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Evaluating The Utility Of Spect/ct Imaging Of Angiosome Perfusion In Diabetic Patients With Critical Limb Ischemia

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Evaluating the Utility of SPECT/CT Imaging of Angiosome Perfusion in Diabetic

Patients with Critical Limb Ischemia

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EVALUATING THE UTILITY OF SPECT/CT IMAGING OF ANGIOSOME PERFUSION IN DIABETIC PATIENTS WITH CRITICAL LIMB ISCHEMIA.

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Impaired lower extremity perfusion is a hallmark of peripheral arterial disease (PAD) and is particularly problematic in diabetic patients, who suffer from high rates of PAD, ulceration, and lower extremity amputation. The ability to non-invasively detect deficits in microvascular perfusion within vascular territories, or angiosomes, of the feet may provide information related to tissue viability and guide therapeutic interventions. In this study, we sought to apply single photon emission computed tomography (SPECT)/CT imaging to quantify volumetric microvascular perfusion within specific angiosomes containing non-healing foot ulcers in diabetic patients with critical limb ischemia (CLI). Additionally, we sought to assess the value of SPECT/CT perfusion imaging for predicting limb salvage in CLI patients undergoing lower extremity endovascular revascularization.

Forty-one diabetic patients (mean age, 66±12 yrs) with non-healing ulcers and nine healthy control subjects (mean age, 50±10 yrs) underwent SPECT/CT imaging of the feet following a resting injection of technetium-99m (^{99m}Tc)-tetrofosmin (dose, 550.6 ± 37 Mbq). CT images of diabetic feet were segmented into five angiosomes and used for quantifying relative radiotracer uptake, expressed as standardized uptake values (SUVs). SUVs were assessed for each CLI patient in the angiosome containing the non-healing ulcers, while average whole foot perfusion was assessed for healthy control subjects. Percent change in SPECT SUVs of ulcerated angiosomes was quantified following

endovascular revascularization in patients, and 3-, 6-, and 12-month limb salvage outcomes were assessed.

SPECT/CT imaging allowed for visualization of perfusion deficits under resting conditions. ^{99m}Tc-tetrofosmin SPECT/CT imaging of angiosome foot perfusion demonstrated a significant difference in baseline perfusion values (SUVs) between diabetic patients with CLI and healthy control subjects (p = 0.02). Analysis of baseline SPECT/CT imaging and ankle-brachial index (ABI) measurements in CLI patients and healthy control subjects demonstrated a significant and positive relationship between SPECT/CT angiosome perfusion and ABI (p = 0.01; r = 0.41). Serial evaluation of relative changes in SPECT angiosome foot perfusion following revascularization revealed significant quantitative changes in perfusion after treatment, whereas ABI measurements did not demonstrate significant changes after revascularization. Changes in SPECT/CT-derived angiosome perfusion significantly differed between patients with and patients without amputation in the 3 (p = 0.01), 6 (p = 0.03), and 12 (p = 0.03) months following revascularization.

SPECT/CT imaging provides a useful non-invasive tool for evaluating microvascular perfusion within specific angiosomes of the foot under resting conditions. SPECT/CT imaging also allows for serial assessment of sensitive changes in angiosome microvascular perfusion following revascularization that are undetected by ABI. Perfusion imaging with SPECT/CT offers a novel quantitative imaging approach for assessing the efficacy of revascularization strategies targeted at restoring perfusion to non-healing wounds of the foot and may assist with predicting limb salvage outcomes in CLI patients undergoing revascularization. Future application of SPECT/CT perfusion

imaging may provide additional value for detection and targeting of ischemic tissue for

therapeutic interventions in the PAD patient population.

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I would like to thank my family: my fiancé, my grandmother, my brothers, and my cousin for supporting me throughout the writing this thesis and my life in general. Thank you for always being there for me, encouraging me, and believing in me.

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INTRODUCTION

Peripheral Arterial Disease

Peripheral arterial disease (PAD) is a manifestation of atherosclerosis and is commonly defined as a partial or complete obstruction of at least one noncoronary blood vessel (3, 4). PAD affects approximately 8 million Americans (5) and it is estimated that the worldwide prevalence of PAD is 10 percent (6). It is estimated that PAD affects greater than 200 million people worldwide (3); however, it is likely that many more may have PAD given that individuals may be asymptomatic. PAD has an increased prevalence among males and the elderly. Furthermore, the prevalence of PAD increases above 10 percent in individuals over the age of 60 (3). A study approximated the annual cost of PAD related treatment at 4.37 billion dollars (7). PAD patients are at a high risk for having a cardiovascular event and the majority die of cardiac or cerebrovascular-related events (8).

PAD manifests in a wide spectrum of symptoms. Individuals with PAD can be asymptomatic or have clinical manifestations ranging from intermittent claudication to limb-threatening ischemia, rest pain, non-healing ulcers, and gangrene. The most common presentation of PAD is a patient suffering from intermittent pain or cramping in the legs and/or feet during exercise or exertion that is relieved with rest, which is referred to as intermittent claudication. The more severe end of the PAD spectrum, rest pain and non-healing ulcers, and/or gangrene is called critical limb ischemia (CLI). Outcomes for patients with CLI are alarming, at 1 year, 10 percent will experience a fatal cardiovascular event and 25 percent will have had an amputation (9). Aggressive treatment is needed for these patients since progression to amputation is common. CLI

results in a mortality rate of 46 percent at 5 years and amputations occur in 27 percent of patients within the first year (10).

Asymptomatic patients are typically identified after a suspicious physical exam that identifies weak or absent pedal pulses. Suspected PAD can be confirmed with a commonly used clinical tool called an Ankle-Brachial Index (ABI). The resting ABI is obtained by measuring systolic blood pressures at the arms (brachial arteries) and ankles (dorsalis pedis and posterior tibial arteries) in the supine position by using a Doppler device. The ABI of each leg is calculated by dividing the higher of the dorsalis pedis or posterior tibial pressure by the higher of the right or left arm blood pressure. The normal range for an ABI is 0.9-1.3. Patients with lower extremity claudication will commonly score below 0.9, however, patients with calcified vessels, especially diabetic patients will often have falsely elevated ABIs because of the inability to properly compress their vessels during this exam.

Apart from physical exam there are several scoring systems that exist to classify PAD patients according to their symptoms, with the most commonly used system in the United States being the Rutherford system, outlined in Figure 1 (1).

Fontaine		Rutherford		
Stage	Clinical	Grade	Category	Clinical
I	Asymptomatic	0	0	Asymptomatic
IIa	Mild claudication	I	1	Mild claudication
IIb	Moderate to severe claudication	I	2	Moderate claudication
		I	3	Severe claudication
III	Ischemic rest pain	II	4	Ischemic rest pain
IV	Ulceration or gangrene	III	5	Minor tissue loss
		III	6	Major tissue loss

Figure 1. Classification systems for PAD patients (1).

Risk factors for PAD echo those of coronary artery disease, namely, family history, diabetes mellitus (DM), smoking, hypertension, hyperlipidemia (9). However,

DM and smoking are the most strongly associated with worse PAD outcomes outside of other risk factors. DM, in particular, is associated with more clinical complications, such as lower extremity amputations and higher mortality (8).

Role of Diabetes Mellitus in PAD

In 2010, there were 25.8 million people in the United States with DM (11). In 2011, it was estimated that 350 million people worldwide were affected by DM (12). The prevalence of DM in the United States has increased by an alarming 382 percent from 1988 to 2014 (13) and is strongly related with the rise in obesity, where 85.2 percent of individuals with type 2 DM are overweight or obese (14). Diabetic foot complications are the leading cause of hospitalization and major or minor amputation, and these complications represents up to 40 percent of health expenditures in DM patients (12). DM is a risk factor for PAD, with PAD prevalence rates of 10-40 percent in the DM patient population (15). DM doubles the chance of developing PAD and it has been shown that a 1 percent increase in glycated hemoglobin A1c (HA1c), an indicator of long-term glycemic exposure, is associated with a 26 percent increase in developing PAD (6, 16). In comparison with patients without DM, PAD is more likely to progress in patients with DM.

In addition to an increased prevalence of PAD in the setting of DM, DM patients also have a 12-15 percent risk of developing foot lesions, making DM an important risk factor for limb amputation (17). Alarmingly, patients with concomitant PAD and DM are 5 to 10 times more likely to have disease progression requiring an amputation (6). DM foot lesions may start as an uncomplicated area, but risk for amputation increases with the development of infection in soft tissue (cellulitis) and bones (osteomyelitis). Eight

five percent of amputations are preceded by foot ulcers that subsequently evolve to severe infection and gangrene, ultimately resulting in 5-8 percent of patients with DM foot complications undergoing major amputation within 1 year of presentation (12). Additionally, patients with concurrent PAD and DM foot ulcers experience high mortality rates, where 50 percent of patients will be dead within 5 years of clinical presentation (15).

