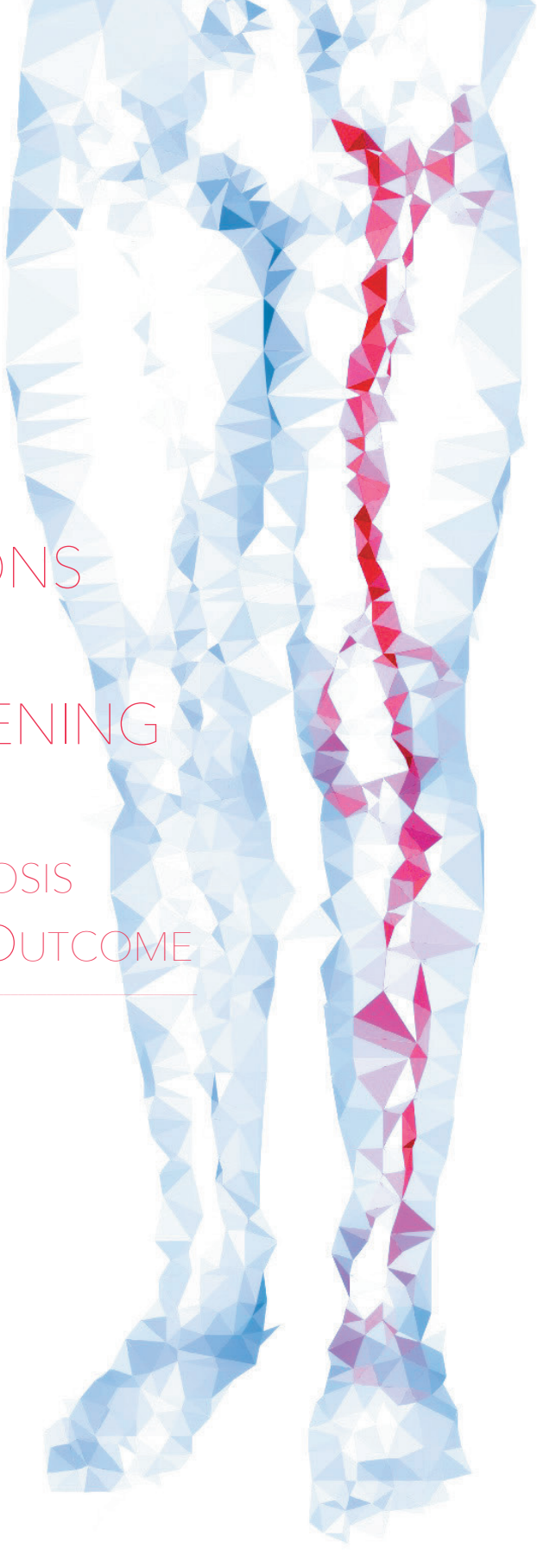
Louise C.D. Konijn

ARTERIAL CALCIFICATIONS IN CHRONIC LIMB-THREATENING ISCHEMIA:

Analysis, Prognosis And Treatment Outcome



Thesis

ARTERIAL CALCIFICATIONS IN CHRONIC LIMB-THREATENING ISCHEMIA: ANALYSIS, PROGNOSIS AND TREATMENT OUTCOME

Louise Cornelia Divera Konijn

ARTERIAL CALCIFICATIONS IN CHRONIC LIMB-THREATENING ISCHEMIA: ANALYSIS, PROGNOSIS AND TREATMENT OUTCOME
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ARTERIËLE CALCIFICATIES IN PATIENTEN MET KRITIEKE ISCHEMIE VAN DE ONDERSTE EXTREMITEITEN: ANALYSE, PROGNOSE EN BEHANDELINGSRESULTATEN

(met een samenvatting in het Nederlands)

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Voor mijn ouders...

Table of contents

PART III. OUTCOME OF CHRONIC LIMB-THREATENING ISCHEMIA TREATMENT

PART IV. SYNOPSIS AND GENERAL DISCUSSION

PART V. ADDENDA

PART I.

Overview

CHAPTER 1.

General Introduction and Outline of this Dissertation

CHAPTER 1. GENERAL INTRODUCTION AND OUTLINE OF THIS DISSERTATION

1. Chronic limb-threatening ischemia

Definitions

Peripheral arterial disease (PAD) is caused by stenoses and occlusions of the arteries of the lower extremities, leading to chronic inadequate tissue perfusion. PAD can be asymptomatic, but can also cause symptoms from intermittent claudication to rest pain and tissue loss. The most severe stage of PAD is chronic limb-threatening ischemia (CLTI), characterized by pain in the foot at rest and/or tissue necrosis (non-healing ulcers and gangrene) [1]. The definition CLTI should be reserved for patients with a chronic character of the complaints of more than 2 weeks, in contrast to acute limb ischemia usually caused by an acute thrombus.

CLTI is classified as Fontaine 3-4 or Rutherford 4-6 [2, 3]. These classifications rely on perfusion characteristics alone. Disease insights have evolved and therefore a new risk stratification system was proposed in 2015 based on three key factors; wounds, ischemia and foot infection (WIfI criteria) [1, 4].

Epidemiology

After approximately 5 years around 10% of all patients with PAD evolve to CLTI [5, 6]. The annual incidence of CLTI ranges from 0.26% - 0.48% and the prevalence is about 1% [5-7].

If the disease has progressed to CLTI, the prognosis is poor. The 5-year mortality is up to 60% [2, 6, 8-16]. Patients with CLTI not only have vascular pathology in the lower limbs, but CLTI is an expression of a systemic vascular disease in which the patients also have significant coronary heart disease and carotid stenoses. Coronary artery disease (CAD) is the main cause of death of CLTI patients, followed by strokes [16, 17]. The relative risk of dying among patients with CLTI versus patients without CLTI was 3.1 for death from all causes and 5.9 for death from cardiovascular disease [18-21].

The incidence of PAD and CLTI is still increasing. This is caused by the increasing prevalence of diabetes mellitus, chronic kidney disease, metabolic syndrome and the continuing habit of smoking [5, 22-27].

Ankle-brachial index

Assessment of the ankle-brachial index (ABI) is often the first step after clinical examination. The ABI is a non-invasive hemodynamic evaluation of the peripheral circulation. The ABI is the ratio from the systolic blood pressure in an artery of the ankle to the systolic blood pressure in an artery of the arm. A normal ABI ranges between 0.90 and 1.30 [28, 29]. An ABI of less than 0.90 has traditionally been diagnostic for PAD, with a sensitivity of 75% and a specificity of 86% [30]. An ABI lower than 0.4 is diagnostic for CLTI.

Before, a high or immeasurable ABI (>1.40 / immeasurable) was seen as unreliable and falsely elevated due to severely calcified arteries. Toe pressure indices are advised in cases of high/immeasurable ABIs [1]. More recently, this high or immeasurable ABI has shown to be a manifestation of an even more devastating form of CLTI with a poorer prognosis than CLTI patients with a low ABI [31-33]. This high or immeasurable ABI is caused by stiffened arteries and limits remodeling, amongst others caused by annular / medial arterial calcifications and fibrosis [34].

Imaging

Duplex ultrasonography, digital subtraction imaging (DSA), CT angiography (CTA) and Magnetic Resonance angiography are all used to visualize the vascular tree. Because of the central role of CT and CTA in our investigations we discuss this method more extensively.

CTA is currently the most widely used diagnostic exam and gives 3D information of the arteries of the patient and their arterial pathology, shown in voxels based on Hounsfield Units (HU). On a CTA of the legs, contrast opacification decreases from the aorta to the anterior tibial artery. CTA is accurate for assessment of PAD in the arteries of the lower extremities. In a meta-analysis, CTA could correctly identify hemodynamically significant lesions and also accurately distinguished between less and more than 50% stenoses and occlusions [35].

The disadvantages of a CTA are the need to use of iodine contrast which can cause nephrotoxicity, overstaging of calcified lesions due to blooming artifacts [36] and incomplete contrast opacification of the arteries of the lower extremities. The latter is due to the fact that the arteries in the lower extremities have a scan length of approximately 125 cm and have stenosis and occlusions making the arrival time of the contrast at the different locations unpredictable [37].

2. Arterial wall calcifications

Arterial calcifications have been a study subject for several decades. The full pathologic process of arterial calcifications has not been revealed. Previously, arterial wall calcifications were seen as merely a static deposition of hydroxyapatite/phosphate in the vessel wall as a side product of atherosclerotic disease and therefore relatively insignificant [38]. Now it has become clear that arterial calcifications are part of a dynamic process that probably cannot only be stopped but also reversed [39-44] and plays an important role in vascular diseases.

Epidemiology of arterial calcification

Arterial calcifications in general are associated with a 3-to-4-fold higher risk for all-cause mortality and cardiovascular events [45]. Arterial calcifications increase with age due to exposure to environmental factors and genetics [46]. Arterial calcifications are defined according to the histologic location in the arterial wall; intimal and medial wall calcifications. Lower extremity intimal and medial arterial calcifications in both patients at risk for vascular disease as well as in patients with known vascular disease have different risk factor profiles [47-51], which suggests these are two separate disease entities. Intimal arterial calcifications are associated with classic risk factors such as hyperlipidemia and smoking. In contrast, medial arterial calcifications (MAC) are associated with diabetes mellitus, chronic kidney disease and aging [52] while smoking appears to protect against MAC [47, 50, 53].

Calcifications of the intimal layer of the arterial wall

The intimal wall consists of endothelium with a basal lamina layer and subendothelial layer. The internal elastic lamina is the boundary with the medial layer of the arterial wall but the lamina itself is mostly seen as part of the medial layer [54]. Intimal wall calcifications are based on calcified atherosclerotic disease located in the intimal layer of the arterial wall.

Atherosclerosis is considered a pathological process due to accumulation of fat in the arterial wall leading to stenoses and occlusions. Classically in the histology of atherosclerotic disease, lesions were classified by a system based on an orderly, linear pattern of lesion progression (Type I – VI) [55]. It was previously thought that arterial intimal calcifications were present in only the most severe lesions (Stary type 5b and VII lesions). We now know that arterial calcification can be present in all forms of atherosclerosis, independent of severity [56]. Therefore in 2000, the American Heart Association Classification to define arterial wall plaques on histology was modified and based on morphologic description [56]. A calcified lesion

is one of the main categories, but the major change in the classification system is that calcification can occur as a descriptive term into each of the other 6 main categories.

Yet, two types of atherosclerotic lesions are recognized. The more atherosclerotic lesions based on lipid accumulation and the more fibrotic type which sometimes is seen as an end stage of the more atherosclerotic type, but is seen by others as an independent expression of atherosclerotic disease. The fibrotic type of lesion consists of connective tissue and smooth muscle cells. The more atheromatous type of lesion begins with subintimal lipid accumulation. An inflammatory response is turned on by pro-inflammatory cytokines. Subsequently, macrophage invasion follows, proliferation of smooth muscle cells and dysfunction of extracellular matrix. Finally, plaque apoptosis follows that induce osteogenic cell differentiation [39, 57-59]. The final disbalance in pro-calcifying and anti-calcifying factors lead to binding of calcium crystals in the atherosclerotic plaque [60-62]. Calcifications occur in both the fibrotic lesions and the atheromatous lesions.

Calcification of the medial layer of the arterial wall

The medial layer of the arterial wall consists of the internal elastic lamina and of vascular smooth muscle cells (VSMC) interspersed with elastin-rich extracellular matrix. The outer layer is called the external elastic lamina, and separates the tunica media from the tunica adventitia.

On histology can be seen that medial calcifications occur preferably alongside the internal elastic lamina [63], but also occur in the VSMCs. Medial calcifications are prevalent in muscle type conduit arteries [64], and appear as linear, often thin and annular calcifications on histology.

MAC develops in an environment or reduced calcium inhibitors, oxidative stress and senescence [39]. Noteworthy, chronic kidney disease and diabetes mellitus both are diseases in a pro-oxidative state (under oxidative stress) [65], and have extensive medial calcifications. A special subpopulation of VSMCs called 'calcifying vascular cells' exhibit osteoblastic characteristics [43] and excrete matrix vesicles produced by the smooth muscle cells. These matrix vesicles themselves excrete calcium which forms as extracellular clumps of hydroxyapatite deposited along elastin fibers. On top of this, these matrix vesicles stimulate vascular smooth muscle cells undergo transdifferentiation to an osteo/chondrogenic cell type and produce calcium as well [66, 67].

MAC is also found in uncommon monogenetic calcifying diseases such as pseudoxanthoma elasticum (PXE), arterial calcifications due to deficiency of CD73 (ACDC) and generalized arterial calcification of infancy (GACI). In these patients,

medial calcifications are frequently called 'Mönkeberg's medial calcific sclerosis' [68]. For the purposes of this dissertation the term MAC will be used. MAC are presumably a separate metabolic disease, causing arterial stiffness and limits remodeling of the arterial wall. This might attribute to obstructive disease in PAD [39, 69]. Therefore, a dominant role for MAC in the development of CLTI has been suggested [70]. Recently, it was shown that MAC drives atherosclerosis in the peripheral arteries [71].

3. Calcifications of the lower extremity arteries

A good oversight of lower extremity arterial calcifications per decade of age, location and the relation to ABI in a random population sample is lacking.
The only peripheral arteries that have been investigated for the prognostic value of calcifications are the abdominal aorta and iliac arteries. The calcifications seen on the lateral x-ray of the lumbar spine have been shown to be an excellent predictor for cardiovascular disease and mortality [72]. Recently these results were confirmed in the UK biobank cohort [73]. In another study, a high annularity score of calcifications of the abdominal aorta was predictive for all-cause mortality in patients without clinically evident vascular disease (3.66 (0.86–15.65)) [48]. Conversely, iliac arterial annularity scores were not significantly related to mortality. In the PESA study it was assessed that abdominal aortic/iliac calcifications are the first spots of calcifications that occur in the body [74]. This was later confirmed by Jadidi *et al.,* who also showed that when considering the whole aorta, calcifications start in the aortic iliac region [75].

There are some histologic studies where a random sample of the population has been investigated. Kamenskiy *et al.* describes a large tissue donor cohort of 431 patients ranging from 13 to 82 years of age [53]. They found that calcification of the femoropopliteal artery is common and can occur in young subjects who do not have intimal disease. Prevalence dramatically increases after 40 years of age. More calcification is associated with older age, diabetes mellitus, dyslipidemia, and high body mass index. Tobacco use has negative association with calcification. Calcified arteries are stiffer, larger in diameter, more diseased, and have more discontinuous elastic fibers. This is in line with the Pathobiological determinants of atherosclerosis in Youth study which concerns 2876 obductions. In the abdominal aorta, calcifications were already found in 1.5% of 15-year-olds [76]. Furthermore, they found that in the abdominal aorta at the age of 30-34 years 12.6% had calcified plaques and 65% had fibrous plaques, while in the thoracic aorta only 21% had fibrous plaques of which 0.8% were calcified.

In another study of a general elderly population, Vos *et al*. described the lower extremity specimen of 14 cadavers with a median age of 87 [77]. The atherosclerotic lesions they found were mostly non-atheromatous lesions classified as pathological intimal thickening or fibrocalcified plaque (80%). They found that atherosclerotic plaque and intimal calcification decreases from proximal to distal in the lower extremities, while medial calcifications increase in the same direction.

Symptomatic vascular calcifications in PAD and CLTI

For many years arterial wall abnormalities in PAD were thought to be similar to the coronary arteries with the classic 'atherosclerotic culprit lesion'. Histological studies have now shown that PAD is based on a different pathophysiological process [78]. In patients with PAD predominantly the atherosclerotic nonatheromatous fibrotic lesions were found, with only a few small atheromas. Second, extensive medial wall calcifications were found in the crural arteries more than in the femoropopliteal arteries [79]. The third abnormality found in PAD are thromboembolic and calcium particles which can occlude the lumen of the lower extremity artery, probably leading to CLTI. Spontaneous recanalization of the artery may occur [77, 79]. In patients with PAD or CLTI, arterial calcifications are directly associated with treatment outcome and mortality [80-82].

CLTI is the most severe subform of PAD and therefore there are differences between PAD and CLTI patients. At first, patients with CLTI had more severe arterial calcifications than patients with intermittent claudication [83]. Second, CLTI patients have a dominant pattern with calcifications located in the medial wall; even more than 70% of calcifications found in histologic samples of amputated legs are medial calcifications [70, 79, 84]. Since medial wall calcifications in CLTI are highly prevalent and might be an important factor in the evolution of PAD to it most severe form CLTI. Therefore, it is of clinical relevance to be able to differentiate medial as well as intimal calcifications.

Femoropopliteal and infrapopliteal/below-the knee arteries seem to differ in pathology with significantly more atherosclerosis in the femoropopliteal arteries than the infrapopliteal arteries. Hunter's canal, through which the femoral artery runs, is a location prone to atherogenesis [85]. Therefore, more intimal wall calcifications can be expected. These intimal lesions are not the atherosclerotic type, but there appears to be a pattern with 93% of the fibrocalcific lesion type [86]. Recently, a histopathologic post-mortem study that analyzed especially CLTI patients showed that medial calcifications are significantly more common in the crural than in femoropopliteal arteries (OR 2.89, p=0.08) [79].

Arterial calcifications on imaging

Arterial calcifications can be visualized with different imaging techniques. Plain film x-ray and CT scan are the most widely used while calcification imaging has been described with ultrasound and MRI but these later are rarely used for this purpose.

Calcifications can be detected on a non-contrast CT based on voxels that exceed 130 HU. The differentiation between medial and intimal calcifications can also be made on CT, as demonstrated in a histologically validated study in the intracranial internal carotid artery [87]. Based on three different characteristics of arterial calcifications; in other words; annularity, thickness and longitudinal continuity, differentiation between intimal (dotted, thick and patchy) and medial (annular, thin and continuous) calcifications in the carotid siphon was possible on CT [49, 87]. However, for other vascular regions this score has not been validated. See **Figure 1** for examples of medial and intimal types of calcifications on a CT of the lower extremities.

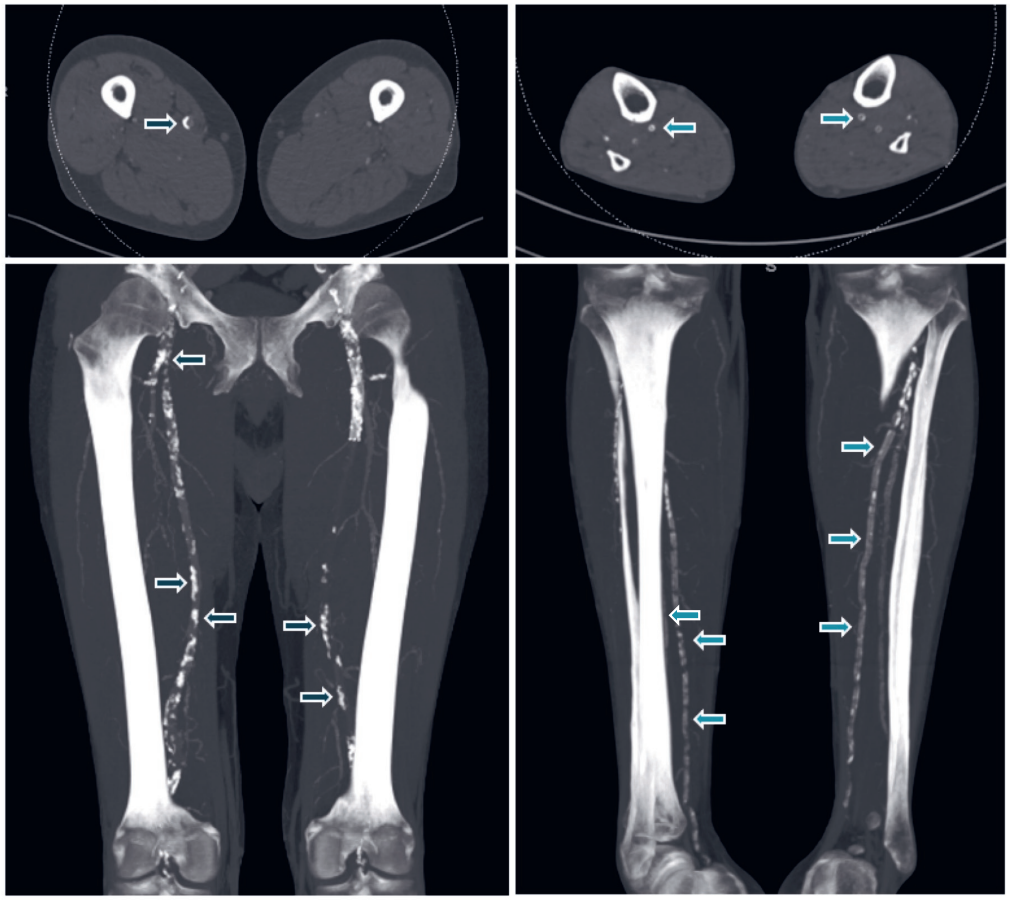
Already in 1950, Lindbom *et al.* visualized vascular calcifications of the legs on plain x-ray images and distinguished medial, intimal and internal elastic calcifications in the arteriograms of dissected specimens and compared this with their histological images [88]. In 1978, another study group successfully distinguished intimal from medial calcifications on plain photographs [89]. The latter study used these same imaging characteristics based on annularity, thickness and continuity. However, calcification patterns in the peripheral arteries are not examined based on 3D imaging methods like CT.

CTA and CT calcium score are both informative investigations for patients with cardiovascular diseases. Both methods use HU-values for material differentiation. CTA of the peripheral arteries in the lower limbs uses a cut-off value for iodinated blood of > 200 HU, a value maintained by many studies [90-93]. Over- and under estimation of this process is likely, since amongst others the HU-values of intravenous contrast overlaps with calcium.

Novel options are offered since the introduction of dual-energy, also called multi-energy or spectral CT (SCT). SCT is mainly used to improve dose efficiency compared to conventional CT [94, 95], but can also be used for spectral separation of materials and material quantification. Based on the attenuation coefficient as a function of energy μ_e, a linear combination of separate attenuation coefficient functions can be expressed [96]. The combined values of these attenuation factors are

material specific and therefore, theoretically, every material can be distinguished. Spectral CT opens up new possibilities for quantification of arterial wall disease.

Figure 1. Examples of calcification measurements in the arteries of the lower extremities. Axial thin and coronal maximum intensity projection (MIP) images.
Left two images: Severe patchy femoropopliteal calcifications (dark blue arrows). N.B. a stent has been placed in the left proximal femoral artery.
Right two images: Severe continuous annular and thin crural calcifications (light blue arrows).



4. Survival of CLTI and treatment strategies

Long-term survival CLTI patients

Although the prevalence of CLTI is increasing, the prognosis of patients with CLTI has improved over the years. Amputation rates and mortality have slightly declined [11], but are still high. Amputation rates decreased between 2000 and 2008 [97] from 263 to 188 per 100 000 [98]. This decrease is caused by improved treatment strategies.

Treatment strategies in CLTI

International guidelines recommended first to treat patients with CLTI by focusing on improving tissue perfusion in salvageable extremities [6]. In subsequent guidelines, reducing the risk of cardiovascular events was added by adequate patient management. These strategies include best pharmacological therapy, smoking cessation, healthy diet, weight loss and regular physical exercise [1].

Tissue perfusion can be improved through arterial revascularization. There are two options: open surgical procedure and endovascular procedures [6]. Between these two there are no significant differences in outcome [1, 99]. However, an endovascular approach is recommended in patients with poor physical condition, unfavorable anatomy for surgery, or advanced age [100]. In general, revascularization treatment is shifting towards endovascular procedures.

Endovascular treatment options include percutaneous transluminal angioplasty (PTA) with or without the placement of a bare metal stent (PTA±BMS). Over time, the implanted BMS often leads to in-stent restenosis caused by intimal hyperplasia [101-104]. Therefore, drug-eluting stents (DES) were developed.

Introduction of DES in cardiology

In-stent restenosis after BMS placement was identified early in cardiology. Subsequently, DES was developed and implemented. The first generation of DES (Sirolimus eluting stents (Cypher®) and paclitaxel eluting stents (Taxus®)) has been used in cardiology since 2003 and has been shown to reduce the risk of restenosis. Comparing paclitaxel-coated DES to BMS, no additional mortality risks have been found. A meta-analysis by Palmerini *et al*. resulted in a HR 1.00 of paclitaxel-coated DES compared to BMS (95% CI 0.82-1.20) [105]. However, after several years of use, in 2010, there was a debate about the long-term safety of drug-eluting balloons (DEB) and DES because of a relatively high myocardial infarction rate after treatment with paclitaxel-coated DES. Post-mortem histological examination showed that these risks were caused by late in-stent thrombosis, which in turn increased the number of

myocardial infarctions and death [106]. Afterwards, first-generation paclitaxel-coated DES were no longer in use in cardiology and replaced by the second-generation paclitaxel-, zotarolimus- and everolimus-coated DES. These second-generation stents had thinner struts so that reendothelialization of these struts was better accomplished. Indeed, these second-generation DES showed improved results with lower late in-stent thrombosis rates and subsequently even lower mortality rates, comparing first to second-generation DES [105, 107, 108].

The use of DES in PAD/CLTI

To prevent in-stent restenosis in BMS in the treatment of PAD and CLTI, first-generation paclitaxel-coated DES were introduced in 2009 [101-104] later followed by Second-generation DES (amongst others Eluvia-DES) with good short-term 2-year results [109]. Long-term follow-up data are still awaited.

In 2009, the Percutaneous transluminal Angioplasty versus Drug-eluting stents for Infrapopliteal lesions (PADI) Trial was initiated [110], to compare the performance of first-generation paclitaxel-coated DES and the standard treatment of percutaneous transluminal angioplasty with bail-out BMS (PTA±BMS) below the knee (BTK) in patients with CLTI. This randomized clinical trial showed a significantly better patency and a higher amputation-free and event-free survival at 5-years with paclitaxel-coated DES (TAXUS Liberté) compared with the current reference treatment PTA±BMS [14, 111]. These results are consistent with other studies that show lower restenosis rates in infrapopliteal lesions treated with DES in their follow-up [103, 112, 113].

However, in 2019 the American Food and Drug Authority in 2019 warned for the use of paclitaxel-coated drug-eluting devices because of possible adverse effects as found in two meta-analyses. These studies found an increased risk of 5-years mortality in patients treated with paclitaxel-coated drug-eluting balloons (DEB) and DES in the superficial femoral and popliteal artery [114], and a significantly worse 1-year amputation-free survival in patients treated with DEB below the knee [115]. This was followed by an international debate among interventional radiologists [116, 117]. So, we felt compelled to review our data to answer the question whether the use of paclitaxel-coated DES indeed led to poorer long-term survival.

Cost-effectiveness of DES

The use of DES has shown lower restenosis rates below the knee in multiple studies [101, 103, 104, 112, 118, 119]. Paclitaxel-coated DES in infrapopliteal CLTI improve both clinical and morphological long-term results compared to PTA with

bailout bare metal stenting (BMS) in patients with CLTI below the knee, as was shown in the PADI Trial [14, 111]. There are however no data on the cost-effectiveness of paclitaxel-coated DES in patients with infrapopliteal CLTI.

A few studies have been published about the costs of drug-coated devices, but these used different materials such as sirolimus- or everolimus-eluting stents, different locations such as the superficial femoral artery or only compared in-patient hospital costs [120-122].

These studies found that in the short-term, costs of DES were higher compared to the standard therapy by BMS. The study of Locham *et al.* compared the short-term costs for the Zilver-PTX DES in lesions above the knee to BMS. In a univariate analysis, the total median costs of endovascular revascularization were similar between DES versus BMS ($13,243 vs. $13,342, p=0.76) [122].

A meta-analysis showed that the use of DES up to 2 years after placement with patency as the primary outcome costed an average of $14,820 per patent limb, while BMS costed $14,237, and balloon angioplasty costed $9,918 [121]. Long-term studies regarding the real-world cost-effectiveness outcomes of DES are needed.

OBJECTIVES OF THIS DISSERTATION

Mortality rates in patients with CLTI have improved somewhat in recent decades. This is probably due to the more systemic use of anti-atherosclerotic medication; however, the prognosis of CLTI patients is still extremely poor. Remarkably, this persistent poor prognosis is mainly caused by cardiovascular diseases that apparently have a different cause than atherosclerosis.

A distinct process which can co-occur with atherosclerosis is MAC. These calcifications are increasingly recognized as an independent cause of arterial disease. The focus of this dissertation is on these calcifications. CLTI patients were compared with an asymptomatic control group. The prognostic significance of these calcification patterns and the extent of these calcifications in arterial areas other than the lower extremities were investigated.

Careful assessment of the amount of calcium present is important, but the use of cut-off values based on HU-values is not reliable and therefore other methods should be considered.

In previous studies, we have shown that for the treatment of CLTI patients, paclitaxel-coated DES give the best results. However, recent studies have published alarming data that the use of paclitaxel-coated DES could lead to higher mortality rates. So, we revisited our data with a focus on the paclitaxel dose. Furthermore, since there is currently no long-term cost-effectiveness data on the use of paclitaxel-coated DES we investigated this topic.

OUTLINE OF THIS DISSERTATION

This dissertation is divided into four parts, each containing several different chapters focusses on several aspects of CTLI.

PART I. OVERVIEW

Part I (***Chapter 1***) gives a comprehensive introduction and outline of this dissertation.

PART II. ARTERIAL CALFICATIONS IN CLTI PATIENTS

Arterial calcifications in the lower extremities are frequently found on a CT scan in asymptomatic people. We investigated the occurrence and patterns of calcifications in this group in ***Chapter 2.*** This group was used as control to compare with patients with CLTI.

In ***Chapter 3***, we compared calcification burden and pattern as seen on CT scans of the lower extremities, between patients with CLTI and controls. We also wanted to determine whether calcification burden and pattern were different in femoropopliteal and the crural arteries.

In ***Chapter 4,*** we evaluated the predictive value of these CT characteristics of lower extremity arterial calcification on both 7-year amputation-free survival and 10-year all-cause mortality in patients with CLTI.

Although CLTI is mostly treated with anti-atherosclerotic medication there still is a considerable burden of residual vascular disease. In ***Chapter 5*** we set out to investigate whether vascular calcification could be involved in this residual burden.

Accurate and reproducible assessment of arterial calcifications of the arterial wall is important for clinical management. Hounsfield Units (HU) based methods however are unreliable due to overlap in HU-value of different materials leading to over and under detection of these materials. In ***Chapter 6*** we investigated a novel material decomposition method by spectral CTA to solve this problem.

PART III. OUTCOME OF CHRONIC LIMB-THREATENING ISCHEMIA TREATMENT

In the PADI study we showed that CLTI patients treated with paclitaxel-coated DES compared with bare metal stents had better patency and less amputations. However, the American Food and Drug Authority (FDA) in 2019 warned for the use of paclitaxel-coated DES because two meta-analyses showed decreased survival using these stents.

In ***Chapter 7*** we reevaluated the 10-year survival of the PADI study related to the paclitaxel dose that our patients received.

Previous results from the PADI study favored paclitaxel-coated DES with less in-stent restenosis and less amputations. However, a cost-effectiveness study on paclitaxel-coated DES has not yet been carried out and has therefore been reported in ***Chapter 8.***

PART IV. SYNOPSIS AND GENERAL DISCUSSION

In ***Chapter 9*** a synopsis is provided. Finally, in ***Chapter 10*** we discuss the conclusions that can be drawn from the data presented in this dissertation and the remaining challenges for the future.

A Dutch summary can be found in ***Chapter 11.***

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Abbreviations

BMI	=	Body-mass index
BMS	=	Bare metal stent
CAD	=	Coronary artery disease
CKD	=	Chronic kidney disease
CT	=	Computed Tomography
CLTI	=	Chronic limb-threatening ischemia
DES	=	Drug-eluting stent
DM	=	Diabetes mellitus
eGFR	=	Electronic glomerular filtration rate(mL/min/1.73m^2)
Non-PAD	=	Patients without known peripheral arterial disease
OR	=	Odds ratio
PAD	=	Peripheral arterial disease
PADI Trial	=	Percutaneous transluminal Angioplasty and Drug-eluting stents for Infrapopliteal lesions in chronic limb ischemia Trial
PADI Imaging Trial	=	Percutaneous transluminal Angioplasty and Drug-eluting stents for Infrapopliteal lesions in chronic limb ischemia Imaging Trial
PTA	=	Percutaneous transluminal angioplasty
PXE	=	Pseudoxanthoma Elasticum
RCT	=	Randomized clinical trial

PART II.

Arterial Calcifications In CLTI Patients

CHAPTER 2.

CT Calcification Patterns of Peripheral Arteries in Patients Without Known Peripheral Arterial Disease☆

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STRUCTURAL ABSTRACT

Background and objectives

In the last few years histologic studies of peripheral arteries have shown that both intimal and medial calcifications are found in patients in an early, asymptomatic stage and that differentiation between medial and intimal calcifications is possible. The aim of this study was to assess the computed tomography (CT) calcification characteristics in peripheral arteries and to explore potential patterns in subjects without peripheral arterial disease (PAD).

Materials and methods

Retrospectively, 204 patients without known PAD were studied. The thin slice CT-imaging characteristics severity, annularity, thickness and continuity were scored in the following arteries: plantar and dorsal, crural, femoropopliteal, iliac and the abdominal aorta. Interrelation was assessed using linear regression and significance was tested by Chi-Square tests.

Results

In the crural arteries two calcification patterns with strong associations were found. Pattern 1: continuous-annular 93.5 % (29/31), continuous-thin and thin-annular both 73 % (27/37, $p < 0.001$) and pattern 2: thick-discontinuous 91.7 % (44/48), thick-dotted 68.8 % (33/48), patchy-dotted 59.3 % (16/27, $p < 0.001$). Similar associations were found in the femoropopliteal artery, but not in the plantar, dorsal, iliac arteries and aorta.

Conclusions

In the crural and femoropopliteal arteries at least two morphological patterns can be distinguished on CT that, compared to a CT-histologically validated score, may represent an intimal and medial calcification pattern.

Key words: chronic limb-threatening ischemia, atherosclerosis, medial calcifications, intimal calcifications

INTRODUCTION

Knowledge about calcifications in peripheral arteries has considerably increased the last few years [1-5]. Several histologic studies on people without vascular symptoms or early vascular disease have shown that vascular calcifications already exist in a very early stage [6, 7] and that the importance of these calcifications increases when overt peripheral arterial disease (PAD) or chronic limb-threatening ischemia (CLTI) is present [8-10]. These histologic studies have shown that in peripheral arteries both intimal and medial calcifications are highly prevalent. Medial and intimal calcifications represent different pathophysiological pathways but the precise meaning of different calcifications is not yet fully understood. These two types of calcifications can be easily distinguished with histology but up to now differentiation with imaging techniques have only been described by Orr et al for plain x-ray techniques of the legs and recently by Kockelkoren et al for computed tomography (CT) scan of the head [10, 11]. In the latter study, based on three different characteristics of vascular calcifications i.e., annularity, thickness and longitudinal continuity, differentiation between intimal (dotted, thick and patchy) and medial (annular, thin and continuous) calcifications in the carotid siphon was possible on CT [11, 12].

The aim of this study was to assess the CT calcification characteristics in peripheral arteries in subjects without PAD.

MATERIALS AND METHODS

Study approval

The medical ethical board of the University Medical Center Utrecht (UMCU) waived review of this study because of its non-invasive, retrospective character (study number: 17-897/C). This study was also approved by the ethical board of the Haga Teaching Hospital (study number: T18-003).

Patient selection

204 patients of a university hospital and a large community teaching hospital in the Netherlands were included. Patients were scanned in the period between June 2011 and March 2017.

The Picture Archiving and Communication System (PACS) data system was retrospectively searched for 18F-FDG full-body Positron Emission Tomography (PET)/CT performed for melanoma, unknown fever or lymphadenopathy. These

scans were linked to medical record data on cardiovascular risk factors and symptomatic PAD status.

150 consecutive patients of the university hospital were selected who were scanned for melanoma staging (n=91), infection (n=33), unknown fever (n=23) and lymphadenopathy (n=1). Two patients were excluded; one because of incomplete scanning, the other because clinical data could not be retrieved, none had known symptomatic PAD. In total, 148 patients of the university hospital were included. 72 consecutive patients of the community teaching hospital who were all scanned for melanoma staging were selected for this study. Fifteen patients were excluded due to incomplete scanning of the upper and lower extremity, while one paediatric patient was excluded, resulting in 56 patients. Finally, full body calcification distribution was investigated in 204 patients without any known PAD from both hospitals (For inclusion chart, see **Supplemental Figure 1**).

Baseline measurements, definitions and characteristics

The following variables were included for analysis: age, gender, diabetes mellitus (DM), weight, current smoking status, systolic blood pressure, diastolic blood pressure and renal function (eGFR). Obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2. Hypertension was defined as a systolic blood pressure at admission > 140mmHg or diastolic blood pressure of > 90 mmHg. Chronic kidney disease (CKD) was defined as an eGFR lower than 60 mL/min/1.73 m^2.
Mean age was 61.3 years (range 22 - 90; SD 16). There were 52.0% male participants. The most prevalent risk factor was hypertension, followed by obesity, smoking, CKD and DM. The demographic characteristics of the total cohort are shown in **Table 1.**

Imaging and Measurements

1.Scanning protocol

All patients underwent imaging with a standard whole-body PET/CT protocol. According to the standard PET-CT scan protocols in our hospitals, no intravenous contrast was used.

In the university hospital, whole-body PET/CT scans were performed on a Siemens Biograph 40 scanner (Siemens Healthineers, Erlangen, Germany). One series of the protocol was a low-dose (approximately 434 mGray*cm for a 70 kg adult) whole-body CT scan from the toes to the vertex of the skull. All images were obtained during one single session scanning time, with a slice thickness of 0.625 mm. CT data were obtained with a spiral acquisition and automated dose modulation (maximum 100 mA and 120 kVp; reconstruction slice thickness 3 mm).

Patients in the large community hospital were scanned on a Siemens PET/CT Biograph 64 scanner (Siemens Healthineers, Erlangen, Germany). This low-dose whole-body CT scan from the toes to the vertex of the skull used approximately 411 mGray*cm for a 70 kg adult. All images were obtained during a single session scanning time, spiral acquisition and automated dose modulation (120 kVp, maximum 160 mA for the heaviest patient; reconstruction slice thickness 3 mm). In both hospitals, only the low dose whole-Body CT scan was used for this study, FDG-PET images were not used for analysis.

2.Systematic assessment of calcification patterns

The calcification measurements were done according the recently developed and CT-histologically validated score for the carotid siphon by Kockelkoren et al [11]. The observers were blinded to the patients' clinical data during scoring. Measurements of 20 patients were performed together by a radiology resident (LK) and an experienced radiologist (PdJ) which had extensive experience in the use of this scoring system. During this training, measurements were checked for comparability and consensus was achieved through discussion. The remaining measurements were performed by LK.

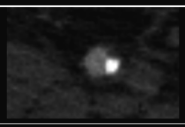
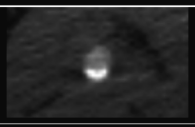
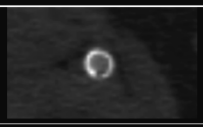
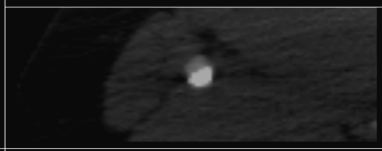
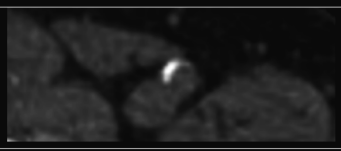
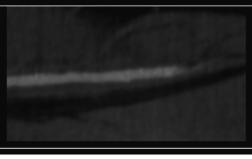
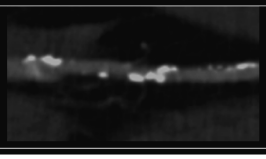
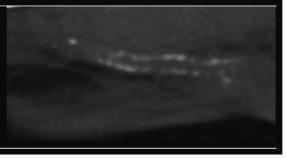
Bone window settings were used for evaluation of calcification (Window Settings: Window = 300 Hounsfield Units; Width = 1600 Hounsfield Units). The following five vascular beds were studied: i.e., plantar and dorsal arteries, crural arteries, femoropopliteal arteries, iliac arteries and the abdominal aorta. In all vascular beds the following imaging characteristics were scored. The severity of the calcifications was scored by a previously tested reproducible semi-quantitative scoring system (inter-observer kappa 0.54-0.99), and is divided into four categories; absent, mild, moderate and severe [13]. The severity was scored in the longitudinal plane. 'Absent' was scored when there were no calcifications, 'mild calcification' in case of 1-3 small calcifications, 'moderate calcification' in case of 4-8 small calcifications and less than three large calcifications, 'severe calcification' in case of more than 9 small calcifications or at least three large calcifications on the CT slides of the artery. The definition of sizes of different small and large calcifications were stated on an extension of previous work [13-16]. Small calcifications are defined as calcifications that are visible on one or two slices, while large calcifications are visible on more than three slices. This applies to both the femoropopliteal and crural arteries. When scoring the severity of calcifications, the other calcification characteristics were not taken into account. Annularity was scored as absent, dot(s),

<90°, 90-270° or 270-360°, thickness as absent, ≥1.5mm or <1.5mm and continuity as indistinguishable, irregular/ patchy or continuous. A discontinuous pattern was noted as indistinguishable or irregular/patchy. Annularity was scored in the axial plane, thickness and continuity in the longitudinal plane. Regarding the calcification characteristics; the most extensive calcification characteristic found in the arterial territory determined was scored; e.g., for annularity the most annular calcification; for thickness the thickest calcification; and for continuity the most continuous calcification. See **Table 1** for an overview of the scoring system.

Statistical analysis

Data analysis was carried out using SPSS Statistics for Windows, Version 24.0 (IBM Corporation, Armonk, United States). Demographics were determined and normal distribution was tested by Quantile-quantile (QQ) plots. Continuous baseline variables were characterized using means and standard deviations; categorical variables were characterized by proportions. Regarding the calcification characteristic severity, this was recoded into a binary variable present or absent and afterwards percentages were calculated. Presence of calcifications was analyzed by stacked figures (age categorized per 20 years) for all morphological characteristics. Linear regression was used to test the difference between the expected frequencies and the observed frequencies for annularity, thickness and continuity in all arterial territories. Significance was tested by two-sided Chi-Square tests. A p-value less than 0.05 was considered to be significant.

