Experimental Assessment of Autologous Lymph Node Transplantation as Treatment of Postsurgical Lymphedema

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Background: The authors’ objective was to test whether the transplantation of an autologous lymph node into a nodal excision site in sheep would restore lymphatic transport function and reduce the magnitude of postsurgical lymphedema.

Methods: As a measure of lymph transport, iodine-125 human serum albumin was injected into prenodal vessels at 8 and 12 weeks after surgery, and plasma levels of the protein were used to calculate the transport rate of the tracer to blood (percent injected per hour). Edema was quantified from the circumferential measurement of the hind limbs.

Results: The transplantation of avascular lymph nodes at 8 (n = 6) and 12 weeks (n = 6) produced lymphatic function levels of 12.3 ± 0.5 and 12.6 ± 0.8, respectively. These values were significantly less (p < 0.001) than those measured at similar times in the animals receiving sham surgical procedures (16.6 ± 0.7, n = 6; and 16.1 ± 0.7, n = 6, respectively). When vascularized transplants were performed, lymphatic function was similar to the sham controls and significantly greater (p < 0.001) than that of the avascular group (8 weeks, 15.8 ± 0.9, n = 8; 12 weeks, 15.7 ± 1.0, n = 10). Lymph transport correlated significantly with the health of the transplanted nodes (scaled with histologic analysis) (p < 0.0001). The vascularized node transplants (n = 18) were associated with the greatest clinical improvement, with the magnitude of edema in these limbs exhibiting significantly lower levels of edema (p = 0.039) than nontreated limbs (n = 18).

Conclusions: The successful reimplantation of a lymph node into a nodal excision site has the potential to restore lymphatic function and facilitate edema resolution. This result has important conceptual implications in the treatment of postsurgical lymphedema. (Plast. Reconstr. Surg. 124: 777, 2009.)

Lymphedema is a frequent surgical complication associated with cancer-related lymph node dissection.1 The exact nature of the surgically induced deficit is still uncertain. Under normal conditions, lymph flow is restored fairly quickly following vessel injury because of the robust regenerative capacity of the lymphatic vessels (lymphangiogenesis).2 However, it is clear that the removal of lymph nodes greatly increases the chance of developing lymphedema, especially if this is combined with radiotherapy.3 With this in mind, we developed a sheep model that permits quantitation of edema and lymphatic function after the removal of a single popliteal lymph node.4 In this report, our objective was to remove a lymph node and examine the impact of autologous vascularized and nonvascularized lymph node transplantation on lymphatic function and edema formation.

MATERIALS AND METHODS

A total of 50 randomly bred male and female Dorset sheep (30.6 ± 0.7 kg) were used in this

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investigation. All experiments outlined in this article were approved by the ethics committee at Sunnybrook Health Sciences Centre and conformed to the guidelines set by the Canadian Council on Animal Care and the Animals for Research Act of Ontario. Subcutaneous Temgesic (Animal Resources Center, McGill University, Montreal, Quebec, Canada) was given postoperatively for pain management. Antibiotic (Duplocillin; Veterinary Medicine Distribution Center, St. Hyacinthe, Quebec, Canada) was given intramuscularly 1 day before surgery and again 2 days postoperatively.

Nodectomy Followed by Avascular/Node Graft Insertion

The animals were anesthetized initially by intravenous injection of sodium pentothal. For surgical maintenance, 2.0 to 3.5% isoflurane was delivered through an endotracheal tube by means of a Moduflex (Dispomed, Joliette, Quebec, Canada) machine with a Hallowell respirator (Hallowell EMC, Pittsfield, Mass.). An 8- to 10-cm vertical skin incision was made over the lateral aspect of the popliteal region. An opening was made in the popliteal fat and prenodal and postnodal lymphatics were tied off with a silk suture and the node excised. Once the nodes from both hind limbs were removed, each was placed into the contralateral popliteal fat pad as a free graft without vascular reconnection.

Nodectomy Followed by Lymph Node Transplant by Microvascular Anastomosis

Lymph nodes were harvested together with the intact fat pads to preserve the delicate nutrient vessels supplying the node. The vascular pedicle formed by the lateral saphenous vein and the medial circumflex femoral artery was dissected free with the aid of Carl Zeiss (Oberkochen, Germany) loupes (3.5 × 400) before ligation and clipping of the vessels. The animal was then turned over and the contralateral popliteal node was excised. Once the nodes from both hind limbs were removed, each was placed into the contralateral popliteal fat pad as a free graft without vascular reconnection.

Sham Control Group

In this group of animals, the popliteal fat pad was exposed but undisturbed. A saline-soaked piece of gauze was applied to the wound, and it was left in this state for 3 hours to duplicate the time needed for the vascularized node transplants.