In several large observational studies, PAD was present in up to 50 percent of the patients with diabetic foot ulcers and was an independent risk factor for amputation (15). The poor outcomes of ischemic foot ulcers in those with DM is thought to be due to a combination of factors including, but not limited to, the anatomic distribution of vascular lesions rendering them more difficult to treat, the association with other abnormalities such as infection, neuropathy, renal failure, and abnormalities in other vascular territories such as coronary and cerebral arteries (15). Wound healing is further disturbed by the complex interaction of several factors, such as poor glycemic control, microvascular dysfunction, impaired collateral vessel formation, abnormal mechanical loading of the ulcer, and co-morbidities (15). It is estimated that early detection and appropriate treatments may prevent up to 85 percent of these amputations (11). A non-invasive imaging approach able to assess microvascular function in PAD patients is needed but more so in those with concomitant diabetes for whom the combination of pathology results in severe and harsh outcomes in a short period of time.

Overview of Diagnostic Tools

Several non-invasive tools have been used to detect and evaluate PAD, including the ankle-brachial index (ABI), toe-brachial index (TBI), Transcutaneous oxygen

pressure (TcPO$_2$), duplex ultrasound, magnetic resonance (MR) imaging, computed tomography (CT) angiography, single-photon emission computed tomography (SPECT), and positron emission tomography (PET).   Currently, the ABI is the most recognized and most widely applied functional measurement used for diagnosis and therapy monitoring (18). In patients with a history or physical examination suggestive of PAD, the ABI has good validity as a first-line test in the diagnosis of PAD, as shown by vascular imaging, with sensitivities ranging from 68 to 84 percent and specificities from 84 to 99 percent (18). However, ABI measurements have disadvantages. Specifically, ABI is only truly effective at evaluating large vessel obstructions and therefore has decreased sensitivity in the setting of microvascular disease, can be falsely elevated in patients with calcified arteries, and has poor reproducibility due to inter-user variability (7, 19). Of note, approximately 30 percent of CLI patients have normal or near-normal ABI measurements (19). Additionally, the accuracy of using the ABI for predicting healed foot ulcers has been shown to be low, with a sensitivity of 0.48 (95% CI, 0.36-0.61) and specificity of 0.52 (95% CI, 0.42-0.63) (11).

Where ABI fails to obtain an accurate measurement in patients with calcified arteries, toe-brachial index (TBI) may prove to be a better choice. TBI is obtained in a similar manner to ABI but uses great toe systolic pressures in the place of ankle and foot pressures. TBI is useful when ABI measure are thought to be inaccurate or cannot be obtained because digital arteries are not compressible (19). However, similarly to ABI, TBI do not localize disease and is not a viable tool in many patients with progressed disease who have already had their great toes amputated.

TcPO$_2$ has been used to assess microcirculation and assist in PAD diagnosis (19). TcPO2 uses a non-invasive diagnostic tool applied to the epidermal surface of the skin to determine the pO2 level in tissues (20). However, TcPO$_2$ has limited accuracy in the setting of edema, thick skin, infection, and is time consuming (19). The greatest weakness of TcPO$_2$ as an assessment tool is its ability to only measure superficial tissue viability (7). For ulcer healing, the combined sensitivity and specificity of TcPO$_2$ have been shown to be 0.72 (95% CI, 0.61-0.81) and 0.86 (95% CI, 0.68-0.95), respectively (11).

For lower extremity anatomical assessment, duplex ultrasound, CT angiography and MR angiography are routinely used in clinical practice to detect PAD and identify the location and extent of arterial obstruction. Due to the non-invasive nature, lower risks, and lower costs, duplex ultrasound is frequently the primary imaging modality used in patients with PAD; however, ultrasound is not without limitations. Specifically, ultrasound only permits evaluation of blood flow in major vessels and is not useful for the assessment of collateral vessel flow (7). Additionally, ultrasound is particularly challenging in the obese patient with arterial calcifications due to image shadowing (19). Clinical studies have shown that the quality of ultrasound depends on the user's level of experience and that some arterial segments are more easily visible than others. (21). For example, in some cases (i.e. obesity, gas interpositions), the iliac arteries are more difficult to visualize and alternative methods, such as CT angiography, MR angiography, and digital subtraction angiography should be considered (22). CT and MR angiography can both offer valuable anatomical information. The great advantage of CT angiography remains the visualization of calcifications, clips, stents, and bypasses (22). CT

angiography requires exposure to radiation and iodinated contrast material and its utility is reduced when it comes to calcified vessels, particularly in the smaller vessels of the calf and foot (19). In comparison to CT angiography, MR angiography does not require radiation or iodinated contrast (19). However, MR angiography does use gadolinium contrast agents, which cannot be used in cases of severe renal insufficiency (GFR, 30 mL/min per 1.73 m2) (22), and MR is hindered in the presence of non-compatible pacemakers or metal implants (including stents).

Digital subtraction angiography (DSA) remains the gold standard for imaging tools in assessing PAD since it allows for high spatial resolution imaging of vascular occlusions (6). DSA is an invasive procedure and carries the risk of complications, including hematoma, pseudoaneurysm, dissection and arteriovenous fistula (6). Additionally, DSA requires intra-arterial delivery of iodinated contrast, and is limited in its inability to characterize vessel walls and thrombus (6). Replacing DSA with a non-invasive alternative may reduce contrast-induced nephrotoxicity, patient discomfort, and reduce patient costs. Considered as the gold standard for decades, DSA is now reserved for patients undergoing peripheral interventions, especially concomitant to endovascular procedures (22). CT and MR angiography are now replacing DSA as routine secondary imaging methods since they permit a non-invasive assessment of the localization and extension of vascular lesions and facilitate accurate planning of endovascular and/or surgical treatment (10).

Assessment of Lower Extremity Perfusion

Since impaired lower extremity perfusion is the main pathophysiological mechanism of PAD, imaging tools capable of non-invasively evaluating tissue perfusion

can be invaluable for ongoing assessment of disease progression and treatment success. The ability to detect microvascular foot abnormalities may be particularly valuable in the setting of diabetes, where patients commonly present with concomitant macro- and microvascular disease. Although studies have attempted to assess perfusion, imaging modalities such as ultrasound and $TcPO_2$ only look at 2D slices, superficial perfusion, or have not been readily adopted owing to lengthy acquisition times and reproducibility concerns (20, 23). Additionally, these tools do not permit quantitative volumetric assessment of tissue oxygenation or perfusion within different vascular territories of the foot. Therefore, a clinical need exists for a non-invasive imaging tool capable of assessing global changes in foot tissue perfusion.

SPECT/CT perfusion imaging of the lower extremities can be performed under resting conditions and provides quantitative assessment of microvascular perfusion within three-dimensional (3D) vascular territories, or angiosomes, of the foot. The ability to identify volumetric abnormalities in microvascular perfusion under resting conditions can be of significant value in the CLI patient population since many of these patients are incapable of ambulating for exercise stress testing. SPECT/CT perfusion imaging can be performed without the need of an intravenous contrast agent, thus offering a useful non-invasive approach for evaluating perfusion in diabetic patients who also commonly present with impaired renal function.

Nuclear imaging modalities provide high sensitivity and offer potentially novel methods for the investigation of PAD. SPECT and PET are the primary nuclear imaging modalities. SPECT imaging also allows for the simultaneous evaluation of multiple radiotracers targeted at various physiological processes. PET imaging provides increased

sensitivity and resolution and generally involves perfusion radiotracers with shorter half-lives, resulting in lower levels of ionizing radiation exposure to patients; however, SPECT is more established, less expensive, and more widely available (5). Both SPECT and PET not only allow for the physiologic assessment of PAD but may also permit evaluation of molecular events associated with disease progression or treatment response. Although SPECT and PET provide higher sensitivity, they both also have lower spatial resolution when compared with CT and MR (submillimeter to ~1 mm resolution) (5). However, the recent emergence of hybrid SPECT/CT and PET/CT systems has allowed for the combination of high sensitivity physiologic SPECT and PET imaging with high resolution anatomic imaging of CT to optimally localize and quantify radiotracer uptake. Additionally, these hybrid imaging systems now permit correction of attenuation and partial-volume effects, allowing for more precise radiotracer quantification within anatomically defined regions of interest (24).

