Ultrasound in Med. & Biol., Vol. ■■, No. ■■, pp. ■■–■■, 2017

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2017.11.014

● *Original Contribution*

MICROVENOUS REFLUX IN THE SKIN OF LIMBS WITH SUPERFICIAL VENOUS INCOMPETENCE

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(*Received* 15 *September* 2017; *revised* 10 *November* 2017; *in final form* 27 *November* 2017)

Abstract—This study investigated whether microvenous reflux can be detected in limbs with chronic venous disease using superb microvascular imaging (SMI) and colour Doppler imaging. Participants with venous disease (limbs, n = 26) and without venous disease (limbs, n = 10) were studied. The skin in the medial gaiter region was imaged using both SMI and colour Doppler to identify reflux in the small vessels in response to distal augmentation. The diameters and depths of responsive vessels were measured. In limbs with venous disease, reflux in response to provocation was visualised with SMI in a greater number of vessels (12/26 versus 4/26) and smaller vessels than with colour Doppler. Reflux in the superficial skin veins was demonstrated in one control participant (1/10) using SMI and in none using colour Doppler (0/10). Our study indicates that microvenous reflux is demonstrable in limbs with venous disease and that SMI is more sensitive than colour Doppler. (E-mail: kate.thomas@otago.ac.nz) **© 2017 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Microvenous, Reflux, Superb micro-vascular imaging, Chronic venous disease.**

INTRODUCTION

Chronic venous disease (CVD) is widely accepted to be a result of progressive valvular incompetence (Cina et al. 2005). The natural history of varicose veins has traditionally been described as a descending disease (Ludbrook and Beale 1962); however, evidence supports the theory that varicose vein development is an ascending condition (Bernardini et al. 2010) initiating from perforator reflux (Labropoulos et al. 2006), or develops at any level irrespective of the site and the function of the valves involved (Labropoulos et al. 1997, 2005). The focus of earlier work is related to the changes in competence of valves in the larger superficial veins of the leg (>2 mm diameter). Although it had previously been thought that there were no valves in smaller tributaries, we now know that even smaller veins, down to microvenous vessels of less than 100 μm in diameter, contain abundant valves (Aharinejad et al. 2001; Braverman and Keh-Yen 1983; Caggiati et al. 2006; Phillips et al. 2004). A study by Vincent et al. (2011) demonstrated the presence of reflux in the venous microvasculature, using retrograde resin venography in amputated limbs. Microvalves were identified using this technique: If the resin passed through a valve and was present on both sides of the valve, it was deemed incompetent, compared with a competent valve in which the resin was held up at the valve. In individuals with reflux in larger vessels, such as the great saphenous vein, as well as microvalve incompetence, the refluxing resin was shown to extend back into the skin microcirculation. One suggestion based on this work is that microvenous reflux is required for the development of the skin changes of chronic venous insufficiency (Vincent et al. 2011). However, to substantiate this, an *in vivo* technique to image the microvenous system and microvalve function is required.

One approach is to use colour Doppler ultrasound, but historically this has been difficult because of both the limitation in discrimination of such small vessels and the sensitivity required to detect the low flow in these small vessels. In 2016, Toshiba introduced an innovative approach called superb microvascular imaging (SMI; Toshiba Medical Systems Europe, Zoetermeer, Netherlands), which uses very slow flow filtering technology that separates signals derived from low velocity flow in tiny vessels from overlaying tissue motion and effectively preserves even the subtlest low flow components (Gent 1997). Colour Doppler on the other hand uses fixed echo cancellation to differentiate between stationary reflectors and red blood cells.

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It removes very low velocity components of tissue motion; this technique therefore makes it difficult to distinguish real blood flow from motion artifacts at low velocities.

This study investigated the ability of this new SMI technology to detect flow in the microvenous network of the skin and in particular, examined whether reflux could be detected within these small vessels. If this proved feasible, then SMI may be a tool for the early detection and evaluation of the progression of CVD. Additionally, this would improve knowledge and support the understanding of reflux in the microvenous system in the intact limb.

