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Keywords
ultrasound, ultrasound-spectral Doppler, veins, peak velocity, vector flow imaging

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ABSTRACT
Purpose Spectral Doppler ultrasound (SDUS) is used for quantifying reflux in lower extremity varicose veins. The technique is angle-dependent opposed to the new angle-independent Vector Flow Imaging (VFI) method. The aim of this study was to compare peak reflux velocities obtained with VFI and SDUS in patients with chronic venous disease, i.e., pathological retrograde blood flow caused by incompetent venous valves.

Materials and Methods 64 patients with chronic venous disease were scanned with VFI and SDUS in the great or the small saphenous vein, and reflux velocities were compared to three assessment tools for chronic venous disease. A flow rig was used to assess the accuracy and precision of the two methods.

Results The mean peak reflux velocities differed significantly (VFI: 47.4 cm/s vs. SDUS: 62.0 cm/s, p < 0.001). No difference in absolute precision (p = 0.18) nor relative precision (p = 0.79) was found. No correlation to disease severity, according to assessment tools, was found for peak reflux velocities obtained with either method. In vitro, VFI was more accurate but equally precise when compared to SDUS.

Conclusion Both VFI and SDUS detected the pathologic retrograde flow in varicose veins but measured different reflux velocities with equal precision. VFI may play a role in evaluating venous disease in the future.

Introduction
Ultrasound is used to evaluate venous diseases of the lower extremities [1–3]. Pathological retrograde blood flow, i.e., reflux, in the superficial veins of the lower extremities indicates chronic venous disease. B-mode ultrasound is used for anatomical mapping, color Doppler ultrasound for detecting reflux, and spectral Doppler ultrasound (SDUS) for quantification of reflux duration and velocities [4]. Duplex ultrasound (B-mode and color Doppler ultrasound) is sufficient in patients presenting solely with varicose veins, while a more severe condition with additional findings, like edema and trophic skin changes due to chronic venous insufficiency, may war-
Materials and Methods

Patients

64 patients with varicose vein disease were prospectively included in a tertiary venous center from August 1, 2016 to January 31, 2017 after informed consent (Table 1). The study was approved by the Danish National Committee on Biomedical Research Ethics and the local ethics committee (H-15007065), as well as by the Danish Data Protection Agency (2012-58-0004).

VFI

The VFI technique is based on the transverse oscillation ultrasound method and can estimate blood flow velocities in an angle-independent manner [8]. In VFI, a conventional pulse for Doppler estimation is emitted, and by using a special estimator and changing the apodization in receive, the axial and transverse velocity components are found simultaneously. The blood flow is visualized in a color box, a color-wheel indicates the direction of the blood flow, and superimposed arrows in the color box ease the interpretation of the flow direction and magnitude (Fig. 1).

Data collection and data processing

The VFI and SDUS recordings were obtained with the same commercial ultrasound scanner (BK3000, BK Ultrasound, Herlev, Denmark) and a 2-8 MHz linear transducer (8L2, BK Ultrasound, Herlev, Denmark). VFI peak velocities acquired from data files were exported and analyzed off-line with an algorithm developed in-house for MATLAB (MathWorks, Natick, MA, USA) after the patients had completed their participation in the research project. The SDUS peak velocities were acquired from screenshots of spectrograms and analyzed off-line in a professional quality vector graphics editor (Inkscape, C/O Software Freedom Conservancy, Brooklyn, NY, USA).

Flow phantom

In a flow phantom (Cole-Parmer centrifugal pump, Vernon Hills, IL, USA), velocities of 50 and 60 cm/s were examined with VFI and SDUS to establish the accuracy and precision of the two techniques.

Table 1 Characteristics of study population (patients).

