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Could MRI visualize the invisible? An Italian single center study comparing magnetic resonance lymphography (MRL), super microsurgery and histology in the identification of lymphatic vessels

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Abstract. – OBJECTIVE: Aim of this study is to evaluate the possibility of limb magnetic resonance lymphography (MRL) to differentiate lymphatic vessels from pathological veins, collect a specimen of the identified lymphatic vessel during operations of super microsurgical lymphatic-venular anastomosis (s-LVA) and perform immunohistochemical stainings to confirm the nature of the collected vessels.

PATIENTS AND METHODS: Twenty patients presenting lymphedema were enrolled in this study. Five patients reported lower limb lymphedema and 15 patients reported upper limb lymphedema. All patients had the indication for s-LVA and underwent preoperative MRL imaging of the affected limb. A total of 57 lymphatic vessels were identified by MRL and used to guide s-LVA: all these vessels have also been used to perform an intraoperative biopsy for immunohistochemical evaluation.

RESULTS: A total of 53/57 vascular structures resulted compatible with lymphatic vessels at the immunohistochemical study performed with D2-40 antibody; 3/57 specimen showed the absence of the D2-40 antibody. A significant association was found between preoperative MRL and immunohistochemical marker D2-40 on collected specimen.

CONCLUSIONS: Most of the articles in the international literature report the concomitant presence of both lymphatic and venous vessels at MRL. However, no one in literature describes the possibility to differentiate venous vessels from lymphatic vessels, and this is a crucial issue for the correct evaluation of the lymphatic system in patients with limb lymphedema undergoing a future surgical correction. In the present study, MRL allowed to identify active lymphatic vessels. MRL was predictive to determine preoperatory lymphatic vessels and to perform successful s-LVA in lymphedema patients. This is the first study to prove the nature of the vessels identified at the preoperative MRL with immunohistochemical stainings.

Key Words:
Lymphedema, MRL, D2-40.

Introduction

Lymphedema is a pathology characterized by a limb accumulation of subcutaneous protein-rich fluid due to a lymphatic system disorder. While primary lymphedema is characterized by congenital abnormalities, secondary lymphedema is caused in most cases by obstruction or stenosis of lymph vessels caused by surgical oncology[1].

Lymphatic-venular anastomosis (LVA) is a microsurgical technique effective to improve limb circumference and to alleviate dermal sclerosis[2,3]. Super microsurgical lymphatic-venular anastomosis (s-LVA) is an advanced technique that allows to perform an anastomosis on even smaller vessels, and showed good results in lymphedema patients to reduce limb circumference and cellulitis[4].

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To perform LVA or s-LVA, the preoperative lymphatic mapping is necessary for surgical planning: the ideal imaging technique to evaluate lymphedema has been, for many years, lymphoscintigraphy, using a radionuclide with various ^{99m}Tc-labelled molecules[5]. Recently, Indocyanine Green (ICG) lymphography has been introduced[6].

Magnetic Resonance Lymphography (MRL) performed with injections of gadolinium-based contrast agent has been widely described in the literature with gadolinium contrast enhancement, injecting the contrast in the interdigital spaces. Its use is mainly related to the lymphedema and the imaging of the lymphatic system, and it is crucial to understand lymphatic vessels role in the disease development and to plan the operation. The main difficulty of MRL imaging is the differential diagnosis between lymphatic vessels and venous vessels.

In the present paper, the authors have intended to verify the accuracy of preoperative MRL performing a biopsy of the lymphatic vessels for immunohistochemical study (D2-40 antibody) during operations of s-LVA in lymphedema patients.

Patients and Methods

The present study included 20 female patients with a mean age of 57.6 years old affected by lymphedema (3 cases of primary lymphedema, 17 cases of secondary lymphedema). These patients underwent to a MRL before operation of s-LVA. During the s-LVA operations specimen of the MRL-presumed lymphatic vessels were collected and sent to the laboratory for immunohistochemical stainings.