The first nuclear medicine studies examining lower extremity skeletal muscle blood flow in PAD patients evaluated the clearance rates of ^{24}Na-Chloride, ^{133}Xe, and ^{99m}Tc-pertechnetate after an intramuscular injection of these radiotracers (25-27). The development of nuclear medicine later led to two-dimensional imaging of microspheres and albumin that were radiolabeled with ^{99m}Tc, ^{131}I-sodium, and ^{111}In to measure lower extremity perfusion (28-30). These techniques were not ideal as they required intra-arterial injections in order to evaluate blood flow during first pass circulation. The need for techniques that did not require intra-arterial injections eventually led to the use of thallium-201 (^{201}Tl) as a perfusion agent for SPECT imaging. ^{201}Tl is a diffusible tracer with properties similar to potassium, thereby allowing for transport into viable cells via

the sodium–potassium pump (31). This makes the tracer uptake a reliable measure of both myocardial and skeletal muscle perfusion (32-36). ^{201}Tl perfusion imaging can provide an estimate of flow over a wide physiologic range since it can be performed after an intravenous injection at rest or during exercise and has a high first-pass extraction (~85%) (33). Whole-body ^{201}Tl scintigraphy has been shown to be useful for evaluating perfusion abnormalities in the lower extremities of PAD patients at rest and during exercise, and for the detection of perfusion abnormalities in asymptomatic patients presenting with normal ankle brachial indices (35-37). PAD severity has traditionally been assessed by determining ratios of activity between non-stenotic and stenotic legs or by normalizing activity in lower extremity regions of interest to whole-body activity in the presence of bilateral disease (32, 37, 38). With the emergence of 3D SPECT imaging systems, it was possible to more accurately detect and localize regions of lower extremity ischemia under both rest and stress conditions with ^{201}Tl (34). One of the first lower-extremity imaging studies to use ^{201}Tl SPECT for the investigation of PAD evaluated stress profile curves from multiple transverse images in the leg during reactive hyperemia with normalization of regional activity to whole-body activity (34). Although ^{201}Tl was shown to be effective in many studies, its long half-life and imaging characteristics were less favorable when compared with technetium-99m (^{99m}Tc)-labeled radiotracers (5). This has resulted in the implementation of newer ^{99m}Tc-labeled perfusion tracers that reduce radiation exposure and provide better image quality (7). ^{99m}Tc-labeled compounds demonstrate little redistribution, which allows for injections during exercise and measures of peak exercise perfusion at a delayed imaging time (39). Additionally, the biodistrubtion and kinetics of these compounds make it possible to perform lower

extremity perfusion measurements in combination with myocardial perfusion assessment (38). One ^{99m}Tc-labeled tracer in particular, ^{99m}Tc-sestamibi, has been incorporated in several studies examining lower extremity perfusion in PAD. It has revealed improved sensitivity for detecting differences in resting perfusion between the lower extremities of patients with unilateral disease and greater sensitivity when compared with Doppler ultrasound for detection of PAD (5). Preliminary data from our lab have shown that SPECT/CT imaging with ^{99m}Tc-tetrofosmin also has potential for assessing regional differences in lower extremity perfusion in PAD patients with abnormal CT angiography and ABI findings (5). SPECT/CT has detected perfusion abnormalities in the calves of diagnosed PAD and asymptomatic patients, with a sensitivity (91%) and specificity (94%) for the diagnosis of PAD while correlating well with angiographic and Doppler findings (34, 37, 40).

To date, non-invasive imaging approaches for evaluating foot perfusion in PAD patients have been primarily limited to assessment of superficial tissue or require physiologic stressors. PET/CT imaging may be an option to assess lower extremity perfusion but thus far has not been applied for evaluating foot perfusion and remains costly due to the need for more expensive instrumentation, including an onsite cyclotron or portable generator for isotope production (41-43). SPECT/CT perfusion imaging which could be easily combined with clinically indicated myocardial perfusion imaging can be performed under resting conditions and provides quantitative assessment of microvascular perfusion within 3D angiosomes of the foot containing non-healing ulcers that are targeted for revascularization. The ability to identify volumetric abnormalities in microvascular perfusion under resting conditions can be of significant value in the CLI

patient population since many of these patients have poor tolerance for and are unable to ambulate for treadmill exercise stress testing. SPECT/CT perfusion imaging can be performed without the need of an intravenous contrast agent, thus offering a useful non-invasive approach for evaluating lower extremity perfusion in high risk diabetic patient who also commonly present with coexisting impaired renal function. SPECT/CT imaging may allow for improved assessment of diabetic patients at baseline and following treatment, such as lower extremity revascularization. Our research team at Yale University was the first to demonstrate the feasibility of using SPECT/CT imaging to track serial changes in resting microvascular perfusion within 3D muscle groups of the lower extremities (44). Additionally, we have developed and clinically translated SPECT/CT image analysis tools for quantifying regional differences in skeletal muscle perfusion into the calf muscles of patients with unilateral PAD (5).

PET imaging can also assess lower extremity blood flow in PAD patients (41, 42, 45-47). The primary PET radiotracer implemented in patient studies has been ^{15}O-H$_2$O. It can freely diffuse into tissue and has a short half-life (~2 min), making it useful for repeated measurements of blood flow in a single visit, at rest and during exercise, or during vasodilator stress (41-43, 45, 47-49). An ^{15}O-H$_2$O rest–stress PET study found significant differences in flow reserve within the calves of PAD patients when compared with healthy volunteers (41). Another study found significantly reduced exercise-induced muscle blood flow in the distal legs of PAD patients who were referred for lower extremity amputation (45). These findings suggested that ^{15}O-H$_2$O PET imaging may be a valuable tool for determining subsequent amputations (45). Another group, Kalliokoski et al., showed that PET assessment of blood flow and oxygen uptake in lower extremity

skeletal muscle may be a useful tool for evaluating responses to exercise (43). However, they observed substantial variability in baseline PET flow measurements and therefore additional assessment tools such as angiography may be required for thorough evaluation of PAD patients. PET imaging of preclinical animal models of PAD have been used to evaluate lower extremity rest and stress blood flow and have demonstrated a high correlation between PET and microsphere-derived blood flow values (46, 50). In a murine model of PAD, PET imaging with ^{13}N-ammonia has been used to assess acute and chronic changes in lower extremity perfusion, showing a close correlation between PET perfusion results and histologic analysis of tissue fibrosis and necrosis (46). Although PET imaging has proven to be useful for evaluating the lower extremities, the short half-life of available tracers can also present a limitation for PET imaging. PET stress imaging has traditionally required the use of exercise ergometers that are attached to scanners, as images must be acquired during or immediately after exercise. The development of an ^{18}F-labeled perfusion agent (flurpiridaz) for myocardial perfusion assessment, however, may assist with exercise PET imaging of both the heart and the lower extremities. The longer half-life (~110 min) allows for tracer injection during peak treadmill exercise and then serial evaluation of myocardial and skeletal muscle stress perfusion. The high extraction fraction of ^{18}F-flurpiridaz in the myocardium may offer an advantage for evaluating lower extremity skeletal muscle blood flow but this possibility still needs to be established (51).

In the present study, we further translate SPECT/CT imaging to evaluate resting microvascular perfusion within 3D foot angiosomes in diabetic patients with CLI using a recently developed approach for segmentation of CT attenuation images of the foot (52).

In addition to our novel approach for 3D segmentation of the foot and analysis of

angiosome perfusion, we translate previously developed image registration tools to assess

serial changes in perfusion within segmented angiosomes following revascularization

(53).

STATEMENT OF PURPOSE

Background and Motivation

Impaired lower extremity perfusion is a hallmark of peripheral arterial disease (PAD) and is particularly problematic in the setting of diabetes mellitus (DM), which is associated with high rates of PAD, foot ulceration, and lower extremity amputation. The ability to non-invasively detect deficits in microvascular perfusion within three-dimensional (3D) vascular territories, or angiosomes, of the feet may provide information related to tissue viability and guide future therapeutic interventions. In this study, the overarching goal was to evaluate the utility of single photon emission computed tomography (SPECT)/CT imaging for quantifying volumetric microvascular perfusion within specific angiosomes of the foot containing non-healing ulcers in DM patients with critical limb ischemia (CLI).

Hypotheses and Aims

<u>Hypothesis 1</u>: SPECT/CT imaging of microvascular perfusion in foot angiosomes provides a quantitative approach for assessing underlying pathophysiology of the foot under resting conditions.

Aim 1: Compare resting angiosome perfusion between healthy volunteers and diabetic patients with critical limb ischemia using SPECT/CT imaging.

