Improvements in high resolution ultrasound for postoperative investigation of capillary microperfusion after free tissue transfer

P. Lamby a,∗, L. Prantl b, S. Schreml c, K. Pfister d, M.P. Mueller b, D.-A. Clevert e and E.M. Jung f

a Department of General Surgery, University Medical Center Regensburg, Regensburg, Germany
b Plastic and Reconstructive Surgery, Department of Trauma, University Medical Center Regensburg, Regensburg, Germany
c Department of Dermatology, University Medical Center Regensburg, Regensburg, Germany
d Department of Vascular Surgery, University Medical Center Regensburg, Regensburg, Germany
e Interdisciplinary Ultrasound Center, Department of Clinical Radiology, University of Munich, Grosshadern Campus, Munich, Germany
f Institute of Diagnostic Radiology, University Medical Center Regensburg, Regensburg, Germany

Abstract. Introduction: High resolution ultrasound (US) techniques as implemented in the latest generation of US machines provide imminently better resolution compared to previous high resolution models. This improvement is based on advanced transducer technologies as well as updated post-processing procedures. Furthermore, matrix linear transducers providing frequencies from 6 to 15 MHz are now available. The aim of the study was the evaluation of these new techniques for the immediate postoperative investigation of microcirculation after free tissue transfer by supplemental use of Contrast-Enhanced Ultrasound Imaging (CEUS).

Patients and methods: To this end, we investigated 12 patients who underwent free tissue transfer in order to cover tissue defects in various body regions. We utilized the new GE Logiq E9 equipped with a linear 6–9 MHz and a matrix 6–15 MHz probe as well as the GE Logiq 9 with the previous version of the linear 6–9 MHz probe. Both machines provide the modalities of SRI®, Cross Beam® and THI®. The perfusion curves were quantitatively analyzed using digital cine sequences (Qontrast®, Bracco, Italy). Furthermore, two independent investigators evaluated the digitally recorded images with respect to the resolution of details based on a scale ranging from 0 to 5, and after application of 2.4 ml SonoVue® (Bracco, Italy), evaluated the image quality regarding the representation of tissue perfusion.

Results: None of the free flaps showed clinical or laboratory signs of flap failure during the hospital stay. Several flaps showed typical perfusion patterns relating to the flap type. The combination of SRI®, Cross Beam® and THI® allows, in most cases, a more exact differentiation of tissue graft outlines and tissue composition, in particular the tissue texture, compared to the use of B-scan only. In addition, the high resolution matrix technology combined with the broader spectrum of 6–15 MHz considerably improves the representation of image details compared to multifrequency probes with 6–9 MHz. The use of updated post-processing procedures as well as new transducer technologies in CEUS also results in improved resolution and thus achieves a higher score compared to previous models.

Conclusion: At present, these new US technologies combined with the updated 6–9 MHz probe provide the optimal assessment of perfusion in cutaneous, subcutaneous and deeper tissue layers. The additional use of new multifrequency 6–15 MHz matrix probes improves the resolution in the B-mode to an even higher degree.

Keywords: Contrast-enhanced ultrasound, free flap, microcirculation, matrix technologies

∗Corresponding author: Philipp Lamby, MD, Department of General Surgery, University Medical Center Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany. E-mail: philipp.lamby@klinik.uni-regensburg.de.
1. Introduction

The immediate evaluation of microvascular tissue flaps with respect to microcirculation after transplantation is crucial for optimal monitoring and outcome [1–8]. Ultrasound (US) techniques as Color-Coded Doppler Sonography® (CCDS) or Power Doppler® are able to detect the vessel course up to the anastomosis and allow an assessment of the quality and precision of its perfusion [9–12]. The accuracy of the investigation also depends on the available resolution of the ultrasound transducer.

However, using conventional US it is not possible to detect vessels within a tissue graft using linear probes with frequencies from 6 to 15 MHz, particularly those in subcutaneous and deeper vessel plexus [7]. To depict microcirculation, contrast-enhanced US (CEUS) techniques are necessary, e.g. Contrast Harmonic Imaging (CHI). Using second generation contrast agents [6], the dynamic detection of microvascular perfusion is possible, even with a mechanic index below 0.2 and a bolus injection of maximum 2.4 ml SonoVue® [7,12–16].

