Assessment of Tissue Perfusion in the Lower Limb
Current Methods and Techniques Under Development

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Peripheral arterial disease (PAD) affects 27 million individuals in Europe and North America. This condition causes a severe restriction of blood flow that can lead to critical limb ischemia (CLI), a condition characterized by rest pain, ulceration, or gangrene, which is associated with limb loss in <25% of cases.

The ischemic limb can be revascularized by endovascular techniques (angioplasty/stenting) or surgical bypass. Imaging modalities, including duplex ultrasound, MR angiography, computer tomographic angiography, or intra-arterial angiography, are used to assess the extent of disease, plan intervention, and confirm the patency of major blood vessels after intervention. These techniques do not provide information about perfusion at the tissue level.

An early relatively crude method for assessing ischemic limbs, venous occlusion plethysmography relies on the principle that the volume of the limb is dependent on the arterial inflow when venous drainage is occluded. Under resting conditions, ≤70% of blood flow to the limb is directed to skeletal muscle, with the remainder to the skin circulation. Venous occlusion plethysmography provides a global indication of limb perfusion but gives no anatomic information and cannot delineate segmental perfusion deficits. It is influenced by numerous factors, such as ambient temperature and arteriovenous shunting that may lead to erroneous readings.

Objective measurement of limb perfusion would particularly benefit patients with CLI but most attempts at addressing this have used patients with claudication. The latter can lead to CLI but is a less severe form of PAD that is often treated conservatively and is characterized by pain only when walking as opposed to pain at rest or gangrene. An accurate, noninvasive method of measuring limb perfusion would aid the diagnosis and treatment of PAD and could be combined with conventional angiography to provide both functional and anatomic information. Some think, for example, that targeted revascularization of specific crural vessels that supply the ischemic/gangrenous area of the limb (ie, using the angiosome concept) would achieve better healing than simply restoring in-line flow to the foot via any available vessel. Accurate assessment of segmental tissue perfusion would help to delineate areas of ischemia, to determine the severity of perfusion deficits when there is diffuse rather than focal disease of the larger vasculature, to guide interventions and provide an objective means of measuring improvements accurately.

Medical conditions, such as diabetes mellitus, an important cause of limb loss and functional disability, affect the microcirculation, as well as the larger vessels. Conventional imaging does not allow reliable assessment of the microcirculation, and could, therefore, underestimate the degree of functional limb ischemia if the main vessels are patent. Similarly, the effect of the collateral circulation (an important compensatory mechanism for maintaining limb perfusion when the main vessels are occluded) cannot be quantified. An accurate method for assessing the functional effect of collaterals on tissue perfusion would be invaluable for determining the efficacy of novel treatments, such as angiogenic cell therapy, that aim to promote collateralization.

Here, we provide a critical review of the current and emerging technologies for the assessment of limb perfusion (Table).

Current Techniques

Laser Doppler

Laser Doppler flowmetry (LDF) and laser Doppler imaging (LDI) measure the speed and concentration of blood cells (blood flux and not blood flow; Figure 1) and, therefore, cannot be used to compare perfusion in different individuals.

LDF requires direct contact and is only effective over a relatively limited area (∼1 mm). LDI assesses a larger area of skin from a distance using a scanning motion that incorporates the entire lower limb and foot. The wavelength of laser, speed of scanning, and distance between the scanner and the leg affect tissue penetration and must be kept constant to allow meaningful comparison of serial measurements.