Table 1. Classification system for calcification characteristics in the peripheral arteries of the lower extremity. Window Settings: window = 300 Hounsfield Units; width = 1600 Hounsfield Units (bone window).

Characteristic	Subcategories			
Annularity (cross-sectional)	Dot(s)	<90°	90-270°	270-360°
				
Thickness (cross-sectional)	Thick ≥ 1.5mm		Thin < 1.5mm	
				
Continuity (longitudinal)	Indistinguishable	Irregular/Patchy	Continuous	
				

RESULTS

Prevalence

When only the presence or absence of calcifications was considered, a wide range was seen between the different arterial territories. The lowest prevalence of calcifications was found in the plantar and dorsal arteries 8.3% (17/204) with an increasing prevalence in the crural arteries 24.0% (49/204), femoropopliteal 30.4% (62/204, iliac arteries 31.9 % (65/204), while the highest prevalence was found in the abdominal aorta 41.2% (84/204). See **Table 2.**

Table 2. Demographic characteristics of the non-vascular cohort (n = 204)

Age (years)	61.3 ± 16.4
20 – 40 (years)	25 (12.3%)
40 – 60 (years)	64 (31.4%)
60 – 80 (years)	91 (44.6)
80 – 100 (years)	24 (11.8%)
Gender (male)	106 (52.0%)
Systolic blood pressure (mmHg)	136 ± 21
Diastolic blood pressure (mmHg)	79 ± 12
Hypertension	94 (46.1%)
Body Mass Index (kg/m^2)	27.4 ± 5.8
Obesity (>30 kg/m^2)	86 (42.2%)
Smoking	59 (28.9%)
Diabetes Mellitus	17 (8.3%)
Glomerular filtration rate (mL/min/1.73m^2)	69 ± 22
Chronic kidney disease (eGFR<60 mL/min/1.73m^2)	37 (18.1%)

Continuous variables described as mean ± SD, categorical variables as number (%).

Correlation of different CT morphological calcification characteristics

Linear regression analysis of the different characteristics of calcifications on CT was performed for all different vascular territories. A compilation of these results is given in **Table 3.** Overall, we observed that clusters of CT characteristics that have been validated for intimal (dotted, thick and patchy) or medial calcifications (annular, thin and continuous) have a high level of agreement with each other, especially in the crural segments and to a lesser extent in the femoropopliteal segments. To specify: in the crural arteries, the majority of thin calcifications also had annular and continuous calcifications (73.0%, 27/37, p<0.001). In addition, patients with continuous calcifications had annular calcifications in almost all cases (93.5%, 29/31, p<0.001). On the other hand, patients with dotted non-annular calcifications had in 68.8% (33/48, p<0.001) of cases thick lesions and in 59.3% patchy calcifications (16/27, p<0.001). Patchy and thick calcifications also had a large overlap (47.9%, 23/48, p<0.001).

In the femoropopliteal arteries, the various media characteristics in particular showed a mutual association annular/continuous 80.5% (33/41), annular/thin 64.3% (27/42), continuous/thin 78.6% (33/42). There was no significance between the

different medial and intimal characteristics, for example thick and continuous only 8.3% had overlap between these characteristics (See **Table 4** for the crural arteries and **Supplemental Tables** for all other vascular territories).
These two different patterns of clusters of CT characteristics were also present in the other vascular beds but the associations were less strong (See **Supplemental Tables 1 A – D**).

Table 3. Distribution of different CT calcification patterns.

	Medial calcification pattern			Intimal calcification pattern		
Arteries	Annular/ Thin	Continuous/ Thin	Annular/ Continuous	Dotted/ patchy	Dotted / thick	Patchy / Thick
Plantar and dorsal	9/25 (36%)	12/25 (48.0%)	9/12 (75.0%)	3/5 (60.0%)	9/10 (90.0%)	2/10 (20.0%)
Crural	27/37 (73%)	27/37 (73%)	29/31 (93.5%)	16/27 (59.3%)	33/48 (68.8%)	23/48 (47.9%)
Femoropopliteal	27/42 (64.3%)	33/42 (78.6%)	33/41 (80.5%)	7/44 (15.9%)	23/64 (35.9%)	38/64 (59.4%)
Iliac	5/20 (25.0%)	8/20 (40.0%)	11/19 (57.9%)	6/89 (6.4%)	16/113 (14.2%)	86/113 (76.1%)
Abdominal aorta	10/19 (52.6%)	11/19 (57.9%)	51/60 (85.0%)	5/63 (7.9%)	16/123 (13.0%)	58/123 (47.2%)
Thoracic aorta	3/17 (17.6%)	5/17 (29.4%)	11/13 (78.6%)	2/62 (3.1%)	19/88 (21.6%)	60/88 (68.2%)

Data presented in n%, tested by linear regression, $p<0.001$.
For full details see **Supplementals**.

Morphological calcification characteristics at different ages

Most of the calcifications first appeared between the ages of 40 and 60, and in older age the severity of the calcification increased dramatically with the percentage of severe calcifications going from a few percentages between 40-60 years to as high a percentage of 80% between 80-90 years. In small numbers we also observed calcifications in our youngest age groups. See **Figure 1** for this overview and **Supplemental Table 2** for a more detailed overview of different severity categories (absent, mild, moderate, severe) in different age categories. Annularity, thickness

and continuity started to increase from approximately 60 years of age and are given for all arteries in the stacked graphs of **Supplemental Figures 2, 3, 4.**

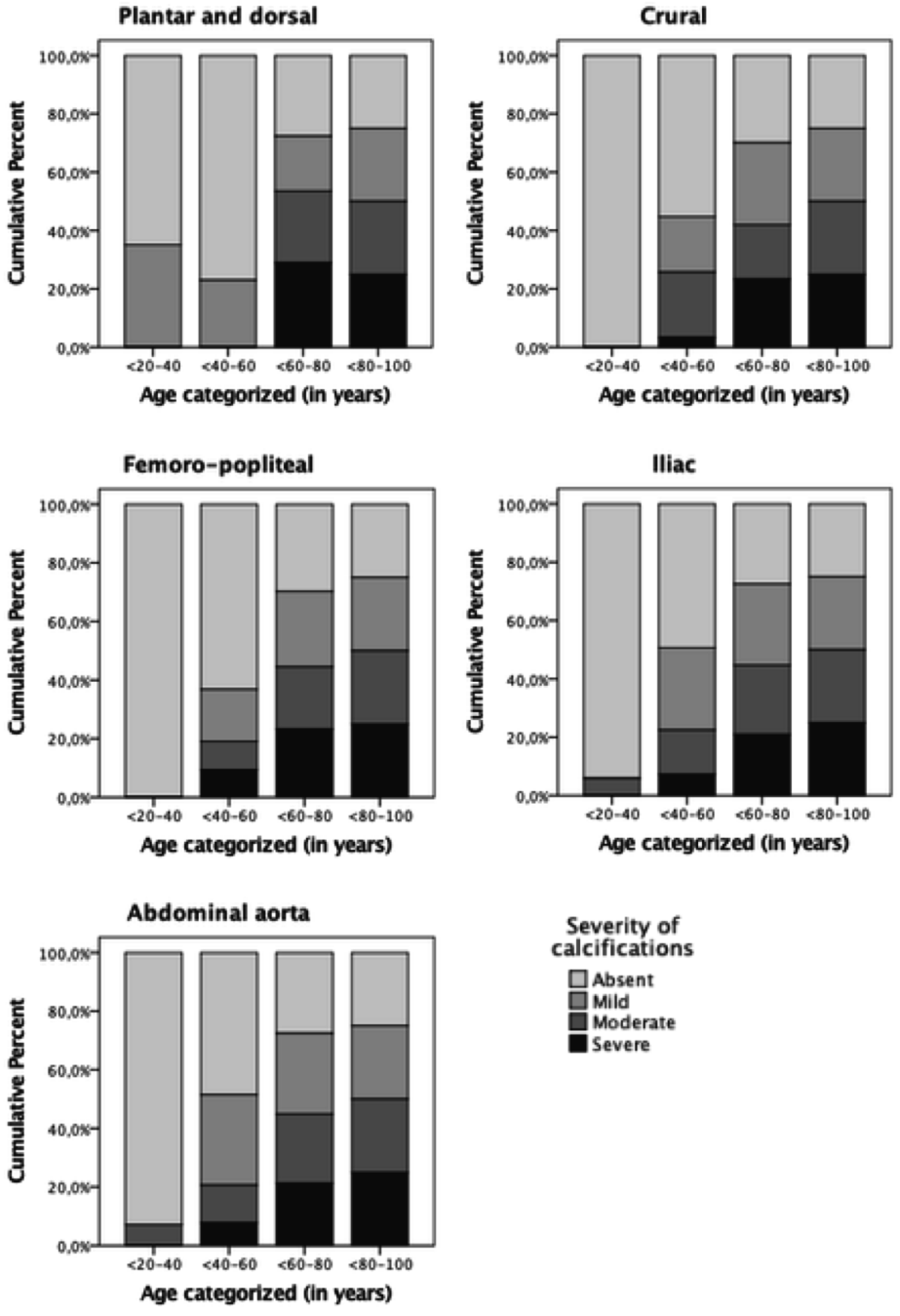
Table 4. Distribution of annularity, thickness and continuity in the crural arteries.

	Annularity of calcifications in crural arteries					
Thickness	No calcifications	Dot(s)	<90°	90-270°	270-360°	Total
Absent/not evaluable	118 (99.2%)	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	119 (100%)
Thick (>1.5mm)	0 (0.0%)	33 (68.8%)	8 (16.7%)	3 (6.3%)	4 (8.3%)	48 (100%)
Thin (≤1.5mm)	0 (0.0%)	6 (16.2%)	0 (0.0%)	4 (10.8%)	27 (73.0%)	37 (100%)
	Annularity of calcifications in crural arteries					
Continuity	No calcifications	Dot(s)	<90°	90-270°	270-360°	Total
Dot(s)	118 (80.8%)	24 (16.4%)	1 (0.7%)	2 (1.4%)	1 (0.7%)	146 (100%)
Irregular/ Patchy	0 (0.0%)	16 (59.3%)	6 (22.2%)	4 (14.8%)	1 (3.7%)	27 (100%)
Continuous	0 (0.0%)	0 (0.0%)	1 (3.2%)	1 (3.2%)	29 (93.5%)	31 (100%)

	Continuity of calcification in crural arteries			
Thickness	Indistinguishable	Irregular/patchy	Continuous	Total
Immeasurable	119 (100.0%)	0 (0.0%)	0 (0.0%)	119 (100%)
Thick (>1.5mm)	21 (43.8%)	23 (47.9%)	4 (8.3%)	48 (100%)
Thin (≤1.5mm)	6 (16.2%)	4 (10.8%)	27 (73.0%)	37 (100%)

Data are numbers of calcifications, with percentages in parenthesis, $p < 0.001$.

Figure 1. Severity of calcifications in different vascular beds categorized by age



DISCUSSION

This current study shows a strong correlation between the annular, thin and continuous CT calcification characteristics on the one hand and dot-like, thick and patchy on the other in the femoral and crural arteries in this cohort without any known PAD. These two separate calcification patterns indicate medial and intimal calcifications. We also showed a wide range of calcification severity in the different vascular territories with a broad age spread. This article adds to the limited literature in non-PAD patients on the prevalence of calcification, severity and patterns for peripheral arterial territories and helps to understand the role of calcification in the development of PAD.

Up to now few attempts have been made to extract information from CT imaging characteristics of arterial calcifications. Criqui *et al.* did show that the density of calcifications was related to less severe clinical outcome [17]. Williams et al showed that coronary calcification with a high Agatston score consisting of one piece of calcium, compared to a comparable Agatston score consisting of many pieces, had a much worse outcome [18]. Finally, annularity was investigated by Hendriks et al, and showed that the degree of annularity of the abdominal aorta was predictive for all-cause mortality [19].

Based on previous work we believe that these two patterns of calcification represent intimal and medial calcifications, based on the work of Orr in 1978 and more recently, in 2017 by Kockelkoren *et al.* Orr showed that plain x-ray images of vascular calcifications could differentiate between intimal and medial calcifications.[10] Intimal calcifications were considered to be irregular, discontinuous or patchy and dense clumps while medial calcifications were continuous, diffuse fine grained and circumferential. In 39 arteries from autopsies based on these characteristics, 92% of calcifications were classified correctly. Plain x-ray images however are two-dimensional and not so easy to standardize and quantify. Recently Kockelkoren *et al.*, using similar characteristics as Orr, showed in a histology-validated study that the CT characteristics annularity, thickness and morphology could reasonably well differentiate the two types of calcifications in the carotid siphon [11]. Annular, thin and longitudinal continuous being characteristic for medial and dotted, thick and irregular/patchy being characteristic for intimal calcifications. If there was little calcium, the results were less convincing, probably due to the fact that early calcifications can look comparable for both types of calcifications. The advantage of CT is its 3-dimensional information, easy to

standardize and quantitate and wide availability. This CT-based classification system was used in the peripheral arteries. The high correlation between these CT characteristics indicated that these characteristics may be used to distinguish between different types of calcifications. We were unable to show a good correlation between the CT characteristics in the iliac, abdominal aorta and plantar and dorsal arteries. In the latter, probably the calcifications are too small to discern CT characteristics reliably. Regarding the iliac artery and the abdominal aorta, a possible explanation could be that in these arteries intimal disease or atherosclerosis is by far the most dominant type.

The two types of vascular calcification probably represent two different diseases. Intimal calcifications are related to atherosclerotic disease and represent calcified plaques. Medial calcifications are located in the medial layer of the vessel wall, frequently are annular and lead to a stiff vessel wall increasing pulse pressure. Medial calcifications are probably caused by a disbalance between pro- and anti-calcifying agents and are found in chronic kidney disease, diabetes and aging [2].

With a good imaging method, we are able to differentiate between intimal and medial calcifications and epidemiological studies can be conducted to gain more insight into the clinical significance of these calcifications. Such studies have recently been conducted for the calcification of the carotid siphon [11, 12].

In patients with PAD or CLTI, arterial calcifications are directly associated with treatment outcome and mortality [20-22]. In CLI, the majority of calcifications found in histologic samples of amputated legs are calcifications located in the medial wall [8, 9]. Peripheral arterial calcification in this patient group is independently associated with the severity of the disease; patients with CLTI had more severe vascular calcifications than patients with intermittent claudication [5]. Since medial wall calcifications in CLTI are highly prevalent and might be an important factor in the evolution of PAD to it most severe form CLTI, it is of clinical relevance to be able to differentiate medial as well as intimal calcifications.

Recently, we showed that the bisphosphate etidronate can halt the progression of medial calcification in patients with Pseudoxantoma Elastica (PXE) [23, 24]. With regard to this possible future treatment of media calcifications in patients with PAD/CLI, we must first identify the severity of media calcifications and the specific patient groups that will benefit most from this. This study was a first step in these investigations. Since in many clinical cases patients with PAD and CLTI already

undergo CT angiography in the standard path of care, and the measurement of severity and annularity is easy to perform, we do not expect an increase in the burden on radiology departments if these techniques might be added to standard of care.

Several limitations deserve mention. We did not perform a histologic CT-imaging correlation study to validate our study. Such a study is considered as reference standard. However, we have done a similar carotid artery study which we used as a reference for this study. Another limitation of this study is that we have looked at the presence or absence of symptomatic PAD, while subclinical PAD could already be present. This could mean that a small number of patients with subclinical PAD might have been included.
Another concern is that histological studies have shown that intimal and medial calcifications often occur simultaneously, making differentiation difficult in some patients and the score mainly represents the dominant pattern in a subject.

Conclusions

In the femoropopliteal and crural arteries two clusters of CT calcification characteristics can be found that, compared to the CT-histologically validated score, indicate intimal and medial arterial calcification. Since both types of calcifications have different pathophysiological pathways, it is important to be able to differentiate between these calcification types.

Conflicts of interest statement

All authors declare that they have received no grants, contracts, other forms of financial supports or relationships with the industry relevant to this paper.

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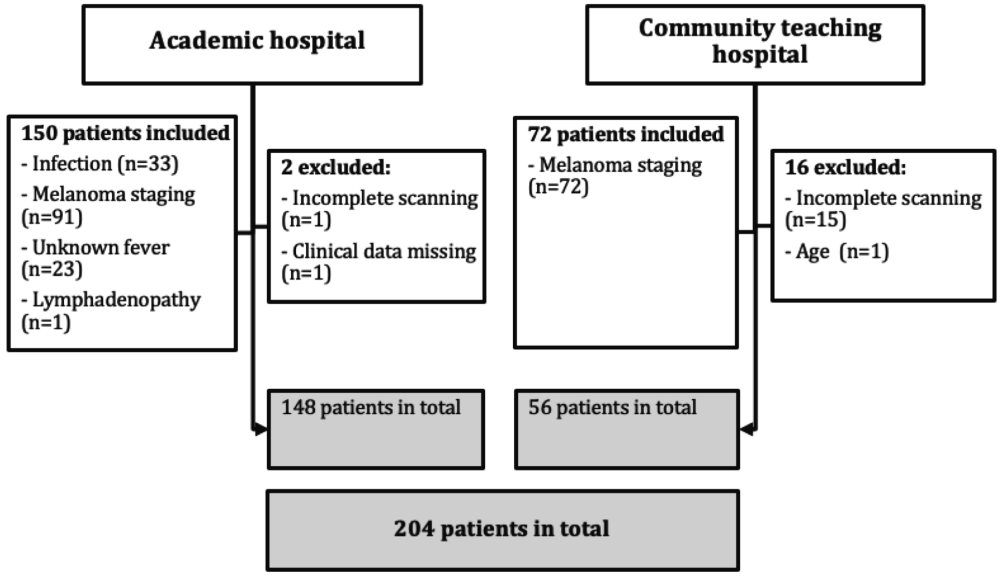
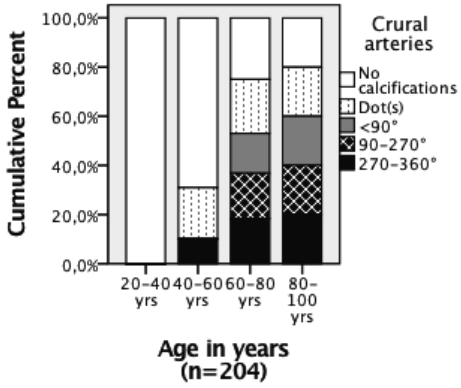
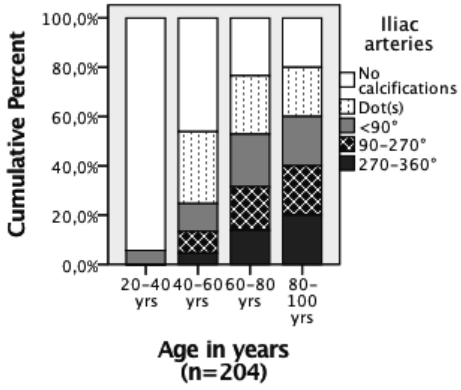
SUPPLEMENTAL FIGURES AND TABLES

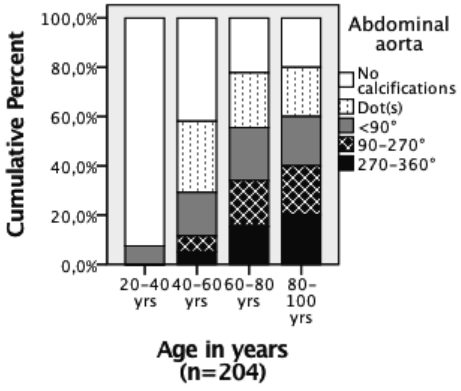
Supplemental Figure 1. Patient inclusion flow-chart.



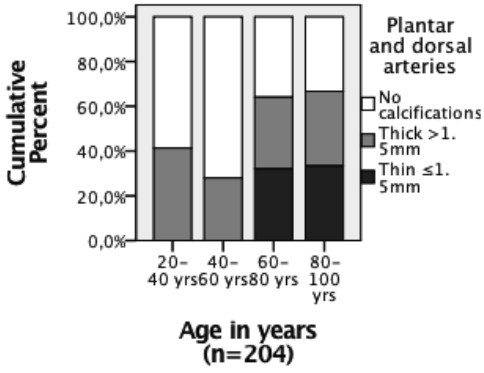
Supplemental Table 2. Severity of calcifications in all arteries categorized by age.

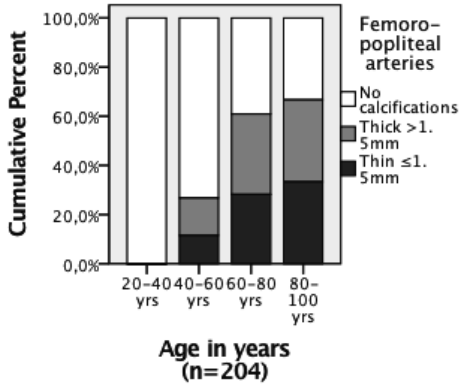


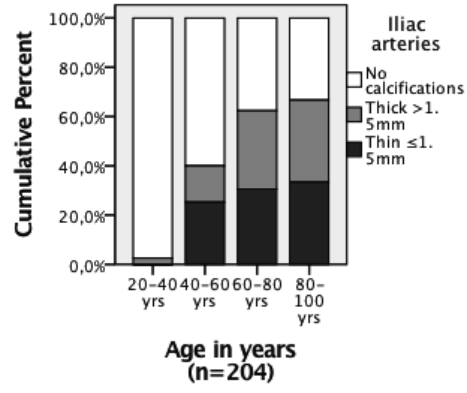


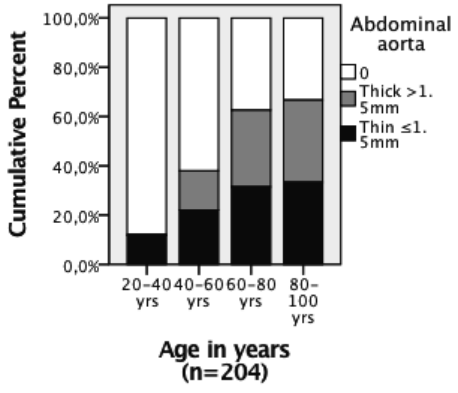


Supplemental Figure 3. Thickness of calcifications

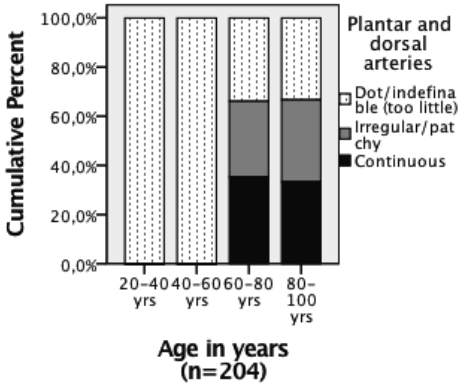


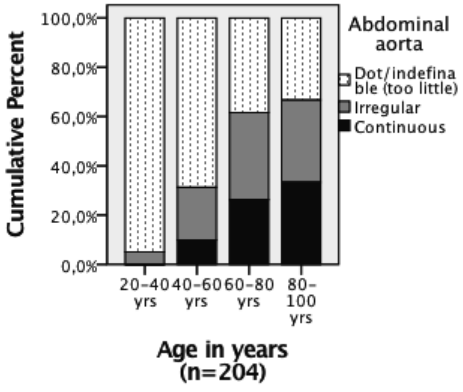






Supplemental Figure 4. Continuity of calcifications.





Supplemental Tables 1 A-D. Linear regression for morphological characteristics of all arteries except crural arteries (crural arteries are shown in the main article).

Supplemental Table 1A. Plantar and dorsal arteries

			Annularity of calcifications in plantar and dorsal arteries					Total
			No calcification	Dot(s)	< 90°	90-270°	270-360°	
Thickness of calcifications in plantar and dorsal arteries	Absent	N	169	0	0	0	0	169
		%	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
	Thick >1.5mm	N	0	9	0	1	0	10
		%	0.0%	90.0%	0.0%	10.0%	0,0%	100.0%
	Thin ≤1.5mm	N	0	10	4	2	9	25
		%	0.0%	40.0%	16.0%	8.0%	36.0%	100.0%

			Annularity of calcifications in plantar and dorsal arteries					Total
			No calcification	Dot(s)	< 90°	90-270°	270-360°	
Continuity of calcifications in plantar and dorsal arteries	Dot(s)	N	169	15	2	1	0	187
		%	90.4%	80%	1.1%	0.5%	0.0 %	100.0%
	Irregular/patchy	N	0	3	1	1	0	5
		%	0.0%	60.0%	20.0%	20.0%	00%	100.0%
	Continuous	N	0	1	1	1	9	12
		%	0.0%	8.3%	8.3%	8.3%	75.0 %	100.0%

			Continuity of calcifications in plantar and dorsal arteries			Total
			Dot(s)	Irregular/patchy	Continuous	
Thickness of calcifications in plantar and dorsal arteries	Absent	N	169	0	0	169
		%	100.0%	0.0%	0.0%	100.0%
	Thick >1.5mm	N	8	2	0	10
		%	80.0%	20.0%	0.0%	100.0%
	Thin ≤1.5mm	N	10	3	12	25
		%	40.0%	12.0%	48.0%	100.0%
Total		N	187	5	12	204
		%	91.7%	2.5%	5.9%	100.0%

Supplemental Table 1B. Femoropopliteal arteries.

			Annularity of calcifications in femoropopliteal arteries					Total
			No calcifications	Dot(s)	<90°	90-270°	270-360°	
Continuity of calcifications in femoropopliteal arteries	Dot(s)	N	97	18	4	0	0	119
		%	81.5%	15.1%	3.4%	0.0%	0.0%	100.0%
	Irregular/patchy	N	0	7	16	18	3	44
		%	0.0%	15.9%	36.4%	40.9%	6.8%	100.0%
	Continuous	N	0	0	3	5	33	41
		%	0.0%	0.0%	7.3%	12.2%	80.5%	100.0%

			Annularity of calcifications in femoropopliteal arteries					Total
			No calcifications	Dot(s)	<90°	90-270°	270-360°	
Thickness of calcifications in femoropopliteal arteries	Absent	N	97	0	1	0	0	98
		%	99.0%	0.0%	1.0%	0.0%	0.0%	100.0%
	Thick >1.5mm	N	0	23	15	17	9	64
		%	0.0%	35.9%	23.4%	26.6%	14.1%	100.0%
	Thin ≤1.5mm	N	0	2	7	6	27	42
		%	0.0%	4.8%	16.7%	14.3%	64.3%	100.0%

			Continuity of calcifications in femoropopliteal arteries			Total
			Dot(s)	Irregular/patchy	Continuous	
Thickness of calcifications in femoropopliteal arteries	Absent	N	97	1	0	98
		%	99.0%	1.0%	0.0%	100.0%
	Thick >1.5mm	N	18	38	8	64
		%	28.1%	59.4%	12.5%	100.0%
	Thin ≤1.5mm	N	4	5	33	42
		%	9.5%	11.9%	78.6%	100.0%

Supplemental Table 1C. Iliac arteries

			Annularity of calcifications in iliac arteries					Total
			No calcifications	Dot(s)	<90°	90-270°	270-360°	
Thickness of calcifications in iliac arteries	Absent	N	71	0	0	0	0	71
		%	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
	Thick >1.5mm	N	0	19	41	46	7	113
		%	0,0%	16.8%	36.3%	40.7%	6.2%	100.0%
	Thin ≤1.5mm	N	0	2	8	5	5	20
		%	0,0%	10.0%	40.0%	25.0%	25.0%	100.0%

			Annularity of calcifications in iliac arteries					Total
			No calcifications	Dot(s)	<90°	90-270°	270-360°	
Continuity of calcifications in iliac arteries	Dot(s)	N	71	15	4	1	0	91
		%	78.0%	16.5%	4.4%	1.1%	0.0%	100.0%
	Irregular/patchy	N	0	6	42	45	1	94
		%	0.0%	6.4%	44.7%	47.9%	1.1%	100.0%
	Continuous	N	0	0	3	5	11	19
		%	0.0%	0.0%	15.8%	26.3%	57.9%	100.0%

			Continuity of calcifications in iliac arteries			Total
			Dot(s)	Irregular/patchy	Continuous	
Thickness	Absent	N	71	0	0	71
		%	100.0%	0.0%	0.0%	100.0%
	Thick >1.5mm	N	16	86	11	113
		%	14.2%	76.1%	9.7%	100.0%
	Thin ≤1.5mm	N	4	8	8	20
		%	20.0%	40.0%	40.0%	100.0%

Supplemental Table 1D. Abdominal aorta

			Annularity of calcifications in the abdominal aorta					Total
			No calcifications	Dot(s)	<90°	90-270°	270-360°	
Thickness	Absent	N	62	0	0	0	0	62
		%	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
	Thick >1.5mm	N	0	16	30	32	45	123
		%	0.0%	13.0%	24.4%	26.0%	36.6%	100.0%
	Thin ≤1.5mm	N	0	2	2	5	10	19
		%	0.0%	10.5%	10.5%	26.3%	52.6%	100.0%

			Annularity of calcifications in the abdominal aorta					Total
			No calcifications	Dot(s)	<90°	90-270°	270-360°	
Continuity	Dot(s)	N	62	13	5	1	0	81
		%	76.5%	16.0%	6.2%	1.2%	0.0%	100.0%
	Irregular/patchy	N	0	5	25	29	4	63
		%	0.0%	7.9%	39.7%	46.0%	6.3%	100.0%
	Continuous	N	0	0	2	7	51	60
		%	0.0%	0.0%	3.3%	11.7%	85.0%	100.0%

			Continuity of calcifications in the abdominal aorta			Total
			Dot(s)	Irregular/ patchy	Continuous	
Thickness	Absent	N	62	0	0	62
		%	100.0%	0.0%	0.0%	100.0%
	Thick >1.5mm	N	16	58	49	123
		%	13.0%	47.2%	39.8%	100.0%
	Thin ≤1.5mm	N	3	5	11	19
		%	15.8%	26.3%	57.9%	100.0%

Supplemental Table 2. Severity of calcifications in all arteries categorized by age.

			Age (years)				
			0-20	20-40	40-60	60-80	80-100
Plantar and dorsal arteries	Absent	N	0	24	63	65	17
		%	0.0%	14.2%	37.3%	38.5%	10.1%
	Mild	N	0	1	1	6	5
		%	0.0%	7.7%	7.7%	46.2%	38.5%
	Moderate	N	0	0	0	4	1
		%	0.0%	0.0%	0.0%	80.0%	20.0%
	Severe	N	0	0	0	16	1
		%	0.0%	0.0%	0.0%	94.1%	5.9%
Crural arteries	Absent	N	0	25	53	36	4
		%	0.0%	21.2%	44.9%	30.5%	3.4%
	Mild	N	0	0	5	15	2
		%	0.0%	0.0%	22.7%	68.2%	9.1%
	Moderate	N	0	0	4	5	6
		%	0.0%	0.0%	26.7%	33.3%	40.0%
	Severe	N	0	0	2	35	12
		%	0.0%	0.0%	4.1%	71.4%	24.5%
Femoro-popliteal arteries	Absent	N	0	25	49	22	1
		%	0.0%	25.8%	50.5%	22.7%	1.0%
	Mild	N	0	0	6	18	4
		%	0.0%	0.0%	21.4%	64.3%	14.3%
	Moderate	N	0	0	2	10	5
		%	0.0%	0.0%	11.8%	58.8%	29.4%
	Severe	N	0	0	7	41	14
		%	0.0%	0.0%	11.3%	66.1%	22.6%
Iliac arteries	Absent	N	0	24	35	11	1
		%	0.0%	33.8%	49.3%	15.5%	1.4%
	Mild	N	0	0	10	11	0
		%	0.0%	0.0%	47.6%	52.4%	0.0%
	Moderate	N	0	1	11	28	7
		%	0.0%	2.1%	23.4%	59.6%	14.9%
	Severe	N	0	0	8	41	16
		%	0.0%	0.0%	12.3%	63.1%	24.6%

CHAPTER 3.

Differences in Lower Extremity Arterial Calcification Patterns in Patients with Chronic Limb-Threatening Ischemia Compared to Control Patients

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STRUCTURED ABSTRACT

Background and objectives

The most severe type of peripheral arterial disease (PAD) is chronic limb-threatening ischemia (CLTI). In CLTI, calcification of the vessel wall plays an important role in symptoms, amputation rate and mortality. However, calcified arteries are also found in asymptomatic persons (non-PAD patients). We investigated whether the calcification pattern in CLTI patients and non- PAD patients are different and could possibly explain the symptoms in CLTI patients.

Materials and methods

130 CLTI and 204 non-PAD patients underwent a CT of the lower extremities. This resulted in 118 CLTI patients (mean age 72±12, 70.3% male) that were age-matched with 118 non-PAD patients (mean age 71±11, 51.7% male). The characteristics severity, annularity, thickness and continuity were assessed in the femoral and crural arteries and analyzed by binary multiple logistic regression.

Results

Nearly all CLTI patients have calcifications and these are equally frequent in the femoropopliteal (98.3%) and crural arteries (97.5%), while the non-PAD patients had in just 67% any calcifications with more calcifications in the femoropopliteal (70.3%) than in the crural arteries (55.9%, $p<0.005$).

The crural arteries of the CLTI patients had significantly more complete annular calcifications (OR 2.92, $p=0.001$.) while in the non-PAD patients dot-like calcifications dominated. In CLTI patients, the femoropopliteal arteries had more severe, irregular / patchy and thick calcifications (OR 2.40, 3.27, 1.81, $p\leq0.05$, respectively) while in non-PAD patients, thin continuous calcifications prevailed.

Conclusions

Compared with non-PAD patients, arteries of the lower extremities of CLTI patients are more frequently and extensively calcified. Annular calcifications were found in the crural arteries of CLTI patients while dot-like calcifications were mostly present in non-PAD patients. These different patterns of calcifications in CLTI point at different etiology and can have prognostic and eventually therapeutic consequences.

Key words: chronic limb-threatening ischemia, calcification pattern

INTRODUCTION

Recent studies have shown that calcification across the vascular wall of patients with peripheral arterial disease (PAD) and chronic limb-threatening ischemia (CLI) play an important role [1-3]. These calcifications are of clinical importance since they are associated with symptoms, treatment outcome and mortality [4-6]. These studies have also shown that in PAD and CLI, two different types of calcifications can be found: 1) calcifications based on calcified atherosclerotic plaques located in the intimal layer of the vessel wall and 2) calcification of the tunica media and internal elastic lamina of the vessel wall as a separate metabolic disease, causing stiffness and limit remodeling [7].

Medial calcifications are not only present in the arteries of the lower extremities but also for example in the carotid siphon and in the arteries of the breast [8-10]. However, medial calcifications are almost absent in the coronary arteries. Although the differentiation between intimal and medial calcification on a CT scan is not completely reliable, annular calcifications most likely represent medial calcifications.

Medial wall calcification is an active metabolic process with bone progression proteins, instead of solely deposition of bisphosphonates in the arterial walls [7, 11]. Medial calcifications can be reversible over time as showed in the arteries of the breast, for example after kidney transplantation, but not all patients have a clear cause for regression of these calcifications [8, 12]. Thus, regression of calcifications can presumably occur partially. Indeed, a histological study of the arteries of the lower limbs found osteoclasts, however scarce [13]. Extensive regression of calcifications therefore does not seem plausible.

It has been shown that medial calcifications can be halted by bisphosphonate etidronate in patients with Pseudoxanthoma Elasticum [14, 15], a rare monogenetic disease resulting in medial calcifications of the arteries. Since we know that CLTI patients with complete annular calcifications, most likely medial arterial calcifications, have worse survival than patients without these calcifications, treatment of these patients with etidronate could therefore be a therapeutic option.

Since it is known that vascular calcifications also occur in asymptomatic people [16], we wanted to compare CLTI patients with these asymptomatic patients and investigate whether the calcification pattern perhaps could be associated with the symptomatology in CLTI patients.

Therefore, the primary aim of this present study was to examine differences in presence, severity and characteristics of arterial calcifications in the lower extremities between CLTI patients and patients without known PAD using CT.

MATERIALS AND METHODS

CLTI patients

This study consisted of data from two trials with CLTI patients. The PADI trial (Percutaneous transluminal Angioplasty and Drug-eluting stents for Infrapopliteal lesions in critical limb ischemia), and the PADI Imaging Trial.
From the PADI trial extensive study details and mid- to long-term results have been published elsewhere [17-20]. Briefly, the PADI Trial was an investigator-initiated prospective, multicenter RCT in CLTI patients due to infrapopliteal pathology to assess the value of drug eluting stents (DES) compared to the current reference treatment with bare-metal stents (BMS). Included in the PADI trial were 137 patients. DES provided better 6-month patency rates and less amputations after 6 and 12 months. From these 137 patients 87 patients underwent a CT angiography of the lower extremity and were included in the present study. Patients were scanned on a 256-slice CT scanner (Siemens Definition Flash Scanner, Siemens Healthineers, Forchheim, Germany). Slice thickness was set between 0.625 and 1 millimeter.

The subsequent PADI Imaging Trial was an investigator-initiated prospective study developed to investigate atherosclerosis and arteriosclerosis in the whole body in patients with CLTI. Patients with CLTI were recruited in the outpatient clinic of the department of vascular surgery in a large teaching hospital in The Hague, the Netherlands. Patients were excluded from the study if they were unable to give consent, if they were younger than 18 years, if patients were allergic for intravenous contrast. All included patients gave written consent. Extensive clinical assessment, routine cardiovascular laboratory tests and a whole-body spectral CT were done including a CT angiography of the lower extremities, which was used for this current study. All 43 participants in the PADI Imaging Trial enrolled to date were included in the current study. A total of 130 CLTI patients from the PADI Trial and PADI Imaging Trial were analyzed in this study.

Non-PAD patients

As control group we used patients from a different study without known vascular disease stated in their electronic patient file like coronary arterial disease (CAD), or surgical procedures such as carotid desobstruction. Patient details have been published elsewhere [21]. In short, patients with an indication for a full-body PET-CT because of melanoma, infection, unknown fever or lymphadenopathy were selected. These scans were matched to medical record data for cardiovascular risk factors and symptomatic PAD status. 204 patients without known PAD were included in this study.

Study approval

The medical ethical board of the participating centers approved the prospective PADI Imaging Trial (Unique identifier number: NL64059.098.17) and to re-use data from the PADI trial (ClinicalTrials.gov trial register Unique identifier number: NCT00471289) as a post hoc-analysis.

Regarding the patients without PAD, the medical ethical board of the University Medical Center Utrecht (UMCU) waived review because of its non-invasive, retrospective character (study number: 17-897/C). This study was also approved by the ethical board of the Haga Teaching Hospital (study number: T18-003).

Baseline measurements and definitions

The following variables were included for analysis: age, gender, diabetes mellitus (DM), weight, current smoking status, systolic blood pressure, diastolic blood pressure and renal function (eGFR). Obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2. Hypertension was defined as a systolic blood pressure at admission > 140mmHg or diastolic blood pressure of > 90 mmHg. Chronic kidney disease (CKD) was defined as an eGFR < 60 mL/min/1.73 m^2, mildly decreased kidney function to renal failure (G3a-G5) [22]. We also showed severely decreased kidney function (G4-G5) with a cut-off value of <30 mL/min/1.73 m^2, since this is the best prognostic factor in patients with CLTI [23].

Calcification assessment

All patients underwent a CT scan of the lower extremities and were evaluated on 3mm slice thickness reconstructions. For evaluation of arterial calcifications, bone settings were used for all CT exams (Window Settings: Window = 300 Hounsfield Units; Width = 1600 Hounsfield Units). This made it possible to distinguish between calcium and other densities on both CT angiographies and non-contrast CT. Both the femoropopliteal and crural arteries were scored. Vascular calcification patterns were examined in a semi-quantitative way; severity (absent, mild, moderate, severe), annularity (absent, dot(s), <90°, 90-270°, 270-360°), thickness (absent, ≥1.5mm, <1.5mm), and continuity (indistinguishable, irregular / patchy or continuous). In case more patterns were present, the most dominant characteristic was chosen to score. **Figure 1** and **2** provide different types of calcifications and illustrative examples of the CT scoring system. Extensive details on the calcification measurements are described elsewhere [21] and is based on the recently developed and CT-histologically validated score for the carotid siphon by Kockelkoren *et al.* with a

proven reproducible scoring system (inter-observer kappa 0.54-0.99) [24, 25]. All measurements were performed by a radiology resident with approximately than 4 years of experience with the scoring system (LCDK) who was blinded to the patients' clinical data. In one of our other studies with a similar set of CLTI patients with Fontaine 3 and 4, test-retest reliability was calculated based on second reader measurements scored by a senior radiologist (WPThM) with over 40 years of radiological experience. Cohen's weighted Kappa tests (Kw) showed good agreement of interreader test-retest reproducibility. Kw values were for severity 0.72 (95%CI 0.55-0.90, p<0.001), annularity 0.77 (95% CI 00.58-0.95, p<0.001), thickness 0.65 (95% CI 0.29-1.01, p<0.001) and continuity 0.62 (95% CI 0.31 – 0.94, p<0.001).

Figure 1. A. Wall layers in a normal artery from inside to outside: endothelium, tunica intima (internal elastic membrane and fibrocollagenous tissue), tunica media (smooth muscle), tunica adventitia (external elastic lamina and fibrocollagenous tissue). B. Calcifications in the intimal wall: thick and non- annular. C. calcifications in the medial wall and in the internal elastic lamina: thin and annular.

