



**HAL**  
open science

## Effects of autotransplanted lymph node fragments on the lymphatic system in the pig model

K. S. Blum, C. Hadamitzky, K. F. Gratz, R. Pabst

► **To cite this version:**

K. S. Blum, C. Hadamitzky, K. F. Gratz, R. Pabst. Effects of autotransplanted lymph node fragments on the lymphatic system in the pig model. *Breast Cancer Research and Treatment*, 2009, 120 (1), pp.59-66. <10.1007/s10549-009-0367-4>. <hal-00535347>

**HAL Id: hal-00535347**

**<https://hal.science/hal-00535347v1>**

Submitted on 11 Nov 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

## Effects of autotransplanted lymph node fragments on the lymphatic system in the pig model

K. S. Blum · C. Hadamitzky · K. F. Gratz · R. Pabst

Received: 2 December 2008 / Accepted: 5 March 2009 / Published online: 20 March 2009  
© Springer Science+Business Media, LLC. 2009

**Abstract** Secondary lymphedema often develops after removal of lymph nodes in combination with radiation therapy, in particular in patients with breast cancer, inguinal cancer, cervical cancer and melanoma. No convincing treatment for the prevention and therapy of acquired lymphedema exists so far, therefore we wanted to show the reintegration of transplanted avascular lymph node fragments in the lymphatic system and positive effects of the transplanted fragments on the restoration of the lymphatic flow in this study. A total of 26 minipigs underwent lymphadenectomy of both groins. A minimum of one lymph node was retransplanted. The lymph nodes were cut into small pieces and retransplanted in the left groin ( $n = 17$ ) or in both groins ( $n = 9$ ). Different retransplantation techniques were investigated, transplantation of large versus small fragments, with and without capsule. The lymph flow was evaluated 5 and 8 months after surgery, using SPECT/CT and Berlin Blue. The results were confirmed by dissection. The lymph node transplants were assessed histologically. In contrast to the lymph flow in the transplanted groin, the lymph flow in the non-transplanted groin was often malfunctioning. Large lymph node fragments were found reintegrated in the lymphatic system more often than small

slices of lymph node fragments. About 5 months after surgery impairment of lymph flow was seen especially after retransplantation of small slices of lymph node fragments. In seven out of eight minipigs a dermal backflow developed in the non-transplanted groin, 8 months after surgery. Only one minipig of these groups developed dermal backflow in both groins. All lymph node fragments showed an organized structure histologically. Autologous lymph node transplantation has positive effects on the regeneration of lymph vessels and restoration of lymph flow after lymphadenectomy.

**Keywords** Lymph node transplantation · Lymphedema · Dermal backflow · SPECT/CT

### Introduction

Lymph nodes (LN) are located at strategic sites of the body and integrated in the lymphatic system, playing an important role in regulating the extravascular fluid volume and immune responses in the body. On the way through the body the lymph fluid is screened for foreign agents and particles are filtered in the various LN before leaving the lymph vessel and entering the venous system. The pathophysiology of lymphedema development is very complex and not entirely clear [1]. Surgical removal of LN often leads to an impaired lymph flow, because of the damage to the lymphatic network. This causes lymphatic insufficiency and accumulation of fluid in the subcutaneous tissue, if the transport capacity of the lymphatic system is overloaded [2]. Additionally, hemodynamic factors seem to be involved in the maintenance of edema, which is a reversible condition at the beginning [3]. Dermal backflow is considered to be a pathognomonic symptom of lymphatic

K. S. Blum (✉)  
Institute of Diagnostic and Interventional Neuroradiology,  
OE 8210, Medical School of Hannover, Carl-Neuberg-Str. 1,  
30625 Hannover, Germany  
e-mail: blum.katrin@mh-hannover.de

K. S. Blum · C. Hadamitzky · R. Pabst  
Institute of Functional and Applied Anatomy,  
Medical School of Hannover, Hannover, Germany

K. F. Gratz  
Clinic for Nuclear Medicine, Medical School of Hannover,  
Hannover, Germany

insufficiency, caused by lymphatic valve insufficiency which leads to retrograde lymph flow from the deep vessels to the superficial lymphatic system [4]. Fibrosis and sclerosis of the dermal structures and hypertrophy of subcutaneous adipose tissue are the morphologic correlate of irreversible lymphedema [5, 6]. Although the introduction of sentinel lymph node biopsy has reduced postoperative complications after cancer treatment, secondary lymphedema is still one of the major complications, e.g., after breast cancer treatment, in particular when the axillary lymph nodes have to be removed in combination with radiotherapy [7–9].