LDF/LDI was first used in the 1980s to assess the severity of ischemia in PAD, but these modalities only identify ischemic

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Table. Advantages and Disadvantages of Various Modalities for Assessing Perfusion

<table>
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<th>Imaging Modality</th>
<th>Pros</th>
<th>Cons</th>
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<td>VOP</td>
<td>Noninvasive</td>
<td>Global indicator of limb perfusion, no anatomic information</td>
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<tr>
<td></td>
<td>No ionizing radiation</td>
<td>Temperature, arteriovenous shunting and inadequate venous drainage may lead to erroneous readings</td>
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<tr>
<td>LDF/LDI</td>
<td>Noninvasive</td>
<td>Low-depth penetration of laser light (marker of skin perfusion)</td>
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<tr>
<td></td>
<td>No ionizing radiation</td>
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<tr>
<td></td>
<td>Performed at the bedside</td>
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<tr>
<td>TcPo2</td>
<td>Noninvasive</td>
<td>Marker of skin perfusion</td>
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<tr>
<td></td>
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<td>Measurements affected</td>
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<tr>
<td></td>
<td>Performed at the bedside</td>
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<td>PET</td>
<td>Absolute quantification of perfusion representing gold standard for perfusion measurements</td>
<td>Exposure to ionization radiation limiting serial measurements</td>
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<td></td>
<td></td>
<td>Significant cost and expertise required</td>
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<td>CEUS</td>
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<td></td>
<td>Easily accessible</td>
<td>Similar to LDI with the addition of intravenous contrast administration</td>
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<tr>
<td>ICGA</td>
<td>Noninvasive</td>
<td>Similar to LDI with the addition of intravenous contrast administration</td>
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<td>MRI: ASL, BOLD, and DCE</td>
<td>Noninvasive</td>
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<td></td>
<td>No ionizing radiation</td>
<td>Longer scan time with inherent problems of motion artifact (ASL)</td>
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<td></td>
<td>Easy access to imaging facilities</td>
<td>Variation in transit time in patients with peripheral vascular disease can introduce an important source of error that may be difficult to correct to assess perfusion accurately (ASL and DCE)</td>
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<td>Quantifies perfusion in different muscle groups (ASL and DCE)</td>
<td>No absolute quantification of perfusion (BOLD)</td>
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<td></td>
<td>Uses endogenous contrast agents (ASL and BOLD)</td>
<td>Uses exogenous contrast agents with risk of nephrogenic systemic fibrosis (DCE)</td>
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ASL indicates arterial spin labeling; BOLD, blood oxygenation level dependent; CEUS, contrast-enhanced ultrasound; DCE, dynamic contrast enhanced; ICGA, indocyanine green fluorescence angiography; LDF/LDI, Laser Doppler flowmetry/laser Doppler imaging; PET, positron emission tomography; TcPo2, transcutaneous oxygen pressure; and VOP, venous occlusion plethysmography.

when compared with normal limbs and cannot quantify the severity of perfusion deficit. More recent studies show that LDF is a good discriminator of skin flap perfusion and can predict the likelihood of healing after limb amputation. LDI is used in preclinical animal models of limb ischemia (e.g., the murine hindlimb ischemia model) for high throughput testing of interventions, including angiogenic therapies. The technique allows longitudinal acquisition of data but is limited by motion artifact, ambient temperature variations, and perfusion measurements, which are weighted toward the larger vessels that are shallow and reside on the surface of the limb musculature.

Transcutaneous Oxygen Pressure

The measurement of transcutaneous oxygen pressure (TcPo2) is a noninvasive technique that allows longitudinal assessment of local oxygen diffusion from the capillary bed through the skin epidermis and provides an indication of the amount of oxygen delivered to the tissue. TcPo2 measurements can reflect the severity of PAD, assess ulcer healing potential, and determine the optimal level for limb amputation. A TcPo2 <20 to 30 mm Hg is consistent with CLI and levels <40 mm Hg are associated with poor healing after amputation. Inhalation of 100% oxygen would normally result in TcPo2 >100 mm Hg, but in patients with CLI the TcPo2 does not rise >30 mm Hg. Oxygen therapy resulting in <10 mm Hg rise in limb TcPo2 is associated with an >60% chance of an amputation at that level not healing. A rise of >40 mm Hg in TcPo2 after limb revascularization is considered significant and increases the chances of tissue healing.