<u>Hypothesis 2</u>: SPECT/CT imaging of angiosome microvascular perfusion does not correlate with a standard clinical index, the ankle-brachial index (ABI), which is commonly used to assess macrovascular disease of the lower extremities.

Aim 2: Evaluate the relationship between SPECT/CT imaging of angiosome microvascular perfusion and the ankle-brachial index, a clinically validated tool for the assessment of PAD.

Hypothesis 3: SPECT/CT imaging allows for sensitive detection of regional changes in microvascular perfusion within specific ulcerated angiosomes following lower extremity revascularization and may predict short-term and long-term clinical outcomes.

Aim 3: Evaluate the utility of SPECT/CT imaging for assessing relative changes in angiosome microvascular perfusion following lower extremity revascularization in diabetic patients with critical limb ischemia and relate serial changes in angiosome perfusion to rates of limb salvage.

METHODS

Subject Recruitment

A prospective single-center study was performed in 41 adult patients (age, 66 ± 12 years) with peripheral arterial disease and diabetes. All patients suffered from critical limb ischemia in the form of non-healing foot ulcers or resting pain in the foot and/or calf. Inclusion criteria for patients included patients 18 years or older with previously diagnosed diabetes (type I or II), based on any of the following criteria: fasting plasma glucose greater than 126 mg/dl on 2 separate occasions, glycated hemoglobin (HbA$_{1c}$) greater than 6.5%, two-hour plasma

Figure 2. Patient Selection.

glucose ≥200 mg/dL in an oral glucose tolerance test; previously established history of peripheral arterial disease via abnormal Ankle-Brachial Index (<0.9 or ≥1.3), history of lower extremity rest pain, tissue loss, or presence of non-healing ulcer. Upon enrollment, demographic information, clinical history, and location and severity of any non-healing ulcers were documented. Patients scheduled for lower extremity peripheral angiography as part of their assessment and treatment were prospectively enrolled into the study. An illustration of our subject enrollment considerations is shown in Figure 2.
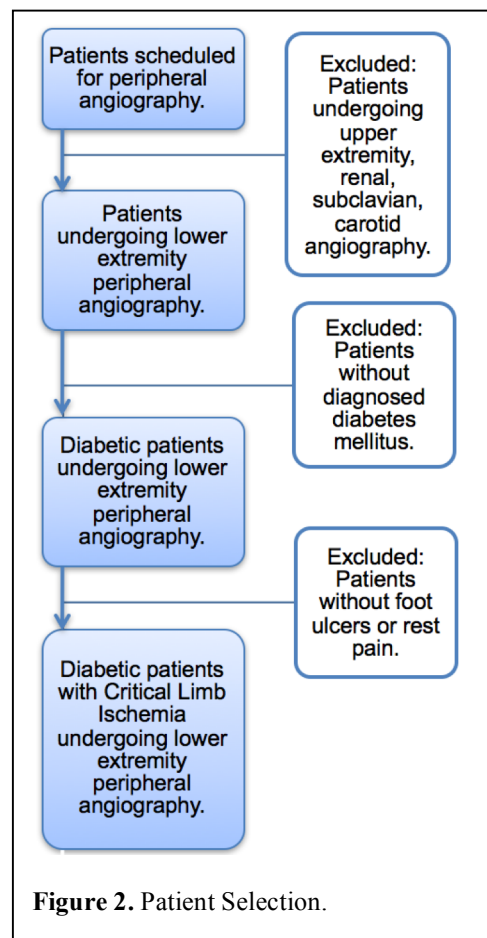
Healthy control subjects (n=9) were also recruited from Yale University, Yale-New Haven Hospital, and the greater New Haven area using a research flyer. They were screened using a standard medical history questionnaire and a physical activity questionnaire. Subjects who did not have any exclusion criteria including preexisting coronary artery disease, peripheral arterial disease, diabetes, cancer, hypertension, smoking history, proceeded with standard PAD screening. PAD screening included Ankle-Brachial Indices (ABIs) of both lower extremities. Additionally, in order to screen for major coronary disease, we recorded subjects' resting blood pressure and heart rate. Inclusion and exclusion criteria are described in Figure 3.

|	Inclusion Criteria	Exclusion Criteria
Healthy Control Subjects	• 18 years or older • Normal ABIs	• Enrolled in another study • Unable to give informed consent • Cardiovascular Disease • Smoking history
CLI Patients	• 18 years or older • Diabetes Mellitus • Established PAD • Scheduled for revascularization	• Unable to lay supine for ~20 minutes

Figure 3. Healthy Control and CLI subject inclusion and exclusion criteria for enrollment.

Yale University's Human Investigation Committee and Radiation Safety Board approved the study protocol and informed consent was obtained from all patients and healthy volunteers.

Clinical SPECT/CT Imaging Protocol

All patients and healthy volunteers underwent baseline single photon emission computed tomography (SPECT)/CT perfusion imaging of the lower extremities at Yale-New Haven Hospital's Nuclear Cardiology clinical laboratory using a standard clinical imaging protocol, following the American Society of Nuclear Cardiology guidelines. All SPECT/CT images were acquired at the level of the ankle and foot using a conventional hybrid SPECT/4-slice CT imaging system with large field-of-view sodium iodide (NaI) detectors (Infinia Hawkeye, GE Healthcare) using a 360º step and shoot acquisition with a 140.5 keV±10% window, 3º projections, and 30 seconds per stop. All subjects received a low dose (14.9 ± 1.0 mCi, 551.3 ± 37 MBq) intravenous injection of technetium-99m (^{99m}Tc)-

Figure 4. SPECT/CT Imaging Protocol.
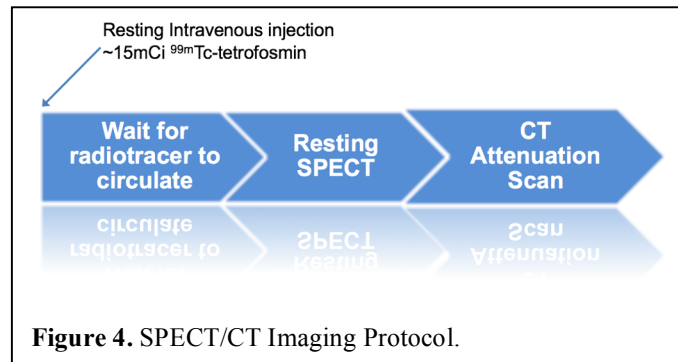
tetrofosmin under resting conditions and underwent SPECT/CT imaging 15 minutes following radiotracer injection (Figure 4). Immediately following the SPECT acquisition, CT images were acquired with a slice thickness of 5 mm, at 140 kVp, and 2.5 mA for the purposes of attenuation correction and future image segmentation of foot angiosomes.

Diabetic patients with CLI who were scheduled for lower extremity revascularization therapy (balloon angioplasty and/or stenting) underwent additional ^{99m}Tc-tetrofosmin SPECT/CT perfusion imaging 1 to 3 days post-procedure, prior to hospital discharge, to examine relative changes in microvascular perfusion in the angiosome containing the non-healing foot ulcer.

All SPECT images were reconstructed using iterative reconstruction applying corrections for attenuation, scatter, and resolution loss. SPECT/CT images were reconstructed using system software (Xeleris, GE Healthcare, Buckinghamshire, UK) capable of generating co-registered functional (SPECT) and anatomical (CT) maps of the feet. SPECT images were reconstructed and fused with CT attenuation images.

SPECT/CT Image Analysis

SPECT/CT images were analyzed using an image analysis toolkit (BioImage Suite, http://www.bioimagesuite.org) developed at Yale University and previously utilized for regional assessment of lower extremity tissue perfusion and oxygenation (2, 5, 44, 52, 54, 55) Low-dose CT attenuation images were used to segment and define five angiosomes of the feet
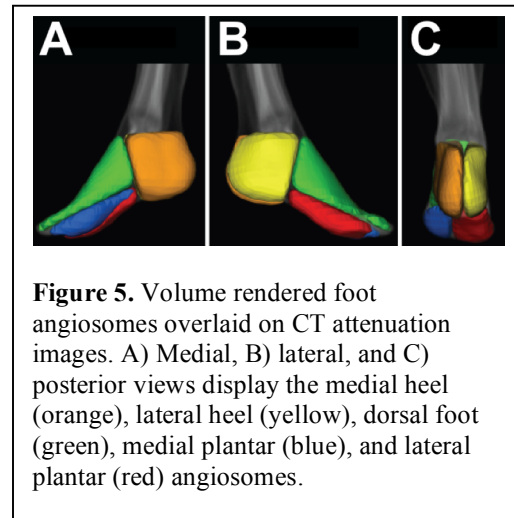

Figure 5. Volume rendered foot angiosomes overlaid on CT attenuation images. A) Medial, B) lateral, and C) posterior views display the medial heel (orange), lateral heel (yellow), dorsal foot (green), medial plantar (blue), and lateral plantar (red) angiosomes.