MATERIALS AND METHODS

Study population and demographic characteristics

We studied 2 groups of limbs: (i) 10 control limbs in 10 patients (normal limbs with no venous disease and no reflux on duplex ultrasound), and (ii) 26 limbs with clinical CVD and ultrasound-detected reflux in 24 patients, further referred to as CVD limbs. Demographic characteristics are presented in Table 1. We used the Clinical Etiology Anatomy Pathophysiology (CEAP) classification system for this study. The distribution of the clinical class *C* according to the CEAP classification in all participants is presented in Figure 1. The study was approved by the University of Otago Human Ethics Committee (Health) (Dunedin, New Zealand), reference number H15/111, and conformed to the standards set by the Declaration of Helsinki. Participants were informed of experimental procedures and provided written informed consent.

Experimental protocol

The participants were examined on a bed (Tanzanite AMC2520, Forme Medical, Carrum Downs, Victoria, Australia) tilted to 45° reverse Trendelenburg, with the limb being assessed relaxed, and the contralateral limb bearing weight. This setup allowed for optimal venous filling. The sonographer initially swept the transducer across the medial gaiter region to identify an area demonstrating reflux on augmentation in larger subcutaneous tributaries. Adequate gel for offsetting and preventing any transducer compression of the skin vessels was used. Once an appropriate area was identified, the transducer was manually kept constant in position by the sonographer throughout the assessment. The same chosen site was investigated with colour Doppler and SMI at rest and during several provocation manoeuvres. Video clips were recorded for each manoeuvre with each imaging modality, colour Doppler and SMI.

Table 1. Participant demographic characteristics

	No venous disease	Venous disease
Limbs	10	26
Women	9	10
Men	1	16
Average age (y)	34	56
Age range (y)	22–53	34–81

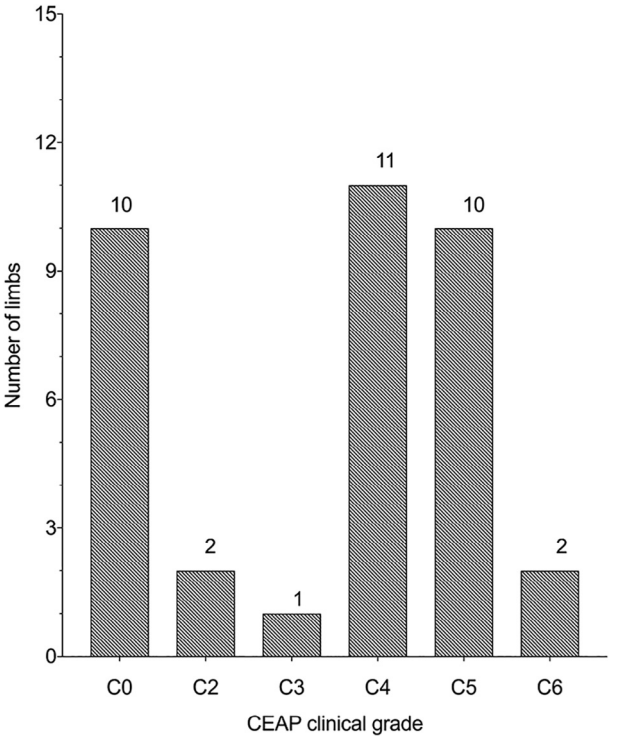

Fig. 1. Distribution of Clinical Etiology Anatomy and Pathophysiology classification in all limbs.

A linear array PLB-1005 BT 12–14 MHz transducer was selected, and a venous ultrasound preset was selected on the Toshiba Aplio 500 ultrasound machine (Aplio 500, Toshiba Medical Systems, Europe). Depth, focal zone and gain were all optimised for each participant. Transmission frequency was always 18 MHz, and the mechanical index was always less than 1.8. Once the linear array transducer was secured in an appropriate location and the image was optimised, the provocation manoeuvres were performed.

