doi: 10.48095/ccachp2022116

3D color doppler ultrasound for postoperative monitoring of vascularized lymph node flaps

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Summary

Background: Vascularized lymph node transfer (VLNT) is a relatively well-established microsurgical treatment for lymphedema that is especially beneficial for advanced cases in which lymphovenous anastomosis is not indicated due to lymphatic vessel sclerosis. When VLNT is performed without a skin paddle, such as a buried flap, the possibilities for postoperative monitoring are limited. The aim of our study was to evaluate the use of ultra-high-frequency color Doppler ultrasound with 3D reconstruction in a pedicled axillary lymph node flap. **Methods:** Flaps were elevated in 15 Wistar rats based on the lateral thoracic vessels. We preserved the axillary vessels to maintain the rats' mobility and comfort. The rats were divided into three groups as follows: Group A, arterial ischemia; group B, venous occlusion; and group C, healthy. **Results:** Ultrasound and color Doppler scan images revealed clear information on flap morphology changes and pathology if it was present. Surprisingly, we detected venous flow in group A rats, supporting the pump theory and venous lymph node flap concept. **Conclusion:** We conclude that 3D color Doppler ultrasound is an effective method for monitoring buried lymph node flaps. 3D reconstruction makes it easier to visualize the flap anatomy and detect pathology if it is present. Moreover, the learning curve for the technique is short. Our setup is user-friendly even in the inexperienced hands of a surgical resident, and images can be reevaluated at any time if necessary. The use of 3D reconstruction removes the complications associated with observer-dependent monitoring of VLNT.

Key words

lymphedema – microsurgery – VLNT – ultrasound – dopller – monitoring

Jonas F., Alejandro K.D.P., Kesa P., et al. 3D color doppler ultrasound for postoperative monitoring of vascularized lymph node flaps. Acta Chir Plast 2022; 64(3–4): 116–120.

Introduction

Vascularized lymph node transfer (VLNT) is a relatively well-established microsurgical treatment for lymphedema and is especially beneficial for advanced stages, where suitable lymphatics to perform lymphovenous anastomosis (LVA) are not available [1]. VLNT is a composite free flap containing vascularized lymph nodes and perinodal fat. The flap can be harvested with or without a skin paddle, depending on the donor site or preferences.

Plastic surgeons have developed a variety of techniques over the years for postoperative monitoring after free tissue transfer. However, most of the monitoring techniques, such as clinical observation, cannot be used for buried flaps. The only possible clinical monitoring procedure for buried flaps to date is a limited assessment for indirect signs of flap failure, such as swelling. Swelling might be apparent only when the flap contains sufficient tissue [2]. Other specific indirect signs of a successful transfer, such as circumference and skin tightness reduction, are not helpful because these signs are not immediately apparent in case of VLNT [3,4].

3D Color Doppler is a well-known technique to evaluate the pattern of blood vessels and takes advantage of the already three-dimensional (3D) data in the summation images [5,6]. Color Doppler is gaining popularity among microsurgeons for the planning of reconstructive microsurgeries and free flap monitoring. Currently, Color Doppler is the gold standard for the monitoring of buried free flaps. Another viable option is the use of an implantable Doppler, although this has previously been associated with a high false positivity rate [7].

We evaluated 3D ultra-high-frequency ultrasound with color Doppler in an animal model of pedicled axillary lymph node flaps in our search for an ideal method for VLNT monitoring [8–10]. Ultra-high-frequency ultrasounds are relatively new but are gaining popularity among microsurgeons, especially among those performing lymphatic micro-and supermicrosurgery. These devices can provide images with very high resolution up to 30 μm. This level of resolution also allows clear visualization of the lymphatic vessels [11].

Materials and methods

In our experimental study, we used a rat model with a buried axillary lymph node flap pedicled on the lateral thoracic artery and vein. Ultrasound imaging in a B-mode, color Doppler and pulsed wave Doppler (PWD) allowed for the noninvasive monitoring of the pedicled axillary lymph node flaps of three groups of rats, namely, group A (arterial ischemia), group B (venous occlusion) and group C (healthy).

B-mode (brightness mode) ultrasound can be viewed as a two-dimensional image and provides structural information. Color Doppler allows for the measurement of the blood flow through the flap. In our experimental study we focused on the blood flow through the flap pedicle, hilar blood flow of the lymph nodes and so was evaluated their viability. The anatomical study of lymph node flaps was performed assessing the flap morphology including the volumetry and lymph node count.