<table>
<thead>
<tr>
<th>Dichotomous variables</th>
<th>Continuous variables</th>
<th>Mean (range)</th>
<th>St. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (sex in percent)</td>
<td>64 (67 % females, 33 % males)</td>
<td>Age</td>
<td>56.98 (29-90)</td>
</tr>
<tr>
<td>Symptomatic side</td>
<td>31 right lower extremities, 33 left lower extremities</td>
<td>BMI</td>
<td>25.76 (19-37)</td>
</tr>
<tr>
<td>Clinical score in CEAP</td>
<td>59 with varicose veins (C2), 3 with edema (C3), 2 with skin changes (C4)</td>
<td>Venous segmental disease score</td>
<td>1.04 (1-3)</td>
</tr>
<tr>
<td>Etiology in CEAP</td>
<td>63 with primary disease, 1 with secondary disease</td>
<td>Prior surgery</td>
<td>0.55 (0-5)</td>
</tr>
<tr>
<td>Anatomy in CEAP</td>
<td>63 with pathology in superficial veins, 1 with pathology in both the superficial and deep veins</td>
<td>Depth in mm</td>
<td>9.38 (2-20)</td>
</tr>
<tr>
<td>Pathophysiology in CEAP</td>
<td>63 with reflux and 1 with reflux and obstruction</td>
<td>Diameter in mm</td>
<td>5.91 (3-12)</td>
</tr>
<tr>
<td>Advanced CEAP</td>
<td>59 with disease in the greater saphenous vein above the knee, 5 with disease in the lesser saphenous vein</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: St. dev. = standard deviation, BMI = body mass index, CEAP = Clinical-Etiology-Anatomy-Pathophysiology classification, C2 = clinical – varicose veins, C3 = clinical – edema, C4 = clinical – skin changes
under controlled settings. The tested in vitro velocities were selected to correspond to the mean peak reflux velocity of the two techniques found in the in vivo study, where the mean in vivo peak reflux velocity was approx. 50 cm/s for VFI and 60 cm/s for SDUS. Ten replicate measurements were made with both methods at two velocity settings. A constant flow was examined with the transducer at a fixed examination depth (2.5 cm) and insonation angle (VFI 90° vs. SDUS 60° due to 30° beam steering). The reference velocities were measured by a magnetic flow meter (MAC1100, Danfoss, Nordborg, Denmark) with a stated accuracy of ± 1%.

The in vivo setup

64 patients with chronic venous disease of the lower extremities underwent evaluation of peak reflux velocity estimations in the great or the small saphenous vein. A standardized approach was used to provoke the retrograde blood flow and to obtain the velocities [28]. For VFI, an insonation angle approximating 90° was applied, while for SDUS, an insonation angle of 60° was applied due to 30° beam steering. There was no difference in the position of the transducer on the surface of the skin for corresponding estimations with the two methods, as the difference in insonation angle was solely due to the beam steering (Fig. 1). The great saphenous vein was examined in the mid-to-distal-thigh region, and the small saphenous vein was examined in the proximal-to-mid-calf region.

A previously described setup for evaluating deep venous reflux [29], and reproduced for examination of insufficient great and small saphenous veins was used [4, 5, 28]. Each patient underwent evaluation of an insufficient vein of the symptomatic extremity. The patients were weight bearing on the opposite extremity than the one examined during the recordings to ensure that the leg examined with US remained relaxed during the examination. Reflux was provoked by the cuff compression-decompression system (Rapid Cuff Inflation System, Hokanson Bellevue, WA, USA) that inflated a 12-cm cuff to 80 mm Hg, which compressed the lower leg for 3 s prior to rapid decompression in 0.3 s. During decompression, the peak reflux velocity was recorded. The recordings were replicated three times with 60 s intervals between recordings as suggested by others [30]. Between measurements, the transducer was lifted from the skin, and the settings were optimized. This procedure was performed for VFI as well as for SDUS. For both methods, the reported reflux velocity for each patient was an average estimate over the three replicated recordings. A similar setup has previously been described for evaluating antegrade venous blood flow velocities [21].

Manual angle correction by tilting the transducer was not performed to avoid influencing the blood flow velocity by incidentally compressing the examined vein. All recordings were performed by one medical doctor (TB), and optimal settings were set for each of the two techniques. The mean pulse repetition frequency was 5.09 kHz for VFI and 5.08 kHz for SDUS, both ranging from 3 kHz to 7 kHz. For SDUS, the D-gain was set at 60 %, the D-frequency at 7 kHz, the B-frequency at 3.8 MHz, the B-gain at 50 %, while these specifications were not recorded for VFI.

