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Role of the Cadaver Lab in Lymphatic Microsurgery Education: Validation of a New Training Model

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ABSTRACT

Background: Microsurgical transplantation of vascularized lymph nodes (VLNT) or lymphatic vessels (VLVT) alongside derivative lymphaticovenous procedures are promising approaches for treatment of lymphedema. However, clinically relevant training models for mastering these techniques are still lacking. Here we describe a new training model in human cadaver and validate its use as training tool for microsurgical lymphatic reconstruction.

Methods: 10 surgeons with previous exposure to microsurgery were trained in a controlled environment. Lymphatic vessel mapping and dissection in 4 relevant body regions, harvesting of five different VLNTs and one VLVT were performed in 5 fresh-frozen cadavers. The number of lymphatic vessels and lymph nodes for each VLNT were recorded. Finally, the efficacy of this model as training tool was validated using the Dundee Ready Education Environment Measure (DREEM).

Results: The average cumulative DREEM score over each category was 30.75 (max = 40) while individual scoring for each relevant category revealed highly positive ratings from the perspective of teaching (39.3), training 40.5 (max = 48) and self perception of the training 30.5 (max = 32) from all participants. The groin revealed the highest number of lymphatic vessels (3.2 ± 0.29) as all other regions on the upper extremity, while the gastroepiploic VLNT had the highest number of lymph nodes (4.2 ± 0.37).

Conclusions: This human cadaver model represents a new, reproducible “all-in-one” tool for effective training in lymphatic microsurgery. Its unique diligence in accurately reproducing human lymphatic anatomy, should make this model worth considering for each microsurgeon willing to approach lymphatic reconstruction.

Introduction

Extremity lymphedema causes significant morbidity in terms of physical limitations and infection risk [1, 2]. The development of microsurgical techniques (lymphaticovenous anastomosis - LVA) to restore lymph flow by bypassing obstructed lymphatic pathways into subdermal venules have offered a functional repair for the disease with consistent clinical outcomes reported world-wide [3–5]. More recently, vascularized lymph node transfers (VLNT), vascularized lymphatic vessels transfers (VLVT) or VLNT combined with simultaneous LVA have emerged as novel microsurgical approaches for lymphatic reconstruction [6, 7]. Although the selection criteria of LVA versus VLNT in the treatment of extremity lymphedema remains a highly debated subject, these techniques yielded promising results in terms of pain and swelling alleviation in uni- or bilateral extremity lymphedema across several centers [8, 9].

However both VLNT, VLVT and LVA are advanced procedures requiring highly trained microsurgical skills, available only in specialized centers [10]. This is a major limitation for the increasing number of lymphedema patients worldwide in urgent need of treatment. Furthermore, adequate standardized training models applicable to all microsurgical approaches for lymphedema are lacking [10].

Several rodent or porcine models have been proposed for training in both LVA and VLNT, however major anatomical disparities limit their importance with regard to their clinical relevance [11–14].
Previous studies showing the ability of either patent blue violet (PBV) dye or indocyanine green (ICG) to stain cadaveric lymphatic vessels opened new avenues for designing training models in lymphatic microsurgery [15, 16]. While ex-vivo and animal models are of utmost importance for attaining required microsurgical competency, cadaveric models remain unparalleled tools for mastering the relevant clinical anatomy for each microsurgical technique before entering the operating room [17].

Experimental models for lymphatic microsurgery using either ex-vivo or live animal settings have been described either as stand alone training tools or as part of established international training programs in reconstructive microsurgery. Validated cadaveric training models including all actual microsurgical approaches available for lymphatic reconstruction have not yet been reported.

Here, we describe a cadaver model that fits well either as part of a systematic program or as stand alone tool for training in lymphatic microsurgery and analyze its academic efficacy in terms of learning effectiveness and skill acquisition in a defined environment, using a standard educational proficiency scoring system (DREEM - Dundee Education Environment Measure).

**Materials and methods**

**Course setup**

The training curriculum is based on an intensive two days hands-on module introduced by short theoretical sessions. 14 hours of practical training on fresh frozen cadavers, divided into lymphatic vessel and subdermal venule dissection as preparation for LVA (5 hours) and VLNT/VLVT harvesting (9 hours) build the practical part of the course.