Animal model

Female Wistar rats (body weight 210–320 g, age range of 45–60 days) were purchased from the Charles River Laboratory, Germany. All experiments were approved by the Institutional Animal Care and Use Committee (MSMT-9993/2017-4). The rats were housed in the animal facility of the Center for Advanced Preclinical Imaging in individually ventilated cages (Tecniplast Inc., Buguggiate VA, Italy) (12/12 light/dark cycle, 22 ± 1 °C, 60 ± 5% humidity) and supplied with food and water *ad libitum*.

Surgical techniques

The rats were anesthetized with a mixture of 50 mg/kg ketamine and 10 mg/kg xylazine prior to the surgical intervention. The animals were then placed in the supine position, the region of the right axilla was shaved, and the skin was disinfected by an antiseptic alcohol solution. A short incision was made in the anterior axillary line. The pectoralis muscle was identified and retracted, and dissection was performed in a subpectoral plane. The flap was meticulously dissected from the surrounding tissue after identifying the axillary lymph nodes in the perinodal fat. The lateral thoracic artery and vein were identified distal to the flap, and dissection of the flap was performed in a flow-through fashion. The lateral thoracic artery and vein were ligated and transected distally to the flap. The distal part of the flap was mobilized, and all muscular branches were identified, ligated and transected in a distal-to-proximal manner. The flap was then gently retracted to visualize the brachial and axillary vessels and the origin of the lateral thoracic vessels. The gentle traction allowed us to avoid exceeding the incision beyond the level of the clavicle. The proximal part of the pedicle was then dissected from the adventitia to simulate a microsurgical approach. Once the flap was elevated, the flow through the pedicle was assessed using an operating microscope (Fig. 1).

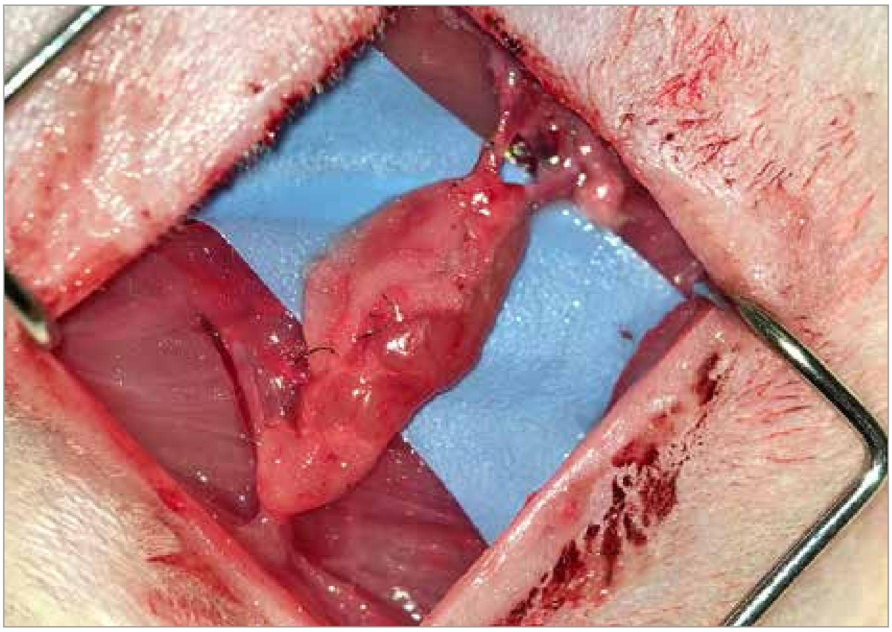

Fig. 1. Vascularized axillary lymph node flap elevated with all branches meticulously ligated. Lymph nodes in perinodal fat with a vascular pedicle based on the lateral thoracic bundle are clearly visible.

Rat models of VLNT

The lateral thoracic artery was ligated to simulate arterial ischemia in group A rats (N = 5). The lateral thoracic vein was ligated to simulate venous occlusion in group B rats (N = 5), and the pedicle was left intact in group C rats (N = 5), which served as healthy controls. The apex of the perinodal fat was fixed by a single stitch to the subcutaneous tissue at the level of the lower pole of the incision to ensure a constant flap position during follow-up and to avoid pedicle kinking. Finally, the wound was flushed with saline, and the skin was closed with monofilament, nonabsorbable polypropylene 4-0 interrupted sutures (Resorba, Germany).

Ultrasound imaging

The rats were kept fully anesthetized during the measurements by using an isoflurane vaporizer system (Vevo Compact Dual Anesthesia System) that pro-

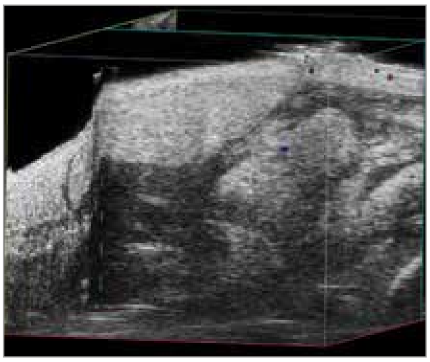

Fig. 2. Flap of a rat with simulated arterial ischemia. Low venous blood flow was detected inside the flap in a color Doppler mode.

vided 1.2 L/min airflow with 3% isoflurane for the initial anesthesia and 2% evaporated isoflurane during the measurements.