Provocation tests

Figure 2 shows the setup of the experiment. Following a few seconds of baseline recording, a pneumatic cuff (custom-made) rapidly inflated and deflated around the foot to induce a distal augmentation. A similar period of recording was obtained using augmentation of a proximal calf cuff (TD312, Hokanson, Bellevue, WA, USA), a Valsalva manoeuvre and a plantar flexion isometric contraction induced by pressing toes downward against a fixed barrier. Ultrasound cine loops were recorded throughout the assessment with both colour Doppler and SMI.

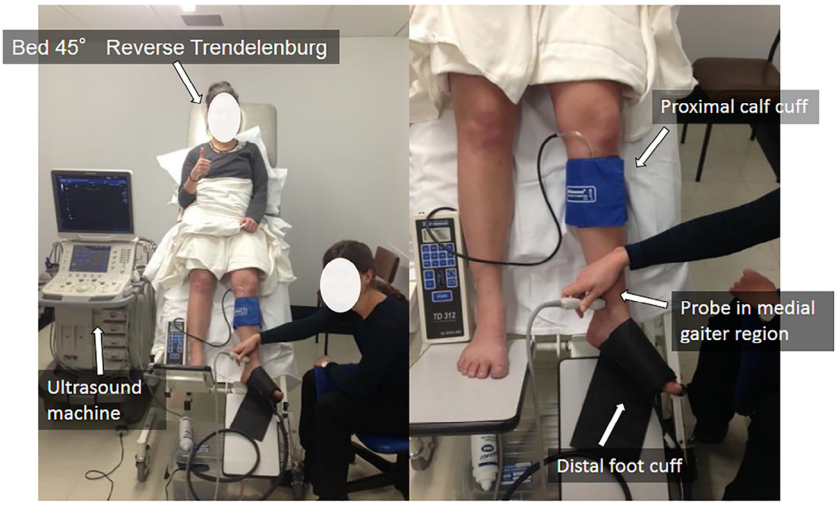

Fig. 2. An example of the experiment setup.

Image analysis

Analysis was performed using Image J (1.51o, Image J Software, Bethesda, MD, USA), a post-processing software tool. The most optimal distal augmentation response demonstrating reflux was selected for each limb with each imaging modality, and still images were captured for analysis in Image J. Our hypothesis was that any colour or SMI signal seen during augmentation was assumed to be reflux, and any signal demonstrated after completion of the augmentation manoeuvre was assumed to be resumption of forward flow. Regardless of direction (as SMI is non-directional), flow was determined semi-quantitatively as *present* or *absent* during each manoeuvre. Subsequent quantitative analyses involved assessing the number of vessels seen to reflux, and further analyses were performed using Image J. Calibration was carried out for a known distance displayed by the ultrasound machine. This allowed the measurement of the diameter of each refluxing vessel and the depth of the vessel from the leading edge of the skin surface. An example of how these measurements were made is shown in Figure 3.

Statistical analysis

Statistics were performed using GraphPad Prism 5 (Prism 5, GraphPad, San Diego, CA, USA). Unpaired *t*-tests were carried out to compare the number of refluxing vessels and the minimum size of refluxing vessels using colour Doppler and SMI. A Mann-Whitney test was performed for the number of refluxing vessels detected in deep and superficial layers.

RESULTS

At rest and without provocation, flow was not demonstrable in small venous vessels by either modality in normal skin or in that with CVD C2–C6. Provocation manoeuvres consistently showed responsive flow in the skin of limbs with CVD C2–C6 but not in the skin of the control limbs. However, excessive movement artifact occurred with proximal calf augmentation, Valsalva manoeuvres and isometric plantar flexion, which precluded meaningful analysis. Further detailed analysis was therefore limited to cine loops of distal augmentation in each limb. For this analysis we used 10 control limbs and 26 venous disease limbs (Table 1).