Magnetic Resonance Lymphography

Before MRLA, interdigital injection of gadolinium-based contrast agent was performed using the most commercially available and widely diffused paramagnetic contrast medium: Gadobenate Dimeglumine (Gd-BOPTA, Multihance, Bracco Imaging, Milan, Italy). The solution to inject was prepared with 15 mL of gadobenate dimeglumine (that corresponds to one bottle) and 1.5 mL of lidocaine (1% solution), then the mixed agent was injected intracutaneously into the interdigital webs of the dorsal foot, with four injections for each limb. The volume injected into each point was 0.7 to 0.8 mL.

All magnetic resonance (MR) exams were performed with a 1.5 Tesla MR unit (Signa Twin Speed Hdxt; General Electric Healthcare, Milwaukee, WI, USA), with a maximum gradient strength of 23 mT/m and a slew rate of 80 mT/m/ms (software release 15.0_0947A). Patients were supine, feet first, with both legs on a ramp pillow in order to obtain a parallelism with the main magnetic field and to position them on the most homogeneous area of the B0. To obtain a large anatomical coverage and a good signal-to-noise ratio, a 7000-elements phased array peripheral vascular receiving coil (Flow 7000) for the study of the lower extremity and a 8 channel Body Array for the upper extremity were used; both of them are built by USA Instrument. The fingers appeared from the holes of the coil, letting them to be out for an easy access during the injections of the contrast agent. To reduce the hyperintensity artifacts we paid attention to avoid direct contact of the coil surface with the patient extremity, using some small pillows. Patients were instructed on the procedure in order to obtain complete collaboration.

After positioning a survey and calibration from all the stations, three for the lower extremity (foot-ankle calf, calf knee, thigh hip) and two or three for the upper extremity (hand-wrist forearms, elbow arm shoulder) were performed. Subsequently, before the injection of the contrast agent, a coronal 3D SSFP Balanced (Fiesta, GE) ECG-triggered with spectral fat saturation (SPECtral Inversion At Lipidi, SPECIAL, GE) was acquired; the technical parameters used were: TR 4.0 ms, TE 1.9 ms, TI 90 ms, FOV 40 × 40 cm, Matrix 224 × 192, slice thickness 2.2 mm, NEX 0.53 (Half Fourier) and Bandwidth kHz. The ECG trigger was acquired with a Peripheral Gating (PG, GE) and a Delay Time set for a systolic phase acquisition in order to obtain a no contrast venography (the high flow artery experienced some flow dephasing reducing their signal); we also obtained a good image to visualize the lymphoedema.

For dynamic MRL, 3D fast spoiled gradient-recalled echo T1-weighted images with a fat saturation technique (T1 high-resolution isotropic volume excitation) were acquired in every station at 5, 10, 15, 20, 25, 30, 35 and 40 minutes approximately after contrast injection. The technical parameters were: f TR/TE 5.0 ms/2.1 ms; TI 17 ms, flip angle 25 , FOV 480 × 480 mm, matrix 448 × 320, slice thickness 2.2 mm, NEX (signal average number) 2 and Bandwidth kHz, acquisition time 0 min 40 sec. The 3D MRL were then reconstructed from the post-contrast

coronal images at each time point using a MIP technique. The exam ended acquiring, in every station, a coronal 3D Rapid Acquisition with Relaxation Enhancement heavily T2-weighted with Driven Equilibrium, in order to reduce the acquisition time by reducing the Repetition Time without affect the images, (FRFSE T2, GE) with the following parameters: TR 2000 ms, TE 679 ms, FOV 48x48 cm, Matrix 320 × 256, Thickness 3.6 mm, NEX 1 and Bandwidth kHz; with these images the lymphoedema is clearly visible and measurable. The examination time for one patient was approximately 1.5 hours. No systemic or local complications were observed during or after the examination.

Image Interpretation

The images analysis was then performed using Multi Planar Reconstruction of the subtracted images and with Thin-Slab Maximum Intensity Projection in order to better visualize the lymphatic vessels.

Super Microsurgery

After the visualiation of the lymphatic vessels using MRLA, superficial veins mapping with Accuvein device was performed to identify the microsurgical sites. Under local anaesthesia with 1% xylocaine containing adrenalin a skin incision was performed on each site, subcutaneous tissue was dissected under a high magnification operating microscope. Lymphatic collectors and adjacent subcutaneous veins were identified. Vessels were dissected and 4 or 5 11/0 endoluminal sutures with a 50-micron needle were performed between lymphatic and venous vessels (Figure 1). Afterwards, the skin was stitched up with continuous 6/0 resorbable microsutures.

