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PhD THESIS

ANGIOSOME CONCEPT IN PERIPHERAL ARTERIAL DISEASE

A B S T R A C T

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ABSTRACT

The present thesis aims to reveal a current analysis of available clinical evidence on targeted infragenicular revascularization according to the Concept of Angiosomes in the treatment of patients with critical ischemia and diabetes, connected with retrospective observations from the database of a single centre in Romania, and to demonstrate the effects of the implications of peripheral diabetic neuropathy in infragenicular endovascular revascularization with data from two medical centres in Belgium.

In the general part, I reviewed the literature that has represented the basis of this research for the studied topic.

Peripheral arterial disease (PAD) affects between 12% and 20% of the world's population aged below 65 years of age. Critical ischemia (CI), the most advanced stage of BAP, characterized by resting pain and ulcer or gangrene, a disease that has an amputation rate of over 30% and a mortality of up to 25% at one year. The prevalence of diabetic ulcers in the diabetic population is increasing from 2.8% to 10.0% and 14-43% of these patients will require amputation with a survival rate of 50-60% per year. Although 85% of amputations can be prevented, the major amputation rate has decreased very little in the last 20 years.

The introduction of endovascular techniques and active pharmaceutical devices has brought a real benefit to patients with diabetes and critical ischemia due to the low potential for intra- and post-operative infection, low complication rate, short hospital stay, possibility of multi-arterial revascularization, faster healing and low intra- and post-operative death rate. The last 10 years have focused on researching topographic revascularization of the gambier arteries guided by the localization of ischemic foot wounds of the death rate.

The literature published in the last 15 years has investigated the idea that endovascular revascularization or surgical bypass based on the Angiosome Concept can provide practical details on the vascular map of the foot and, consequently, can generate a better clinical result in the evolution of patients with peripheral arterial disease and, in particular, in the treatment of patients with critical limb ischemia.

If, until now, in the current practice of revascularization of the lower limbs, endovascular or surgical, the choice of the best outflow artery based on angiographic image has been accepted, now a new strategy is being approached based on advanced research in critical ischemia, namely the Angiosome Concept.

The Angiosome Concept was defined by Taylor and Palmer in 1987 by extending the literature published by several previous anatomists in the field of reconstructive surgery. They separated the body into distinct three-dimensional anatomical territories of perfused and drained tissue from source arteries called angiosomes. They have shown that the arteries that supply blood flow to these areas of tissue are responsible for feeding the skin, subcutaneous tissue, fascia, muscles, and bone. They called these composite units angiosomes and concluded that the arterial map they described should be the basis for the logical planning of incisions and flaps. In 2006, Attinger et al. used differently coloured methyl methacrylate solutions, injected them into the lower leg arteries of 50 dissected corpses and demonstrated the distribution of angiosomes.

They describe 6 angiosomes that come from 3 main arteries with multiple arterio-arterial connections and concluded that their detailed information on the vascular anatomy of the ankle and foot should guide surgeons in planning foot reconstruction and informed choice of the most effective revascularization for a certain wound.

Their research found that there are 3 angiosomes of the plantar foot (supports of the posterior tibial artery), 2 angiosomes of the ankle and hind leg (peroneal artery) and the angiosome of the dorsum of the foot (anterior tibial artery). Each angiosome is bordered by collateral vessels.

These collateral vessels provide an indirect interconnection between multiple angiosomes.

The Angiosome Concept is an anatomical description rather than a physiological model. Arterio-arterial connections allow blood to flow to the entire leg even when one or more arteries are occluded. Direct revascularization on affected angiosomes has led to higher limb rescue and wound healing rates. However, many factors must be taken into account in choosing the target artery for revascularization and the establishment of the angiosomal arterial network and its collaterals should be precisely objectified.

It has been suggested that, in diabetic patients, the occurrence of (neuro-)ischemic problems (diabetic foot) is related to a combination of distal atherosclerotic macroangiopathy and an induced impairment of microcirculation functionality because of neuropathy and local sepsis. The collateral vascular network is severely damaged in diabetic patients with critical ischemia and end-stage renal disease, thus interrupting communication between angiosomes.

The network of collaterals depends on age, the underlying pathology that causes critical ischemia, and the location of the angiosome itself. The two extremes are the middle-aged, atherosclerotic, non-diabetic patient with trophic lesions of the forefoot and, on the other hand, the elderly, diabetic patient or with long-term renal disease with neuro-ischemic tissue defects at the heel level.

In patients with diabetic terminal arterial disease, so-called “irregular atherosclerosis” occurs with acute septic thrombosis and loss of small collaterals. The absence of collateral vessels emphasizes the need for a more distal, selective revascularization, which improves the perfusion in the skin.

Clinical reports suggest an advantage of direct revascularization, and its importance in diabetic patients is considered to be greater due to different basic pathophysiology. In diabetic patients, the middle tunic is affected rather than intimated, which leads to a situation in which not only the source artery is affected, but also the collaterals and anastomoses between the angiosomes. It should be noted that indirect revascularization may be appropriate when sufficient collateral is present.

In recent years, a large number of diagnostic methods have been proposed to highlight direct arterial flow. Although they offer advantages for clinicians, none of them is superior in terms of specificity and sensitivity in the prognosis of ischemic tissue regeneration.

Taylor & Palmer, in 1987, dividing the body into three-dimensional anatomical blocks of tissue provided by a particular artery, laid the foundations of the Angiosome Concept (6 vascular angiosomes of 3 main arteries); following that, in 2006, Attinger et al., using methyl methacrylate injection in the arteries of the legs, demonstrated the distribution of angiosomes in 50 corpse feet. Their theory should guide surgeons in the most effective revascularization for a particular wound to save the lower limb. The posterior tibial artery irrigates 3 angiosomes of the plantar foot, the peroneal artery provides 2 angiosomes of the ankle and hind leg, and the anterior tibial artery contributes to the angiosome of the dorsum of the foot.