Comparing imaging modalities

In limbs without venous disease, distal augmentation-induced microvenous flow was not seen with colour flow imaging and in only 1 of 10 limbs when using SMI. In contrast, in the venous disease group, flow in at least one small vein was observed in 17 of 26 limbs with colour Doppler and in all 26 limbs imaged using SMI. Furthermore, the average number of refluxing vessels visualised

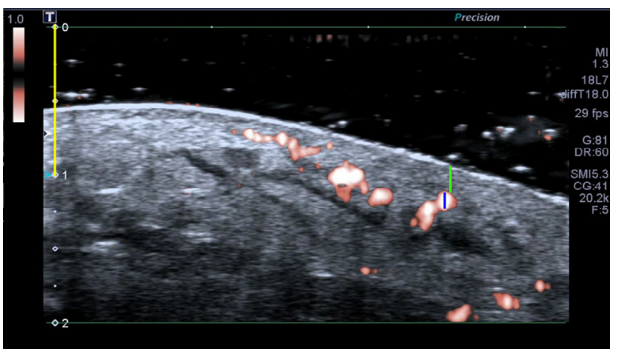

Fig. 3. Example measurements performed using Image J analysis software. *Yellow line* represents calibration of 1-cm depth. *Blue line* indicates the diameter of a vessel. *Green line* indicates the depth of vessel from skin surface.

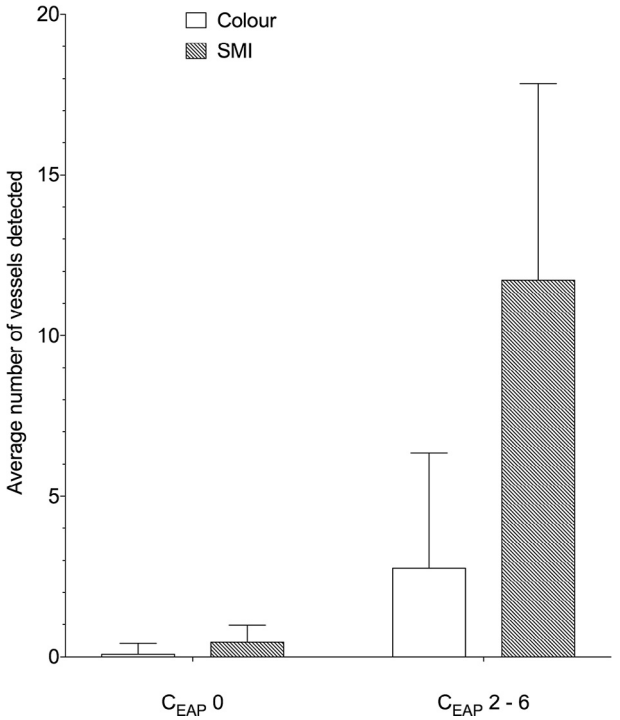

Fig. 4. The average number of refluxing vessels detected in no venous disease (C 0) and venous disease (C 2–6) comparing both imaging modalities; colour Doppler and SMI. SMI, superb microvascular imaging.

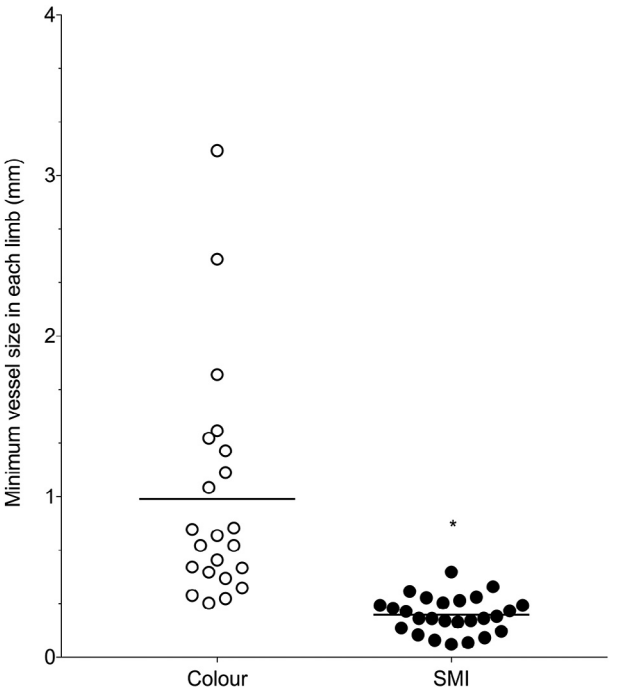

Fig. 6. The minimum size of refluxing vessels that were detected with colour Doppler and SMI for each participant in the venous disease group. *Significantly different between groups, $p = 0.0001$. Minimum vessel size was 0.3 mm for colour Doppler and 0.1 mm for SMI. SMI, superb microvascular imaging.