Preliminary studies [7,12–16] using a multifrequency linear probe demonstrated that CEUS is a viable alternative to the previous standard procedures, such as fluoroscopy, for the assessment of the cutaneous perfusion. In addition, CEUS provides the ability to assess the tissue morphology of deeper tissue layers and to analyze quantitatively the tissue perfusion by recording digital picture sequences and by using time intensity curves (TIC-analysis) [7,16].

The latest developments in US probe technology plus new software for perfusion analysis have significantly improved the assessment of the blood flow as well as the contrast agent dynamic by using time to peak analysis by dint of cine sequences [17].

New matrix probes using improved technologies allow CEUS with frequencies from 6 to 15 MHz, although these innovative techniques have not yet been tested for contrast-enhanced postoperative monitoring of tissue grafts. Previously evaluated CEUS techniques have functioned with 2–5 MHz convex probes and 6–9 MHz linear probes [7,12–16].

The aim of this study was to investigate these new technologies for the postoperative evaluation of tissue perfusion of free microvascular grafts in plastic surgery.

2. Methods

2.1. Patients

An experienced radiologist examined 12 patients (7 males, 5 females) 5 to 10 days after microvascular flap transplantation. The age of subjects ranged from 24 to 65 years (mean age 44.83 ± 11.81). From October 2007 to February 2008, one single plastic surgeon performed 7 parascapular flaps, 2 latissimus dorsi flaps, 2 radialis flaps and 1 anterolateral thigh flap.

2.2. Ultrasound and post-processing

For US investigation we utilized the GE Logiq 9 equipped with a linear 6–9 MHz probe (9L) compared to the new GE Logiq E9 (E-series) equipped with updated post-processing procedures, an updated version of the 6–9 MHz probe (9L-D) as well as a matrix 9–15 MHz transducer (ML 6–15-D) (Figs 1A and 1B).

The update of the E-series transducers includes, among other improvements, single crystal technology, which provides a better bandwidth due to excellent polarization of significant clearer crystal materials.
Fig. 1A. B-scan with 6–15 MHz using SRI®, Cross Beam® and THI®. Depiction of flap tissue over middle and deeper layers although metal clips inserted in the cutaneous layer.

Fig. 1B. Contrast-enhanced ultrasound (CEUS) using updated postprocessing procedures and transducer technologies. GE Logiq E9 with matrix linear probe ML 6–15-D.
In addition, acoustic amplifier technology is utilized, which transforms the loss of energy due to the elimination of disturbing signal reflections and feeds it to the receiving transducer signal. These innovations result in a better acoustic noise-to-signal relationship, better sensitivity as well as a better frequency bandwidth. Furthermore, the ML 6–15 as a matrix probe is equipped with crystal architecture composed of vertical and horizontal crystal arrays, thus allowing for a more detailed spatial resolution.

For CEUS the GE Logiq E9 uses the principle of amplitude modulation [18]. Tissue reflects transducer signals of different strength in a linear correlation. Due to the elasticity of contrast micro bubbles, amplified transducer signals result in stronger contrast agent echoes compared to tissue echoes (Fig. 2). Using modulated transducer signal amplitudes the US machine can differentiate between echoes from tissue and those from contrast micro bubbles and thus is able to subtract the tissue reflections in order to more precisely depict contrast enhancement.

The contrast agent SonoVue® (Bracco, Italy) was injected as a bolus of 2.4 ml through a cubital vein followed by 10 ml NaCl solution. Tissue perfusion could be detected in the form of micro bubbles using Contrast Harmonic Imaging (CHI) with the True Agent Detection® modality of pulse inversion imaging over 2 min in low MI-technique (Mechanical Index, MI < 0.2). Appositional Color-Coded Doppler Sonography (CCDS) and Power Doppler were performed as well (Fig. 3).

The perfusion was investigated for each tissue layer separately. First, GE Logiq E9 with the 6–9 MHz probe was used in order to evaluate the effect of Speckle Reduction Imaging® (SRI®), Cross Beam® and Tissue Harmonic Imaging (THI®) [7,10–14,16–18]. Afterwards, the 6–15 MHz matrix probe in combination with SRI®, Cross Beam® and THI® was compared (Fig. 4).