(lateral plantar, medial plantar, lateral calcaneal, medial calcaneal, dorsal foot; Figure 5). Average radiotracer uptake was assessed from co-registered SPECT images within the CT-defined three-dimensional (3D) angiosome containing the non-healing ulcer. Average SPECT intensity values for the angiosome containing the non-healing ulcer were normalized to injected dose of radiotracer (mCi) and patient body weight (kg) to generate standardized uptake values (SUVs). In healthy volunteers who did not have foot ulceration, average SPECT activity was assessed across the entire foot of their dominant leg.

In diabetic patients who underwent SPECT/CT perfusion imaging before and after lower extremity revascularization, serial images were co-registered using an automated approach of rigid and non-rigid image registration previously developed and published within the principal investigator's laboratory thus ensuring serial perfusion analysis of the same 3D angiosome before and after revascularization (52, 53). Changes in angiosome perfusion were ultimately expressed as the percent change in SUV from baseline to post-revascularization.

Evaluation of Clinical Outcomes

Rates of re-intervention, wound healing, and limb salvage were evaluated in CLI patients at 3, 6, and 12 months following lower extremity revascularization. Percent changes in SPECT angiosome perfusion were compared between patients who underwent an amputation versus patients who did not undergo an amputation in the first 3, 6, and 12 months following lower extremity revascularization.

^{99m}Tc-tetrofosmin SPECT/CT Imaging and Radiation Safety

Due to concerns regarding the amount of ionizing radiation that patients receive with diagnostic imaging, it is prudent that all measures be taken to maintain the ALARA (as low as reasonably achievable) principle. A low-dose (~15 mCi) injection of ^{99m}Tc-tetrofosmin, which possesses a relatively short half-life of 6 hours, is associated with ~3 mSv of ionizing radiation, while CT attenuation scans of the feet immediately after the SPECT acquisition are associated with ~0.3-0.4 mSv, thus making total exposure similar to that of PET/CT perfusion imaging. Total radiation doses remained well within current standard clinical limits. Of note, CT images were acquired at the level of the foot and ankle where no vital organs were exposed to X- rays and associated radiation risk was

low.

Statistical Analysis

Unpaired t-tests were used to identify differences in baseline SPECT perfusion values (i.e. SUVs) and baseline ABIs between healthy volunteers and diabetic patients with CLI. Pearson's correlation coefficient was used to assess the relation between baseline SPECT perfusion values and baseline ABI measurements. A paired t-test was used to identify differences between the SPECT and ABI responses to revascularization in diabetic patients. Unpaired t-tests were used to identify differences between SPECT perfusion changes for patient groups (amputation or no amputation) at each outcome time point (3, 6, and 12 months after revascularization). All statistical analyses were performed using commercially available software (GraphPad Prism v6.0 for Mac OS X, GraphPad Software, La Jolla, CA). Statistical significance for all analyses was set at P < 0.05. All values are expressed as means ± SD unless stated otherwise.

RESULTS

A total of 41 diabetic patients with CLI and 9 healthy control subjects were recruited into the study. Subject group characteristics are summarized in Table 1. The healthy control subjects presented with normal ABIs and did not have a history of diabetes or other cardiovascular co-morbidities. The CLI patients were on average obese, presented with abnormal ABI measures, and had numerous cardiovascular co-morbidities, such as a history of smoking.

Research Subject Demographics		
	Control Subjects (n = 9)	CLI Patients (n = 41)
Age, yrs	50 ± 10	67 ± 12 *
Body Mass Index	27.5 ± 3.7	30 ± 6.4
Ankle-Brachial Index	1.05 ± 0.1	0.6 ± 0.2 *
Toe-Brachial Index	N/A	0.4 ± 0.2
Glycated Hemoglobin	N/A	9.2 ± 2.0
Smoker	0	22
Hypertension	0	38
Hyperlipidemia	N/A	28
Diabetes	0	41
Coronary Artery Disease	N/A	20
Chronic Kidney Disease	N/A	14
Stroke	0	9

Values are mean ± SD.
CLI = Critical Limb Ischemia

Table 1. Demographics of diabetic patients with CLI and healthy control subjects. *, significantly different from control subjects ($p < 0.0.5$).

Of the 41 CLI patients initially scheduled for endovascular revascularization procedure, only 30 underwent their scheduled procedure. Only 24 of the initial 41 CLI patients recruited underwent post-procedure SPECT/CT imaging. Patient retention is illustrated in Figure 6.

Figure 6. CLI Patient Recruitment.
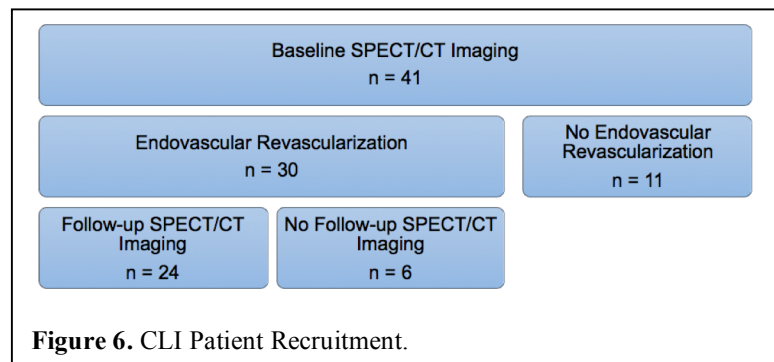
Significant differences in ABI measurements between CLI patients (0.62 ± 0.22, n = 29) and healthy control subjects (1.05 ± 0.11, n = 9) confirmed the presence of PAD in the diabetic patient population (p < 0.0001; Figure 7). Of note, only 29 of 41 recruited CLI patients had ABI measurements available.

SPECT/CT Imaging of Baseline Angiosome Perfusion

Figure 7. Comparison of ABI in healthy control subjects and diabetic patients with CLI. Healthy control subjects demonstrated significantly higher ABI measures compared to CLI patients.

^{99m}Tc-tetrofosmin SPECT/CT imaging of perfusion within specific angiosomes containing non-healing ulcers demonstrated a significant difference in baseline perfusion values (SUVs) between diabetic patients with CLI and healthy control subjects (p = 0.02; Figure 8).

SPECT/CT perfusion imaging revealed the appearance of decreased foot perfusion in CLI patients who presented with multiple signs of lower extremity disease (Figure 9), including non-healing foot ulcers (Figure 9A, 9B), decreased pressure waveforms (Figure 9C), and arterial occlusion (Figure 9D).

