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A Novel Lymphaticovenular Anastomosis Rat Model

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Abstract:	Introduction: Lymphaticovenular anastomosis (LVA) has become an important procedure for the surgical treatment of lymphedema. In the past, the anatomy of the lymphatic system of animal models has been reported. However, to our knowledge there have been few reports of animal models of LVA including training model. In this study, we report on a relatively simple and ideal animal LVA model based on peritoneal lymph ducts and veins. Patients and Methods: For 10 rats, diameters of lumbar lymphatic ducts and iliolumbar veins in the peritoneal cavity on both sides were measured and LVA was performed. In addition, we measured the diameters of 28 lymphatic ducts and veins in 8 patients who had previously undergone LVA and compared the results with those obtained this rat model. Results: The mean diameter of the lymphatic ducts was 0.61 mm and the iliolumbar veins were 0.81 mm. On the other hand, the mean diameters of the 28 lymphatic ducts and veins of the 8 patients in whom we performed LVA were 0.58 and 0.76 mm, respectively. The differences in the diameters of the lymph vessels and veins between the rats and patients were not statistically significant. Conclusion: We report on an LVA model involving the use of the lumbar lymphatic duct and iliolumbar veins of rats. The diameter, nature, and placement of the anastomosis using this model are very similar to that noted during real human surgery. We believe that our rat model will be useful as a practical training model for LVA and in studies on postoperative changes in LVA.

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Introduction

Since years, liposuction involving removal of lymphatic fluid and fatty tissue or surgical resection of extra subcutaneous tissue has been performed for lymphedema.[1-4] In the field of plastic surgery, microsurgical procedures have become increasingly common. In line with this, lymphaticovenular anastomosis (LVA) has become an important procedure for the surgical treatment of lymphedema.[5-7] However, the postoperative success or failure rates after LVA are difficult to determine, unlike that for normal free flap transfer, because significant symptoms such as ischemia or congestion are not noted. Thus, an estimate of operative result is performed through a change of the edema symptom that various elements coexist, feed-back of operation results are difficult. In addition, LVA are different from conventional microsurgery, and there are little training models, and improvement of operation technique is difficult from this point of view. And the operative methods of LVA vary greatly by institution, and there are many methods for creating an anastomosis such as end-to-end, end-to-side, and side-to-side.[8] In addition, many other specific anastomosis styles have been reported.[9-10] However, it is difficult to compare various anastomosis method in clinical situation and doesn't understand about ideal anastomosis method. We hypothesize that an experimental animal model would be necessary to perform a detailed examination of the postoperative course of the LVA. In the past, the anatomy of the lymphatic system of animal models has been reported.[11-13] However, to our knowledge there have been few reports of animal models of LVA including training model.[14] In this study, we report on a relatively simple and ideal animal LVA model based on peritoneal lymph ducts and veins.

Methods

We selected the lumbar portion of the lymphatic duct for use in our LVA model because the comparative diameter was large based on the rat’s systemic lymphoid dissection and did not require a major aggressive operative approach such as thoracotomy. In addition, we chose the iliolumbar vein as the recipient vein, as it was present in the neighboring region. Ten male rats were dissected for the purpose of LVA. Wistar rats weighing 450–550 g were used as the LVA model. For all the 10 rats, body

weights and diameters of the lumbar lymphatic ducts and iliolumbar veins in the peritoneal cavity on the right and left sides were measured and bilateral LVA was performed using the lumbar portion of the lymphatic duct and iliolumbar vein. General anesthesia was administered via intraperitoneal infusion of pentobarbital sodium at a dose of 50–70 mg/kg. The time of the LVA was measured using a stopwatch. In addition, we measured the diameters of 28 lymphatic ducts and veins in 8 patients of LVA performed previously, and the results were compared to those the rat model in this study. The diameters of all lymph ducts and veins were measured using a stainless steel ruler (Shinwa Measuring Tools Corp.), calibrated to 0.05 mm (Figure 1).

Data analysis

A *T* test was performed to compare the differences in the diameter of lymph vessels and veins between the rats and patients. $P < 0.05$ was considered as a statistically insignificant difference in the mean diameter of lymph vessels and veins between the rats and the patients.

