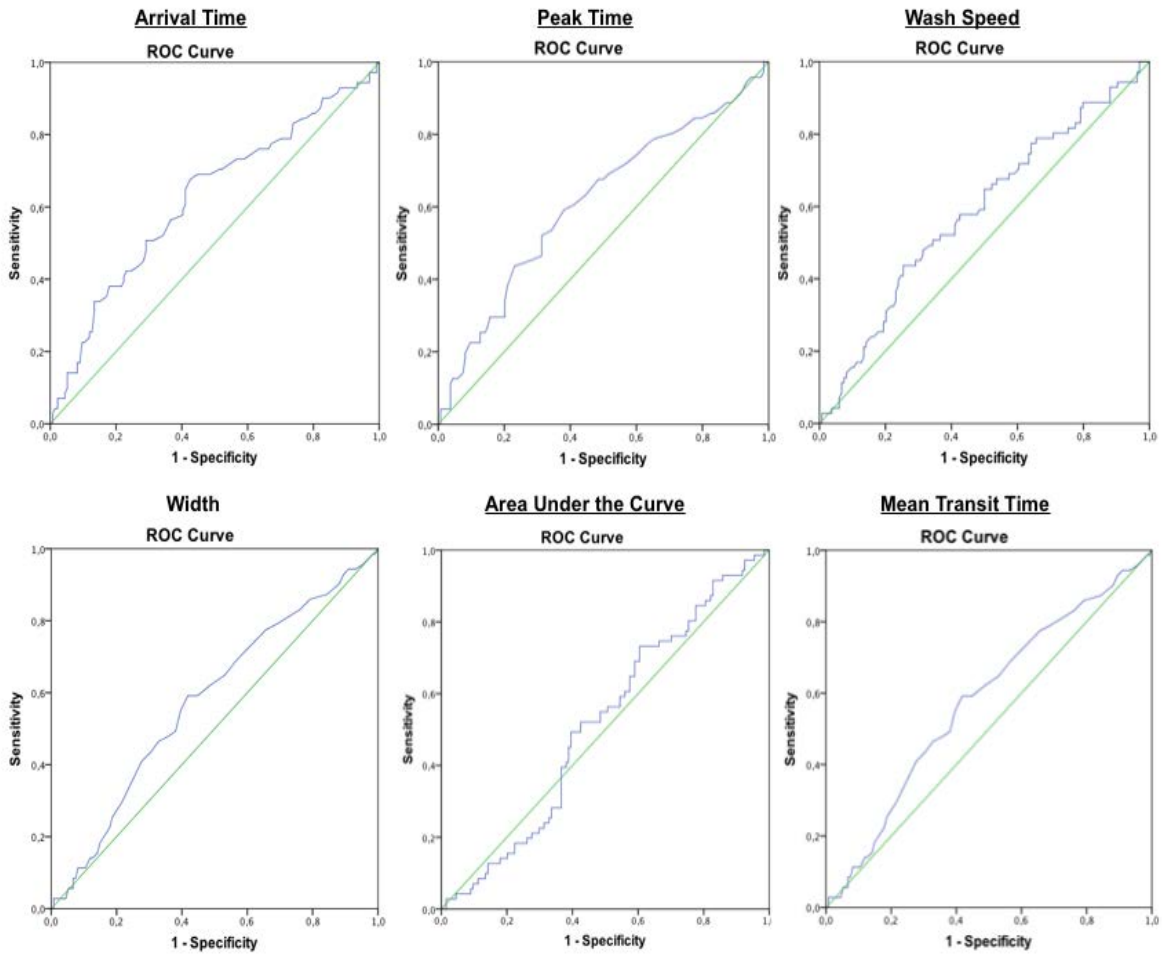

Figure 30. Receiver Operating Curves for the post-EVT PA parameters prediction ability for a TTH<30 days. EVT: Endovascular Treatement; PA: Perfusion Angiography; TTH: Time To Heal.

	TTH>30 days	TTH<30 days	Sensitivity	Specificity	*p value*
AT>6 s	76%	24%	64.8%	59%	0.001
PT>5.2 s	72%	28%	43.7%	76.9%	0.002
WS>20	71.4%	28.6%	56.3%	25.4%	0.007
W>3.6 s	72.9%	27.1%	59.2%	58.2%	0.018
MTT>4.1 s	77%	23%	80.3%	35.1%	0.022
ΔPT>1.5 s	46%	54%	40.9%	80.8%	0.001
ΔW>0.6 s	55.8%	44.2%	57.6%	60%	0.021
ΔMTT>1.7s	44%	56%	42.4%	81.7%	<0.001

Table 15. Chi Square analysis for cut-off points, which were retrieved from ROC curves.

The differences were also explored for the TTH at 40 and 120 days, as shown in Tables 16 and 17 respectively, without observing statistically significant differences.

Finally, figure 31 shows Kaplan-Meier curves comparing the TTH of ulcers with the cut-off points of each PA parameter analyzed.

	TTH>40 days	TTH<40 days	*p value*
AT	6.1 ± 2.7	7 ± 2.9	0.018
PT	4.6 ± 1.5	4.9 ± 1.6	0.126
WS	36.9 ± 30.9	32.9 ± 28.1	0.333
W	3.7 ± 1.1	3.8 ± 1.1	0.5
AUC	6434 ± 7001	5613 ± 4662	0.321
MTT	4.9 ± 1.6	5.2 ± 1.7	0.157
AUC/s	0.6 ± 0.6	0.5 ± 0.4	0.194
ΔAT	-2.9 ± 2.6	-2.6 ± 3.3	0.603
ΔPT	0.5 ± 1.6	0.4 ± 5.3	0.837
ΔWS	9.7 ± 24.1	6.1 ± 19.7	0.046
ΔW	0.5 ± 1.2	0.7 ± 1.4	0.28
ΔAUC	3378 ± 6738	2648 ± 4198	0.381
ΔMTT	0.6 ± 1.6	1 ± 1.9	0.126
ΔAUC/s	0.3 ± 0.6	0.2 ± 0.3	0.187

Table 16. Differences in post-EVT and ΔPA parameters for TTH at 40 days. Data are shown as mean±SD. Patients with good post-EVT image (N=209) for post-EVT and good both pre- and post-EVT (N=190). Units of AT, PR W and MTT are expressed in seconds. AT: Arrival Time; AUC: Area Under the Curve; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; TTH: Time To Heal; W: Width; WS: Wash Speed.

	TTH>120 days	TTH<120 days	*p value*
AT	6.2 ± 2.9	6.6 ± 2.8	0.324
PT	4.8 ± 1.7	4.8 ± 1.5	0.995
WS	41.2 ± 37.5	32 ± 25.4	0.097
W	3.7 ± 1.2	3.7 ± 1.1	0.879
AUC	7381 ± 8218	5487 ± 4715	0.098
MTT	5.1 ± 1.7	5.1 ± 1.6	0.992
AUC/s	0.6 ± 0.7	0.5 ± 0.4	0.076
ΔAT	-2.8 ± 2.5	-2.7 ± 3.2	0.759
ΔPT	0.8 ± 1.7	0.4 ± 4.5	0.519
ΔWS	9.6 ± 28.4	7.3 ± 18.8	0.523
ΔW	0.7 ± 1.2	0.6 ± 1.3	0.606
ΔAUC	4120 ± 7446	2546 ± 4618	0.081
ΔMTT	0.8 ± 1.7	0.8 ± 1.8	0.927
ΔAUC/s	0.4 ± 0.6	0.2 ± 0.4	0.097

Table 17. Differences in post-EVT and ΔPA parameters for TTH at 120 days. Data are shown as mean±SD. Patients with good post-EVT image (N=209) for post-EVT and good both pre- and post-EVT (N=190). Units of AT, PR W and MTT are expressed in seconds. AT: Arrival Time; AUC: Area Under the Curve; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; TTH: Time To Heal; W: Width; WS: Wash Speed.

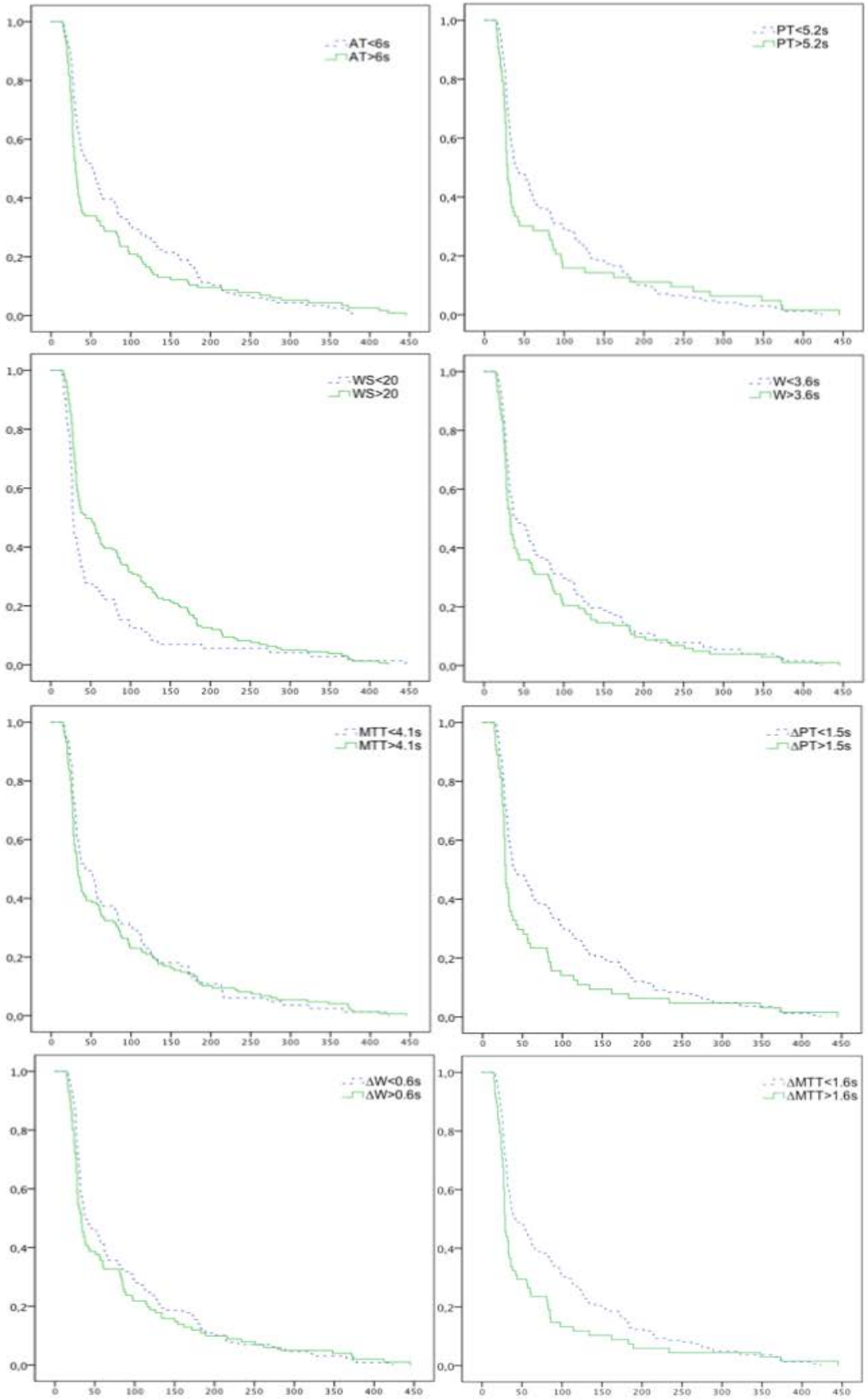

Figure 31. Kaplan-Meier survival curves comparing the TTH of ulcers (days) with the cut-off points of each PA parameter with statistical differences. AT and ΔMTT appear as the better predictors, according to the logistic regression analysis. AT: Arrival Time; AUC: Area Under the Curve; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; TTH: Time To Heal; W: Width; WS: Wash Speed.

5.5.1. Multivariate analysis and confounding factors

The multivariate analysis was made with probable confounding factors such as sex, age, BMI, DM, hypertension, AF, CKD, ESRD, ICM, CVD, autoimmune disease and WIfI stage(1) at baseline. The Odds Ratio (OR) values of the statistically significant cut-off points are shown in Table 18. The cut-off values of the parameters AT>6 s, WS>20, MTT>4.1 s, ΔPT>1.5 s and the ΔMTT>1.7 s maintained the null benefit out of the confidence interval and persisted with statistically significant differences.

After that step, a logistic regression revealed the ΔMTT of more than 1.7 seconds and the AT over 6 seconds to be independent predictors of wound healing. The predicted ability for these two parameters is represented in figure 32, which had an area under the curve of 0.65.

	OR	95% CI	*p value*
AT>6 s	2.64	(1.08-6.42)	0.033
PT>5.2 s	2.42	(0.98-6.02)	0.056
WS>20	0.34	(0.14-0.87)	0.024
W>3.6 s	2.28	(0.95-5.5)	0.067
MTT>4.1 s	3.19	(1.11-9.11)	0.030
ΔPT>1.5 s	3.067	(1.19-7.88)	0.020
ΔW>0.6 s	2.36	(0.96-5.824)	0.062
ΔMTT>1.7s	3.21	(1.23-8.42)	0.017

Table 18. Multivariate analysis with possible confounding factors. Used to select the best predictors of wound healing. AT: Arrival Time; AUC: Area Under the Curve; MTT: Mean Transit Time; PT: Peak Time; TLR: Target Lesion Revascularization; W: Width; WS: Wash Speed.

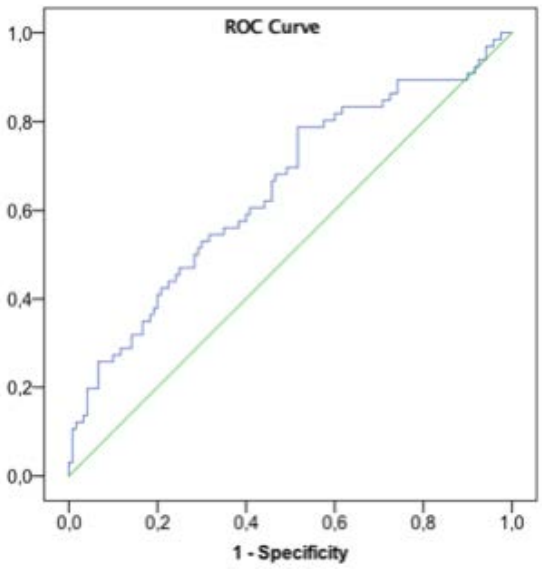

Figure 32. Multivariate ROC curve of independent predictor of wound healing: ΔMTT>1.7s and AT>6s; area under the curve = 0.65. AT: Arrival Time; MTT: Mean Transit Time.