Exercise, manual lymph drainage, compression garments and intermittent pump therapy are common, symptomatic treatment options requiring a good compliance and lifelong treatment [10–12]. Microsurgical lymphovenous or lymph to lymph vessel anastomoses are possible surgical interventions, but without significant longterm benefit to the patients [13–16]. Liposuction can reduce the volume in upper extremity lymphedema, especially in combination with compression garments, although the lymph flow is not cured [5, 17]. The urgent need for adequate animal models to prevent lymphedema, has been stressed recently [1]. Studies with LN transplantation alone and in combination with growth factor therapy showed an improvement of lymphedema after lymphadenectomy in mice [18]. These results are not unexpected, since a role of LN in the regulation of peripheral fluid homeostasis is obvious [19]. The preventive effects of residual LN was shown recently and was obvious since the biopsy of the sentinel lymph node only reduced the occurrence of lymphedema [20]. In a previous study we established a technique to document the size and topography of auto-transplanted avascular LN fragments in the minipig by using SPECT/CT [21]. In a pilot study just published, the advantage of using SPECT/CT to localise lymph nodes draining the area in patients under breast cancer treatment has been documented [22].

In the present study the aim was to show that the transplanted avascular LN tissue is reintegrated in the lymphatic system and that LN retransplantation is able to restore the lymphatic flow. Different surgical procedures were performed with the LN before retransplantation: transplantation after cutting the LN into small or large pieces, enlargement of the surface of LN fragments through a butterfly flap (transverse incision close to the capsule of the other end of the LN and a flap of both halves), transplantation of LN fragments sewn as a tandem and transplantation of LN fragments after removal of the capsule (Fig. 1). The repopulation of LN fragments with lymphocytes was analyzed histologically to show the functionality of the tissue.

## Materials and methods

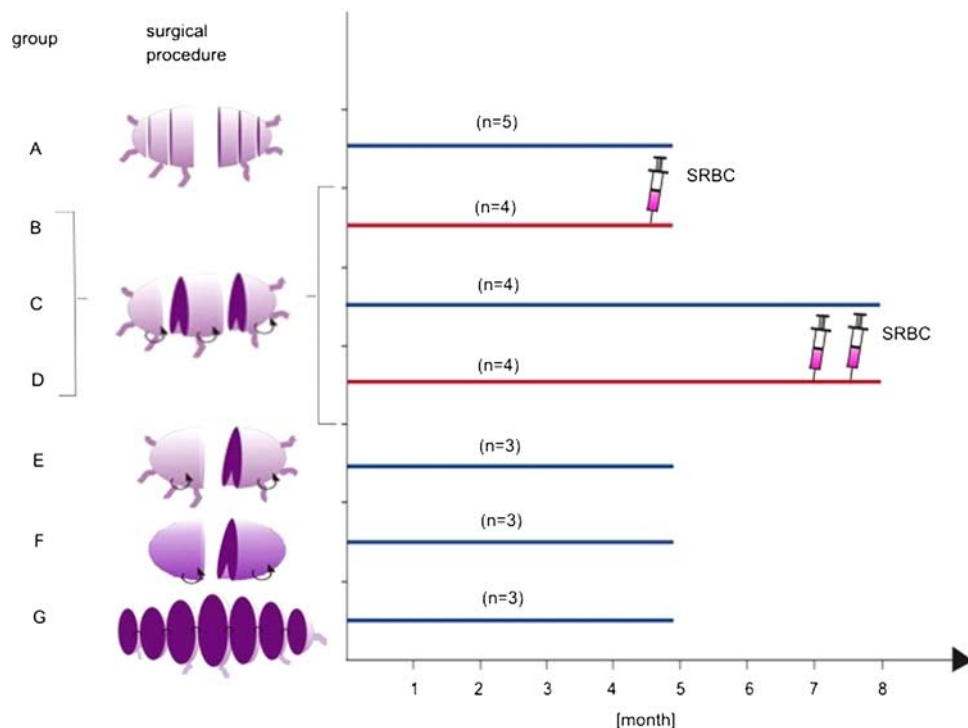
### Animals

Twenty-six female Göttingen minipigs (Reliehausen, Germany), 2–4 months old and with a mean weight of  $8.5 \pm 2$  kg, were fed mixed pig food and water ad libitum and kept under specific pathogen free (SPF) conditions. Surgery was performed after an adaptive phase of 2 weeks. The experiments had been approved by the local government (Ref. No. 509.6-42502-04/809).

