

# QUANTITY OF LYMPH NODES CORRELATES WITH IMPROVEMENT IN LYMPHATIC DRAINAGE IN TREATMENT OF HIND LIMB LYMPHEDEMA WITH LYMPH NODE FLAP TRANSFER IN RATS

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**Purpose:** This study was conducted to investigate the correlation between the number of vascularized lymph nodes (LN) transferred and resolution of hind limb lymphedema in a rat model. **Methods:** Unilateral hind limb lymphedema was created in 18 male Sprague–Dawley rats following inguinal and popliteal LN resection and radiation. A para-aortic LN flap based on the celiac artery was subsequently transferred to the affected groin. The three study groups consisted of Group A (no LN transfer), Group B (transfer of a single vascularized LN), and Group C (transfer of three vascularized LNs). Volumetric analysis of bilateral hind limbs was performed using micro-CT imaging at 1, 2, and 3 months postoperatively. Lymphatic drainage was assessed with Tc<sup>99m</sup> lymphoscintigraphy preoperatively and at 3 months postoperatively. **Results:** A statistically significant volume reduction was seen in Groups B and C compared to Group A at all time points. Volume reduction of Group A vs. Group B at 1 month ( $8.6\% \pm 2.0\%$  vs.  $2.7\% \pm 2.6\%$ ,  $P < 0.05$ ), 2 months ( $9.3\% \pm 2.2\%$  vs.  $-4.3\% \pm 2.7\%$ ,  $P < 0.05$ ), and 3 months ( $7.6\% \pm 3.3\%$  vs.  $-8.9\% \pm 5.2\%$ ,  $P < 0.05$ ). Volume reduction of Group A vs. Group C at 1 month ( $8.6\% \pm 2.0\%$  vs.  $-6.6\% \pm 3.1\%$ ,  $P < 0.05$ ), 2 months ( $9.3\% \pm 2.2\%$  vs.  $-10.2\% \pm 4.6\%$ ,  $P < 0.05$ ), and 3 months ( $7.6\% \pm 3.3\%$  vs.  $-9.1\% \pm 3.1\%$ ,  $P < 0.05$ ). Of note, comparison of Groups B and C demonstrated greater volume reduction in Group C at 1 ( $P < 0.02$ ) and 2 ( $P = 0.07$ ) months postoperatively. **Conclusions:** LN flap transfer is an effective procedure for the treatment of lymphedema. The number of vascularized LNs transferred correlates positively with the degree of volume reduction. © 2015 Wiley Periodicals, Inc. *Microsurgery* 36:239–245, 2016.

Lymphedema represents a condition characterized by the pathologic accumulation of protein-rich fluid in the interstitium.<sup>1</sup> The most common cause in the United States is the multimodal management of malignancies.<sup>2,3</sup> Most current treatment protocols for lymphedema are based on conservative therapy aimed at suppressing symptoms. Although providing symptom relief, these non-invasive modalities do not provide a curative solution.

Innovations and advances in surgical technique and management of lymphedema in recent years can be categorized into debulking procedures, physiologic procedures including lympho-lymphatic, lympho-venous anastomoses, lymphatic vessel transplantation,<sup>4–9</sup> and vascularized lymph node transfer.<sup>10–16</sup> Clinical benefits have been demonstrated and few hypotheses have been

proposed regarding the mechanisms of action of the lymph node (LN) flap.<sup>13,14</sup> While technical modifications of LN flap transfer have been reported to improve the efficacy of the procedure,<sup>17</sup> other aspects of the intervention have not been sufficiently investigated, such as donor-site morbidity, impact of the number of transferred LNs on resolution of symptoms, the occurrence of lymphangiogenesis, as well as immunologic activity and function of transferred LNs.<sup>18,19</sup>

This study was conducted to investigate the outcome of lymph node flap transfer with different numbers of lymph nodes included in treatment of hind limb lymphedema in a rat model using micro-CT imaging and lymphoscintigraphy for analysis.

## MATERIALS AND METHODS

All animal procedures were performed at Chang Gung Memorial Hospital (CGMH) complying with the animal research guidelines. Total of 30 rats were used in this study.

### Unilateral Hind Limb Lymphedema Rat Model

Unilateral hind limb lymphedema was established in male Sprague–Dawley rats (350–400 g) based on a published protocol with 80% success rate.<sup>20</sup> A total of 18 rats were anesthetized with 2.5% Isoflurane. 0.1 mL, 10% Evan's Blue (Sigma–Aldrich, Saint Louis, MI) was injected into the subcutaneous tissue of the paw to identify the inguinal and popliteal lymph nodes as well as accompanying lymphatic ducts. All these structures were

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