Immunohistochemistry

Specimens were formalin fixed and paraffin embedded. Consecutive sections were obtained from each sample, dewaxed with xylene and rehydrated in descending ethanol series. Endogenous peroxidases were blocked with 3% hydrogen peroxide. Non-specific binding sites were blocked by a 5 min incubation with Blocking Reagent (IHC SelectTM kit, Millipore, Billerica, MA, USA) followed by a brief wash in PBS (Phosphate Buffered Saline). The sections were then incubated overnight with the primary antibody D2-40, a monoclonal IgG1 specific for the lymphatic endothelium (Dako, Santa Clara, CA, USA, diluted 1:20 in PBS containing 1% bovine serum albumin)[7]. The sections were then incubated 10 min in the dark with the secondary antibody (biotinylated goat anti mouse IgG of the same kit). The reaction was revealed by a 10 min incubation with streptavidin horseradish peroxidase diluted in Tris Buffered Saline followed by a 2 min incubation with 3,3'-diaminobenzidine Substrate Kit for Peroxidase (VECTOR), which contains a nickel solution that converts the brown color characteristic of 3,3'-diaminobenzidine in black. Sections were counterstained with Mayer's hematoxylin and mounted with Eukitt (Sigma, St Louis, MO, USA).

Data Analysis

After data acquisition, image post-processing and subsequent analysis were performed by two experienced radiologists, who reached an agreement by consensus. At MRLA examination affected lymphatic vessels were distinguished from a vein because of their caliber (diameter) and their morphology.

Differences between groups were evaluated using chi-square test. Association between variables were tested by univariate regression analysis. p-values < 0.05 were considered to be significant if not otherwise specified.

Results

No complications were observed after the examination, in particular, no complications were observed during or after intracutaneous injections of Gd-BOPTA. The lymphedema showed an epifascial distribution with a high signal intensity on Coronal 3D SSFP Balanced images. Affected lymphatic vessels were distinguished from veins because of their caliber and their morphology; in particular the diameter of the ectasic lymphatic vessels was smaller than the one of the adjacent vein and greater than the one of the lymphatic vessels of the unaffected extremity when visualized, whereas the morphology was tortuous (beaded appearance) in comparison with the morphology of the veins. The beaded appearance of the lymphatic vessels extending from the injection site was reliably detected 5-10 minutes after the injection and, in the majority of the cases, the lymphatic vessels could be detected, with the strongest enhancement at 35-40 minutes after contrast injection. Collateral vessels with dermal backflow (an area of progressive dispersion of the contrast