### Surgery and postoperative immune stimulation

Premedication, anesthesia and wound prophylaxis was performed as described before [21]. In group A ( $n = 5$ ) the superficial inguinal LN of the left groin was excised and cut into six small pieces ( $\sim 8 \times 5 \times 2$  mm). Three LN fragments were placed in an artificial subcutaneous pouch and a medial pouch was filled with the other three fragments (Fig. 1). In the animals of group B ( $n = 4$ ), group C ( $n = 4$ ) and group D ( $n = 4$ ) the excised, superficial LN of the left side was cut into three pieces. Enlargement of the surface was achieved through a transverse incision close to the capsule of the other end of the LN and a flap of both halves of the LN before reimplantation (like a butterfly flap). Those pieces were then placed in three subcutaneous pouches, two in the groin (medial and lateral) and one piece in an area 3 cm distal to the groin at the lateral side of the leg (Fig. 1). In all four groups the LN of the right side was removed as control. The superficial inguinal LN of both sides of the minipigs of group E ( $n = 3$ ) were cut transverse into two pieces. The same procedure was performed with the superficial LN of the minipigs of group F ( $n = 3$ ), with the difference that the whole capsule was removed before embedding them in two artificial, subcutaneous pouches in the left and right groin (Fig. 1). In the minipigs of group G ( $n = 3$ ) the LN was cut into small slices in a transverse direction. The slices were sewn as a tandem (Fig. 1) and the chain of LN fragments was placed in the subcutaneous tissue of both groins, similar to the method described by Fu et al. [23].

To evaluate the effect of immune stimulation on the regeneration of the LN fragments and on the development of a lymphedema 0.25 ml of sheep red blood cells (SRBC) (Virion/Serion GmbH, Würzburg, Germany) were injected into the draining area of the inguinal LN at the medial and lateral side of both feet. This procedure was performed with all animals of groups B and D 4 and 7 months after surgery, respectively (Fig. 1). All retransplantation areas were closed by atraumatic sutures and the skin was sutured. The wound healing was checked regularly.



**Fig. 1** Overview of study groups, surgical procedures and antigen stimulation. Minipigs of groups *A–D* underwent LN transplantation of the left groin only. In groups *B* and *D* the minipigs received additional stimulation with SRBC (2 and/or 4 weeks before SPECT/CT). SPECT/CT was performed 5 months after surgery in groups *A*, *B* and *E–G*. The LN of group *A* was divided into six pieces before transplantation. The LN in the minipigs of groups *B–D* was cut into

three pieces and enlarged by a “butterfly flap”. The “butterfly flap” was also performed with the LN of groups *E* and *F*, but the LN was divided into only two pieces in both groups. In group *F* the capsule was removed from the LN in addition. The LN of minipigs of group *G* was cut into small slices and those slices were attached to each other with one stitch

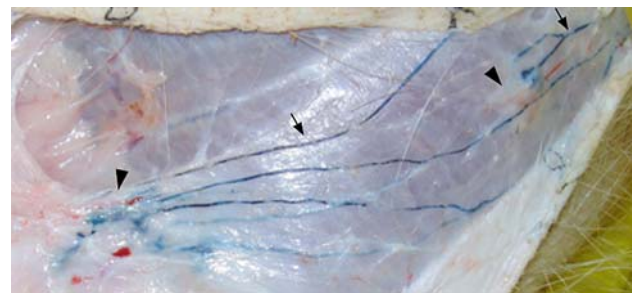
#### Radiotracer administration and Tc-99 m-NC-SPECT/CT lymphoscintigraphy

The regeneration of the LN fragments and the integration of these fragments into the lymphatic system were evaluated with Tc-99 m-NC-SPECT/CT (combined Technetium-99 m-Nanocolloid-single photon computed tomography and transmission computed tomography) lymphoscintigraphy in combination with the Berlin Blue technique to assess the situation in situ, utilizing methods previously described [21].

SPECT and CT images were fused for anatomical orientation and displayed as additional set of images (SPECT data, CT data and SPECT/CT data).

#### Postmortem examination

Five months after surgery the animals reached a mean weight of  $16.5 \pm 2.3$  kg. The skin of the proximal hind leg and the groin area of the minipigs were opened carefully. Lymphatics running to the area of the implanted LN tissue could easily be identified by their blue color in the animals which had received Berlin blue (Fig. 2). With a hand probe



**Fig. 2** Draining area of the left groin (macroscopic view). Afferent Berlin blue filled lymph vessels (arrows) are draining via lymph node transplants (arrowheads). (Color figure online)

(C-Track automatic equipped with Omni Probe-Gamma-sonde, Tc-99 m-collimation, AEA Technology QSA, Brunswick, Germany) the radioactivity was localized. The radiotracer enriched tissue was excised and frozen in liquid nitrogen.