5.6. Correlations of PA parameters with TASC and ATS

Non-statistical associations were found between ATS and the clinical outcomes, measured as a TTH of 30 days. Table 19 shows the results from the student T test for ATS after the EVT and the variation of the ATS.

		TTH>30 days	TTH<30 days	*p-value*
Post EVT ATS	FP	0.4 ± 0.8	0.4 ± 0.8	0.724
	BTK	1 ± 2	1.2 ± 2.3	0.427
	Limb	1.4 ± 2.2	1.6 ± 2.4	0.538
Increments of ATS	FP	-4.1 ± 4.5	-4.1 ± 4.3	0.961
	BTK	-5 ± 3.7	-5.5 ± 3.9	0.415
	Limb	-9.1 ± 5.4	-9.6 ± 4.9	0.529

Table 19. ATS predicting power for wound healing. ATS post-EVT and the increments of ATS are not related to a higher proportion of ulcer healing in 30 days. ATS: Abano Terme Score; BTK: Below-the-knee; EVT: Envovascular Treatment; FP: Femoro-popliteal;

On the other hand, a specific database was built with all the cases with PA runs suitable for analysis (following the described protocol and without artifacts). On this database a N=537 of PA analysis was obtained. In this group, the ATS appeared to be correlated with PA parameters from the spearman's rho test, exposed in Table 20. Although there were significant correlations with TASC classifications, both in FP and BTK regions, the ATS was more strongly correlated and a unique value could be given to the whole limb (which was the best correlated value). Concretely the AT and the AUC/s showed a medium-moderate grade of correlation with the ATS for the whole limb.

	AT	**PT**	**WS**	**W**	**AUC**	**MTT**	**AUC/s**
TASC of FP	0.28	-0.20	-0.18	-0.20	-0.33	-0.23	-0.40
TASC of BTK	0.38	-0.13	-0.14	-0.13	-0.22	-0.16	-0.34
ATS of FP	0.35	-0.12	-0.17	-0.11	-0.25	-0.14	-0.36
ATS of BTK	0.35	0.01*	-0.17	-0.01*	-0.16	-0.04*	0.30
ATS of the limb	0.41	-0.14	-0.21	-0.14	-0.31	-0.18	-0.45

Table 20. Spearman's rho correlation between PA parameters and classification systems: TASC and ATS. *: PT, W and MTT showed a non-significant correlation with ATS of BTK (p>0.05). AT: Arrival Time; ATS: Abano Terme Score; AUC: Area Under the Curve; BTK: Below-the-knee; EVT: Envovascular Treatment; FP: Femoro-popliteal; MTT: Mean Transit Time; PT: Peak Time; W: Width; WS: Wash Speed.

5.7. Non-invasive diagnostic methods

The distribution of the TcPO2 is represented in the histogram of figure 33. Note that due to the retrospective nature of the study only few cases had a TcPO2 measure. The values of the TcPO2 at baseline, one and six months (expressed in mean ± SD) are 22 ± 15.8 mmHg, 50.9 ± 11.9 mmHg and 32.2 ± 17.2 mmHg, respectively.

From the Spearman's rho analysis at baseline no significant associations were found. However, at the 30 days follow-up control of TcPO2 significant correlations were found for AT after the EVT (sr=0.23, p=0.029), the ATS of BTK (sr=0.25, p=0.020) and for the whole limb (sr=0.24, p=0.023); all of which are low-medium degrees of correlation.

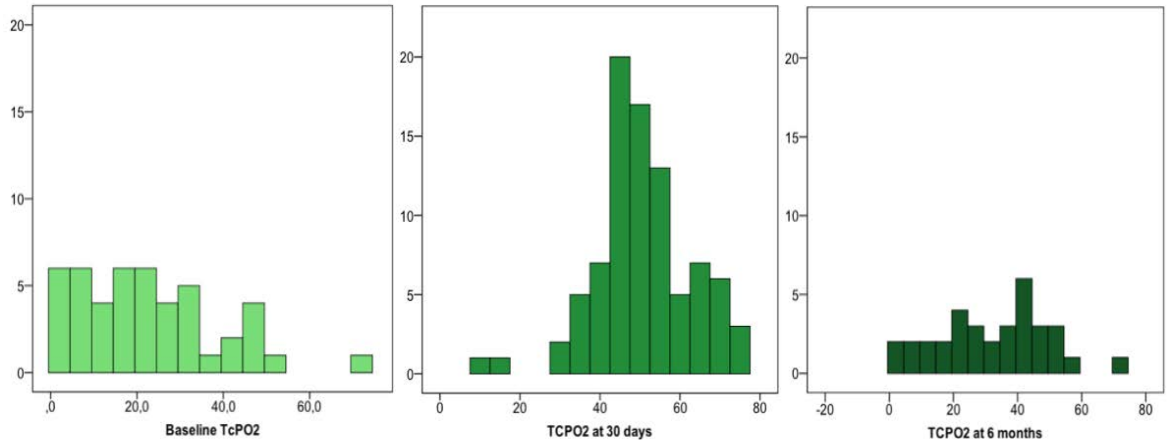

Figure 33. Histograms of TcPO2 measures at baseline (N=46), at 30 days (N=87) and at 6 months (N=34) of follow-up. TcPO2: TransCutaneous Pressure of Oxygen.

5.8. Effect of incomplete plantar arch on PA parameters

The comparison between the PA parameters in patients with a native pedal-plantar arch and those in patients with a previous surgically cropped plantar arch (from a minor amputation) was made with the specific database with both pre- and post-EVT values evaluated as different cases (N=537). We found 25 cases of angiographies with a cropped plantar arch.

As it is shown in Table 21, the parameters that were significantly related with an incomplete plantar arch were those that refer to the slope of the PA curve: the PT (p=0.005), the WS (p=<0.001), the W (p:0.043) and the MTT (p=0.003).

	Native	Cropped	*p-value*
AT	7.8 ± 3.1	6.5 ± 2.4	0.057
PT	4 ± 1.3	4.8 ± 1.7	0.005
WS	44.6 ± 23.1	26.5 ± 11.9	<0.001
W	3.3 ± 1	3.7 ± 1.2	0.043
AUC	5357 ± 7431	4654 ± 4021	0.639
MTT	4.2 ± 1.4	5.1 ± 1.7	0.003
AUC/s	0.4 ± 0.4	0.4 ± 0.2	0.646

Table 21. Relation between the PA parameters and the state of the plantar arch, whether it is in its native state or it is surgically cropped. Data are shown as mean ± SD. AT: Arrival Time; AUC: Area Under the Curve; MTT: Mean Transit Time; PA: Perfusion Angiography; PT: Peak Time; W: Width; WS: Wash Speed.

6. DISCUSSION

The aim of this project is to solve the current challenges that physicians face when performing an EVT in a CLI patient. These are partially solved by the WIfI clinical staging(1), which addresses the doubts centered on the outpatient scenario rather than the operating room uncertainties. Indeed, a diagnostic tool aiding the interventing physician as the WIfI stage helps the outpatient clinic is still lacking. Therefore, PA parameters may play a central role in objectively quantifying the degree of distal blood irrigation to the lower limbs, ultimately simplifying the decision-making process during revascularization based on a threshold value capable of predicting the healing of an ulcer or the avoidance of amputation. Solving the knowledge gap between revascularization and clinical outcomes may simplify revascularization strategies and treatment aggressiveness. Furthermore, a numeric and simpler classification method of the arterial occlusive disease burden in the limb, like the ATS, will allow for comparisons with other cases, increase precision in reports and accurately determine the optimal treatment for each patient. As the WIfI consensus document(1) states, a more precise classification to describe the disease burden is needed to predict outcomes and allow comparisons between patients and therapies.

6.1. Results compared with other perfusion angiography studies

Statistically significant changes were found between EVT results of the TASC, ATS and PA parameters, which supports their validity in the evaluation of distal blood irrigation of the lower limbs.

While no other risk factor or classification stage at baseline predicted a higher healing rate before 30 days, with an AT > 6 seconds after the EVT, the ulcer is 2.64 times more likely to heal in less than 30 days, and if the ΔMTT is > 1.7 seconds, that Odd Ratio is 3.21. The MTT seems to take importance on the difference between before and after EVT, but if the pre-EVT run of the PA is not adequate, the MTT post-EVT over 4.1 seconds should be used, being then 3.19 times more prone to heal before a month. The PT and the ΔPT also showed significant differences which further enforces the validity of MTT as they are measuring almost the same feature on the curve: while the PT measures the time elapsed between the AT and the maximum peak of contrast intensity in the ROI, the MTT measures the time elapsed between the AT and the point of the center of gravity in the time-density curve (see Figure 20 explaining the source of the PA parameters). Otherwise, PA parameters in the revascularization of rest pain patients did not reveal any significant differences regar-

ding clinical outcomes. Not surprisingly, while PA parameters convey information describing local tissue perfusion at the moment of the revascularization, clinical outcomes of EVT in patients with rest pain rely on reintervention rate and limb salvage, which involves the whole hemodynamic status of the limb. However, the observed results in ulcer patients might be applicable as a composite with other factors to patients with rest pain; an alternative that deserves investigation in a larger population of rest pain patients.

There are four main studies exploring the feasibility and the clinical significance of PA, from 2015 to 2017. While three of them explored PA functionalities in the foot as the target organ of the revascularization, describing the injection technique with a long sheath placed at the mid-part of the popliteal artery;(181) the forth one used PA software to explore the effect of certain stenosis measuring only the artery, injecting the contrast medium from the groin(187). Similarly to the latter, Kostrzewa et al.(188) explored flow-limiting stenosis with a different parametric color coding software based on angiography (syngo iFlow, Siemens Healthcare GmbH, Erlangen, Germany). Unfortunately, the differences between the parameters and the processing principles used render any comparison unfeasible. The previous studies used the former version of the software (Interventional Workspot R1.1 or 1.0.1) we employed in our project (Interventional Workspot 1.3.1 / 2D Perfusion 1.1.6). This change in software version impeded the measurement of the peak density value, taken as a main variable in their studies, but considered prone to movement artifacts and subject to variability of tissue composition, anatomy and foot positioning.(189) From 9 to 15 cc of non-ionic iodinated contrast material were injected at a rate of 3 cc/sec (except one in which hand injections were performed(187)), totaling from 300 to 320 mg I /mL (while in our study the total dose was of 270 mg I/mL). These differences should not have induced any bias in the results.

The study population of those studies was of 18(181), 21(187,189) and 89(183) patients. In none of them a follow-up for clinical outcomes was made. Thus, our study is the first to evaluate the clinical outcome of EVT in CLI after PA analysis.

With the use of dedicated footrest for immobilization during PA, the rate of invalid runs due to artifacts was relatively low (ranging 5-14%). The rate of invalid runs in our study, where we did not use more than fixing the foot with a tape, was slightly higher: 16% when using only the completion angiography and 24.2% when both pre- and post-EVT runs were used. However, those exclusion rates did not impede reaching the intended results stated in the study's objectives.

Choosing the proper ROI is matter of debate, and while some groups compare the hindfoot and forefoot regions(189), others suggest that the ROI should include the hindfoot

while excluding the forefoot (as the area distal from the mid-metatarsal region) in order to avoid potential small movement artefacts(181). The technique in itself has been considered to have limitations to describe local ischemia at the site of an ulcer or to quantify local improvement after PTA.(181) In spite of all, knowing the three-dimensional impreciseness when referring to the underlying tissue of an ulcer and assuming more risk to get movement artifact on the run, we thought that the most representative measure of the selective perfusion of an ulcer would be measuring over it, independently of its location in the foot. Our preference is for the antero-posterior or lateral projection depending on the location of the ulcer. Further, the vast majority of ulcers were located at the toes and on the antero-posterior projection the amount of tissue overlapping to the target tissue was negligible.

Previous studies have been controversial regarding the best parameter on which to base the decision-making during the procedure. While Murray et al.(189) only found a significant increase after successful angioplasty in the AUC (29.4%, p=0.03); Reekers et al.(183) described an average increment of 21% for maximal peak density (not measured with our software's version) and 48% for AUC. On the other hand, Hinrichs et al.(187) had correlated the PT pre- and post-intervention with the ABI ($r = -0.53$, $p = 0.0081$) whilst the peak density (PD) and the AUC failed to demonstrate significant correlation with improved ABI.(187) Consistently, our results have effectively shown an increment of the AUC of 2832±5053 ($p<0.001$), but without any prediction power on clinical outcomes. AUC/s, that intended to correct the effect of the possible variability in duration of the angiographic run, did not demonstrate predictive value on ulcer healing. On the contrary, the PT>5.2 s (Sensitivity: 43.7%, Specificity: 76.9%, p:0.002) and the ΔPT>1.5s (Sensitivity: 40.9%, Specificity: 80.8%, p:0.001) showed clinical relevance in our study, as Hinrichs had suggested. However, a ΔMTT>1.7 s (OR: 3.21; 95% CI: 1.23-8,42, p:0.017) and an AT>6 s (OR: 2.64; 95% CI: 1.08-6.42, p:0.033) have appeared in the multivariate analysis as better predicting factors for a TTH<30 days than PT>5.2 s (OR: 2.42; 95% CI: 0.98-6.02, p:0.056) or ΔPT>1.5 s (OR: 3.067; 95% CI: 1.19-7.88, p:0.02).

Another parameter measured by Reekers et al.(183) was the capillary resistance index. We could not measure this parameter in our study because it is calculated from the maximal peak density before and after the administration of tolazoline (a non-selective competitive ∝-adrenergic receptor antagonist).