Two ultrasound artifacts were noticed during collection of VFI data, also known from conventional Doppler ultrasound, i.e. blooming and aliasing [31, 32]. Blooming is random noise covering the vessel wall and surroundings due to incorrect adjustment of color gain. In the VFI examination, blooming artifacts were avoided by adjusting the vector gain to the highest setting possible, to fill the lumen with flow signal without noise from the surrounding soft tissue. Aliasing arises in pulsed Doppler systems when the Doppler shift of the moving blood scatterer is more than half the pulse repetition frequency (PRF) corresponding to the Nyquist limit [33]. Flow direction and velocity will be displayed reversed due to the wrap-around effect. When aliasing artifacts were encountered during the VFI reflux velocity estimation, the PRF was adjusted to a higher setting [33].

![Fig. 1](image-url) a Longitudinal SDUS scan of superficial lower extremity vein with valve incompetence causing reflux. The range gate covers the vessel without including vessel boundaries and 30° beam steering is applied reducing the insonation angle from 90° to 60°. The spectrogram for the SDUS data is shown at the bottom of the figure. b Corresponding longitudinal VFI scan. The VFI region of interest was placed corresponding to the position of the SDUS range gate. A 2D vector velocity map is displayed with VFI to depict the directions and magnitudes of the blood flow. A color wheel is provided and superimposed arrows on the color-coded pixels ease the velocity interpretation. Velocity curve for VFI data is shown at the bottom of the figure.
Velocity estimations with conventional US were performed with combined B-mode and SDUS. Aliasing was avoided by adjusting the scale of the spectrogram by increasing the PRF and lowering the baseline. The depicted velocity curve was thus continuous without wrapping. Also, when adjusting the spectral gain as recommended, the flow curve was distinguishable from the background noise [33].

**Tools for grading and assessing venous disease**

The included patient population underwent a range of classifications and questionnaires, patient- and physician-based, used for the characterization and severity assessment of chronic venous disease. The Clinical-Etiology-Anatomy-Pathophysiology classification, and Venous Segmental Disease Score were used for characterization by objective measures, while the Visual Analog Scale, Aberdeen Varicose Vein Questionnaire and Venous Clinical Severity Score were used to assess the severity of disease in each patient by subjective measures [34–36]. The former two were used for describing the patient population (▶ Table 1), while the latter three were used to correlate disease severity and peak reflux velocities measured with VFI and SDUS. The Visual Analog Scale score represented pain over the last 14 days, and ranged from 0 to 100 [34]. The Aberdeen Varicose Vein Symptom Severity Score ranged from 0 to 100 [35], and the Venous Clinical Severity Score is a 30-point score evaluating 10 items [1, 35, 36].

Additionally, demographic information and previously performed venous interventions, vessel diameter and vessel depth were obtained (▶ Table 1).

**Statistics**

In the phantom setup, VFI and SDUS measurements were summarized by their bias (mean deviation from truth), relative bias (in % of true velocity), the absolute precision (standard deviation), and relative precision as measured by the coefficient of variation and error factor. Biases were compared between VFI and SDUS using two-sample t-tests and standard deviations by use of folded F-tests. There is no valid statistical test for comparing two coefficients of variation, and therefore, differences in relative precision were assessed by comparing the error factors (i.e., the standard deviations of log-transformed data). Similar tests were applied to assess differences in performance at the two different velocities for each technique in turn.

In vivo measurements of VFI and SDUS were summarized as the mean, standard deviation and coefficient of variation in each of the 64 patients and compared between the two methods using paired t-tests. Agreement between VFI and SDUS peak reflux velocities was further analyzed in Bland-Altman plots and histograms of absolute and relative differences between VFI and SDUS peak reflux velocities (▶ Fig. 2). The relative difference was calculated as:

\[
\text{Differences in mean in } \% = \frac{(\text{SDUS velocity} - \text{VFI velocity})}{\text{mean velocity}} \times 100 \tag{eq. 1}
\]

Linear regression was used for assessing proportional bias in the Bland-Altman plots. The null hypothesis was that there was no proportional bias.