**Participants**

10 participants with either plastic, vascular, or general surgery background all with previous clinical exposure to microsurgery participated at the course. Participants were divided into groups of two for all practical sessions. Each group had its own anatomical specimen and completed the VLNT and the VLVT models, once as operator and once as assistant. Lymphatic vessel staining and dissection was performed individually by each participant on the allotted side. Each pair of participants was assigned a tutor from the faculty to assist and explain all exercises.

**Human anatomical specimens**

Five cadavers (two males, three females) with a mean age of 76.3 years (70-80 years) from the XXXX Human Body Donation Program were used. Prior, approval from the Internal Ethics Committee of the above-mentioned program was obtained. All cadavers were selected according to their medical files to exclude a history of any type of surgery in any of the regions of interest for the study (head and neck, axilla, upper extremity/hand, groin, lower extremity/foot, abdominal cavity). After anonymization and complete hair removal, the cadavers were deep frozen at −40°C for 24 hours, then transferred to −20°C freezers for long-term storage (Isocab Freezer Company, Pontsstraat, Belgium). Before dissection, the cadavers were removed from the freezers into rooms with a controlled temperature (19 °C) and allowed to thaw for a total of 72 hours. All dissections were performed in the dissection rooms of the University Medical Center, XXXX. After dissection, all incisions and wounds were properly closed to restore the integrity of the body. The bodies were refrigerated until final disposal by incineration, according to the local regulations.

**Injection technique**

Intradermal injections of either 25 mg/ml PBV (Guerbet GmbH, Sulzbach, Germany) or 5 mg/ml ICG (Verdyeye, Diagnostic Green GmbH, Aschheim-Dornbach, Deutschland) were performed 30 minutes before lymphatic vessel mapping and dissection in both the upper and lower limbs of each cadaver. Dissection was performed using prismatic loupe magnification and operative microscope (Zeiss, Oberkochen, Germany), surgical instruments (Aesculap, Tuttingen, Germany) and if appropriate, supermicrosurgical instrumentation (S&T AG, Schaffhausen, Switzerland). Intradermal injections were performed in the dorsum of the hand, medial upper arm, dorsum of the foot and the medial proximal thigh respectively. To assure consistency, all injections were performed using the same protocol. A total of 0.2 ml PBV and ICG (per injection site) were performed on the dorsum of the hand. The same PBV and ICG volume was injected approximately 14 cm distal from the axilla, along the proximal third of the bicipital groove, followed by the dorsum of the foot (2 cm proximally to the 2nd interdigital space) and 20 cm distal to the mid-inguinal point toward the groin.

**Dissection of lymphatic vessels**

Within approximately 30 minutes from injection, lymphatic vessels of all injected body areas were mapped as identified with PBV or ICG using near infrared scanning (PDE System, Hamamatsu Photonics K.K., Shizuoka, Japan) and dissected using loupe magnification, supermicrosurgical instruments and a table-top operative microscope (Carl Zeiss GmbH, Jena, Germany) (Figures 1 and 2). Optionally, several veins (branches of the saphenous-femoral junction and collateral branches of the brachial veins) were dissected as for LVA. On the dorsum of the foot, lymphatic vessels were dissected after harvesting the VLVT whereas after identification of its vascular pedicle, the flap was sutured back on to the donor site to assure a stable background for further dissection.

The number of lymphatic vessels in all dissected regions were documented and are presented as mean ± standard error of the mean (SEM) for each anatomical region.

**Vascularized lymph node transfer models (VLNT)**

Five common VLNTs were harvested in each cadaver, including submental, supraclavicular, lateral thoracic, groin and right gastroepiploic flaps. All VLNTs except the
gastroepiploic flap were harvested bilaterally. The vascular anatomy and harvesting technique for the lymph node flap models presented in this study have been described in detail elsewhere [18, 19]. However, for replicability of the model, these techniques have been adapted to the cadaver lab conditions and their brief descriptions are summarized below.

**Submental VLNT**

After flap marking, skin incision was initiated at the cranial border of the flap. Next, the marginal mandibular branch of the facial nerve was identified and protected. The facial artery above the margin of the mandible was exposed and followed caudally until its junction with the submental artery, which was dissected proximally to distally along its axis, taking care to preserve all the soft tissues around it and thereby assuring the incorporation of maximum number of lymph nodes (Figure 3A). In order to preserve the submental artery perforators supplying the skin paddle, the anterior belly of the digastric muscle was included in the flap.