Direct revascularization (DR) refers to the selective reconstitution of a straight line of blood flow from the abdominal aorta to the lesion angiosome. In contrast, indirect revascularization (ID) refers to the reconstitution of a straight line of blood flow to ankle or foot angiosomes that are not involved in tissue injury; thus, the indirect infusion through collateral vessels is performed.

The theory that direct revascularization improves the healing and saving rate of limbs in patients with chronic ischemia is supported by numerous studies published in the last 20 years.

New Global Vascular Guidelines (GVGs) introduce the new concept of direct revascularization, thus developing a new strategy to improve the clinical success of revascularization, greatly influenced by the clinical stage of the disease, endovascular technique, and physician experience.

The angiographic study of the arteries of the foot is a key point in defining the possible targets of revascularization; the target arterial pathway must be evaluated by high quality imaging.

It is often difficult to pinpoint the exact angiosomes; toe injuries, for example, which account for more than half of the lesions encountered in diabetic ischemic foot, should have a double blood supply from the pedestrian artery interconnected with the posterior tibial artery through the plantar arch.

Most analyses comparing direct to indirect revascularization are retrospective and not randomized, and the results of meta-analyses reveal that direct revascularization increases the rate of wound healing and limb rescue.

Clinical evaluation is essential in choosing the surgical approach for debridement or secondary suturing. Adequate blood supply is the key factor in the surgery of patients with CI, and if there is good blood flow from the source artery that feeds each angiosome, the safest incision is along the border between adjacent angiosomes because each part of the incision has maximum blood flow. In patients with CI, much of the surgery involves minor amputations: performing amputations of the forefoot and midfoot in patients who have intact circulation with integrated dorsal and plantar blood flow has minimal risk. On the contrary, in the case of forefoot amputations in patients with pure dorsal or plantar blood supply, it is essential that blood flow and arterial-arterial connections be complete and well-preserved during surgery.

The three key points of viable tissue availability, direct or indirect flow and biomechanical needs should be carefully considered before choosing the appropriate type of amputation. Too proximal amputation, disrupting the metatarsal connection between the dorsal and plantar vascular systems may exacerbate the postoperative ischemia of the flap fed by indirect blood flow.

Terashi et al. proposed a modified transmetatarsal amputation procedure, designed to keep the soft tissue between the metatarsal bones (the vascular complex with muscles, periosteum and vessels) in order to maintain better circulation at the ends of the abutment. For debridement and minor amputations in these patients, a “stress-free” surgical approach should be considered. Closure of the wound with primary intent should be avoided, as any pressure points related to tissue torsion, skin extension and suture can lead to post-operative focal ischemic necrosis. A secondary intentional closure should be pursued. The edges are closed only with one or two subcutaneous sutures and a dermal substitute (collagen) can be used to cover the exposed bone in transverse amputations. Any surgical treatment of foot injuries in patients with CI should be conditioned by blood supply, according to the concepts of flow-guided and tension-free revascularization.

The clinical picture of neuro-ischemic diabetic wounds is very complex and often independent of the success of surgical revascularization. Without strict management of all risk factors that influence tissue regeneration, minor or even major amputations (approximately 28%) of the neuro-ischemic diabetic foot can be reached, regardless of the type of direct or indirect revascularization. At least 25% of diabetic ulcers will not heal without a control of the risk factors of critical ischemia: ischemia, metabolic factors, local pressure, and neuropathy.

The guidelines of the Society of Vascular Surgery, of the American Podiatric Medical Association, and of the Society of Vascular Medicine support the multidisciplinary approach in the prevention of diabetic ulcer, off-loading, and control of foot pressure, early diagnosis of sepsis and osteomyelitis, excisional arterial debridement, and control.

Diabetes and nutritionists play a key role in preventing the onset of neuro-ischemic diabetic wounds. Off-loading devices reduce pressure and help heal ulcers regardless of the type of revascularization. Collaboration between vascular surgeons and physiotherapists is essential. The role of the infectious disease physician in working closely with vascular surgeons is vital. Wound infection can have devastating consequences in post-revascularization healing and, therefore, it should be diagnosed and treated urgently.

Elraiayah et al., in a recent analysis, have shown that careful debridement performed as quickly as possible can save important areas of tissue and this process can be done in collaboration with general surgeons and orthopaedists.

The Special part of this thesis is structured on three studies that provide an overview of the entire research: 1. Infragenicular endovascular revascularization based on the Angiosome Concept; 2. Clinical implications of peripheral diabetic neuropathy in the approach of primary infrapopliteal angioplasty for neuro-ischemic foot wounds; 3. Comparative investigation on the polymers of pharmacologically active balloons used in infrapopliteal angioplasty based on the Angiosome Concept.

The main objectives of the thesis are:

- Evaluation of the clinical utility of targeted endovascular revascularization on angiosomes using simple or pharmacologically active balloons and its clinical outcome in the treatment of patients with critical diabetic ischemia of the limbs who had localized disease in the infrapopliteal arteries;
- Evaluation of the clinical effects of diabetic peripheral neuropathy (DPN) in patients with critical ischemia treated by primary infrapopliteal angioplasty for neuro-ischemic wounds Rutherford 5, leg.