▶ Fig. 2  a Bland-Altman plot with mean difference of 14.55 cm/s and limits of agreement from -48.51 to 77.61 cm/s. As the scatter has a higher spread toward the right of the figure, at higher mean velocities, the conic shape is a visual sign of increasing variability at increasing mean velocities. b Bland-Altman plot with mean difference of 29.32 % and limits of agreement from -73.24 to 131.88 %. The variability in velocity differences at different mean velocities did not occur when depicted relatively using ▶ eq. 1. c Histogram of absolute differences in SDUS and VFI peak reflux velocities with a normally distributed curve superimposed. d Histogram of relative differences in SDUS and VFI peak reflux velocities with a normally distributed curve superimposed.
A significance level of 0.05 was chosen. SPSS version 24 (IBM, Armonk, NY, USA) and SAS enterprise guide version 6.1 (SAS Institute, Cary, NC, USA) were used for the statistical analyses.

Results

Flow phantom

The bias of VFI and SDUS at 50 cm/s did not differ (p = 0.515), while a lower bias for VFI compared to SDUS was found at 60 cm/s (p < 0.001) (Table 2). All measurements were above the true velocity, so in all reported cases the bias was identical to the accuracy (mean absolute difference). No significant differences were found in absolute and relative precision between the two techniques at the two velocity settings (Table 2). The accuracy of VFI differed for the two velocity settings (p < 0.01), as for SDUS (p < 0.001). The coefficients of variation of VFI were similar to SDUS at the two velocity settings: VFI (50 cm/s: 2.15 %; 60 cm/s: 2.11 %) and SDUS (50 cm/s: 3.1 %; 60 cm/s: 1.94 %) (Table 2).

In vivo

In the clinical setup, both methods detected pathological retrograde flow for all patients. VFI and SDUS estimated different peak reflux velocities (p < 0.001), while no differences in absolute precision (p = 0.177) or relative precision (p = 0.789) were found (Table 3). A significant although weak correlation was found between the peak reflux velocities obtained with the two methods (r = 0.28, p = 0.023). No significant correlations were found between the peak reflux velocities determined by either VFI or SDUS and the tools for assessing chronic venous disease symptom severity (Visual Analog Scale, Aberdeen Varicose Vein Questionnaire, and Venous Clinical Severity Score).

The mean difference in VFI and SDUS peak reflux velocities was 14.55 cm/s, and the relative mean difference was 29.32 %. The absolute and relative velocity differences were depicted as a function of the mean velocity of the two techniques in the Bland-Altman plots (Fig. 2a,b), and corresponding histograms with normal distribution of the data (Fig. 2c,d). The variability in differences seemed to increase with increasing velocities in the Bland-Altman plot of absolute velocities. Hence, limits of agreement were better estimated in relative terms, in which case they ranged from −73.24 % to 131.88 %. It was not possible to reject the null hypothesis of no proportional bias (linear regression of absolute mean: p = 0.263; linear regression of relative mean: p = 0.270), and no skewness in the data was present in either of the histograms.

Discussion

The main findings in vitro suggest that VFI performed slightly more accurately than SDUS, while the precision was equal for the two techniques (Table 2), as no difference in absolute nor relative precision was found at the two velocity settings. The phantom results are in line with previously published studies and indicate that VFI is comparable to SDUS for the estimation of velocities expected for venous reflux evaluation, even though the measurements in vitro were conducted with a fixed and known angle, which favored SDUS.

Table 2  In vitro results – biases, standard deviations, error factors and coefficients of variation of VFI and SDUS.