We agree that intensity related parameters (considered as those parameters measuring the blood volume passing through the foot) are probably measuring both micro- and macrocirculation;(183) but the shape related parameters (PT, MTT, W, WS) are possibly more dependent on the tissue hemodynamics and measuring then the microcirculation.(181) Hereby,

two conclusions can be extracted: they are of no less importance(183) and they are probably more precise since they depend solely on the 90 % of the pixels in the ROI.(183) The latter fact is indeed also applicable to the intensity related parameters, so it is plausible that AT>6s is one of the independent predicting factors for the healing of ulcers. Along the same lines, there is the finding that the ATS (analyzing merely the macro-circulation) appears to be correlated with AT, AUC/s and AUC (sr:0.41, sr:0.45 and sr:0.31, respectively), with a mild-to-moderate degree of correlation maybe due to the fact that they are still based on the 90% measuring of the microcirculation.

Although PA contains microvascular information, it could asses the macrovascular information. The application of the PA technology to the main arteries in order to explore the effect on the big vessels had been explored by Kostrzewa et al.(188) They used a similar technique to the PA (the parameter color coding), which allows to further interpret DSA series and better visualize contrast medium dynamics through a particular stenosis or occlusion. Nevertheless, they could not show a strong correlation between parametric color coding and the clinical parameters of ABI and the ultrasound peak systolic velocity ratio.

6.2. Internal and external validity

The number of deaths and of cases with missing data before healing was not large enough to be a potential source of confounding factors. Even if we considered all missing cases as bad outcomes in healing (as we did in an out of study analysis) the results would be similar in terms of significance and in main outcomes. Regarding the study population, we can disclose that the kind of patients included and treated were the typical CLI patients found in a diabetic foot clinic, patients with high prevalence of DM and multiple comorbidities. Since the center did not have hemodialysis facilities, the prevalence of ESRD in our study population (9.2%) was similar to the general prevalence of ESRD, with the prevalence of CKD stages 3 to 5 in Europe being of 11.86%.(190)

The WIfI classification in the 30-days follow-up wass in the 96% in stages 1 or 2. Subsequently we expected the general behaviour of the clinical predictions on those stages. Thus, the likelihood of wound healing at 1 year was found to be 94.1±2.0% for stage 1 wounds,(21) which is similar to the 96.6% limb salvage found in our study. The same consistance was observed in the validation of Cull et al.,(22) where the WIfI clinical stages 1 and 2 showed to be predictive of 1-year limb amputations of 3% and 10%, respectively.

Another potential factor affecting the results was the particularly aggressive treatment strategy for revascularization undertaken in our study center. The relatively low percentage of stent and drug eluting technologies is a consequence of the bailout strategy for both. They are usually applied on a clearly bad result on a first treatment or in an early failure of EVT with clinical relevance. Comparing our results with the OPG described by Conte et al.(165), our series were significantly better. While the OPG are 71%, 84% and 80% for Amputation Free Survival (AFS), (freedom from amputation and survival at 1 year), our results at 6 months were 92.4%, 96.6% and 95.8%, respectively. The latter comparison is made with the general population OPG, but our study population may better reflect an anatomical high risk (78.2% of patients presenting infrapopliteal lesions in TASC) in which the OPG for the AFS is 68%. Other results on clinical outcomes were reported by Egorova et al.(139), with AFS at 1 year (2007) of 48-81% and the rates of major amputations ranging from 10 to 38% (freedom from amputation of 62-90%), both much higher than the rates in our study.

A limitation that we should considered is that the iodinated contrast agent is a liquid that advances by dilution in the bloodstream and not in phases (like the behavior of a gas, for example). This implies that there will be an overlap between the speed of the blood flow and the speed of dilution of the contrast agent, resulting in a slight shorter arrival speed and a lower wash-in rate than the real blood and oxygen exchange rate. It has been observed in the same way that iodinated contrast agents are not hemodynamically inert when explored on coronary blood flow, they induce a flow reduction followed by a hyperemic response.(191) A last consideration on the properties of the contrast medium on the blood stream, is that during the first pass of the contrast, there is a diffusion into the interstitial space, which may suppose a non-controlled disturbance of the measured contrast density.(191)

6.3. Interpretation of the results, possible confounding factors and clinical translation.

The results may seem paradoxical since larger perfusion times appear to be related to better clinical outcomes. However, all of the PA parameters are the mean value of the curve on each pixel on the ROI and the vast majority of pixels of the ROI are in the tissue rather recording the flow in the big vessels. Therefore, we should shift our mind from the hemodynamics in the large vessels to flow in a later stage in the tissue. In the tissue there are the small vessels – not visible to the human naked eye – that define the shape of the PA curve. As it can be seen in figure 34, the vast majority of vessels in a selected ROI will rely on microcirculation rather than on big arteries that could be identified by conventional

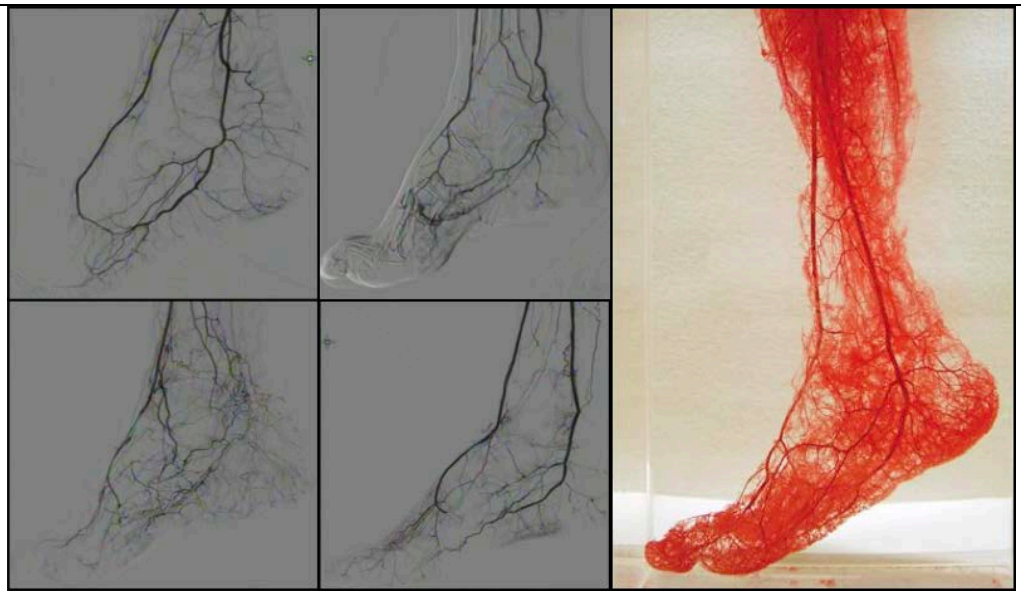

Figure 34. Plastination of microcirculation in the foot. The conventional DSA angiographies (left) of CLI patients after treatment provide good assessments, but are far from showing the complete microcirculatory tree existing after the plantar arch that can be seen on the plastination model (right). CLI: Critical Limb Ischemia; DSA: Digital Subtraction Angiography.

angiography. Subsequently PA, which works with a bidimensional image and tridimensional information, is focused on the study of small vessels with interferences from the big vessels. It is noteworthy that all PA parameters (except the AUC) measure the shape of the curve in terms of time and independently from the absolute contrast density values (at least in the PA software version used in the current study). Thus, it would be intuitively consistent to consider the flow behavior in the foot rather than the total amount of contrast (blood) arriving to the foot.

Although an AT > 6 seconds appeared to predict a higher rate of healing before 30 days, there could exist a certain degree of vasospasm and endothelial dysfunction following the aggression of the EVT. Especially in those technically complex cases that are more prone to bad clinical outcomes, it could be justified (assuming the global improvement after the EVT) to lower the mean AT among the whole study population. Notwithstanding, rather than not acting, this hypothetic effect could event blur a real meaning of an AT>6 seconds. If the degree of vasospasm or the endothelial dysfunction could be analyzed, it could improve the diagnostic power of AT in predicting a good revascularization outcome. Vasospasm and acute endothelial dysfunction should be considered as confounding factors since they would modify the hemodynamics in the foot without occlusive lesions. This could be seen in the worsening

of the PA in some patients with very mild lesions at the beginning of the treatment, as it is shown in figure PA5 (see annexes). Consequently, we could expect some grade of worsening of the readouts just because of vasospasm due to the treatment itself. This fact could be a reason why the Δ parameters are not the most exact measures, and consequently the post-treatment parameter shall become the more useful one.

6.4. Comparison with other diagnostic methods

As suggested by Venermo et al.,(93) all diagnostic methods have their own strengths and pitfalls. The clinician should then combine several methods to get the better information about the patient prognostic and potential improvements of revascularization. An ideal diagnostic method to serve as an intraoperative decision making should (1) be able to predict clinical outcomes; (2) have no limitations due to calcifications; (3) non-invasive or not requiring extra-intervention; and, (4) able to be performed during revascularization to assist the peri-procedure decision making. Additionally, it should: (5) be reproducible; (6) easy to do during the practice; (7) provide anatomical data; and, (8) provide histological tissue information. Extending on the two latter properties, an ideal test should also be able to measure separately the macrovascular and the microvascular disease and identify the location of the culprit of the disease; or at least, even if a test would be sensible to both vascular beds simultaneously, it should allow the identification the site of the effect measured. The histological information is referred to the distribution of the perfusion to the ulcers or towards the specific regions of tissue that we intend to treat. In table 22 we summarized the existing diagnostic methods with an approach to describe the main attributes above mentioned. Among all diagnostic methods, the PA appears to be a powerful tool, despite the availability of the technology is not still widespread. Lack of access is a common situation for all novel technologies while they are standardized and clinically tested.

The pressure-related quantitative diagnostic methods (ABI, AP and TP) present the inconvenience of a high variability and lack of measurement precision becoming not very enlightening for follow-up analysis, but they perform well for screening and for an initial diagnosis. In addition, they could not assist during procedures due to the prolonged time needed for a measure and the lack of histological or precise angiosome related improvement of EVT. Despite TP pressure overcomes the limitation of the arterial wall stiffness due to calcifications and its clinical significance is well-known, it still does not give objective information about the perfusion to a concrete ulcer. On the other hand, the pressure-related qualitative methods as PVRs and PPG do not differ from the conventional angiography in the

sense of being roughly estimated subjective values, and are not suitable for intraprocedural assessment. Thus, they do not afford a threshold value, nor comparisons between patients or interventions (inter-observer and intra-observer variations).

TcPO2 has two main drawbacks, the need of a temperature-controlled environment and the time-consuming nature of the test. However, it is a well-known and clinically proven test with a clear threshold of 25 mmHg with high sensitivity (85%) and specificity (92%) for ulcers to heal. The PA parameters did not result in better predicting power, since the AT>6 seconds and the ΔMTT>1.7 seconds showed a sensitivity of 64.8% and 42.4%, respectively; while their specificities were 59% and 81.7%. However, the TcPO2 cannot be used during procedures nor distinguish among different angiosomes or specific histological measures. This latter issue has been solved by the tissue oxygen saturation mapping, which is able to measure quickly (only 10 seconds/point) the oxygen saturation through all the tissue. It is then a really promising method with the theoretical ability of being sensible to detect vasospasm and endothelial dysfunction, but it is still clinically unproven. Last but not least, the mild correlation between TcPO2 and AT (sr=0.23, p=0.029) could indicate that both methods are not measuring exactly the same pathophysiological process. Otherwise, if it were the case that they both measure perfusion, the two methods will have to be applied at different times (intraprocedural for AT and ambulatory for TcPO2). In the latter hypothesis, the lower sensitivity and specificity of the PA could be explained by a subgroup of patients that having had the same angiographic result, an eventual greater post-procedure endothelial dysfunction did not lead to a good clinical result as the angiographic result could predict.

Based on a similar principle of the TcPO2 mapping, there are several methods able to assess the skin perfusion: Hyperspectral imaging (HI), O2C, MOXYs and ICG-FI. None of them has any large clinical follow-up study testing patient-centered clinical outcomes. Only HI has showed predicting potential for ulcer healing (sensitivity=80% and PPV=90%), as Nouvong et al.(106) reported. Comparatively, those are better results than those obtained by TcPO2 and PA, and further HI could be applied during the procedure. However, HI is sensibly limited by common factors such as deep tissue ischemia or infection (osteomyelitis), wounds, scars or callus, because it only measures surface (1 cm) perfusion. Nonetheless, it could have its own meaningful use in the ischemic assessment and procedure decision making; particularly in prevention and follow-up.

Another similar measuring method is the O2C, able to measure not only the oxygen saturation until 8 mm, but also hemoglobin and the blood flow, and it could be used during procedures. However, the only clinical data available with from this technique aimed to predict the prognosis of amputation levels.

MOXYs could be hard to standardize due to its invasiveness, incompatibility with infection or ulcers, and the thin surface able to be measured (from 2 to 4 mm). There are no studies measuring clinical outcomes although it is sensible to revascularization.

To end with surface perfusion measuring methods there is the ICG-FI. Despite the exploration lasts 5 minutes and that it needs extra intervention procedures, it requires special environment and temperature conditions. This method showed strong correlations with TP and TcPO2, but clinical follow-up data is not yet available.

As of yet, none of the above-mentioned methods gives actual anatomical information. The linkage between ulcer perfusion and the anatomical distribution of steno-occlusions is partially solved by the angiosome theory. Unfortunately, no method could give any more personalized assessment than attaching to the theoretical distribution of the angiosomes without the presence of anatomical variations of the tibial arteries. The PA measurement, however, is based on the ROI traced just adapted to the target tissue (ulcer). Furthermore, the PA will always be associated with a conventional angiography which does have the anatomical information of the steno-occlusions in the limb. The clue then is to be able to categorize the subjective information about the lesion's distribution contained in the angiography, such as it is the aim of the ATS (despite that no strong correlation was found with TcPO2 or PA). In that case we could be able to compare patients and set an anatomical revascularization threshold.

The other diagnostic methods containing anatomical information are DUS, MSOT (multispectral optoacustic tomography), CTA and MRI angiography; with just the latter being able to measure the tissue perfusion (but without any clinical outcomes data). All of them, specially the three last ones, are confined to the treatment planning period because they cannot be performed during the EVT. DUS on the other hand could be performed periprocedurally, it is highly correlated with angiography and gives real hemodynamic information; however, no correlation with the microcirculation state or the clinical long-term outcomes are known. Furthermore, it is very time-consuming and operator dependent, which becomes a problem to compare among patients. A new modality of ultrasound imaging is the MSOT, and while it could give a trustworthy vasculature picture of 14 mm-depth, no clinical or feasibility studies are yet available.