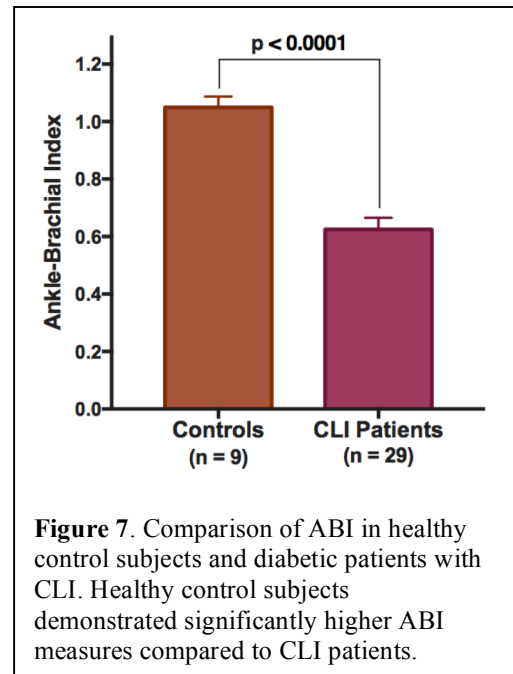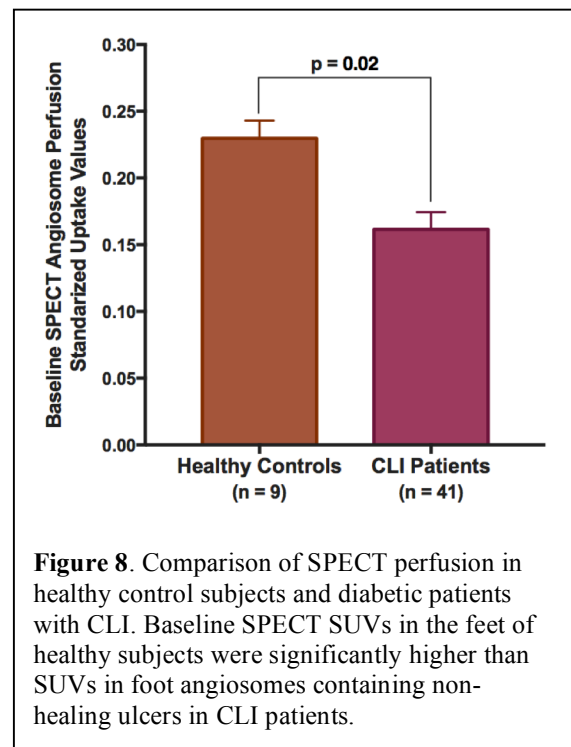**Figure 8**. Comparison of SPECT perfusion in healthy control subjects and diabetic patients with CLI. Baseline SPECT SUVs in the feet of healthy subjects were significantly higher than SUVs in foot angiosomes containing non-healing ulcers in CLI patients.
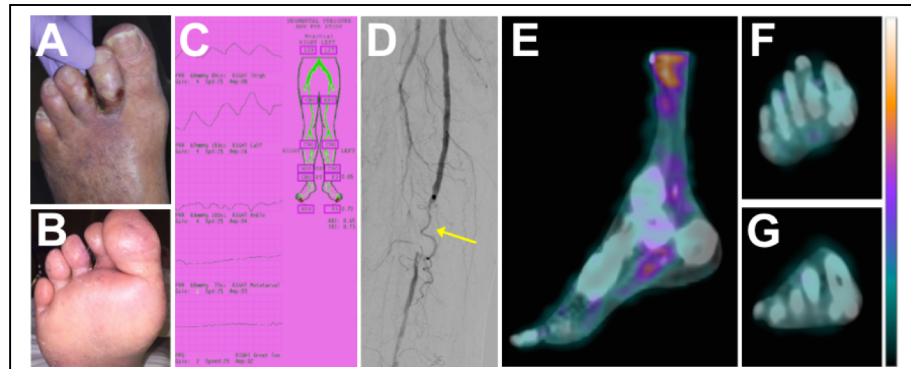
Figure 9. A) Dorsal and B) plantar views of a non-healing ulcer in a patient with C) decreased pressure waveforms and D) superficial femoral artery occlusion, as identified by angiography (occlusion denoted by yellow arrow). E) Sagittal, F) axial, and G) coronal SPECT/CT images reveal decreased resting perfusion in the distal foot (2).

Correlation of SPECT/CT Perfusion Imaging with Standard Assessment Tools

Analysis of baseline SPECT/CT imaging and ABI measurements in CLI patients and healthy control subjects demonstrated a significant and positive relationship between SPECT/CT angiosome perfusion and ABI (p = 0.01; r = 0.41; Figure 10).

Evaluation of Serial Changes in Angiosome Perfusion Using SPECT/CT Imaging

SPECT/CT imaging of CLI patients undergoing lower extremity endovascular

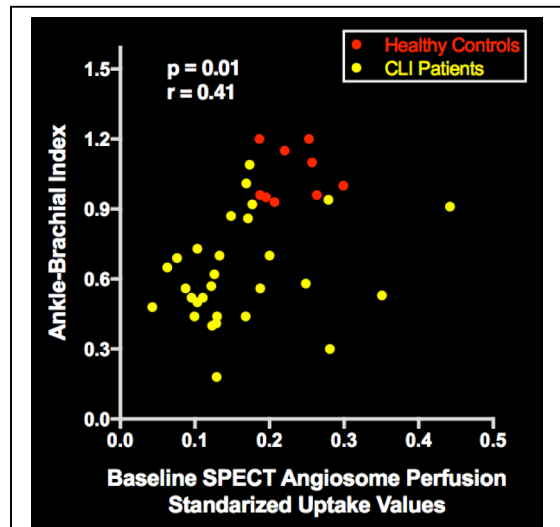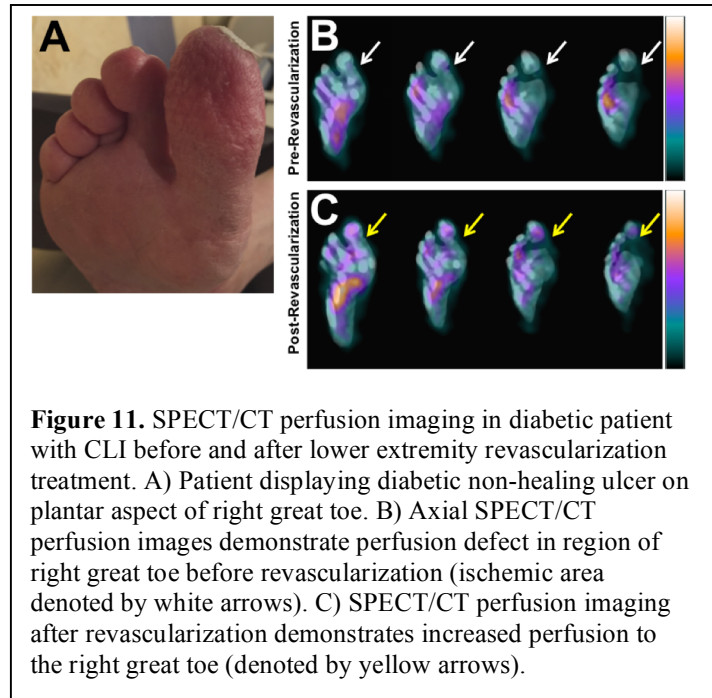**Figure 10**. Relationship between ABI and baseline SPECT perfusion. ABI was significantly and positively related to baseline SPECT SUVs when collectively assessing healthy controls and CLI patients; however, more scatter among CLI patient data points suggests a weaker relationship in the setting of DM. n=9 healthy controls, n=29 CLI patients.

revascularization demonstrated the ability to detect resting deficits in angiosome foot

perfusion before intervention within areas of ulceration (Figure 11A, 11B), as well as allowed for visualization of improvements in angiosome perfusion following revascularization (Figure 11C).

Serial evaluation of relative changes in SPECT angiosome foot perfusion

Figure 11. SPECT/CT perfusion imaging in diabetic patient with CLI before and after lower extremity revascularization treatment. A) Patient displaying diabetic non-healing ulcer on plantar aspect of right great toe. B) Axial SPECT/CT perfusion images demonstrate perfusion defect in region of right great toe before revascularization (ischemic area denoted by white arrows). C) SPECT/CT perfusion imaging after revascularization demonstrates increased perfusion to the right great toe (denoted by yellow arrows).

following revascularization revealed significant quantitative changes in perfusion after treatment (Figure 12) whereas ABI measurements did not demonstrate significant changes after revascularization (Figure 13).

Figure 12. Evaluation of serial changes in SPECT SUVs in CLI patients undergoing revascularization. SPECT SUVs showed a significant increase following lower extremity revascularization.

Figure 13. Evaluation of serial changes in ABI in CLI patients undergoing revascularization. ABI measures did not significantly increase following lower extremity revascularization.
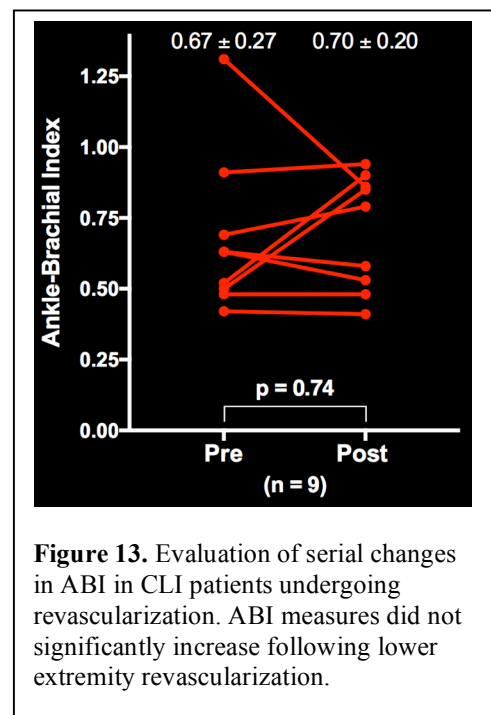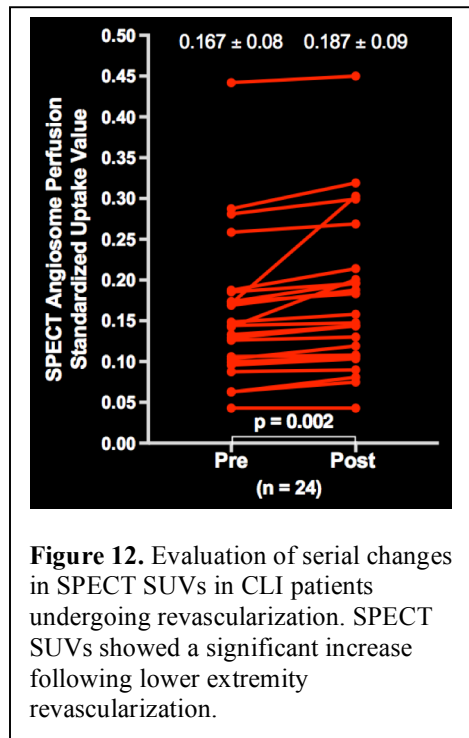
Of note, only 24 of 41 recruited CLI patients had post-procedure SPECT/CT imaging and only 9 of 41 recruited CLI patients had ABI measurements taken before and after revascularization procedure.