![Principle of Amplitude Modulation](image)

Fig. 2. Amplitude modulation as used in GE Logiq E9. The transducer alternately sends signals of different strength. Tissue echoes show linear correlation to signal variations. However, due to elasticity, reflections of micro bubbles react stronger and are nonlinear. The Logiq E9 can differentiate between linear and nonlinear signals. (Permission, GE Healthcare, Chalfont St. Giles, UK.)
Fig. 3. Micro- and macrovascularisation detected with GE Logiq E9, 6–9 MHz after bolus injection of 2.4 ml contrast agent (CA) SonoVue® using Color-Coded Doppler Sonography (CCDS), Power Doppler, B-scan and Contrast Harmonic Imaging (CHI).

Fig. 4. B-scan with linear 6–9 MHz compared to matrix 6–15 MHz using SRI®, Cross Beam®, THI® and Power Doppler®. GE Logiq E9.
In the next step, we investigated the CEUS modality of both US machines using linear 6–9 MHz transducers. The precondition for a dynamic and quantitative perfusion analysis was digitally recorded cine loops saved as Audio Video Interleave (AVI) files. Thus, it was possible to interpret data collected by two different US machines. An additional post-processing procedure was performed using Qontrast® Software (Bracco, Italy). This application allows a color-coded visualization of the perfusion thereby considerably facilitating the interpretation over several tissue depths [17]. Moreover, color coding allows retrospective analyses including parameters such as flow volume or time to peak (Figs 5 and 6).

Two independent investigators evaluated the digitally recorded images with respect to the resolution of details in the transplanted tissue graft based on a scale ranging from 0 to 5 (0 = not assessable; 1 = insufficient; 2 = limited; 3 = sufficient; 4 = good; 5 = optimal). After application of the contrast agent, the image quality with respect to tissue perfusion and the sensitivity for detecting possible complications was considered as well (Fig. 7).

2.3. Statistics

All data were analyzed using Sigmasstat 3.5 (Chicago, IL, USA). Values are given as medians with minimum, maximum, 25%- and 75%-percentiles (min; max; $\text{q}_{25}$; $\text{q}_{75}$). Friedman repeated measures analysis of variance (ANOVA) on ranks was performed to detect differences in the median values between the different conventional ultrasound methods. In order to isolate groups that differ from each other, a multiple pairwise comparison using the Tukey test was performed. Scores from contrast-enhanced ultrasound
Fig. 6. Relative blood volume analysis with color coding software application Qontrast® (Bracco, Italy). GE Logiq E9, 6–9 MHz.

Fig. 7. Comparison of CEUS depicted with linear 6–9 MHz transducer and matrix 6–15 MHz transducers. GE Logiq E9.
measurement were analyzed using the Wilcoxon signed rank test. Results obtained from both investigators were additionally compared using the Spearman rank correlation. A p-value of below 0.05 was considered to be significant throughout the paper and is marked with an asterisk in the graphs.

3. Results

None of the microvascular flaps needed to be revised and no graft failure occurred. Furthermore, all patients tolerated the application of the contrast agent without complications. Several types of free flaps showed a typical perfusion pattern matching the corresponding tissue structure. Therefore, high perfusion values were registered for small flaps with a thin subcutaneous fat portion and large anastomosis vessels, e.g. radialis flaps.

3.1. B-scan (investigator 1)

The 6–9 MHz probe in combination with SRI®, Cross Beam® and THI® showed no significant improvement compared to B-scan only. Nevertheless, there was a tendency towards higher scores with additional ultrasound modalities. Broadening the spectrum (Fig. 9) (from 6–9 MHz to 6–15 MHz) resulted in significantly higher (p < 0.05; Table 1) scores than seen for 6–9 MHz B-scan only or 6–9 MHz SRI®