Ultrasound imaging was performed using a Vevo 3100 high-frequency micro-ultrasound system (FUJIFILM, VisualSonics, Inc., Canada). The rats were positioned on a heated platform (FUJIFILM, VisualSonics, Inc., Canada) to enable the monitoring of vital functions (ECG and breathing) during imaging. All four paws were conductively connected with four electrodes, and the table temperature was set to 37.7 °C. The rats were carefully shaved before imaging using a shaver and depilatory gel to avoid skin artifacts. A bubble-free ultrasound gel was applied to facilitate ultrasound transmission from the transducer to the skin. A linear transducer Mx400 (FUJIFILM, VisualSonics, Inc., Canada) (50 µm / 110 µm axial/lateral resolution, 30 and 24 MHz center frequency at B-Mode and Color Doppler Mode, respectively) was chosen and placed on the area of interest and held in position by a clamp mounted on the Vevo Rail System to enable motorized scanning with an adjusted step size of 110 µm. Respiratory gating was enabled to minimize artifacts from breathing during the scan, and the persistence function (averaging of images) was set to the maximum. Color Doppler images were acquired as follows: sensitivity, 5; gate, 3; PRF, 9-12; power, 100%; Doppler gain, 37 dB; Doppler angle, 0 or –15°; and wall filter, low. Pulsed wave Doppler (PWD) was utilized to measure blood flow using the following settings: Doppler gain, 35 dB; PRF, 3–15 (depending on blood flow velocity); and beam angle, 0 or –15°. The Doppler angle was adjusted individually for each measured flow.

The proposed monitoring was performed immediately after surgery, 24 hours after surgery, and one week postoperation.

All obtained records were postprocessed using VevoLAB software, and the numerical data obtained by B-Mode and color Doppler were expressed as the mean ± SD.

Results

During the first two measurements (immediately after surgery and after 24 hours) we observed the presence of artifacts due to the presence of the residual air and suture material. Although the flap viability evaluation was possible in the indicative way, we decided to use the measurements obtained one week postoperation for our study purposes to provide reliable data.

Group A – arterial ischemia

The lymph node flaps of rats in the induced arterial ischemia group were analyzed using postprocessing software, and a volumetric study was performed. The flaps of the rats in group A had an average volume of 241 ± 95 mm³ (mean ± SD). The mean venous flow in the flap pedicle was –324.4 ± 108.3 mm/s, and virtually no flow inside the nodes was measured (Fig. 2).

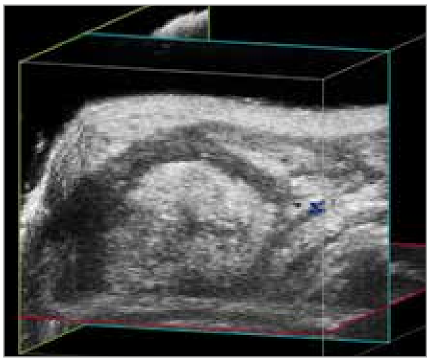

Fig. 3. A 3D visualization of the flap in animals with simulated venous occlusion. Color Doppler mode did not detect any blood flow within the whole flap.

Group B – venous occlusion

The average volume of the flaps of the rats in group B was 252 ± 68 mm³. No measurable blood flow in nodes was observed in rats with evoked venous occlusion. We did not detect any measurable flow in rats in group B in either the pedicle vein or the hila of the axillary lymph nodes (Fig. 3).

Group C – healthy group

A flap volume of 188 ± 35 mm³ was observed in group C rats, which was lower than that of group A and B rats. Inside each flap, 6.5 ± 1.7 (mean ± SD)

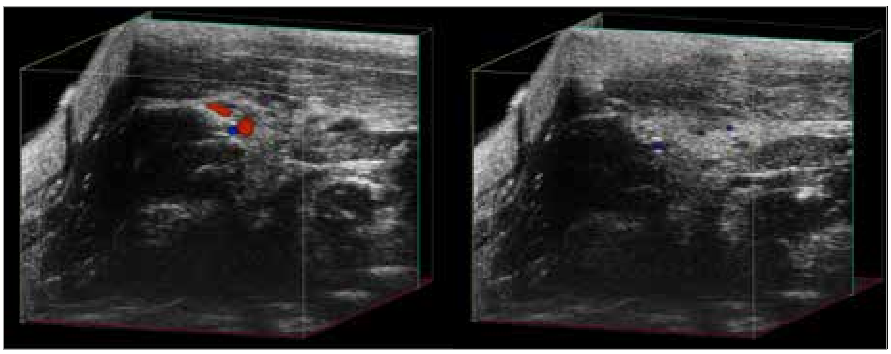

Fig. 4. Color Doppler imaging indicated significant blood flow in the pedicle (left) of the healthy rat flap (control group C) and vascularized lymph nodes inside the flap (right).