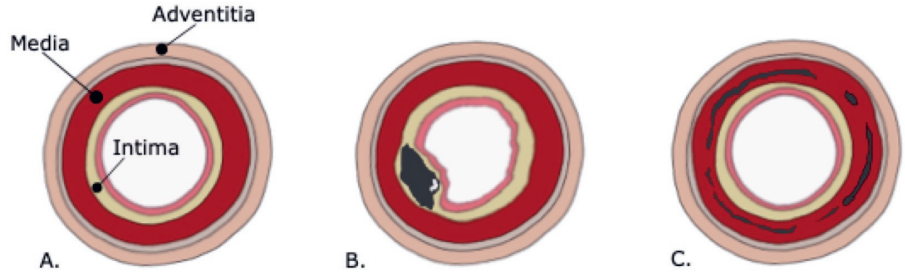
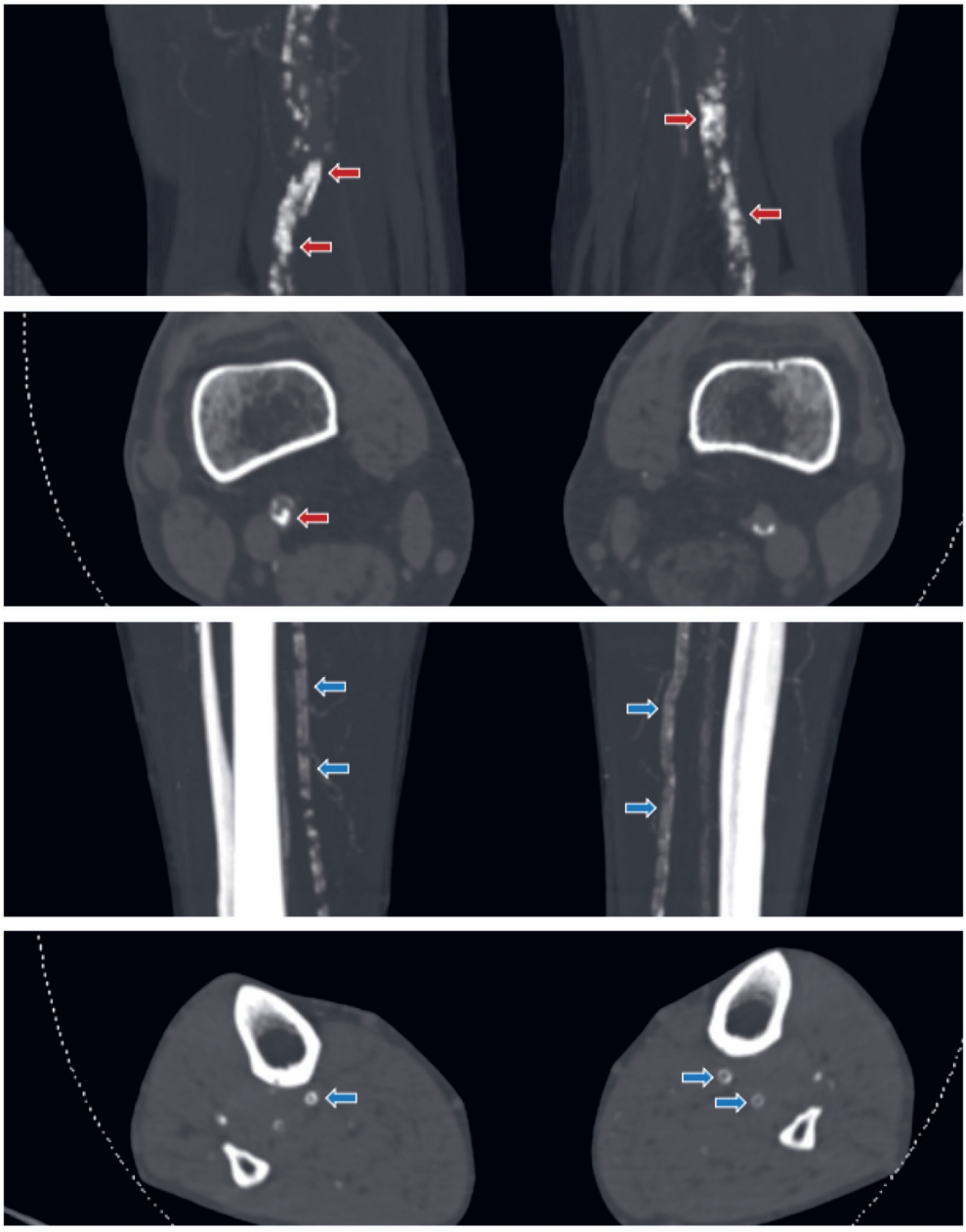


Figure 2. Examples of different calcification patterns in patients with CLTI. Shown are coronal MIP and axial 3mm CT angiography images of the lower extremities. **A.** Top two figures: the red arrows in femoropopliteal arteries showing irregular / patchy, thick and non-annular calcifications corresponding to a dominant intimal calcification pattern. **B.** Bottom two figures: the blue arrows in the crural arteries showing continuous, thin and annular calcifications corresponding to a dominant medial calcification pattern.



Statistical considerations

1.Case matching

Due to an age difference of approximately 9 years in the CLTI (cases) and non-PAD patients (controls), age matching was performed. The intention was to construct patient groups with a comparable age, as the occurrence of arterial calcification is age-dependent. The match tolerance (fuzz) factor was set on 5 years. There were 118 matches, 98 patients were excluded of further analysis. This resulted in a final sample of 236 patients, whereof 118 patients with CLTI and 118 patients without clinical known PAD. Matching was performed blinded to outcomes.

2.Statistical analysis

Descriptive data are presented as mean ± standard deviation (SD) for normally distributed continuous variables, for non-normally distributed continuous variables as median (interquartile range (IQR)). Regarding categorical variables, characteristics were presented as number (percentages). Groups were compared using the Chi-squared test for categorical variables and the student's t test for continuous variables. The prevalence of different calcifications characteristics (severity, annularity, thickness and continuity) in the femoropopliteal and crural arteries was plotted graphically for both absolute numbers and percentages.

Regarding evaluation of similarities and differences between these CT calcification characteristics for both the femoropopliteal and crural arteries, patients without calcifications were excluded from this analysis (4 CLTI patients and 57 non-PAD patients). Of the remaining 114 CLTI patients and 61 non-PAD patients with any calcifications, type and pattern analysis were performed.

Binary multiple logistic regression was performed to evaluate these different CT calcification characteristics. To adjust for confounding, correction for gender was performed. A p-value of less than 0.05 was considered to be significant. Data analysis was carried out using SPSS version 27.0 (IBM Corporation, New York, United States).

RESULTS

Baseline characteristics

Mean age was 74 years (range 40-95; SD 11). There were 61% (n=236) male patients. Patients with CLTI were significantly more likely to be male, had a higher prevalence of diabetes mellitus, smokers, hypertension and lower eGFR. Baseline characteristics and comorbidities of both CLTI and non-PAD are shown in **Table 1.**

Prevalence of lower extremity arterial calcifications

The prevalence of any calcification in the crural arteries was 97.5% (115/118) in CLTI patients and 55.9% (66/118) in non-PAD patients, p<0.005. In the femoropopliteal arteries, the prevalence of calcifications was 98.3% (116/118) in CLTI patients and in non-PAD patients 70.3% (83/118), p<0.005.

Differences in lower extremity arterial calcification patterns between CLTI and non-PAD patients

Patients without calcifications were excluded for further analysis. This resulted in the CLTI patient group in the loss of only 3 (2.5%) patients without crural arterial calcifications and only 2 patients (1.7%) without femoropopliteal arterial calcifications. In the non-PAD group, 52 (44.1%) patients without crural arterial calcifications were excluded from analysis and 35 (29.7%) non-PAD patients without femoropopliteal arterial calcifications. Baseline characteristics of these subpopulations were not markedly different on any of the variables compared to the primary case-match cohort of 236 patients. These baseline characteristics are shown in **Supplemental Table 1**. A further subdivision in baseline characteristics between patients with calcifications and without calcifications in non-PAD patients, showed that patients with calcifications in this group were older, more likely to be male, smoker, and had more often hypertension. See **Supplemental Tables 2 and 3.**

Table 1. Baseline variables of the age-matched CLTI patients and the control non-PAD patients.

	Non-PAD (n=118)	**CLTI (n=118)**	**p-value**
Age (years)	71±11	72±12	0.461
Sex (male)	61(51.7%)	83(70.3%)	0.003*
BMI	27.9(6.0%)	25.2(3.9%)	0.053
Diabetes mellitus	11 (9.6%)	68 (57.6%)	<0.001*
History of PAD	0(0%)	71(60.7%)	
Stroke	0(0%)	12 (10.3%)	
CAD	0(0%)	45 (38.1%)	
Smoking	32(30.2%)	63(54.3%)	<0.001*
eGFR	67(93)	61(142)	0.069
Chronic kidney disease (eGFR<60)	30(37.5%)	50(62.5%)	<0.006*
Severely decreased kidney function (eGFR<30)	10(43.5%)	13(56.5%)	<0.510
Systolic blood pressure	137±20	153±26	<0.001*
Diastolic blood pressure	78±13	83±12	<0.001*
Hypertension	54(45.8%)	64(69.6%)	<0.001*
Any Calcification			
Crural	66/118 (55.9%)	115/118(97.5%)	<0.001*
Femoropopliteal	83/118(70.3%)	116/118(98.3%)	<0.001*

Values are mean ± SD, median (IQR) or n (%) as appropriate.
Abbreviations: BMI=body mass index; PAD=peripheral arterial disease; CAD= coronary artery disease; eGFR=estimated glomerular filtration rate(mL/min/1.73m2). *= statistically significant p-value.

Comparing all patients with lower extremity arterial calcifications in the crural arteries, most calcifications were severe (CLTI vs non-PAD: 71.3% (82/115) vs 60,0% (39/65) without a significant difference between these two patient groups. The majority of femoropopliteal arteries were also severely calcified with 78.4% (91/116) in CLTI patients compared to 60.2% (50/83) in non-PAD patients, here a significant OR was found (OR 2.40, 95% CI 1.29-4.48, p=0.006). See **Figure 3A and 3B** for stacked graphs and **Table 2 and 3** for corresponding logistic regression analysis. See **Supplementary Tables 4** for the severity of calcifications by decade.

Figure 3A. CT calcification characteristics in the crural arteries as percentage of total number of age-matched patients. Red: non-PAD patients (n=65). Dark blue: CLTI patients (n=115).

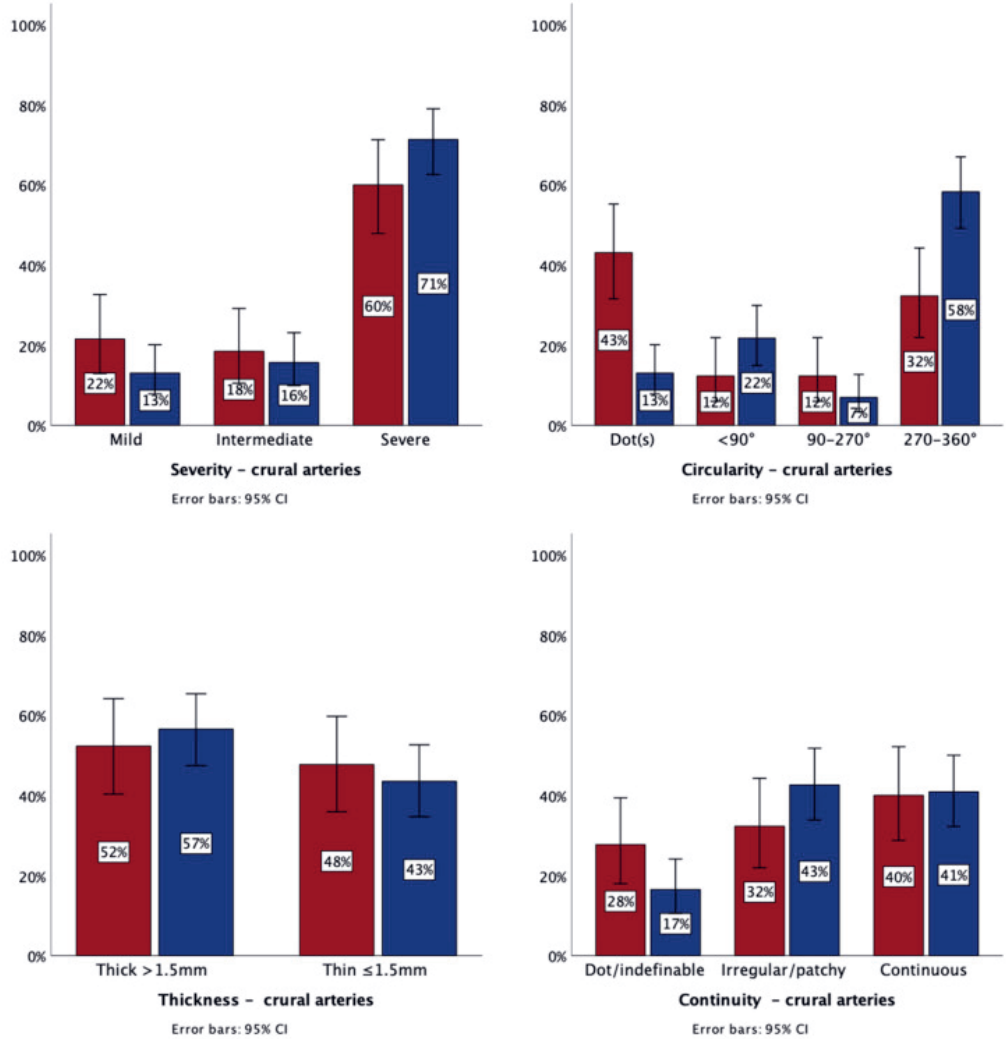


Figure 3B. Severity, annularity, thickness and continuity in the femoropopliteal arteries as percentage of total number of patients. Red: non-PAD patients (n=83), dark blue: CLTI patients (n=116).

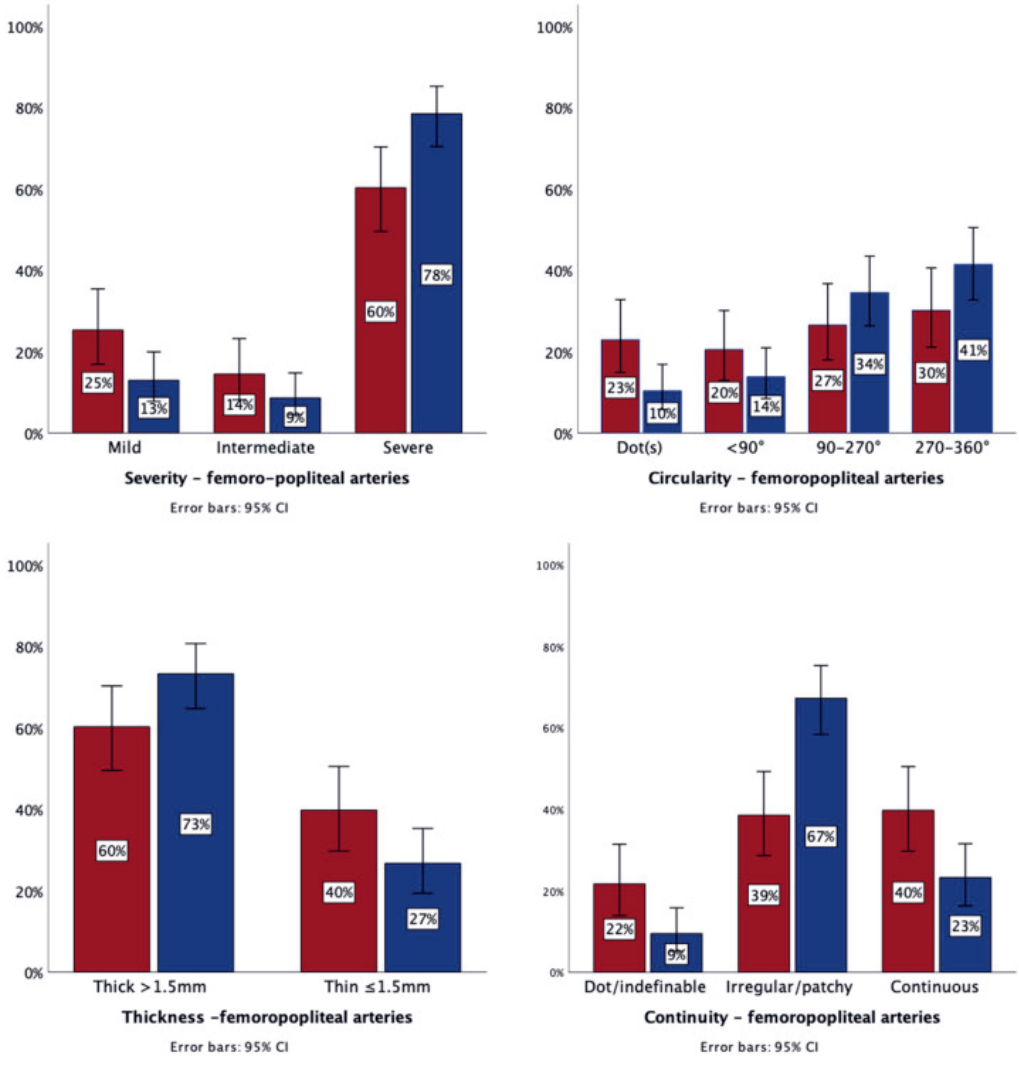


Table 2. Multivariate logistic regression analysis (sex-adjusted) was performed to determine the different calcification characteristics in the crural arteries associated to age-matched non-PAD (n=65) and CLTI patients (n=115). The patients without calcifications were excluded from this analysis.

Variables in the Equation	OR	95% CI	Standard error of the mean	P-value
Severity				
Mild	0.55	0.24-1.22	0.391	0.140
Intermediate	0.82	0.37-1.83	0.398	0.627
Severe	1.60	0.87-3.14	0.280	0.122
Annularity				
Dot(s)	0.20	0.10-0.41	0.351	<0.005*
<90°	1.20	0.84-4.69	0.430	0.121
90-270°	0.53	0.15-1.50	0.504	0.231
Complete annularity	2.92	1.55-5.54	0.304	0.001*
Thickness				
Thick (≥1.5mm)	1.08	0.58-1.98	0.273	0.820
Thin (<1.5mm)	0.90	0.49-1.66	0.280	0.729
Continuity				
Irregular / patchy	1.50	0.79-2.84	0.305	0.212
Continuous	0.86	0.46-1.61	0.293	0.644

*= statistically significant p-value.

Table 3. Multivariate logistic regression analysis was performed to determine the different calcification characteristics in the femoropopliteal arteries correlated to age-matched non-PAD (n=83) and CLTI patients (n=116). The patients without calcifications were excluded from this analysis.

Variables in the Equation	OR	95% CI	Standard error of the mean	P-value
Severity				
Mild	0.44	0.21-0.91	0.366	0.028*
Intermediate	0.56	0.23-1.36	0.398	0.200
Severe	2.40	1.29-4.48	0.280	0.006*
Annularity				
Dot(s)	0.39	0.18-0.85	0.391	0.019*
<90°	0.62	0.29-1.32	0.376	0.213
90-270°	1.46	0.79-2.71	0.306	0.232
Complete annularity	1.64	0.90-2.98	0.293	0.105
Thickness				
Thick (≥1.5mm)	1.81	1.09-3.30	0.277	0.053
Thin (<1.5mm)	0.55	0.30-1.01	0.293	0.053
Continuity				
Irregular / patchy	3.27	1.82-5.89	0.283	<0.005*
Continuous	0.44	0.24-0.81	0.302	0.009*

*= statistically significant p-value.

Next, the different calcification characteristics of annularity, thickness and continuity were compared between CLTI and non-PAD patients and its results are schematically shown in **Figure 4**.

In the crural arteries, CLTI patients had significantly more complete annular calcifications (58.3% (67/115)) than in non-PAD patients (32.3%,21/65, OR 2.92, 95% CI 1.55-5.44, p=0.001). Dotted (non-annular) calcifications are more frequently found in non-PAD patients, with an OR of 0.20. No significant OR were found for all other calcification characteristics with respect to the crural arteries.

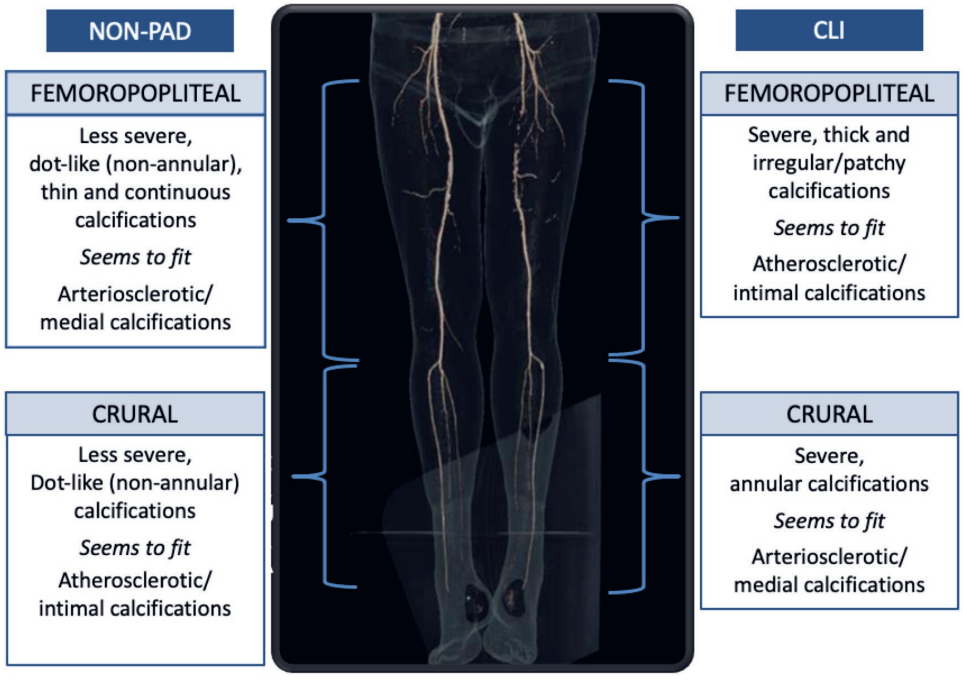
In the femoropopliteal arteries, CLTI patients had irregular / patchy calcifications in 67.8% (78/116), significantly more than non-PAD patients with 38.6% (32/83), OR 3.27, 95% CI 1.82-5.89, p<0.005). Also, CLTI patients had significantly more thick calcifications in the femoropopliteal artery with 73.3% (85/116), compared to non-PAD patients with 60.2% (50/83, OR 1.81, 95% CI 1.09-3.30, p=0.05). All other calcification characteristics the femoropopliteal arteries did not yield significant differences.

Sub analyses CKD and DM

Since DM and CKD are present in a high percentage of CLTI patients and have a substantially worse prognosis than patients without these characteristics, sub analyses were performed to test the specific subcategories DM and CKD. However, the patient numbers of these sub analyses were low (see baseline **Table 1** for an overview of these numbers). As a result, p-values were not calculated.

There were no substantial differences between patients with and without DM in non-PAD patients and those with CLTI. In the CLTI group with CKD, annularity was more frequent (65.4 % versus 54.3%) but the difference with non-CKD CLTI patients was small.

Figure 4. Schematic representation of the different calcification patterns between CLTI and non-PAD patients.



DISCUSSION

In this matched case-control study, the features of arterial calcifications in the arteries of the lower extremities between patients with CLTI and non-PAD patients without known vascular disease were investigated using CT imaging.

The principal finding of this study is that in the crural arteries CLTI patients have predominantly annular calcifications, while in non-PAD patients these calcifications are mainly dot-like. Second, almost all patients with CLTI have calcifications in the femoropopliteal and crural arteries, while the controls without known vascular disease had any calcifications in less than two third of the cases with a considerable difference between the femoropopliteal and crural arteries.

Annular calcifications are mainly found in the crural arteries of CLTI patients have been more frequently related to medial arterial calcifications, while dot-like calcifications are mainly found in the crural arteries of non-PAD patients and seem related to atherosclerotic intimal calcifications. There are however no histologic-CT

imaging correlation studies available for the crural arteries. Yet, a histologic-CT imaging study done in the intracranial internal carotid artery (carotid syphon) confirmed that annular calcifications are indeed more likely located in the medial layer of the arterial wall, while the dot-like ones were related to calcifications in atherosclerotic plaques [10].

Our findings are consistent with several histopathological studies showing that in the crural arteries, medial arterial calcifications play a prominent role in CLTI patients. Soor *et al.* showed that the crural arteries in the majority of CLTI patients contain medial calcifications (51-100% annularity) and do not have severe atherosclerosis [3]. O'Neill *et al.* found medial calcifications in 72% of the arteries of the amputation specimen, while atheroma's were present in only 23% [26]. More recently, a histopathological post-mortem study analyzing especially CLTI patients showed that medial calcifications occur significantly more frequently in the crural than in femoropopliteal arteries (OR 2.89, p=0.08) [2]. These findings are quite different from the findings in the coronary arteries where medial calcifications are rarely found. In our cohort, only 3 patients had no crural vessel calcifications (2.5%) and only 2 patients (1.7%) had no femoropopliteal arterial calcifications. An occlusive thrombus and/or partially atheromatous wall changes may have played a role in these few patients with CLTI.

Previous studies have shown that patients with medial calcifications have a poor prognosis [27-29]. Recently, we also showed that CLTI patients with complete annular calcifications in the lower extremity had a significantly worse all-cause mortality compared to the group without complete annular calcifications [30]. Another study has shown that patients with a higher degree of annular calcification in the abdominal aorta is associated with a higher all-cause mortality (29).

It was recently suggested that medial calcification and atherosclerotic intimal disease can both be present in the arteries of the lower extremities in PAD patients, and that medial calcification prevents the arteries of the lower extremities from remodeling causing obstructive disease in PAD [31]. In our study, we were unable to demonstrate the presence of the two types due to the fact that when describing the characteristics, we always noted only the most common type. As a result, there was no room for less common calcifications. It may be that atherosclerotic disease could lead to much less severe obstructive disease only in the crural arteries, and the presence of both atherosclerotic and medial disease could lead to much more severe PAD.

In the femoropopliteal arteries of CLTI patients we found remarkably severe, irregular / patchy and thick calcifications, while in non-PAD patients, mild, thin, dot-

like and continuous calcifications are found. So, these findings are different from those in the crural arteries where in CLTI patients annular medial type of calcifications were found and in non-PAD patients a dot-like atherosclerotic type. Calcification pattern differs in the different vascular territories.

We did not find a difference in calcification pattern between diabetic and non-diabetic patients in CLTI and non-PAD patient groups. Such a difference was found in CKD patients but the difference was small and could not explain the high percentage of annular lesions. However, we should be careful with the interpretation since the numbers in this sub analysis were small.

Intimal calcifications are mainly related to atherosclerotic disease and medial calcifications are a metabolic disease due to an imbalance between pro- and anti-calcifications. These two diseases therefore justify different therapeutic approaches. Intimal calcifications warrant more classical anti-atherosclerotic medication while medial calcification could be halted by calcification inhibitors such as etidronate. The recent TEMP trial was conducted in a group of PXE patients with severe medial calcified arteries [14, 15]. After one year of treatment with etidronate, progressive calcification in the femoral arteries was reduced compared to controls, without significant side effects such as osteonecrosis being found. Therefore, we think that a similar study could be considered to perform in this severely affected patient group of CLTI patients.

Strengths and limitations

One of the major strengths is that this study compared arterial calcifications in patients with and without symptomatic peripheral vascular disease using CT. The use of CT makes prediction and longitudinal studies of PAD severity through calcification characteristics easier in the long term. A second important strength is that age has been corrected by means of case-matching. Age is a known important confounder of CLTI research, and case-matching reduced the possibility of confounding.

Yet, the study also has its limitations. First, it remains difficult to measure an entire artery with an arbitrary three-, four- or five-point score. This is certainly the case for CLTI patients with a fair number of calcifications in the specific lower extremity arterial territory and therefore both (medial and intimal) patterns are often present. In this case, the most dominant characteristic was chosen to score.

Second, it is remarkable that of the patients without known PAD, there are relatively many patients with severe calcifications. This means that we may have chosen a too low cut-off point for severity of calcifications. Third, the CLTI group

underwent contrast enhanced CT and the non-PAD group unenhanced CT and therefore we might have underestimated the calcification burden in the CLTI group and missed some thin annular calcifications because these may be less recognizable on CT with contrast than CT without contrast. If so, the reported association and findings could be stronger.

Finally, we cannot be completely sure that the non-PAD patients did not have any atherosclerotic disease since no angiograms were made and therefore the lumen was not examined. Yet no clinical symptoms were present in these patients and therefore these patients were considered non-PAD patients.

Conclusions

This study shows that in the arteries of the lower extremities in CLTI patients, any arterial calcification is almost always present. In non-PAD patients however, only two third of patients have any calcifications. Most calcifications are usually severe. In the crural arteries CLTI patients have an annular type of calcifications as seen in medial calcifications, while non-PAD patients have a dot-like type of calcifications as seen in atherosclerotic disease. As medial calcifications are increasingly considered treatable, our findings may contribute to the development of a treatment strategy for these difficult-to-treat CLTI patients.

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Institutional Review Board Statement

These investigations were carried out following the rules of the Declaration of Helsinki of 1975.
Written informed consent for the PADI Trial and PADI Imaging Trial was obtained in all participants.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

SUPPLEMENTAL TABLES

Supplemental Table 1A. Baseline variables of the sub populations with any calcifications in the crural arteries of the initially age-matched CLTI patients and the control non-PAD patients.

	Non-PAD	**CLTI**	**p-value**
Crural	**66/118 (55.9%)**	**115/118(97.5%)**	**<0.001**
Age (years)	75 (9)	72(11)	
Sex (male)	37(56.9%)	80(69.6%)	0.089
BMI	28(6.07)	25.26(3.94)	0.05*
Diabetes mellitus	9(13.8%)	67(58.3%)	<0.001*
History of PAD	0(0)	69(60.5%)	<0.001*
Stroke	0(0)	12(10.5%)	
CAD	0(0)	45(39.1%)	
Smoking	20(33.9%)	60(54.0%)	0.012*
eGFR	60(76)	61(142)	0.784
Severely decreased kidney function (eGFR<30)	9(40.9%)	13(59.1%)	0.936
Chronic kidney disease (eGFR<60)	26(34.7%)	49(65.3%)	0.117
Hypertension	32(49.2%)	29(82.9%)	0.001*

Values are mean ± SD, median (IQR) or n (%) as appropriate.

Abbreviations: BMI=body mass index; PAD=peripheral arterial disease; CAD= coronary artery disease; eGFR=estimated glomerular filtration rate(mL/min/1.73m^2). *= statistically significant p-value.

Supplemental Table 1B. Baseline variables of the sub populations with any calcifications in the femoropopliteal arteries of the initially age-matched CLTI patients and the control non-PAD patients.

	Non-PAD	**CLTI**	
Femoropopliteal	**83/118(70.3%)**	**116/118(98.3%)**	**<0.001***
Age (years)	74(8)	72(12)	
Sex (male)	47(56.6%)	82(70.7%)	0.41
BMI	27.9(6.08)	25.24(3.89)	0.05*
Diabetes mellitus	10(12.3%)	67(57.8%)	<0.001*
History of PAD	0(0)	69.0(60.0%)	<0.001*
Stroke	0(0)	12(10.4%)	
CAD	0(0)	45(38.4%)	
Smoking	22(30.6%)	62(54.4%)	<0.001*
eGFR	63(84)	61(142)	
Severely decreased kidney function (eGFR<30)	9(40.9%)	13(59.1%)	0.617
Chronic kidney disease (eGFR<60)	21(30.0%)	49(70.0%)	0.173
Hypertension	38(45.8%)	30(83.3%)	<0.001*

Values are mean ± SD, median (IQR) or n (%) as appropriate.

Abbreviations: BMI=body mass index; PAD=peripheral arterial disease; CAD= coronary artery disease; eGFR=estimated glomerular filtration rate(mL/min/1.73m^2). *= statistically significant p-value.

Supplemental Table 2. Baseline variables of the control group consisted of non-PAD patients, subdivided in patients with and without calcifications in the crural arteries.

		Non-symptomatic Crural arteries							
		No calcification				Calcification present			
		Mean	SD	N	N %	Mean	SD	N	N %
Age		66	11			75	9		
Gender	Male			23	44.2%			38	57.6%
	Female			29	55.8%			28	42.4%
BMI		25.96	4.24			25.59	5.23		
DM	No			47	90.4%			58	87.9%
	yes			5	9.6%			8	12.1%
PAD				52	100.0%			66	100.0%
stroke	No			0	0.0%			0	0.0%
	Yes			0	0.0%			0	0.0%
CAD	No			0	0.0%			0	0.0%
	Yes			0	0.0%			0	0.0%
Smoking	No			35	76.1%			39	65.0%
behaviour	yes			11	23.9%			21	35.0%
GFR		73	20			62	22		
Hypertension	No			31	59.6%			33	50.0%
	Yes			21	40.4%			33	50.0%

Supplemental Table 3. Severity of calcifications in the crural arteries, stratified by age (decades).

	Non-symptomatic				Chronic limb-threatening ischemia			
	Severity - crural arteries				Severity - crural arteries			
	No	Mild	Intermediate	Severe	No	Mild	Intermediate	Severe
Age per decade (years)	N	N	N	N	N	N	N	N
40	5	0	1	0	0	2	0	4
50	11	1	0	0	2	3	2	4
60	15	6	3	7	0	7	11	11
70	18	5	2	19	1	3	4	32
80	4	2	5	13	0	0	1	26
90	0	0	1	0	0	0	0	5

Supplemental Table 4. Severity of calcifications in the femoropopliteal arteries, stratified by age (decades).

	Non-symptomatic				Chronic limb-threatening ischemia			
	No	Mild	Intermediate	Severe	No	Mild	Intermediate	Severe
Age per decade (years)	N	N	N	N	N	N	N	N
40	6	0	0	0	0	2	0	4
50	8	1	0	3	1	3	2	5
60	8	6	4	13	1	7	4	17
70	11	10	3	20	0	3	4	33
80	2	4	5	13	0	0	0	27
90	0	0	0	1	0	0	0	5

CHAPTER 4.

Arterial Calcification and Long-term Outcome in Chronic Limb-Threatening Ischemia Patients

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STRUCTURED ABSTRACT

Background and objectives

Within five years after presentation 50-60% of patients with chronic limb-threatening ischemia (CLTI) have died or had an amputation. We assessed the predictive value of lower extremity arterial calcification on computed tomography (CT) characteristics on both 7-years amputation-free survival and 10-years all-cause mortality in patients with CLTI.

Materials and methods

Included were 89 CLTI patients (mean age 73.1 ± 11.6 years) who underwent a CT angiography of the lower extremities. In the femoropopliteal and crural arteries based on a CT score the following calcification characteristics were assessed: severity, annularity, thickness and continuity. The predictive value of different arterial calcification characteristics was analyzed by age- and sex-adjusted multivariate Cox regression analysis.

Results

Complete annular calcifications were common (femoropopliteal 43.7%, n=38; crural, 63.2%, n=55). Mean survival was 278.4 weeks (95% CI 238.77-318.0 weeks). Patients with complete annular calcifications had a higher all-cause 10-year mortality (femoropopliteal unadjusted HR 1.64, p=0.04 and adjusted for age and sex HR 1.68, p=0.04; crural unadjusted HR 1.92, p=0.02, adjusted for age and sex HR 2.29, p=0.006) than patients with other calcification characteristics.

Conclusions

Annularity of calcification of both femoropopliteal and crural arteries is a predictor for 10-year all-cause survival, its hazard being even higher than the traditional prognostic risk factors for CLTI and therefore could be involved in the poor survival of these patients.

Key words: chronic limb-threatening ischemia, peripheral artery disease, medial calcification, intimal calcification, amputation-free survival, all-cause mortality, prediction models, computed tomography

INTRODUCTION

Chronic limb-threatening ischemia (CLTI) defined as Rutherford categories 4, 5 and 6 (corresponding with Fontaine 3 and 4) is associated with high rates of amputations (amputation rate at 1 year: 20.5-38.0%) and cardiovascular diseases [1-4], and this contributes greatly to a high mortality in CLTI patients. Within five years after presentation 50-60% of patients will have died [5-8]. The burden of CLTI is likely to increase due to increasing age of the western population, the increasing prevalence of diabetes mellitus, chronic kidney disease, metabolic syndrome and the continuing habits of smoking [9-12].

Why the prognosis of these patients is so poor is still not well understood. Many studies in CLTI focus on the outcome of treatment strategies of the legs (PREVENT III score, Finnvasc score, BASIL prediction model) [13-19] or risk factors [20], but prognostic studies are still limited. In a previous study we showed that a high/immeasurable ankle-brachial index (ABI) is an independent risk factor for poor amputation-free survival in patients with CLTI [20], and it is suggested that medial calcification is in part responsible for this stiffness [20-23].

Vascular calcifications have gained renewed attention in the last few years. The main approach to assess the role of calcifications has been to quantify the amount of calcification [24], but there has been much less interest in imaging characteristics of these calcifications as seen on computed tomography (CT) scans. A CT imaging score was proposed to analyze the carotid siphon on three imaging characteristics; annularity, thickness and continuity and these characteristics were used to improve prediction models [25]. Recently, one of our recent studies showed that in the lower extremities different types of arterial calcifications can be distinguished on CT [26].

Different arterial calcification patterns have also proven to have different risk factors and treatment outcome, which may indicate different pathophysiological mechanisms [27-31]. We hypothesized that the specific arterial calcification characteristics on CT scan can have a significant influence on the prognosis in CLTI patients, but up to now this has not been proven.

Hence, the aim of this study was to assess the predictive value of CT characteristics of lower extremity arterial calcification on both 7-years amputation-free survival and 10-years all-cause mortality in patients with CLTI.

MATERIALS AND METHODS

Study approval

The Percutaneous transluminal Angioplasty versus Drug-eluting stents for Infrapopliteal lesions (PADI) trial was registered in the ClinicalTrials.gov trial register under the number NCT00471289 and informed consents were obtained. The study was conducted according to the Declaration of Helsinki. The present study is a post-hoc analysis of this multi-center trial.

Patients

Data from the PADI trial which included patients with CLTI, were used. Detailed study design and results of this study have been published elsewhere [32-35]. In short, the PADI trial is a randomized controlled trial to investigate drug eluting stents (DES) for the treatment of infrapopliteal lesions in patients with CLTI in comparison with the current reference treatment. Patients (n=149) were included between October 2007 and February 2013. At 6 months after primary treatment, patency results were imaged by CT angiography, digital subtraction angiography or duplex sonography. A CT angiography was performed in 87 patients who are the subject of this study.

Clinical parameters and definitions

Patients were included with a Rutherford score of 4, 5 or 6. These patients suffer from ischemic rest pain, forefoot ulceration and ulceration with tissue necrosis. Symptoms had to be present longer than 2 weeks [5]. The following patient characteristics were recorded; sex, age, smoking status, diabetes mellitus (DM), coronary artery disease (CAD), stroke, the use of anticoagulant medication, hematocrit value and estimated Glomerular Filtration Rate (eGFR per mL/min/1.73 m^2). Regarding smoking status, patients were classified as smokers, ex-smokers or non-smokers. Chronic kidney disease (CKD) was defined as an eGFR < 45 mL/min/1.73 m^2. This is consistent with CKD stage 3B, 4 and 5: mild to moderate decrease in renal function to renal failure. In specific, dialysis dependent renal function was stated CKD class 5 (eGFR < 15 mL/min/1.73 m^2), which selects patients with the most severe degree of renal failure [36]. An experienced nurse measured blood pressures. Hypertension was defined as a systolic blood pressure ≥ 140mmHg or if the patient was taking antihypertensive medication. The ankle-brachial index was calculated from the highest systolic blood pressure of the dorsalis pedis and posterior tibial arteries divided by the highest systolic blood pressure on the

ipsilateral or contralateral brachial arterial systolic pressure. Overall, a single pressure measurement of each limb artery was performed.

Follow-up criteria and definitions

Follow-up consisted of annual assessments up to 7 years after treatment or until a clinical end-point was reached; amputation (through or above the ankle) or death. Regarding the completion of follow-up data until April 2019 (520 weeks) for this current study, municipal basic records were checked regarding death and date of death. Major amputation was defined as amputation through or above the ankle joint. Amputation-free survival was defined as survival free of amputations in the follow-up period.

Systematic assessment of calcification patterns

All 87 patients were evaluated on a CT angiography with 3mm slice thickness reconstructions. Measurements were done by a radiology resident with extensive experience with the scoring system (LK), who was blinded to the patients' clinical data and outcome.

Bone window settings were used for evaluation of arterial calcifications (Window Settings: Window = 300 Hounsfield Units; Width = 1600 Hounsfield Units). This made it possible to distinguish well between calcium and other densities on the CT angiography. Calcification measurements were also not hindered by an acute occluded artery, because the thrombi responsible for these occlusions have lower densities than calcifications. If a stent was present due to previous treatment, this part of the artery was not included in the scoring of calcifications.

The calcification measurements were done according to the recently developed and CT-histologically validated score for the carotid siphon (inter-observer kappa 0.54-0.99) [25, 37]. This scoring system has recently been applied in a study of the peripheral arteries and has shown that it makes a good distinction between different calcification characteristics on CT in the peripheral arteries. Extensive information and details about the CT calcification measurement procedure and the associated scoring system are also described herein [26].