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of the different ultrasound methods</th>
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<td>B-Scan</td>
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<td>SRI Cross Beam (6–9 MHz)</td>
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<td>SRI Cross Beam THI (6–9 MHz)</td>
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<td>SRI Cross Beam THI (6–9 MHz)</td>
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<td>SRI Cross Beam THI (6–15 MHz)</td>
<td>q = 4.938</td>
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<td>CEUS L9 (6–9 MHz)</td>
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<td>CEUS E9 (6–9 MHz)</td>
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Notes: Friedman repeated measures analysis of variance (ANOVA) on ranks showed a statistically significant difference in the median values among the treatment groups (p < 0.001). Multiple pairwise comparisons using the Tukey test were performed to isolate the groups that differ from each other. Difference of ranks (DOR), q-values and respective p-values are given. Boxes left of the diagonal line (represented by empty boxes) show values obtained for investigator 1 (white boxes) and boxes on the right those for investigator 2 (grey background), respectively. Significant results are marked with asterisks and written in bold type. The analysis of the data form both investigators showed the same significant differences between the marked measurement groups except for SRI Cross Beam THI (6–9 MHz) vs. CEUS L9 (6–9 MHz). Data from contrast-enhanced ultrasound were analyzed using Wilcoxon signed rank test. Scores for CEUS E9 were significantly higher than those for CEUS L9 (p < 0.001).
with Cross Beam® (Fig. 8A). Scores for 6–15 MHz SRI® with Cross Beam® and THI® amounted to 4.50
(min = 4.00; max = 5.00; \( x_{25} = 4.00; x_{75} = 5.00 \)) whereas scores for B-Scan only amounted to 3.00
(min = 2.00; max = 3.00; \( x_{25} = 2.00; x_{75} = 3.00 \)). The addition of 6–9 MHz SRI® and Cross Beam®
did not lead to significantly higher scores as compared to B-scan only. Scores for 6–9 MHz B-scan with
SRI® and Cross Beam® amounted to 3.00 (min = 3.00; max = 4.00; \( x_{25} = 3.00; x_{75} = 3.00 \)).

3.2. Contrast-enhanced ultrasound (investigator 1)

The use of GE Logiq E9 resulted in significantly higher \((p < 0.001; \text{Table 1})\) scores than the use of GE
Logiq 9. Scores for GE Logiq E9 amounted to 4.00 (min = 3.00; max = 4.00; \( x_{25} = 4.00; x_{75} = 4.00 \))
and to 3.00 (min = 2.00; max = 3.00; \( x_{25} = 2.00; x_{75} = 3.00 \)) for GE Logiq 9 (Table 2; Figs 8A,
10–13).

3.3. B-scan (investigator 2)

The 6–9 MHz probe in combination with SRI®, Cross Beam® and THI® did not show any significant
improvement compared to B-scan only. However, there was a tendency towards higher scores when
adding the modalities mentioned above. The use of the 6–15 MHz probe (Fig. 9) resulted in significantly
higher \((p < 0.05; \text{Table 1})\) scores than seen for 6–9 MHz B-scan only or 6–9 MHz SRI® with Cross

Fig. 8A. Scores for US assessment by investigator 1. Dashed lines with asterisks denote significant difference \( \ast p < 0.05 \).
Fig. 8B. Scores for US assessment by investigator 2. Dashed lines with asterisks denote significant difference \( *p < 0.05 \).

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<td>SRI, CB, THI 6–15 MHz</td>
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<td>CEUS Logiq E9 6–9 MHz</td>
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<td>Investigator 2</td>
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Notes: Score ranging from 0 to 5 (0 = not assessable; 1 = insufficient; 2 = limited; 3 = sufficient; 4 = good; 5 = optimal). Comparison of SRI®, Cross Beam (CB) and THI in B-scan mode using GE Logiq E9 with 6–9 MHz and 6–15 MHz. Validation of contrast-enhanced ultrasound (CEUS) using GE Logiq 9 and GE Logiq E9 with 6–9 MHz.
Fig. 9. High resolution of details particularly in subcutaneous and middle layers after bolus injection of 2.4 ml SonoVue®. GE Logiq E9, matrix 6–15 MHz.

Fig. 10. Case of a 38-year-old patient with an osteocutaneous flap of the left fore foot. Investigation of the perfusion with CHI after bolus injection of 2.4 ml SonoVue® i.v. GE Logiq 9, 6–9 MHz.
Fig. 11. Case as shown in Fig. 10. CHI after bolus injection of 2.4 ml SonoVue® i.v. New digital imaging techniques with improved depiction of details from subcutaneous to deep layers. GE Logiq E9, 6–9 MHz.

Fig. 12. Case as shown in Fig. 10. Quantitative color-coded perfusion analysis using Qontrast®. Assessment of relative blood flow (RBF) and time to peak (TTP). GE Logiq 9, 6–9 MHz.
Fig. 13. Case as shown in Fig. 10. Quantitative color-coded perfusion analysis using Qontrast®. Assessment of relative blood flow (RBF) and time to peak (TTP) (GE Logiq E9, 6–9 MHz).