Assessment of Edema

The hind legs were shaved and leg circumference measurements were taken at a point 10 cm distal to the hock (tarsus). This landmark was highlighted with a skin marker for circumference measurements postoperatively. The limb circumferences were divided by the original (presurgical value) and expressed as percentage change over time. To compare the edema outcomes in the various groups, the percentage change in limb circumference was plotted against time, and graphical integration of the area under the curves was calculated using the trapezoidal rule. To allow comparisons of experiments conducted over different times (8 and 12 weeks), an edema coefficient was calculated by dividing the areas under the curves with the duration of the experiment in days.

Fluoroscopy

In 17 sheep (19 limbs) at 8 or 12 weeks after surgery, 1 to 3 ml of lipiodol (Therepex; E-Z-EM Canada, Inc., Anjou, Quebec, Canada), a radiographic contrast medium, was injected into an upstream popliteal prenodal vessel and the lymphatics/nodes were visualized at various times after surgery using a mobile fluoroscopy system (BV Pulsera; Philips Healthcare, Andover, Mass.).

Histologic Assessment of Lymph Nodes

Popliteal nodes were fixed in 10% formalin, cut into 5-mm sections, and paraffin embedded. Sections (6 μm) were placed on slides and then stained with hematoxylin and eosin. One representative node slide per limb was stained for the T-cell marker CD3 using a 1:200 dilution of a rabbit anti-human CD3 antibody (Dako Canada, Inc., Mississauga, Ontario, Canada). This was followed by sequential incubation with biotinylated link antibody, peroxidase-conjugated streptavidin and then visualized with diaminobenzidine (Dako Canada). The sections were then counterstained with Harris’s hematoxylin and treated with acid alcohol and ammonia water. Lymph nodes were scaled qualitatively after transplantation on a scale of 0 to 3, where 3 = normal-appearing nodes; 2 = nodes with some abnormality (evidence of ischemic damage and loss of cellularity); 1 = partial...
nodes or nodes with severe damage (fibrosis); and 0 = lymph nodes absent (tissue resorbed).

Quantitative Assessment of Lymphatic Function

A schematic illustrating the experimental model is provided in Figure 1. Evans blue dye (1% in saline) was injected subcutaneously above the hoof to enhance visualization of the prenodal lymphatic vessels. An incision was made through the skin and subcutaneous tissues over the lower lateral aspect of the hind limb, and a single vessel was cannulated with a 26-gauge angiocatheter. Saline (100 μl) was injected into the cannula over 30 seconds to check for leaks. Radiolabeled human serum albumin (iodine-125 human serum albumin, 2 mg in a 200-μl volume) was then injected over a 60-second period into the prenodal lymphatic vessel and flushed with 100 μl of saline. Blood samples were taken from a neckline inserted into the jugular vein at time 0, 15, and 30 minutes and then every 30 minutes up to 4 hours.

Quantitative studies were performed under anesthesia at 8 and 12 weeks in the various experimental groups described earlier. The plasma concentrations of the radioactive protein tracer were used in a mass balance equation (equation 1) to calculate the lymphatic mass transport rate ($B_{in}$) averaged over the 4-hour collection period:

$$B_{in} = \frac{[C_p(t_f) \exp(K_{exp}t_f) - C_p(0)](K_{exp}V_p)}{\exp(K_{exp}t_f) - 1}$$

where $C_p(t_f)$ = the concentration of the tracer at 4 hours, $C_p(0)$ = the concentration of tracer at time 0, and $K_{exp}$ = the coefficient of elimination of tracer from plasma. Because our previous experience indicated that this coefficient did not differ significantly between animals of various ages and weights, an average value was derived from 41 animals used in previous studies. Because the volume of distribution of the tracer (plasma volume, $V_p$) would differ between animals, we adjusted the plasma recoveries to reflect this. Based on data derived in previous studies from our group, we plotted the plasma volumes derived from 41 animals against their weights. We used a regression analysis of these data (equation 2) to calculate a plasma volume in each sheep. The values for $B_{in}$ derived from equation (1) were divided by the total radioactivity injected to give percentage injected per hour.

Fig. 1. Schematic diagram illustrating the basic principle behind the method to quantify lymphatic function. To aid in the visualization of the popliteal lymphatic system, Evans blue dye has been injected into an upstream prenodal duct. $^{125}$I-HSA, iodine-125 human serum albumin.
Statistical Analysis

All data were expressed as the mean ± SEM. The data were assessed with regression analysis, Kruskal-Wallis one-way analysis of variance, or t test (unpaired) as appropriate. We interpreted values of \( p < 0.05 \) as significant.