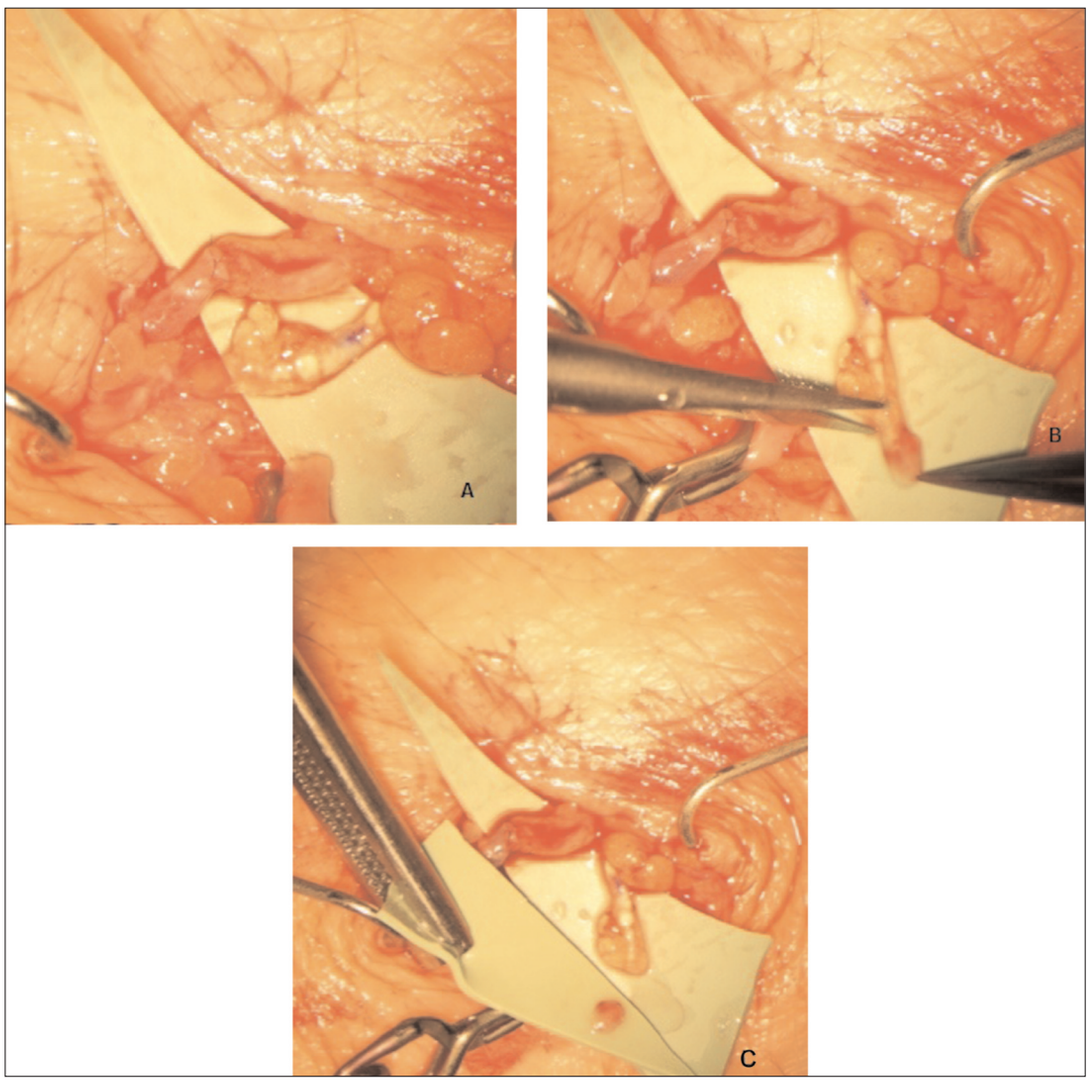

Figure 1. Intraoperative image of the super microsurgical lymphatic-venular anostomosis (s-LVA) during the collection of biopsy specimen.

medium between lymphatic vessels in the soft tissues), indicating proximal lymph flow obstruction with alternate pathways of transport, were generally seen after 15-20 minutes (Figure 2). The mean diameter of a dilated lymphatic vessel was 2.20 ± 0.5 mm. The mean diameter of a venous vessel was 2.4 ± 0.2 mm.

No complications were showed after s-LVA; mean hospitalization was 2 days. No correlation between age and results was found. A total of 57 (2.85 per patient mean) lymphatic vessels were identified at the preoperative MRL; among these 53/57 specimen collected of these vessels resulted positive at the immunohistochemical marker D2-40 (Figure 2, Figure 3). A significant association was found between preoperative MRI and immunohistochemical marker D2-40 on collected specimen (Chi-square = 40.421, DF = 1, Significance level $p < 0.0001$, contingency coefficient 0.644).

Discussion

Lymphoscintigraphy has been for many years the gold standard imaging in lymphoedema patients; however, radiation exposure, long examination time needed, side effects such as pulmonary embolism have limited its clinical applicability[8].