#### Image analysis

SPECT/CT data were evaluated qualitatively by two different investigators according to modified criteria specified

**Table 1** Comparison and evaluation of SPECT/CT results of minipigs of groups A and B (5 month after surgery) and groups C and D (8 months after surgery)

Minipig	Transplants recovered	Right groin		Left groin	
		Impaired lymph flow	Dermal backflow	Impaired lymph flow	Dermal backflow
Group A					
1	2 hot spots	+	–	–	–
2	2 hot spots	–	–	–	–
3	1 hot spot	–	–	–	–
4	2 hot spots	–	–	–	–
5	2 hot spots	+	–	+	–
Group B					
6	2 hot spots groin 1 hot spot knee	+	++	–	–
7	1 hot spot knee 1 hot spot groin	+	++	+	–
8	2 hot spots groin	+	++	+	–
9	1 hot spot groin	+	–	–	–
Group C					
10	1 hot spot	+	++	–	–
11	1 hot spot	+	–	–	–
12	1 hot spot	+	++	+	–
13	1 hot spot	+	++	–	–
Group D					
14	0 hot spot	+	++	–	–
15	1 hot spot	+	++	–	–
16	2 hot spots	+	++	+	++
17	2 hot spots	+	++	–	–

Only the LN of the left groin was transplanted. The lymph flow in the right groin was often malfunctioning, especially in minipigs of groups B and D, which were injected with SRBC. Different numbers of LN transplants were recovered. SPECT/CT correlated with results from dissection

by Weissleder and Weissleder [24]. The criteria evaluated quantitatively were: LN transplants and lymph flow in the groin. In the animals of groups A–D the lymphatic flow of the left hind leg (LN transplantation in the groin) was compared with the lymphatic flow of the right hind leg (control group with lymphadenectomy of the right groin). Due to hygienic reasons it was impossible to perform SPECT/CT with living minipigs, therefore rendering a quantitative analysis of lymphatic flow impossible. To evaluate the changes in lymphatic flow with and without LN transplantation, the qualitative analysis of scintigraphic images alone is sufficient to detect morphologic changes [24].