Secondary objectives of the thesis:

- Description of the polymeric materials and comparison of the medical objectives obtained in infrapopliteal angioplasty targeted on angiosomes using a simple two-layer, polyethylene-based flask and a drug-coated flask containing a multiblock copolymer of polyethylene, poly (kilohehexethylene), polyisoprene and poly (1,3-butadiene) covered by Paclitaxel.

STUDY 1: Targeted infrapopliteal endovascular revascularization, based on the concept of angiosomes: how did it affect our clinical outcome? An observational study.

This study examines the relevance of direct balloon angioplasty based on the Angiosome Concept in the treatment of diabetic patients with critical limb ischemia. The analysis of 51 patients with critical limb ischemia and tissue damage highlights that the use of the Angiosome Concept in the endovascular treatment of patients with Type II Diabetes and critical limb ischemia has been associated with improved wound healing, leg rescue, and amputation-free survival.

This retrospective non-randomized observational study aimed to evaluate the clinical utility of targeted endovascular revascularization on angiosomes using simple or pharmacologically active balloons and its clinical outcome in treating patients with critical diabetic limb ischemia with localized infrapopliteal arteries.

A retrospective analysis was performed in 51 patients with critical lower limb ischemia (76.5% men and 23.5% women); they were revascularized based on the AC (28 direct, 12 indirect, and 11 both). Forty-three patients were treated with a single commercial flask, while the other 8 patients were treated with pharmacologically active flasks. The study cohort consisted of patients in the Rutherford 5 and 6 categories. Data were collected retrospectively between November 1, 2018 and November 1, 2019; the end of its research and one-off investigations was on 1 November 2020. The average follow-up period was 12 months; 4 patients were excluded from the follow-up because they did not participate in the follow-up investigation.

The ankle-brachial index (ABI) was measured pre- and post-operatively. Patients with elevated false rates of medial sclerosis and calcifications (more than 1.3) were excluded from the calculation. A dorsal and plantar measurement was performed on the foot pre- and post-operatively to evaluate the angiosomes of the dorsal and plantar foot.

In the follow-up clinical investigation, the characteristics of the wound were recorded, as well as the local pain, ABI was obtained, as well as duplex ultrasound as an assessment of the permeability of revascularization; the size of the wound was assessed and documented by photography, the wounds were classified at the time of the first investigation using the Wound, Ischaemia and Foot Infection (WIFI) scores from the Society of Vascular Surgery.

The criteria for inclusion in the study were: patients from Rutherford stage 5-6 or Leriche-Fontaine IV with neuro-ischemic wounds, the need for infragenicular revascularization by endovascular technique, the number of viable arteries for revascularization, the number of angiosomes affected by the wound, comorbidities, critical ischemia, complications of the underlying disease, smoking, age, and ABI.

Exclusion criteria: iodine allergy, liver failure, pregnancy, hyperthyroidism, pulmonary arterial hypertension, end-stage renal disease and dialysis, acute ischemia, fem-popliteal aneurysmal pathology, extensive leg gangrene, systemic sepsis, previous infra-genicular bypass and thrombosis, chronic treatment with cortisone or cytostatics, dementia or psychotic behaviour, total absence of collateral of the foot ("Desert foot") and diagnosed thrombophilia.

In the section presenting the results of the study are presented a comparison between the two types of angioplasty used as objectives; the parameters that were evaluated 12 months after the clinical intervention are wound healing, leg rescue, and amputation-free survival.

The following direct correlations were found: strong (Leg salvage at 12 months vs Survival at 12 months and Healing at 12 months, respectively Leg salvage at 12 months), moderate (Haemodialysis vs renal failure, number of angiomas vs wounds at PTA and angiosomal levels vs ATA plaque, respectively) and weak (insulin-deficient diabetes vs sex and angiosomes vs rescue at 12 months, respectively), while indirect correlations were also strong (Death vs Rescue of the legs at 12 months and Death vs Healing at 12 months, respectively), moderate (Wound at level Per.A vs ATA and minor amputation vs ABI pre) and weak (Dyslipidemia vs Haemodialysis and wound respectively on both feet vs Diabetes OralAd).

The overall one-year wound healing rate was 90.2%; it was found that the highest rate of wound healing was achieved in the case of indirect revascularization (95.7%) and the lowest in the case of simple balloons (89.5%). It seems that the indirect procedure has a more effective influence in wound healing, but the difference between the two groups is not important. The overall one-year foot rescue rate was 88.3%; it was found that the leg salvage rate was reached in the case of direct revascularization (89.5%), while the lowest in the case of the indirect procedure (87.0%). No significant differences were observed between the two groups at the end of this investigation.

The overall one-year amputation-free survival rate was 91.8%, and the amputation-free survival rate was reached in the case of direct intervention (92.1%) and lower in the case of the indirect method (91.3%). No significant difference was observed between the two groups 12 months after this investigation.

These results may support a correlation between patients treated by direct revascularization and Wifl degrees – in their cases, the highest value (3) was not found for any of the three parameters; most of these patients being included between those with W21f11 and W21f12. On the other hand, it should be noted that no patient in the indirect intervention group had the best value for the Wifl score.

The Discussions were conducted on the basis of the Global Vascular Guidelines (GVG), according to which there is widespread controversy around the benefits of performing angiosome-guided revascularization for several reasons. First, there are only a limited percentage of cases in which foot injuries can be attributed to an individual angiosome without any ambiguity, especially due to injuries to the toes, which have a double blood supply, the anterior tibial artery and the posterior tibial artery (ATA and PTA) are present in more than half of the cases. Second, from a technical point of view, there is the question of the availability of the target artery of that angiosome. And, then, there is the discussion about the comparative haemodynamic and clinical efficacy of "direct" versus "indirect" revascularization.