In short, CT calcification characteristics were measured on the affected leg with CLTI. Both the femoropopliteal and crural arteries were scored. Arterial calcification patterns were examined in a semi-quantitative way; severity (absent, mild, moderate, severe), annularity (absent, dot(s), <90°, 90-270°, 270-360°), thickness (absent, ≥1.5mm, <1.5mm), and continuity (indistinguishable, irregular/patchy or

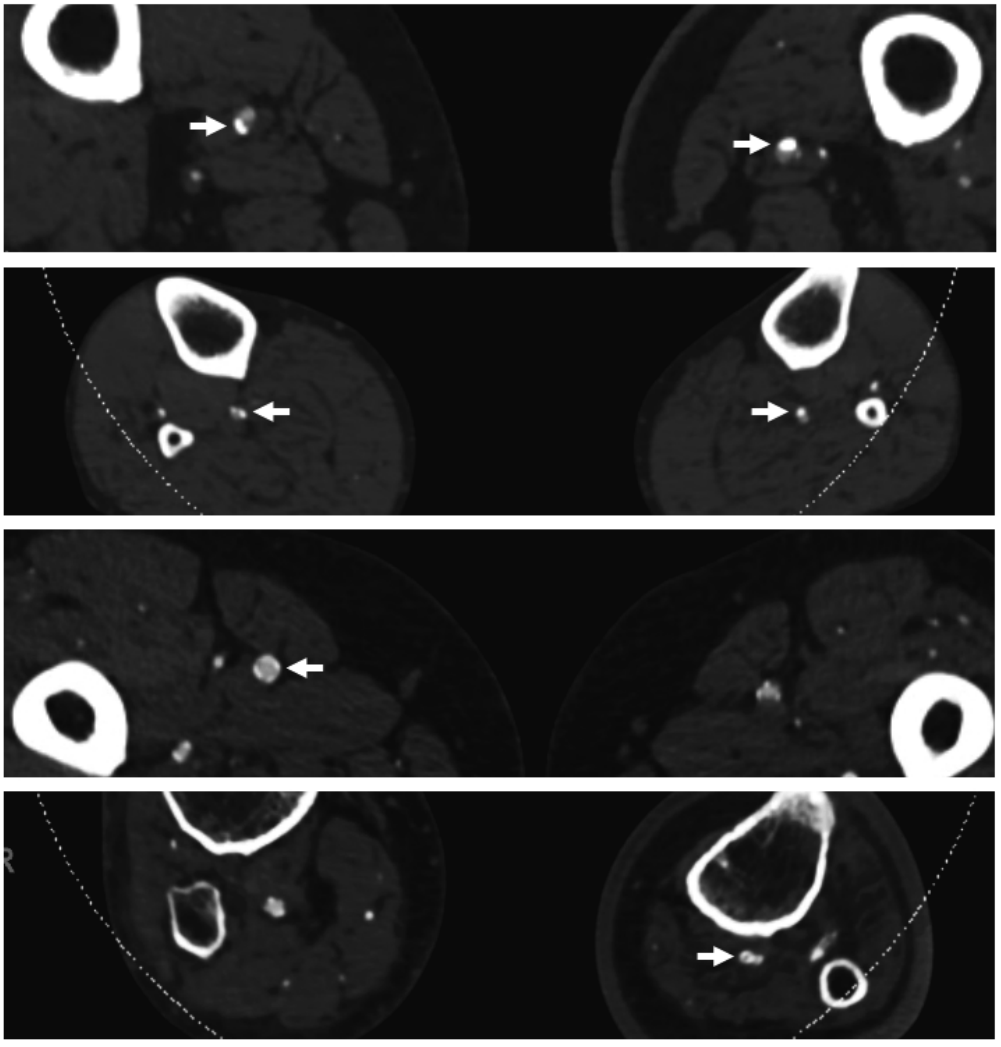
continuous). See **Figure 1** for examples of different calcifications of the arteries involved.

Statistical analysis

Baseline characteristics as well as CT imaging characteristics of arterial calcifications were used to describe the characteristics of the study populations; means with standard deviations (SD) for continuous variables, counts and percentages for categorical variables. Normal distribution was tested by QQ-plots. The variables severity, annularity, thickness and continuity were converted into dichotomous variables due to the relatively low patient numbers in the 'less severe' categories, into severe vs non-severe, annular vs non-annular, thick vs thin and continuous vs non-continuous. Survival of patients was calculated up to ten years after the first inclusion; 7-year amputation-free survival and 10-year all-cause mortality for all patients were presented using Kaplan-Meier plots. Comparisons between the different characteristics were assessed based on the Log-Rank/Mantel Cox test for significance.

Univariate analyses and age- and sex-adjusted effects were calculated by hazard ratios with 95% confidence intervals (CI) for the different CT imaging characteristics for both 7-year amputation-free survival and 10-year all-cause mortality. A full univariate model and age- and sex-adjusted was created including the CT calcification characteristics and the factors of the known best performing prognostic model for CLTI (the prevent III model). The factors of the prevent III model include dialysis-dependent renal function, tissue loss at baseline, advanced age (>75 year), history of coronary disease and hematocrit (≤ 30%). A p-value of less than 0.05 was considered to be significant. Data analysis was carried out using SPSS version 24.0 (IBM Corporation, New York, United States).

Figure 1. Axial CT angiography slides at the level of the femoral and crural arteries. **Top two figures**: examples of incomplete annular (<270°) and thick calcifications (indicating dominant intimal calcifications). **Bottom two figures**: examples of complete annular (>270°) calcifications and thin calcifications (indicating dominant medial calcifications).



RESULTS

Study population

In total there were 87 patients with a mean age of 73.11 ± 11.63 years. There were 73.6% male patients. Baseline characteristics are shown in **Table 1**.

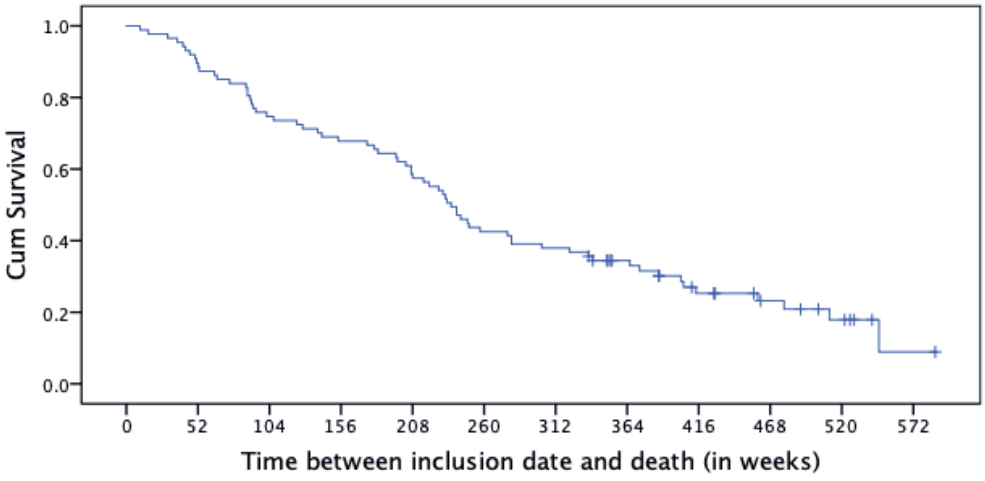
Table 1. Baseline clinical characteristics of all included patients.

Variables		N (%)/mean±SD
Age (years)		73.1 ± 11.6
40-59 y		10 (11.5%)
60-79 y		47 (54.0%)
80-99 y		30 (34.5%)
Sex (male)		64 (73.6%)
Smoking habit	Smoker	21 (24.1%)
	Ex-smoker	20 (23.0%)
Hypertension		39 (70.9%)
History of CAD		33 (37.9%)
History of stroke		9 (10.3%)
History of DM		53 (60.9%)
eGFR (mL/min/1.73m^2)		59 ± 27
CKD (eGFR<45 mL/min/1.73m^2)		26 (30.2%)
Dialysis-dependent renal function (eGFR<15 mL/min/1.73m^2)		4 (4.6%)
Rutherford category at baseline	4	15 (17.2%)
	5	55 (63.2%)
	6	16 (18.4%)
ABI at baseline		0.85 ± 0.27
ABI at baseline (categorized)	ABI<0.7	41 (47.1%)
	0.7<ABI<1.4	37 (42.5%)
	ABI>1.4 or immeasurable	9 (10.3%)
Initial treatment (PTA±BMS or DES)	PTA±BMS	45 (51.7%)
	DES	42 (48.3%)

Abbreviations: CAD = coronary artery disease, DM = diabetes mellitus, eGFR = glomerular filtration rate (mL/min/1.73m^2), CKD = chronic kidney disease, ABI = ankle-brachial index, PTA = percutaneous transluminal angioplasty, BMS = bare-metal stents, DES = drug-eluting stents.

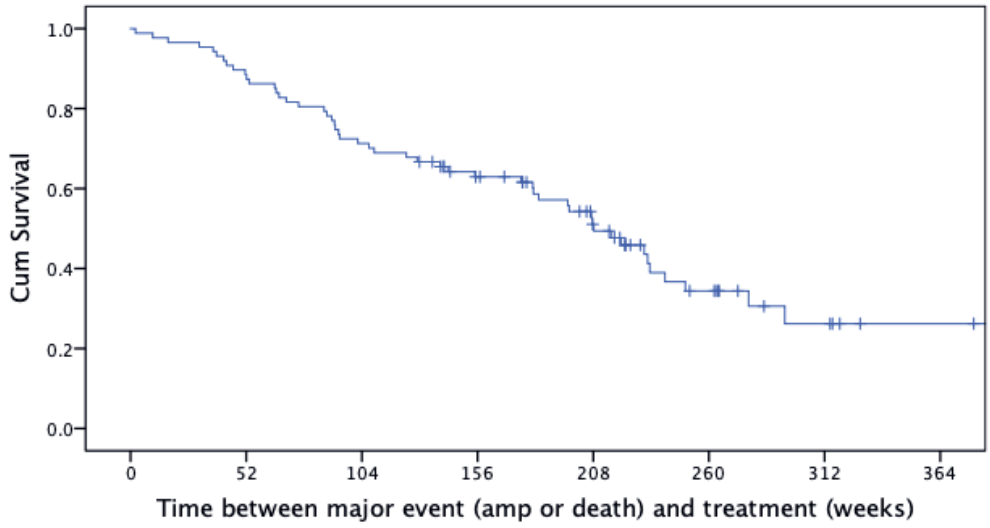
Mean survival was 278.4 weeks (95% CI 238.77-318.0 weeks) with a maximum follow-up of 520 weeks. After 10 years, the majority of CLTI patients had died 67/87 (77.0%). 7-year amputation-free survival was 23/87 (26.2%). Mean amputation-free survival was 215.1 (95% CI 185.1-245.1 weeks). See **Figure 2A and 2B** for all-cause mortality and amputation-free survival Kaplan-Meier curves.

Figure 2A. 10-years Kaplan Meier all-cause survival curve of all CLTI patients.



Time	0	52	104	156	208	260	312	364	416	468	520	572
Number at risk (censored)	87	78	65	59	51	37	33(6)	24(3)	15(4)	10(2)	6(5)	1(1)

Figure 2B. Kaplan Meier amputation-free survival of all CLTI patients.



Time	0	52	104	156	208	260	312	364
Number at risk (censored)	87	77	62(7)	48(9)	31(9)	14(6)	6(4)	2(2)

CT imaging calcification patterns

The results of the assessment of calcification CT imaging characteristics (severity, annularity, thickness and continuity) for both femoropopliteal and crural arteries are presented in **Table 2**.

The majority of the femoropopliteal and crural arterial calcifications were severe. Thickness, annularity and continuity could already be present at lower age but increased considerably with age. See stacked **Figures 3A and 3B**, age divided into strata of 20 years. There were no significant differences in calcification patterns between initial treatment strategy (PTA±BMS / paclitaxel-coated DES).

Table 2. Severity, annularity, thickness and continuity of calcifications in femoropopliteal and crural arteries.

		Femoropopliteal N (%)	**Crural N (%)**
Severity	Absent	2 (2.3%)	2 (2.3%)
	Mild	5 (5.7%)	4 (4.6%)
	Moderate	6 (6.9%)	10 (11.5%)
	Severe	74 (85.1%)	71 (81.6%)
Annularity	No calcifications	2 (2.3%)	2 (2.3%)
	Dot(s)	8 (9.2%)	6 (6.9%)
	< 90	10(11.5%)	18 (20.7%)
	90-269	29 (33.3%)	6 (6.9%)
	270-360	38 (43.7%)	55 (63.2%)
Thickness	0mm	2 (2.3%)	2 (2.3%)
	>1.5mm	60 (69.0%)	63 (72.4%)
	≤1.5mm	25 (28.7%)	22 (25.3%)
Continuity	Dot(s)	6 (6.9%)	3 (3.4%)
	Irregular/patchy	58 (66.7%)	49 (56.3%)
	Continuous	23 (26.4%)	35 (40.2%)

Values were presented as number (percentages).

Figure 3A. Stacked graphs showing the different calcification characteristics, divided into age-strata per 20 years for the crural arteries.

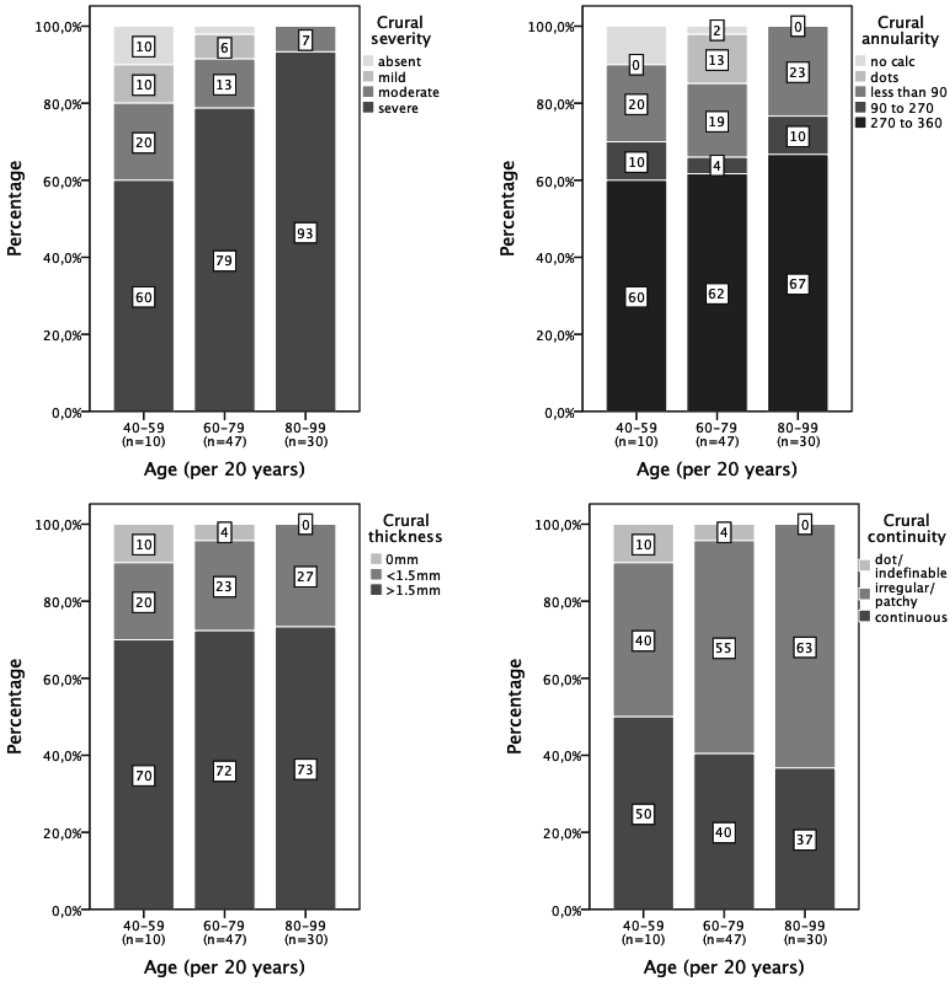
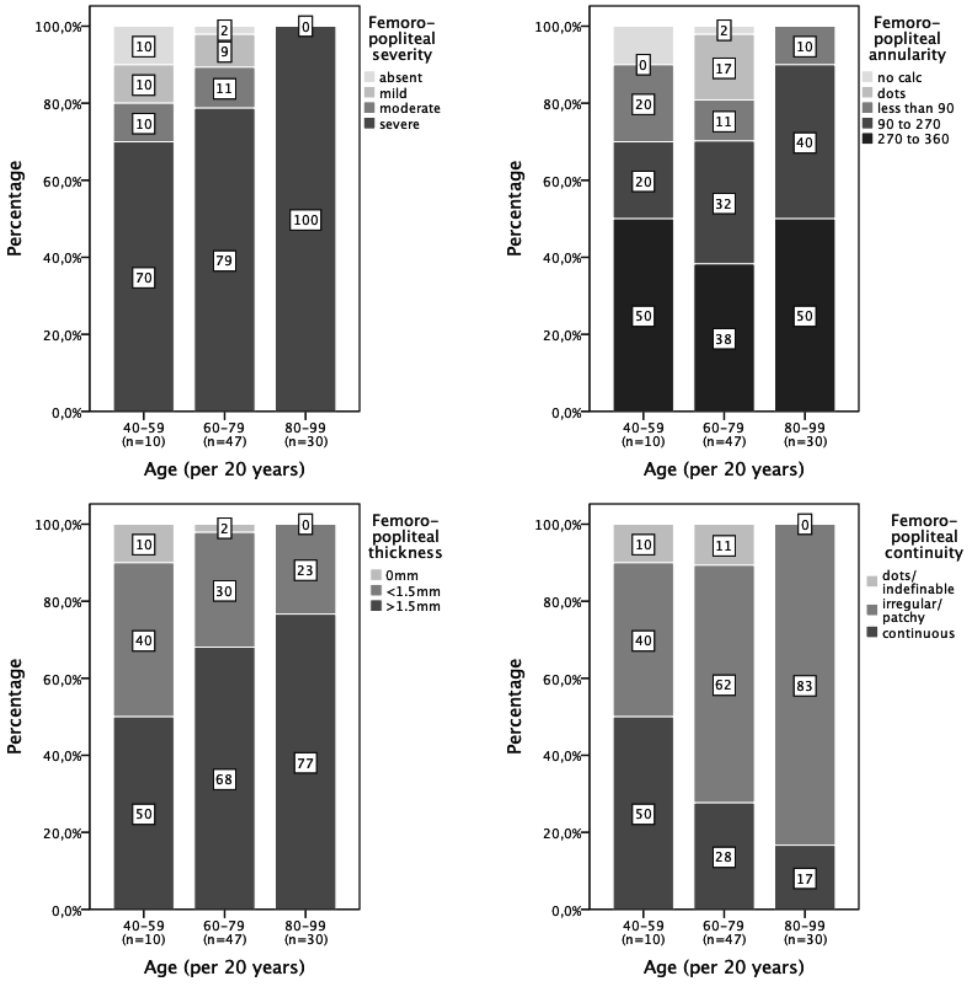


Figure 3B. Stacked graphs showing the different calcification characteristics, divided into age-strata per 20 years for the femoropopliteal arteries.



Prognostic value of calcification characteristics and factors from the prevent III model

Age- and sex-adjusted effects were calculated for both 7-years amputation-free survival and 10-years all-cause mortality (see **Table 3**).

For annularity of calcifications in the femoropopliteal arteries, both univariate and age- and sex-adjusted HR showed significant HR for predicting 10-year all-cause mortality (unadjusted HR 1.64, 95% CI 0.99-2.73, p=0.04 and adjusted for age and sex HR 1.68, 95% CI 1.01-2.80, p=0.04; crural unadjusted HR 1.92, 95% CI 1.01-3.34, p=0.02, adjusted for age and sex HR 2.29, 95% CI 1.28-4.13, p=0.006). Crural

calcifications also had a significant predictive value on 7-years amputation-free survival after adjusting for age and sex (see **Table 3**).

Severity of calcification showed in univariate analysis a higher hazard ratio (HR) than the classic prognostic factors used for CLTI as reported in the prevent III model (10-year survival rate: femoropopliteal HR 2.75, 95% CI 1.09 – 6.91, p=0.01 and crural HR 2.13, 95% CI 1.00-4.51, p=0.03). After adjusting for age and sex, these differences lost significance. Also, for 7-year amputation-free survival, severity did not influence the outcome significantly. Thickness and continuity had no significant HR for both univariate and age- and gender-adjusted.

Of the prevent III factors, only advanced age was significant for both 7-year amputation-free survival (HR 1.40, 95% CI 1.05-1.9, p=0.02) and 10-year all-cause mortality (HR 1.38, 1.06-1.80, p=0.02).

Table 3. Cox Regression analyses for 7-years amputation-free survival and 10-year all-cause mortality, multivariate corrected for age and sex.

		7-years amputation-free survival			10-year all-cause mortality		
		HR	95,0% CI	P	HR	95,0% CI	P
Severity	Femoropopliteal	0.78	0.34-1.77	0.50	2.03	0.79-5.13	0.14
	Crural	1.00	0.47-2.15	0.99	1.55	0.71-3.35	0.26
Annularity	Femoropopliteal	1.54	0.88-2.71	0.13	1.68	1.01-2.80	0.04*
	Crural	1.96	1.05-3.65	0.03*	2.29	1.28-4.13	0.006*
Dialysis-dependent renal function		1.27	0.88-1.83	0.20	0.85	0.52-1.40	0.53
Tissue loss at baseline		1.08	0.84-1.39	0.56	1.16	0.91-1.47	0.23
Advanced age (>75 year) †		1.37	1.02-1.85	0.04*	1.38	1.05-1.82	0.02*
History of coronary disease		1.42	0.80-2.55	0.89	1.26	0.74-2.16	0.39
Hematocrit (≤ 30%)		1.80	0.67-4.89	0.24	1.05	1.02-1.08	0.84

* = p-value< 0.05.

† = advanced age (>75year) only adjusted for sex.

DISCUSSION

Principal findings

In this present study, CT imaging characteristics of calcifications of the femoropopliteal and crural arteries in a cohort of CLTI patients were investigated. Annularity was a good predictor for 10-year all-cause survival in both femoropopliteal and crural arteries and for 7-year amputation-free survival only in the crural arteries. Severity, thickness and continuity of calcifications were of no predictive value. Already in our youngest age category 40 – 59 years, the majority of femoropopliteal and crural arteries had severe calcifications, which increased gradually per age group. Calcifications were also thicker, more annular and more continuous on a higher age.

These findings provide insights into the importance of CT imaging characteristics of arterial calcifications compared to the classical known risk factors of CLTI and can contribute to improving prognostic modelling for CLTI patients.

Different arterial calcifications and locations

Histologic studies show two different types of calcifications; medial and intimal. Where in earlier days was thought that arterial intimal calcifications were present in only the most severe (type 5b lesions) [38], we now know that arterial calcification can be present in all forms of atherosclerosis, despite its severity. Medial arterial calcification is hydroxyapatite deposited along elastin fibers in the medial wall where vascular smooth muscle cells undergo transdifferentiation to an osteo/chondrogenic cell type [39]. One of the calcification characteristics of medial calcification is annularity. The predictive value of annular calcifications for mortality found in this study is therefore most likely caused by these medial calcifications [40].

Femoropopliteal and infrapopliteal/below-the knee arteries seem to differ in pathology with significantly more atherosclerosis in the SFA than the BTK arteries. Hunter's canal, through which the femoral artery runs, is a location prone to atherogenesis [41]. Therefore, more intimal wall calcifications can be expected. Recently, a histopathologic post-mortem study that analyzed especially CLTI patients showed that medial calcifications are significantly more common in the crural than in femoropopliteal arteries (OR 2.89, p=0.08) [42].

Our current study result fits well with these observations. Since we can expect full annular calcification, which is more common in the crural arteries, can lead to a higher odds ratio on survival compared to the femoropopliteal arteries where less complete annular calcification occurs.

Annularity of arterial calcifications

Annularity of calcifications, probably localized in the medial layer of the arterial wall, as a predictor for all-cause mortality has been described in other arterial locations previously. Hendriks *et al.* showed that a greater annularity of the calcifications in the aorta-iliac arteries is associated with a higher mortality risk [28]. In addition, in another study conducted in the internal carotid artery it was shown that greater annularity was associated with less neurological symptoms [43]. Annularity of the vascular calcifications has also been studied by correlating histology with CT imaging characteristics. Kockelkoren *et al.* showed in the intracranial internal carotid artery that complete annularity was mainly located in the medial layer of the vessel wall [25]. However, CT–histologic correlation studies for the arteries of the lower extremity are lacking.

How annular vascular calcifications can influence all-cause mortality is not completely understood but it has been thought that annular calcifications can damage the elasticity and cause stiffening of the vessel wall. This in turn can cause an increase in pulse wave velocity and damage the small vessels of organs such as kidney and brain [44, 45]. Furthermore, it has been postulated that annular calcifications can prevent vascular remodeling making coping with concomitant atherosclerotic disease much more difficult [40].

Arterial calcification and aging

We showed that in CLTI the calcifications were more severe in older patients which increased gradually by age group. Previously we also showed that in asymptomatic patients, arterial calcifications gradually increased per age group [26]. Zettervall *et al.* showed that patients with CLTI had much more severe calcifications than patients with intermittent claudication independent of the severity of the disease [46]. So, it seems likely that asymptomatic patients, patients with claudication and CLTI patients have increasingly more severe calcifications and that calcifications are more severe with a higher age.

Strengths and limitations

This study has several strengths. To our knowledge, this study is the first prognostic cohort which studied calcification patterns as seen on CT scans in CLTI patients in relation to long-term survival. Other strengths include the high event rate and completeness of the mortality data at 10-year follow-up.

This study also has its limitations to mention. First, since this study is a follow-up study of a randomized clinical trial (PADI trial), the treatment strategy may have

affected our outcome. The initial PADI Trial has randomized for PTA±BMS and paclitaxel-coated DES. We are aware of the current warnings by amongst others the FDA given after a meta-analysis concerns paclitaxel-coated DES and DEB. Therefore, we conducted an additional study investigating 10-year follow-up, this showed no significant difference in survival between our PTA±BMS and DES patients. Also, we did not observe any dose-related adverse effects on survival of CLTI patients treated with paclitaxel-coated DES in a recently published additional analysis [35]. We also rigorously searched the current literature to a possible influence of paclitaxel on (arterial) calcium. However, we found no evidence in current literature that calcifications are affected by these low doses of paclitaxel. Therefore, we do not expect the previous treatment strategies in this study cohort to have affected our current study results.

The second limitation of the study is that the PADI study has used as exclusion criterium an impaired renal function (eGFR<20 ml/min/1.73 m^2), excluding patients with a likely high calcification load that, if included, may have increased our odds ratios for calcification in our study. If so, the reported association and findings could be stronger.

Conclusions

In conclusion, annularity of calcifications in the arterial wall is a good predictor for 10-year all-cause mortality and 7-year amputation-free survival of CLTI patients, especially in the crural arteries and is easy to use in daily clinical practice. Severity, thickness and continuity of calcifications are not predictive for survival. The predictive value of annularity is higher than the traditional prognostic risk factors for CLTI.

Recognizing the influence of arterial calcifications on long-term survival in CLTI patients adds to the current prognostic risk factors known in this poorly performing patient cohort.

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Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Conflicts of interest statement

All authors declare that they have received no grants, contracts, other forms of financial supports or relationships with the industry relevant to this paper.

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CHAPTER 5.

SYSTEMIC ARTERIAL CALCIUM BURDEN IN PATIENTS WITH CHRONIC LIMB-THREATENING ISCHEMIA

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STRUCTURAL ABSTRACT

Background and objectives

5-year mortality of chronic limb-threatening ischemia (CLTI) is 50-60% and coronary artery disease (CAD) is the main cause of death of CLTI patients, followed by stroke. The aim of this study is to quantify and qualify the calcium load in different arterial territories in patients with CLTI.

Materials and methods

Prospectively, 60 patients with CLTI were included and received a full-body CT scan. 6 patients were excluded. Different arterial territories (the peripheral lower extremity arteries, coronary arteries, extracranial and intracranial carotid arteries, thoracic and abdominal aorta) were analyzed. Analysis and interrelations of both quantitative and semi-quantitative CT measurements was performed.

Results

Mean age was 72 years (range 47-95; SD 11.4). Almost all CLTI patients had calcified arterial beds (femoropopliteal 100%, crural 98.1%, coronary 100%, carotid bifurcation 96.2%, internal carotid artery 98.1%, thoracic aorta 96.2%, abdominal aorta 92.3%). Nearly all arterial territories had severe calcifications. 57% had a high coronary Agatston score (>1000), and 35% extremely high (> 2000). Calcifications in the lower extremity were significantly correlated to CAC score, carotid artery bifurcation calcification score, and to a lesser extent correlated to annular calcifications in the aorta. High and extremely high total CAC scores were strongly correlated with severe lower extremity arterial calcifications and severe carotid and intracranial internal carotid artery, thoracic and abdominal aorta calcifications in patients with CLTI patients.

Conclusions

In CLTI patients nearly all arterial territories are severely calcified, suggesting that systemic calcification plays an important role in the poor outcome of this disease.

Key words: chronic limb-threatening ischemia, arterial calcifications, medial wall calcifications, intimal wall calcifications, atherosclerosis, stroke, coronary artery disease

INTRODUCTION

Although chronic limb-threatening ischemia (CLTI) is considered the most severe stage of peripheral arterial disease (PAD) leading frequently to lower extremity amputation, data suggest that it is a systemic cardiovascular disease since acute coronary syndrome, stroke and heart failure are leading to a high morbidity and mortality. A recent systematic review and meta-analysis showed that in patients with non-reconstructible CLTI in the year of diagnosis 27% had a major amputation and 18% died. The five-year mortality of CLTI patients is 50-60% [1-11] and CAD is the main cause of death followed by stroke [11, 12]. The prevalence of PAD including CLTI is still increasing to date [13, 14]. Mortality rates in patients with CLTI improved somewhat in the last decade, probably due to more systematic use of anti-atherosclerotic and anti-hypertensive medication but the prognosis of patients with CLTI still remains very poor. Given the effective drugs it may well be that the high residual risk is not fully explained by atherosclerosis.

Evidence is accumulating that, at least in the lower extremities, a distinct process co-occurs with atherosclerosis, which is medial arterial calcification (MAC). In MAC deposits of hydroxyapatite occur in the medial layer of the arterial wall. These calcifications are increasingly recognized as being prevalent in PAD and they maybe an independent cause of vascular events. The typical concentric calcifications in MAC contribute to arterial stiffening and thereby contribute to hypertension, heart failure and pulse pressure related organ damage in susceptible organs like the kidneys and brain. We hypothesized that extensive calcifications would be present in the arterial tree of CLTI patients. If so, they could be responsible for some of the remaining vascular disease burden after adequate anti-atherosclerotic and anti-hypertensive treatment.

Therefore, the aim of this current study is to quantify and qualify the calcium load in different arterial territories (the peripheral lower extremity arteries, coronary arteries, extracranial and intracranial carotid arteries, thoracic and abdominal aorta) on full-body CT scans in patients with CLTI and assess interrelations.

MATERIALS AND METHODS

Ethical approval

The medical Ethical Committee Zuid-West Holland Leiden/The Hague gave approval to perform the prospective PADI Imaging Trial in the Haga Teaching Hospital, the Hague, the Netherlands (Unique identifier number: NL64059.098.17) as

well as the institutional board of the Haga Teaching Hospital. Written informed consent for the PADI Imaging Trial was obtained in all participants.

Study population

60 CLTI patients with a Rutherford 4, 5 and 6 (Fontaine 3 and 4) were recruited in the outpatient clinic by the vascular surgeon and were included in this study. Six patients were excluded; two patients due to low Fontaine stage (IIB) and four patients due to incomplete scanning. The remaining 54 patients were included for analysis. Extensive clinical assessment contained the following variables: age, gender, diabetes mellitus (DM), weight, current smoking status, systolic blood pressure, diastolic blood pressure, and renal function (eGFR). Obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2. Hypertension was defined as a systolic blood pressure at admission >140 mmHg or diastolic blood pressure of >90 mmHg. Chronic kidney disease (CKD) was defined as an eGFR < 60 mL/min/1.73 m^2, mildly decreased kidney function to renal failure (G3a-G5) [15]. Severely decreased kidney function (G4-G5) was defined as a cut-off value of <30 mL/min/1.73 m^2, [16].

CT scanning protocol

All included CLTI patients were scanned on a 256-slice CT scanner (Siemens Definition Flash Scanner, Siemens Healthineers, Forchheim, Germany). The scanning protocol consisted a full-body protocol divided into two scans. At first, a low-dose prospective ECG-triggered coronary CT without contrast (flash) was performed from the vertex of the skull to the pubic bone. The arms were placed next to the body in slender patients, in large patients the arms were stretched on the abdomen to get as much of the arms as possible in the field-of-view. Patients were scanned with a standard 80 and 120 keV with dose modulation on. The rotation time was 0.28, pitch 3.4 and collimation 0.6). Slice thickness was set on 0.65 millimeter.

The second scan was from the vertebral body L1 to the feet. The scan was performed with intravenous contrast (90ml Xenetix, 300/100ml NaCl), with an injection time of 5ml/sec. Contrast timing was triggered on the aorta with a test bolus of 15ml contrast mixed with 40ml NaCl with a delay of 18 seconds. The standard kV was 80 and 120 with dose modulation. The rotation time was 0.5, pitch 0.6 and collimation 0.6.

Overview of arterial calcification assessment

For this study, we used quantitative calcium score as well as a semi-quantitative morphologic CT score. When achievable, both were performed. A

radiology resident (LCDK) with more than four years of experience in both quantitative as semi-quantitative score was blinded to the patient's clinical data during the scoring process and performed all measurements. Independently of this, a subset of 30 patients was scored with the semi-quantitative morphologic CT score by a senior radiologist (WPThM) with more than 40 years of experience to evaluate test-retest reproducibility.

Calcifications were measured in the femoropopliteal and crural arteries, coronary arteries, carotid artery bifurcation and intracranial carotid arteries, thoracic and abdominal aorta.

Quantitative calcium score

Quantitative arterial calcium scores were measured by the method described by Agatston et al. [17]. The software package Syngo.Calcium Scoring (Siemens Healthineers, Erlangen, Germany) was used. Calcium was detected with a Hounsfield Units (HU) above 130 on a non-contrast CT. Total volume/mm^3 and calcium scores were calculated. Stents can cause metal artifacts, however, in bone setting we were able to locate calcium outside the stents and this was measured by hand as accurately as possible.

Semi-quantitative morphologic CT score

Semi-quantitative morphological calcification measurements were done according to the recently developed and CT-histological validated score for the carotid siphon (inter-observer kappa 0.54-0.99) [18, 19]. This scoring system has recently been applied in a study of the peripheral arteries of the lower extremities and has shown that these characteristics for intima and media calcifications cluster in the same way in the femorocrural arteries as in the carotid syphon [20, 21]. Severity was scored as absent, mild, moderate and severe. Annularity was scored as absent, dot(s), <90°, 90-270°, 270-360°. Thickness as absent, ≥1.5mm and 1.5mm. At last, continuity was scored as absent/indistinguishable, irregular/patchy and continuous.

1. Lower extremity artery calcification measurements

The femoropopliteal artery was defined as the superficial femoral artery in direct continuation with the popliteal artery. The crural arteries were defined as the tibioperoneal trunk and the anterior tibial artery.

Due to the presence of intravenous contrast no quantitative measurements could be obtained. Semi-quantitative measurements were performed on the affected

leg with CLTI. See **Supplemental Figure 1** for examples of extensive calcifications in the femoropopliteal and crural arteries.

2. Coronary arterial calcification measurements

All coronary arteries were measured independently as well as added up to a total score. Quantitative Agatston scores and total calcium scores were measured. CAC scores were divided into 0, 0-100, 101-400, 401-1000, according to Rumberger *et al.* [22]. High and extremely high CAC scores were defined as total CAC ≥ 1000 and total CAC ≥ 2000, respectively [23, 24]. See **Supplemental Figure 2A** for examples of severely calcified coronary arteries. Semi-quantitative measurements were not performed.

3. Carotid artery calcification measurements

The extracranial carotid artery was measured within 3 cm proximal and distal at the carotid bifurcation (CB). Both left and right arteries were measured. Semi-quantitative and quantitative scores were used. CB scores were classified into terciles (0-800, 801-1600, 1601-2400) to perform logistic regression analyses. See **Supplemental Figure 2B** for examples of calcifications at the carotid artery bifurcation.

The intracranial internal carotid artery (iICA) (frequently known as carotid syphon) was measured with the semi-quantitative score. Quantitative measurements could not be done in this software package because of the adjacent petrous bone. See **Figure 2C.**

4. Aortic calcification measurements

Of the semi-quantitative scores only annularity of the aorta was assessed at two levels. The ascending thoracic aorta was measured just before the origin of the left subclavian artery. Second, the abdominal aorta was measured below the level of the diaphragm, above the run-off of the celiac trunk.

Quantitative scores could not be assessed in the aorta, because the particles of calcium were too large for the calcium scoring software since this was developed for the coronary arteries and a cut-off was used for the detection of calcium. See **Supplemental Figure 2D** for examples of calcifications of the thoracic and abdominal aorta.

Statistical considerations

1.Interrater reliability

Test - retest reproducibility was assessed for the semi-quantitative score (severity, annularity, thickness and continuity) in the lower extremities. Since the data to be tested consisted of ordinal data, the weighted Cohen's Kappa test (K_w) was used to compare the reliability of the two observers of calcification measurements. K_w values were interpreted based on the guidelines of Landis and Koch *[25].*

2.Statistical analysis

All continuous variables were presented as means and standard deviations if normal distributed. Normal distribution was tested with QQ-plots. If not normally distributed, for example CAC scores, data were presented as medians with quartiles (P25-P75). Categorical data are presented as frequencies and percentages (%). Correlation was assessed by Spearman's correlation coefficients (since the data were not normally distributed) of calcifications of the femoropopliteal and crural arteries compared to the coronary, carotid arteries and aorta. We interpreted a negligible Spearman's correlation between 0.00-0.20, weak between 0.21-0.40, moderate between 0.41-0.60, strong between 0.61-0.80 and very strong between 0.81-1.00. Additional analyses were performed for the high and extremely high CAC scores using binary logistic regression analysis, shown in odds ratios (OR). A p-value less than 0.05 was considered to be significant.
Statistical analyses were conducted using SPSS Statistics version 27.0 (IBM Corporation, Armonk, New York).

RESULTS

Interrater reliability of morphologic calcification measurements in the lower extremities

Cohen's weighted Kappa tests showed good agreement of inter reader test-retest reproducibility for the morphologic calcification measurements in the lower extremities. K_w values were for severity 0.72 (95%CI 0.55-0.90, p<0.001), annularity 0.77 (95% CI 00.58-0.95, p<0.001), thickness 0.65 (95% CI 0.29-1.01, p<0.001) and continuity 0.62 (95% CI 0.31 – 0.94, p<0.001).

Baseline characteristics

Baseline characteristics and comorbidities of the included CLTI patients are shown in **Table 1.** As can be expected with these CLTI patients, 58.5% (31/54) had a

history of PAD, 38.9% (21/54) of CAD, 13.2% (7/54) of stroke. Patients in these cohort were treated according to the current guidelines amongst others of best pharmacological therapy and treated, if necessary, with statins (78%) and anti-hypertensive medication (67.3%).

Table 1. Baseline table of the included patients with CLTI (n=54).

Baseline characteristics		**Mean (SD)**	**N(%)**
Age (years)		72(11)	
Gender (male)			36(66.7%)
Length (cm)		172(10)	
Weight (kg)		74.7(17.4)	
BMI (kg/m^2)		25.05(4.08)	
Diabetes Mellitus			24(44.4%)
Stroke			7(13.2%)
CAD			21(38.9%)
History of PAD			31(58.5%)
Smoking	No smoking		2(3.8%)
	Former smoker		18(34.6%)
	Smoker		32(61.5%)
Current usage of warfarin			16(30.2%)
Current usage of antihypertensive medicine			35(67.3%)
Current usage of statins			32(78.0%)
Fontaine baseline	3		22(40.7%)
	4		32(59.3%)
Systolic blood pressure upper extremity (mmHg)		162(27)	
Diastolic blood pressure upper extremity (mmHg)		84(22)	
Systolic blood pressure ankle (mmHg)		84(58)	
Hypertension (mmHg)	<140		7(13.5%)
	≥140		45(86.5%)
eGFR (ml/min/1.73m^2)		72(27)	
CKD (eGFR <60 mL/min/1.73m^2)		17(31.5%)	
CKD (eGFR <30mL/min/1.73 m^2)		5(9.3%)	
ABI categorized	<0.7		42(77.8%)
	0.7-1.40		3(5.6%)
	>1.40/immeasurable		9(16.7%

Abbreviations: BMI=body mass index; PAD=peripheral arterial disease; CAD= coronary artery disease; eGFR=estimated glomerular filtration rate(mL/min/1.73m^2); CKD=chronic kidney disease; ABI= ankle-brachial index.

Calcifications in different locations in CLTI patients

1.Lower extremity arterial calcifications

Results of the semi-quantitative assessment of the femoropopliteal and crural arteries are given in **Table 2**. Calcifications in the femoral arteries were mostly severe (70.4%), 90-270° (50.0%), thick (83.3%) and irregular/patchy in 74.9%. There were no patients without calcifications in the femoropopliteal arteries. In the crural arteries, there was only one patient without calcifications (1.9% of all patients). Calcifications varied in severity. Calcifications were mostly thin in 74.1% of patients. There was no clear pattern with annular, continuous or irregular/patchy calcifications.

2.Coronary arterial calcifications

The results of the semi-quantitative and quantitative calcification assessments of the coronary arteries are presented in **Table 3**. There were no patients without coronary calcifications. The coronary arteries of CLTI patients are severely calcified. The median total CAC score is 1484.9 (P25-P75: 342.6-2386.8), with 16 lesions (SD 9) per patient. In descending order, the LAD was the most severe calcified artery with a CAC score of 499.5(94.5-875.5), followed by the RCA with a CAC score of 222.0 (16.58-936.0), the Cx with a CAC score of 134.1(8.2-466.5) and the LM with a CAC score of 63.9 (0-185.4).

3. Carotid bifurcation (CB) and intracranial internal carotid artery (iICA)

The CB was assessed quantitative and semi-quantitative and the results are given in **Table 4.** Most of the calcifications consisted of 2 pieces for both left and right. The CB had a high calcification score with a relatively higher left-side calcification score of 472.6 (P25-P75: 107.8-874.8) versus right with 282.9 (P25-P75**:** 96.7-605.9)**.** Most calcifications at the CB were almost annular (between 90-270°) in 41.5%, thick in 84.3% and irregular/patchy in 59.6%. Only 11.8% were thin.

Most of the calcifications in the iICA were 90-270° in 48.1% (26/54) or complete annular in 27.8% (17/54), and thick in 68.7% (37/54).

4.Aorta calcifications

The semi-quantitative data are given in **Table 5.** In the thoracic aorta there were only 3.8% of patients (n=2) without calcifications. Many of the calcifications were annular (36.5%, n=19). The same pattern could be seen in the abdominal aorta with only 7.7% of patients without calcifications (n=4) and a substantial proportion of patients with annular calcifications (30.8%).

Table 2. Semi-quantitative calcification measurements in the femoropopliteal and crural arteries.

Artery	Calcification characteristic		N	%
Femoropopliteal arteries	Severity	Absent	0	0.0%
		Mild	8	14.8%
		Moderate	8	14.8%
		Severe	38	70.4%
	Annularity	Absent	0	0.0%
		Dot(s)	5	9.3%
		<90°	11	20.4%
		90-270°	27	50.0%
		271-360°	11	20.4%
	Thickness	Absent	0	0.0%
		Thick ≥1.5 mm	45	83.3%
		Thin <1.5 mm	9	16.7%
	Continuity	Indistinguishable	7	13.0%
		Irregular/patchy	41	75.9%
		Continuous	6	11.1%
Crural arteries	Severity	Absent	1	1.9%
		Mild	16	30.2%
		Moderate	20	37.7%
		Severe	16	30.2%
	Annularity	Absent	1	1.9%
		Dot(s)	13	24.5%
		<90°	15	28.3%
		90-270°	10	18.9%
		271-360°	14	26.4%
	Thickness	Absent	2	3.7%
		Thick ≥1.5 mm	12	22.2%
		Thin <1.5 mm	40	74.1%
	Continuity	Indistinguishable	24	44.4%
		Irregular/patchy	12	22.2%
		Continuous	18	33.3%

Table 3. Calcifications at the coronary arteries and the aorta.