## Results

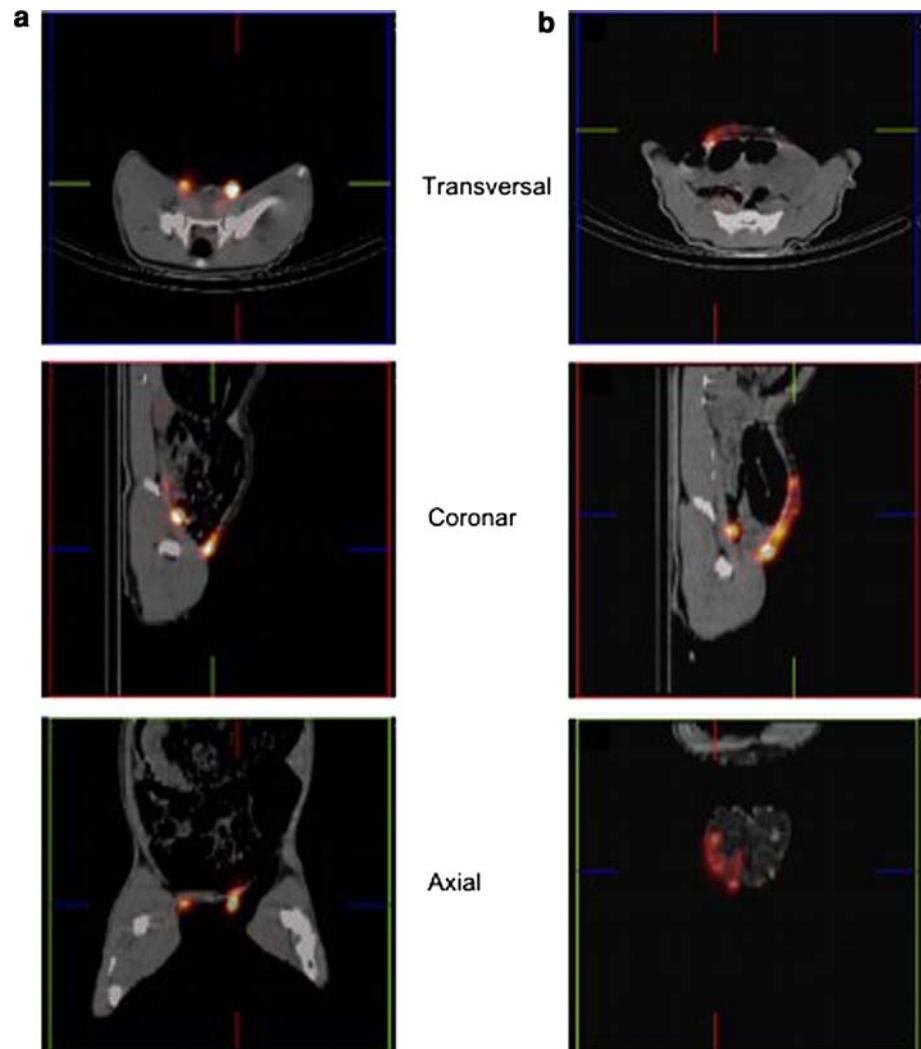
### Tc-99 m-NC-SPECT/CT

No significant increase of the diameter of the hind legs was noticed during the 5 or 8 months after surgery. The wound

healing was checked regularly and no infection or hypertrophic scar tissue development was reported.

Differences in the drainage of the Tc-99 m-NC were seen in the lymphoscintigraphy of both hind limbs of the minipigs of groups A–D. As displayed in Table 1, only one minipig of group A developed a dermal backflow in the right groin region. In most animals of this group two Tc-99 m-NC enhancing hot spots and accumulation of the tracer in the LN fragments were observed in the left groin (Fig. 3a). In contrast to group A, in all animals of group B dermal lymph vessel collaterals were observed in the right groin, whereby three animals had a dermal backflow (Table 1). Only in one minipig of group B was enhancement of Tc-99 m-NC found in all three regions of LN transplantation. Two animals showed two hot spots. In all minipigs of groups C and D it was difficult to exactly localize the transplanted LN fragments in the groin. Although macroscopic and microscopic analysis showed lymphatic tissue in the groin, those fragments could not be visualized exactly with SPECT/CT. In the macroscopic and microscopic investigation the size of the LN fragments was

**Fig. 3** Differences in lymph flow displayed with SPECT/CT. **a** LN transplant in the left groin with efferent lymph flow. Regular lymph flow in the right groin after lymphadenectomy 5 months post surgery. **b** Dermal backflow of the tracer in a minipig 8 months after lymphadenectomy



much smaller at 8 months (group C and D) than at 5 months (group A and B) after transplantation. Dermal backflow in the right groin was found in all animals of group D and in three of four animals of group C (Fig. 3b). Only one minipig of group D developed a dermal backflow in the left groin (minipig 16) and a collateralization of lymph vessels was found in minipig 12, group C. The other animals of group C and D showed no impairment of lymphatic drainage in the transplanted left groin.

In all minipigs of group C one hot spot in the left groin was found, although there was just a slight increase of radioactivity in the LN fragments compared to the accumulation of tracer in the subcutaneous tissue of the right groin. Two Tc-99 m-NC enhancing hot spots in the left groin, resembling the transplanted LN fragments, were recovered in two minipigs of group D, one hot spot was found in one animal of the same group (Table 1). In one animal of group D no hot spot was found in the groin,

although lymphatic tissue was determined in the left groin in the microscopic analysis.

The effect of different surgical procedures was investigated in the minipigs of group E, F and G. The inguinal LN of both groins were removed, processed and retransplanted. In group E lymph vessel collaterals were seen in the right groin of all three minipigs (Table 2). No significant dermal backflow was found. As shown in Table 2 different numbers of radiotracer enhancing hot spots were found in the left and right groin. All animals of group F showed collateralization of lymph vessels, animal 22 and 23 only on one side, and animal 21 on both sides. Dermal backflow led to difficulties in determining the transplanted LN fragments, but at least one radiotracer enhancing hot spot was found in each groin of the other two minipigs of this group (Table 2). No circumscribed hot spot was detected in either groin of all animals of group G. Minipig 24 developed dermal backflow in both groins, minipig 25 in the right

**Table 2** SPECT/CT results 5 months after autologous LN transplantation of both groins

Minipig	Transplants recovered		Right groin		Left groin	
	Right	Left	Impaired lymph flow	Dermal backflow	Impaired lymph flow	Dermal backflow
Group E						
18	1	2	–	–	+	–
19	2	0	–	–	+	–
20	2	0	–	–	+	–
Group F						
21			+	–	+	++
22	2	1	+	–	–	–
23	2	2	–	–	+	–
Group G						
24			+	++	+	++
25			+	++	–	–
26			+	–	+	–

The best restoration of the lymph flow was seen in the minipig of group E, with transplantation of two LN pieces in combination with the “butterfly flap”. Similar results were seen in minipigs of group F, which underwent the same procedure but with the capsule removed. In group G dermal backflow was often detected. In animals with dermal backflow and in all animals of group G LN transplants could not be detected with SPECT/CT

groin, and a development of lymph vessel collaterals was found in both groins of minipig 26.