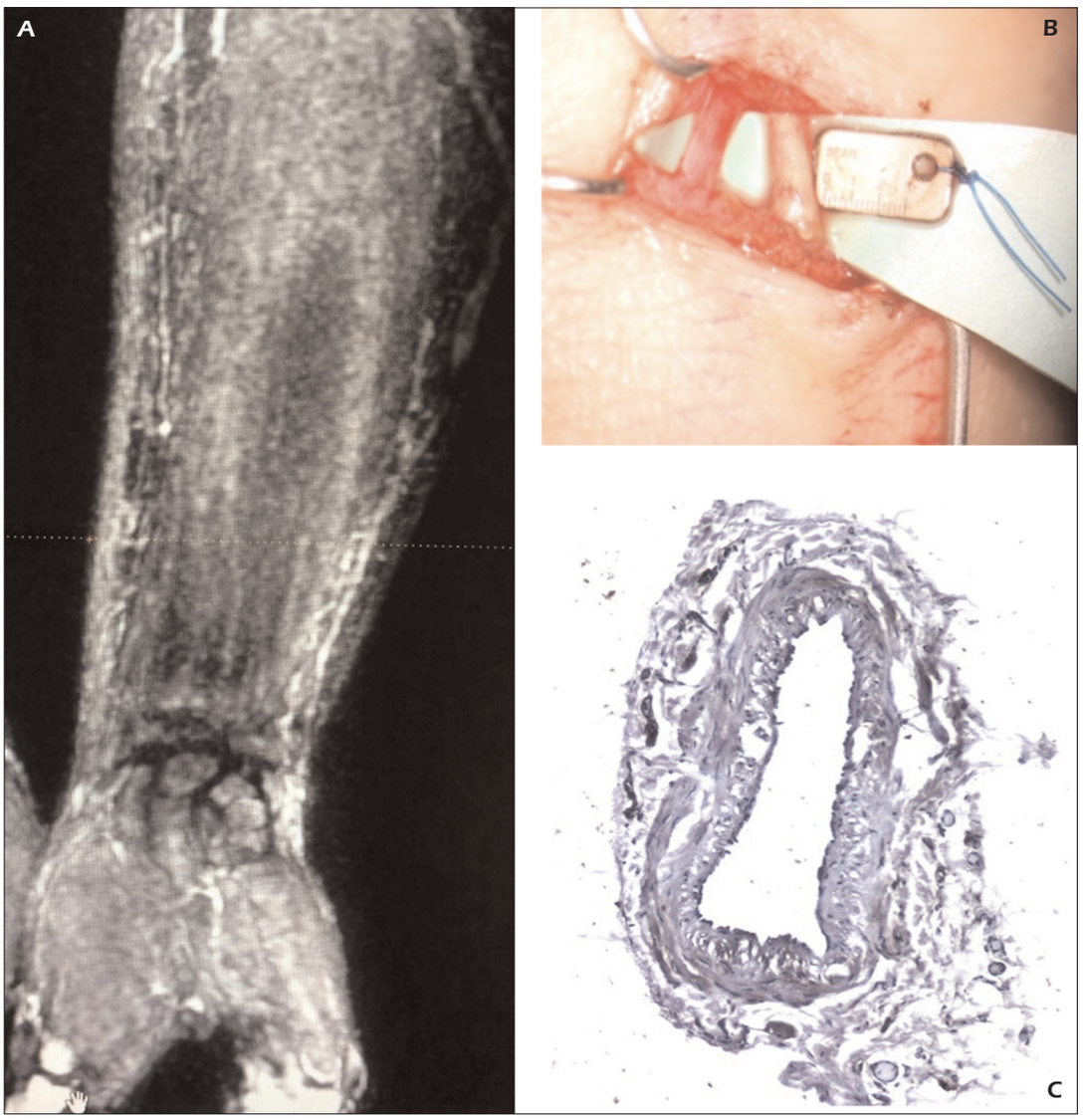

Figure 2. ***A,*** Preoperative magnetic resonance lymphography (MRL) of the affected limb. ***B,*** Intraoperative s-LVA image. ***C,*** Histological staining of the collected lymphatic vessel.

Lohrmann et al[9-11] in their papers reported the visualization of venous vessels at MRL, as contrast may be captured by both lymphatic and venous capillaries: venous vessels resulted to be contrast-enhanced faster than lymphatic vessels, which resulted slower.

At the Lymphoedema Mondial Congress in Rome in 2013, and at the International Lymphoedema Congress in Genova in 2014, many criticisms have been raised against the use of the MRL and the potential discrimination between lymphatic and venous vessels.