Considering the big picture among current diagnostic methods, the results obtained and the clinical applicability of PA, we constructed an algorithm (figure 35) to integrate this information and make it applicable into routine clinical practice. A particular consideration, when the described threshold values of PA are not reached, is that it relays on the clinical judgement of the attending physician. In the case that the intended procedure for revascularization is achieved, the viability of the limb should be first considered, or the patient

	Able to predict clinical outcomes	*Limitations due to calcifications*	*Invasive*	*Peri-procedural*	*min/measure*	*Anatomical information*	*Histological information*
ABI	+	++	**No**	**No**	**10**	**No**	**No**
AP	+	++	**No**	**No**	**10**	**No**	**No**
PVRs	-	-	**No**	**No**	**10**	**No**	**No**
PPG	-	-	**No**	**No**	**10**	**No**	**No**
TP	++	-	**No**	**No**	**10**	**No**	**No**
TcPO2	++	-	**No**	**No**	**20-40**	**No**	**No**
TcPO2 mapping	**N/A**	-	**No**	**N/A**	**<5**	**No**	**Yes**
Hyperspectral imaging	++	-	**No**	**Yes**	**<5**	**No**	**Yes**
O2C	**N/A**	-	**No**	**No**	**N/A**	**No**	**Yes**
MOXYs	**N/A**	-	**Yes**	**Yes**	**N/A**	**No**	**No**
ICG-FI	**N/A**	-	**No**	**No**	**15**	**No**	**Yes**
PA	++	-	**No**	**Yes**	**<5**	**No**	**Yes**
DUS	**N/A**	+	**No**	**Yes**	**30-40**	**Yes**	**Yes**
MSOT	**N/A**	**N/A**	**No**	**No**	**N/A**	**Yes**	**Yes**
CT angiography	**N/A**	++	**Yes**	**No**	**20**	**Yes**	**No**
MRI angiography	**N/A**	++	**No**	**No**	**30-60**	**Yes**	**Yes**

Table 22. Compared diagnostic methods. AP: ankle pressure; DUS: duplex ultrasound; ICG-FI: indocyanine-green fluorescence imaging; MOXYs: micro-oxygen sensors; MSOT: multispectral optoacustic tomography; O2C: oxygen-2C; PA: perfusion angiography; PPG: photopletysmography; PVRs: pulse volume recordings; TP: toe pressure.

should be rescheduled for a reintervention, even applying vasodilators as a bailout therapy. On the other hand, if the intended procedure for the EVT could not be achieved, it would depend on technical issues of the procedure to decide rescheduling the patient or reconsidering the risk of a reintervention over the one of an above the ankle amputation.

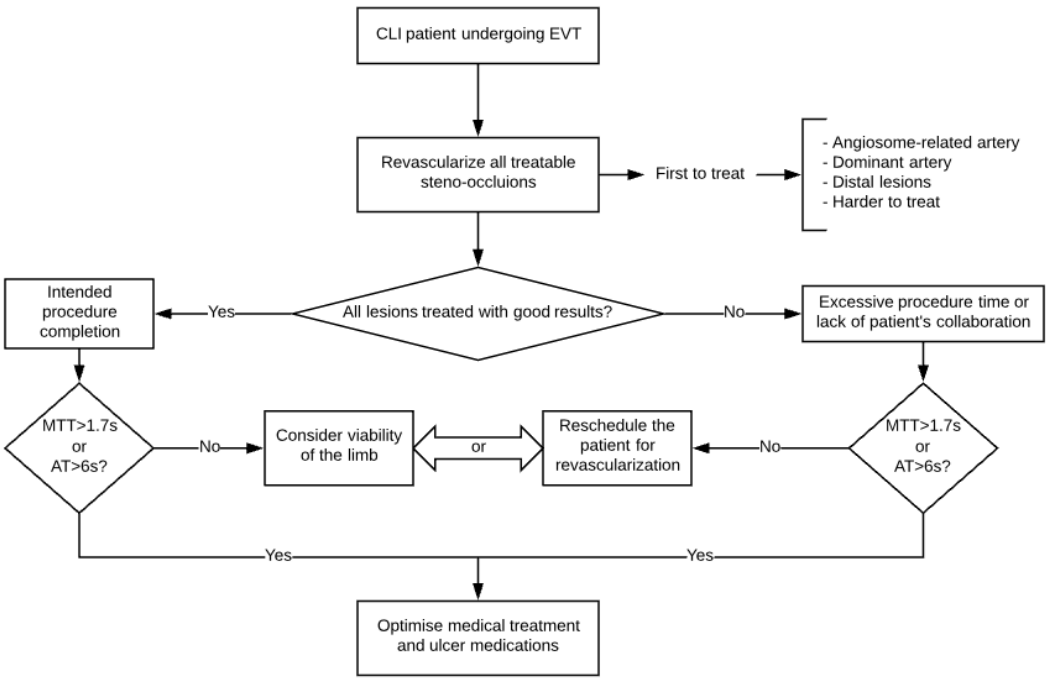

Figure 35. Decision algorithm to apply PA information during the intervention. AT: Arrival Time; CLI: Critical Limb Ischemia; EVT: Endovascular Treatment; MTT: Mean Transit Time; PA: Perfusion angiography.

6.5. Secondary results

6.5.1. Disease burden anatomical classification.

The current most used classification to describe the steno-occlusive lesions in PAD is the TASC, but it has several pitfalls. To begin with, it is not easy to remember, its aim is focused on the election of the revascularization technique (open surgery or endovascular) rather than the hemodynamic description of a limb; furthermore, the classification does not raise a single value for the whole limb. Concretely in the BTK TASC classification, the concept of the target vessel is ambiguous. Beside the lesions of the other arteries, the target vessel in the current study had been chosen as the angiosome related vessel, since this is the vessel with the major interest to treat. Nevertheless, anatomical variations should be considered and in patients with rest pain the target tibial will be the one with less severe lesions. In the post-treatment angiography, the target vessel had been chosen as the one with

the best angiographic result. Therefore, it is hard to clearly determine a case to a BTK TASC C or D, in the sense of the amount of calcium needed to consider a D classification. With the herein described context, without any better performance of other systems like Bollinger's(192) or Graziani's(193,194) scores, a simpler anatomic classification could be really helpful to describe, compare and even set a revascularization goal. Better correlation with the clinical outcome was demonstrated by Bargellini et al.(194), who compared JVSC foot and calf scores with TASC and Graziani's classification and found that only the JVSC foot scores (but not the calf score) where related to better healing time; both with lower preprocedural (mean score, 7 in healed patients and 7.8 in non-healed patients; $p = .049$) and postprocedural (mean score, 5.5 in healed patients and 6.3 in nonhealed patients; $p = .047$). Given that the territories that describe the ATS and the JVSC foot score are in fact complementary and both systems are numerical, this could suppose an advantage for its clinical application.

Ideally the classification to be used should be easy to remember and apply, based on hemodynamic criteria, avoiding as much as possible arbitrariness in the definition and with a certain degree of clinical relevance. This has been the aim of the herein described Abano Terme Score (ATS). ATS appeared to be an easy to describe and to remember score system that is moderately correlated with the AT and the AUC/s when applying the ATS for the whole limb. Those were a Spearman rho correlation of 0.41 for the AT and -0.45 for the AUC/s. Although they are not excellent, they are better than the TASC classification. Furthermore, although it has not been related to the primary endpoint of this study (TTH<30 days), the ATS is actually sensible to the EVT. The ATS mean score pre-EVT was 10.6±5.8 and post-EVT was 1.43±2.3 ($p<0.001$). A good point of the ATS is that it would be simple and always applicable to every patient undergoing a conventional angiography, without requiring any extra intervention or technology use.

Recently though, an expert global panel as part of the Global Vascular Guidelines on Chronic Limb Threatening Ischaemia designed the GLASS score. GLASS is part of the Patient, Limb, Anatomy paradigm presented in the Global Vascular Guidelines. GLASS involves choosing the target artery pathway for endovascular revascularization from the origin of the SFA (common and deep femoral disease are considered part of inflow and considered corrected before more distal revascularization) to the foot with the aim of establishing inline flow. Disease severity in FP and infrapopliteal (IP) segments are graded separately on features including duration of disease, stenosis or occlusion, and level of calcification. FP and IP grades are combined within a matrix to determine GLASS stage. The GLASS stage is believed to likely correlate with endo-vascular immediate technical

success rates and 12-month limb-based patency. GLASS has been presented at several congresses and symposia (European Society of Vascular Surgery, Lyon, France in 2017, the Society of Vascular Surgery VAM in San Diego in 2017, and in Boston in 2018, and in the Charing Cross Symposium in London in 2018). In a recent study Kodama et al.(18) demonstrated the GLASS can predict technical failure, AFS and major adverse limb events (MALE). However, only the baseline angiography was used, so no treatment endpoint could be extracted from those studies. On the other hand, the GLASS feasibility study,(32) showed inter-observer variation in applying the classification system. Further trials and investigations should be done to select the most powerful score and which one is more relevant to clinical daily practice.

For more information see section 11.5 about the submitted article to the European Journal of Vascular & Endovascular Surgery: Novel Abano Terme Scores and its application in the treatment of critical limb threatening ischemia.

6.5.2. TcPO2 correlation with perfusion angiography

The decrease of the TCPO2 at the 6-months follow-up from 50.9±11.9 mmHg to 32.2±17.2 mmHg (just slightly higher mean than the one before the revascularization) seems to indicate an important restenosis rate. Nonetheless, this has no clinical implications nor could be compared to the Objective Performance Goals (OPG) defined by Conte et al.(159) because it is not directly a stenosis nor an occlusion. The TCPO2 showed a mild but statistical correlation with the AT (sr=0.23, p=0.029), which seems consistently indicate that both tests measure the oxygen arrival to the tissues. Additionally, the TCPO2 was correlated with the ATS for BTK (sr=0.25, p=0.020) and for the whole limb (sr=0.24, p=0.023).

6.5.3. Effects of infra-popliteal anatomical variations

There were no significant associations between the presence of an anatomical variation in tibial arteries and rest pain clinical presentation. However, since a tendency for this association had been seen (17.7% among the patients with ulcers and 29.7% in those with rest pain, p= 0.146), it worth giving an extra attention to the possibility of an anatomical variation when treating a patient with rest pain. A possible explanation for this is the fact that an anatomical variation usually implies the existence of an hypoplasic artery. Subsequently, the remaining two arteries are responsible for a higher proportion of whole foot perfusions; being one over two instead of one over three arteries. In this situation, a steno-occlusion in one infra-popliteal artery will affect one half rather than one third of the blood supply, which

supposes a more severe ischemia present in the rest pain. Finally, there is a higher risk of failure for revascularization when an anatomical variation is not well noticed before revascularization. It is striking that we detected significantly more anatomical variations than those described in the literature, where the proportion is from 9 to 12 %. It is not easy though to precisely describe anatomical variations over occluded and extremely diseased arteries.

6.5.4. Effects of a cropped plantar arch

A special consideration of the application of the PA should be made for patients with a previous cropped plantar arch. In those patients PT, WS, W and MTT appeared to be statistically different. This endorses the hemodynamic main importance of the plantar arch patency for the tissue perfusion in the foot.

6.6. Limitations and further possibilities

We have had a lack of standardization of the projections and the PA protocol in the study, since the objective was to explore the technique for daily practice. The same problem has occurred with the proportion of missing cases during follow-up. Over those data we made exploratory statistics to test the PA predicting value of clinical results after EVT. This approach could sometimes produce random findings.(195)

Due to the retrospective nature of the data collection, the vast majority of patients have not clearly recorded the active or former smoking habits. Thus, the lack of that potentially significant information could be hiding another confusion factor.

Another limitation is that the follow-up only lasted 6 months, while the suggested duration for follow-up of CLI revascularizations is 1 year;(1) however in our study, the effects of revascularization were studied in a shorter period of time (30 days).

A particular characteristic for our studied population is the divergence in the patient centered outcomes, which are significantly better than those described in other large series and RCTs. It would be probably useful to compare the results in this study with other less aggressively treated patients, and compare the significance of the PA among patients in all stages of disease burden and degree of treatment.

From a conceptual point of view, we should understand the differences between the blood flow through the big arteries, as those in which we could do a balloon dilatation (dorsalis pedis, lateral plantar, plantar arch, lateral tarsal, etc), and in the small arteries, whose blood flow is affected by MAC. It is at tissue level where the oxygen and the nutrients carried in the blood are ultimately needed. With this picture in mind we can easily imagine that every

diagnostic method will be better exploring one or another step-in perfusion, as the complete process of oxygen and nutrients delivered to the tissues. The real implication in CLI clinical outcomes of Mönckeberg's disease and the management of SHPT with phosphate binders, active vitamin D analogs and Ca^{2+} mimetics should be explored. In other words, the management through other strategies rather than the interventional one should be investigated, such as the use of novel specific drugs or cellular therapies to improve the microvascular circulation and ulcer tissue angiogenesis.

It would be really useful to compare the ATS with other CLI endpoints (like limb salvage or freedom from TLR) and other well-known diagnostic methods like TcPO2 and the duplex ultrasound. Probably a global limb threshold could predict limb salvage and the ATS for BTK could predict outcomes after a femoropopliteal bypass (as a bypass outflow quantification). The ATS is simple to describe and it is easy to obtain a numeric value to the PAD disease burden in the limb; both considering the limb entirely or above- or below-the-knee separately.

The PA software itself could also be substantially improved by making a software not distortable by movement, or being able to make an automated selection over the course of a single vessel for specific measures. It could even be equipped with machine learning processing to develop specific treatment recommendations and with predictions of tissue perfusion improvement.

7. CONCLUSIONS

The conclusions of this thesis are:

1. The PA parameters are sensible to revascularization of CLI patients and to the real perfusion of the foot.

2. The threshold values of PA parameters to predict clinical improvement are:

   2a. The best predicting factors for the healing of an ulcer in less than 30 days were AT>6 seconds in the post-treatment PA run and presenting an ΔMTT > 1.7 seconds (or an MTT > 4.1 seconds in the post-treatment run).

   2b. No significative translation from perfusion angiography parameters to clinical outcomes (freedom from reintervention) were seen in Rutherford 3-4 patients.

3. A classification hereby called “Abano Terme Score” appeared to be simple and useful for clinical practice, being mild-moderately correlated not only with AT, AUC and AUC/s, but also with the TcPO2, which also showed the same strength correlation with AT.