RESULTS

Lymphatic Function

Measurements of lymphatic function for all groups are illustrated in Figure 2. The transport rates of the protein tracer for the sham group averaged 16.6 ± 0.7 percent per hour at 8 weeks and 16.1 ± 0.7 percent per hour at 12 weeks. These values were similar to those observed in limbs that had not been subjected to any surgical procedures (17.2 ± 0.6 percent per hour, taken from our previous report)\(^4\) and indicated that all of the surgical procedures excluding the actual removal of the nodes had little impact on lymphatic function.

The removal of a lymph node and replacement with an avascular node from the contralateral side gave lymphatic function values at 8 (12.3 ± 0.5 percent per hour) and 12 weeks (12.6 ± 0.8 percent per hour) after surgery that were significantly lower than those of the sham group (\( p < 0.001 \), unpaired \( t \) test). In contrast, the replacement of the excised node with a vascularized node transplant resulted in lymph transport that was significantly greater than that of the avascular group (\( p < 0.001 \), unpaired \( t \) test). Indeed, lymphatic function approached sham levels (15.8 ± 0.9 percent per hour at 8 weeks and 15.7 ± 1.0 percent per hour at 12 weeks).

Correlation of Lymphatic Function with Health of Lymph Nodes

In the sham group, all nodes rated 2 or 3 on histologic assessment, indicating that the nodes were generally healthy appearing. Examples are illustrated in Figure 3, above, right and center, right. Those in the avascular transplant group fared poorly, with seven of 12 rating 0, three rating 1, and only two

\[
y = 21.77x + 649.68 \quad (2)
\]
classified as 2. An example of a node ranking 1 is illustrated in Figure 3, below, right. The vascularized node transplants were generally more successful. At 8 weeks, the ratings were four of eight, 3; three of eight, 2; and one of eight, 0. At 12 weeks, the results were four of 10, 3; two of 10, 2; and four of 10, 0.

Figure 3 also shows a significant correlation between the health of the lymph nodes and lymphatic transport function \( (p = 0.0002, \text{Kruskal-Wallis one-way analysis of variance}) \). These data include all values from the avascular and vascularized transplant groups at both 8 and 12 weeks \( (n = 30) \) and demonstrate that the subjective node rankings have different levels of lymphatic function \( (p < 0.0001) \). \( (\text{Above, right}) \)

Example of normal node ranked 3 (hematoxylin and eosin stain). \( (\text{Center, right}) \)

Example of node ranked 2 (hematoxylin and eosin stain). There is evidence of ischemic injury with some loss of cellularity and lipid accumulation in the medulla (black arrow). \( (\text{Below, right}) \)

Example of node ranked 1 (hematoxylin and eosin stain). In this case, there is evidence of significant injury, with major portions of the node replaced by fibrous tissue (red arrow). In addition, the capsule is much thicker than normal (white arrow). In the inset, the vascularized data have been subdivided into two groups: those preparations in which the nodes were scaled 2 or 3 \( (A; n = 13) \) and those in which the nodes were scaled 0 or 1 \( (B; n = 5) \). Lymphatic function was significantly greater in the transplant group with the healthiest appearing lymph nodes \( (p < 0.0001, \text{unpaired } t \text{ test}) \). Numbers in parentheses represent the number of animals in each series. HSA, human serum albumin.

**Edema**

In the sham group, we noticed no change in the limb circumference over time. In all cases in

\[ \text{Figure 3. Relationship between lymphatic function and lymph node health after transplantation. These data include all values from the avascular and vascularized transplant groups at both 8 and 12 weeks (n = 30) and demonstrate that the subjective node rankings have different levels of lymphatic function (p = 0.0002, Kruskal-Wallis one-way analysis of variance). This association was monotonic in that increasing lymph transport was associated with increasing node rank scores (p < 0.0001). (Above, right) Example of normal node ranked 3 (hematoxylin and eosin stain). (Center, right) Example of node ranked 2 (hematoxylin and eosin stain). There is evidence of ischemic injury with some loss of cellularity and lipid accumulation in the medulla (black arrow). (Below, right) Example of node ranked 1 (hematoxylin and eosin stain). In this case, there is evidence of significant injury, with major portions of the node replaced by fibrous tissue (red arrow). In addition, the capsule is much thicker than normal (white arrow). In the inset, the vascularized data have been subdivided into two groups: those preparations in which the nodes were scaled 2 or 3 (A; n = 13) and those in which the nodes were scaled 0 or 1 (B; n = 5). Lymphatic function was significantly greater in the transplant group with the healthiest appearing lymph nodes (p < 0.0001, unpaired t test). Numbers in parentheses represent the number of animals in each series. HSA, human serum albumin.} \]
which a popliteal node was placed into the nodal excision site without vascular connections, the limb became edematous. The average percentage increase in leg circumference at day 1 was 12.7 percent, with maximum values attained within the first week after surgery. By 12 weeks on average, the edema was still higher than the presurgical levels (data not shown).