**Supraclavicular VLNT**

After marking the flap the skin was incised along the lateral margin of the pectoralis major. The dissection was deepened proximally into the axilla. After identification of the axillary vein and artery, the proximal part of the lateral thoracic pedicle was identified. The intercostobrachial nerve crossing the lateral thoracic pedicle toward the arm was identified and preserved (Figure 3C). Approximately 2-3 cm distal from the crossing of this nerve, the lateral thoracic pedicle enters the fat layer containing the 2 to 4 lateral thoracic axillary lymph nodes.

**Lateral thoracic VLNT**

After marking the flap the skin was incised along the lateral margin of the pectoralis major. The dissection was deepened proximally into the axilla. After identification of the axillary vein and artery, the proximal part of the lateral thoracic pedicle was identified. The intercostobrachial nerve crossing the lateral thoracic pedicle toward the arm was identified and preserved (Figure 3C). Approximately 2-3 cm distal from the crossing of this nerve, the lateral thoracic pedicle enters the fat layer containing the 2 to 4 lateral thoracic axillary lymph nodes.

**Groin VLNT**

This flap followed the exact anatomy of the superficial circumflex iliac artery perforator (SCIAP) flap and was marked raised from lateral to medial in a suprafascial plane as described by Hong et al. [20]. The entry point of the
medial septo-cutaneous perforator of the SCIA into the skin paddle of the flap was identified. The perforator was followed up to the main trunk of the SCIA, which could be found medially to the lateral inguinal lymph node group that are included in the flap (Figure 3D).

**Gastroepiploic VLNT**

After minilaparotomy, under loupe magnification, the right gastroepiploic vessels were localized and lymph nodes located along their axis in the omental tissue, were identified. After dividing the ascending segmental gastric branches the flap was dissected off the transverse colon (Figure 4A). Finally, the flap pedicle was prepared medially to the level of the right gastroepiploic vessels, which were ligated and cut. The flap was then split down the middle to provide two gastroepiploic VLNTs for simultaneous transplantation (Figure 4B).

The number of lymph nodes harvested with each of the five flaps were counted and are presented as bilateral mean ± standard error of the mean (SEM) for each cadaver and anatomical region.

**Vascularized lymphoadiposal flap model (VLVT)**

Based on the VLVT principle initially described by Koshima et al, we developed for training purposes a similar lymphoadiposal flap model based on the first dorsal intermetatarsal artery (FDMA) in the fresh frozen cadaver following the initial flap design proposed by his group [8]. After transcutaneous marking of the lymphatic vessels in the dorsum of the foot using both PBV and ICG as described above, the skin was excised in a subdermal layer (Figure 5A). After exposing the lymphatic vessels, the flap was incised down to and through the fascia and raised in this layer from medial to lateral. Once at least one FDMA perforator was identified (Figure 5B), the FDMA was ligated and flap excised (Figure 5C).

**Data analysis**

All data were recorded and analyzed using Numbers® (Apple Inc., Cupertino, USA). All quantitatively measured variables are presented as mean ± standard error of the mean (SEM). Custom error bars and reference lines representing mean values over each data set were used when appropriate.

**Evaluation**

To analyze its immediate teaching impact, at the end of the course all participants underwent an MCQ exam containing 20 questions, each question with a single correct answer (max. points = 20). Additionally, each participant was asked to complete an auto-evaluation form, summarizing the entire learning experience as related to the topics, all experimental models presented and the overall course structure and organization.

For assessing the teaching efficacy of the cadaveric model, all participants were asked to complete the DREEM evaluation questionnaire using a standard online anonymised survey platform with 8 out of 10 participants (80%) responding to our request.

**Dundee ready education environment measure (DREEM)**

The DREEM score is a standard tool designed to measure the overall educational environment for health professionals and comprises of 50-statement closed questions questionnaire which we adapted to fit our requirements, divided into five categories: (1) student's perception of learning (12 questions); (2) student's perception of teachers (11 questions); (3) student academic self perceptions (4) (iii) students perception of “OR-working” atmosphere (12 questions) and (5) student's social self perceptions (8 questions) [21]. Each individual question is scored from “Strongly agree” (4 points), “Agree” (3 points), “Unsure” (2 points), “Disagree” (1 point) and “Strongly disagree” (0 points). The higher the score the more positive the evaluation, except the questions 11, 19, 20, 42, 47, 50 which require reverse coding. The DREEMM has a maximum score of 200 representing an ideal
The results of the DREEM scoring are presented as mean values ± standard error of the mean (mean ± SEM) either for all categories for each participant as overall mean scores (Figure 6A), student evaluation of teaching (Figure 6B), student academic self perception (Figure 6C) and student perception of the course environment (Figure 6D).