In conclusion, I pointed out that, in cases of critical limb ischemia, direct endovascular revascularization leads to significantly better wound healing and leg rescue rates compared to indirect revascularization. It should be admitted that the treatment of

critical ischemia cannot be achieved by surgery alone, even if it is successful, and post-operative care, including wound management and medication, is of the utmost importance. They are invaluable because the healing power of the wounds is diminished and the immune system is compromised, especially in patients with diabetes or end-stage kidney disease. The Angiosome Concept is feasible for most patients treated with an endovascular approach, although only 27.45% of tissue lesions are located in a single angiosome. Factors that are associated with a worse wound healing time are a few angiosomes affected by > 3 and PTA indirectly. Therefore, observing the Angiosome Concept in decision-making seems to bring better wound healing and foot rescue rates, especially in PTA directly. It is possible, if the wound extends over more than one angiosome in the region of the heel or forefoot; all the angiosomes involved could be targeted for a better clinical outcome. Based on our study, the Angiosome Concept plays a significant role in endovascular treatment because indirect revascularization leads to the most unsatisfactory clinical results.

STUDY 2. Clinical implications of diabetic peripheral neuropathy in the approach of primary infrapopliteal angioplasty for neuro-ischemic foot wounds

Peripheral diabetic neuropathy (PDN), a current complication of diabetes frequently accompanied by chronic ischemia which affects at least 60% of diabetic patients, is associated with 80% of diabetic foot ulcers and approximately 37% have latent ischemic features. Fifteen percent of patients with diabetes will develop leg ulcers, and 14-43% will require amputation.

The medical records of 287 diabetic patients (304 ischemic limbs) from two medical centres in Belgium, treated endovascular at the infragenicular IC level, with or without obvious neuropathic symptoms, between January 2009 and March 2020, were selected and analysed retrospectively. Patient selection, a common intervention protocol and follow-up were carried out uniformly by a multidisciplinary team treating the diabetic foot, with the approval of the local ethics committee. All studied limbs showed ischemic symptoms that were clinically evaluated using Eco-Doppler and transcutaneous oximetry (TcPO₂). In all cases selected by CI, punctual revascularization was recommended for tissue recovery and limb rescue. In cases of unsuccessful attempts at endovascular techniques, second-line surgical options were implemented. These patients were considered technical failures and were excluded from the follow-up. There were 195 (68%) men, and the average age was 75.9 years (range 44–97 years). A total of 267 ischemic limbs (88%) were associated with type 2 diabetes and 37 (12%) with type 1 diabetes. A total of 228 patients (75%) were insulin-required at the time of revascularization, and 237 interventions (78%) were performed in patients diagnosed with diabetes for > 5 years. More than 2/3 of patients had been diagnosed with diabetes for more than 10 years and 246 (81%) members had different stages of peripheral neuropathy associated with critical ischemia.

Given the homogeneous distribution of clinical presentations and atherosclerotic risk factors in all three groups of patients, the study was designed to compare the results of post-angioplasty in terms of permeability, epithelialization (clinical success) and limb rescue rates (major objectives) at specific time intervals from the primary endovascular approach. Patient survival was also assessed as a secondary endpoint. The initial clinical evaluation of all neuro-ischemic limbs was performed in all cases (arteriopathy and neuropathy); associated diabetic characteristics (ankle-brachial index – ABI); measurements of AI (ankle index) or toes (index finger), vascular ultrasound and TcPO₂ foot coupled with computed tomography angiography or magnetic resonance imaging, with detailed visualization of pedal arch. In all cases, a consistent multidisciplinary approach by the “diabetic team” with similar pre- and post-operative drugs and wound care was implemented. Endovascular revascularization focused on opening a straight ilio-plantar arterial axis into the accessible arteries of the legs technically as the “target artery pathway”, with or without angiosomal orientation (optional targeted wound revascularization) following digital subtraction angiography. A plan with lower resistance was used in all cases of total chronic recanalization (RCT) due to endo- or extra-luminal passages. Multi-level arterial disease has been a common finding among this cohort of neuro-ischemic diabetic patients. The characteristics of the targeted infragenicular

atherosclerotic lesions and the extent of the associated calcifications (evaluated according to the GLASS classification) are summarized graphically.

Atherosclerotic lesions were scored using a semiquantitative assessment scale as follows: “mild” (category 1); “moderate” (category 2, <50% of the length of the lesion); and “severe” (category 3, > 50% of vessel length), including annular and continuous calcifications. When present (especially in diabetic patients in group 3), category 3 calcification was associated with CTO (complex total occlusion) of the tibial and plantar trunk.

A uniform protocol for treating the wound was applied to all three groups of patients, regardless of the period of inclusion. This multidisciplinary protocol included urgent debridement, local sepsis control, rapid revascularization, wound dressings, discharge devices, and possibly negative wound pressure therapy depending on each clinical presentation. All patients underwent surveillance by a multidisciplinary “diabetic foot team”, which included a regular clinical evaluation with vascular ultrasound, ABI, neuropathic evaluation of the Semmes-Weinstein monofilament, and periodic evaluation of TcPO₂. Post-operative follow-up was scheduled one month after discharge and every six months thereafter.

The severity of sensory neuropathy was assessed using the Semmes-Weinstein monofilament test. Technical success has been defined as direct topographic revascularization to allow direct arterial flow from the aortic to the plantar arches. A residual stenosis of up to 30% was allowed on control angiography. Permeability was checked using periodic ultrasound, ABI and TcPO₂. Clinical success was defined as a postoperative increase in ABI > 0.10 (if applicable), adding a substantial improvement in wound recovery (at least two Rutherford categories) with or without minor amputations of the forefoot or toes. Limb salvage did not involve any request for a major amputation of the limb and was revealed because the patient’s functional autonomy was restored.