4. The arrival time, as the parameter of the perfusion angiography, showed a mild but statistical correlation with the TCPO2.

5. The parameters related with the perfusion curve shape (PT, WS, W and MTT) were significantly related with the presence of an incomplete plantar arch.

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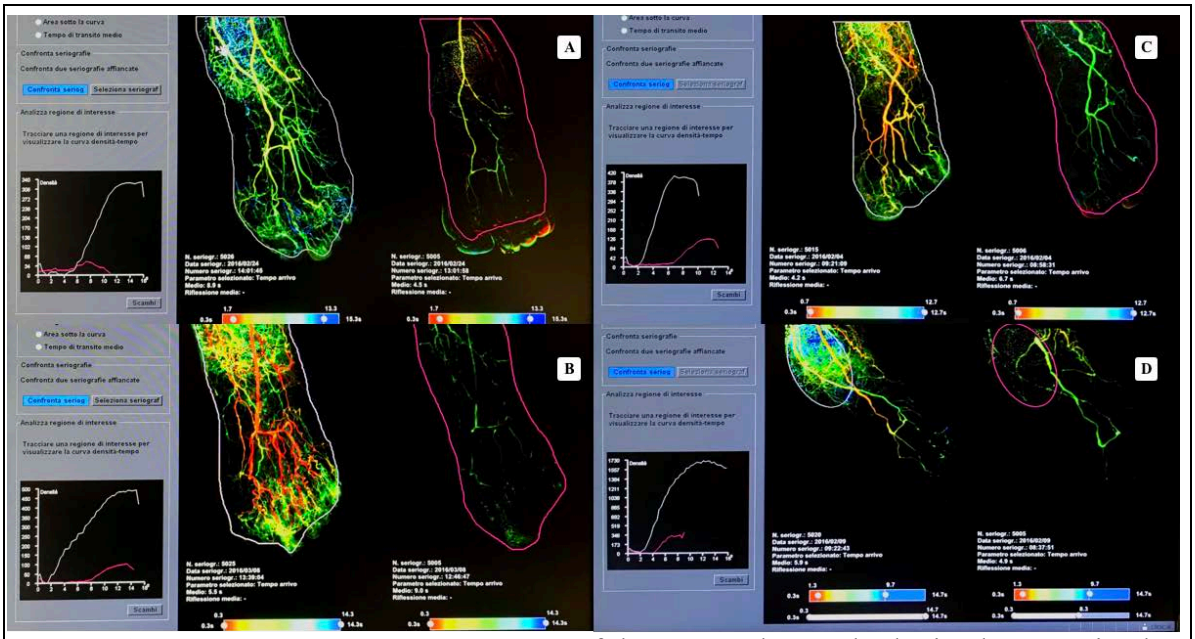
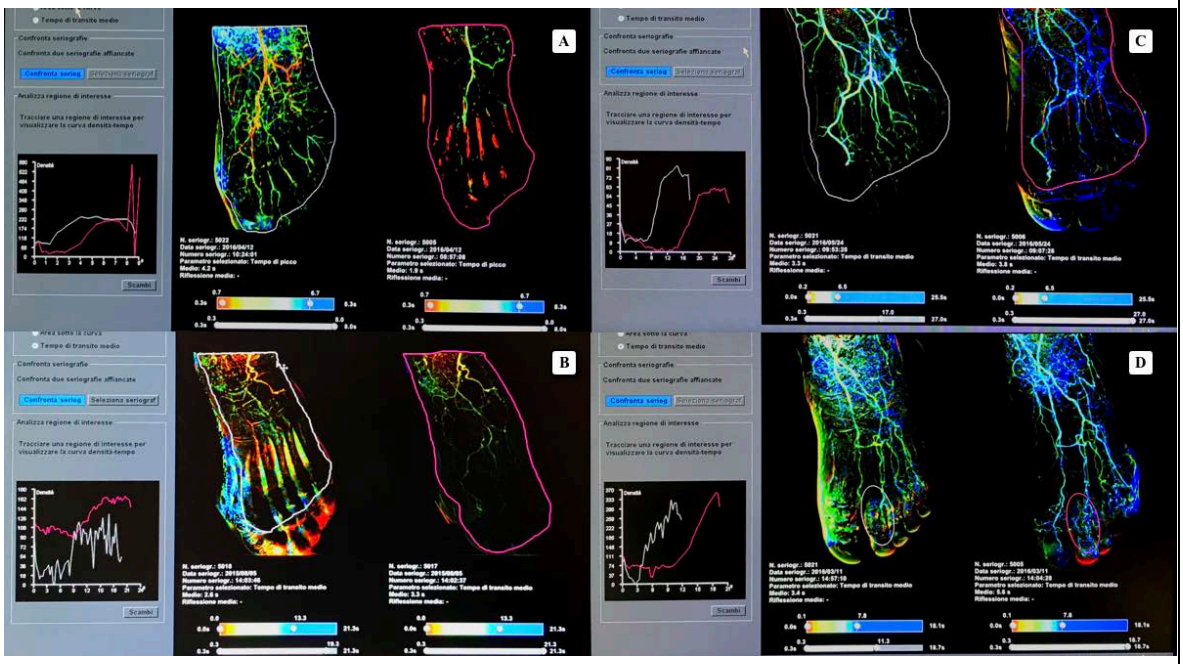
11. ANNEXES

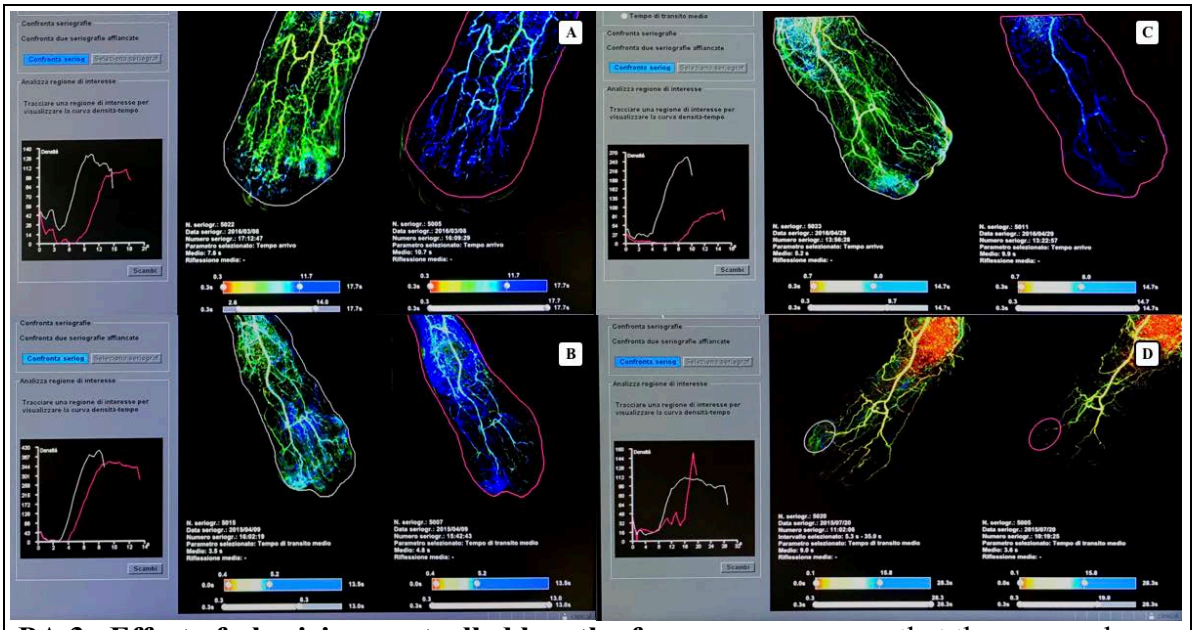
11.1. Examples of perfusion angiography images



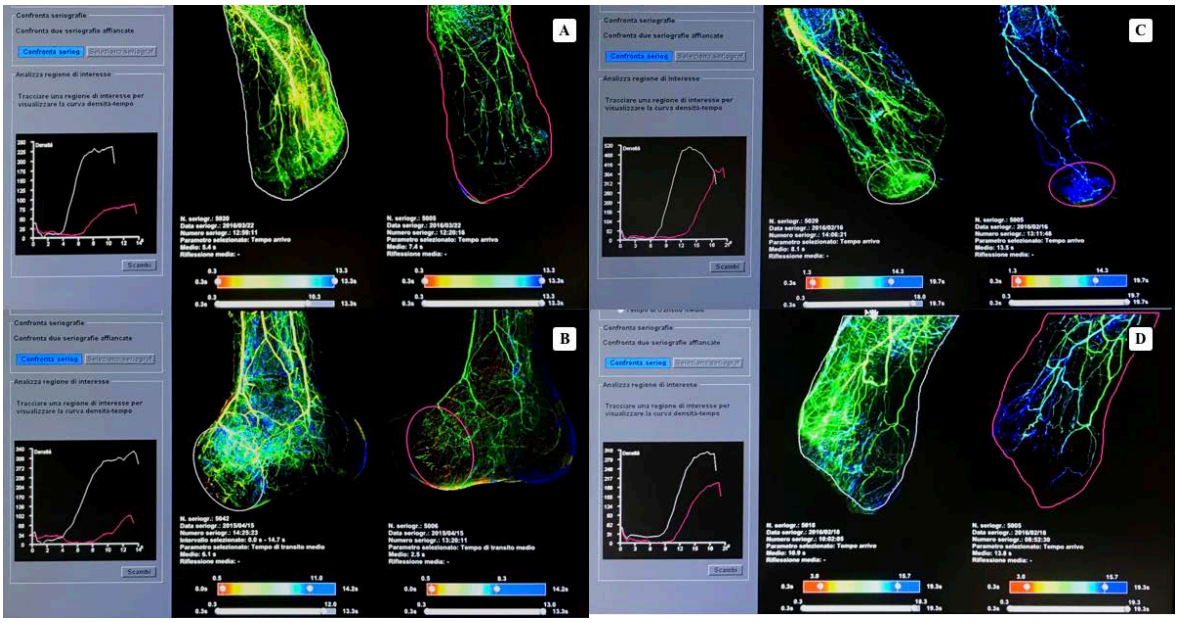
PA 1. Clearly good results. In every case of these ones the result obtained was optimal, both considering the color-coded image and the time-density plot, but this alone would still be a subjective impression nor could be stated the exact amount of improvement necessary to obtain satisfying clinical outcomes.



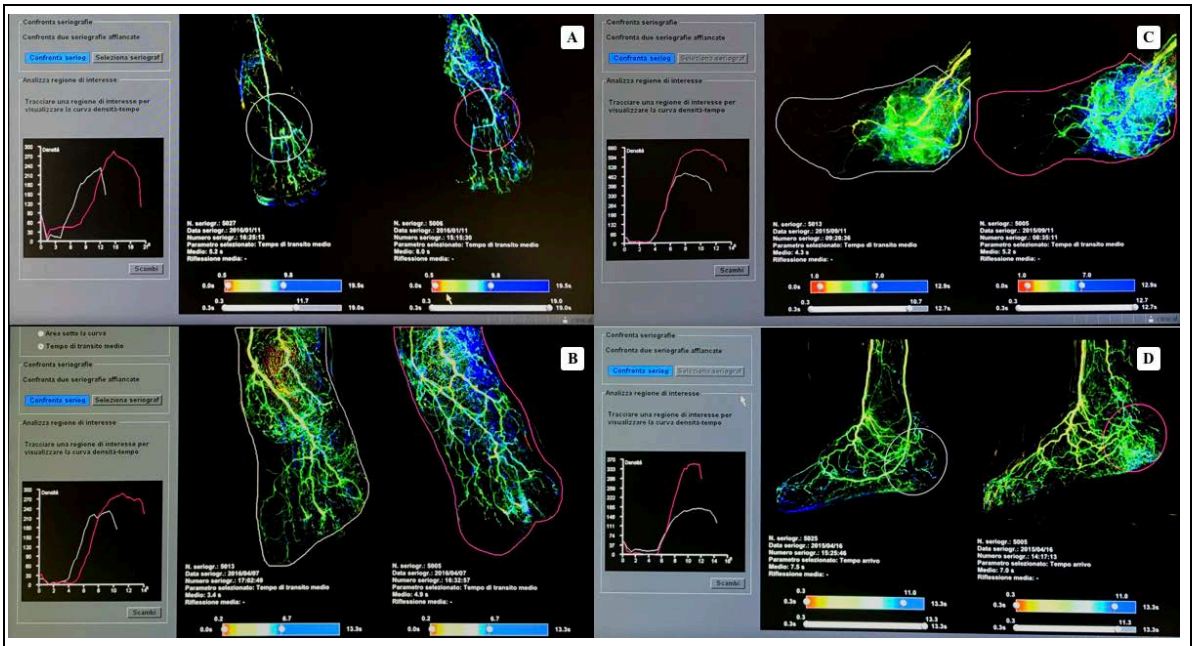
PA 2. Artifacts. Movement artifacts could be identified in these pictures by a high peak-valley (A), movement in the bone (B) or solely in the toes (C), that can be corrected excluding the artifact from the ROI. In other cases, artifacts could not be seen in the image but still be affecting to the curve's shape (D), which cannot be corrected either since it could be affecting parameter values.