**Results**

**Occurrence of lymphatic vessels**

Using PBV and ICG mapping we identified and dissected lymphatic vessels in upper and lower extremities in all five cadaver specimens (Figures 1, 2, and 7A). The average number of lymphatic vessels found at the dorsum of the hand...
was comparable with the ones in the bicipital groove (1.7 ± 0.26 vs. 1.2 ± 0.24, mean ± SEM). The groin revealed the highest number of lymphatic vessels as compared with the dorsum of the foot (3.2 ± 0.29 vs. 2.1 ± 0.27, mean ± SEM) and all other regions on the upper extremity thereof.

Occurrence of lymph nodes

Lymph nodes were detected in all VLNTs harvested bilaterally in 4 anatomical regions (submental, supraclavicular, lateral thoracic, groin) and unilaterally in the case of the gastroepiploic VLNT for all cadaver specimens (Figure 7B).

Immediate impact of the course

All participants completed the two-day training course. With an overall auto evaluation mean score of 18.2 ± 0.44 points (mean ± SEM), the level of overall feedback was excellent among all trainees.
All participants reported meeting their individual learning goals at least ‘moderately well’, with the majority rating this as ‘very well’ (4/10 participants) or ‘extremely well’ (4/10). Similarly, the participants rated the course as either ‘extremely useful’ (6/10 participants), ‘very useful’ (2/10 participants), or moderately useful (2/10 participants) for
their future surgical careers. Comments regarding this aspect noted that this training course gave a great insight into the technical pearls of all microsurgical lymphatic reconstruction procedures actually performed in the clinic as well as the surgical treatment of lymphedema in general.

**In depth evaluation of educational environment**

The average cumulative DREEM score over each of the five categories for all participants of 30,75 (max = 40) with no significant difference between each trainee (Figure 6A) stands as notable argument of the adequate educational environment provided by this course when used as instrument to evaluate the efficacy of the fresh-frozen cadaver model for teaching microsurgical lymphatic reconstruction. Separate in depth analysis of each relevant category revealed further positive rating from each participant either from the perspective of teaching (Figure 6B), the training atmosphere (Figure 6C) and the self perception of training thereof (Figure 6D). All these provide evidence for the effectiveness of the cadaver training in this setting.

**Discussion**

Both active and passive mechanisms have been found responsible for the intraluminal propagation process of PBV and ICG in vivo [22, 23]. Based on these findings, we speculated that ex-vivo “fresh-thawed” lymphatic vessels could react in the same way, leading to identification of “healthy” lymphatic vessels ideal for microsurgical training purposes. Indeed, our injection protocol revealed lymphatic vessels in the upper and lower extremities. An average of two lymphatic vessels were successfully dissected in each of the four anatomical areas of all five anatomical specimens (Figure 6A).

Understanding the VLNT anatomy is instrumental before entering the operating room and the cadaver model provides the ideal mirroring of a given clinical scenario. It is at this level that cadavers show their undeniable utility and unicity as training tools. In this study we developed five models of the most widely used clinical VLNTs. Back table dissection of all VLNTs revealed an average of 2.9 lymph nodes across flaps, a finding resembling other existent studies [23]. The gastroepiploic VLNT was harvested using a minimal median laparotomy. However, the cadaver model can be easily extended to include the laparoscopic technique.

In this study, analysis of the number of identified lymphatic vessels or lymph nodes provides an overview of their overall occurrence while serving as evidence to support the repeatability of the described model.

The described lymphoadiposal flap is to the best of our knowledge, the first reproduction of a VLVT model in fresh frozen cadavers following the flap design initially described by Prof. Koshima for treatment of secondary lymphedema. Contemplating the medley of actual microsurgical training opportunities, the lack of programs providing microsurgical lymphatic reconstruction becomes evident [24–25].

Dry lab and in-vivo animal models are important but insufficient for attaining the required level of competence before entering the operating room [26]. Training on human anatomical specimens is in our opinion, a key factor and concluding step of a training ladder for each surgeon willing to approach microsurgical lymphatic reconstruction.