All the data were subjected to a statistical analysis – “intention to treat”. Data are presented as mean +/- standard deviation. The Kaplan-Meier life table system was used to assess results on primary and secondary permeability, clinical success (wound healing), limb rescue, and survival rates.

These parameters were further compared between groups using the log-rank test (Mantel-Cox). Estimates are reported with 95% confidence intervals (CIs). Time to event data were studied between groups using Cox proportional hazard regression; the results were reported as hazard ratios (HR) and 95% CI.

A $p < 0.05$ was established as statistically significant. The main patient characteristics and individual risk factors were compared using the Chi-square test. All analyses were performed using the Prism statistical software package (GraphPad, La Jolla, CA, USA).

In the RESULTS section, primary infrapopliteal angioplasty was successful in 243 members (80%). For each group, technical success was confirmed in 52/58 (89%) cases in group 1, 137/167 (82%) in group 2, and 54/79 (68%) in group 3. After each designation, Direct topographic revascularization, iliac, femoral, and/or associated popliteal angioplasties were performed in 93 (38%) of all successful infrapopliteal PTAs. Two-thirds of these multilevel interventions were performed in step-by-step approaches to reduce the overall amount of contrast management per procedure (chronic renal failure was present in 45% of cases). Associated inframalleolar PTAs were performed in a total of 122 (51%) cases, concomitantly with the main tibial gestures. Of the 61 initial technical failures, 21 (35%) involved unsuccessful passage of the guidewire through highly calcified arterial lesions and 7 (11%) were related to impossible balloon access over the guide positioned in similarly densely calcified media.

Six suboptimal balloon swellings (10%), 19 (32%) inadequate residual stenoses (> 30%), four dissections limiting tibial artery flow and inability to follow interventions (6%) and

another four (6%) other “elastic recoils” with lumen collapse and TAP thrombosis have also been encountered.

For all initially failed angioplasty procedures, 19 alternative surgical revascularizations, 21 adjuvant endovascular interventions (endarterectomy or ultrasound recanalization), five venous arterializations of different levels, two medical and complementary wound approaches, and 14 inevitable major amputations were required.

An overall complication rate of 12% was observed. In 9 (3%) cases, major complications were observed: two limbs initially showed features of acute ischemia requiring rapid surgical revascularization; two patients developed myocardial infarction; three patients had transient renal failure with contrast agent with temporary dialysis; and two others developed inguinal hematomas that required prompt surgical haemostasis.

In the remaining 28 (9%) cases, minor complications with limited clinical repercussions were documented: three arterial perforations with flow restriction; nine transient arterial spasms; two distal emboli resolved by surgery and endoaspiration; six superficial inguinal haematomas with spontaneous local resolution; two uncomplicated anginas; and six with self-limiting renal dysfunction.

The 30-day survival rate was 99% (a case of myocardial infarction). The mean follow-up was 11.8 ± 0.6 months (range 3–26.5 months), during which time 27 patients died (gr. 1, $n = 5$; gr. 2, $n = 13$; and gr. 3, $n = 9$ members) and 12 members (gr. 1, $n = 2$; gr. 2, $n = 6$; and gr. 3, $n = 4$) were lost for follow-up before 12 months. Over a year, 168 (69%) of the 243 successfully treated members showed wound healing: 39/52 (76%) in grade 1, 97/137 (71%) in grade 2 and 32/54 (59%) in gr.3. In general, 31% (75/243) of wound recurrences were observed after the initial healing (gr. 1, $n = 10$; gr. 2, $n = 38$; and gr. 3, $n = 27$) in the first two years tracking. Interestingly, among the 27 gr. 3 of recurrent ulcers in 21 limbs, the initial arterial reconstruction was obvious and only the treatment of local ulcer complementary to severe neuropathy was administered.

At one year, the mean increases in TcPO₂ were 28.7 ± 2.6 mmHg (range 23–44 mmHg) in gr. 1, 23.1 ± 3.0 mmHg (range 21–39 mmHg) in gr. 2 and 17 ± 2.1 mmHg (range 15–30 mmHg) in gr. 3. Comparing these data as categorical variables, a significant difference was found between gr. 1 and gr. 2 ($p = 0.048$) and gr. 1 and gr. 3 ($p = 0.012$), although no clinically significant difference was found between gr. 1 and gr. 2. The ABI assessment was applicable in 220 (72%) of the studied members.

Comparisons of logarithmic rank (Mantel-Cox) tests of primary permeability values after 36 months between gr. 1 and gr. 3 and gr. 2 and gr. 3 were significant, but not between gr. 1 and gr. 2. Analysis of the correlation of secondary permeability rates after 36 months between gr. 1 and gr. 3, gr. 2 and gr. 3, and gr. 1 and gr. 2 showed insignificant differences. Freedom rates from amputation (i.e., limb rescue) were estimated at 87% after 12, 24 and 36 months for gr. 1, 65% for gr. 2 and 54% for gr. 3, over the same time periods. Comparison of limb rescue estimates between gr. 1 and gr. 3 after 36 months and gr. 2 and gr. 3 were found to be significant, but not between gr. 1 and gr. 2. Estimates of wound healing (i.e., clinical success) were 77 %, 65% and 61% after 12, 24 and 36 months for gr. 1, 72%, 55% and 51% for gr. 2 and 60% and 36% for gr. 3 ($p = 0.034$), at the same time intervals.