Most of the articles present in the international literature report the concomitant presence of both lymphatic and venous vessels in MRL. Lohrmann et al[7] and Ruehm et al[12] reported some suggestions to differentiate lymphatic and venous vessels. However these criteria may be not enough, due to the concomitant dysfunction of deep and superficial venous circulation, the identified structures may easily be dysfunctional venous vessels (closed or thrombosed veins, varicose veins or valvular insufficiency). Further resonance imaging of lymphatic vessels may be even more doubtful on nonedematous limbs[9]. The most of the published papers suppose to identify the lymphatic vessels mostly due to their ectasic morphology, without any laboratory confirmation.

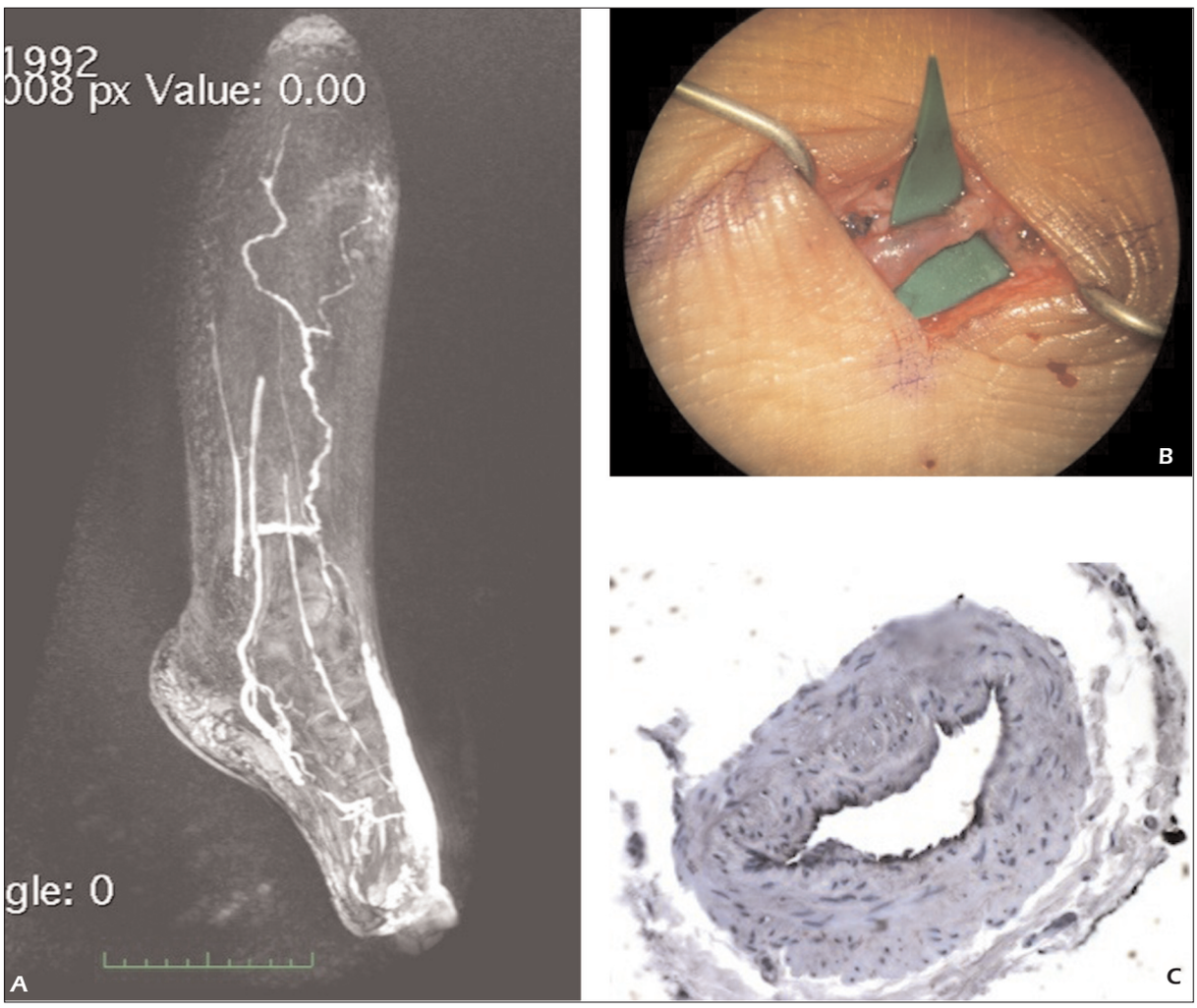

Figure 3. Another case with preoperative MRL of the affected limb. ***B,*** Intraoperative s-LVA image. ***C,*** Histological staining of the collected lymphatic vessel.