TcPo2 measurements vary in different tissues and under different physiological conditions. Barriers to diffusion, such as edema, increased oxygen consumption secondary to inflammation, and vasoconstriction, may lead to falsely low TcPo2 readings falsely. Readings are also affected by temperature, with a 1°C reduction in the thermistor temperature resulting in a 2% to 3% lower TcPo2 reading.

Indocyanine Green Fluorescence Angiography

This technique has been used for many years to image retinal vessels, and more recently in the assessment of microanastomoses in surgical flap reconstruction surgery, detection of arteriovenous malformations, and measurement of hepatic blood flow. Indocyanine green is a fluorescent dye that is activated by near-infrared laser light at ≈780 nm. This allows a penetration of 3 mm from the surface of the skin to assess the subdermal microcirculation. A semiquantitative measure of perfusion is obtained by measuring pixel fluorescence intensity in different areas of the image from the limb. Perfusion measured by indocyanine green fluorescence angiography correlates with LDI in the hindlimb ischemia model and predicts the likelihood of amputation healing in man. This technique can identify patients with peripheral arterial occlusions who have extensive collateralization. Lack of differences between the 2 groups when ankle-brachial pressure index has been used as the discriminator suggests that indocyanine green fluorescence angiography may be a more sensitive method for assessing perfusion in the limb than ankle-brachial pressure index.
The main limitation of all of these techniques is that they only provide information on perfusion in superficial tissues. Analysis of skin perfusion misses the muscle compartments, which determines symptomatic and functional limitations in PAD. Changes in skin perfusion in PAD are only reliably detected in the later stages of limb ischemia, whereas disturbances in muscle microcirculation is seen much earlier. Muscle perfusion in patients with end-stage CLI requiring amputation correlates well with skin perfusion measured by TcPo2 and LDF. Reliable measurement of muscle perfusion may, therefore, allow intervention at an earlier stage where a more positive outcome in terms of limb salvage is achievable.

Cutaneous blood flow is highly variable and, therefore, is a poor marker of limb perfusion. Autonomic neural dysfunction, as seen in diabetes mellitus, and the presence of infection, for example, can significantly alter skin perfusion.

Emerging Techniques

Positron Emission Tomography

This imaging modality was originally used to measure perfusion and absolute blood flow to the brain and heart but has also been used to quantify blood flow to the musculature of the leg. Myocardial perfusion, assessed by positron emission tomography (PET), correlates extremely well with measurements made using radiolabeled microspheres in animals, a technique that is considered the gold standard in the laboratory setting. Oxygen, in water for injection or mixed with air/CO2 for inhalation, is currently used in man for imaging perfusion using PET. A small number of PET studies have assessed blood flow, oxygen consumption, and oxygen extraction in the lower limb and shown that patients with PAD have decreased blood flow and oxygen consumption in the affected limb after exercise when compared with the contralateral control leg. After stimulation with exercise or intra-arterial administration of vasodilators, the increase in blood flow (hyperemia) is less pronounced in patients with PAD when compared with young healthy volunteers (Figure 2). These studies must, however, be interpreted with caution because they neither use age-matched controls nor remeasure perfusion after limb revascularization. PET does not offer the same spatial and temporal resolution as MRI, but its close correlation with microsphere-derived perfusion measurements in mouse and canine models of ischemia renders it the gold standard in perfusion imaging. PET requires a lengthy scan time and exposure to ionizing radiation precluding serial investigations. Nitrogen-ammonia has been used extensively as a PET agent for assessment of cardiac perfusion but not for the measurement of muscle perfusion in the lower limb. Nitrogen-ammonia and Oxygen both have relatively short half-lives of 10 and 2 minutes, respectively, which limits their versatility. Oxygen is better suited as a perfusion probe in the lower limb because it is freely diffusible and not affected by metabolism. Fluorine flurpiridaz tracer is an alternative, versatile radioisotope for measuring perfusion because of its longer half-life (110 minutes) and high extraction rate, but its use in the clinical setting is still under investigation.