A similar comparison of wound healing between gr. 1 and gr. 3 after 36 months and gr. 2 and gr. 3 was found to be significant, but not between gr. 1 and gr. 2. Of all patients included in this study, there were 36 (12%) major amputations (14 early failures and another 22 for disappointing evolution during the follow-up period). Survival without amputation was not influenced after three years due to the presence and severity of peripheral limb neuropathy ($p = 0.344$), probably attributed to uniform supervision of the multidisciplinary team.

Survival estimates from this study were 91%, 77%, and 56% after 12, 24, and 36 months, respectively, for gr. 1, 88%, 73%, and 53% for gr. 2, and 79%, 63% and, respectively, 49% for gr. 3, at the same time intervals.

The present study showed a significant difference in the results of primary infragenicular angioplasty performed in diabetic patients with CLI without PDN or with early

to moderate peripheral neuropathic symptoms compared to those with severe NPD. Due to the uneven calcium score and occlusive disease of the vessel attached to the tibial and plantar trunks, primary infrapopliteal angioplasty was successful in 89% of cases in gr. 1, in 82% in gr. 2 and in only 68% of the members in gr. 3. The highest neuropathic damage and arterial calcifications and proved to be the lowest clinical benefit at 36 months: 35% of primary permeability, 36% wound healing and 54% limb preservation rates.

As one of the original findings of this study, the primary comparison of permeability between gr. 1 vs gr. 3 and gr. 2 vs gr. 3, was added to tissue healing and limb rescue the differentiation proved to be significant, but without statistical weight for gr. 1 vs gr. 2.

These findings seem to support the hypothesis that severe PDN (equivalent to gr. 3 of diabetic patients) may be an independent risk factor for worsening haemodynamic and clinical outcomes of infrapopliteal angioplasty in diabetic patients with CLI.

The primary PTA approach has been increasingly suggested to be beneficial in BTK and revascularization under the ankle, providing low aggressiveness, limb rescue rates comparable to surgery, and increasing applicability, even in tibial atherosclerotic lesions of the foot and difficult to cross.

Relevant literature reports that, although most diabetic foot ulcers appear to be neuropathic, ischemic involvement may be suspected in > 60% of these presentations. Although considered distinct pathologies, diabetic neuropathy and angiopathy have a common origin triggered by arteriolar thickening “vasa-vasorum” and “vasa-nervorum” accelerated by hyperglycaemia.

The present study was limited by the small number of cases registered and its retrospective design. It should be noted that the technical skills and characteristics of PTA technology could have been unquestionably improved during the 10-year observation period of this research, with a plausible influence on overall technical success rates and other statistical data.

Due to the solid standardization of the measurements, the degree of neuropathic involvement (explored only using the Semmes-Weinstein monofilament test) and the accuracy of the TcPO₂ assessment were, however, exposed to foreseeable technical difficulties. I also recognize that specific strategies for BTK angioplasty, detailed anatomical features of neuroischemic wounds, and other individual local and systemic risk factors may have further influenced tissue recovery and limb preservation rates and may not be further detailed in this DPN observational study.

In diabetic neuro-ischemic limbs, the evaluation of peripheral neuropathy appeared to be useful and required independent screening and stratification, in parallel with that of CLI. The presence of severe neuropathic involvement may jeopardize the clinical benefit of successful infrapopliteal angioplasty in terms of permeability, tissue healing, and limb preservation, but without a significant survival benefit in these patients.

NDP requires adequate multidisciplinary control and distinct assessment as an additional risk factor for tissue damage and limb loss, in parallel with the current revascularization of CLI.

STUDY 3. Comparative investigation of polymers of pharmacologically active balloons used in infrapopliteal angioplasty based on the concept of angiosomes

Endovascular surgery involves simple balloon angioplasty or stent angioplasty. The medium- and long-term results vary depending on the location of the lesions, but the trauma to which the patient is subjected is much lower. The materials used in the manufacture of angioplasty balloons have a great impact on the final properties of the balloon. The first angioplasty balloons were made of polyvinyl chloride (PVC); they were thick-walled and designed for low pressure. In the mid-1980s, PVC was replaced by cross-linked polyethylene (PE) and polyethylene terephthalate (PET), both of which were able to withstand higher pressures. The most recent materials used for angioplasty balloons were polyurethane (PU) and nylon.

Simple balloon angioplasty (POBA) is used for percutaneous transluminal angioplasty (PTA). The strength of the material is between 1.9 atm for a diameter equal to 2

mm and 6.3 atm for a diameter equal to 6 mm. It is often used in various operations above and below the knee. It is a single-use device that cannot be resterilised.

On the other hand, a drug-coated balloon (DCB), indicated in the PTA, has provided lasting and safe results in many clinical trials, as well as for complex types of patients and lesions. The manufacturer states that over 3,500 patients were enrolled in 21 clinical trials and more than 200,000 patients were treated using this DCB for the treatment of femur-popliteal disease, 75% of patients did not require reoperation at 5 years.

The simple balloon used for POBA contains an inner layer based on high density polyethylene, while the outer material is based on low density polyethylene. The second flask to be investigated is a DCB based on a multiblock copolymer in the form of a mixture of polyethylene, poly (ciloheylethylene), polyisoprene and poly (1,3-butadiene) coated with Paclitaxel.

The thermal behaviour of two flasks was evaluated compared to a Mettler-Toledo DSC1 instrument (Nanikon, Switzerland) with an inert gas purge (nitrogen) flow rate of 50 ml/min between 30-200°C with a heating rate of 10°C/min using 40 µL aluminium crucibles with perforated lids.