Clinical Outcomes

Long-term follow-up of CLI patients at 12 months demonstrated 68 percent of patients had an at least one major (above the ankle) or minor (below the ankle) ampuation (Figure 14A). 19.5 percent of patients were deceased at 12 months (Figure 14B). 19.5 percent of patients had evidence that their initial wounds had healed (Figure 14C). Note, this is not to say they did not develop subsequent wounds. 48.7 percent of patients had

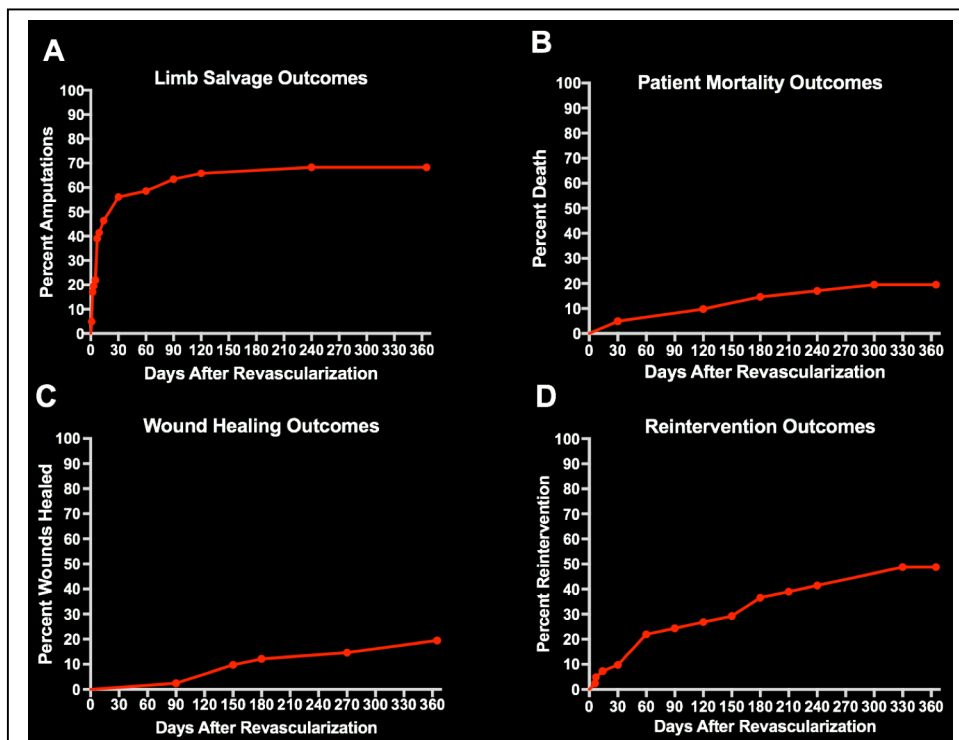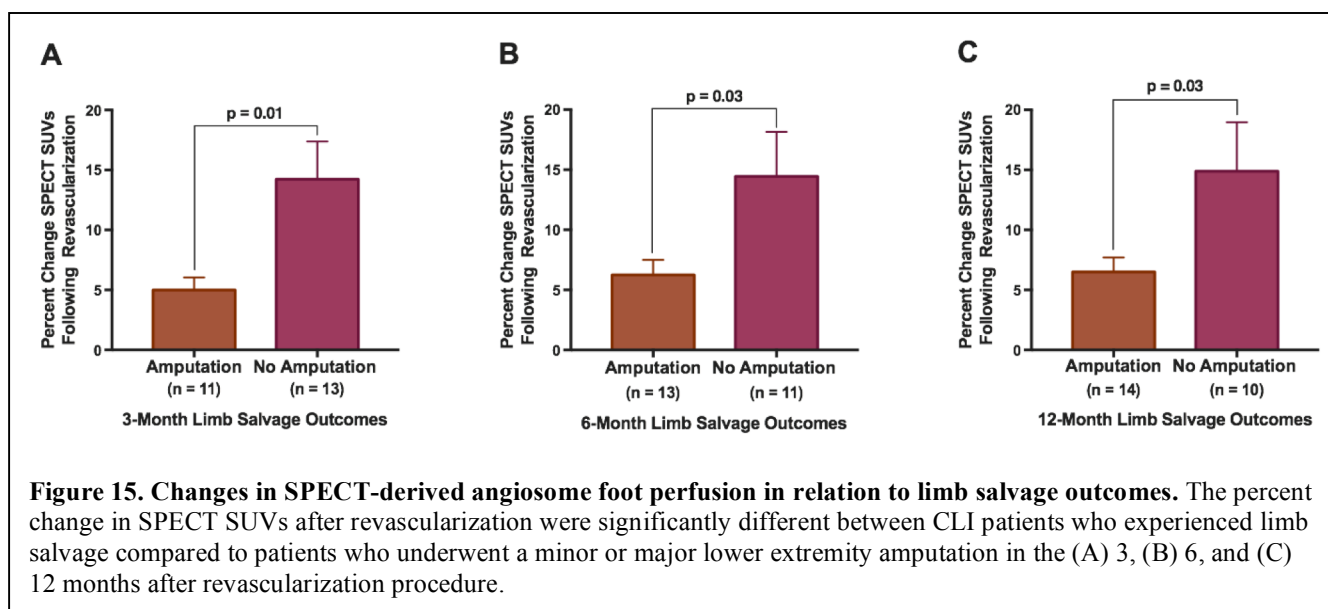**Figure 14.** CLI patients' clinical outcomes 12 months after baseline SPECT/CT imaging. (A) 12 months after baseline imaging, 68 percent of CLI patients had undergone a major (above the ankle) or minor (below the ankle) amputation, (B) 19.5 percent were deceased, (C) 19.5 percent experienced wound healing, and (D) 48.7 percent of patients required at least one re-intervention procedure. n=41 CLI patients.

undergone at least one revasculariztion intervention due to restenosis, new symptoms such as ischemic rest pain, or new non-healing wounds (Figure 14D).

Of the 24 CLI patients who had post-procedure SPECT/CT imaging, 11 had an amputation within 3 months after revascularization (Figure 15A). There was a significant difference between the percent changes in angiosome perfusion following revascularization in patients with (4.99 ± 3.44%) and patients without (14.2 ± 11.3%) amputation in the first 3 months post-procedure (Figure 15A; p = 0.01).

In the 6 months after procedure, 13 of the 24 CLI patients who underwent revascularization had undergone an amputation. Again, the percent changes in SPECT angiosome perfusion were significantly higher in the patients who had not undergone an amputation (14.4 ± 12.3%) in the first 6 months post-revascularization compared to patients who did have an amputation (6.24 ± 4.53%) (Figure 15B; p = 0.03).

In the 12 months after revascularization, 14 of the 24 CLI patients who underwent revascularization had undergone an amputation. Again, the percent changes in SPECT perfusion were significantly higher in patients who had not undergone an amputation

Figure 15. Changes in SPECT-derived angiosome foot perfusion in relation to limb salvage outcomes. The percent change in SPECT SUVs after revascularization were significantly different between CLI patients who experienced limb salvage compared to patients who underwent a minor or major lower extremity amputation in the (A) 3, (B) 6, and (C) 12 months after revascularization procedure.

(14.8 ± 12.8%) in the first 12 months after revascularization versus patients who

underwent amputation (6.50 ± 4.46%) (Figure 15C; p = 0.03).