Contrast-Enhanced Ultrasound

Contrast-enhanced ultrasound (CEUS) with microbubble contrast agents (measuring 2.5 μm in size) can assess muscle
Assessment of perfusion in skeletal muscle by CEUS is performed with a linear ultrasound transducer probe placed around the calf and a region of interest (ROI) selected in which only muscle is seen and bone is excluded. The probes used usually have a depth of 4 cm beneath the skin and are held in place by hand, keeping the ROI in view. Microbubble contrast is administered intravenously, either as a bolus injection or by slow continuous infusion with the assumption that signal intensity is a direct measure of microbubble contrast concentration in the ROI (Figure 3). It is not known which method of administration is superior. Changes in muscle perfusion are provoked to help assess perfusion and flow reserve. This can be done using a cuff inflated around the thigh to suprasystolic pressure to occlude the arterial circulation. The cuff is subsequently deflated producing a reactive hyperemia; alternatively the subject exercises to induce functional hyperemia in the muscle and changes in perfusion. In both scenarios, signal intensity can be used to quantify perfusion (Figure 4), but the stressors to increase muscle blood flow in the lower limbs are more difficult to achieve in patients with CLI when compared with claudicants. Parameters, such as time to peak (TTP) contrast, maximal contrast, areas under time–signal intensity curves, and gradients, have been used to assess perfusion. TTP, maximal signal intensity, and area under the time–signal intensity curves are lower in patients with PAD when compared with healthy controls.

CEUS has been used to assess collateralization, an important compensatory mechanism in patients with PAD. Patients with extensive collateralization (assessed by angiography) have a shorter TTP when compared with those that collateralize poorly. In contrast, no difference in ankle-brachial pressure index was detectable between patients with good and poor collateralization.

CEUS shows promise for measuring tissue perfusion because it is cheap, easily available, and does not use ionizing radiation, but it remains to be validated against other gold standard modalities, such as ¹⁵Oxygen PET. To date, it has only been used to measure perfusion in the gastrocnemius and soleus muscle groups. Its use for other calf muscle compartments is limited by the presence of bone. As with all ultrasound techniques, CEUS is operator dependent and can be limited by artifacts created by patient movement. Although microbubble contrast agents are safe to use and only needed in small doses, they are rapidly lost in the circulation.

MRI
This is a promising modality for simultaneously assessing limb perfusion and the larger vasculature using conventional MR angiography. This noninvasive technique does not require ionizing radiation, thereby allowing serial assessment of the patient.

Figure 2. Positron emission tomography (PET) of the calf using ¹⁵Oxygen-labeled water in healthy volunteers and patients with peripheral arterial disease (PAD). At rest, little difference is seen between the 2 groups. Reactive hyperemia provoked with intraarterial infusion of adenosine shows a change between the groups with a lower response in patients with PAD. Reprinted from Schmidt et al with permission of the publisher. Copyright © 2003, Society of Nuclear Medicine and Molecular Imaging, Inc.

Figure 3. Contrast-enhanced ultrasound with continuous infusion in a healthy volunteer (A) and patient with peripheral arterial disease (PAD; B). Images taken at rest (top) and after reactive hyperemia (bottom) provoked using a pedal ergometer. The signal intensity time course curves for each subject are seen on the right. There is little difference at rest, but the reactive hyperemic response is attenuated in the PAD patient when compared with volunteer. Reprinted from Lindner et al with permission of the publisher. Copyright © 2008, Elsevier.
Arterial Spin Labeling MRI

This technique, developed to measure perfusion of the brain, has been used in the kidney, heart, and more recently, skeletal muscle. ASL uses radiofrequency pulse sequences to place a magnetic tag onto the water component of blood proximal to the ROI. A T1-weighted image acquired in the ROI, after a delay to allow for inflow of tagged blood, is subtracted from a control image with no magnetic tagging. The difference in T1 signal between control and tag image is proportional to blood flow and can be used to construct perfusion maps over a designated time period. ASL MRI can be considered analogous to PET perfusion imaging, but unlike PET, only uses blood as an endogenous tracer.