Comparative stress-strain curves were obtained by gradually applying the load of the samples and measuring the strain, from which the stress and strain can be determined. A Netzsch dynamic-mechanical analyser, type DMA 242 C (Selb, Germany), was used in voltage mode, under air atmosphere, at 25°C to obtain the curves.

The perforation test is used to determine the penetration or puncture resistance of the materials. ASTM F1306 describes the procedure; an Instron 1011 universal testing machine (Canton, MA, USA) with the following parameters was used: crosshead speed (2 mm/sec) and probe size (1.6 mm diameter).

Ankle-arm index measurements were made pre- and postoperatively. Patients with elevated medial sclerosis and calcifications (ABI above 1,3) were excluded from the sample. A dorsal and a plantar measurement were performed on the foot pre- and post-operatively to evaluate the angiosomes of the dorsal and plantar foot. Each infusion measurement sequence lasted 272 s; sequential dorsal and plantar measurements were performed after an interval of 5 min. In the follow-up clinical investigation, the characteristics of the wound were recorded, as well as the clinical symptoms, ABI was performed, as well as vascular ultrasound as an assessment of the permeability of revascularization; the size of the wound was assessed and documented by photography, the wounds were classified at the time of the first investigation using the WIFI Score from the Society of Vascular Surgery.

Following this score, the risk of amputation after one year was determined. In addition, the healing time of the wound was assessed for all patients followed. At this time, wound healing had been defined as complete epithelialization. Patients who presented with follow-up lesions were defined as unhealed.

A comparison of the time to wound healing and the wound healing rate was performed according to the revascularization method (direct and indirect).

To address the type of endovascular angioplasty and plantar arch permeability, as well as individual collateralization, intraoperative digital subtraction angiographies were noted according to the classification of the plantar arch, as suggested by Kawarada.

Statistical analysis was performed using statistical software SPSS v. 27.0.0.0 64-bit edition (IBM SPSS Inc., Chicago, IL, USA) and Excel v. 1808 from Microsoft Office Professional Plus 2019 (Microsoft, WA, US). Data are presented as categorical variables and frequency distributions. The Kolmogorov-Smirnov test was used to test the distribution of variables. Variables with normal distribution were presented as mean and standard deviation. Statistical differences were determined using bidirectional ANOVA analysis followed by Bonferroni post-test. Sixty-seven variables were included; only those with a p value < 0.5 were included in the multivariate analysis. No attempt was made to replace the missing values. The impact of the reference variables on the late outcome was based on the Cox proportional hazards method.

The physical and mechanical properties of materials depend on their chemical structure and are very important for the application of products. The most important

properties of polymers that are used for different balloons are surface smoothness, puncture resistance and tear strength, but just as important is ensuring a certain length and diameter or an exact size of the wall thickness.

The DSC thermograms reveal a very good stability of the polymeric materials in the studied temperature range. The glass transition temperature of these polymers is well below 20°C, as in the case of low- or high-density polyethylene, polycaprolactone, polypropylene and glass fibres. The high endothermic peak in the DCB curve around 105°C can be attributed to the loss of H₂O and other volatile compounds, such as aldehyde, ketone and ether residues.

The stress-strain curves contain different parts: the first contains the growth-flow ratio (the slope that gives the Young's modulus), the second is hardening to deformation, the third is strangulation and the last is fracturing. Based on these curves, the Young's modulus, i.e., a measurement of the rigidity of elastic materials, can be seen to have obtained higher values in the radial direction than in the axial direction and in the DCB material than in the simple balloon.

The results of the puncture resistance for the two materials are presented graphically. It can be seen that the perforation load is higher for the simple balloon, while DCB showed a good performance against perforation, but below the level of the simple balloon, both in the deflection distance and in the penetration force.

Pre- and post-operative patient data were retrieved from a database. The comparison between the two types of balloons angioplasty were evaluated for 12 months after the clinical intervention in terms of wound healing, leg rescue and survival without amputation.

Cox proportional risk analysis showed that PTA ATA may be associated with hypertension and renal impairment, while PTA ATP with hypertension and renal impairment. Also, the number of major amputations may be associated with age, while the number of minor amputations may be associated with age and hypertension.

The safety and efficacy of PTA directly versus indirectly have been investigated comparatively by many other research groups. In our study, PTA directly was associated with wound healing and limb rescue; no significant association was found between indirect PTA and medical goals.

Our treatment focuses on the identification and specific treatment of ischemic ulceration of the foot, aims to support the body's own healing processes, prevents wound infection, reduces microbial load and eliminates areas of infection and tissue necrosis.

The overall one-year wound healing rate was 90.2%; it was found that the highest wound healing rate was achieved in the case of DCB (100%) and the lowest in the case of simple balloons (88.4%). It seems that pharmacologically active balloons have a more effective influence in wound healing. Ang et al. described the progress and development of drug-impregnated balloons as an emerging alternative treatment in the peripheral and coronary arteries; the authors highlighted four key elements of these balloons: the drug, the excipients, the platform, and the coating process. Acute drug transfer occurs almost immediately after positioning and swelling of the balloon and releases the antiproliferative drug from its surface on the vessel walls, most being bound to the hydrophobic binding sites of the wall and a small portion being transported by diffusion.

Two main possibilities for restoring leg arterial flow are well known in literature: endovascular procedures (arterial flow is restored using balloons or stents that enlarge and re-permeabilise blocked or narrowed arteries – minimally invasive) and bypass, revascularization, which involves a bypass surgery to nourish ischemic muscles and tissues. Our study observed a leg-saving procedure, and the comparative results are plotted.