<table>
<thead>
<tr>
<th>Velocities</th>
<th>Accuracy</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phantom Velocity [cm/s]</td>
<td>Mean VFI [cm/s]</td>
<td>Mean SDUS [cm/s]</td>
</tr>
<tr>
<td>50 cm/s</td>
<td>56.65</td>
<td>57.10</td>
</tr>
<tr>
<td>60 cm/s</td>
<td>72.01</td>
<td>72.01</td>
</tr>
</tbody>
</table>

Abbreviations: SDUS = spectral Doppler US, VFI = Vector Flow Imaging, st. dev. = standard deviation, EF = error factor, CV = coefficient of variation.
The main in vivo findings showed that pathologic retrograde venous blood flow in the great or small saphenous vein could be identified in all patients with both techniques. The VFI and SDUS technique estimated significantly different peak reflux velocities with no differences in absolute or relative precision (Table 3). VFI and SDUS velocities have previously been compared in different vessels including the deep veins of the lower extremities [21], the portal vein [13], the ascending aorta [37], arteriovenous fistulas [18], the heart [38], and the carotid arteries [15, 26, 39]. In most of these studies, SDUS velocities were above the VFI velocities as in this study. Equal precision of VFI compared to SDUS in vivo has likewise been reported previously for blood flow estimation in e.g. the deep veins of the lower extremities [21], the carotid arteries [39], and the ascending aorta [40].

Increasing bias between VFI and SDUS at increasing peak velocities has been indicated by other papers concerning previous VFI implementation [21, 37, 40]. Hansen et al. reported an increasing bias for increasing velocities, and suggested a correction algorithm for the systematic bias [40]. No systematic bias between the two methods was present for venous reflux estimation (Fig. 2b).

In this study, reduced precision for VFI and SDUS was found in vivo compared to in vitro. This has also been reported for velocity estimations in the flow phantom with constant versus pulsatile flow [41], as for in vitro estimation compared to in vivo [21, 40, 42, 43]. The main reason for the difference in this study is probably that the in vitro flow was constant, while the in vivo blood flow was pulsatile.

Even though the venous reflux in vivo was provoked in the standardized setup, variables influenced the velocity estimates. The instrumental settings of the ultrasound scanner, variation in placement of the transducer on the patient, echo cancelling of moving tissue, and patient specific parameters including cooperation and habitus had an impact on velocity estimates of both methods. Operator-dependent instrumental factors, like placement of range gate, range of velocity scale, Doppler gain, beam steering, and insonation angle were additional concerns in the peak velocity estimation with SDUS [7, 41, 44–47].

Reflux duration is used for diagnosing chronic venous disease in the saphenous veins [1], though no significant discrimination power has been found [5]. Konoeda et al. and Yamaki et al. have suggested peak reflux velocities for discriminating between early to moderate stage chronic venous disease and more advanced chronic venous disease [4, 5]. In both studies, peak reflux velocities were significantly higher for more advanced disease, and almost identical average peak reflux velocities were reported for the early (C1-C3) and the advanced stage chronic venous disease group (C4-C6 in the Clinical-Etiology-Anatomy-Pathophysiology classification) (Table 1). In this present study, the average SDUS peak reflux velocity was higher compared to recently reported average peak reflux velocities, despite the patients almost exclusively presenting with early stage chronic venous disease in this study opposed to the compared studies (Table 1). On the contrary, the mean VFI peak reflux velocity of 47.2 cm/s was close to the mean SDUS peak reflux velocities of 43.8 cm/s reported by Konoeda et al. and of 43.4 cm/s reported by Yamaki et al. in the early to moderate stage chronic venous disease group [4, 5]. The two studies came from the same institution and may have been performed with identical examination techniques, which can explain the high agreement in average peak velocities [41].

In this study, no correlations were found between peak reflux velocities of VFI and SDUS and the three symptom grading systems for chronic venous disease (Visual Analog Scale, Aberdeen Varicose Vein Questionnaire, and Venous Clinical Severity Score). Neither the Clinical-Etiology-Anatomy-Pathophysiology classification nor the Venous Segmental Disease Score was used as most of the patients were identical in these two classifications, that is C2 in the Clinical-Etiology-Anatomy-Pathophysiology classification and single vessel involvement in the Venous Segmental Disease Score (Table 1). The lack of correlation in this study can be explained by the fact that the included patient population was too homogeneous.