Different methods of ASL are available for perfusion imaging, depending on how the tagging is achieved. Continuous ASL (CASL) involves continuous saturation or tagging of arterial water proximal to the imaging slice. This allows for a steady state in muscle magnetization to be reached, which maximizes signal contrast between the control and tagged images. CASL is hampered by magnetization transfer effects that overestimate perfusion and make multislice imaging more complex. Pulsed ASL avoids this problem by tagging over a spatial region close to the imaging slice in pulses and acquiring the tag image between pulses after a postlabeling delay. The magnetization transfer effect is less of a problem with pulsed ASL because less RF power is needed. There is, however, variable delay between tagging and blood reaching the imaging slice, which makes absolute quantification of perfusion more difficult. Alternative sequences, including QUIPPS (I & II) and Q2TIPS, have been developed to tackle this problem. Pulsed ASL is also limited by a lower signal/noise ratio when compared with CASL. A method known as pseudocontinuous ASL aims to circumvent this problem using short trains of RF pulses to mimic CASL, improving labeling efficiency and achieving a higher signal/noise ratio when compared with pulsed ASL (Figure 5).

Like CEUS, reactive hyperemia or exercise is required to unmask perfusion deficits with ASL. This technique allows absolute quantification (mL/g per minute) of perfusion in the tissues, as well as measurement of parameters, such as peak hyperemic flow and TTP in a ROI over time. Although ASL has not been validated for the measurement of perfusion in the limb in man, comparison with radiolabeled microsphere measurements in the rat hindlimb ischemia model and in the porcine cardiac model has shown good correlation for perfusion. TTP increases with the severity of PAD, and peak hyperemic flow decreases. Analysis of individual muscle groups in the calf reveals that peak hyperemic flow is preserved in the soleus with increasing severity of PAD when compared with the other muscle groups. This may be explained by the dual blood supply of the soleus, which receives blood from both the peroneal and the posterior tibial arteries. ASL shows that patients with PAD have lower muscle perfusion after exercise when compared with age-matched controls.

ASL provides an objective, quantifiable measure of muscle perfusion without ionizing radiation or gadolinium-based contrast agents but has limitations, including a low signal/noise ratio (because magnetically tagged blood represents only 0.5%-1% of the full tissue signal), relatively long scan times and patient movement causing image artifacts, calculations required to quantify perfusion are complex, vascular artifact from large blood vessels causes erroneous measurements, and transit time (the time taken for blood to travel from the tagging region to the ROI) is an important source of error that can be potentiated by disease in the peripheral arteries. Echo planar imaging provides faster acquisition but is associated with magnetic field distortions, making quantification of the MRI signal complex.

Blood Oxygenation Level-Dependent MRI

First developed in 1990 for mapping brain activity, BOLD MRI uses the saturation state of hemoglobin as an endogenous contrast agent. The paramagnetic effect of deoxyhemoglobin causes a decrease in the BOLD signal in T2* gradient echo planar imaging MRI sequences (Figure 6).

As with CEUS and ASL MRI, hyperemia is also required to elicit BOLD signal changes because of the relatively low resting blood flow in skeletal muscle capillary beds. Reactive hyperemia offers a more consistent and easily reproducible method to obtain T2* signal intensity changes when compared with functional hyperemia. BOLD measurements can be taken through a single section of the calf at rest, after thigh cuff inflation (arterial occlusion), during reactive hyperemia and during return to baseline. Figure 7 depicts the typical T2*...
values obtained before and after cuff inflation and illustrates the parameters that can be measured using the curve to determine oxygenation/perfusion, including minimum T2* during ischemia (T2*min), TTP, and maximum T2* (T2*max) during reactive hyperemia.

BOLD MRI has been used to detect changes in muscle perfusion in patients with PAD and healthy controls.44,54–56 Patients with PAD have significantly reduced T2*max and a prolonged TTP when compared with age-matched healthy controls.54 After angioplasty, the T2* signal intensity returns to baseline faster and there is a trend toward a higher T2*max value and faster TTP, when compared with preangioplasty of superficial femoral artery lesions in patients with intermittent claudication,55 indicating that arterial revascularization has an effect on the BOLD MRI signal.