The overall one-year foot rescue rate was 86.3%; the highest wound healing rate was achieved for DCB (100%) and the lowest for simple balloons (83.7%). Wound healing, lower limb rescue and preservation are different therapeutic goals from improving gait, and there are often time constraints. Tukiainen et al. determined a persistent outcome and anticipatory factors for extreme surgery through teamwork of vascular and plastic surgery to save the legs in patients with extensive tissue defects of CLI in 2157 cases of vascular or

endovascular revascularization; found good and very good rates in saving legs at 1 and 5 years (73 and 66%), survival (91 and 63%) and survival without amputations (70 and 41%); and concluded that endovascular interventions are a solution for advanced limb rescue in patients with HF and/or a major tissue defect.

The overall one-year survival rate without amputation was 92.2%; it was found that the highest wound healing rate was achieved for DCB (100%) and worse for single balloon PTA (90.7%). Based on the definition of Benoit et al., survival without amputation is a composite goal of mortality and amputation and is the preferred outcome measure in HF. On the other hand, Lin et al. considered the endovascular approach to be the best therapy in HF.

The DCB used by our team has already been described as a device with a significant advantage in surviving without amputation after treatment of the femoropopliteal artery in a 4-year follow-up, compared to simple balloons.

Based on the theory of Beropoulos et al., Wound-Ischemia-foot Infection (WIFI) is a possibility to classify the prediction of amputation risk in patients with CLI. The Society of Vascular Surgery often uses this parameter to have a predictive ability after lower extremity revascularizations and after intrapopliteal endovascular interventions in CLI.

Our results showed a strong correlation between patients treated with DCB and WIFI degrees. The WIFI classification system of the Society for Vascular Surgery appeared useful to predict one-year amputation, reintervention, restenosis, and wound healing in patients with HF undergoing various procedures for endovascular infrapoplite revascularization. A number of correlations were calculated to observe any association between the evaluated parameters and an inverse proportionality was found between diabetic patients treated with oral antidiabetics and the presence of wounds in both limbs.

The present research compared two materials in terms of material structure (physical and mechanical tests) and in terms of their application as balloons used in angioplasty.

In the CONCLUSIONS section, I point to the fact that endovascular revascularization using POBA vs DCB highlights significant differences by using multivariate analysis. Overall, one-year wound healing, limb rescue, and survival rate without amputation were higher for DCB compared to POBA in this study. According to our feasibility rates, the results do not indicate significant differences between direct and indirect angioplasty. The attachment of Paclitaxel to polymeric surfaces can be altered by several parameters, such as substrate topography, roughness, and macromolecular chain structure. Various investigations on its binding affinity to different surfaces have led to the development of drug-containing balloons with increased efficacy in angioplasty.

In the FUTURE PERSPECTIVES section, it is justified to ask questions about the future of this research. Scientific progress in the field of vascular surgery and in the new DCB modelling technique will lead to superior polymeric materials capable of providing better values for medical purposes. It is very important to study the type of balloon and the drug used and to compare the results from the point of view of the vascular surgeon. Finally, a collaboration between surgeons and chemists would lead to the creation of virtual models based on silico studies to imagine new polymeric materials capable of being used in the manufacture of tomorrow's DCB.

In the last section, CASE PRESENTATIONS, I try to exemplify the evolution of direct/indirect endovascular revascularization according to the Angiosome Concept of 5 clinical cases.

THE GENERAL CONCLUSIONS of my thesis are:

- The theory of targeted revascularization according to the Concept of Angiosomes, launched in numerous studies and supported by our analyses, reveals benefits in wound healing, especially in patients with critical ischemia and diabetes.
- In cases of critical limb ischemia, direct endovascular revascularization leads to significantly better wound healing and higher leg rescue rates compared to indirect revascularization. It must be recognized that the treatment of critical ischemia cannot be achieved by surgery alone, even if it is successful, and postoperative care, including wound management and medication, is of the utmost importance. These are invaluable because the healing power of wounds is diminished and the immune system is compromised, especially in patients with diabetes or end-stage kidney disease.
- The AC is feasible for most patients treated with endovascular approach, although only 27.45% of tissue lesions are located in only one angiosome.
- The factor that is associated with a worse wound healing time is the number of angiosomes > 3 and indirect PTA.
- Therefore, observing the concept of angiosome in decision-making seems to bring better rates of wound healing and foot rescue, especially in direct endovascular revascularization.
- It is possible if the wound extends over more than one angiosome in the region of the heel or forefoot; all the angiosomes involved could be targeted for a better clinical outcome. Based on our study, the Concept of Angiosomes plays a significant role in endovascular treatment because indirect revascularization leads to the most unsatisfactory clinical results.
- In diabetic neuro-ischemic limbs, the evaluation of peripheral neuropathy appeared to be useful and required independent screening and stratification, in parallel with that of critical ischemia. The presence of severe neuropathic involvement may jeopardize the clinical benefit of successful infrapopliteal angioplasty in terms of permeability, tissue healing, and limb preservation, but without a significant survival benefit in these patients.
- When present, PDN requires adequate multidisciplinary control and distinct assessment as an additional risk factor for tissue damage and limb loss, in parallel with the current revascularization of CLTI.
- Critical ischemia is a complex pathology, and in each patient many variables are interconnected in a complex scenario (wound, vascular anatomy, collateral network), in which the choice of the best revascularization strategy is often challenging.
- Endovascular revascularization using POBA vs DCB highlighted significant differences by using multivariate analysis. Overall, one-year wound healing, limb rescue, and survival rate without amputation were higher for DCB compared to POBA in this study.
- Our analysis demonstrates and encourages the multidisciplinary management of patients with critical ischemia and diabetes.