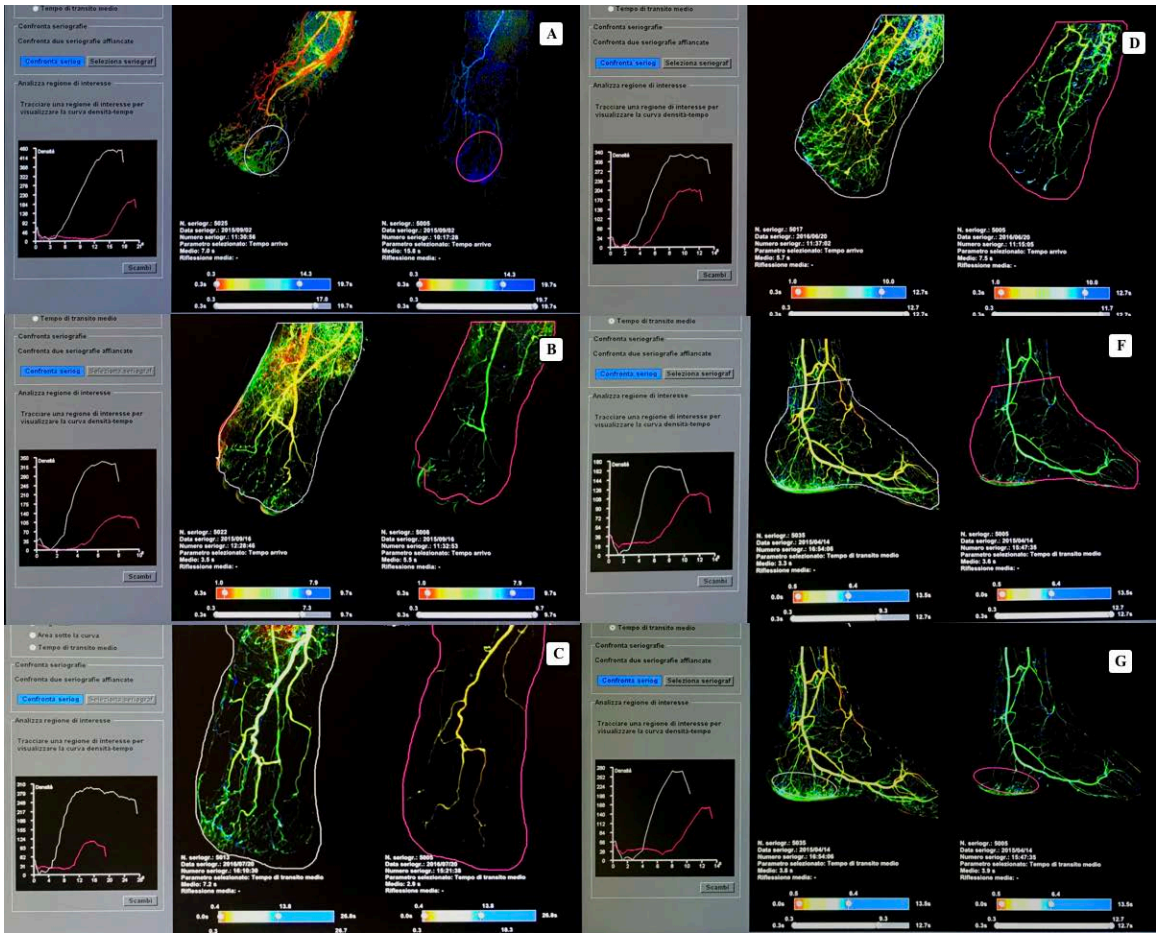
PA 3. Effect of physician-controlled length of exposure, suppose that the area under the curve could not be compared. These examples show an exposure length differences of 5.6 s (A), 5 (B), 4.7 s (C) and -9.3 (D), which usually uses a longer exposure before the treatment because a delayed arrival of the contrast media.



PA 4. Perfusion in wound-blush. When a wound-blush effect, a typically sing of good prognosis, the time-density curve adopts usually a high-plateau shape (A and B). However, it cannot be triggered and it is not constant (C and D), being better detected in the color-shape image and without any significative difference from conventional angiography.



PA 5. Vasospasm. When an after treatment a distal vasospasm occurred, a diminishment of the maximum density is observed; either with a faster arrival of the contrast (A and B) or just stopping the ascent of the curve (C and D).



PA 6. Patient-dependent shape of the curve is shown in these samples, as a parallel ascending curves (A and D) or similar shape of the whole plateau (C and B). It can also be seen in the same patient different ROIs (F and G), where both initial and completion runs have similar shapes.

11.2. Presented abstract: Hemodynamic significance of perfusion angiography parameters.

Gómez, E[1, 2]; Mestres, G[1]; Palena, LM[2]; Manzi, M[2]

[1]Hospital Clínic de Barcelona. [2]Policlinico Abano Terme

Introduction: Perfusion angiography (PA) is a post-processing software algorithm that does not require digital subtraction angiography (DSA) or contrast medium injection. Given that their significance is unknown, PA parameters are not being used in decision-making processes.
Objectives: Our study is a first attempt to demonstrate that PA parameters may provide valuable hemodynamic significance.
Material used: On consecutive patients undergoing endovascular treatment (EVT) at Policlinico Abano Terme (Italy) for critical limb ischemia (CLI), standard angiographic studies of the limb and PA of the foot before undergoing EVT and thereafter were performed, measuring all its parameters (arrival time, time to peak, wash-in rate, width, area under the curve, and mean transit time).
Methodology: Demographic data and PA analysis on the foot were measured and TASC classification in femoro-popliteal (FP-TASC) and below the knee (BTK-TASC) segments were assigned accordingly.
Results: 74 consecutive patients were studied. Mean age was 71, 74% were men. 6 patients were excluded due to PA artefacts. All PA parameters showed significant improvements between PA performed previously and after EVT ($p<0.03$), according to angiographic findings. Wash-in rate was inversely related with both FP-TASC ($p=0.026$) and BTK-TASC ($p=0.009$) classifications, and arrival time had a direct relation with BTK-TASC ($p=0.032$). The differences in BTK-TASC after EVT revealed a negative correlation with arrival time ($p=0.005$) and width ($p=0.045$).
Conclusions: Arrival time appears to be the most related PA parameter with BTK-TASC (for an isolated angiography and for differences after EVT). Wash-in rate seems to be more representative of the whole TASC classification of the limb, both FP-TASC and BTK-TASC.

Presented at the SITE 2017 and SCACVE congress 2017

11.3. Presented abstract: Perfusion angiography in the prediction of wound healing in endovascular treatment of critical limb ischemia.

Gómez Jabalera, Efrem1,2; Mestres, Gaspar2; Bellmunt, Sergi3; Palena, L Mariano1; Manzi, Marco11

Policlinico Abano Terme; 2Hospital Clínic de Barcelona; 3Hospital Universitari Vall d'Hebron

Introduction: Current endovascular treatments (EVT) of critical limb ischemia (CLI) have no clear treatment goals. Perfusion Angiography (PA) is an image-processing software that analyzes density per pixel over time, and it could predict the clinical success of EVT.

Methods: Consecutive patients undergoing EVT for CLI were analyzed with PA before and after treatment. Exclusion criteria were poor PA image quality, no ulcer, death and loss at follow-up. Demographic and clinical data were recorded and clinical follow-up was performed to trace the time to heal (TTH) of the ulcers. The PA parameters were measured on the contrast time-density curve: Arrival Time (AT), Peak Time (PT), Wash-in Rate, Width, Area Under Curve and Mean Transit Time (MTT). Two cohorts based upon a TTH of less (group A) or more than 30 days (group B). We used Student-t test for independent variables and for changes before and after EVT and cut-off values from ROC curves were identified.

Results: From January2015 to July2016, 332 consecutive patients were studied and 123 were excluded. Mean age was 72 years and 67.5% were men. 133 patients had Rutherford 5 and 76 had Rutherford 6 lesions, with similar distribution in both groups. We found significant differences between groups in the following after treatment PA parameters: AT (+1.2seconds, 95%CI:2.01-0.39; p = .004), PT (+0.56seconds, 95% CI: 1.01-0.11; p = 0.014) and MTT (+0.5seconds, 95%CI: 1-0.08; p = .022), and also on the improvement of MTT (+0.64 seconds, 95% CI: 1.16-0.1; p = .02). Cut-off values were: AT > 6 seconds (Sensitivity = 64.8%, Specificity = 59%, p = .001), PT > 5.2 seconds (Sensitivity = 43.7%, Specificity = 76.9%, p = .002), and improvement of MTT > 1.7 seconds (Sensitivity = 42.4%, Specificity = 81.7%, p = .016).

Conclusion: AT, PT and MTT can predict CLI wound healing in less than 30 days.

Presented at SCACVE 2018 and the 32nd ESVS congress 2018

11.4. Presented abstract: Angiography based Abano Terme Score: a novel descriptive system for below the groin arterial disease.

Efrem Gómez-Jabalera[1,2], Sergi Bellmunt Montoya[3], Jaume Dilmé Muñoz[4], Vicenç Artigas Raventós[5], Luis Mariano Palena[2], Marco Manzi[2]

[1]Hospital Clínic Barcelona, Vascular Surgery, Barcelona, Spain, [2]Policlinico Abano Terme, Interventional radiology, Abano Terme, Italy, [3]Hospital General Universitari Vall d'Hebron, Vascular surgery, Barcelona, Spain, [4]Hospital de la Santa creu i Sant Pau, Vascular Surgery, Barcelona, Spain, [5]Universitat Autònoma de Barcelona, Surgery, Barcelona, Spain

Introduction: The TASC classification is the most frequently used system to describe lower limb angiographic lesions. However, its lack in hemodynamic and clinical information render it a suboptimal system for daily practice. The calf and foot score of the Joint Vascular Society Council (JVSC) provides a grading system of bypass grafting outflow and foot steno-occlusions. Inspired on the latter score, our aim is to describe the novel classification system Abano Terme Score (ATS), which adequately assesses the anatomy and burden of steno-occlusions in the whole limb, to ultimately select the best therapy for each patient.

Methods: The JVSC classification2 (0:no stenosis>20%; 1:21%-49% stenosis; 2:50%-99% stenosis; 2.5:<half the vessel length occluded; 3:>half the vessel length occluded) was adapted to each limb's arteries: superficial femoral artery (SFA), popliteal, anterior tibial (ATA), posterior tibial (PTA) and peroneal arteries (PA). The limb-ATS was obtained by adding the above the knee (ATK) and the below the knee (BTK) scores. The ATK-ATS was calculated by adding the squares of the scores of SFA and popliteal artery. The BTK-ATS was calculated by multiplying the scores of ATA and PTA, adding the score for PA afterwards. Retrospective cohort analysis was done over endovascular treatment of critical limb ischemia patients which were studied with perfusion angiography; their baseline co-morbidities recorded, and pre-treatment and completion angiographies were performed and classified according to TASC (ATK and BTK) and ATS (ATK-ATS, BTK-ATS and whole limb-ATS). Perfusion angiography (Philips 2D Perfusion 1.1.6.) runs were performed in both angiographies and the arrival times (AT) were measured. Changes throughout treatment, perfusion angiography and TcPO2 at baseline and 1-month post-intervention were recorded. We assessed statistical post-revascularization changes and explored correlations (Spearman's rho, sr) between TASC, ATS, AT and TcPO2.

Results: From January 2015 to July 2016, 246 patients received endovascular treatment and were studied with perfusion angiography (68.6% men, mean age of 72.2±10.4 years). In this cohort 92.8% were diabetics, 50.2% had chronic kidney disease and 9.2% end-stage renal disease. At 6 months, ulcer healing rate was 70.6%, amputation free-survival was 96.6%, and mortality of 4.2%. Student t-test showed statistical differences ($p< 0.001$) between ATS values before and after treatment in ATK region (from 4.7±4.6 to 0.3±0.7), BTK region (from 6±3.9 to 1.09±2.1) and on the whole limb (from 10.6±5.8 and to 1.43±2.3). The TcPO2 at baseline and at 1-month were 22±15.8mmHg, 50.9±11.9mmHg, respectively; with significant correlations between TcPO2 and the BTK-ATS ($sr=0.25$, $p=0.020$) and with whole limb-ATS ($sr=0.24$, $p=0.023$). Comparing both pre-treatment and completion perfusion angiographies, we found correlations between the AT and the ATK-TASC ($sr=0.28$, $p< 0.001$), the BTK-TASC ($sr=0.38$, $p< 0.001$) and the whole limb-ATS ($sr=0.41$, $p< 0.001$).

Conclusion: ATS is a simpler, easier and more thorough classification method than current systems available to describe the steno-occlusive burden of a target limb. It varies with revascularization, and appears to be mild-moderately correlated with TcPO2 and perfusion angiography (better than TASC in the latter). Further studies are warranted to validate and compare the ATS with other anatomical and hemodynamic diagnostic methods like doppler ultrasound and other patient-centered outcomes.

Presented at the 33rd ESVS congress

11.5. Article submitted to the European Journal of Vascular & Endovascular Surgery:

Novel Abano Terme Score and its application in the treatment of critical limb threatening ischemia

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original Article-Other
Keywords:	Critical limb threatening ischemia; Peripheral Artery Disease Classification; Prognosis; Anatomical Score; Arterial Disease Burden; Global Limb Anatomic Staging System
Corresponding Author:	Efrem Gómez-Jabalera, MD, FEBVS Hospital Nostra Senyora de Meritxell Escaldes-Engordany, Escaldes-Engordany ANDORRA
First Author:	Efrem Gómez-Jabalera, MD, FEBVS
Order of Authors:	Efrem Gómez-Jabalera, MD, FEBVS
	Luis Mariano Palena, MD
	Sergi Bellmunt Montoya, MD, FEBVS, PhD
	Jaume Dilmé Muñoz, MD, FEBVS, PhD
	Vicenç Artigas Raventós, MD
	Marco Manzi, MD
Abstract:	Objectives The Global Limb Anatomic Staging System (GLASS) attempts to describe critical limb threatening ischemia (CLTI) patterns to correlate with patient centered outcomes. Although, the system intended to direct evidence-based revascularization, it has several limitations. Aiming to improve GLASS, we defined a novel scoring system capable of quantifying the disease burden of a patient and adding prognostic value, which we called the Abano Terme Score (ATS). Methods Following the scoring system of the Joint Vascular Society Council, we assigned a grade to each artery of the limb. Retrospective cohort analysis was performed on endovascular treatments of Rutherford 5 CLTI patients with 6-month clinical follow-up; their baseline co-morbidities were recorded, and pre-treatment and completion angiographies were classified according to the GLASS and ATS. We assessed statistical post-revascularization changes and associations between GLASS, amputation free survival (AFS), ulcer healing rates and ATS. Results From January 2015 to July 2016, 162 patients with Rutherford 5 ulcers received endovascular treatment (67.9% men, mean age of 73±10.3 years). At 6 months of follow-up, there was a 93.2% ulcer healing rate, a 97.5% AFS, a 6.8% of mortality. We found statistical differences between ATS and GLASS values before and after treatment. Pre-intervention femoro-popliteal ATS (ATS-FP) was correlated with ulcer healing rates (7.5±5.6 for the non-healing group and 4.4±4.6 for the healing group; p=.036). Also, the post-intervention ATS-FP for the group with amputations was higher (1.25±0.96) than for the AFS group (0.35±0.7; p=.014). No correlations for those endpoints were found with the below-the-knee ATS (ATS-BTK) nor the GLASS classification. Conclusion The ATS-FP provides a more complete classification method that has better prognostic value than current scores presently available to describe the steno-occlusive burden of a target limb. Further studies are warranted to validate the ATS-BTK in larger patient groups and for the whole limb.