Beam® (Fig. 8B). Scores for 6–15 MHz SRI® with Cross Beam® and THI® amounted to 4.00 (min = 4.00; max = 5.00; x_{25} = 4.00; x_{75} = 5.00) whereas scores for B-scan only amounted to 2.00 (min = 2.00; max = 3.00; x_{25} = 2.00; x_{75} = 3.00). The combination of 6–9 MHz SRI® and Cross Beam® did not lead to significantly higher scores compared to B-scan only. Scores for 6 MHz B-scan with SRI® and Cross Beam® amounted to 3.00 (min = 2.00; max = 3.00; x_{25} = 3.00; x_{75} = 3.00).

3.4. Contrast-enhanced ultrasound (investigator 2)

The use of GE Logiq E9 resulted in significantly higher ($p < 0.001$; Table 1) scores than the use of GE Logiq 9. Scores for GE Logiq E9 amounted to 4.00 (min = 3.00; max = 5.00; x_{25} = 4.00; x_{75} = 4.00) and to 3.00 (min = 2.00; max = 3.00; x_{25} = 2.00; x_{75} = 3.00) for GE Logiq 9 (Table 2; Figs 8B, 10–13).

4. Discussion

The detection of capillary perfusion for the evaluation of the microcirculation of tissue grafts poses a challenge for sonographic imaging technology. The quality of conventional US modalities like CCDS or Power Doppler does not meet the stringent requirements for the adequate depiction of such fine structures [7,16].

The use of high resolution linear probes, however, represents the tissue architecture in a much more reliable way and even allows the detection of complications such as lymphedema, local hematomas or
abscess capsules. The assessment of the perfusion using conventional US modalities is only reliable for the anastomosis vessels [7,16]. Even after contrast enhancement, only the arterial and venous vessels can be detected using CCDS or Power Doppler but not the capillary perfusion. CEUS is thus a mandatory requirement for the reliable evaluation of the microperfusion.

The parallel use of fundamental B-scan, CEUS and True Agent Detection Mode® in combination seems to have a markedly favorable effect and makes a dynamic reproduction of the morphology and perfusion possible. The quality of the CEUS subtraction methods is not only dependent on the probe’s technology, but is also strongly influenced by the electronic post-processing procedure. Thus, updated post-processing methods using a 6–9 MHz linear probe seem to substantially improve the detailed detection of the microcirculation of free tissue transplants including the subcutaneous plexus.

The recording of raw digital data as cine sequences makes a quantitative perfusion analysis possible and allows the investigation of several perfusion parameters, e.g. time to peak and relative blood volume [17]. Color-coded perfusion imaging with Qontrast® could facilitate the detection of the perfusion of deeper tissue layers and thus the detection of more poorly perfused areas at an earlier stage. Furthermore, the correlation to the morphology of the tissue is crucial since a fatty fraction is expected to show less perfusion, whereas a muscular fraction with low perfusion would be classified as a serious pathological finding.

Combining Speckle Reduction Imaging® (SRI), Cross Beam® and THI® enables the highlighting of the contours of several tissue structures. Initially after surgery, the use of SRI®, Cross Beam® and THI® seems to be beneficial for identifying lymphedema, hematoma or signs of an incipient infection [1–7]. In addition, combining CEUS with the perfusion analysis facilitates the detection of such postoperative complications since hyperperfusion can differentiate an infection from a hematoma, which regularly shows low perfusion.

Particularly when patient positioning is restricted, or metal clips or other fixtures inhibit other diagnostic modalities, the high resolution US with perfusion analysis is a reliable option for postoperative monitoring.

Currently, the best way to represent the perfusion is using a 6–9 MHz probe which offers the highest quality available in harmonic imaging techniques. In the future, the development of a high resolution US probe compatible with contrast agents would be ideal. Presently, modern technological development is moving towards CEUS using transmission frequencies up to 15 MHz, which would allow a more detailed imaging of the vessel plexus, but must also incorporate the ability to analyze the deeper tissue layers as well.

5. Conclusion

To date, the combination of high resolution B-scan with SRI®, Cross Beam® and THI® using transmission frequencies of 6–15 MHz as well CEUS using digital perfusion analysis seems to be the most effective and reliable option for tissue graft monitoring.

References