#### Macroscopic examination

Macroscopic examination of both groins showed various amounts of LN fragments of different size. There was no significant difference between the size of recovered LN fragments comparing groups A and B (5 months after transplantation) and comparing the size of LN fragments of the group C to G (8 months after transplantation). The LN fragments of group A and B were bigger in size compared to those of groups C, D, E, F and G. In the animals 8 months after transplantation very small LN resembling tissue were often found, sometimes only consisting of one germinal center.

The location of transplanted LN fragments in the right groin was consistent with the Tc-99 m-NC accumulating spots in the SPECT/CT in all animals of groups A–C and in all animals, except one, of group D. In all Tc-99 m-NC enhancing regions in the animals of groups E–G lymphatic tissue was found. Although no hot spot was detected in some animals of groups E and F and all animals of group G, lymphatic tissue was found in the groins of these animals. Reticular fibers and scar tissue were observed in all animals and a network of Berlin Blue filled, twisted and partially dilated lymph vessels in the animals with lymph vessel collaterals. Dermal backflow was indistinguishable from lymph vessel collateralization in the macroscopic inspection.

#### Discussion

The removal of LN in the treatment of cancer by surgery is one major factor causing breast cancer-related lymphedema. Other significant predisposing factors for breast cancer-related lymphedema development are radiotherapy, the number of removed LN and the tumor size [25]. The removal of the LN damages the axillary lymphatic system and impairs lymph drainage. As described by Hadamitzky and Pabst, adequate models to study lymphedema development are missing, although there is an urgent need for such animal models [1]. In the present study we showed that lymphadenectomy results in a development of lymph vessel collaterals and dermal backflow in minipigs 8 months later. The dermal backflow seems to be pathognomonic for lymph vessel insufficiency, which results from valvular incompetence, often seen in patients with lymphedema [3]. Another predisposing factor of lymphedema development is infection of the draining area. [26]. This situation was simulated by administration of SRBC. Especially 5 months after surgery, minipigs developed a dermal backflow after SRBC injection (group B) much faster than the control group (group A). This difference was not as distinct 8 months after surgery. One possible reason for this finding is that the dermal backflow is already established at this time. Although there was disturbance of the lymphatic flow no differences in leg-volumes were detected, which can be explained by a too short period of investigation. This phenomenon was also described in mice by Tamela et al, who documented a subcutaneous

lymphedema with MRI, but without changes in the volume of the leg [18].

Five months after LN autologous transplantation, most of the LN fragments can be localized with SPECT/CT, which suggests that the fragments are integrated in the lymphatic system and are working as a filter, collecting the nanocolloid. Eight months after surgery the LN fragments appear much smaller in size than 5 months after surgery. This is not influenced by the application of SRBC, although the LN fragments seem more organized in the minipigs which had received SRBC. The smaller size can be partially explained by the transplantation procedure. A further possible explanation is the replacement of necrotic areas with subcutaneous fat. In the LN fragments 5 months after surgery necrotic areas are found between and at the edge of germinal centers. In most LN fragments 8 months after transplantation no necrotic areas are found in the transplants, suggesting the replacement of necrotic areas by subcutaneous tissue.

The positive effect of autologous LN transplantation on the restoration of lymphatic drainage and the reintegration of transplanted LN fragments in the lymphatic vasculature has been documented [18, 27]. In contrast to those studies we performed an avascular transplantation, because it had been shown that hypoxia induces the expression of VEGF, which is chemotactic for monocytes and macrophages. Macrophages in turn secrete many lymphangiogenic factors such as VEGF-C and VEGF-D (for review see Saharinen [28]). The results clearly show differences in lymph flow between the transplanted region and the control region 5 and 8 months after transplantation. With some exceptions the lymph flow in the transplanted region was almost normal 5 and 8 months after surgery. In the control region most animals developed impairment of the lymphatic flow beginning with the development of lymph vessel collaterals up to a dermal backflow in the region. The results from SPECT/CT of groups E–G show that the transplantation of small slices of LN fragments, which are connected with one stitch, results in dermal backflow in 50% of cases. A possible explanation is the development of scar tissue between the fragments, which constricts and inhibits lymphangiogenesis. The best results are seen in the animals of group E, in which the LN was cut transversely into two pieces before transplantation. This technique seems to have the advantage that small pieces have in total a large surface area, which can subsist by diffusion. The capsule can function as a large lymph vessel and lymphangiogenesis can start from there, sprouting toward the capsule.