WHAT THIS PAPER ADDS: *Current anatomical classifications for peripheral artery disease are difficult to memorize and hard to apply in everyday cases. Besides lacking prognostic value, they are impractical when it comes to guiding revascularization threshold requirements. To overcome these limitations, and with the aim of providing a handy and representative description of the hemodynamic status of a limb, we designed a scoring system: the Abano Terme Score.*

INTRODUCTION

Peripheral artery disease (PAD) mainly affects arteries of the lower limbs. The most advanced stages of the disease, formerly known as critical limb ischemia, is now termed chronic limb-threatening ischemia (CLTI)(1). The diagnosis of CLTI conveys a bleak prognosis including morbidity and mortality. Data from recent trials (2006 – 2010) demonstrates that, without revascularization, the risk of major amputation is of 30-50% in the first year(2), while revascularization can reduce this risk to a 10-38%.(3) Consequently, in order to provide a descriptive and predictive stratification of PAD patients, the Society for Vascular Surgery designed the WIfI classification (Wound, Ischemia, foot Infection). This classification ranks patients (before intervention) by their lower extremity risk with respect to the natural history of the disease and also by the benefit of having revascularization.(4) Consistently, several studies have validated the WIfI classification as a predictor of amputation free-survival (AFS)(5–9) and for time to wound healing.(10)

The 2017 ESC guidelines for PAD(11) proposed an algorithm to approach the revascularization strategy, but without detailed anatomical considerations nor angiographic endpoints for the treatment. Accordingly, and in the search for an Evidence-Based Revascularization (EBR) strategy, the Global Vascular Guidelines on the management of CLTI(12) proposed a new Global Anatomic Staging System (GLASS) to correlate arterial disease patterns from groin to ankle and technical success in a patient centered outcome base. Previously used anatomical classifications(13–16) had failed to demonstrate that correlation,(17) and were quite unpractical to describe CLTI patients with complex, multilevel, and distal disease. In a recent study Kodama et al.(18) demonstrated the GLASS can predict technical failure, AFS and major adverse limb events (MALE). However, only the baseline angiography was used, so no treatment endpoint could be extracted from those studies.

On the other hand, the Joint Vascular Societies Council (JVSC)(19), when studying distal bypass grafting outcomes, developed an angiographic scoring system with stronger correlation to clinical success.(17) The JVSC score proposed a grading system according to lesion severity of each evaluated artery, ultimately assigning a numeric value to each artery. This strategy might allow to

create a simple classification to facilitate outcome prediction and allow for comparisons between patients and therapies, as the WIfI consensus document suggested.(4) Here, our aim was to create a novel scoring system capable of better and univocally quantifying the arterial disease burden of a patient, and ultimately endowed with prognostic value, which we called the Abano Terme Score (ATS). The objective of this study was to explore the predictive ability of ATS on patient-centered outcomes such as AFS and the healing rates of the ulcers.

METHODS

Study design

This study looks at a retrospective cohort of patients with Rutherford 5 CLTI and a minimum follow-up period of 6 months after the endovascular treatment (EVT) performed at the diabetic foot clinic of the Interventional Radiology Department in the Policlinico Abano Terme (Abano Terme, Veneto, Italy). Patients with a Rutherford classification different than 5 and those who died or were missing during follow-up have all been excluded from analysis. We selected cases taking advantage of the randomization conducted for another study in the same population, which was only 1 random case every 4 consecutive patients (to adjust dedicated time to daily schedule).

Interventions

Interventions were performed through a percutaneous antegrade approach of the common femoral artery, under local anesthesia. Initial standard treatment was plain old balloon angioplasty, followed by drug eluting technologies or stenting according to the treating interventionist's discretion. Standard digital subtraction angiographies (DSA) were performed (Philips Allura Xper FD20, Philips Healthcare®, Best, the Netherlands) pre- and post-EVT. The DSA projections performed were those described by Manzi et al.(20) The protocol for contrast injection consisted in 9 mL of Visipaque 270 mg/ml (Iodixanol, GE Healthcare Inc.®, Princeton, NJ) administered at a speed of 3 ml/s using a coupled injector. Patients with borderline end-stage renal disease (ESRD) or allergy to iodinated contrast media were studied with CO_2 angiography instead. Reinterventions and target lesion revascularizations (TLR) were performed according to clinical justification.

Variables

Demographic data, medication, cardiovascular risk factors and co-morbidities were recorded, as well as laboratory tests (serum creatinine levels, red and white blood cell count) performed prior to and following the EVT. Baseline Rutherford degree(21), WIfI clinical stage(4) estimating the likelihood of benefit from revascularization and $TCPO_2$ (mmHg) were also annotated.

At 1- and 6-months follow-up $TCPO_2$, TLR, the ulcer healing (complete epithelization of the ulcer) and date of event were recorded. WIfI stage(4), the AFS, the occurrence of any major adverse limb events (MALE) and major adverse cardiovascular events (MACE) were also recorded. Any intervention in the index limb was considered as a MALE; and MACE corresponded to any clinical coronary syndrome, stroke or any peripheral artery intervention.

Data collection

Data was collected by the main investigator. Patients who missed the follow-up visit at the diabetic foot clinic were phone called twice during the period of data collection in order to rule-out MALE or death.

Hemodynamic angiography based "Abano Terme Score"

The Abano Terme Score was designed to facilitate the classification and refine the description, while also providing a hemodynamic based classification, of lesions throughout the limb. As previously stated, it adapts the JVSC classification described by Toursarkissian et al.(19). The JVSC classification gives a score from 0 to 3 on the basis of the most severe stenosis in each artery of the foot (dorsalis pedis, lateral plantar and medial plantar) and the calf (anterior tibial, posterior tibial and peroneal) depending on the following scores:

- 0 points in a stenosis of 0-20%
- 1 point in a stenosis of 20-50% or in tandem stenosis of <20%
- 2 points in a stenosis of >50% or in tandem stenosis of <50%
- 2.5 points in an occlusion of < 50% of the vessel length
- 3 points in an occlusion of > 50% of the vessel length

Our intention in designing the ATS was to adapt this scoring to the whole limb. Therefore, based on the same grading criteria, we assigned a grade to each artery from the groin to the ankle (superficial femoral artery, popliteal, anterior tibial, posterior tibial and peroneal arteries). We only added an exception for no flow-limiting dissections or mild recoiling after angioplasty, assigning 1 point to these case-scenarios.

The global scoring for the whole limb was obtained by adding up the scores given to the femoro-popliteal (FP) and the below-the-knee (BTK) regions. To obtain the score of the FP region, we adopted a mathematical equation to weight the hemodynamic effect of a steno-occlusion in each artery. Hence, we added the squares of the scores of the superficial femoral artery (SFA) and the popliteal artery (Pop). In the BTK area we multiplied the scores of the anterior (ATA) and posterior (PTA) tibial arteries, and then the score for the peroneal artery (Per) was added. Thus, the formula to calculate the global score is:

$$limb\ ATS = SFA^2 + Pop^2 + (ATA \times ATP) + Per$$

Where the global limb's ATS is the result of adding the femoro-popliteal ATS (ATS-FP) and below-the-knee ATS (ATS-BTK) calculated values. The rationale behind this strategy is that the presence of lesions in the FP region are hemodynamically more deleterious, while the lesions in one tibial artery could be properly compensated by the other tibial artery if the latter has no stenosis. The peroneal artery alone, otherwise, could not replace completely both tibial arteries. Further examples are showed in figures 1 and 2.

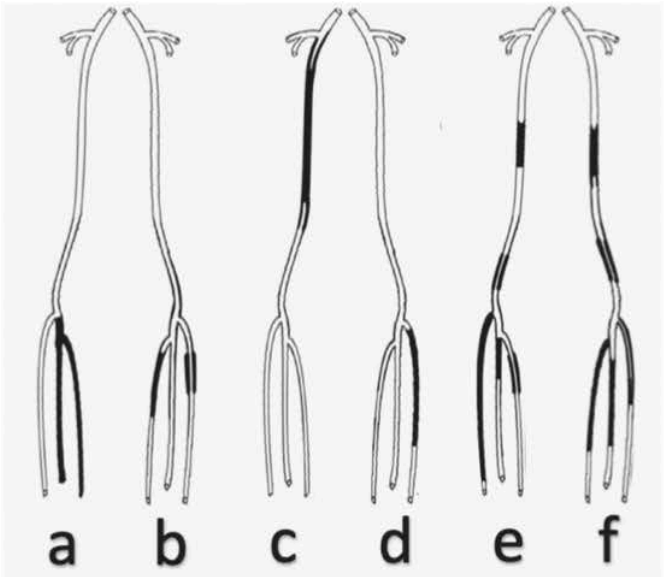


Figure 1. Examples of the Abano Terme Scoring with diagrams. An ATS < 6 ensures a TAP free from stenosis, as it is in the case of the first picture [ATS(a) = 0+0+(0x3)+3 = 3]. In the second case (b), although there are fewer lesions, there is not a thorough TAP to the foot: ATS(b) = 0+0+(3x2)+0 = 6. Third (c) and forth (d) cases intend to demonstrate the difference in burden of disease between a long occlusion of the superficial femoral artery [ATS(c) = 3^2+0+(0x0)+0 = 9] and an isolated occlusion in BTK [ATS(d) = 0+0+(3x0)+0 = 0]. On the other hand, while cases a, b or c would represent typical CLTI patients, case c would be more typically related to a patient with claudication. To compare the ATS with other classification systems; the fifth case (e) would correspond to a Graziani's grade 6 [ATS(e) = 2.5^2+2^2+(3x2)+2.5 = 18.75]; which is lower than that of case f [ATS(f) = 2.5^2+2^2+(3x2.5)+3 = 20.75]; however, both cases could be graded with a GLASS stage of II. (ATS = Abano Terme score; GLASS = Global Limb Anatomic Staging System; TAP = target artery pathway).

Other specific considerations are that the tibioperoneal trunk is considered along with the posterior tibial artery; and the prior FP bypasses are included as a native FP region, with eventual stenosis or occlusions. Finally, in case of anatomical variations, such as hypoplasia of the anterior or the posterior tibial arteries, the score value is given to the artery that contributes blood supply to the theoretical angiosome of the hypoplastic artery (usually the peroneal artery).(22)

Endpoints and groups for analysis

The independent variables of the study were ATS and GLASS classifications before and after the EVT. The dependent variables for the main endpoint were the healing rate and the AFS. We have also explored correlations between ATS and $TCPO_2$ measurement at 1-month of follow-up.

Statistical analysis

Descriptive and frequency statistical analysis were obtained and comparisons were made using IBM SPSS

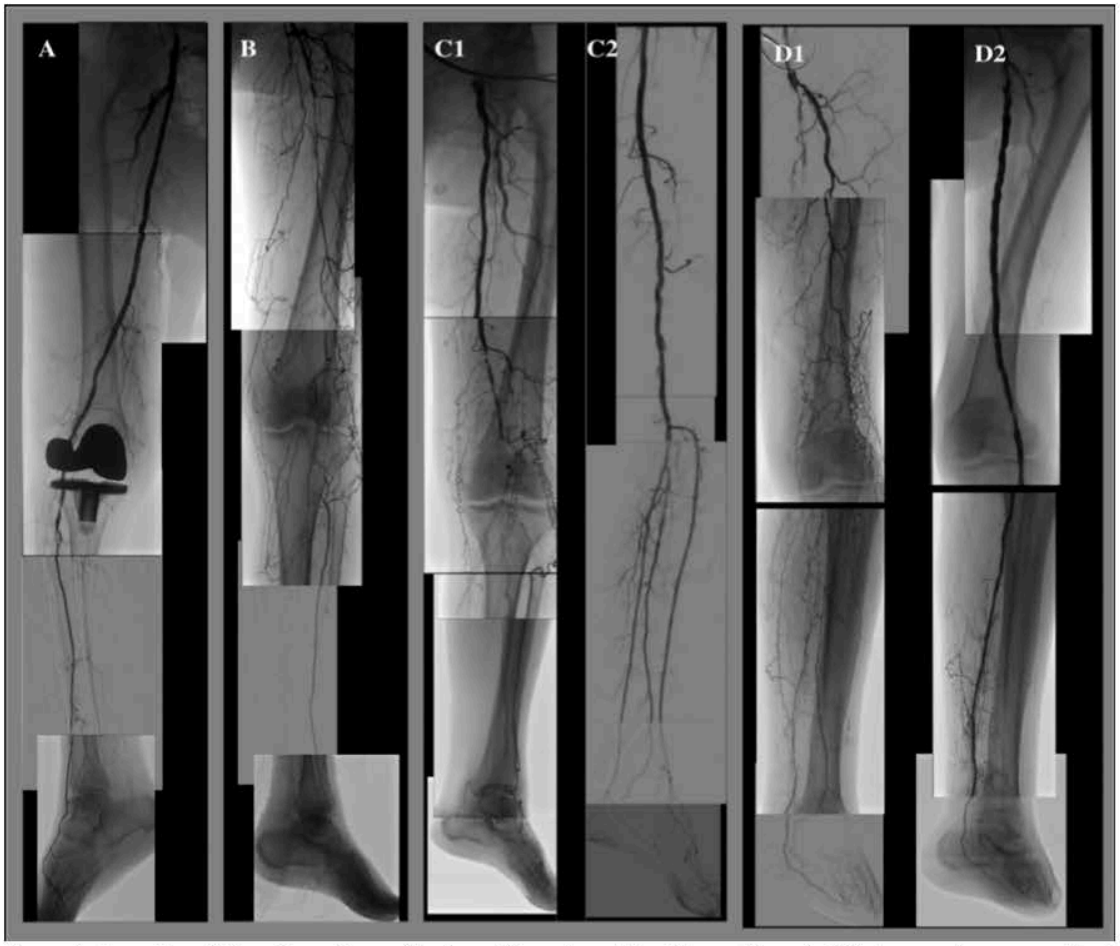


Figure 2. Examples of the Abano Terme Scoring with angiographies. Composition of DSA images from conventional angiographies. The projections vary from the above the knee and the ones below the knee. The two cases on the left represent the descriptive power of the ATS to quantify the disease burden in a limb. While case A has an ATS of 3 [ATS(A) = 0+0+(0x3)+3], case B has an ATS of 21 [ATS(B) = 3^2+3^2+(0x3)+3]. The other two examples on the right show the ATS' ability to describe the global improvement throughout a procedure. The case (C) had an ATS of 13 [2^2+3^2+(0x3)+0] prior to intervention and a 0 (0+0+0+0+0) post-intervention. Finally, the last case (D) had a basal ATS of 28 [3^2+3^2+(3x2.5)+2.5] that was reduced to 3 [0+0+(3x0)+3] after treatment. (ATS = Abano Terme score; DSA = digital subtraction angiography).