Changes in BOLD T2* signal intensity correlate with LDF and TcPo2 (measure skin perfusion) in the ischemic-reactive hyperemic model56; tissue oxygenation (measured by myoglobin proton MR spectroscopy)57; and perfusion measurements made using venous occlusion plethysmography adapted for MRI and ASL MRI.58

Although the source of the BOLD signal in peripheral skeletal muscle is not fully understood, it is generally accepted that the signal is mainly related to tissue oxygenation state. BOLD MRI, unlike ASL, does not allow absolute quantification of perfusion but instead relies on ratios in signal changes for semiquantitative measurements. BOLD does, however, produce a better signal:noise ratio, which allows superior spatial and temporal resolution when compared with ASL imaging.

**First-Pass Dynamic Contrast-Enhanced MRI**

This MRI technique measures cardiac perfusion and has been adapted for assessing perfusion in skeletal muscle.59 T1-weighted MRI sequences capture the transit of intravenously administered paramagnetic contrast agents, such as gadolinium through tissues, and measure changes in signal intensity in the muscle. Mathematical algorithms for tracer kinetics have been investigated to estimate myocardial blood flow, but this has yet to be used in the clinical setting.60

A reduction in DCE MRI signal intensity, which correlated significantly with radiolabeled microsphere measurements of perfusion, has been demonstrated in the presence of ischemia in the rat hindlimb model.61 In man, DCE MRI measures detectable changes in muscle perfusion after thigh cuff-induced arterial occlusion of a healthy limb when compared with the uncuffed contralateral limb.62,63 A small DCE MRI study, lacking age-matched controls, has suggested that patients with PAD may have a lower perfusion index than healthy individuals.64

The most significant limitation associated with DCE MRI is the need for exogenous gadolinium-based contrast agents with their inherent risk of nephrogenic systemic fibrosis in patients with chronic kidney disease.

**Kinetic Models Used in Contrast-Based Techniques**

Kinetic models are needed to quantify perfusion using contrast-based imaging modalities to differentiate between the tracer in the arterial system and in the tissues. In PET imaging,13 Oxygen is freely diffusible into tissue from the intravascular space, which means a simple single compartment model can be applied to quantify muscle perfusion.65 For DCE MRI and ASL, the modeling is more complex and quantifying perfusion is dependent on deconvolving the arterial input function, calculated from a major artery proximal to the ROI, from the tissue concentration. A nonlinear relationship between arterial input function concentration and signal intensity can introduce errors. Partial volume, signal truncation, and magnetization transfer effects can also lead to inaccuracies. Finally, the bolus transit time, which is dependent on factors such as cardiac output and rate of injection, can also introduce variability.66
Conclusions
An accurate, reproducible, and noninvasive imaging technique capable of providing an objective measure of tissue perfusion in the lower limb would be an invaluable tool for managing patients with PAD. Traditional methods of assessing perfusion such as venous occlusion plethysmography, TcPo₂, and PET each have limitations and are likely to be superseded with modern imaging modalities, such as MRI and CEUS. Such imaging modalities would find use in (1) mapping of areas of poor tissue oxygenation and perfusion to allow informed planning for surgical or endoluminal interventions (eg, selecting a target vessel that would be most likely to reperfuse the most ischemic areas of the limb); (2) rapid assessment of improvements in muscle perfusion/oxygenation after intervention on the affected limb; (3) selective targeting of injections of novel therapeutic agents in the ischemic limb (eg, angiogenic cells and cytokines); (4) diagnosis of poor muscle oxygenation and perfusion in cases where there is uncertainty as to the cause of symptoms and signs in the limb.

Successful validation of these novel imaging techniques would present the opportunity of quantitative assessment of muscle perfusion and may ultimately help to improve limb salvage rates in patients with limb ischemia.

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None.

References


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