Figure 4, left illustrates the averaged edema data from the vascularized node transplant series ($n = 44$ limbs). In these experiments, lymph nodes from both hind limbs were excised; one of the limbs received a vascularized node transplant ($n = 22$) and the other limb ($n = 22$) did not. In both groups, the limb circumference changed significantly over time ($p < 0.001$, repeated-measures linear regression). In the limbs that received a vascularized transplant, the average percentage increase in leg circumference at postoperative day 1 was 12.1 percent, with peak edema also occurring during the first week. Edema in the contralateral nodectomy limbs peaked at the same time. Thereafter, limb circumference declined steadily in both limb groups, with values from the vascularized transplant limbs significantly less than in the “untreated” limbs ($p = 0.023$, repeated-measures linear regression).

Figure 4, above, center illustrates a case in which lymphatic transport in the transplant side was 17.0 and the node was classified as 3. The edema in the transplant limb recovered completely, whereas the contralateral side (with node removed) was still edematous by the end of the experiment. In the example in Figure 4, above, right, the transplant was not successful (lymphatic function was 12.6 and the node was classified as 0). In this case, the edema (limb circumference) in the transplant side was similar to that of the contralateral limb and did not resolve over the course of the study.

![Fig. 4. Development of edema following excision of popliteal lymph node with no nodal replacement (open circles, $n = 22$) or after the transplantation of an autologous vascularized node (closed circles, $n = 22$) (left). SE bars have been omitted from the averaged data for clarity. In both groups, the limb circumference changed significantly over time ($p < 0.001$, repeated-measures linear regression). Values from the vascularized transplant limbs were significantly less than those in the untreated limbs ($p = 0.023$, repeated-measures linear regression). (Above, center) An example in which the nodal transplant procedure reduced edema generation over the course of the experiment compared with the nontreated limb, and at 21 days no edema could be measured. In this case, lymphatic function was 17.0 percent injected per hour and the node was scaled as 3. (Above, right) An example in which the transplant was largely unsuccessful at ameliorating edema although, by the end of the experiment, the limb circumference was less than the contralateral untreated side. In this animal, lymphatic function was 12.6 percent injected per hour and the node was scaled as 0.](image)
Figure 5 illustrates the relationship between the coefficient of edema and lymphatic function combining all of the available data from the vascularized and avascular node transport limbs \( (n = 30) \). Although there is a tendency for the limbs with the highest lymphatic function to be associated with the lowest levels of edema, the variability in the edema data prevented this relationship from being significant (regression analysis). The inset illustrates averaged edema coefficients for these groups in addition to the limbs from which the nodes were harvested for transplant (labeled node excision). These comparisons indicate that the vascularized node transplants \( (n = 18) \) were associated with the lowest levels of edema. The edema coefficients in this group were significantly lower than those in the nontreated limbs (node excision) \( (p = 0.039, \text{ unpaired } t\text{ test}) \). On average, the vascularized group had considerably lower edema coefficients than the avascular series \( (n = 24) \), but these effects just failed to reach statistical significance \( (p = 0.055, \text{ unpaired } t\text{ test}) \). In this regard, it is important to note that the vascularized group included animals in which the transplants were successful and unsuccessful (based on histologic assessment). Consequently, the differences between the two groups would be somewhat muted.

**Fluoroscopy Studies**

In the surgical sham series, fluoroscopic analysis revealed nodal tissue, well-defined prenodal vessels, and two or more postnodal ducts (Fig. 6, above, left). In all other groups (vascular and avascular node transplants and following node excision with no transplant), fluid continuity had been reestablished by 8 weeks. This indicated regeneration of lymphatic vessels. In those preparations in which an avascular node was implanted into the excision site, a distinct lymph node was not observed in most cases (Fig. 6, above, right). With vascularized nodal transplants, a node was visualized in some (Fig. 6, below, left) but not all limbs (Fig. 6, below, right). There were also no obvious differences between the 8- and 12-week time points in any of the groups.