Arterial territory	**Arterial calcification characteristic**		**Median (P25-P75)**	**N (%)**
Coronary arteries	Total calcium volume (mm^3)		1216.3(290.9-1891.8)	
	Total CAC score		1484.9(342.6-2386.8)	
		Subcategorized total CAC scores		
		0		0 (0.0%)
		1-100		6 (11.1%)
		101-400		8(14.8%)
		401-1000		8(14.8%)
		1001-2000		14(25.9%)
		>2000		18(33.3%)

Abbreviations: CAC= coronary arterial calcifications
Continuous variables are reported as median (P25-P75) and categorical variables as n(%).

Table 4. Arterial calcifications in the carotid arteries at the carotid bifurcation and the carotid siphon in patients with CLTI (n=54 patients).

Arterial territory	**Arterial calcification characteristic**		**Median (P25-P75)**	**N (%)**
Carotid bifurcation	Annularity	Absent		2(3.8%)
		Dot(s)		4 (7.5%)
		<90°		10(18.9%)
		90-270°		22(41.5%)
		271-360°		15(28.3%)
	Thickness	Absent		2(3.9%)
		Thick ≥1.5 mm		43(84.3%)
		Thin <1.5 mm		6(11.8%)
	Continuity	Indistinguisable		15(28.8%)
		Irregular/patchy		31(59.6%)
		Continuous		6(11.5%)
	Left volume (mm^3)		375.7(94.0-741.3)	
	Left score		472.6(107.8-874.8)	
	Right volume (mm^3)		248.1(83.8-505.4)	
	Right score		282.9(96.7-605.9)	
iICA	Annularity	Absent		1(1.9%)
		Dot(s)		1(1.9%)
		<90°		9(16.7%)
		90-270°		26(48.1%)
		271-360°		17(31.5%)
	Thickness	Absent		1(1.9%)
		Thick ≥1.5 mm		37(68.5%)
		Thin <1.5 mm		16(31.5%)
	Continuity	Indistinguisable		7(13.0%)
		Irregular/patchy		24(44.4%)
		Continuous		23(42.6%)

Abbreviations: CB = external carotid artery at the carotid bifurcation; iICA=intracranial internal carotid arteries.
Continuous variables are reported as median (P25-P75) and categorical variables as n(%).

Table 5. Annularity of arterial calcifications in the aorta in patients with CLTI (n=54).

Arterial territory	Arterial calcification characteristic		Median (P25-P75)	N (%)
Aorta	Thoracic aorta annularity	Absent		2(3.8%)
		Dot(s)		4(7.7%)
		<90°		10(19.2%)
		90-270°		17(32.7%)
		271-360°		19(36.5%)
	Abdominal aorta annularity	Absent		4(7.7%)
		Dot(s)		8(15.4%)
		<90°		14(26.9%)
		90-270°		10(19.2%)
		271-360°		16(30.8%)

Continuous variables are reported as median (P25-P75) and categorical variables as n(%).

Correlation analysis

1. Lower extremities: correlations between lower extremity arteries and different arterial territories

Spearman's correlation coefficients were calculated between the semi-quantitative calcification scores in the arteries of the lower extremities and the quantitative and semi-quantitative calcification measurements in the coronary arteries, carotid arteries and aorta. See **Supplemental Table 3 – 6.**

Femoropopliteal and crural calcification severity both had a moderate to strong correlated with coronary calcifications (R=0.60 and R=0.53 respectively). Both femoropopliteal and crural severity was also moderate to strong correlated to carotid bifurcation calcification (R=0.62 and R=0.53 respectively). Interestingly, crural severity had a moderate correlation with annularity of calcifications in the iICA (R=0.55, p<0.001).

With regard to annularity of calcifications in the lower extremities, particularly moderate correlations were found in the crural arteries when compared to total CAC scores (total CAC volume R=0.47, p<0.001). The calcification load at the left carotid bifurcation had a stronger correlation than the right carotid bifurcation for both femoropopliteal and crural arteries. A moderate correlation of crural annularity with annularity was also found in the iICA (R=0.48, p<0.001).

Correlations between thickness of calcifications in the lower extremities were negligible to weak (see **Supplemental Table 5).**

Continuity of calcifications in the femoropopliteal artery showed significantly higher correlations than crural arteries (femoropopliteal 0.45, p<0.001 vs crural 0.22, p=0.11). Next to the earlier described strong correlation for crural severity and annularity, crural continuity of calcifications were also moderately associated with annular iICA calcifications (0.51, p<0.001).

2.Coronary arteries: high and extremely high CAC score

Additionally, patients with high and extremely high CAC scores were evaluated. Patients were stratified according coronary arterial calcification (CAC) scores; CAC <1000, high with a CAC score of 1000-2000 and extremely high with a CAC score >2000. For baseline characteristics of these sub categories, see **Supplemental Table 7.** Patients with an extremely high CAC score (CAC > 2000) had the highest percentage of CAD, PAD, CKD and high or immeasurable ABI.

Binary logistic regression analysis was performed to determine the ORs between CAC<1000, CAC 1000-2000 and CAC >2000 and the calcium measurements of lower extremity calcification, carotid calcifications and aortic calcifications. See **Supplemental Table 8.** CAC<1000 had weak to moderate odds ratios with calcifications score and morphology in the different arterial territories.

Significantly higher OR were found in patients with high and extremely high CAC scores. In patients with the most severe calcifications in the femoropopliteal arteries the OR was 11.70 (p<0.005), the most severe calcifications in the crural arteries 9.19 (p=0.007) and the complete annular calcifications with an OR of 6.08, p-value 0.030. In the carotid bifurcation significant OR were found in the highest calcification categories of 1600-2400 for both left and right, however these were not significant.

Regarding CAC >1000, patients had higher OR for complete annular abdominal aortic calcifications (OR 4.44, p=0.04).

In patients with an extremely high CAC score (CAC >2000), the OR did improve in the femoropopliteal arteries and aorta even more. For complete annular calcifications in the thoracic aorta, the OR went from 3.15 to 3.61 with a significant p-value of 0.036. For abdominal complete annular calcifications, the OR did even improve from 4.44 up to 7.50 (p-value=0.0003). There was no improvement in the crural arteries or carotid arteries. The extremely high OR in severity of calcifications in the femoropopliteal arteries with a *rho* of 1.53^9, can be explained because all patients had severe calcifications.

DISCUSSION

This study provides an overview of systemic calcifications in patients with CLTI. The main finding of our study is that nearly all arterial beds are severely calcified in patients with CLTI and that there are moderate to high correlations between severe lower extremity arterial calcifications and severe systemic calcification. In addition, CLTI patients with high CAC scores (>1000) were significantly correlated with most severe and complete annular calcifications in the crural and femoropopliteal arteries.
We will discuss our findings in the context of the different arterial territories.

Correlations lower extremities with systemic calcifications

1. High and extremely high CAC scores

In the coronary arteries 57% of CLTI patients had an Agatston score of >1000 and 35% a Agatston score > 2000. Recent results of the CAC Consortium show these values represent a distinct group of patients at the highest risk for all-cause mortality and cardiovascular mortality [24]. In this cohort with a clinically indicated CT scan only 4.3% had an Agatston score >1000. Patients with an Agatston score of zero had a mortality rate per 1000 person years for CHD, CVD, cancer and all-cause mortality of 0.1, 0.3, 0.8 and 1.6 compared with patients with an Agatston score of >1000 of 5.1, 8.0, 4.6 and 18.8 respectively. In general, calcifications in the coronary arteries are ascribed to atherosclerotic disease however in histologic-CT correlation studies cases with Agatston scores >1000 are generally lacking. So, in CLTI the high coronary disease burden can be explained by the extremely high calcium scores and the histologic nature of these calcifications is uncertain. In dialysis patients it has been described that the coronary calcification score is less related to obstruction and maybe the etiology of these high calcium scores is different [26]. In our logistic regression analysis, high and extremely high were related to severe calcification in all arterial beds. This points at a common mechanism of calcification in these arterial beds.

2.Carotid arteries

In the carotid bifurcation we found thick annular calcifications in 40%, while thin ones were rare. This fits with atherosclerotic type of calcific lesions. However, calcification scores at the carotid artery bifurcation are not convincingly related to the degree of strokes [27, 28]. It has been stated that the inverse relationship of calcifications at the carotid artery bifurcation and strokes indicates the stabilizing effect of calcifications to protect against stroke [29].

Quantitatively, in the carotid bifurcation, we found a mean calcification load score on the left of 473, while on the right this was 283. Recently, in a cohort of patients with unstable angina or positive stress test for myocardial ischaemia the quantitative carotid score was 59 and 23 respectively on the left and right side [30]. So, this score in CLTI patients is about 10 times higher, but the difference between left and right remains. This could mean that the mechanism by which calcification occurs remains similar but that the calcification process itself is much more unleashed in CLTI patients than in other atherosclerotic patients. In other words, CLTI patients may react much stronger with calcification on a certain stimulus than other vascular patients.

Calcifications in the iICA are prognostic for stroke [31]. In a recent study that also differentiated between media and intimal calcifications, a dominant media pattern was related to cardiovascular risk factors, as well as intimal iICA calcification [32]. Since we found high annular calcified iICAs indicative of MAC, our results are consistent with these results and may explain the high stroke rate.

Remarkably, there was a strong correlation between the lower leg calcifications and the iICA calcifications. In the lower leg, histological studies of amputated legs have shown extensive medial calcifications. In the iICA, it was shown in a CT histology correlation study that more than 70% of the calcifications seen on CT in this region are due to internal elastic lamina calcification fitting medial calcifications. Perhaps a similar mechanism is here at play.

3.Annularity of the aorta

Complete annular calcification of the ascending (thoracic) aorta, also the definition porcelain aorta, was found in 36.5% of the CLTI patients. Due to lack of a clear definition, few data are available about the prevalence of these annular calcifications in the general population [33]. Recently, assessment of the extent of the calcification has become relevant for the transaortic valve replacement procedure and it was shown that in this severely cardiovascular compromised population 18% had a porcelain aorta. The calcifications seen in a porcelain aorta are thought to be caused by both atherosclerotic disease and by MAC frequently occurring simultaneously. The prognostic value of the complete annular thoracic aorta calcification is unknown at this time.

Complete annular calcification was also found in the abdominal aorta in 34% of our cases. Calcifications in the abdominal aorta have extensively been investigated on lateral x-rays made for bone mineral density assessment. These calcifications have been linked to all cardiovascular disease and mortality and incident coronary heart

disease myocardial infarction and stroke [34, 35]. Recently it was shown that abdominal aorta calcification was shown to be a better predictor than the Framingham risk score. Annularity of the calcification of the abdominal aorta adds to this risk [36].

The purpose of our study was to investigate whether calcification could explain the residual disease burden after adequate treatment with anti-atherosclerotic and anti-hypertensive treatment. Patients in these cohort were treated according to the current guidelines amongst others of best pharmacological therapy and treated, if necessary, with statins (78%) and anti-hypertensive medication (67.3%).

We found convincing evidence that there is excessive systemic calcification. In most arterial territories these calcifications are linked to a high risk of cardiovascular disease or mortality [31, 32, 36-45]. So, these calcifications could explain the high residual cardiovascular disease burden in CLTI patients.

We cannot be sure about the type of the calcification. As of now probably both intimal and medial calcifications are involved and lead to excessive calcification. Intimal calcifications are not only calcified lipid lakes of complex atherosclerotic lesions but also fibrocalcific type of lesions without lipids that play an important role in abdominal aorta, iliac and femoropopliteal artery calcifications and are probably present in the peripheral vessels in a very early stage [46]. Furthermore, medial calcifications can originate in the internal elastic lamina but also in the vascular smooth muscle cells and up to now the precise relation between both is uncertain.

Possibly in the very last phase of the calcification process, which is similar for intimal and medial calcifications, the inhibition of the calcification process is compromised in CLTI patients, leading to excessive calcification. A single systemic nature of the calcification process has been suggested before. Takx *et al.* showed in a cluster analysis that calcification of different vascular territories cluster very well, but do not cluster with traditional cardiovascular risk factors [47].

For CLTI patients, therapies blocking calcifications are needed to test the hypothesis that the calcifications itself are a cause of disease. Recently it was shown that etidronate could halt calcifications in all arterial beds in patients with pseudoxantomata elastica [48]. This rare monogenetic vascular calcifying disease is like several other rare monogenetic calcifying diseases treated with etidronate or is subject of investigation with this medication. In all these diseases anorganic pyrophosphate (PPi), the strong calcification inhibitor is diminished causing vascular calcification. Etidronate, a stable PPi analogon, can replace PPi. Several other non-

surgical treatment strategies are currently being investigated and could in the future contribute to a better treatment of CLTI patients [49, 50].

Strengths and limitations

The main strength of this study is that we studied the calcification process in CLTI patients comprehensively in nearly all large vascular territories in the body. As far as we know, such assessment in CLTI patients has not occurred.

This study also has its limitations. There has not been a systematic quantitative assessment of all territories due to the use of contrast or lack of a good quantitative program. However, the semi-quantitative assessment was done in all territories and provided convincing evidence for extensive calcification in all territories. Second, we did not take into account renal function and diabetes as a possible cause of this calcification.

Conclusion

In conclusion, nearly all arterial territories are severely calcified in patients with CLTI and calcifications in the lower extremity were significantly correlated to CAC score, carotid artery bifurcation calcification score, and to a lesser extent correlated to the annular calcifications in the aorta. Secondly, high and extremely high total CAC scores were strongly correlated with severe lower extremity arterial calcifications and severe carotid and intracranial internal carotid artery, thoracic and abdominal aorta calcifications in patients with CLTI patients.

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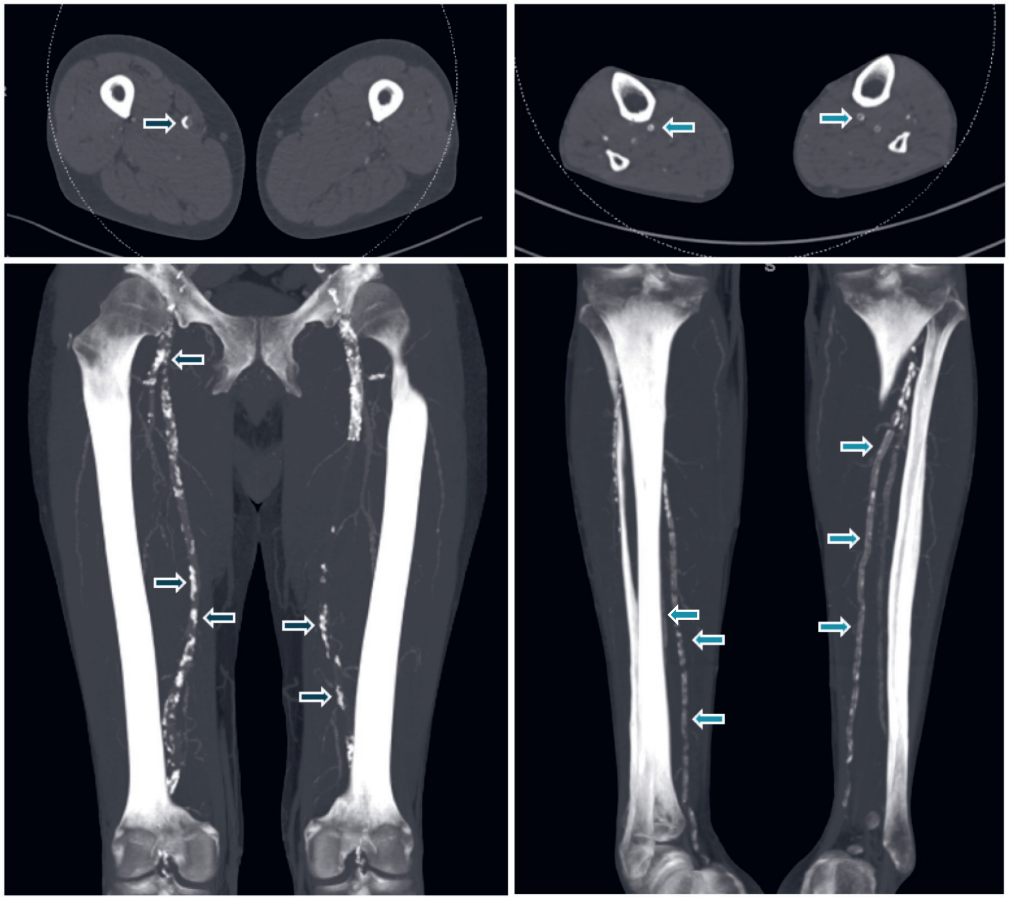
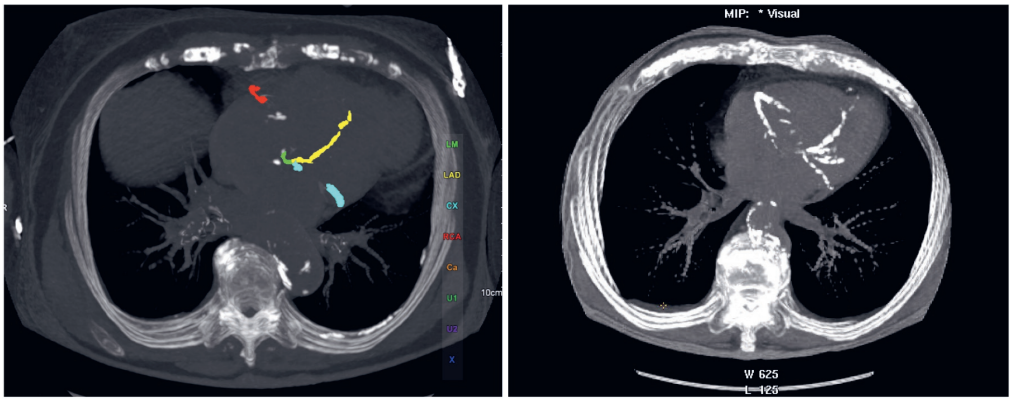
SUPPLEMENTAL FIGURES AND TABLES

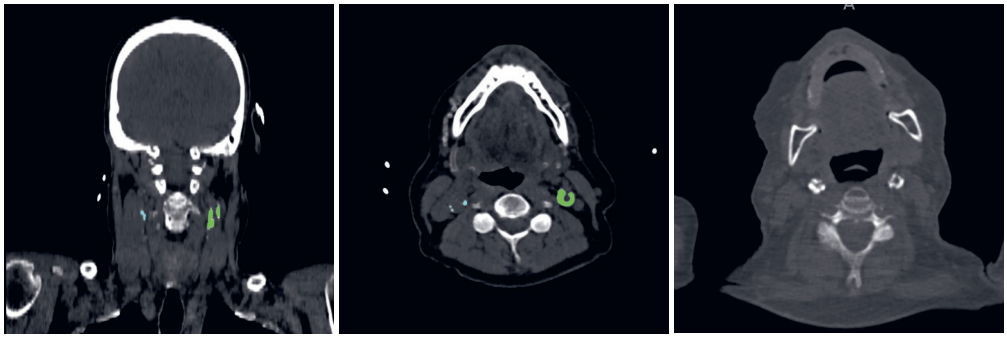
Supplemental Figure 1. Examples of calcification measurements in the arteries of the lower extremities. Axial thin and coronal maximum intensity projection (MIP) images.
Left two images: Severe patchy femoropopliteal calcifications (dark blue arrows). N.B. a stent has been placed in the left proximal femoral artery.
Right two images: Severe continuous annular and thin crural calcifications (light blue arrows).



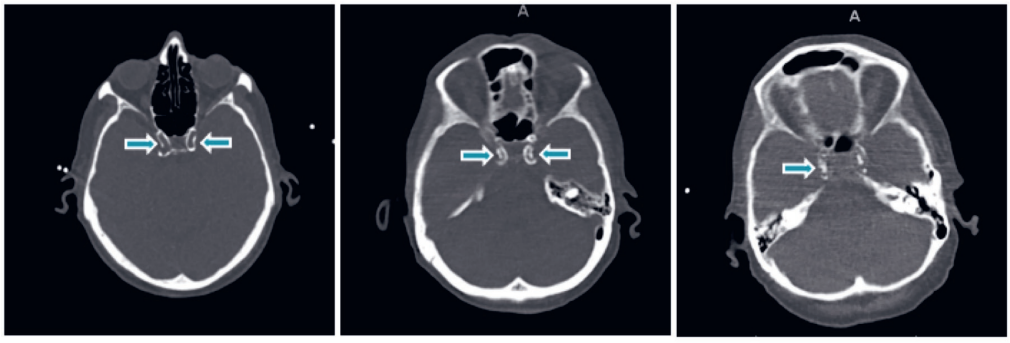
Supplemental Figure 2. Examples of calcification measurements in CLTI patients. **A.** Left: Maximum intensity projection (MIP) Image with marked calcium scores in the LM (green), LAD (yellow), CX (light blue) and RCA (red) in CLTI patients. Right: complete MIP view of the heart with extensive calcifications



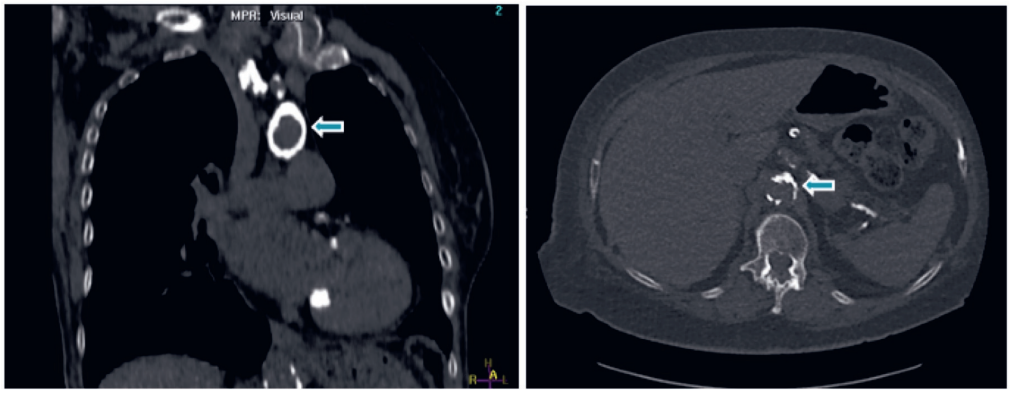
B. Extensive calcifications at the left (green) and right (blue) carotid artery at the level of the carotid bifurcation (axial and coronal planes)



C. Examples of iICA calcification measurements at the level of the carotid syphon (axial CT planes). Left and middle image: continuous annular calcifications. Right image: non-continuous/patchy calcifications. See blue arrows.



D. Examples of aorta calcification measurements. Left: complete annular calcifications in the ascending thoracic aorta at the level of the left subclavian artery. Right: 90-270° calcifications in the abdominal aorta at the level of the diaphragm.



Supplemental Table 3. Spearman's correlation coefficients were performed to determine the different variables correlated to severity in the femoropopliteal and crural arteries.

		Femoropopliteal severity		**Crural severity**	
Arterial territory	**Variables in the Equation**	***R***	**p-value (2-tailed)**	***R***	**p-value (2-tailed)**
Coronary artery	Total n. lesions	0.47	<0.001*	0.39	0.005*
	Total calcium volume (mm^3)	0.60	<0.001*	0.53	<0.001*
	Total CAC score	0.57	<0.001*	0.55	<0.005*
CB	CB left volume	0.60	<0.001*	0.52	<0.001*
	CB left score	0.60	<0.001*	0.44	<0.001*
	CB right volume (mm^3)	0.62	<0.001*	0.53	<0.001*
	CB right score	0.62	<0.001*	0.49	<0.001*
iICA	Annularity	0.45	<0.001*	0.55	<0.001*
	Thickness	0.15	0.28	0.18	0.22
	Continuity	0.47	<0.001*	0.44	0.001*
Aorta	Thoracic annularity	0.33	0.014*	0.36	0.009*
	Abdominal annularity	0.41	0.002*	0.28	0.05

Abbreviations: CB= external carotid artery at the level of the carotid bifurcation, iICA=intracranial internal carotid artery

Supplemental Table 4. Spearman's correlation coefficients were performed to determine the different variables correlated to annularity in the femoropopliteal and crural arteries.

		Femoropopliteal annularity		Crural annularity	
Arterial territory	**Variables in the Equation**	***R***	**p-value (2-tailed)**	***R***	**p-value (2-tailed)**
Coronary artery	Total n. lesions	0.31	<0.02*	0.39	0.004*
	Total calcium volume (mm^3)	0.35	<0.01*	0.47	<0.001*
	Total CAC score	0.30	<0.03*	0.47	<0.001*
CB	CB left volume	0.45	<0.001*	0.46	<0.001*
	CB left score	0.43	0.002*	0.46	<0.001*
	CB right volume (mm^3)	0.33	<0.02*	0.39	0.006*
	CB right score	0.31	<0.03*	0.38	<0.006*
iICA	Annularity	0.32	<0.02*	0.48	<0.001*
	Thickness	0.24	0.09	0.24	0.09
	Continuity	0.33	0.01*	0.37	0.007*
Aorta	Thoracic annularity	0.19	0.18	0.31	0.02*
	Abdominal annularity	0.24	0.08	0.31	0.02*

Abbreviations: CB= external carotid artery at the level of the carotid bifurcation, iICA=intracranial internal carotid artery

Supplemental Table 5. Spearman's correlation coefficients were performed to determine the different variables correlated to thickness in the femoropopliteal and crural arteries.

		Femoropopliteal thickness		**Crural thickness**	
Arterial territory	**Variables in the Equation**	***R***	**p-value (2-tailed)**	***R***	**p-value (2-tailed)**
Coronary artery	Total n. lesions	0.12	0.40	0.30	0.03*
	Total calcium volume (mm^3)	0.27	0.05	0.19	<0.19
	Total CAC score	0.25	0.08	0.19	0.19
CB	CB left volume	0.08	0.54	0.06	0.67
	CB left score	0.09	0.49	0.07	0.63
	CB right volume (mm^3)	0.14	0.33	0.06	0.69
	CB right score	0.14	0.33	0.06	0.66
iICA	Annularity	0.14	0.30	0.04	0.77
	Thickness	0.05	0.73	0.10	0.50
	Continuity	0.02	0.87	0.01	0.92
Aorta	Thoracic annularity	0.07	0.63	0.15	0.29
	Abdominal annularity	0.12	0.40	0.09	0.50

Abbreviations: CB= external carotid artery at the level of the carotid bifurcation, iICA=intracranial internal carotid artery

Supplemental Table 6. Spearman's correlation coefficients were performed to determine the different variables correlated to continuity in the femoropopliteal and crural arteries.

		Femoropopliteal continuity		Crural continuity	
Arterial territory	**Variables in the Equation**	***R***	**p-value (2-tailed)**	***R***	**p-value (2-tailed)**
Coronary artery	Total n. lesions	0.47	<0.001*	0.40	0.003*
	Total calcium volume (mm^3)	0.24	0.08	0.38	0.005*
	Total CAC score	0.25	0.08	0.41	0.003*
CB	CB left volume	0.45	<0.001*	0.22	0.11
	CB left score	0.44	<0.001*	0.21	0.13
	CB right volume (mm^3)	0.35	0.01*	0.30	0.04*
	CB right score	0.35	0.01*	0.29	0.04*
iICA	Annularity	0.34	0.01*	0.51	<0.001*
	Thickness	0.32	0.02	0.38	0.005*
	Continuity	0.35	0.01*	0.32	0.02*
Aorta	Thoracic annularity	0.33	0.02*	0.36	0.007*
	Abdominal annularity	0.27	0.04*	0.30	0.03

Abbreviations: CB= external carotid artery at the level of the carotid bifurcation, iICA=intracranial internal carotid artery

Supplemental Table 7. Baseline characteristics according coronary arterial calcification (CAC) scores

		CAC<1000 (n=23)	**CAC 1000-1999 (n=12)**	**CAC >2000 (n=19)**
		Mean(SD)/n(%)	**Mean(SD)/n(%)**	**Mean(SD)/n(%)**
Age (years)		69 (12)	74 (10)	75(10)
Gender	Male	14(60.9%)	8(66.7%)	14(77.8%)
Length (cm)		171(11)	172(10)	172(10)
Weight (kg)		76.3(19.2)	71.2(13.3)	75.1(18.1)
BMI (kg/m^2)		25.71(4.41)	23.90(2.32)	25.024.59)
Diabetes Mellitus		10(43.5%)	4(33.3%)	10(52.6%)
Stroke		3(13.6%)	2(16.7%)	2(10.5%)
CAD		8(34.8%)	3(25.0%)	10(52.6%)
History of PAD		12(54.5%)	5(41.7%)	14(73.7%)
Smoking status	Former smoker	7(30.4%)	4(40.0%)	7(36.8%)
	Smoker	16(69.6%)	5(50%)	11(57.9%)
Current usage of warfarin		8(34.8%)	2(18.2%)	6(31.6%)
Current usage of hypertensive medicine		16(72.7%)	8(72.2%)	11(57.9%)
Current usage of statins		12(70.6%)	9(100%)	11(73.3%)
Fontaine baseline	3	7(30.4%)	7(58.3%)	8(42.1%)
	4	16(69.6%)	5(41.7%)	11(57.9%)
Systolic BP upper extremity (mmHg)		160(29)	167(26)	160(24)
Diastolic BP upper extremity (mmHg)		86(21)	91(34)	77(10)
Systolic BP ankle (mmHg)		77(43)	72(62)	106(69)
Hypertension (mmHg)	<140	3(13.6%)	1(8.3%)	3(16.7%)
	≥140	19(86.4%)	11(91.7%)	15(83.3%)
eGFR (ml/min/1.73m^2)		77(29)	69(24)	68(27)
CKD	eGFR<60	6(26.1%)	3(25.0%)	8(44.4%)
	eGFR<30	2(8.7%)	1(8.3%)	2(10.5%)
ABI categorized	<0.7	20(87%)	11(91.7%)	11(57.9%)
	0.7-1.40	1(4.3%)	1(8.3%)	1(5.3%)
	>1.40	2(8.7%)	0(0.0%)	7(36.8%)

Abbreviations: BP=blood pressure, CAD=coronary artery disease

Supplemental Table 8. Logistic regression analysis was performed to determine the different variables correlated to CAC scores < 1000, >1000 and >2000.

		CAC <1000 (n=23)			CAC >1000 (n=12)			CAC >2000 (n=19)		
Arterial territory	**Variables in the Equation**	**Odds Ratio**	**95% CI**	**p-value (2-tailed)**	**Odds Ratio**	**95% CI**	**p-value (2-tailed)**	**Odds Ratio**	**95% CI**	**p-value (2-tailed)**
Crural arteries	Most severe	0.12	0.02-0.58	0.009*	9.19	1.82-46.34	0.007*	4.00	1.16-13.83	0.029*
	Complete annular	0.15	0.03-0.76	0.022*	6.08	1.19-31.01	0.030*	4.80	1.27-18.09	0.021*
Femoropopliteal arteries	Most severe	0.08	0.02-0.35	<0.001*	11.70	2.75-49.88	<0.001*	1.53^9	0.00-	0.998
	Complete annular	1.16	0.31-4.39	0.83	0.90	0.24-3.42	0.877	0.68	0.16-2.93	0.600
CB score (stratified)	1601-2400 (Right)	0.50	0.09-2.82	0.43	1.62	0.27-9.70	0.602	0.97	0.16-5.87	0.97
	1601-2400 (Left)	0.30	0.31-2.84	0.29	2.54	0.25-26.18	0.434	2.00	0.26-15.53	0.51
iICA	Complete annular	0.18	0.05-0.74	0.017*	5.10	1.24-20.93	0.024*	6.04	1.68-21.72	0.006*
	Thick	3.43	0.94-12.50	0.062	0.32	0.09-1.16	0.083	0.54	0.16-1.83	0.325
	Continuous	0.29	0.90-9.36	0.038*	3.24	1.00-10.49	0.05	2.34	0.75-7.7	0.141
Aorta	Thoracic complete annular	0.34	0.10-1.14	0.080	3.15	0.93-10.70	0.066	3.61	1.09-11.98	0.036*
	Abdominal complete annular	0.21	0.05-0.85	0.029*	4.44	1.08-18.32	0.039*	7.50	1.99-28.27	0.003*

CHAPTER 6.

Feasibility of Arterial Wall Calcification Quantification by Material Decomposition per Voxel using Spectral Computed Tomography Angiographies in the Femoral Arteries. A Proof-of-Concept Study

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STRUCTURAL ABSTRACT

Background and purposes

Accurate quantification of calcification load in the lower extremity arteries by computed tomographic angiography (CTA) cannot be applied, among other things, due to intraluminal contrast. The aim was to assess technical feasibility of calcium-hydroxyapatite (CaHa) quantification in the femoral arterial wall based on material decomposition of spectral CTA (SCTA) images and compare these findings with the usual Hounsfield Unit (HU) cut-off value-based methods.

Methods

SCTAs of the lower extremities were obtained from three patients and the femoral arteries were segmented. Based on the attenuation coefficient of the photoelectric effect (μ_{pe}) and Compton scattering (μ_{cs}), X-ray attenuation decomposition (XAD)-plots and material compositions were calculated. < 350 HU (no calcium) and < 130 HU (no calcium) values were used as an overlay on the XAD-plot data. Material decomposition data of CaHa and blood-iodine were compared with the usual HU cut-off values.

Results

Based on HU-based calculations below the CTA cut-off value of CaHa (350 HU), between 17.17 % and 90.92% CaHa was missed, when compared to the CaHa detected by this new material decomposition method. Below the value of 130 HU, these missed CaHa percentages were between 0.80 - 8.54% of all CaHa detected by the material decomposition method.
Second, in the artery without visually detectable calcium, as much as 1.1% CaHa of the segmented volume was detectable by the material decomposition method.

Conclusions

With the material decomposition technique by XAD-plots of spectral CT angiography it was feasible to quantify CaHa in the arterial wall of the femoral arteries on CTA.

Key words: Dual-energy CT, spectral CT, multi-energy CT, spectral x-ray, material decomposition, Agatston score, CT angiography

INTRODUCTION

CT angiography and a CT calcium score are both informative investigations for patients with cardiovascular diseases. Both methods use Hounsfield Units (HU) for material differentiation on a CT scan. Often values between 250 HU and 350 HU are used on CT angiography of the lower extremities for iodinated blood [1-4]. For the CT calcium score on unenhanced CT all voxels >130 HU are considered calcium. In clinical practice these cut-off values work reasonably well for unenhanced CT although, due to overlap of HU-values and partial volume effects these measurements are still imprecise. For CTA the situation is even more challenging. In a recent study that attempted to obtain a calcium score from CT angiography iodine containing blood was set between 250 and 350 HU while calcium was set as more than 350 HU [5]. A calcium score determined in this way obviously underestimates the calcium load and it was deemed too unreliable for practical use [6, 7].

However, novel options are offered since the introduction of dual-energy, also called multi-energy or spectral CT (SCT). SCT can improve dose efficiency compared to conventional CT [8, 9], but it can also be used for separation of materials and material quantification. There are several spectral methods to determine the exact material composition of a voxel [10]. It can be assessed by post-reconstruction image space, using ϱZ decomposition [11], basis material decomposition [12], K-edge imaging [13] and projection- and image-space approaches with decomposition algorithms [14, 15]. Le Huy *et al.* quantified hydroxyapatite in a phantom study, based on the least squares parameter estimation method [15].

Materials can also be calculated by material decomposition per voxel based on Compton scatter (CS) and photoelectric absorption (PE), first described in 1976 [16]. They found that the attenuation coefficient as a function of energy (μ_e) can be expressed as a linear combination of separate attenuation coefficient functions for CS and for PE [16]. The combined values of these attenuation factors are material specific. Thus, this material decomposition method could obviate the need to rely on arbitrary HU-based cut-off values to the arteries for analyzing arterial pathology *in-vivo* and was therefore tested in this study.

The aims of this study are to show that 1) it is feasible to quantify calcium hydroxyapatite (CaHa) in the arterial wall of iodinated blood vessels based on the material decomposition technique determined by spectral CT angiography (SCTA), and 2) that conventional CT angiography techniques leads to different calcium values.

MATERIALS AND METHODS

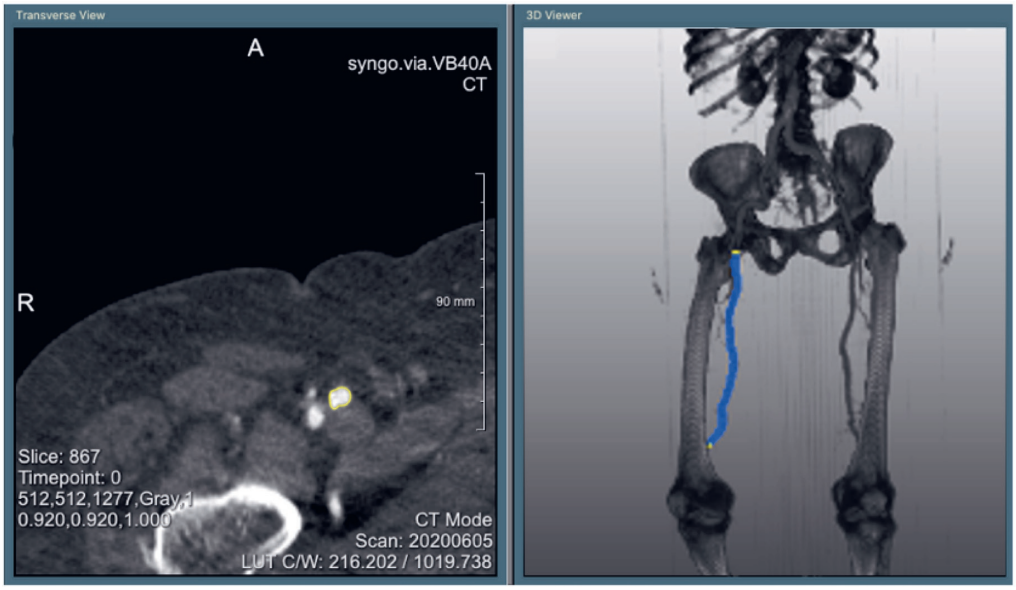
Scanners and technical parameters

For this investigation, three anonymous spectral CT angiography (SCTA) datasets of lower extremity arteries acquired on a IQon Spectral CT (Philips, Best, The Netherlands) were used. The mAs was set at approximately 78-100 mAs. Virtual mono-energetic (monoE) reconstructions of 50keV, 80keV and 100 keV were calculated. The 50 and 100keV were used for X-ray attenuation decomposition (XAD) calculations. The 80keV scan was only used to verify these calculations. 90 ml Xenetix (iodine concentration 300 ml/mg, flow (2ml/sec)) was injected intravenously. Slice thickness was set between 0.625 and 1 mm. The voxel sizes were different for all three patients. Case 1 had a voxel size of 0.7 x 0.799 x 0.799 mm (spacing between slices x pixel spacing on the x-axis and y-axis). Case 2 had a voxel size of 0.7 x 0.588 x 0.588 mm and case 3 0.7 x 0.596 x 0.596 mm. This accounts for a volume per voxel of 0.447 mm^3 in case 1, a volume per voxel of 0.242 mm^3 for case 2 and a volume per voxel of 0.249 mm^3 in case 3.

Arterial segmentation

The automatic tubular tracking method was built in the MeVisLab framework (MeVis Medical Solutions AG, Bremen, Germany). Based on the method proposed by Friman *et al.* arterial segmentation was performed [17]. After the automatic segmentation, two medical doctors (RAPT and LCDK) with both more than 5 years of experience in radiology, corrected the segmentations on points where the vessel tracking proved unsatisfactory. See **Figure 1** for an example of these segmentations.

Figure 1. Example of the segmentation of a femoral artery in the MeVisLab software in the axial 2D and coronal 3D viewer. The automatic segmentation is visible as a blue outline on the right image. The proximal and distal segmentations were corrected by hand (see yellow outlines). An example of the manual correction to the automatic segmentation is shown as the yellow outline on the left axial image.



XAD-plots and material decomposition method

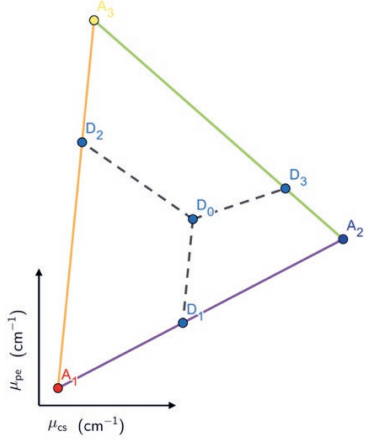
The photon cross section values of materials were taken from reports from the National Institute of Standards and Technology (NIST) and the International Commission on Radiation Units and Measurements (ICRU) [18, 19]. Materials were represented as photoelectric effect attenuation coefficient in cm^{-1} (μ_{pe}) and Compton scattering attenuation coefficient in cm^{-1} (μ_{cs}). The Raleigh scattering component was included in the photoelectric effect. With these two variables, the location of the material could be indicated on the XAD-plot (see **Figure 2A** and **Figure 2B**). The lines in the XAD-plots indicate the pure materials based on μ_{pe} and μ_{cs}, where the relative distance along the line concerns the volume fractions of the materials. The voxels close to the intersection of the x-axis and y-axis are the voxels that contain the least of the relevant material, the voxels further away from the intersection point are the voxels that contain the most of the relevant material.

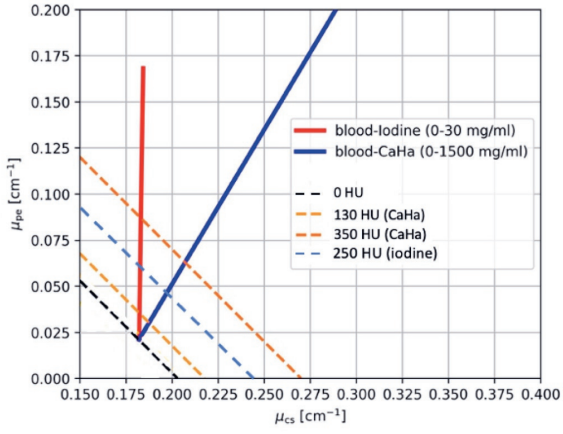
By material decomposition, materials can be calculated as a fraction of a voxel. In the calculations, the *number of voxels per material* are all voxels completely filled

with a certain material of all segmented voxels. Since voxel size differed for all three cases, the conversion of the absolute number of voxels to a volume (in mm^3 was then made to improve readability and comparability of the three cases.