Here we have standardized a cadaver training model containing a complete set of all modern microsurgical approaches for lymphedema. The data from the DREEM analysis in terms of both overall scoring as well as in depth analysis of each relevant category provides evidence on the effectiveness of this model as training tool in lymphatic microsurgery (Figure 6A–D). The DREEM measure tool is the result of a Delphi panel of nearly 100 health care educators around the world in an effort to define a more precise tool for quantification of the learning environment efficacy in the undergraduate phase of the medical academic development. Similar tests, such as the postgraduate hospital educational environment measure (PHEEM) have been conceived using similar methodology, to address the postgraduate level [27]. However when compared to DREEM, the PHEEM tools refers mainly to longer educational timeframes such as residency programs, the questionnaire including items (e.g. contract of employment, racism, no-blame culture, etc) which in our setting and for our evaluation purposes were not considered relevant [27]. Furthermore, although the DREEM is applied to pregraduate students, several studies document its successful use to evaluate the learning quality of postgraduate programs or independent practical courses for medical doctors even in small sample size [28, 29]. While the authors are aware that there is no ideal tool to objectively evaluate the learning efficacy of a practical microsurgical skill acquisition course, due to its design, evaluation methods and existing evidence, we considered the DREEM questionnaire the most suited one to evaluate the educational efficacy of the presented cadaver model.

The results presented in this study have to be seen also in light of certain limitations. The small size of the trainee group affecting the overall scientific relevance of the study. However, according to the recommendations from Swift et al. on DREEM reporting, using averages and appropriate parametric significance analysis for each relevant subcategory in the context of a single group study, can allow reliable data interpretation provided the overall responder group represent at least 75% from the study sample, a condition which our study complies to (e.g. 8 out of 10 participants responded to the DREEM survey) [30]. It is difficult to know the extent to which the degree of pervious microsurgery exposure contributed to the ease the trainees acquired the skills to perform all exercises. The absence of a validated evaluation of each participant’s microsurgical expertise before the course, is a factor that that could have influenced the response rating and thereby the data homogeneity. Certainly, all participants rated the program highly and noted that it was useful for their future careers, particularly if they want to treat patients with lymphedema. We don’t know how the model would have performed
outside the course. Therefore we see here a clear need for its future independent evaluation in studies with more participants divided by years of surgical experience, in order to determine if all components in terms of explanation, technical demonstrations, practical sessions etc. are optimal to ensure good skill acquisition. The course comprised also of a limited theoretical part which might have diluted the accuracy of the model's evaluation process. However because the intensive hands-on sessions occupied the most of the course and the theoretical input was solely related to the models and their potential clinical relevance, this possible limitation becomes at least arguable. Lastly, the scarce availability of human fresh frozen cadavers, high costs as well as the lack of immediate feedback after performing the models may represent one major limiting factor for this model. However, we strongly believe that training using human specimens should be considered one fo the fundamental steps of the training ladder for each reconstructive microsurgeon willing to approach lymphatic reconstruction.

One other possible drawback to our lymphatic microsurgery training model is that it is limited to microsurgical procedures. Patients with advanced stages of lymphedema can benefit from LVA, VLNT, VLVT or a combination thereof to improve the lymphatic flow in the affected limb. However, there often remains a solid component (fibro-adipose tissue) requiring additional volume reduction surgery to remove it. The additional reduction of the solid compartment represents nowadays an important widely accepted concept for the surgical treatment of lymphedema where either selective liposuction procedures or, in other specific cases, modified Charles procedures in combination with microsurgical procedures are employed to treat advanced stages of disease [31–34]. While this is beyond our scope of a lymphatic microsurgical training model, it is important to acknowledge that complete lymphedema surgical treatment requires an array of procedures that may be applied depending on the needs of the individual patients.

Conclusion

Here we describe a complete training model for lymphatic microsurgery in fresh-frozen cadavers geared up with all modern microsurgical reconstruction approaches for lymphedema. Furthermore, we provide evidence on its efficacy as teaching tool within the context of a practical course with the ability to fill up an existent gap toward a more efficient training of the next generation lymphatic surgeons.

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Disclosure statement

All the authors have read and agree to abide by the Journal of Investigative Surgery Conflict of Interest policy. We acknowledge that we must disclose any conflict of interest, along with a description of any personal business interest, affiliation, or activity with any entity which may give rise to a conflict of interest. The authors of this study have no conflicts of interest to disclose.

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