**DISCUSSION**

The results in this study support the notion that the lymph node may be beneficial in the treatment of lymphedema. The data from Figures

![Figure 5](image-url)
Fig. 6. Fluoroscopic images of popliteal fossa area. (Above, left) Image of sham preparation at 12 weeks illustrating popliteal lymph node, prenodal, and postnodal vessels. (Above, right) Image taken 8 weeks after an avascular node transplant. In this example, no lymph node is present because of tissue resorption. Regenerating lymphatics have provided some fluid continuity between the prenodal and postnodal ducts. (Below, left) Image showing successful vascularized transplant 8 weeks after surgery. The lymph node can be clearly observed, and prenodal and postnodal ducts have connected to the transplant and provided fluid continuity. (Below, right) Image illustrating an unsuccessful vascularized transplant 12 weeks after surgery. The lymph node has been resorbed, but some fluid continuity has been established by the regenerating vessels. In all examples, the prenodal and postnodal lymphatics are labeled and the area around the lymph nodes (or where the node was originally located) is circled in white.

4 and 5 (inset) indicate that, overall, the vascularized transplant group (which had high levels of lymphatic function) had the lowest edema measures. These edema values were significantly lower than those in the contralateral node excised limbs (lacking lymph nodes and with lower lymphatic transport) and were considerably lower than those in the avascular transplants (also with lower lym-
phatic function), although the latter comparison just failed to register significance.

That the replacement of the node had relatively greater impact on lymphatic function than it did on edema resolution may be attributable, at least in part, to limitations related to edema quantification. The circumference of the limb is not a very sensitive indicator of tissue water elevation. In addition, estimating the area under the curve was the only obvious method of reducing the edema measures to one number. This approach was misleading in some cases. For example, if edema resolved quickly in the vascularized transplanted limb relative to the contralateral node excision limb, the area under the curve (edema coefficient) in the former would be relatively low in comparison. However, if resolution of the edema took much longer in the transplanted side, the coefficient tended to be considerably higher (i.e., more tissue water) despite the eventual good outcome. Nonetheless, the replacement of an excised lymph node with a vascularized node transplant resulted in the restoration of lymphatic function to control levels in many cases and in approximately one-half of these preparations, edema resolved completely.

Importance of the Lymph Node in Tissue Fluid Balance

Negative interstitial pressures in the limbs provide some hydrostatic buffering effect (safety factor), as interstitial fluid pressure must rise before edema develops. If lymph transport is impaired and tissue pressures increase, the magnitude of this “edema safety factor” is reduced, as the limb is now closer to the threshold at which clinical edema may occur. After lymph node removal, lymphangiogenesis occurs and the ducts regenerate. In many cases, the functional properties of the newly formed lymphatic vessels appear to be sufficiently developed to prevent clinical edema; however, a lymph transport deficit likely still exists.

In this regard, the lymph node appears to have a physiologic function beyond its recognized immunologic duties. The nodes appear to separate the lymphatic system into higher (prenodal) and lower (postnodal) pressure areas because protein-free fluid is absorbed from the lymph into the blood capillaries within the nodes to establish an equilibrium of Starling forces across the lymph-blood barrier. This is important because lymphatic vessels contract and provide much of the energy required for lymph propulsion. The pressure range over which the prenodal and postnodal ducts operate is different for these vessel types. Without the dissipation of lymphatic pressure within the lymph nodes, intralymphatic pressures in the downstream vessels would presumably be higher, and this would force these vessels to “pump lymph” under nonoptimal conditions. The combination of lymphatic damage and loss of lymph nodes likely consumes a significant portion of the safety factor discussed above and pushes the tissue compliance curve closer to the threshold associated with clinical edema.

Lymph Node Transplantation as Therapy for Lymphedema

Several surgical approaches for lymphedema therapy have been investigated. These include lymphaticovenous anastomoses, lymphatic vessel transplantation (lymphatic-to-lymphatic anastomoses), omental grafts that contain lymphatics, implantation of lymph node fragments, and vascularized lymph node transplantation. Provided the vasculature is reconnected, data in the literature suggest that autotransplanted lymph nodes seem to survive well. In an experimental lymphedema model in dogs, node transplantation was associated with improved lymph drainage and a reduction of edema.

Becker and colleagues transplanted femoral nodes into the axillary region of 24 postmastectomy lymphedema patients using microsurgical procedures followed by manual drainage (physiotherapy) during the first 3 months. Overall, edema resolution was good, especially in those individuals in which the lymphedema was of the shortest duration. In 10 of 24 cases, the edema appeared to be completely resolved. However, isotopic lymphangiography in 16 patients suggested that node transplants were successful in only 31 percent of individuals. Nevertheless, although the surgical complexity and potential donor-site morbidity issues complicate the application of lymph node transplantation in clinical practice, such treatment started early may have the potential to reduce or prevent the development of lymphedema at its inception.
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REFERENCES