The results show a protective effect of LN fragment transplantation against development of dermal backflow. A possible explanation is that lymph vessels sprout from and toward the LN fragments and shorten the period of lymph

vessel reconnection. The potential of lymphatic tissue to guide lymph vessel sprouting is known from the development of LN and the lymphatic system [29]. The protective effect of the LN fragments against foreign antigens also seems evident, regarding the results of SPECT/CT of group A compared to B and C compared to D. Another important function of the LN fragments seems to be the regulation of fluid homeostasis, as described before [30, 31]. We are convinced that these functions make this model a promising method to prevent impairment of lymph flow and to restore lymphatic flow after lymphadenectomy. Long term results have to show whether lymphedema can be prevented by lymph node transplantation. Further studies are necessary to evaluate the effect of preoperative radiation as in breast cancer patients on the lymph flow and the transplanted LN fragments.

**Acknowledgment** The excellent technical assistance of Andrea Herden and Karin Westermann and the polishing of the English by Sheila Fryk are gratefully acknowledged. This study was partly supported by the German Research Foundation (Cluster of Excellence “From Regenerative Biology to Reconstructive Therapy, REBIRTH”).

## References

1. Hadamitzky C, Pabst R (2008) Acquired lymphedema: an urgent need for adequate animal models. *Cancer Res* 68:343–345. doi: [10.1158/0008-5472.CAN-07-2454](https://doi.org/10.1158/0008-5472.CAN-07-2454)
2. Lawenda BD, Mondry TE, Johnstone PA (2009) Lymphedema: a primer on the identification and management of a chronic condition in oncologic treatment. *CA Cancer J Clin* 59:8–24. doi: [10.3322/caac.20001](https://doi.org/10.3322/caac.20001)
3. Mortimer PS (1998) The pathophysiology of lymphedema. *Cancer* 83:2798–2802. doi: [10.1002/\(SICI\)1097-0142\(19981215\)83:12B+<2798::AID-CNCR28>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0142(19981215)83:12B+<2798::AID-CNCR28>3.0.CO;2-E)
4. Szuba A, Strauss W, Sirsikar SP et al (2002) Quantitative radio-nuclide lymphoscintigraphy predicts outcome of manual lymphatic therapy in breast cancer-related lymphedema of the upper extremity. *Nucl Med Commun* 23:1171–1175. doi: [10.1097/00006231-200212000-00004](https://doi.org/10.1097/00006231-200212000-00004)
5. Brorson H, Svensson H, Norrgren K et al (1998) Liposuction reduces arm lymphedema without significantly altering the already impaired lymph transport. *Lymphology* 31:156–172
6. Ryan TJ (1995) Lymphatics and adipose tissue. *Clin Dermatol* 13:493–498. doi: [10.1016/0738-081X\(95\)00092-T](https://doi.org/10.1016/0738-081X(95)00092-T)
7. Burak WE, Hollenbeck ST, Zervos EE et al (2002) Sentinel lymph node biopsy results in less postoperative morbidity compared with axillary lymph node dissection for breast cancer. *Am J Surg* 183:23–27. doi: [10.1016/S0002-9610\(01\)00848-0](https://doi.org/10.1016/S0002-9610(01)00848-0)
8. Kwan W, Jackson J, Weir LM et al (2002) Chronic arm morbidity after curative breast cancer treatment: prevalence and impact on quality of life. *J Clin Oncol* 20:4242–4248. doi: [10.1200/JCO.2002.09.018](https://doi.org/10.1200/JCO.2002.09.018)
9. Meneses KD, McNees MP (2007) Upper extremity lymphedema after treatment for breast cancer: a review of the literature. *Ostomy Wound Manage* 53:16–29
10. Brennan MJ, Miller LT (1998) Overview of treatment options and review of the current role and use of compression garments, intermittent pumps, and exercise in the management of