DISCUSSION

In the present study, we demonstrate for the first time the utility of SPECT/CT perfusion imaging for non-invasive assessment of angiosome microvascular perfusion under resting conditions, as well as following lower extremity endovascular revascularization. We demonstrate that SPECT/CT imaging is sensitive for detecting perfusion abnormalities in diabetic patients with CLI and that baseline SPECT/CT perfusion imaging correlates loosely with a standard clinical index, the ABI. Additionally, we demonstrate that SPECT/CT imaging allows for serial assessment of relative changes in microvascular perfusion within angiosomes of the foot containing non-healing ulcers following lower extremity revascularization, and that angiosome specific changes in SPECT perfusion may relate to clinical outcomes such as rates of limb salvage.

In the assessment of baseline angiosome foot perfusion, SPECT/CT perfusion imaging demonstrated significantly higher perfusion values in the feet of healthy control subjects when compared to ulcerated foot angiosomes of diabetic patients with CLI (Figure 8), thus indicating sensitivity of SPECT/CT imaging for detecting PAD pathology. The presence of PAD was confirmed in diabetic patients with CLI by using a standard clinical index, the ABI, which revealed significant differences between healthy and CLI patients (Figure 7). Prior studies have utilized SPECT/CT imaging for assessment of PAD patients; however, this study presents a novel image segmentation tool that allows for unique SPECT/CT imaging of regional angiosome foot perfusion under resting conditions in a CLI patient population at high risk for lower extremity amputation (7, 56, 57). Correlational analysis demonstrated a significant and positive

relationship between baseline SPECT SUVs and ABI measurements in the collective

assessment of CLI patients and healthy control subjects; however, the relationship

between SPECT perfusion and ABI was clearly more scattered when looking specifically

at CLI patient data points (Figure 10). This finding is consistent with previous studies

that have demonstrated ABI is limited in utility when assessing the diabetic patient

population, which is likely due to the high prevalence of microvascular disease and

calcification (58). Additionally, the looser relationship between SPECT and ABI within

CLI patients may be suggestive of a mismatch between microvascular perfusion (as

assessed by SPECT) and large vessel obstruction (as assessed by ABI) in the diabetic

patient population.

In addition to evaluating the utility of SPECT/CT imaging for baseline assessment

of angiosome foot perfusion, we have demonstrated the value and sensitivity of

SPECT/CT imaging for assessing the effect of lower extremity revascularization in

diabetic patients with CLI. To facilitate serial assessment of changes in angiosome

perfusion following treatment, we have translated previously developed tools for serial

image registration, which allow for reproducible assessment of perfusion within the same

3D segmented angiosomes (52, 53). Following lower extremity revascularization, our

SPECT/CT imaging approach demonstrated significant changes in angiosome perfusion

(Figure 12) that were not observed when utilizing the ABI (Figure 13), a standard clinical

tool utilized for assessing PAD patients. This data suggests that SPECT/CT perfusion

imaging is sensitive for non-invasively detecting changes in microvascular perfusion

following medical treatment, and that SPECT/CT imaging may offer benefits over the

standard ABI, which primarily assesses macrovascular pressures/patency and can be

limited in utility in the setting of arterial calcification or microvascular disease.

Additionally, the ability of SPECT/CT imaging to evaluate microvascular perfusion

might be of particular relevance in the diabetic patient population since diabetic patients

commonly suffer from concomitant macrovascular and microvascular, with the latter

being a key contributor to the formation and impaired healing process of diabetic foot

ulcers (58).

Long-term follow-up of CLI patient outcomes at 12 months post-revascularization

demonstrated similar clinical outcomes to those previously reported in clinical trials aside

from our amputation rates, which were slightly higher (8). This may be a reflection of

wound severity in our CLI patient populating, in particular, infection and the fact these

patients were already scheduled for intervention perhaps indicating wound severity.

Specifically, 68 percent of patients had an at least one major (above the ankle) or minor

(below the ankle) ampuation (Figure 14A), while 19.5 percent of patients were deceased

by 12 months (Figure 14B) and only 19.5 percent experienced wound healing (Figure

14C). Additionally, 48.7 percent of patients required at least one additional

revasculariztion procedure due to restenosis, new symptoms such as ischemic rest pain,

or new non-healing wounds (Figure 14D). This data is in agreement with prior clinical

studies that have demonstrated similar rates of re-intervention in the CLI patient

population (8, 59).

Prior research studies have applied non-invasive imaging for the evaluation of

responses to medical treatment in PAD patients, however, to date, there has been a lack

of clinical studies that have related findings from non-invasive imaging to clinical

outcomes (60, 61). In the present study, we found that our SPECT/CT imaging approach

may possess strength as a tool for predicting clinical outcomes, such as limb salvage. Specifically, we found that patients who experienced poor improvements in angiosome perfusion following revascularization (i.e. 5 to 6 percent) ultimately experienced a minor or major amputation of the lower extremity, whereas patients experiencing greater improvements in angiosome perfusion (i.e. 14 to 15 percent) experienced limb salvage up to 3, 6, and 12 months after revascularization procedure (Figure 15). These findings suggest that SPECT/CT perfusion may possess significant value for the assessment of treatment responses in PAD patients and could assist clinical decisions in the time period immediately following medical treatment.

To date, non-invasive imaging approaches for evaluating foot perfusion in PAD patients have been primarily limited to assessment of superficial tissue or require physiologic stressors (22). CT and MR angiography can both offer valuable anatomical information, however, CT angiography requires exposure to radiation and iodinated contrast material and its utility is reduced in the setting of calcified vessels, particularly in the smaller vessels of the calf and foot (19). In comparison to CT angiography, MR angiography does not require radiation or iodinated contrast, but MR angiography does use gadolinium contrast agents, which cannot be used in cases of severe renal failure. Additionally, MR is hindered in the presence of non-compatible pacemakers or metal implants (including stents) (19, 22). DSA remains the gold standard for imaging tools in assessing PAD since it allows for high spatial resolution imaging of vascular occlusions, but it is an invasive procedure and carries the risk of complications, including hematoma, pseudoaneurysm, dissection and arteriovenous fistula (6). Additionally, it requires intra-arterial delivery of iodinated contrast, and is limited in its inability to characterize vessel

walls and thrombus (6). PET/CT imaging may be an option to assess lower extremity perfusion but thus far has not been applied for evaluating foot perfusion and remains costly due to the need for more expensive instrumentation (41-43). SPECT/CT perfusion imaging could be easily combined with clinically indicated myocardial perfusion imaging, can be performed under resting conditions, and provides quantitative assessment of microvascular perfusion within 3D angiosomes of the foot containing non-healing ulcers that are targeted for revascularization. Many CLI patients are unable to ambulate for treadmill exercise stress testing and many of these high-risk patients also commonly present with impaired renal function. Therefore, the ability to derive potentially valuable clinical information in CLI patients under resting conditions without the need for an intravenous contrast agent may lead to lower extremity SPECT/CT perfusion imaging becoming a clinically valuable tool in the near future.

One limitation of this study is the imaging approach has not been fully validated. We could validate this approach by evaluating test-retest reliability. In the future we could also expand our imaging approach to assess patients along the entire spectrum of PAD and not just the most severe cases, such as CLI. Additionally, we may investigate the utility of stress SPECT/CT perfusion imaging of the lower extremity which could be combined with clinically indicated cardiac perfusion imaging.

In the present study, we apply SPECT/CT imaging to evaluate resting microvascular perfusion within 3D foot angiosomes in diabetic patients with CLI using a recently developed approach for segmentation of CT attenuation images of the foot (52). In addition to our novel approach for 3D segmentation of the foot and analysis of angiosome perfusion, we translate previously developed image registration tools to assess

serial changes in perfusion within segmented angiosomes following revascularization (53). Lastly, we show SPECT/CT imaging of angiosome perfusion may be able to predict short- and long-term limb salvage outcomes for CLI patients undergoing lower extremity revascularization procedures. Therefore, our results demonstrate that, ^{99m}Tc-tetrofosmin SPECT/CT perfusion imaging may be useful for the evaluation of various types of targeted therapies in PAD, such as revascularization procedures, exercise therapy, and novel gene therapies. Additionally, SPECT/CT imaging may be useful in assessing early response to treatment, thereby providing clinicians with an early indication of treatment efficacy and potentially guiding further treatment. Future application of SPECT/CT imaging may provide additional value for detection of ischemic regions of the foot, as well as assist with risk stratification. Further evaluation of the sensitivity of SPECT/CT imaging in predicting wound healing and limb salvage in a large clinical trial of PAD patients undergoing medical treatment is warranted and could elucidate the true clinical utility of this imaging approach.

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