MRL imaging is a relatively new technique used to map the lymphatic vessels injecting contrast material, accompanied by high-resolution sequential 3D imaging of the affected organ. It is well known that the advantage of this technique is that it allows dynamic monitoring of the lymphatic transit with simple and minimally invasive high-spatial- and high-temporal-resolution imaging techniques to visualize the lymphatic system. However, no one in literature describes the possibility to differentiate venous vessels from lymphatic vessels and this is a crucial issue to correctly evaluate the lymphatic system in patients with limb lymphedema undergoing future surgical correction[13,14].

S-LVA is a super microsurgical technique effective to improve limb circumference, alleviate dermal sclerosis and reduce cellulitis in lymphoedema patients[2,3,15]: to perform s-LVA preoperative lymphatic mapping is necessary for surgical planning.

Our criteria for differential diagnosis between venous and lymphatic vessels were mainly the caliber, the morphology and the beaded appearance of the vessels. The study of the unaffected limb has been essential for the imaging and differential diagnosis: diameter of the ectasic lymphatic vessels was smaller than the adiacent vein and greater than the lymphatic vessels of the unaffected extremity. In comparison with others authors that referred their experience in MRL, their reported criteria to distinguish veins and lymphatic vessels may be not effective, and especially may not be effective on healthy limbs. Liu et al[16] evidenced only 5/23 cases of contrast-enhanced lymphatic vessels with MRL.

In this paper, we performed a biopsy to collect a specimen of the lymphatic vessels presumed at the MRL. Most of these specimen resulted to be lymphatic vessels thanks to immunohistochemical stainings. For this reason, our criteria resulted to be significantly effective limited to the cases of this study: during s-LVA, we found a significant number of lymphatic vessels on the basis of preoperative MRL images. Mitsumori et al[17] reported their experience of MRL on four consecutive patients. Mitsumori et al[17] performed an intravenous systemic and subdermal injection of Gd-based MR contrast and supported the idea to identify all venous vessels that, with the technological removal of these from the structures evidenced with subdermal contrast, should leave only the lymphatic vessels[17]. However, it is possible that a thrombosed vein (e.g. a tied saphenous vein) could receive contrast from a cutaneous injection but not from systemic circulation. Further, no histological evidence of the identified vessels was reported.

MRL with the proper criteria is a feasible technique that studies the limb lymphatic system with the use of commercially available contrast agents. This technique offers a newer minimally invasive procedure to visualize the lymphatic system anatomy with the aim to guide surgical operation. However, further studies enrolling more patients should be performed to determine the kinetics of gadolinium transport in disease states with the high lymphatic flow.

Present diagnostic tools in order to quantify the lymphoedema are limb circumference, self-assessment questionnaires and water displacement[18]: MRL may find its clinical usefulness in the follow-up of the s-LVA and to evaluate the flow into the anostomosis[19]. Further, screening of oncological non-symptomatic patients could highlight possible latent lymphoedema and suggest conservative treatments or super microsurgical noninvasive surgery: treatment in initial lymphoedema has a significantly better prognosis than in the advanced ones[20].

Conclusions

In the present work, MRL revealed to be predictive to identify preoperatory lymphatic vessels and to perform successfully s-LVA in lymphoedema patients. A significative number of lymphatic vessels were found during s-LVA on the basis of preoperatory MRL images. This is the first single-center study that compares super microsurgery, magnetic resonance and immunohistochemistry in order to perform differential diagnosis between lymphatic and venous vessels: our significative results add credit to the imaging technique and let us define MRL as a possible standard diagnostic imaging technique for lymphedema patients.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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