- lymphedema. *Cancer* 83:2821–2827. doi:10.1002/(SICI)1097-0142(19981215)83:12B+<2821::AID-CNCR33>3.0.CO;2-G
11. Mortimer PS (1997) Therapy approaches for lymphedema. *Angiology* 48:87–91. doi:10.1177/000331979704800114
  12. Rockson SG (2001) Lymphedema. *Am J Med* 110:288–295. doi:10.1016/S0002-9343(00)00727-0
  13. Campisi C, Davini D, Bellini C et al (2006) Is there a role for microsurgery in the prevention of arm lymphedema secondary to breast cancer treatment? *Microsurgery* 26:70–72. doi:10.1002/micr.20215
  14. Filippetti M, Santoro E, Graziano F et al (1994) Modern therapeutic approaches to postmastectomy brachial lymphedema. *Microsurgery* 15:604–610. doi:10.1002/micr.1920150816
  15. Weiss M, Baumeister RG, Hahn K (2002) Post-therapeutic lymphedema: scintigraphy before and after autologous lymph vessel transplantation: 8 years of long-term follow-up. *Clin Nucl Med* 27:788–792. doi:10.1097/00003072-200211000-00007
  16. Damstra RJ, Voesten HG, van Schelven WD, et al (2008) Lymphatic venous anastomosis (LVA) for treatment of secondary arm lymphedema. A prospective study of 11 LVA procedures in 10 patients with breast cancer related lymphedema and a critical review of the literature. *Breast Cancer Res Treat* 113:199–206. doi:10.1007/s10549-008-9932-5
  17. Brorson H, Ohlin K, Olsson G et al (2006) Adipose tissue dominates chronic arm lymphedema following breast cancer: an analysis using volume rendered CT images. *Lymphat Res Biol* 4:199–210. doi:10.1089/lrb.2006.4404
  18. Tammela T, Saaristo A, Holopainen T et al (2007) Therapeutic differentiation and maturation of lymphatic vessels after lymph node dissection and transplantation. *Nat Med* 13:1458–1466. doi:10.1038/nm1689
  19. Lammermann T, Sixt M (2008) The microanatomy of T-cell responses. *Immunol Rev* 221:26–43. doi:10.1111/j.1600-065X.2008.00592.x
  20. Szuba A, Pyszel A, Jedrzejuk D et al (2007) Presence of functional axillary lymph nodes and lymph drainage within arms in women with and without breast cancer-related lymphedema. *Lymphology* 40:81–86
  21. Blum KS, Radtke C, Knapp WH et al (2007) SPECT-CT: a valuable method to document the regeneration of lymphatics and autotransplanted lymph node fragments. *Eur J Nucl Med Mol Imaging* 34:1861–1867. doi:10.1007/s00259-007-0458-6
  22. Chevillat AL, Das I, Srinivas S, et al (2009) A pilot study to assess the utility of SPECT/CT-based lymph node imaging to localize lymph nodes that drain the arm in patients undergoing treatment for breast cancer. *Breast Cancer Res Treat*. doi:10.1007/s10549-008-0283-z
  23. Fu K, Izquierdo R, Vandevender D et al (1998) Transplantation of lymph node fragments in a rabbit ear lymphedema model: a new method for restoring the lymphatic pathway. *Plast Reconstr Surg* 101:134–141. doi:10.1097/00006534-199801000-00022
  24. Weissleder H, Weissleder R (1988) Lymphedema: evaluation of qualitative and quantitative lymphoscintigraphy in 238 patients. *Radiology* 167:729–735
  25. Herd-Smith A, Russo A, Muraca MG et al (2001) Prognostic factors for lymphedema after primary treatment of breast carcinoma. *Cancer* 92:1783–1787. doi:10.1002/1097-0142(20011001)92:7<1783::AID-CNCR1694>3.0.CO;2-G
  26. Petrek JA, Senie RT, Peters M et al (2001) Lymphedema in a cohort of breast carcinoma survivors 20 years after diagnosis. *Cancer* 92:1368–1377. doi:10.1002/1097-0142(20010915)92:6<1368::AID-CNCR1459>3.0.CO;2-9
  27. Becker C, Assouad J, Riquet M et al (2006) Postmastectomy lymphedema: long-term results following microsurgical lymph node transplantation. *Ann Surg* 243:313–315. doi:10.1097/01.sla.0000201258.10304.16
  28. Saharinen P, Tammela T, Karkkainen MJ et al (2004) Lymphatic vasculature: development, molecular regulation and role in tumor metastasis and inflammation. *Trends Immunol* 25:387–395. doi:10.1016/j.it.2004.05.003
  29. Blum KS, Pabst R (2006) Keystones in lymph node development. *J Anat* 209:585–595. doi:10.1111/j.1469-7580.2006.00650.x
  30. Porter CJ, Charman SA (2000) Lymphatic transport of proteins after subcutaneous administration. *J Pharm Sci* 89:297–310. doi:10.1002/(SICI)1520-6017(200003)89:3<297::AID-JPS2>3.0.CO;2-P
  31. Adair TH, Guyton AC (1983) Modification of lymph by lymph nodes II. Effect of increased lymph node venous blood pressure. *Am J Physiol* 245:H616–H622