Statistics 22.0® software. Categorical variables were reported as frequencies (percentages) and continuous variables as mean ± standard deviation (SD). Pre-post changes in classification scores (ATS and GLASS) were evaluated with the paired Student-T test; and they were also compared to the healing rate and the AFS. Finally, a Spearman's rho correlation was done to look for correlations between the ATS and the $TCPO_2$. A p-value < .05 was considered as statistically significant.

RESULTS

From January 2015 to July 2016, 1189 patients were treated for CLTI at our facility. As described in figure 3, among the 332 randomized cases, 43 patients were excluded due to absence of ulcers (Rutherford 4 patients), 20 (6.9%) patients died with unhealed ulcers (mean days to the event were 177±167 days) and 19 (6.6%) were lost during follow-up. The final study population was of 162 patients after

excluding the 88 patients with Rutherford 6 ulcers. Three possible reasons were attributed to the missing cases during follow-up: the patient had been managed in another hospital and/or another city (with or without clinical success) and/or could have died. However, outcomes could be confirmed nor discarded via phone call in the final data review.

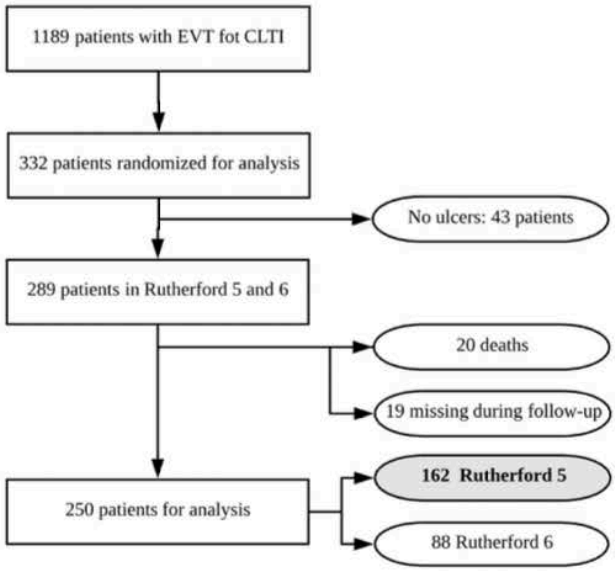


Figure 3. Algorithm of the aforementioned exclusions before obtaining the baseline study population for analysis. Among this population we found 162 cases with ulcers restricted to the toes (Rutherford 5) and 88 with Rutherford 6 classified ulcers.

Patients were mostly men (67.9%), had a mean age of 73±10.3 years, and diabetes mellitus was their most frequent co-morbidity (94.4%). In addition, 53.7% had chronic kidney disease and 8% ESRD. Other baseline comorbidities are described in table 1. At the end of the follow-up (6-months), the ulcer healing rate was of 93.8% (11 non-healed ulcers), AFS was of 97.5% (4 major amputations). No statistically significant differences in laboratory tests performed before and after the EVT were detected.

The distribution of the WIfI clinical stages(4) during follow-up are represented in figure 4. Both ATS and GLASS gradings presented statistically meaningful differences through the EVT (table 2). The mean time to heal for ulcers was 75.9±85.9 days. The TLRs were 17.2% and we observed a 26.1% of MALEs and a 14.5% of MACEs. The rate of major amputations was 3.4%.

The values of the $TCPO_2$ at baseline, one and six months (expressed in mean±SD) are 22±15.8 mmHg, 50.9±11.9 mmHg (N=46) and 32.2±17.2 mmHg (N=34), respectively. Note that due to the retrospective nature of the study only few cases had a $TCPO_2$ measure. From the

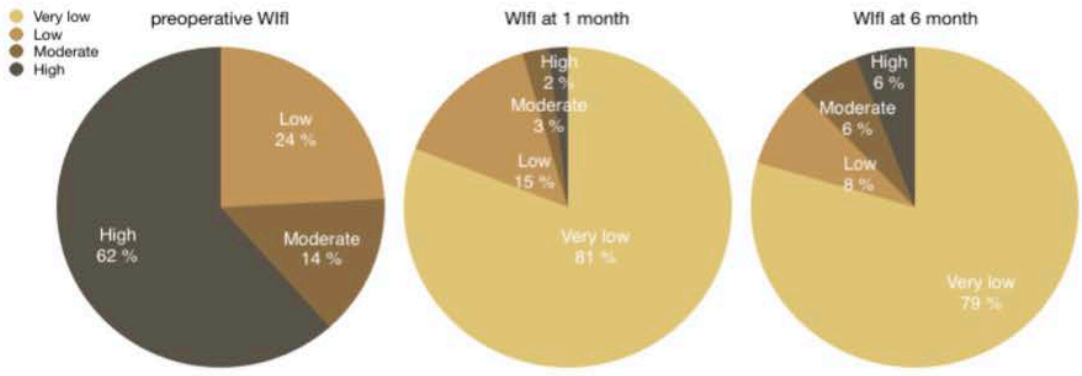


Figure 4. . Evolution of WIfI clinical stage preoperatively and during follow-up for the total population with ulcers.

Table 1. Baseline characteristics of cases for analysis.

	N=162
Men	67.9 %
BMI	26.9 ± 4.2
Hypertension	96.9 %
Atrial Fibrillation	23.5 %
Ischemic cardiomyopathy	43.2 %
Cerebrovascular disease	27.2 %
Autoimmune disease	7.4 %
Previous ipsilateral EVT	36.4 %
Previous ipsilateral bypass	9.9 %
Previous contralateral EVT	29.6 %
Previous contralateral bypass	5.6 %
Major contralateral amputation	2.5 %
Percutaneous coronary intervention	14.2 %
CABG	18.5 %
Simple antiplatelet treatment	73.5 %
Dual antiplatelet treatment	14.2 %
Anticoagulation	18.5 %
Corticosteroid therapy	3.7 %

Data are presented as percentage or mean±SD. BMI = body mass index; CABG = coronary artery bypass grafting; EVT = endovascular treatment.

Spearman's rho at the 30 days follow-up there were significant, although low-medium grade, correlations of the $TCPO_2$ with ATS-BTK (sr=.25, p=.020) and with the global ATS (sr=.24, p=.023) both after the EVT.

Finally, table 3 shows the results of the ATS and GLASS comparisons with healing rates and AFS. There was a significant correlation between high pre-intervention ATS-FP and lower rates of ulcer healing and, conversely, low pre-intervention ATS-FP and higher rates of ulcer healing. Furthermore, there was also a trend relating the global pre-intervention

Table 2. Changes in ATS and GLASS from before to after EVT.

	pre-EVT	post-EVT	*p value*
ATS – FP	4.6±4.7	0.4±0.7	< .001
ATS – BTK	6.8±3.7	1.2±2.3	< .001
ATS limb	11.5±6.2	1.6±2.4	< .001
GLASS	2.1±0.9	0.2±0.6	< .001

Data are presented as mean ± SD. ATS = Abano Terme score; BTK = below-the-knee; EVT = Endovascular Treatment; FP = femoro-popliteal

Table 3. Mean±SD values for ATS and GLASS scores gradings from the results of the Student-T test in predicting the healing of the ulcer and the limb salvage (n=162).

	pre-EVT			post-EVT		
	Non-healing	Healing	*p value*	Non-healing	Healing	*p value*
ATS-FP	7.5±5.6	4.4±4.6	**.036**	0.6±0.8	0.4±0.7	.218
ATS-BTK	6.5±3.6	6.9±3.7	.793	0.5±0.9	1.3±2.3	.292
ATS-LIMB	14±4	11.3±6.3	.152	1.2±0.9	1.7±2.5	.167
GLASS	2.4±0.9	2.1±0.9	.326	0.1±0.3	0.2±0.6	.619
	Major Amputation	AFS	*p value*	Major Amputation	AFS	*p value*
ATS-FP	9.1±6.8	4.5±4.6	**.056**	1.3±1	0.4±0.7	**.014**
ATS-BTK	7.8±5.3	6.8±3.7	.619	0.5±0.6	1.3±2.3	.509
ATS-LIMB	16.8±6.2	11.3±6.1	**.079**	1.8±0.5	1.6±2.5	.916
GLASS	2.8±0.5	2.1±0.9	.148	0±0	0.2±0.6	.536

ATS = Abano Terme Score; BTK = Below-The-Knee; EVT = Endovascular Treatment; FP = Femoro-Popliteal

ATS: 14±4 for the non-healing group and 11.3±6.3 for the healing group (p=.082; from Mann-Whitney U). On the other hand, compared to the AFS, we found a significant correlation between the post-intervention ATS-FP (1.25±0.96) for the group with amputations and (0.35±0.7) for the AFS group (p=.014), and a trend relating the global pre-intervention ATS and ATS-FP with the AFS (p=.056 and p=.079, respectively). No correlations were found between the ATS-BTK and the GLASS classification.

DISCUSSION

CLTI is the most severe expression of PAD and comprises approximately 2-3% of all cases.(23) The goals of treatment for CLTI are to preserve life, limb function, relieve pain, and minimize the frequency and magnitude of interventions. However, there are no clearly established endpoints for treatment therapies.(24) Therefore, the ATS was designed with the intention of simplifying the classification of steno-occlusive burden of disease, to ease the unification of treatment criteria and standardize therapies and has proven to be a feasible and useful tool to predict patient-centered outcomes.

Despite the scope of the problem, since the publication of the Bypass Versus Angioplasty in Severe Ischaemia of the Leg (BASIL) study(25) in 2005, no other randomized clinical trial (RCT) has compared open surgery to endovascular techniques. The high variability of disease burden and severity among patients, together with the rapid evolution of revascularization techniques, have complicated the design of RCTs. Finally, the lack of reliable and validated prognostic indexes has further precluded the undertaking of multicenter registries. The BEST-CLI study, which began in 2017 and aimed at the comparison of outcomes of the best open surgical and the best endovascular revascularization strategies(26) is still ongoing. Therefore, there is a persistent and unmet need to define an EBR and the standard of care in the management of CLTI.(27) For practical purposes, many authors have suggested that the ideal therapeutic strategy is a "complete" revascularization of the tibial vessels.(28) However, this is not always feasible and in these cases, the application of the angiosome theory is a valid strategy to decide which treatment of the vessel will yield the best results.(29).

In current CLTI therapy practice, the success of a procedure is based in several subjective signs. So far, no clear perfusion threshold has been set as a cut-off to ensure successful revascularization and no anatomical references upon which EBR can be set have been defined. Keeping in mind these gaps in knowledge, the ATS is aimed at assisting the interventional physician in the description of anatomical patterns and disease burden of the CLTI patient, thereby allowing a standard of comparison between patients.

The relatively limited proportion of deaths and low number of missing data excludes potential confounding factors affecting our results. Given that WIfI classification stages are between 1 and 2 in the 30-days follow-up in 96% of cases, wound healing rates can be expected at 1 year to be approximately around 94.1±2%,(8) similar to the 97.5% limb salvage rate found in our study.

Although ATS-BTK in cases that did not heal (0.5±0.9) were lower than those with healed ulcer (1.3±2.3), and that

ATS-BTK outcomes in AFS were of 0.5±0.6 for cases ending up with an amputation and 1.3±2.3 for the AFS ones, Mönckeberg's (or small artery) disease may have played a role in these paradoxical results. Identifying Mönckeberg's disease in a certain patient may give way to a more aggressive revascularization strategy, ultimately resulting in the restoration of circulation in many tibial arteries (and lower ATS-BTK) but with persistent unfavourable prognosis due to baseline pathology.

Both the GLASS system and ATS can describe the whole limb with a unique value and are applicable to open and endovascular techniques. Both scores consider the concept of the target artery pathway (TAP) as the in-line flow from the groin to the ankle and foot. However, in our study, only ATS showed significant correlations with ulcer healing rate and AFS. Furthermore, ATS is capable of quantifying TAP: any indirect blood flow to the foot cannot have an ATS below 6, while a perfect in-line blood flow to the foot appears with an ATS between 0 and 3 (see figure 1). These values are consistent with a pre-EVT ATS ≥6 (11.5±6.2) and, given that the TAP was usually achieved, the post-EVT ATS was <3 (1.6±2.4), which might justify the overall good performance of our cohort.

Since ATS has a wider score range than the GLASS, it characterizes more accurately the patient burden of disease. Unlike GLASS classification, ATS is not only sensitive to any post-treatment residual stenosis or flow limiting dissections, but also suitable for anatomical variations, which could account for at least 9–12% of cases.(22,30,31) Moreover, the GLASS classification does not measure the peroneal and the non-TAP tibial artery, both of which probably do influence final foot perfusion. Hence, the application of ATS may shed some light into the complexity of multivessel infrapopliteal disease.

The combination of ATS with other diagnostic methods or classifications may help into the standardization of therapies to achieve the best treatment results. Given that ATS simplifies criteria for classification, it is less prone to interobserver variations. However, it should be explored and compared to the GLASS feasibility study,(32) where inter-observer variation occurred. As a limitation we should mention that this is a retrospective study with some missing data. To this end with, missing data on tobacco use (only 23.5% of the cases could be retrieved), which is decisive in the natural history of PAD, has compelled us to assume that the prevalence found is not representative.

In summary, here we propose a novel grading system the ATS that now requires additional prospective validation studies with a greater number of patients and a more heterogenous patient population.

CONCLUSIONS

ATS is a more thorough classification method than current systems available to describe the steno-occlusive burden of a target limb. It changes upon revascularization, and can have a prognostic value before and after EVT. Further studies are warranted to validate the ATS-BTK and ATS for the entire limb.

ACKNOWLEDGEMENTS

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