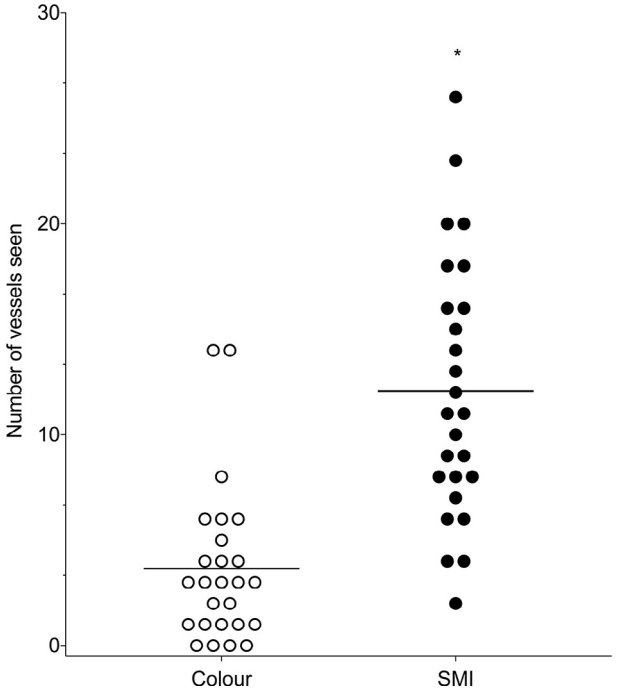

Fig. 5. The number of refluxing vessels for each participant in the venous disease group that were detected using colour Doppler and SMI. *Significantly different between modalities, $p < 0.0001$.

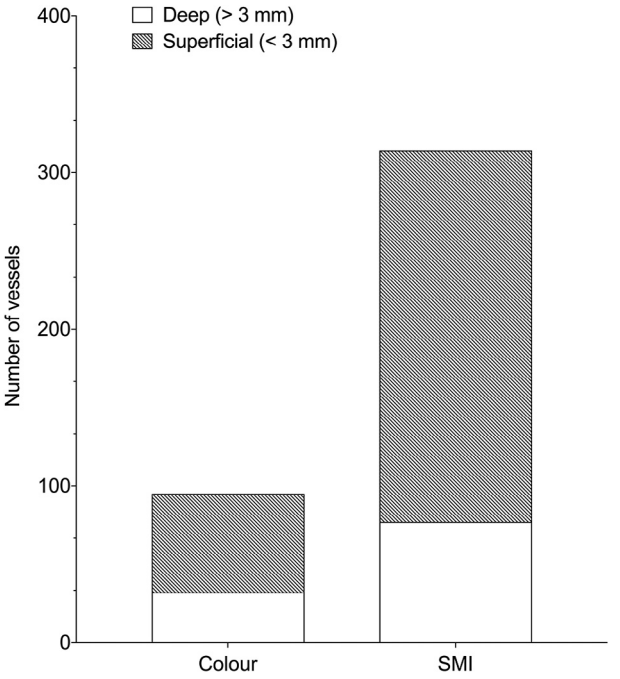

Fig. 7. The total number of refluxing vessels detected with colour Doppler and SMI separated into depth of superficial and deep layers. SMI, superb microvascular imaging.

Fig. 8. Comparison of colour Doppler imaging and SMI in a control participant without venous disease (*first row*) and three patients with venous disease (*rows 2–4*). SMI, superb microvascular imaging.

with SMI (mean 12 vessels, range 2–26) was much greater than with colour duplex alone (mean 4 vessels, range 0–14, $p < 0.001$). The average number of vessels demonstrating reflux in each group is presented in Figure 4.