In the clinical setting, qualitative flow analysis is used more than quantitative flow estimation, e.g. peak velocity estimation [2, 4, 5]. VFI detected reflux in all patients as did SDUS. It must, however, be underlined that this study did not examine the diagnostic accuracy of the two techniques, as all included patients suffered from chronic venous disease presenting with varicose veins caused by valve incompetence in the great or the small saphenous vein. The direction of blood flow is readily interpreted with VFI (Fig. 1). As VFI offers equal precision compared to conventional SDUS, the additional advantages of the technique should be exploited. New insonation windows, improved operator agreement, and applications for quantifying complex flow and volume flow with 3D ultrasound should be examined further [21, 42, 48, 49].

The homogeneous patient population in contrast to previously performed studies with representation from the entire disease spec-

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**Table 3** In vivo results – peak reflux velocities, precisions and correlations with clinical scores of VFI and SDUS.

<table>
<thead>
<tr>
<th>n = 64</th>
<th>VFI</th>
<th>SDUS</th>
<th>p-value</th>
<th>Mean (range)</th>
<th>St. dev.</th>
<th>Correlation of VFI (p-value)</th>
<th>Correlation of SDUS (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PRV [cm/s]</td>
<td>47.42</td>
<td>61.98</td>
<td>&lt;0.001</td>
<td>Visual analog scale (pain last 14 days)</td>
<td>29.96 (1-78)</td>
<td>21.31</td>
<td>0.10 (0.43)</td>
</tr>
<tr>
<td>Mean st. dev. [cm/s]</td>
<td>6.45</td>
<td>9.96</td>
<td>0.177</td>
<td>Aberdeen varicose vein questionnaire</td>
<td>12.36 (2-37)</td>
<td>6.80</td>
<td>0.17 (0.19)</td>
</tr>
<tr>
<td>Mean CV [%]</td>
<td>14.42</td>
<td>13.77</td>
<td>0.789</td>
<td>Venous clinical severity score</td>
<td>5.15 (1-11)</td>
<td>2.36</td>
<td>0.09 (0.48)</td>
</tr>
</tbody>
</table>

Abbreviations: VFI = Vector Flow Imaging, SDUS = spectral Doppler ultrasound, st. dev. = standard deviation, CV = coefficient of variation
trum was a limitation in this study [4, 5]. Surgery has previously been performed in 42% of patients, which may have influenced the results of this study (Table 1). Measurements with the compared techniques were not obtained simultaneously, which also may have influenced the results. The blood flow velocity of a varicose saphenous vein may vary depending on the distance to the nearest sufficient valve, and the reflux velocity provoked by cuff compression may be related to the body mass index. No inter- and intraobserver variations were examined, and no solid ground truth was available for reference. SDUS is operator- and angle-dependent and is liable to over-estimate velocities due to spectral broadening [7, 32, 44]. Whether VFI or SDUS estimates the most accurate peak reflux velocities in vivo can only be clarified with an independent gold standard, e.g. phase contrast magnetic resonance angiography (MRA) [50]. However, superficial veins are small, and valid results would be a challenge given the limited spatial and temporal resolution of MRA [51].

Conclusion

The study showed that both VFI and SDUS detect pathological retrograde venous blood flow in all chronic venous disease patients. No significant difference in the precision of peak reflux velocity was found. The mean peak reflux velocity was lower for VFI compared to SDUS, and the correlation between the VFI and SDUS velocity was low. No significant correlations were found between the VFI or SDUS peak reflux velocities and the clinical assessment tools for chronic venous disease. In vitro, VFI was more accurate and equally precise when compared to SDUS for velocity estimation.

Further research will show if additional features of VFI, e.g. estimation of flow complexity and volume flow, can benefit patients with venous disease.

Conflict of Interest

The Danish National Advanced Technology Foundation and BK Ultrasound supported the project. The author AJA developed and patented the Transverse Oscillation technique and earns royalties from the sale of scanners with the Transverse Oscillation Vector Flow Imaging technique implemented from BK Ultrasound.

References