The materials in this study are defined as *blood-iodine* and *blood-CaHa*. The term 'blood' is added in these definitions, since a voxel can be composed of both blood and CaHa or composed of blood and iodine. The blue *blood-CaHa line* on the XAD plot therefore represents the voxels with the material composition of CaHa. Voxels on or around this line represent voxels with a percentage of CaHa. The red line is the *blood-iodine line*, the voxels on or around this line represent voxels with a certain percentage of iodine.

Figure 2A. The figure illustrates the material decomposition of point D0, used to define the position on the XAD-plot as shown in figure B. **Figure 2B.** The general outline of the XAD-plot is shown. The solid lines show the attenuation coefficients of different concentrations of materials of blood-iodine and blood-CaHa. The dashed lines indicate the HU reference values of water (0 HU), CaHa on a non-contrast CT (Agatston score) (130 HU), CaHa on a CTA (>350 HU) and iodine (>250 HU).





Definitions of material volumes and fractions

The *segmented volume* is the complete volume (in mm^3) of the femoral arterial segmentation included in the material decomposition method (absolute number of voxels x voxel diameters). These are only the voxels to the right of the blood-iodine line in the XAD-plots (see **Figure 2B**).
The *blood-iodine volume* is the volume (in mm^3) of the femoral arterial segmentation included in the material decomposition method (absolute number of voxels x voxel diameters) containing iodine or a mixture of blood and iodine in the voxel, since not all voxels are filled completely with iodine. The *blood-iodine %:* the percentage of blood-iodine of the complete segmented femoral arterial volume.

The *blood-CaHa volume* is the volume (in mm^3) of the femoral arterial segmentation included in the material decomposition method (absolute number of voxels x voxel diameters) containing CaHa voxels or a mixture of blood and CaHa in the voxel since not all voxels are filled completely with CaHa. The *blood-CaHa %* is the percentage of blood-CaHa of the complete segmented femoral arterial volume.

Overlay of HU-values on the XAD-plot

Certain cut-off HU values can also be displayed on the XAD plot, as diagonal lines. In this case, the values of iodine and CaHa used in current clinical practice are shown. On an unenhanced CT - as used with the Agatston score - the cut-off value of calcium is 130 HU. Good contrast opacification in the lower extremities assume a HU-value of between 250 and 350 HU [1-4]. On a CTA, the threshold for measuring CaHa is thus raised to > 350 HU in order not to measure iodine [5]. A theoretical visual explanation is presented in **Figure 2B** regarding these overlapping HU values.

Differences between material decomposition and HU-based CaHa measurements

After calculating material decomposition data, several absolute values and percentages of the blood-CaHa line and blood-iodine line could be calculated. With the HU cut-off values displayed on the XAD plot, it was possible to calculate what percentage of blood-CaHa and blood-iodine would be missed with the HU-based detection versus the detection of CaHa with the material decomposition method. For example, with regard to the CTA CaHa threshold 350 HU, voxels below the dashed 350 HU represent all voxels that are counted as CaHa using the material decomposition method, and not the HU-based method.

Visual conventional CTA reading

After constructing the XAD-plot and calculating the material compositions, the associated SCTA was visually checked for CaHa by a senior radiology resident with 5 years of experience in calcification research (LCDK).

Statistics

Given the sample size of n=3 no statistical analysis was done in this feasibility study.

RESULTS

XAD-plots and SCTA findings of the three cases

The results of the material composition calculations and percentages of material composition of the three SCTAs are shown in **Table 1.** Below, the results per case are discussed.

Table 1. Absolute volumes and percentages calculated by the material decomposition method of the corresponding three SCTA's.

CTA figure	Blood-iodine volume (mm^3)	Blood-iodine %	Blood-CaHa volume (mm^3)	Blood-CaHa %	Segmented volume (mm^3)
Fig. 2A	2214.44	99.01	24.14	1.1	2236.34
Fig. 2B	2011.75	92.6	159.96	7.4	2173.40
Fig. 2C	5523.07	92.8	493.02	8.2	6014.10

Abbreviations: SCTA=spectral computed tomography angiography, CaHa= calcium-hydroxyapatite.
Volumes shown in mm^3, fractions shown as mean/percentages.

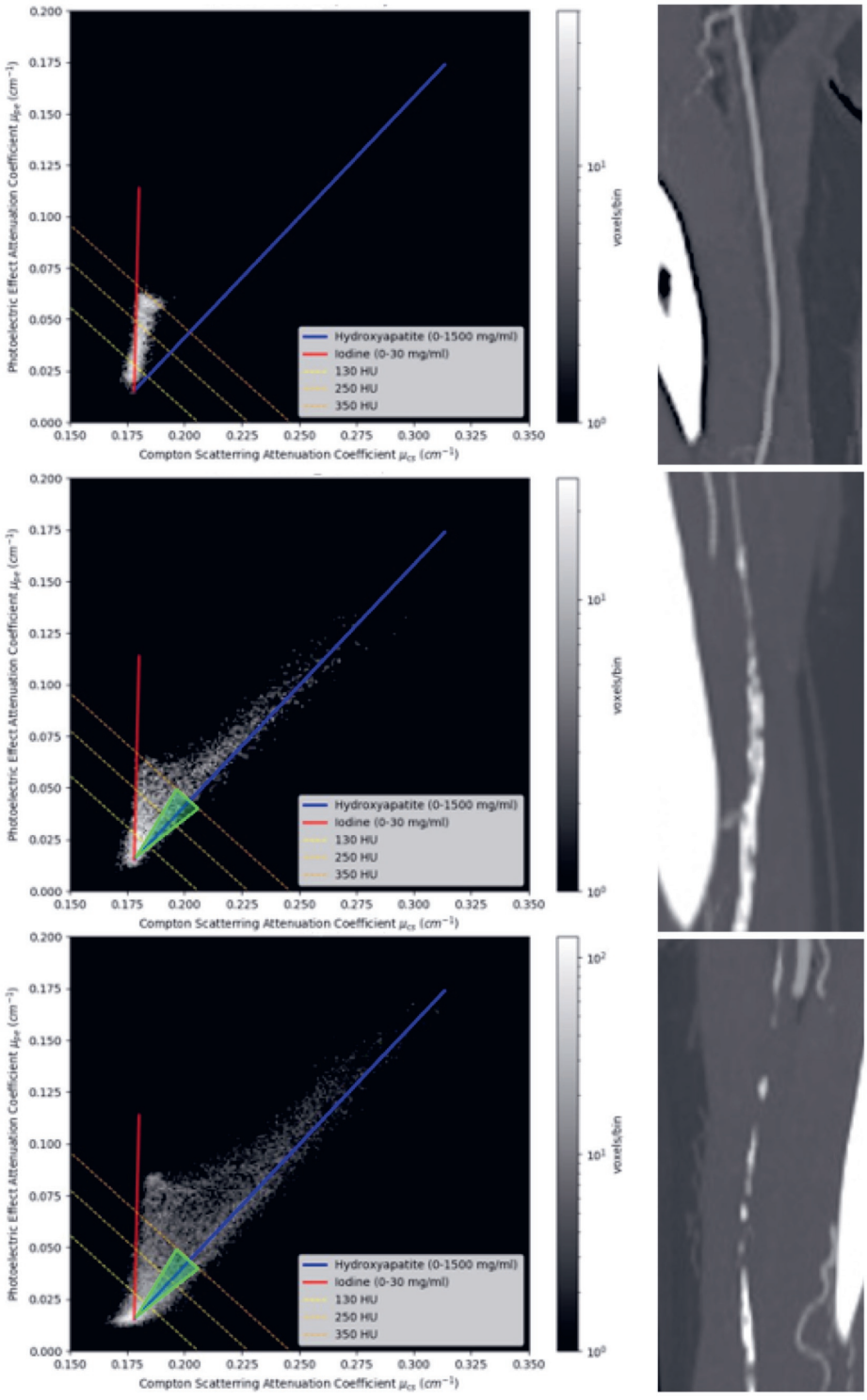
Case 1: A normal femoral artery is shown without any calcification visible on the CTA (**see Figure 3, case 1**).
SCTA/XAD plot: Most of the voxels in the XAD-plot spread along the blood-iodine line. As mentioned above, there was no visual objectification of CaHa. However, in this SCTA there was CaHa measurable with the material decomposition method. The calculated total CaHa volume was equivalent to a total of 24.14 out of 2236.34 mm^3 of all segmented voxels, accounting for 1.1% total CaHa in this segmentation.
The blood-iodine percentage was 99.01% in this segmentation (blood-iodine volume/ segmented volume = 2214.44/2236.34 mm^3).

CTA/HU-values: There was 21.90 mm^3 CaHa with a HU value of less than 350 out of a total of 24.14 mm^3 CaHa identified by the material decomposition method. This equates to a missed CaHa percentage of 90.72% when the HU value of 350 was used.

Case 2: Moderate to extensive calcified femoral artery with open lumen (**see Figure 3, case 2**.
SCTA/XAD plot: In addition to the voxels around the red blood-iodine line, many voxels around the blue blood-CaHa line were found indicating CaHa. The blood-CaHa volume in this segmentation was equal to 159.96 mm^3. Out of the total segmented volume of 2173.40 mm^3, the blood-CaHa percentage was 7.4%.
The blood-iodine percentage in the XAD plot was lower than in case 1 with 92.6% (blood-iodine volume/segmented volume = 2011.75/2173.40 mm^3).
CTA/HU-values: There was a volume of 30.98 mm^3 with an HU-value lower than 350, which equates a missed CaHa rate of 19.42% (30.98 (<350 HU voxels on the blood-CaHa line)/ 159.96 mm^3 (volume of all voxels on the CaHa line)). For a visual explanation of these missed voxels, see the green triangle in **Figure 3, case 2 and 3** indicating these voxels).

Case 3: Extensive calcified femoral artery with occluded Hunters' canal (**see Figure 3, case 3.**
SCTA/XAD plot: Also in this case, there were numerous voxels around the blood-CaHa line. In total there was a blood-CaHa volume of 493.02 mm^3. The total volume in this segmentation was 6014.10 mm^3. Therefore, 8.2% of the total volume consisted of CaHa.
The blood-iodine percentage was 90.84% (blood-iodine volume/ segmented volume = 5523.07/6014.10 mm^3).
CTA/HU-values: The total volume of CaHa identified by the material decomposition method was 493.02 mm^3. Of this volume, there was a volume of 84.66 mm^3 with a < 350 HU on the CTA. Therefore, in case 3 17.17% of the total CaHa volume were missed with the HU-based analysis compared with the material decomposition method.

Figure 3: Overview of various XAD-plots made from the data per case (1,2,3).*



*= ***Case 1*** *(upper image): example of a healthy artery with most of the volume projecting over the blood-iodine line.* ***Case 2*** *(middle image): example of an artery with extensive calcification resulting in a distribution is characteristically spun up between the blood-iodine and blood-CaHa lines.* ***Case 3*** *(lower image): a more extensive calcified artery with an occlusion of the superficial femoral artery. N.B. The green triangles in figure B and C projected over the represents < 350 HU volume (in mm³).*

We can conclude that in all three cases there was a substantial percentage of CaHa with a HU value below 350 missed in the HU-based analyses with a spread between 17.18 - 90.97%. See **Table 2** and **Table 3** for an overview of HU-based calculations.

Table 2. Absolute volumes and percentages derived from the HU-based method, corresponding the three SCTA's.

CTA figure	>130HU volume (mm³)	> 250 HU <= 350 HU volume (mm³)	>350 HU volume (mm³)	Segmented volume (mm³)
Fig. 2A	1963.22	1182.76	64	2236.34
Fig. 2B	2021.67	651.95	841	2173.40
Fig. 2C	5381.89	1215.87	2753	6014.10

Abbreviations: SCTA=spectral computed tomography angiography, HU= Hounsfield Units, CaHa= calcium-hydroxyapatite.
HU cut-off values: >130 HU = CaHa on a non-contrast CT, >250<=350 HU = expected iodine volume, >350 HU = CaHa on a contrast-enhanced CT.

Table 3. Absolute CaHa volumes (in mm³) calculated with the material decomposition method, truncated for 130 HU and 350 HU, the currently in literature known HU-calcium detection values.

CTA figure	Caha < 130 HU volume (mm3)	CaHa >130 HU volume (mm3)	CaHa <350 HU volume (mm3)	CaHa >350 HU volume (mm3)	Total CaHa by MDM (mm3)	Segmented volume (mm3)
Fig. 2A	2.24	21.90	21.90	2.24	24.14	2236.34
Fig. 2B	1.21	158.75	30.98	128.99	159.96	2173.40
Fig. 2C	6.23	486.55	84.66	408.36	493.02	6014.10

Abbreviations: SCTA=spectral computed tomography angiography, HU= Hounsfield Units, CaHa= calcium-hydroxyapatite.
HU cut-off values: >130 HU = CaHa on a non-contrast CT, >350 HU = CaHa on a contrast-enhanced CT. MDM = material decomposition method.

CaHa material decomposition in relation to the HU cut-off for calcium on the Agatston score

In the Agatston score a cut-off value for CaHa is >130 HU. On the material decomposition image, there were also voxels with a value <130 HU laying on the blood-CaHa line. Calculations were also made with this >130 HU cut-off to see which percentage of the voxels were not detected with the HU-based method, but were detected with the material decomposition method.

This accounts for a missed >130 HU CaHa percentage of 8.54% in case 1 (2.24/24.14). In case 2 this percentage accounts for 0.80% (1.21/159.96) and in case 3 1.28% (6.23/493.02). This might be due to an overestimation of calcium in the XAD method, although in our clinical experience there are also calcifications visible on CT with a HU below 130 HU.

DISCUSSION

This study investigated the first technical feasibility of a new material decomposition method using SCTA based on μ_{pe} and μ_{cs} using XAD-plots. We have shown that the material decomposition technique of CaHa and iodine containing blood results in different amounts of these materials in the arteries of the femoral arteries on SCTA, compared to the conventional method with HU-based cut-off values. Whether this bias is in the conventional CTA, the XAD method or both requires further investigation.

XAD plots and SCTA findings

We found CaHa values in the three arteries that matched the visual scoring to some extent, as visually more severely calcified arteries had higher CaHa load. In the patient that had visually no CaHa, however, the material decomposition results showed that a total CaHa volume of 24.14 mm^3 (filling 54 voxels) was present. In other words, all small fractions of CaHa in each voxel added together give enough CaHa in the material composition calculations to fill 54 voxels. Ex-vivo work with the micro-CT has shown that in the legs and in the circle of Willis small amounts of CaHa can be found that are invisible with conventional CT scan but seen on micro-CT [20, 21]. It is possible that this volume of visually non-detectable CaHa voxels represents these small amounts as seen on micro-CT but further studies must confirm this. Another explanation for these 54 voxels could be noise which could cause voxels to get an incorrect attenuation coefficient and therefore incorrect material composition in the calculations. Visually, however, there was no scattering

of, for example, metal artifacts or otherwise. Finally, it could represent a systematic bias in the XAD method.

Another interesting finding of our study was that we even found between 0.80 and 8.54% of voxels CaHa, below 130 HU. Since the Agatston calcium scoring on CT was introduced, an arbitrary cut-off of 130 HU on a non-contrast CT has been established as a fixed value both in current research and clinical patient risk stratification [22-27]. The value of 130 HU is determined to avoid noise with lower HU-values in the calcium score. A study in 2012 by Dhungel *et al.* however investigated the optimal cut-off calcium HU-value by comparing CT to intravascular ultrasound and showed that optimal calcification values were different per patient [28]. They also found a wide spread of calcifications above and below 130 HU, choosing the lowest threshold as 50 HU. Another study found that a lowering of the HU-value to 90 leads to a higher accuracy and the area under the receiver operating characteristic curve [29]. Lowering the threshold to 90 HU would be possible with current scanners since they have a higher signal-to-noise-ratio; however, the specificity decreases from 61% to 52% when lowering the threshold from 130 HU to 90 HU [29, 30]. It remains to be determined, in relation to a proper reference standard, whether SCT with XAD analysis improves the measurement of CaHa on CTA.

Limitations

Several limitations deserve mention; adequate segmentation and the validation. First, adequate segmentation is essential for the calculations of material composition in this method. A correct segmentation method not only ensures the exclusion of as much non-vessel related soft tissues as possible, but ensures also reliable and consistent tracking, even in sections of stenosis or complex morphology due to disease. The used segmentation method is based on the iodine-blood column, which determines the volume of the segmentation. The segmentations were visually checked by two senior radiology residents and corrected if the segmentation did not match the blood-iodine column.

Our study was a technical feasibility study to provide a proof of concept that material decomposition can be applied in the vessels of the lower extremities on CTA. This study is inherently limited by the sample size and absence of a proper reference standard. In order to eventually use this method for diagnostic purposes, phantom studies and subsequent in vivo studies should be carried out.

Third, with respect to the triangle spanning the three materials, the total of the 3 separate volume fractions should be 100%. In our data we arrive at a maximum

deviation of 1% of 100%, because we allow for small deviations due to noise. We cannot therefore explain a small part of the voxels as CaHa, blood or iodine; these voxels can be identified as those voxels outside the triangle. However, the influence of this on our method and data is very small, but should be mentioned here.
We advise to refine this material decomposition method in the future with a maximum margin of error for the intermediate calculations of the volume fractions of no more than 5% deviating from 100% should be used as an additional validation method in the following calculations.

Future research: arterial wall material compositions

As can be seen in the XAD-plots, the lines of the material composition of blood-iodine and blood-CaHa are well spaced if concentrations of iodine or CaHa are not too small. The material composition lines of fat, fibrous and muscular tissue are not shown in the figures, but these are situated closer together and therefore it was not possible at this time to perform material decomposition of the arterial wall other than blood-CaHa and the amount of blood-iodine. Since in principle any material can be calculated with the material decomposition method based on the μ_{pe} and μ_{cs} per material, this method offers opportunities for the future to quantify these arterial wall materials too. If the technical challenges, with noise, spatial resolution, partial value and others can be solved, the degree of arterial pathologies may be quantifiable by CTA *in vivo*.

Conclusions

With the material decomposition technique by μ_{pe} and μ_{cs} using XAD-plots of spectral CT angiography it was feasible to quantify CaHa in the arterial wall of the femoral arteries and iodine containing blood in the vessel lumen. This technique may avoid underdetection of CaHa as is frequently the case with HU-based cut-off values. Further studies needed to be performed to explore the functionality and accuracy of this material decomposition technique.

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PART III.

Outcome of chronic limb-threatening ischemia treatment

CHAPTER 7.

10-YEAR PACLITAXEL DOSE-RELATED OUTCOMES OF DRUG-ELUTING STENTS TREATED BELOW THE KNEE IN PATIENTS WITH CLTI☆

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STRUCTURED ABSTRACT

Background and objectives

Recently, two meta-analyses concluded that there appears to be an increased risk of long-term mortality of paclitaxel-coated balloons and stents in the superficial femoral and popliteal artery, and paclitaxel-coated balloons below the knee. In this post-hoc study of the PADI Trial, we investigated the long-term safety of first-generation paclitaxel-coated drug-eluting stents (DES) below the knee and the dose-mortality relationships of paclitaxel in patients with chronic limb-threatening ischemia (CLTI).

Materials and methods

The PADI Trial compared paclitaxel-coated DES with percutaneous transluminal angioplasty with bail-out bare-metal stents (PTA±BMS) in patients with CLTI treated below the knee. Follow-up was extended to 10 years after the first inclusion and survival analyses were performed. In addition, dose-related mortality and dose per patient weight-related mortality relations were examined.

Results

A total of 140 limbs in 137 patients were included in the PADI Trial. Ten years after the first inclusion, 109/137 (79.6%) patients had died. There was no significant difference between mortality in the DES group compared with the PTA±BMS group (Log-rank p-value= 0.12). No specific dose-related mortality (HR 1.00, 95% CI 0.99-1.00, p=0.99) or dose per weight mortality (HR 1.05, 95% CI 0.93-1.18, p=0.46) relationships were identified in the Cox-proportional Hazard models or by Kaplan-Meier survival analyses.

Conclusions

There is a poor 10-year survival in both paclitaxel-coated DES and PTA±BMS in patients with CLTI treated below the knee. No dose-related adverse effects of paclitaxel-coated DES were observed in our study of patients with CLTI treated below the knee.

Key words: chronic limb-threatening ischemia, below the knee, drug-eluting stents, paclitaxel, mortality, dose-related analysis

INTRODUCTION

Drug-eluting stents (DES) were developed to improve patency with less in-stent restenosis caused by intimal hyperplasia, which is the most common constraint of bare-metal stents (BMS) [1-4]. DES were implemented in 2012 in patients with peripheral arterial disease (PAD). To compare the performance of paclitaxel-coated DES and standard treatment, percutaneous transluminal angioplasty with bail-out BMS (PTA±BMS) below the knee (BTK) in patients with chronic limb-threatening ischemia (CLTI), the Percutaneous transluminal Angioplasty versus Drug eluting stents for Infrapopliteal lesions (PADI) Trial was conducted [5]. This randomized clinical trial (RCT) showed a significantly better patency and a higher amputation-free and event-free survival at 5-years with paclitaxel-coated DES compared with the current reference treatment PTA±BMS [6, 7]. These results are consistent with other studies that show lower restenosis rates in infrapopliteal lesions treated with DES in their follow-up [3, 8, 9].

Recently, two meta-analyses were published that concluded that there appears to be an increased risk of 5-years mortality of paclitaxel-coated drug-eluting balloons and DES in the superficial femoral and popliteal artery [10], and a significantly worse 1-year amputation-free survival in patients treated with DEB below the knee [11]. The vascular areas and devices used in the PADI study are not the same as in the published meta-analyzes and the survival results are not consistent either.

The aim of this study was to extend the PADI Trial long-term follow-up to 10 years after the first inclusion and to evaluate the mortality to assess if the use of paclitaxel-coated DES in our population is safe.

MATERIALS AND METHODS

The PADI Trial

Study approval

The medical ethical board of the participating centers approved the PADI Trial (Unique identifier number: NCT00471289) and written informed consent was obtained from all patients. The present study is a post-hoc study of this RCT.

Patient population

The PADI Trial was an investigator initiated prospective, multicenter RCT that compared the patency and clinical performance of paclitaxel-coated drug-eluting stainless-steel coronary stents (TAXUS Liberté stent system; Boston Scientific Corporation, Natick, United States) versus the current reference treatment with

PTA±BMS in patients with CLTI treated infrapopliteal/BTK. Extensive information of the study protocol and mid- to long-term results can be found in previous publications [5-7, 12].

Briefly, between October 2007 and February 2013, patients with CLTI who were treated BTK were included in three hospitals in the Netherlands. A total of 144 limbs in 141 patients were included. Randomization was performed per limb. Four patients were included for 2 limbs with 1 limb in each study arm. Four limbs/patients were excluded, 1 patient (1 limb) in the DES arm and in the PTA arm 3 patients (3 limbs), because of intermitted claudication (IC) (n=1), renal failure without dialysis (n=1), coagulation disorder (n=1) or a too small vessel for treatment (n=1). In total, 140 limbs in 137 patients were included for analysis of which 74 limbs in 73 patients for paclitaxel-coated DES and 66 for PTA±BMS in 64 patients.

Methods

In the PADI Trial, stenosis of the lesions had to be at least >50%, the lesion length ≤90mm and the arterial diameter between 2 to 6mm. Bail-out BMS was used in case of flow limiting lesions, rest stenosis >50% or occlusion after PTA.
For both the DES and the PTA±BMS group the stent lengths and stent diameters were noted in the case report form. Per patient, a maximum of three DES was placed and the total number of stents was recorded. Per DES, the paclitaxel dose was then calculated on the basis of its length and diameter, according to the TAXUS Liberté product information [13]. For an overview of the used DES sizes in this study, see **Supplemental Table 1.**

The Taxus Liberté stents are manufactured from a 316L stainless steel stent. This stent is coated with a drug-polimer coating (consisting of a mix of paclitaxel and a Translute polymer carrier, directly applied on the stent). Paclitaxel is the active pharmaceutical ingredient, with the molecular formula of C47H51NO14. There is no topcoat layer or primer.

Follow-up

Follow-up in the PADI Trial consisted of computed tomography (CT) angiography, duplex ultrasound and clinical assessment at 6 months. After that, patients were assessed annually for a period of 5 years after treatment by medical history, physical examination and duplex ultrasound of the treated limb. If patients were unavailable for follow-up, the information was gathered from general practitioners by phone or from digital medical records. The 5 years follow-up data have been published previously [7]. Regarding the follow-up data until April 2019

(10 years) for this current report, municipal basic records were checked for death and date of death.

Statistical analysis

Data analysis was carried out using SPSS version 24.0 for Windows (IBM Corporation, New York, United States). Baseline characteristics were assessed to describe the study population.

Continuous variables were calculated as means with standard deviations (SD). Categorical variables were presented as counts and percentages and tested with the Chi-squared tests. P-values were tested two-sided, a p-value of less than 0.05 was considered to be significant.

Survival of patients was assessed up to ten years after the first inclusion and 10-year all-cause mortality for all patients was estimated using Kaplan-Meier plots. Significance was tested by Log-Rank (Mantel-Cox) tests.

The average dose of paclitaxel per stent, the total dose per patient and the dose per body weight ratio (μcg/kg) were assessed. Plotted in a stacked figure are the number of patients per dose (0-700μcg). All Cox-proportional hazard models were performed for 10-year all-cause mortality. Cumulative dose was stratified per 150μcg (0-149, 150-299, 300-449, 450-700μcg) and univariate analyses and age- and sex-adjusted effects were calculated by hazard ratios with 95% confidence intervals (CI).

In addition, separate univariate analyses were performed for paclitaxel-coated DES (dichotomous) and total dose per kilogram body weight (continuous). Multivariate modelling was performed included paclitaxel-coated DES and known risk factors of CLTI: age, smoking, history of PAD, diabetes mellitus, previous stroke or transient ischemic attack, coronary arterial disease and the use of anticoagulant medication.

RESULTS

Baseline characteristics

At the time of treatment, mean age in the DES group was 74.2 ± 12.1 years and in the PTA±BMS group 72.9 ± 11.9 years. 67.1% of the patients in the DES group were male. Overall, patients treated with DES were more likely to have a history of PAD than patients treated with PTA±BMS (72.7% vs 55.4% respectively, p-value=0.03). Patients treated with DES also had a higher percentage of previous amputation (34.8% vs 18.9% respectively, p-value=0.03). Baseline characteristics are summarized in **Table 1.**

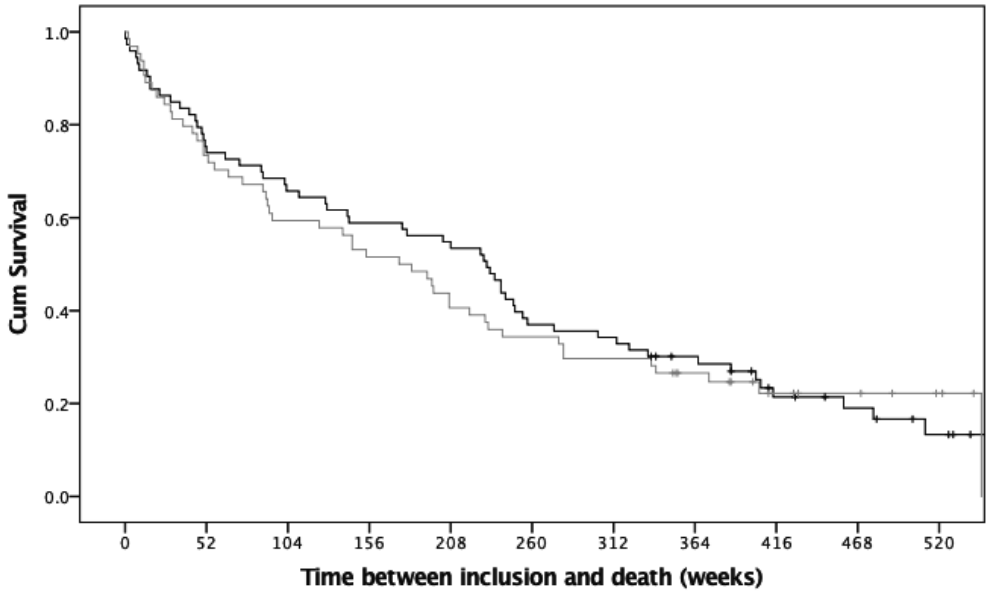
Table 1. Baseline characteristics total group (paclitaxel-DES and PTA±BMS), total 140 limbs in 137 patients, of which 74 DES limbs in 73 patients and 66 PTA±BMS limbs in 64 patients.

		PTA±BMS (n=64)		Paclitaxel-DES (n=73)		p-value
		Mean ± SD	N(%) /min-max	Mean ± SD	N(%) /min-max	
Age (years)		72.9±11.9	40-93	74.2±12.1	40-98	
Sex (male)			47 (73.4%)		49 (67.1%)	0.64
Smoking status	smoker		17 (26.6%)		16 (21.9%)	0.64
	ex-smoker		12 (18.8%)		18 (24.7%)	
Diabetes mellitus			43 (67.2%)		44 (60.3%)	0.36
Previous stroke or TIA			13 (20.3%)		12 (16.4%)	0.60
Coronary artery disease			25 (39.1%)		27 (37%)	0.87
History of PAD			41(55.4%)		48(72.7%)	0.03*
Previous amputation			14(18.9%)		23(34.8%)	0.03*
On anticoagulant medication			58 (90.6%)		67 (91.8%)	0.58
Impaired renal function (eGFR<30)			10 (15.6%)		10 (13.7%)	0.75
Rutherford at baseline	4		8 (12.1%)		10 (13.5%)	0.83
	5		46 (69.7%)		48 (64.9%)	
	6		12 (18.2%)		16 (21.6%)	
Ankle-brachial index		0.81±0.28	0.15-imm.	0.89±0.28	0.22-imm.	

Abbreviations: PTA=percutaneous transluminal angioplasty; BMS= bail-out bare-metal stents; DES = drug-eluting stent; TIA= transient ischemic attack; PAD=peripheral arterial disease; eGFR=electronic glomerular filtration rate(mL/min/1.73m^2); imm.=immeasurable high ankle-brachial index.

Kaplan-Meier curves up to 10 years after the first inclusion are shown in **Figure 1**. After 10 years, the majority patients had died 109/137 (79.6%). There was no significant difference between mortality in the DES and PTA±BMS group, 59/73 (80.8%) and 50/64 (78.1%), respectively (Log-rank (Mantel-Cox) p-value=0.12). Mean survival was 236.7 weeks (95% CI 203.0 – 270.3 weeks) with a maximum follow-up of 520 weeks.

Fig 1. 10-years Kaplan-Meier survival curves for DES (black line) versus PTA±BMS (grey line).



Numbers at risk (numbers censored)

Log-rank (Mantel-Cox) p-value= 0.12

Time(weeks)	**0**	**52**	**104**	**156**	**208**	**260**	**312**	**364**	**416**	**468**	**520**
DES	73	55	48	43	40	27	25	19	11	8 (2)	4 (4)
	(0)	(0)	(0)	(0)	(0)	(0)	(3)	(3)	(2)		
PTA±BMS	64	47	38	33	26	22	19	14	8 (2)	6 (3)	3 (2)
	(0)	(0)	(0)	(0)	(0)	(0)	(3)	(4)			

There was also no significant difference in mortality between the different Rutherford classes included; 4, 5 or 6 (Log-Rank (Mantel-Cox) p= 0.13). However, a non-significant difference in mortality between Rutherford 4 versus Rutherford 5 and 6 was noticeable. Rutherford 4 had a mean survival of 304.3 weeks (95%CI 208.6-

400.0 weeks) versus Rutherford 5 with a mean survival of 199.9 (95% CI 164.0-235.7 weeks) and Rutherford 6 a mean survival of 191.04 (95% CI 129.3-252.8 weeks). Interestingly, a significant Log-Rank (Mantel-Cox) value was found for impaired renal function; p= 0.04.

Mean survival of patients with a renal function lower than 30 mL/min/1.73m^2 was 144.3 weeks (95% CI 70.9-217.6 weeks), while patients with a renal function higher than 30 mL/min/1.73m^2 had a significant longer mean survival of 226.3 weeks (95% CI 192.5-260.0 weeks). Corresponding Kaplan-Meier curves of different Rutherford classes and impaired renal function are shown in **Supplemental Figures 1 and 2**.

Paclitaxel-coated drug-eluting stents and dose

The average number of stents in the DES group was 1.81 ± 0.84. There were no significant differences in comorbidity per total number of stents placed per patient. In the DES group, the stent diameters ranged between 2 and 4mm (mean 2.83 ± 0.40). Length of stents ranged between 16 and 38mm (mean 29.74 ± 4.30). The paclitaxel dose per DES varied between 77.00 and 273.00µcg (mean 182.27 ± 39.05). See **Table 2** for more detailed information.

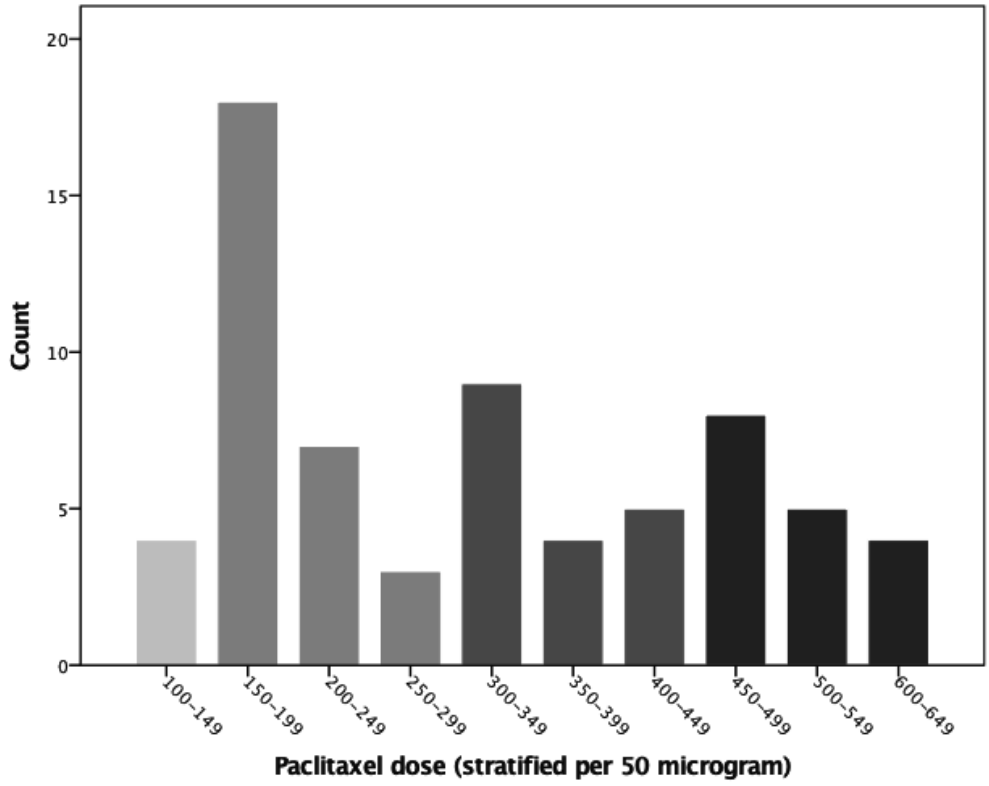
The paclitaxel dose in male patients was slightly higher than in female patients (336.61 ± 158.87 vs 273.74 ± 131.15µcg, respectively). Per added stent the mean, minimum and maximum paclitaxel dose increased. The total paclitaxel dose per patient varied between 116.00 and 645.00µcg with a mean dose of 315.03µcg. A paclitaxel dose between 150 and 200µcg was most frequently observed (see **Figure 2**). Patients had a mean body weight of 75.6 ± 12.9kg. The mean paclitaxel dose per kg was 4.3µcg with a wide spread (min-max 1.3-11.99µcg/kg).

Table 2. Stents, dose, weight and the relation of these parameters for patients treated with paclitaxel-coated DES in the PADI Trial.

		Number (%)	Mean±SD	Min-max
Stents				
Number of stents			1.81 ± 0.84	1.00-3.00
Diameter (mm)			2.83 ± 0.40	2.00-4.00
Length (mm)			29.74 ± 4.3	16-38.00
Dose per stent				
Total placed stents	1	30 (41.1%)	172.69 ± 40.87	116.00-266.00
(numbers and dose	2	29 (39.7%)	366.75 ± 80.30	213.00-532.00
(μcg))	3	14 (19.2%)	521.21 ± 79.15	361.00-645.00
Dose per gender and per weight				
Sex (numbers	Male	49 (67.1%)	336.61 ± 158.87	116.00-645.00
and dose (μcg))	Female	24 (32.9%)	273.74 ± 131.15	116.00-478.00
Weight (kg)			75.6 ± 12.9	47.0-122.0
Dose per patient (μcg)			315.03 ± 151.93	116.00-645.00
Dose/weight (μcg/kg)			4.30 ± 2.28	1.30-10.99

Per patient 3 stents maximum are used. Data are displayed as mean±SD, minimum and maximum.

Figure 2. Total paclitaxel dose per patient. The 4 different gray values are corresponding the 4-fold classes as used in the analysis in **Table 3.**



Cox-regression analysis of paclitaxel dose-response relationships

No significant univariate hazard ratios were found for the use of paclitaxel-coated DES as a dichotomous variable (HR 1.14, 95% CI 0.78-1.66, p=0.50), for the total dose of paclitaxel as a continuous variable (HR 1.00, 95% CI 0.99-1.00, p=0.99) or for any of the stratified dosages (both univariate or age- and sex-adjusted), or dose-weight ratios (HR 1.05, 95% CI 0.93-1.18, p=0.46), see **Table 3**. Thus, no significant effects of paclitaxel dose exposure on mortality were observed up to 10 years of analysis.

Multivariate analyses were carried out to investigate the risk of dose or dose-related effects. The multivariate analysis with all included factors only showed a significant hazard ratio for age (HR 1.08, 95% CI 1.04-1.12, p-value <0.005). See **Table 4.**

Table 3. 10-years all-cause mortality hazard ratios (unadjusted and adjusted for age and sex) for cumulative paclitaxel dosages.

	Univariate			**Adjusted for age and sex**		
	HR	**95,0% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Paclitaxel-DES (dichotomous)	1.14	0.78-1.66	0.50	1.09	0.75-1.58	0.52
Total dose paclitaxel, (continuous) (µcg)	1.0	0.99-1.00	0.90	0.99	0.99-1.00	0.06
Stratified paclitaxel dose (µcg)						
0-149µcg	1.27	0.85-1.87	0.24	1.48	0.86-1.89	0.22
150-299µcg	0.72	0.42-1.21	0.21	0.94	0.55-1.61	0.82
300-449µcg	0.93	0.53-1.65	0.81	0.82	0.46-1.45	0.49
450-700µcg	1.01	0.58-1.78	0.97	0.80	0.45-1.43	0.45
Total dose paclitaxel per weight (continuous) (µcg/kg)	1.05	0.93-1.18	0.46	0.19	0.80-1.05	0.19

Table 4. Cox-regression analysis of paclitaxel dose on mortality in patients with paclitaxel-DES.

Variables in the Equation	HR	95% CI	P-value
Univariate analysis			
Paclitaxel-DES (dichotomous)	1.14	0.78-1.66	0.50
Multivariate analysis			
Paclitaxel-DES (dichotomous)	1.60	0.19-13.77	0.67
Age (years)	1.08	1.04-1.12	<0.005
Smoking	1.03	071-1.50	0.89
History of PAD	1.08	0.59-1.98	0.81
Diabetes mellitus	1.48	0.76-2.88	0.24
Previous stroke or transient ischemic attack	1.41	0.67-2.94	0.37
Coronary artery disease	1.47	0.76-2.83	0.25
Anticoagulant medication	1.77	0.63-4.96	0.28

DISCUSSION

In this present study we investigated the survival of CLTI patients treated BTK at 10 years follow-up and the possible influence of treatment with paclitaxel-coated DES.

The main finding of our study is that there is no significant difference between survival in the DES and the PTA±BMS group at 10-years follow-up. The 10-year survival for both groups was astonishing poor. The second main finding is that no specific paclitaxel-coated DES dose-related mortality and dose per body weight mortality relationships were identified.

Recently, two meta-analyses were published that concluded 1) that there appears to be an increased risk of 5-years mortality of paclitaxel-coated DEB and DES in the superficial femoral and popliteal artery [10] and 2) a significantly worse 1-year

amputation-free survival in patients treated with DEB below the knee [11]. These meta-analyses suggested that DEB and DES may be related to poor outcome and should be used with caution.

By focusing on a selected study group within the PADI Trial, we show that this warning does not seem valid for the use of paclitaxel-coated DES in CLTI patients treated BTK. We believe there are several explanations for the discrepancies between the PADI Trial and the above-mentioned meta-analyses.

First, we only included patients treated with paclitaxel-coated DES in the PADI Trial, while the meta-analyses mainly included patients treated with DEB. DEB have a substantial higher paclitaxel dose than DES. For example, the 4 x 40mm IN.PACT DEB (Medtronic, Inc. Minneapolis, United States) [14] and the LUTONIX DEB (Bard Peripheral Vascular, Inc. Tempe, United States) [15] have a 7.21 and a 3.66 times higher dose than the 4 x 38mm TAXUS Liberté DES (Boston Scientific Corporation, Natick, United States) [13], respectively (see **Table 5**). In addition, the DES has a scaffold which is not present with DEBs.

Second, femoropopliteal and BTK arteries differ in diameter. Thus, different stent size and balloon size is used for these arteries. In general, a larger stent or balloon size means a higher paclitaxel dose. However, this increase in dose is less in stents, and larger in balloons. For example, the paclitaxel dose on a TAXUS Liberté stent with a diameter of 2.75mm and a length of 38mm is 266µcg which increases to 273µcg (3% dose increase) on a stent with a diameter of 4.0mm (30% diameter increase) and an equal length. The LUTONIX DEB of 4.0 x 40mm has a dose of 1000µcg which increases to a dose of 1800µcg (45% dose increase) on the 6.0 x 40mm (33% diameter increase).