vital lymph nodes were detected in group C rats with a measurable Doppler mean velocity of 182.1 ± 69 mm/s. The mean velocity of the blood flow in the pedicle of group C rats was −432.1 ± 112.8 mm/s while the higher blood flow was observed in the axillary vein (−587.6 ± 32.8 mm/s) and in the artery (−436.9 ± 174.2 mm/s). We detected non-compromised blood flow throughout the lateral thoracic bundle, including hilar vessels of the axillary lymph nodes, in group C rats (Fig. 4).

Discussion

Our study demonstrated that 3D color Doppler ultrasound is a powerful and reliable tool for the monitoring of VLNT. Surprisingly, we detected venous flow in group C, the model of arterial ischemia. This finding supports the pump theory, and the presence of hilar and lymph node microcirculation acts as a proof-of-concept for the venous lymph node flap approach [12,13]. We did not detect any blood flow in any of the measurements of rats with venous occlusion. These findings are supported by a study showing the difference between arterial ischemia and venous occlusion in VLNT. Venous occlusion has been proven to be more harmful to lymph nodes due to blood stasis and extravasation, and arterial ischemia has also been found to have a tendency toward drainage recovery [14]. However, more studies are needed to clearly understand the lymph node pathophysiology involved in VLNT.

We decided to begin US/color Doppler imaging one week after the procedure due to the artifacts originating from the presence of free gas after microsurgery and to avoid artifacts from stitches.

The findings of our study are easily transferable to clinical settings. Imaging in humans is easier because of the size of the structures, which allows for easier orientation and flap structure visualization. The limitation of the present study is the use of an animal model, although our model has been proven to be reliable in previous studies. Additionally, the transfer of thorough flap monitoring to daily practice might be challenging for the involved personnel, regardless of how fast the monitoring sessions are.

High-frequency ultrasounds are currently used by lymphedema microsurgeons for lymphatic sclerosis classification and LVA planning and can be used without ICG lymphangiography [15,16]. High-frequency ultrasound is also widely used for perforator flap planning, speeds up processes of pedicle dissection and perforator flap raising, and allows for the harvesting of thin and ultra-thin flaps [17,18].

The obtained 3D reconstructed flap images provided clear information on flap anatomy and circulation. The critical arterial ischemia time of the lymph node has been reported to be 5 hours, and in one case of venous occlusion, it was reported to be 4 hours [14,19]. Therefore, if there are any concerns about flap perfusion, immediate surgical revision should be performed promptly. Authors are beware that the selected timepoints of measurement would be insufficient in clinical settings for potential flap salvage, however for the experimental study we found this timing to be adequate and efficient for the study.

The advancement of 3D reconstruction allows for the assessment of ultrasound scans at any time and at any location because it provides a clear image of the whole flap and surrounding tissues. The 3D scans could theoretically be obtained by any medical professional with proper training. Additionally, preoperative radiological analysis of the donor site and the quantity of lymph node assessment are helpful to improve functional outcomes. It has been reported that the lymph node count positively correlates with flap function and, therefore, limb circumference reduction [20].

One of the biggest advantages of this technique is the potential for performing the telemonitoring of any buried free flap.

Conclusions

We conclude that 3D color Doppler ultrasound is an effective method for the monitoring of buried lymph node flaps. 3D reconstruction makes it easier to visualize the anatomy, is user-friendly even in the inexperienced hands of a surgical resident, and images can be reevaluated at any time if necessary. The use of 3D reconstruction removes the complications associated with observer-dependent monitoring of VLNT.

Disclosure statement: All procedures performed in this study involving human participants were in accordance with ethical standards of the institutional research committee and with the Helsinki declaration and its later amendments or comparable ethical standards.

Funding: This work was supported funded by the Ministry of Health of the Czech Republic, grant number AZV 17-29084A

Roles of authors

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Karla D. P. Alejandro – ultrasound operator, data collection and analysis

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Lukas Lambert and Andrea Burgetova MD, PhD. – radiological advice

Ludek Sefc – data analysis, management of the study

Pavla Ticha – data analysis, consultation

Ondrej Mestak – study design, data collection and analysis, management of the study

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Submitted: 21. 1. 2022
Accepted: 9. 11. 2022