The number of vessels in the venous disease group demonstrating reflux by colour Doppler and SMI is shown in Figure 5. The sensitivity of SMI was greater because of the ability to identify flow in vessels with a smaller minimum size of 0.1 mm demonstrated with SMI (interquartile range: 0.18–0.34 mm) compared to the 0.4 mm vessels observable with colour Doppler (interquartile range: 0.52–1.30 mm; Fig. 6). The location of these additional vessels seen with SMI was predominantly more superficial at a depth <3 mm from the skin surface leading edge; they were not detected by colour Doppler (Fig. 7). There were insufficient numbers to stratify appearances by clinical class. An example of reflux in the CVD group with colour Doppler and SMI is shown in Figure 8.

DISCUSSION

It has been suggested that CVD in the skin of the lower limb is not only a consequence of wall abnormalities and valvular dysfunction in the larger superficial veins but is also associated with changes in the microvenous circulation in the skin (Vincent et al. 2011). Further investigation requires the ability to visualise these vessels. In this study, we have shown that SMI, a new ultrasound-based technology, has the potential to do this. Currently, ultrasound is very limited in detecting flow in the microvenous network. Other promising technologies have been developed to examine microcirculation of the skin, but these are limited in not being able to distinguish the

microvenous component. The challenge is not only to discriminate vessels of such small size, but to examine the very low flow velocities within them. SMI facilitates this by its ability to detect low velocities in superficial tissue. Even with the benefit of SMI, spontaneous flow cannot be visualised in these small venous vessels in a resting state. In addition, it is not only a matter of size and flow velocity: These vessels are low-pressure collapsible tubes. One approach to overcome these limitations has been to challenge or augment the venous microcirculation to enhance flow velocity and distension. This is consistent with similar manoeuvres used in larger veins to demonstrate flow direction, reflux and disease pathophysiology. This successfully allowed for flow to be observed in microvenules using ultrasound.

Detection of flow in this setting is very sensitive to movement artifact, and SMI reduced the impact on imaging quality of some of this. However, the movement caused by augmentation manoeuvres limits the proximity at which this can be done to the area of interest in the skin. An automated foot compression device produced the least interference.

Previously, we used ultrasound contrast microbubble infusion to examine microvenous reflux without success (unpublished observations). Colour Doppler imaging is a readily-available technology, with consistently improving resolution, and this was the reason for comparison with SMI in this study. However, SMI detected a significantly greater number of refluxing vessels and smaller diameter vessels compared with colour Doppler imaging. The smaller vessels seen with SMI were predominantly located in the superficial layer (<3 mm of the skin surface). We believe these are refluxing microvenous vessels and are the vessels of critical interest to confirm *in vivo* the role of the microvenous system in CVD and to test the concept of boundary valves. In this study, the distinction between normal limbs and those with CVD has been shown. Further investigation is required to determine whether differences in microvenous reflux reflect the progression in severity of venous disease.

This study was primarily a feasibility study. Other approaches to look at the changes in microcirculation in CVD have focused on the changes within the capillary bed. Visualisation with capillaroscopy and fluorescent video capillaroscopy is a well-established tool with clinical applications and has contributed to our understanding of the progression of CVD (Fagrell and Intaglietta 1997; Lascasas-Porto et al. 2008). More recent methods with optical methodology (*e.g.,* optical coherence tomography) have also been described (Anderson and Parrish 1981; Vakoc et al. 2009); however these lack any specificity for the microvenous vessels.

SUMMARY

This study has shown that SMI can be used for detecting microvenous reflux in the skin and was more sensitive than colour Doppler imaging. If this microvenous reflux proves to be important in the development of CVD, then SMI may become a useful tool to follow the microvenous changes in the skin.

Acknowledgments—We would like to acknowledge Toshiba Medical Systems (New Zealand) for providing the SMI software throughout the duration of this study. Thanks also go to Rebecca Millen and Matthew Versteeg for their assistance with statistical analysis.

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