Third, IC and CLTI patients have two different stages of PAD, not only with a different Rutherford category (1,2,3 versus 4, 5, 6 respectively), but they also differ significantly in morbidity and survival [16-18].
Finally, there are indications that femoropopliteal and infrapopliteal/BTK arteries differ in pathology with significantly more atherosclerosis in the SFA, while the BTK arteries have more medial calcifications (arteriosclerosis) and intraluminal thrombi [19].

Thus, as alarming as the results of this meta-analyses are, its results cannot be extrapolated to all subcategories of PAD especially in patients with BTK disease.

Table 5. Standardized and commonly used diameters and lengths for both different stents and balloons, so that dose per product could be compared. If the stent diameter or lengths were not available in the product information, the most similar stent was chosen.

				Size 1 (small)		Size 2 (large)	
	Artery	**Dose denstity (μcg/mm²)**	**Length (mm)**	**Diameter (mm)**	**Dosis (μcg)**	**Diameter (mm)**	**Dosis (μcg)**
Stents							
TAXUS Liberté (Boston scientific) [13]	BTK, coronaries	1	38	4.0*	273	-	-
ZILVER-PTX (Cook) [20]	Fempop	3	40	5.0	390	7.0	390
Balloons							
LUTONIX (Bard) [15]	Fempop	2	40	4.0	1000	7.0	1800
IN.PACT (Medtronic) [14]	Fempop	3.5	40	4.0	1969	7.0	3819

*The TAXUS Liberté DES has a maximum diameter of 4.0mm, since this stent was originally developed for use in cardiology.

Strengths and limitations

The major strength of this study is the complete survival analysis up to 10 years after the primary treatment.

This study also has its limitations. The sample size of this study was relatively small and the study design as a post-hoc RCT study has its limitations. However, it is the only RCT with such long follow-up. Therefore, a meta-analysis is not possible. Secondly, for the analysis of the paclitaxel dose, we used the manufacturer's product information. Since we have not performed any checks on the amounts of paclitaxel on the stents, the actual dose in the patient may differ from the doses provided by the manufacturer.

Last, the body weight may have affected the dose body weight analysis. For this study the body weight was used from the electronic patient file. Patients' weight may fluctuate and especially decrease during follow-up, due to long-term disease burden in this severely affected patient category.

Conclusions

In conclusion, the 10-year survival of CLTI patients treated BTK is poor. There were no significant differences between 10-year mortality in patients with CLTI treated BTK with either paclitaxel-coated DES and PTA±BMS. Despite a relative broad range of paclitaxel per body weight in our patients, no specific dose-related mortality and dose per body weight mortality relationships were identified.

Thus, there are no dose-related adverse effects of paclitaxel-coated DES in patients with CLTI treated BTK.

Level of evidence

The PADI Trial: level 1, randomized clinical trial

Sources of funding

The original PADI Trial received an unrestricted research grant from The Netherlands Society for Interventional Radiology. All authors of this current post-hoc study declare that they have received no grants, contracts, other forms of financial supports or relationships with the industry relevant to this paper.

Informed consent

Written informed consent for the PADI Trial was obtained in all participants, this was a post-hoc analysis of the trial.

Author contributions

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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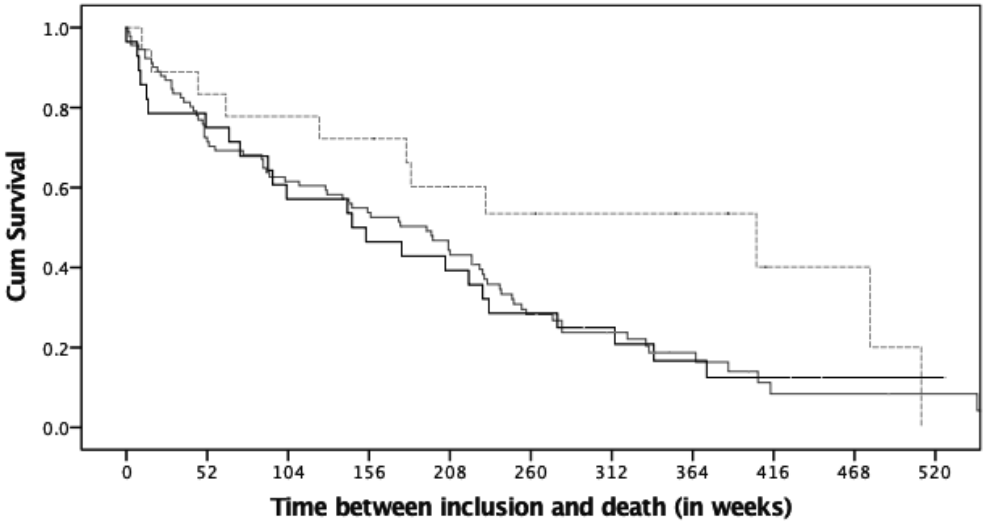
SUPPLEMENTAL FIGURES AND TABLES

Supplemental Figure 1. 10-years Kaplan-Meier survival curves for Rutherford classes. Stippled line: Rutherford 4, continuous grey line: Rutherford 5, continuous black line: Rutherford 6.

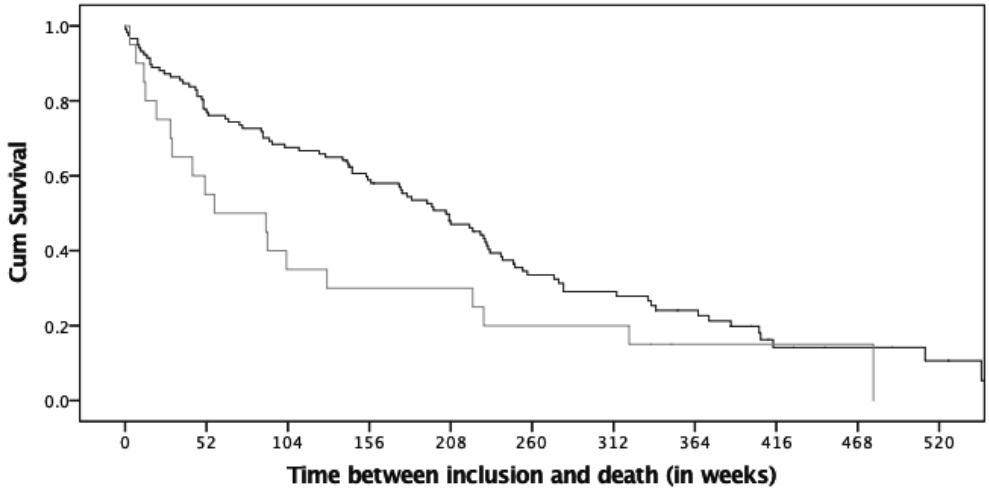


Numbers at risk (numbers censored)

Log-rank (Mantel-Cox) p-value= 0.13

Time(weeks)	**0**	**52**	**104**	**156**	**208**	**260**	**312**	**364**	**416**	**468**	**520**
Rutherford 4	18	15	14	13	9 (0)	8 (1)	7 (1)	6 (3)	2 (0)	2 (0)	0 (0)
	(0)	(0)	(0)	(2)							
Rutherford 5	91	66	56	47	37	22	15	8 (1)	3 (0)	3 (1)	2 (1)
	(0)	(0)	(2)	(2)	(2)	(4)	(4)				
Rutherford 6	28	21	16	13	11	8 (1)	6 (0)	4 (0)	3 (2)	1 (0)	1 (1)
	(0)	(0)	(0)	(0)	(0)						

Supplemental Figure 2. 10-years Kaplan-Meier survival curves for impaired renal function (eGFR<30 mL/min/1.73m^2). Light grey line: eGFR<30 mL/min/1.73m^2, dark grey line: eGFR >30 mL/min/1.73m^2.



Numbers at risk (numbers censored)

Log-rank (Mantel-Cox) p-value= 0.04

Time(weeks)	**0**	**52**	**104**	**156**	**208**	**260**	**312**	**364**	**416**	**468**	**520**
eGFR>30	117 (0)	91 (0)	79 (2)	67 (4)	51 (2)	34 (6)	24 (3)	17 (4)	7 (2)	5 (1)	3 (2)
eGFR<30	20 (0)	11 (0)	7 (0)	6 (0)	6 (0)	4 (0)	4 (2)	1 (0)	1 (0)	1 (0)	0 (0)

Supplemental Table 1. Paclitaxel-DES diameters, lengths and associated paclitaxel doses.

Nominal expanded stent inner diameter (mm)	Nominal unexpanded stent length (mm)	Nominal paclitaxel dose (μcg)
2.25	32	155
2.50	16	77
2.50	24	116
2.50	28	136
2.50	32	155
2.75	38	266
3.00	24	168
3.00	28	196
3.00	32	224
3.00	38	266
3.50	32	224
4.00	38	273

Supplemental Table 2. Diameter and length for both DES and BMS. For DES, dose per stent is also shown.

		DES (n=74)		**PTA±BMS (n=66)**	
		Mean ± SD	**Min-max**	**Mean ± SD**	**Min-max**
All stents	Diameter (mm)	2.83 ± 0.40	2.00-4.00	3.34 ± 0.53	2.50-4.00
	Length (mm)	29.74 ± 4.3	16-38.00	39.17 ± 17.81	30-60
	Paclitaxel dose (μcg)	179 ± 40	77-273.00	-	-

Per patient 3 stents maximum are used. In the control group PTA±BMS all parameters are displayed except dose and dose-related parameters. Data are displayed as mean ± SD, minimum and maximum.

CHAPTER 8.

COST-EFFECTIVENESS OF DRUG-ELUTING STENTS IN PATIENTS WITH CHRONIC LIMB-THREATENING ISCHEMIA: THE PADI TRIAL

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STRUCTURED ABSTRACT

Background and objectives

Drug-eluting stents (DES) improve clinical and morphological long-term results compared to percutaneous transluminal angioplasty (PTA) with bailout bare metal stenting (BMS) in patients with chronic limb-threatening ischemia (CLTI) and infrapopliteal lesions (PADI trial). We per- formed a cost-effectiveness analysis of DES compared to PTA ± BMS in cooperation with Dutch health insurance company VGZ, using data from the PADI trial.

Materials and methods

In the PADI trial, adults with CLTI (Rutherford category C 4) and infrapopliteal lesions were randomized to receive DES with paclitaxel or PTA ± BMS. Seventy-four limbs (73 patients) were treated with DES and 66 limbs (64 patients) with PTA ± BMS. The costs were calculated by using the mean costs per stent multiplied by the mean number of stents used per patient (€750 9 1.8 for DES vs €250 9 0.3 for PTA ± BMS). These costs were compared with the costs of major amputation (€16.000) and rehabilitation (first year €15.750, second year €7.375 and third year €3.600).

Results

The 5-year major amputation rate was lower in the DES group (19.3% vs 34.0% for PTA ± BMS; p = 0.091). In addition, the 5-year amputation-free survival and event- free survival were significantly higher in the DES group (31.8% vs 20.4%, p=0.043; and 26.2% vs 15.3%, p=0.041, respectively). After 1 year, the cost difference per patient between DES and PTA ± BMS is €1.679 in favor of DES and €2.694 after 3 years.

Conclusion

In our analysis, DES are cost-effective due to the higher hospital costs of amputation and rehabilitation in the PTA ± BMS group.

Keywords: Amputation, Infrapopliteal, Cost-benefit, Paclitaxel, Endovascular

INTRODUCTION

Chronic-limb threatening ischemia (CLTI), the most severe manifestation of peripheral arterial disease (PAD), is the leading cause of major amputations (above the ankle), especially in patients with diabetes mellitus and chronic kidney disease [1, 2]. Due to its relation with diabetes mellitus and an aging population, infrapopliteal arterial disease (IAD) is expected to increase in the Western world [3]. Although the prevalence of CLTI is increasing, the number of amputations seems to decline, due to advances in treatment [4]. To avoid amputation, which is associated with high morbidity and mortality and a poor functional outcome [5-8], endovascular revascularization of the affected limb is the main treatment option. Percutaneous transluminal angioplasty (PTA) for IAD alone has a disappointing morphological 1-year outcome with a restenosis rate of nearly 40% [9]. The use of drug-eluting stents (DES) has shown lower restenosis rates below the knee in multiple studies [10-15].

Paclitaxel-coated DES in IAD improve both clinical and morphological long-term results compared to PTA with bailout bare metal stenting (BMS) in patients with CLTI below the knee, as was shown in the PADI (percutaneous transluminal angioplasty and drug- eluting stents for infrapopliteal lesions in critical ischemia) trial [16, 17]. To assess the cost-effectiveness of these DES, we compared two treatment strategies in cooperation with a Dutch health insurance company VGZ: DES and PTA with bailout BMS on the basis of the PADI trial data.

MATERIALS AND METHODS

For our cost-effectiveness analysis, we used the results from the PADI trial. These results have been described elsewhere [16, 17]. In short, adults with CLTI (Rutherford category ≥ 4) [18] and infrapopliteal lesions were randomized to receive primary stenting with DES coated with paclitaxel (Taxus Liberté, Boston Scientific, Marlborough MA, USA) or PTA ± BMS. Stenosis of the lesions had to be > 50%, the lesion length ≤ 90 mm, vessel diameter 2–6 mm and a maximum of three lesions per leg. Bailout BMS was used in case of flow limiting lesions, restenosis > 50% or occlusion after PTA. A total of 144 limbs in 137 patients were included, seventy-five limbs in 74 patients in the DES group and 69 limbs in 67 patients in the PTA ± BMS group. One patient/limb in the DES group (the vessel was too small) and three patients/limbs in the PTA ± BMS group (no chronic limb-threatening ischemia, renal failure without dialysis and coagulation disorder) were excluded. Complete details on inclusion (criteria) and exclusion (criteria) have been published previously [16].

Follow-up consisted of CT angiography (CTA), duplex ultrasound and clinical assessment at 6 months. After that, patients were assessed annually for a period of 5 years after treatment by medical history, physical examination and duplex ultrasound of the treated limb. If patients were unavailable for follow-up, the information was gathered from general practitioners by phone or from digital medical records. Five patients were lost to follow-up, one in the PTA ± BMS group and four in the DES group [17]. Survival and major amputations were recorded in both the DES group and the PTA ± BMS group. Seventy-four limbs (73 patients) were treated with DES, and 66 limbs (64 patients) were treated with PTA ± BMS [16]. The estimated 5-year major amputation rate was lower in the DES arm (19.3% vs 34.0% for PTA ± BMS; p = 0.091) [17]. In addition, the 5-year amputation-free survival and event-free survival were significantly higher in the DES group compared to the PTA ± BMS group (31.8% vs 20.4%, p = 0.043; and 26.2% vs 15.3%, p = 0.041, respectively) [17]. Overall survival rates were comparable.

Our analysis is based on differences in costs due to major amputations between the DES group and the PTA ± BMS group. For the purpose of our analysis, we assumed major amputation was always combined with rehabilitation. Rehabilitation was defined by physical rehabilitation up until self-reliance. Minor amputations are of relatively low costs and have little impact on self-reliance without the need for extensive rehabilitation with limited financial consequences. VGZ (a Dutch health insurance company) provided the costs of major amputation and rehabilitation. The cost of a major amputation was estimated at € 16.000,-. These are average costs based on health declaration codes used by the health insurer. Included in these costs are costs of the operation, hospital stay and nursing costs. The cost of rehabilitation per patient was estimated at € 15.750,- in the first year after amputation, € 7.375,- in the second year and € 3.600,- in the third year. These costs include medical rehabilitation specialized in amputation of the lower extremity, geriatric rehabilitation, home care, nursing aids and paramedic costs (i.e., physical therapy and ergo therapy). Product costs were estimated from the average purchase costs, spent by our hospital during the last 2 years, of bailout bare metal stents and drug-eluting coronary stents that are used in our hospital from four major medical product companies (Abbott, Chicago, IL, USA; Boston Scientific, Marlborough, MA, USA; Cordis, Hialeah, FL, USA; and Medtronic, Dublin, Ireland). The purchase costs were an average € 750,- per DES and € 250,- per BMS. All other materials such as sheaths, balloons and guidewires were assumed to be evenly distributed over both groups. Data of these patient groups are summarized in **Table 1.** Calculations were made per group and per patient at 1-, 2- and 3-year follow-up. For every follow-up

year, the total number of deaths was subtracted from the number of patients with rehabilitation per group in that same year. Total treatment costs per group were calculated using the following formula:

#stents/limb × € /stent × #limbs +#amputations × €/amputation +
(#rehabilitation−#mortality) × € /rehabilitation

Table 1. Patient characteristics and data

	PTA ± BMS	**DES**
Patients with CLTI	64	73
Treated limbs	66	74
Mean implanted stents[a]	0.3	1.8
Major amputations		
First year after treatment	13	8
Third year after treatment	15	10
Cost per stent[b]	€ 250	€ 750
Cost per major amputation[c]	€ 16.000	
Annual rehabilitation cost[c]		
First year after treatment	€ 15.750	
Second year after treatment	€ 7.375	
Third year after treatment	€ 3.600	

CLTI = Chronic limb-threatening ischemia
[a]Data from PADI trial [16]
[b]Mean purchase cost made by our hospital from four major medical product vendors
[c]Provided by Dutch health insurance company VGZ.

For the purpose of our analysis, all patients were considered receiving rehabilitation after a major amputation up until death or up to the end of follow-up. The results were divided by the number of patients per group to give us the costs per patient. The cost difference was calculated using the following formula:

(**€** /PTA ± BMS group / # patients PTA ± BMS group)
− (**€** /DES group / # patients DES group)

The same formulas were used at 2- and 3-year follow-up. The number of years of rehabilitation was based on our 3-year study follow-up period with at least 1-year rehabilitation after an amputation for each patient.

RESULTS

On average, we used 0.3 bare metal stents per treated limb in the PTA ± BMS group and 1.8 drug-eluting stents per treated limb in the DES group. The total stent costs in the PTA ± BMS group were (#stents/limb × €/stent × #limbs):

0.3× €250,− × 66 = €4.950,−
versus
1.8× €750,−× 74 = €99.900,− in the DES group.

In the first year after the procedure ($t = 1$), the costs in the PTA ± BMS group were the stent costs plus the costs per amputation and rehabilitation. Total number of amputations was 13 in the PTA ± BMS group, and 8 in the DES group, respectively. (#stents/limb × €/stent × #limbs + #amputations × €/amputation + #rehabilitation × €/rehabilitation):

€4.950,− + 13 × (€16.000,− + €15.750,−) = €417.700,−.

In the DES group:

€99.900,− +8 × (€16.000,− + €15.750,−) = €353.900,−.

The costs per patient were calculated by dividing the total costs per group by the number of patients in each group. After 1-year follow-up, the costs per patient were higher in the PTA ± BMS group compared to the DES group (€ 417.700/64 = € 6.527,- vs € 353.900/73 = € 4.848,-, respectively). The difference between both groups at 1-year follow-up was thus, € 1.679,- per patient in favor of DES (results after 1-year follow-up are shown in **Table 2**. In the second year after the procedure ($t = 2$), the costs in the PTA ± BMS group were calculated by adding the costs of new amputations and rehabilitations in the second year after treatment (2 in the PTA ± BMS group and 1 in the DES group) to the number of patients who underwent amputation in the first year ($t = 1$) minus the number of deceased patients in the first year after the procedure ($t = 1$) multiplied by the costs of a second year of rehabilitation:

2 × €16.000,– + 2 × €15.750,– + (13–5) × €7.375,–
= €122.500,–.

In the DES group:

1 × €16.000,– + 1 × €15.750,– + (8–3)× €7.375,–
= €68.625,–.

Table 2. Calculations in multiple steps first year after treatment

	PTA ± BMS	**DES**
	N = 64 patients	*N* = 73 patients
#limbs × #stents/limb × €/stent	66 × 0.3 × 250 = € 4.950	74 × 1.8 × 750 = € 99.900
#amputations × (€/amputation + €/rehabilitation)	13 × (16.000 + 15.750) = € 412.750	8 × (16.000 + 15.750) = € 252.000
Total costs	€ 417.700	€ 353.900
Costs per patient	€ 6.527	€ 4.848

DES, Drug-eluting stent; *PTA ± BMS*, percutaneous transluminal angioplasty with bailout bare metal stent

The costs in the PTA ± BMS group in the third year after treatment (*t* = 3) were calculated by adding the costs of new amputations and rehabilitations in the third year 1; to the number of patients who underwent an amputation in the second year after treatment, multiplied by the costs of a second year of rehabilitation and 2; to the number of patients who underwent an amputation in the first year minus the number of deceased patients in the first and second year after treatment, multiplied by the costs of a third year of rehabilitation:

0 × € 16.000,– + 0 × € 15.750,– + 2 × € 7.375,–
+ (13 – 5 – 1) × € 3.600,–
= € 39.950,–.

The same in the DES group:

1 × €16.000,– + 1 × €15.750,– + 0 × €7.375,– + (8 – 3 – 2) × €3.600,–
= €42.550,–.

After 3 years, the total costs reached € 580.150,- in the PTA ± BMS group versus € 465.075,- in the DES group. Per patient:

PTA±BMS: €580.150,– / 64 = €9.065 and
DES: €465.075,– / 73 = €6.371,–.

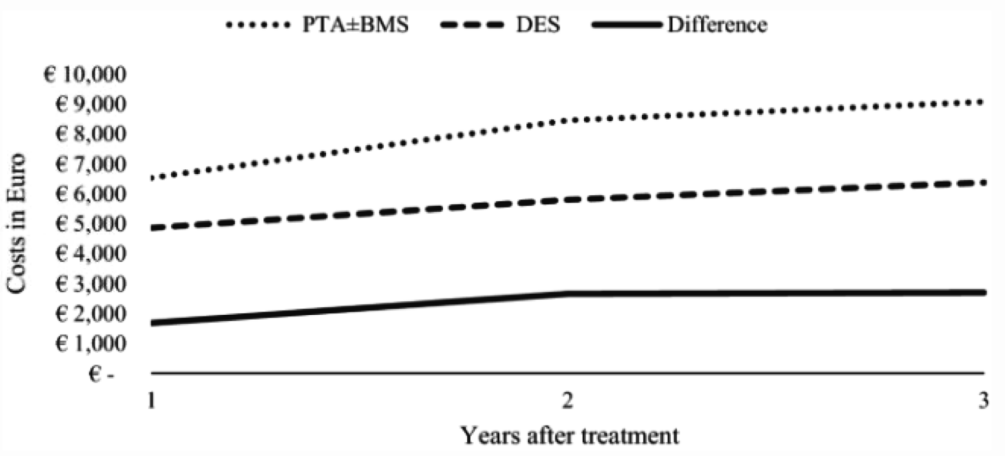
A difference of € 2.694,- per patient in favor of DES. Results of all 3 years are summarized in **Table 3** and **Figure 1.**

Table 3. Results for every year after treatment in both groups in euro.

Year	PTA ± BMS	DES	PTA ± BMS per patient	DES per patient	Difference per patient
1	417,700	353,900	6527	4848	1679
2	122,500	68,625	1914	940	974
3	39,950	42,550	624	583	41

DES, Drug-eluting stent; *PTA ± BMS*, percutaneous transluminal angioplasty with bailout bare metal stent

Figure 1. Cumulative costs per average patient at 1, 2 and 3 years after treatment in euro. The dotted line represents the PTA ± BMS group, the striped line the DES group and the continuous line the difference between both groups. DES = Drug-eluting stent. PTA ± BMS = Percutaneous transluminal angioplasty with bailout bare metal stent



DISCUSSION

The main findings in this study are that although DES below the knee are more expensive to purchase, at 1-, 2- and 3-year follow-up they are increasingly more cost-effective than PTA ± BMS in patients with CLTI due to lesser costs of amputation and rehabilitation.

To our knowledge, this is the first study of the cost-effectiveness of paclitaxel-coated DES for infrapopliteal lesions in CLTI. In addition, it is the first study to combine long-term clinical and morphological outcomes with the costs of paclitaxel-coated DES, to achieve a 3-year cost-effectiveness analysis. A few studies have been published about the costs of drug-coated devices, but these used different materials such as sirolimus- or everolimus-eluting stents, different locations such as the superficial femoral artery or only compared in-patient hospital costs [19-21].

In the PADI trial, DES not only have a better patency, but also a significantly better amputation-free survival and event-free survival compared with PTA ± BMS at 5-year follow-up [17]. Secemsky *et al.* found no difference in overall survival in their analysis of patients with CLTI [22, 23] similar to patients from the PADI trial. Katsanos *et al.* [24] recently published data in a meta-analysis, reporting increased

mortality in patients treated with paclitaxel-coated drug-eluting devices in the superficial femoral artery and popliteal artery. Although worrisome, these results should not be extrapolated to the CLTI patients reported on in the PADI trial. First, there is a difference in the location of the lesions. All our patients in the PADI trial had crural lesions in contrary to the superficial femoral artery and popliteal artery in the meta-analysis from Katsanos. Second, most patients (85%) in the meta-analysis were treated with drug-eluting balloons compared to only drug-eluting stents in the PADI trial. Third, all of our patients had CLTI as opposed to those in the meta-analysis of Katsanos that consists of 89% of patients with intermittent claudication. These patient groups differ dramatically in both morbidity and life expectancy [1, 4, 25-28]. Finally, the dosage of paclitaxel in drug-eluting stents in crural vessels may be ten times as low compared with drug-eluting devices, balloons or stents, in the superficial femoral artery, due to slimmer and shorter devices with a smaller surface area used below the knee.

In this study, multiple DES had to be used to fully cover and successfully treat relatively short lesions (up to 90 mm). This has a negative impact on the total costs. Patients often have long-segment IAD, especially in diabetes [3]. As shown in the IDEAS trial, paclitaxel-coated DES are also more effective for treating long-segment infrapopliteal lesions compared with drug-coated balloons [12]. In this study, we used relatively short and older paclitaxel eluting coronary stents. Newer versions with different mechanical and drug-eluting properties are currently available, while dedicated longer drug-eluting stents are not yet on the market. These latter could further decrease the cost of drug-eluting stent treatment.

Dutch health insurance company VGZ delivered detailed information on costs from a variety of treatments and treatment codes. In our cost-effectiveness analysis, we tried to use as many relevant and objective data on patient costs as possible. Major amputation has the largest impact on the total patient treatment costs, followed by the costs of rehabilitation and materials. This is where the largest differences between the DES group and PTA ± BMS group arise. We are aware that this is a simplified model and that the costs are an average and not an exact representation for an individual patient. For example, costs for minor amputation, psychological treatment or admission to a psychiatric institution in the years after amputation were not taken into account as contributing to rehabilitation costs. In addition, indirect costs leading to an improved cost-effectiveness such as sick leave or quality of life have not been analyzed in this study since these data were not available. This analysis is based on a single health care insurance company in the

Netherlands; therefore, the costs and cost-effectiveness might be different in other hospitals or countries where healthcare costs and device costs may be different.

Conclusions

Long-term morphological and clinical effects of DES below the knee in CLTI are promising. Cost-effectiveness analysis in the Netherlands shows that the higher initial purchase costs of paclitaxel-coated DES eventually outweigh the lower costs of BMS, mainly due to differences in amputation and rehabilitation rate. Paclitaxel-coated DES are € 1.679,- and € 2.694,- less expensive per patient, compared with PTA ± BMS, after 1- and 3-year follow-up, respectively.

Level of Evidence

Level 1b, analysis based on clinically sensible costs and randomized controlled trial.

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Conflict of interest

Hans van Overhagen is PI of the PADI trial, PI of the SAVAL trail and consultant of Boston Scientific. The remaining authors declare that they have no conflict of interest.

Informed Consent

For this type of study, formal consent is not required. For this type of study, informed consent is not required. For this type of study, consent for publication is not required.

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PART IV.

Synopsis and general discussion

CHAPTER 9.

SYNOPSIS

CHAPTER 9 SYNOPSIS

Chronic limb-threatening ischemia (CLTI) is a devastating disease with a 5-year mortality up to 60% [1-11]. Current treatment strategies focus mainly on reducing the risk of 1) luminal thrombosis by interventional strategies and 2) cholesterol-mediated atherosclerosis. A high/immeasurable ABI in patients with CLTI has shown to be an independent risk factor of major amputation and of poor amputation free survival [12]. Medial arterial calcification (MAC) can be the cause of these stiffened arteries and therefore responsible for the high/immeasurable ABI. The PADI Imaging Trial was conducted to provide insight into the nature and clinical relevance of PAD by measuring medial arterial disease and atherosclerosis by both single and dual-energy CT-angiography and applying these techniques in multiple retrospective and prospective cohorts. The studies of this dissertation could lead to novel treatment allocation for various distinct patient phenotypes in PAD.

PART II. ARTERIAL CALCIFICATIONS IN CHRONIC LIMB-THREATENING ISCHEMIA PATIENTS

In ***Chapter 2***, patients without PAD were examined to assess the development of arterial calcifications in different age categories. From 204 patients, the thin slice CT-imaging characteristics severity, annularity, thickness and continuity were scored in the following arteries: plantar and dorsal, crural, femoropopliteal, iliac and the abdominal aorta. In the crural arteries two calcification patterns with strong associations were found. The first pattern showed continuous-annular 93.5 % (29/31), continuous-thin and thin-annular both 73 % (27/37, $p < 0.001$). The second pattern showed thick-discontinuous 91.7 % (44/48), thick-dotted 68.8 % (33/48), patchy-dotted 59.3 % (16/27, $p < 0.001$) calcifications. Similar associations were found in the femoropopliteal artery, but not in the plantar, dorsal, iliac arteries and aorta. We concluded that in the crural and femoropopliteal arteries at least two morphological patterns can be distinguished on CT that, compared to a CT-histologically validated score, may represent an intimal and medial calcification pattern. Since both types of calcifications have different pathophysiological pathways, it is important to be able to differentiate between these calcification types.

Chapter 3 focused on the differences in lower extremity arterial calcification patterns between non-PAD patients and patients with CLTI. 118 CLTI patients (mean age 72±12, 70.3% male) that were age-matched with 118 non-PAD patients (mean age 71±11, 51.7% male). The CT calcification characteristics severity, annularity, thickness

and continuity were assessed in the femoral and crural arteries and analyzed. Nearly all CLTI patients have calcifications and these are equally frequent in the femoropopliteal (98.3%) and crural arteries (97.5%), while the non-PAD patients had in just 67% any calcifications with more calcifications in the femoropopliteal (70.3%) than in the crural arteries (55.9%, p<0.005). The crural arteries of the CLTI patients had significantly more complete annular calcifications (OR 2.92, p=0.001.) while in the non-PAD patients dot-like calcifications dominated. In CLTI patients, the femoropopliteal arteries had more severe, irregular / patchy and thick calcifications (OR 2.40, 3.27, 1.81, p≤0.05, respectively) while in non-PAD patients, thin continuous calcifications prevailed. This study showed that in the arteries of the lower extremities in CLTI patients, any arterial calcification is almost always present. In non-PAD patients however, only two third of patients have any calcifications. Most calcifications are usually severe. In the crural arteries CLTI patients have an annular type of calcifications as seen in medial calcifications, while non-PAD patients have a dot-like type of calcifications as seen in atherosclerotic disease. As medial calcifications are increasingly considered treatable, our findings may contribute to the development of a treatment strategy for these difficult-to-treat CLTI patients.

In ***Chapter 4,*** the predictive value of CT characteristics of lower extremity arterial calcification on both 7-years amputation-free survival and 10-years all-cause mortality in patients with CLTI was evaluated. 89 CLTI patients were included (mean age 73.1 ± 11.6 years) and underwent a CT angiography of the lower extremities. In the femoropopliteal and crural arteries based on a CT score the following calcification characteristics were assessed: severity, annularity, thickness and continuity. The predictive value of different arterial calcification characteristics was analyzed by age- and sex-adjusted multivariate Cox regression analysis. Complete annular calcifications were common (femoropopliteal 43.7%, n=38; crural, 63.2%, n=55). Mean survival was 278.4 weeks (95% CI 238.77-318.0 weeks). Patients with complete annular calcifications had a higher all-cause 10-year mortality (femoropopliteal unadjusted HR 1.64, p=0.04 and adjusted for age and sex HR 1.68, p=0.04; crural unadjusted HR 1.92, p=0.02, adjusted for age and sex HR 2.29, p=0.006) than patients with other calcification characteristics. This study showed that annularity of calcifications in the arterial wall is a good predictor for 10-year all-cause mortality and 7-year amputation-free survival of CLTI patients, especially in the crural arteries and is easy to use in daily clinical practice. Severity, thickness and continuity of calcifications are not predictive for survival. The predictive value of annularity is higher than the traditional prognostic risk factors for CLTI. Recognizing

the influence of arterial calcifications on long-term survival in CLTI patients adds to the current prognostic risk factors known in this poorly performing patient cohort.

In ***Chapter 5*** we assessed the associations of multi-arterial calcium burden in patients with CLTI. CLTI should be regarded as a systemic cardiovascular disease, as acute coronary syndrome, stroke and heart failure lead to high morbidity and mortality in this patient group. 60 patients with CLTI were included in this study, after exclusion of 6 patients, 54 patients remained for analysis. Patients were scanned full-body. The peripheral lower extremity arteries, the coronary arteries, extracranial and intracranial carotid arteries, thoracic and abdominal aorta were analyzed. Quantitatively interrelations of both calcium scoring and CT morphologic semi-quantitative measurements were performed. Mean age was 72 years (range 47-95; SD 11.4). Almost all CLTI patients had calcified arterial beds (femoropopliteal 100%, crural 98.1%, coronary 100%, carotid bifurcation 96.2%, internal carotid artery 98.1%, thoracic aorta 96.2%, abdominal aorta 92.3%). Nearly all arterial territories had severe calcifications. 57% had a high coronary Agatston score (>1000), and 35% extremely high (> 2000). Calcifications in the lower extremity were significantly correlated to CAC score, carotid artery bifurcation calcification score, and to a lesser extent correlated to annular calcifications in the aorta. High and extremely high total CAC scores were strongly correlated with severe lower extremity arterial calcifications and severe carotid and intracranial internal carotid artery, thoracic and abdominal aorta calcifications in patients with CLTI patients. In conclusion, nearly all arterial territories are severely calcified in patients with CLTI. High and extremely high total CAC scores are significantly correlated to calcifications in the thoracic and abdominal aorta calcifications, to femoropopliteal and crural arterial calcifications in patients with CLTI.

Since the arteries of the lower extremities are long and there are numerous plaques to be analyzed, we tested a novel method of automatic hydroxyapatite quantification in ***Chapter 6***. The aims of this study were to show that 1) calcium hydroxyapatite (CaHa) in the arterial wall and iodinated blood in the vessel lumen can be quantified based on the material decomposition technique determined by spectral CT angiography (SCTA), and 2) that the cut-off values of Hounsfield unit as used in CT angiography, the CT calcium score and calcium score obtained from CT angiography leads to over- and underestimation. Three SCTAs of the lower extremities were performed on a SCT scanner. Subsequently, arterial segmentation of the femoropopliteal arteries was executed. Based on the attenuation coefficient of the

photoelectric effect (μ_{pe}) with the included Raleigh component, the attenuation coefficient of Compton scattering (μ_{cs}), XAD plots and material compositions were calculated. In the artery without visible calcium, 1.12% CaHa of all voxels were detectable with the material decomposition method. 350 HU and 130 HU value (CTA and non-contrast CT calcium HU-values, respectively) were used as an overlay on the XAD-plot data. Regarding the 350 HU cut-off value, all three cases a substantial percentage of CaHa voxels was missed with a spread between 17.18 - 90.97%. For 130 HU, 0.80 - 8.54% of calcium voxels were missed in the calcium voxel calculations.

The conclusion of this study was that it was possible to quantify CaHa in the arterial wall of the femoral arteries and iodine containing blood in the vessel lumen with the material decomposition technique by μ_{pe} and μ_{cs} in XAD-plots by spectral CT angiography. This technique avoids over and underdetection of CaHa and iodinated blood as is frequently the case with HU-based cut-off values.

PART III. OUTCOME OF CHRONIC LIMB-THREATENING ISCHEMIA TREATMENT

Chapter 7 focused on 10-year paclitaxel dose-related outcomes of drug-eluting stents treated below the knee in patients with CLTI (the PADI Trial) since two meta-analyses concluded that there appeared be an increased risk of long-term mortality of paclitaxel-coated balloons and stents in the superficial femoral and popliteal artery, and paclitaxel-coated balloons below the knee. The PADI Trial compared paclitaxel-coated DES with percutaneous transluminal angioplasty with bail-out bare-metal stents (PTA±BMS) in patients with CLTI treated below the knee. Follow-up was extended to 10 years after the first inclusion and survival analyses were performed. In addition, dose-related mortality and dose per patient weight-related mortality relations were examined. A total of 140 limbs in 137 patients were included in the PADI Trial. Ten years after the first inclusion, 109/137 (79.6%) patients had died. There was no significant difference between mortality in the DES group compared with the PTA±BMS group (Log-rank p-value= 0.12). No specific dose-related mortality (HR 1.00, 95% CI 0.99-1.00, p=0.99) or dose per weight mortality (HR 1.05, 95% CI 0.93-1.18, p=0.46) relationships were identified in the Cox-proportional Hazard models or by Kaplan-Meier survival analyses. We concluded that the 10-year survival of CLTI patients treated BTK is poor. There were no significant differences between 10-year mortality in patients with CLTI treated BTK with either paclitaxel-coated DES and PTA±BMS. Despite a relative broad range of paclitaxel per body weight in our patients, no specific dose-related mortality and dose per body weight mortality relationships were identified.

Thus, there are no dose-related adverse effects of paclitaxel-coated DES in patients with CLTI treated BTK.

In ***Chapter 8*****,** a cost-effectiveness study was conducted up to a 3-year follow-up and reported since previous short-term results from this PADI study favored drug-eluting stents. In the PADI trial, adults with CLTI (Rutherford category C 4) and infrapopliteal lesions were randomized to receive DES with paclitaxel or PTA ± BMS. Seventy-four limbs (73 patients) were treated with DES and 66 limbs (64 patients) with PTA ± BMS. The costs were calculated by using the mean costs per stent multiplied by the mean number of stents used per patient (€750 9 1.8 for DES vs €250 9 0.3 for PTA ± BMS). These costs were compared with the costs of major amputation (€16.000) and rehabilitation (first year €15.750, second year €7.375 and third year €3.600). The 5-year major amputation rate was lower in the DES group (19.3% vs 34.0% for PTA ± BMS; p = 0.091). In addition, the 5-year amputation-free survival and event- free survival were significantly higher in the DES group (31.8% vs 20.4%, p=0.043; and 26.2% vs 15.3%, p=0.041, respectively). After 1 year, the cost difference per patient between DES and PTA ± BMS is €1.679 in favor of DES and €2.694 after 3 years.

In our analysis, DES are cost-effective due to the higher hospital costs of amputation and rehabilitation in the PTA ± BMS group.

CONCLUSIONS

In summary, this dissertation provides new insights into arteriosclerosis in patients with and without CLTI. Arteries in patients with CLTI are very severely calcified, both in the legs but also in other locations such as the aorta, carotid arteries and arteries in the brain and possibly explaining the high mortality and disease burden in this patient group. In the legs, two calcification patterns can be objectified; a dominant media pattern in the lower legs and a dominant intima calcification pattern in the upper legs. Secondly, this dissertation has shown that the use of paclitaxel-coated DES as a treatment for CLTI patients with stenoses and occlusions of the lower leg arteries is safe and cost-effective.

Recent studies have shown that media calcifications can be reduced and even disappear with drug therapy. Future studies should therefore focus on the influence of this treatment on CLTI patients in order to possibly improve the very poor prognosis of these patients.

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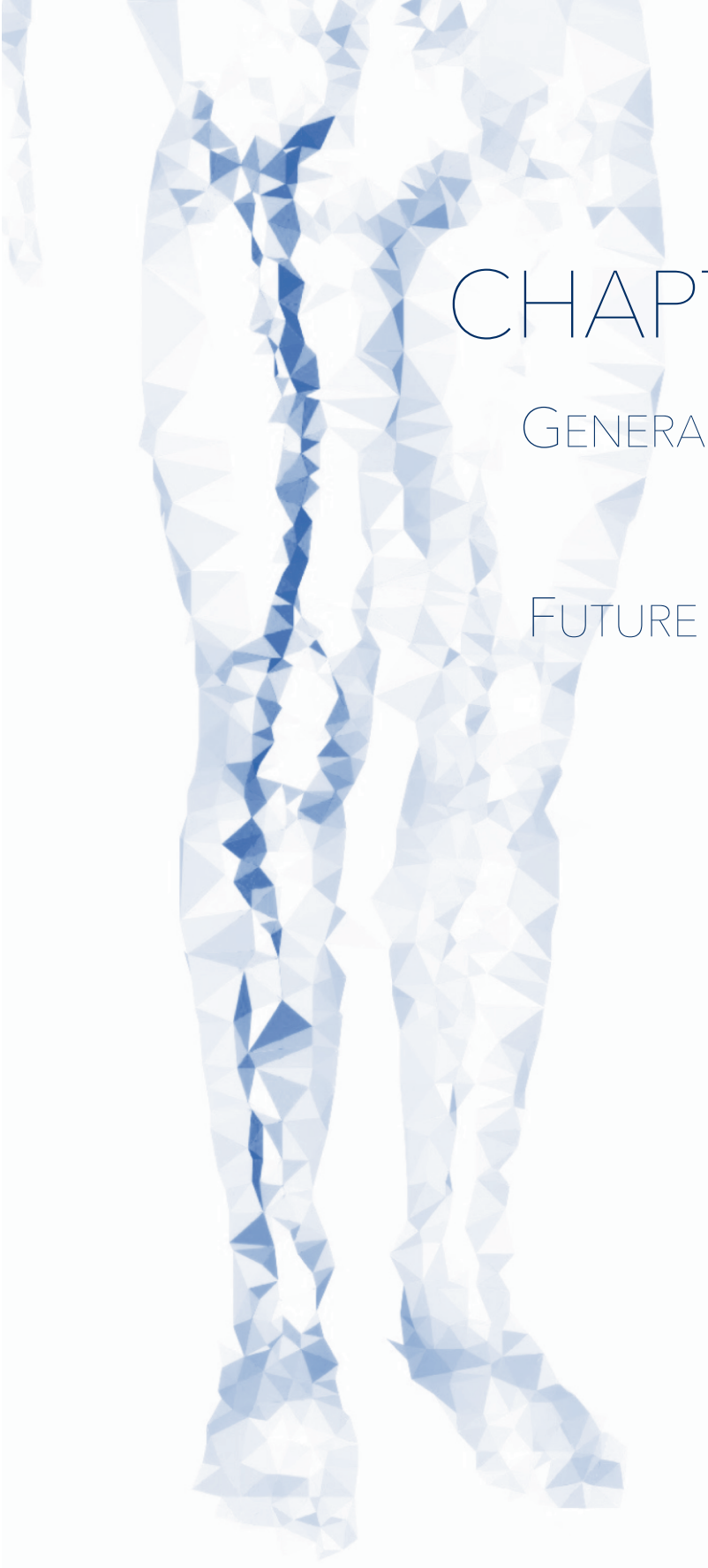


CHAPTER 10.

General Discussion and Future Perspectives

CHAPTER 10. GENERAL DISCUSSION AND FUTURE PERSPECTIVE

This dissertation focuses on patients with chronic-limb threatening ischemia (CLTI). To improve our understanding of this disease, we first investigated the role of vascular calcification severity and patterns in these patients. Second, we described a novel computed tomography (CT) method to improve the quantification of vascular calcifications. Third, we investigated whether patients with CLTI can be treated safely and cost-effectively with drug-eluting stents (DES).

In this discussion, we will place the key findings in a broader context and discuss directions for future research.

Arterial calcifications

For the past 30 years the main focus of calcification research was on the coronary arteries. These calcifications are seen as the inert end stage of atherosclerosis and quantification was a proxy for the atherosclerotic burden of the patient. The calcium score was used for prognostication and stratification, but the calcifications itself are thought to stabilize the plaque. These coronary calcifications are located in the intimal layer of the arterial wall. Later on, it was shown that calcifications can also occur in earlier stages of the disease. The thin cap atheromatous lesion thought to be responsible for acute events, can calcify and these early calcifications may make plaques even more unstable. Besides these plaque calcifications, a second form of atherosclerotic calcifications has been described. This fibrocalcific from without a significant atheromatous component seems unrelated to acute events [1-3]. While the former dominated in the coronary and extracranial carotid arteries, the latter were more recently found dominant in the femoropopliteal arteries and the abdominal aorta [4-6]. The relationship between these two types of atherosclerotic lesions is unclear, although some believe that fibrocalcific lesions are the end stage of fatty atherosclerotic disease.

In the era more than 30 years ago it was better known that there is even another form of arterial calcification. Calcifications can also be found in the medial layer of the arterial wall. They can be located in or originate from the internal elastic lamina or in the vascular smooth muscle cells deeper in the tunica media. The relationship between atherosclerotic intimal forms and non-atherosclerotic medial calcifications is uncertain. These medial calcifications are found in aging, chronic kidney disease, diabetes and in some rare monogenetic calcifying diseases [7]. They are thought to stiffen arterial walls and cause pulse pressure damage to high-flow organs, such as the kidneys and brain. These calcifications are seen as an active

metabolic process that can be stimulated or inhibited by medication. Some researchers distinguish calcification of the heart valves as yet another type of vascular calcification and calcifications also occur in the venous system, but we will not discuss this in this setting.

Chronic limb-threatening ischemia

CLTI is considered the worst version of peripheral arterial occlusive disease. As such, the focus of treatment is on the recanalization of occluded blood vessels in the lower extremities. Initially, this treatment was surgical with bypass, end-atherectomy, or sympathectomy. Increasingly, these methods have been replaced by minimal invasive endovascular methods such as percutaneous transluminal angioplasty (PTA), bare metal stents (BMS), drug eluting balloons (DEB) and DES although the difference in clinical outcome between surgical and endovascular methods is small. Also, anti-thrombotic, anti-atherosclerotic, anti-inflammatory and anti-hypertensive drugs are prescribed. However, despite these methods, amputation cannot be prevented in many patients. Calcifications in the lower extremities are known to be present in CLTI patients, but these have been considered primarily end-stage atherosclerotic disease and as such of little interest. Similarly, recent reviews barely mention calcifications in CLTI patients [8-11].

In medicine, cause and effect are often confused as a result of cross-sectional observations where cause and effect are indistinguishable and therefore many personal convictions and more widespread biases rule. It is in this regard crucial to realize; the prevailing view is that CLTI ultimately causes inert calcification. We pose the opposite as a hypothesis: calcification causes CLTI. A recent publication suggested that atherosclerotic disease in combination with medial calcification could cause severe vascular disease in the lower leg [12]. Increasingly the presence of calcification is emphasized in pathological-histological publications [13, 14]. In addition, the rare monogenetic calcification diseases are increasingly successfully treated with medication (etidronate) that stops and even reverses the calcification process and leads to a better survival of children with Generalized Arterial Calcification of Infancy (GACI) [15-18].

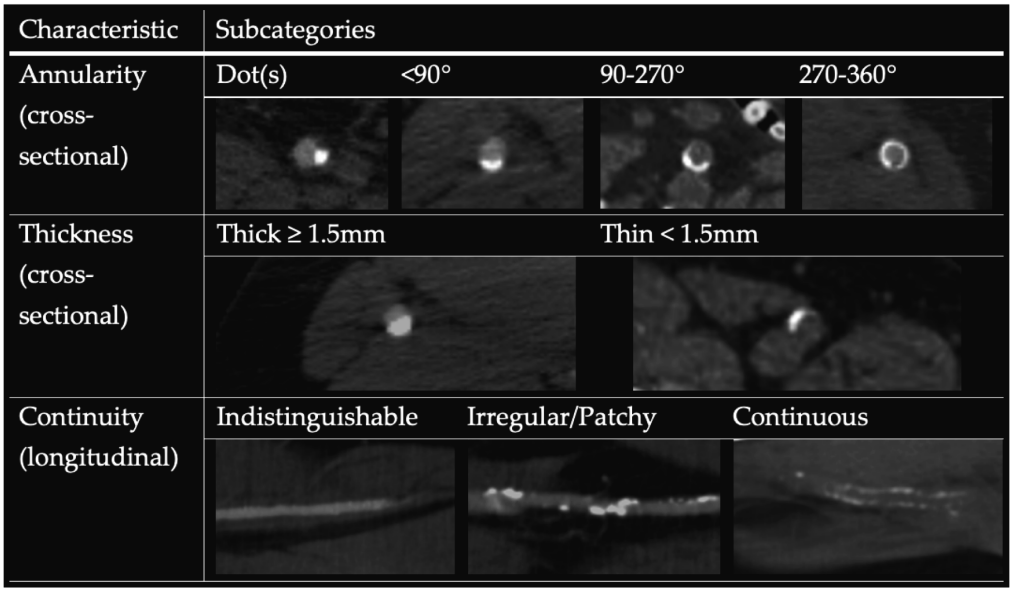
It is well known that CLTI patients not only have vascular disease in the lower extremities, but also vascular disease elsewhere in the body. Although most CLTI patients die from these diseases and not from lower extremity arterial disease, little effort has been made to identify and treat these causes. The prognosis of CLTI patients has improved somewhat, probably due to more systematic treatment with anti-atherosclerotic and anti-hypertensive drugs [8]. Nevertheless, the residual

vascular disease has remained significant. Apparently, this residual vascular disease does not improve on the medication used. Arterial calcifications could explain the systemic adverse findings in optimally treated patients with CLTI. They maybe the cause of more chronic vascular diseases and cannot be treated with anti-atherosclerotic or antihypertensive drugs. We stress the importance of being open to unconventional causes in a disease with such a poor outcome.

Arterial calcifications and patients with CLTI

Thus, the focus of this dissertation is on the role of arterial calcifications in CLTI patients. Arterial calcifications can be found at a very early age in the intracranial internal carotid artery [19] and in the abdominal aorta [20], although no systematic studies have been done in the lower extremity arteries to our knowledge. From the age of 40, in an increasing number of patients, the calcifications begin macroscopically (visible on routine CT) at the iliac bifurcation of the aorta and proceed from there down to the lower leg, while at the same time the calcification process also upwards in the aorta. Distinguishing between normal or abnormal aging and symptomatic and asymptomatic vascular disease is difficult. By the age of 70, almost all people have calcification at some point. To understand calcification load in patients without known peripheral arterial disease, we described the several morphologic calcification characteristics a non-PAD cohort in ***Chapter 2***. Based on a semi-quantitative validated CT histological score [19], we analyzed the calcification pattern in the femoropopliteal and crural arteries based on three characteristics; annularity (circularity), thickness and continuity. Thick, patchy and non-continuous calcifications on CT are CT-histologically proven to be more intimal calcifications, whereas thin, circular and continuous calcifications are proven to be more medial calcifications. See **Figure 1** below.

Figure 1. Classification system for calcification characteristics in the peripheral arteries of the lower extremity. Window Settings: window = 300 Hounsfield Units; width = 1600 Hounsfield Units (bone window).



Patients had a mean age of 61 years. We found a good correlation between the intimal and medial CT calcification features for the lower extremity arteries but not for the aorta/iliac arteries. This suggests that the semi-quantitative score can be used to assess in cohort studies the presence of intimal and medial types of calcifications in the lower extremities and outside the scope of this dissertation this was also confirmed histologically by Vos and colleagues (unpublished data).

In ***Chapter 3***, we compared the arteries of the lower extremities between non-PAD patients and CLTI patients. We found that arterial calcification is almost always present in the arteries of the lower extremities in CLTI patients. However, in non-PAD patients, only two-thirds of patients have calcifications. Most calcifications in both groups are usually severe. In the crural arteries, CLTI patients have annular thin type of calcifications as seen in medial calcifications while non-PAD patients have dot-like type calcifications as seen in atherosclerotic disease. This could indicate an important role for medial arterial calcifications in CLTI patients, as also suggested by histological studies [13].

In ***Chapter 4*** we showed that annular calcifications in both the femoropopliteal and crural arteries of CLTI patients can better predict 10-year all-cause mortality than traditional risk factors. In the abdominal aorta, a similar predictive power has been described for annular calcifications [21].

However, there are some critical caveats to our semi-quantitative calcification score. One of the important findings of recent histological research is that different combinations of calcification occur in different vascular areas. In the intracranial internal carotid artery (siphon), 70% of the calcification burden seen on CT has been shown to be due to calcification of the internal elastic lamina and 30% to atherosclerotic lesions [22]. In the carotid bifurcation and in the coronary arteries, lipid-rich atherosclerotic lesions predominate. In the ascending aorta, atherosclerotic and medial calcifications have been described [23], while in the abdominal aorta mainly both types of atherosclerotic intimal lesions (in plaques and fibrocalcific) are present [6]. Only medial arterial calcifications are described in the breast arteries [24]. In the femoropopliteal arteries, fibrocalcific lesions are found with a few atheromatous lesions and many medial calcifications. In the lower leg, medial calcifications dominate with fewer atherosclerotic lesions [5]. We thus conclude that often more than one type of calcification is present in an artery at the same time and that the dominant type differs between most arteries. Furthermore, changes in the calcification pattern may also occur with age and comorbidity.

Our semi-quantitative method was validated in the intracranial internal carotid artery where atherosclerotic intimal and medial lesions are similar to the calcifications in the peripheral vessels. But recently, as we described above, it was shown that calcifications of the intimal wall of the lower extremity consist not so much of the atheromatous atherosclerotic type, but rather of the other form of the atherosclerotic type, the fibrocalcific lesion. Since this type of intimal lesion is rarely seen in the intracranial internal carotid artery, the effect of this finding on our scoring system is uncertain. It is likely that the scoring system for all vascular territories needs to be validated separately and that based on location and pattern recognition and dominant score will be possible.

In ***Chapter 5***, we showed in full body CT-scanned CLTI patients that severe arterial calcifications were present in all vascular areas. This could explain at least part of the residual burden of vascular disease in these patients. The severity of the calcifications in the different territories showed a strong interrelationship, making extensive systemic disease probable. However, we could not assess whether intimal or medial calcifications are important, as no consistent pattern was found and histology was lacking. Only between the lower leg and the intracranial internal carotid artery, good relationships were found between the semi-quantitative features thickness, annularity and continuity, indicating medial calcifications to be dominant at both locations. This is consistent with histological examination [5].

Furthermore, our study showed that a high ankle-arm index (>1.4) is strongly related to the most severe calcifications and the highest calcium scores. This could explain the extremely poor survival of these patients compared to those with a low ankle-arm index, as we have previously shown [25]. Finally, we found that the left carotid bifurcation had twice as much calcium as the right carotid bifurcation. Such a difference had previously been found in the first quantitative calcium loading study of the carotid bifurcation [26]. Furthermore, extensive literature shows that more strokes occur on the left than on the right [27]. Why there are more calcifications and strokes on the left side remains unclear.

The reason and cause of the calcification process is unknown. Intimal calcifications have been thought to stabilize atherosclerotic plaque, preventing rupture and acute events [28]. Medial calcification has been proposed as a repair mechanism for broken elastin fibers [29]. Hemodynamic factors such as wall shear stress and pulse pressure probably also play an important role in the location and severity of calcifications, but conclusive evidence is lacking. It is well known that the presence of calcification is the best predictor of more calcification. Apparently, there are people who easily calcify and others who do not, which has been known in anthropology and which is evident also in the genetic syndromes. We can speculate that somewhere along an S-curve people react with calcification to injury where there is no calcification on one side of the curve and severe calcification on the other side of the curve, and that both extremes cause problems with reparation and regeneration. In the middle of the curve, these calcification systems are presumably working properly.

Much knowledge about the arterial calcification process has been obtained from several rare monogenetic calcifying diseases such as GACI (OMIM #208000), PXE (OMIM #264800) and ACDC (OMIM #211800). In these diseases, the medial layer of the vessel wall becomes calcified, mainly from the aorta to the peripheral vessels. It has been established that inorganic pyrophosphate (PPi) plays an important role in these diseases. PPi is the strongest endogenous inhibitor of the calcification process in the body. It blocks the last step in the calcification process. Several pharmaceutical companies are developing new medications with a focus on PPi in rare diseases. Lack of PPi could also play a role in CLTI patients, potentially benefiting them in the future from these new drug developments.

New CT imaging technique to quantify arterial wall pathology

A correct quantification of arterial wall calcifications with limited noise and bias is essential for the interpretation of the study results. In the technical note in

Chapter 6 we showed that the material decomposition technique is a new way and potentially superior to the conventional HU-based calcium scoring technique to quantify calcium load. In our feasibility study we focused on one clinical problem. In the clinic frequently a calcium score and CT angiogram are frequently requested together. However, up to now from a CT angiogram no reliable calcium score can be subtracted due to overlap of Hounsfield Unit values between calcium and contrast containing blood. The new technique enabled us to quantify both calcium and contrast containing blood separately providing a potentially more reliable way to assess these substances than the Hounsfield values based cut off methods, but further ex vivo and in vivo studies are needed.

Before this technique can be used in the clinic several limitations need to be resolved. First, the software for the segmentation of the artery must be further developed. In this way, we know with certainty that the entire artery is included in the measurements, and in the further XAD calculations no material from outside the wall is included. Second, a hydroxyapatite phantom-based study needs to be conducted for analyzing reproducibility and bias. This technique has potential not only for calcifications but also for assessment of any material with a certain concentration in the body including the amount of fibrosis, muscle and lipid composition. In a slightly different technique, based on the least squares parameter estimation method, an hydroxyapatite phantom was used by le Huy *et al.* [30]. They quantified hydroxyapatite with an average error of 9.83% relative to the absolute amount of hydroxyapatite in the phantom. These results show the potential of these new CT techniques. However, there is still a lot of room for improvement and this is likely to occur over the next few years.

Safety and cost effectiveness of paclitaxel-coated DES

For patients with CLTI, endovascular or surgical management targeting arterial patency remains important until a treatment strategy is developed that addresses the root cause of PAD/CLTI. Yet endovascular or surgical recanalization often fails to save the lower extremity. This is partly due to restenosis. In general, vascular occlusions and stenoses are treated with PTA. However, PTA alone often results in improved arterial patency initially but is bothered by restenosis at a later stage. BMS were developed to treat flow limiting dissections and hopefully to prevent restenosis. However, restenosis has not been prevented by using bare metal stents in the crural arteries. Restenosis is caused by intimal proliferation and migration of cells from the media to the intima of the arterial wall. Thus, this gave rise to the idea of applying antimitotic drugs to PTA balloons or stents in 2009. One

such antimitosis drug was paclitaxel, which was applied to both balloons and stents. The use of DEB below the knee has been disappointing with even worse clinical outcome in two randomized trials [31, 32]. Better results have been reported both morphologically and clinically of DES [33-35]. No outcome studies on improved survival have been performed.

However, in 2019, two meta-analyses showed increased mortality in patients treated with mainly DEB and also DES in the femoropopliteal arteries [36, 37]. There are two important considerations regarding these findings. First, DEB and DES have quite different dose profiles, dosages and release profiles, so they need to be looked at separately. Second, patients with claudication and CLTI are analyzed together, while the risk profile and possible etiology of the first is very different from the latter. Thus, these types of PAD should be considered separately.

In ***Chapter 7*** we have shown that paclitaxel-coated DES used in the infrapopliteal arteries of CLTI patients are safe compared to PTA±BMS, during a follow up period of 10 years after primary placement. We found a wide spread in paclitaxel dose per patient, but no significant difference in mortality between the DES group and the PTA-BMS group (Log-rank p-value = 0.12). No specific dose-related mortality was found (HR 1.00, 95% CI 0.99-1.00, p-value = 0.99) or dose-by-weight mortality (HR 1.05, 95% CI 0.93- 1.18, p=0.46) relationships. Although this was a post-hoc analysis with limited power, our study shows that the use of DES in the crural arteries of patients with CLTI patients is justified in order to reduce amputation, and to increase amputation-free survival and event-free survival up to 5 years after treatment. It should be noted that the results from using DEB in the femoropopliteal arteries cannot be applied to the use of DES in the crural arteries because the dosage of Paclitaxel is much higher on balloons than on stents especially balloons used for the larger above the knee arteries. Katsanos *et al.* have agreed with our conclusions that paclitaxel-coated DES and DEB are not comparable and added that another important difference between DES and DEB is that the former have a slow release and the later a much faster one [38].

In the last ***Chapter 8***, a cost-effectiveness analysis of the use DES to recanalize the lower leg arteries in patients with CLTI was performed. It shows that in the Netherlands, the higher initial purchase costs of paclitaxel-coated DES ultimately outweigh the lower costs of BMS, mainly due to differences in amputation and rehabilitation rates. Paclitaxel-coated DES are €1.679 and €2.694 cheaper per patient, compared with PTA ± BMS, at 1 and 3 years of follow-up, respectively.

In conclusion, DES seems safe and cost effective.

FUTURE PERSPECTIVES

Research on arterial calcifications could progress if whole-body histology and imaging studies are designed in a wide range of people, from infants to the very old, and from healthy and patients with diabetes and chronic kidney disease. This could provide a solid foundation for our understanding of differences between vascular territories and for a good in vivo scoring system. This would allow us to follow up patients and study the development of calcifications and its clinical consequences. Such a cohort study is necessary because different types of calcifications often occur in different combinations in each vascular area. Although it seems old-fashioned, we still think anatomic and histologic knowledge is essential in medicine.

Another important area of research should focus on developing effective drugs against arterial calcifications. Several pharmaceutical companies are active in this field. Most activities are focused on rare calcifying monogenetic diseases where the financial and tax benefits are extremely high in the United States of America. Inorganic pyrophosphate (PPi) plays a central role in this. This molecule is the strongest inhibitor of calcification in the body and is insufficiently available in these diseases. Some companies try to increase its endogenous production, while others try to prevent the breakdown of the molecule. It will be many years before these drugs are available. However, etidronate is currently available. This bisphosphate and PPi analog can stop the calcification process in PXE patients. In fact, originally developed by the washing machine industry it was first applies in humans as a treatment against ectopic calcification. However, due to a lack of financial incentives, this drug has hardly been researched recently by the pharmaceutical industry. Clinical and non-commercial parties will need to step in to push this option. It is our hypothesis that for healthy vascular aging, arteries need to be degreased and decalcified.

Finally, a good cooperation for CLTI patients must be established. These severely diseased patients should be treated in a limited number of centers and the research focus should be on improving the overall outcome. We propose a focus on the calcification process in the whole body and less on new techniques to recanalize only the arteries of the lower extremity.

MAIN CONCLUSIONS

The main finding of this dissertation is that in patients with CLTI extensive calcification is present in all arterial territories. Patients with CLTI have a medial calcification type in the lower leg, while in control patients the intimal calcification type dominates. Complete annular calcifications found in CLTI patients in the lower extremities have a worse survival than patients without these calcifications. Second, new CT technology both in hardware and software has the potential to quantify and qualify various materials in the arterial wall. Third, DES has been shown to be safe and cost-effective compared to PTA ± BMS in our cohort of CLTI patients treated for lower leg arterial obstruction.

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CHAPTER 11.

DUTCH SUMMARY

(NEDERLANDSE SAMENVATTING VOOR NIET-INGEWIJDEN)

NEDERLANDSE SAMENVATTING (VOOR NIET-INGEWIJDEN)

Perifeer arterieel vaatlijden/ kritieke ischemie

Het ziektebeeld perifeer arterieel vaatlijden wordt veroorzaakt door een vernauwing, of door volledig dichtzittende slagaders van de benen. Hierdoor ontstaat onvoldoende aanbod van zuurstof en voeding naar de weefsels van de benen, waardoor pijn ontstaat bij lopen en weefsel zelfs kan afsterven (ischemie).

De meest ernstige vorm van perifeer arterieel vaatlijden betreft kritieke ischemie. Deze patiënten hebben pijn zelf al in rust of niet-genezende wonden. De symptomen hebben een chronisch karakter; de klachten bestaan vaak al maanden, maar minstens twee weken. Dit in tegenstelling tot acute ischemie waar een bloedvat plotseling dicht zit. De overleving van kritieke ischemie patiënten is de laatste jaren wel wat verbeterd, waarschijnlijk doordat deze patiënten systematischer behandeld worden met anti-atherosclerotische medicatie. Toch blijft de prognose nog zeer slecht en blijven kritieke ischemie patiënten nieuwe vaatziekten krijgen en sterven zij hier ook aan. Blijkbaar is er dus naast atherosclerose ook nog een andere vaatziekte in het spel die niet reageert op behandeling met anti-atherosclerotische medicatie. Kritieke ischemie is dus mogelijk een onderdeel van een systemische ziekte die overal in het lichaam zit in plaats van enkel een focale vaatziekte van de benen en wij denken dat verkalking van alle bloedvaten een belangrijke rol speelt.

Slagaderlijke verkalkingen

De slagaderwand bestaat uit drie lagen. Van binnen naar buiten zijn dit de volgende lagen: de intima, media en adventitia. Slagaderlijke verkalkingen treden op in de binnenste twee lagen; de intima en/of in de media laag.

De eerstgenoemde vorm, de intimaverkalkingen, zijn het gevolg van atherosclerose. Bij atherosclerose zijn de vaatafwijkingen ontstaan door een teveel aan cholesterol in het bloed, waardoor er vet ophoopt in de slagaderwand. Deze vetophoping leidt op zijn beurt weer tot een ontstekingsproces in de slagaderwand. Hierbij vormt zich ook kalk. De tweede vorm van vaatverkalking bevindt zicht in de middelste laag van de vaatwand (media) en ontstaat doordat de balans tussen aanmaak en afbraak van kalk verstoord is. Mediakalk leidt tot vaatwandverstijving waardoor de drukgolf veel te hard doorloopt in weefsel. Normaal hebben soepele bloedvaten een windketelfunctie. Stijve vaten leidt tot chronische vaatschade zoals perifeer arterieel vaatlijden, nierproblemen en mogelijk dementie. Intima- en mediaverkalkingen zijn dus twee verschillende vormen van vaatverkalking en zien

er anders uit als we het weefsel bekijken onder de microscoop, alsook op röntgenfoto's en computertomografie scans.

Voorheen werden slagaderlijke wandverkalkingen gezien als slechts een statische afzetting van hydroxyapatiet/fosfaat in de vaatwand als een nevenproduct van atherosclerotische ziekte en daarom relatief onbeduidend. Nu is duidelijk geworden dat in ieder geval de mediale slagaderlijke verkalkingen deel uitmaken van een dynamisch proces dat waarschijnlijk niet alleen kan worden gestopt, maar ook kan worden omgekeerd en een belangrijke rol speelt bij vaatziekten.

Behandeling perifeer arterieel vaarlijden en kritieke ischemie

De behandeling van perifeer arterieel vaarlijden en kritieke ischemie richt zich op het opheffen van de vernauwingen en volledig dichtzittende slagaders waardoor het weefsel weer voldoende zuurstof en voedingsstoffen kan krijgen. Naast deze lokale behandeling wordt er ook steeds meer gericht op het behandelen van risicofactoren. Dit omvat medicamenteuze therapie tegen atherosclerose, stoppen met roken, gezonde voeding, gewichtsverlies en regelmatige lichaamsbeweging.

Het opheffen van de slagadervernauwing kan onder andere worden bereikt door het gebruik van ballonnen en stents. Ter hoogte van de vaatvernauwing wordt de ballon opgeblazen, waardoor het vernauwde bloedvat wijder wordt gemaakt. Soms kan het nodig zijn om hierna een stent te plaatsen, om de vernauwing open te houden. Stents zijn metalen holle buisjes (stents) die ter plaatse van de vernauwing wordt uitgevouwen. Een nadeel van deze stents is dat uiteindelijk weefsel de stent ingroeit, waardoor het vat weer vernauwt. Een oplossing hiervoor is het aanbrengen van zeer kleine hoeveelheid van een chemotherapeuticum op de stent (drug-eluting stent), zoals bijvoorbeeld paclitaxel, waardoor de stent niet wordt overgroeid met nieuw weefsel. Meerdere van onze eerdere studies in het kader van de PADI-trial lieten zien dat deze stents beter openbleven dan de ongecoate stents.

In dit proefschrift worden meerdere aspecten van kritieke ischemie onderzocht. Het eerste doel van dit onderzoek is om slagaderwandverkalkingen van deze patiënten op Computer Tomografie (CT) scans in kaart te brengen om zo het ziektebeeld beter te begrijpen en de hoog-risico patiënten te identificeren. Het tweede doel van dit onderzoek richt zich meer op de behandeling van deze patiënten en evalueert de veiligheid en kosteneffectiviteit van het gebruik van paclitaxel-gecoate vaatstents vergeleken met het gebruik van vaatstents zonder coating.

DEEL II. SLAGADERLIJKE VERKALKINGEN

Slagaderlijke verkalkingen in de onderste ledematen worden vaak aangetroffen op een CT-scan bij mensen zonder vaatlijden. We onderzochten bij deze groep het voorkomen en de patronen (ringvormig, dikte en lengte) van deze verkalkingen in ***hoofdstuk 2***. Uit de resultaten van deze studie concludeerden we dat slagaderverkalkingen al op vroege leeftijd voorkomen en fors toenemen met de leeftijd. Daarnaast kunnen ten minste twee verkalkingspatronen op CT worden onderscheiden, die lijken te passen bij een intima- en mediaverkalkingspatroon. Aangezien beide soorten verkalkingen verschillende ontstaansmechanismen en relevantie hebben, is het belangrijk om onderscheid te kunnen maken.

In ***hoofdstuk 3*** vergeleken we de verkalkingslast en verkalkingspatronen op CT-scans van de benen tussen patiënten met kritieke ischemie en mensen zonder vaatlijden. We wilden met deze studie ook bepalen of verkalkingslast en -patronen verschillend waren in de bovenbeensslagaders in vergelijking met de onderbeensslagaders. Uit de resultaten blijkt dat slagaderverkalkingen in de benen meestal ernstig zijn bij kritieke ischemie patiënten. In de slagaders van de onderbenen hebben deze patiënten een meer ringvormig type verkalking passend bij mediaverkalkingen, terwijl mensen zonder vaatlijden een focaal/puntvormig type verkalkingen. In de bovenbeensslagaders hebben patiënten met kritieke ischemie juist dikke, irregulaire, niet-ringvormige verkalkingen passend bij intimaverkalkingen.

Aangezien mediaverkalkingen steeds vaker als behandelbaar worden beschouwd, kunnen onze bevindingen bijdragen aan de ontwikkeling van een behandelingsstrategie voor deze moeilijk te behandelen KI-patiënten.

In ***hoofdstuk 4*** evalueerden we de voorspellende waarde van deze CT-kenmerken van slagaderlijke verkalking van de benen op zowel 7-jaars amputatievrije overleving als 10-jaars mortaliteit bij patiënten met kritieke ischemie. Deze studie toonde aan dat ringvormige verkalkingen vooral in de onderbeensslagaders een goede voorspeller zijn voor de 10-jaars mortaliteit door alle oorzaken en de 7-jaars amputatievrije overleving van kritieke ischemie patiënten. Deze meting is gemakkelijk te gebruiken in de dagelijkse klinische praktijk en kan daarvoor bijdragend zijn voor het stratificeren van hoog-risico kritieke ischemie patiënten.

Hoewel kritieke ischemie meestal wordt behandeld met anti-atherosclerotische medicatie wat primair aangrijpt op atherosclerose, is de overleving van kritieke ischemie patiënten nog steeds zeer slecht. Vermoedelijk wordt dit veroorzaakt door een bijkomende, resterende vaatziekte. In ***hoofdstuk 5*** hebben we onderzocht of vasculaire verkalking van de mediawand betrokken kan zijn bij deze restlast. Concluderend uit deze studie blijkt dat bijna alle arteriële gebieden ernstig verkalkt zijn bij patiënten met kritieke ischemie. Daarnaast blijkt dat verkalkingen in de benen significant gecorreleerd waren met de verkalkingsscore van de hartslagaders, de verkalkingsscore in de halsslagaders, en in mindere mate gecorreleerd met ringvormige verkalkingen in de grote lichaamsslagader. Ten tweede waren hoge en extreem hoge totale verkalkingsscores in de hartslagaders sterk gecorreleerd met verkalkingen van de benen en thoracale en abdominale aortaverkalkingen bij patiënten met kritieke ischemie patiënten. Dit duidt zeer duidelijk op een systemische ziekte en kan waarschijnlijk de resterende hoge ziektelast en sterfte bij deze patiëntenpopulatie verklaren.

Een nauwkeurige en reproduceerbare bepaling van slagaderlijke verkalkingen is belangrijk om perifeer arterieel vaatlijden goed te kunnen begrijpen. Op CT worden materialen (en dus ook verkalkingen) gemeten middels Hounsfield Units (HU). Deze methoden zijn echter onbetrouwbaar als gevolg van de overlap in HU-waarden van verschillende materialen. Dit leidt tot over- en onderdetectie van deze materialen. In ***hoofdstuk 6*** onderzochten we een nieuwe methode, de materiaaldecompositiemethode met behulp van spectrale CT-angiografie Met deze methoden kunnen de materialen specifiek worden geïdentificeerd. Uit onze studie blijkt dat deze materiaaldecompositietechniek een goed alternatief is om slagaderverkalkingen van de benen te kwantificeren alsook jodium-bevattend bloed in het vatlumen. Deze techniek vermijdt over- en onderdetectie van verkalkingen en jodiumrijk bloed zoals vaak het geval is bij methoden die werken met op HU-gebaseerde afkapwaarden.

DEEL III. UITKOMSTEN VAN BEHANDELING VAN KRITIEKE ISCHEMIE

In de “Percutaneous transluminal Angioplasty versus Drug eluting stents for Infrapopliteal lesions”-trial toonden we aan dat kritieke ischemie patiënten behandeld met paclitaxel-gecoate stents vergeleken met stents zonder coating een betere doorgankelijkheid hadden en minder amputaties. De American Food and Drug Authority waarschuwde in 2019 echter voor het gebruik van deze paclitaxel-gecoate devices (ballonnen en stents) omdat twee meta-analyses een verminderde

overleving lieten zien na gebruik van deze stents. In ***hoofdstuk 7*** evalueerden we opnieuw de 10-jaars overleving van de trial met betrekking tot de totale dosis paclitaxel die onze patiënten kregen. We concludeerden dat de 10-jarige overleving van kritieke ischemie patiënten die onder de knie behandeld waren met vaatstents in het algemeen slecht is. Er werden geen significante verschillen gevonden in de 10-jarige mortaliteit bij patiënten met KI behandeld werden met paclitaxel-gecoate stents vergelijking met de standaardbehandeling met ongecoate stents.

Eerdere resultaten van de trial gaven de voorkeur aan paclitaxel-gecoate stents met minder nieuwe verstoppingen van de stent door weefseloverwoekering én minder amputaties. Een kosteneffectiviteitsstudie naar het gebruik van paclitaxel-coated stents in patiënten die worden behandeld in de onderbeensslagaders is echter nog niet uitgevoerd en wordt daarom gerapporteerd in ***hoofdstuk 8***. In onze analyse zijn paclitaxel-gecoate stents kosteneffectief vanwege de hogere ziekenhuiskosten van amputatie en revalidatie in de standaard behandelde groep met ongecoate stents.

Conclusies

Samenvattend geeft dit proefschrift nieuwe inzichten in slagaderverkalkingen bij patiënten met en zonder kritieke ischemie. Slagaders bij patiënten met kritieke ischemie zijn zeer ernstig verkalkt, zowel in de benen maar ook op andere locaties zoals de aorta, halsslagaders en slagaders in de hersenen. Dit vormt een mogelijke verklaring voor de hoge sterfte en ziektelast bij deze patiëntengroep. In de benen kan een tweetal verkalkingspatronen worden gevonden; een dominant mediapatroon in de onderbenen en een dominant intima verkalkingspatroon in de bovenbenen.
Ten tweede heeft dit proefschrift laten zien dat het gebruik van paclitaxel-gecoate stents als behandeling van kritieke ischemie patiënten met vernauwingen en verstoppingen van de onderbeensslagaders, veilig en kosteneffectief is.

PART V.

Addenda

ADDENDUM.

Complete reference list

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ADDENDUM.

Review Committee

REVIEW COMMITTEE

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ADDENDUM.

SCIENTIFIC PUBLICATIONS AND SUBMITTED ARTICLES

SCIENTIFIC PUBLICATIONS

Differences in lower extremity arterial calcification patterns in patients with chronic limb-threatening ischemia compared to control patients
Konijn LCD, Takx RAP, Mali WPTM, Veger HTC, van Overhagen H.
J Pers Med. 2021 May 31;11(6):493. doi: 10.3390/jpm11060493.

Arterial calcification and long-term outcome in chronic limb-threatening ischemia patients
Konijn LCD, Takx RAP, de Jong PA, Spreen MI, Veger HTC, Mali WPTM, van Overhagen H.
Eur J Radiol. 2020 Sep 28;132:109305. doi: 10.1016/j.ejrad.2020.109305.

10-year paclitaxel dose-related outcomes of drug-eluting stents treated below the knee in patients with chronic limb-threatening ischemia (the PADI Trial)
Konijn LCD, Wakkie T, Spreen MI, de Jong PA, van Dijk LC, Wever JJ, Veger HTC, Statius van Eps RG, Mali WPTM, van Overhagen H.
Cardiovasc Intervent Radiol. 2020 Jul 28. doi: 10.1007/s00270-020-02602-6.
PMID: 32725411

CT calcification patterns of peripheral arteries in patients without known peripheral arterialdisease
Konijn LCD, van Overhagen H, Takx RAP, de Jong PA, Veger HTC, Mali WPTM.
Eur J Radiol. 2020 May 11;128:108973. doi: 10.1016/j.ejrad.2020.108973.

Cost-Effectiveness of Drug-Eluting Stents in Patients with Critical Limb Ischemia: the PADI Trial
Wakkie T, **Konijn LCD**, van Herpen NPC, Maessen MFH, Spreen MI, Wever JJ, Statius van Eps RG, Veger HTC, van Dijk LC, Mali WPTM, van Overhagen H.
Cardiovasc Intervent Radiol. 2020 Mar;43(3):376-381. doi: 10.1007/s00270-019 02385-5.

SCIENTIFIC SUBMITTED ARTICLES

Systemic arterial calcium burden in patients with chronic limb-threatening ischemia
Konijn LCD, Takx RAP, van Overhagen H, de Jong PA, Mali WPThM.
[Submitted]

Feasibility of arterial wall calcification quantification by material decomposition per voxel using spectral computed tomography angiographies in the femoral arteries. A proof-of-concept study
Konijn LCD, Konings TOM, Schilham AMR, de Jong PA, Mali WPTM.
[Submitted]

ADDENDUM.

Scientific Presentations

SCIENTIFIC PRESENTATIONS

10-year paclitaxel dose-related outcomes of drug-eluting stents in the treatment of critical limb ischemia due to infrapopliteal pathology (the PADI trial)
Cardiovascular and Interventional Radiological Society of Europe (CIRSE) Congress, September 2021, online version

10-year paclitaxel dose-related outcomes of drug-eluting stents in the treatment of critical limb ischemia due to infrapopliteal pathology (the PADI trial)
Cardiovascular and Interventional Radiological Society of Europe (CIRSE) Congress, September 2020, online version

The PADI Trial and PADI Imaging Trial - The Hague. Morphology of vascular calcifications on CT in non-vascular patients and patients with critical limb ischemia
Refereeravond HagaZiekenhuis, november 2019, The Hague

Prevalence, morphological patterns and associations in arterial calcification on whole body computed tomography in subclinical patients
Hagaziekenhuis/Guerbuet onderzoeksavond, march 2019, The Hague

Validation of CT imaging characteristics to differentiate intimal from medial calcifications in peripheral vessels and its application in a non-vascular cohort
ECR 25th European Congress of Radiology 2019, february 2019, Vienna, Austria

Characteristics of lower extremity calcifications and its association with the ankle-brachial index in patients with chronic critical limb ischemia
Onderzoeksdag Hagaziekenhuis 2019, The Hague

Characteristics of lower extremity calcifications and its association with the ankle-brachial index in patients with chronic critical limb ischemia
TCT Congress 2018, september 2018, San Diego, United States of America

Prevalentie en ernst van vasculaire calcificaties
Onderzoeksdag Hagaziekenhuis 2018, The Hague

Validatie van CT-calcificatie karakteristieken en implementatie in een non-vasculair cohort

Onderzoeksdag Hagaziekenhuis 2018, The Hague

Prevalentie en ernst van vasculaire calcificaties
Hagaziekenhuis/Guerbuet onderzoeksavond, march 2018, The Hague

ADDENDUM.

SCIENTIFIC AWARD

SCIENTIFIC AWARD

CVIR Editors' Medal 2021

As reflected by our recently increased impact factor, a wealth of high-quality scientific articles has been submitted to and published in CVIR of late. The difficult task and great honour of reviewing and short-listing the many clinical investigations, experimental papers and review articles published in 2020 has fallen to the associate editors, with the Editor-in-Chief then selecting one outstanding paper to receive the 2021 Editors' Medal.

After much deliberation, we are pleased to announce the winners: the Dutch group of authors who published the PADI Trial, investigating 10-year paclitaxel dose-related outcomes of drug-eluting stents in below-the-knee patients with chronic limb-threatening ischaemia. This paper underlines the importance of long-term systematic follow-up and rigorous post-hoc review of the materials used in interventional radiological practice.

Editors' Medal winner:

10-Year Paclitaxel Dose-Related Outcomes of Drug-Eluting Stents Treated Below the Knee in Patients with Chronic Limb-Threatening Ischemia (The PADI Trial)

Louise C. D. Konijn, Thijs Wakkie, Marlon I. Spreen, Pim A. de Jong, Lukas C. van Dijk, Jan J. Wever, Hugo T. C. Veger, Randolph G. Statius van Eps, Willem P. Th. M. Mali & Hendrik van Overhagen

December 2020 [1]

Congratulations to the winners, and thank you for making CVIR your preferred journal for your submissions!

Klaus A. Hausegger, MD

CVIR Editor-in-Chief

Reference

1. Konijn LCD, Wakkie T, Spreen MI, et al. 10-year paclitaxel dose-related outcomes of drug-eluting stents treated below the knee in patients with chronic limb-threatening ischemia (the PADI trial). Cardiovasc Intervent Radiol. 2020;43:1881–8. https://doi.org/10.1007/s00270-020-02602-6.

ADDENDUM.

Biography

BIOGRAPHY

Louise Cornelia Divera Konijn was born on June 27 1984 in Alkmaar, The Netherlands. She attended secondary school at the Oscar Romero, Hoorn.

September 2005, Louise started studying medicine at the Leiden University Medical Center. During her medical studies she was a voluntary student member of the KNMG student platform, analyzed data from medical students and presented it at educational conferences to improve education and education-related matters. She also worked numerous hours in the anatomical laboratory for macro-anatomy and provided anatomical education for medical students. During her internships she participated in her first research project, a cadaveric study on several anatomical axes of the knee. In December 2012, she obtained her medical degree at the Leiden University Medical Center.

From July 2016 Louise started her Radiology Residency at the Haga Teaching Hospital in The Hague. In the first year of her Radiology Residency, Louise participated in the spin-off of the investigator initiated PADI Trial of the Haga Teaching Hospital. Through a collaboration of the Haga Teaching Hospital and the University Medical Center Utrecht, Louise got the opportunity to initiate the PADI Imaging Trial. This resulted in a PhD trajectory at the Utrecht University under the supervision promotors prof. dr. P.A. de Jong and prof. dr. W.P.Th.M. Mali, and co-promotors dr. H. van Overhagen and dr. R.A.P. Takx. In September 2021 she was awarded the CIRSE Editors' Medal 2021 for her work in dose-related outcomes of drug-eluting stents.

Louise is very proud that she has successfully combined these two trajectories and to present this current dissertation.

Currently, Louise is the junior board member of the abdominal division of the Dutch Society of Radiology. In the summer of 2022, Louise will also complete her radiology training at the UMC Utrecht. In her subspecialty she focusses on abdominal and breast radiology.

ADDENDUM.

Dankwoord

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Beoordelingscommissie

Leden van de promotiecommissie: prof. dr. J.A. van Herwaarden, prof. dr. B.K. Velthuis, dr. I.C. van der Schaaf, prof. dr. J.A. Reekers, prof. dr. M.W. de Haan en dr. W. Spiering, veel dank voor het kritisch lezen en beoordelen van dit manuscript en dat u zitting wil nemen in deze promotiecommissie.

Paranymfen

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