LVA model using the rat

First, after induction of anesthesia, the rats were weighed and placed in the supine position. The abdominal region was shaved. If needed, the four limbs of the rat were secured to the operating table using pins or tape. A 5–6-cm midline abdominal incision was made (Figure 2-a). After the skin incision, we divided both the rectus abdominis muscles into left and right parts and entered the peritoneum for abdominal operation. After the abdomen was opened, we moved intra-abdominal organs such as the stomach, cecum, small intestine, and mesentery into the extraperitoneal space so that the position of the iliolumbar vein could be confirmed near the posterior vena cava and abdominal aorta (Figure 2-b). Next, we identified the lumbar lymphatic duct running directly to the iliolumbar vein near the surface of the posterior vena cava and abdominal aorta (Figure 2-c). An operating microscope was used while dissecting lymphatic ducts and veins and for the LVA. The iliolumbar vein and lumbar lymphatic duct were dissected meticulously to avoid bleeding. The surface of the iliolumbar vein that was surrounded by a dense, fibrous sheath was removed. Two percent lidocaine was applied to relieve

the lymph duct and vessel spasm after the removal of adventitia. The lymphatic duct and vein were then divided for anastomosis by using string or a radio knife. After setting the lymphatic duct and vein on a background sheet, we started the anastomosis. We used 11-0 or 12-0 nylon sutures with 60-μm needles for the anastomosis. Six or eight sutures were usually sufficient to secure the anastomosis. After completion of the anastomosis, we tested its patency by examining countercurrent blood flow in the lymphatic duct and peristaltic motion (Figure 2-d). Finally, we shifted all organs into the abdominal cavity, resutured the rectus muscles, and closed the skin.

Results

The diameters of lumbar lymphatic ducts and iliolumbar veins were measured in all 10 male rats. The mean weight of the rats was 492 g (range, 456–539 g). The diameters of all lymphatic ducts and veins were less than 1 mm. When a vein had a branch, we isolated and used the branch with a diameter nearest to that of a lymphatic duct. The mean diameter of the lymphatic ducts was 0.61 mm (range, 0.5–0.7 mm). The mean diameter of the iliolumbar veins was 0.81 mm (range, 0.7–0.9 mm). All lymphatic ducts exhibit continuous peristaltic motion. The mean duration of bilateral LVA was 42.2 minutes (range, 39–48 minutes). The intraoperative patency rate after anastomosis was 100%. All rats recovered from anesthesia and survived the procedure. The details of the measurements are listed in Table 1. On the other hand, the mean diameters of the 28 lymphatic ducts and veins of 8 patients who had previously undergone LVA were 0.58 (range, 0.3–0.8 mm) and 0.76 mm (range, 0.4–1.0 mm), respectively (Table 2).

The differences in the diameters of the lymph vessels and veins between the rats and patients were not statistically significant ($P = 0.2127$).

Discussion

Mastery of microsurgery is essential for becoming a refined plastic surgeon. Various reports discussing microsurgery training already exist. General training programs for the acquisition of technical microsurgical skills involve the use of silicone tubes and animal models such as chicken vessels and rat femoral arteries and veins. In our institute, we developed a unique training program combining these general training methods, with

which we achieved good results.[15] On the other hand, the diameter of lymph ducts for LVA is usually less than 1 mm, which requires advanced skill compared to that for typical microvascular anastomosis. Compared to microvascular anastomoses, microlymphatic anastomoses have different, difficult characteristics. Lymphatic ducts are transparent, which makes it difficult to define the border between the adventitia and surrounding connective tissue. Because lymphatic ducts are soft and fragile, it is difficult to identify the space between lymphatics, and therefore, an atraumatic operative procedure is needed. In other words, LVA is different from normal microsurgery. We believe that our rat model will be remarkably helpful as a practical training model for LVA creation. However, this model has limitations because it involves the use of animals, and we should keep the use of animals to a minimum. From an ethical perspective, it would be better to use this model at the final stages of training, after having practiced with a supermicrosurgery model that does not involve the use of animals.

Yamamoto et al. reports the LVA model used rat femoral lymphatic vessels and short saphenous vein the model.[16] However, diameter of rat femoral lymphatic vessels were 0.240 ± 0.057mm and very small than human lymphatic vessels for LVA. On the other hand, our rat model has many advantages. One is that the diameters of lymphatic ducts and veins in this model are similar to those of actual lymphatic ducts and veins. The mean diameters of the 28 lymphatic ducts and veins of our 8 patients who had undergone LVA, were very similar to those used in this model. Thus, training of LVA which is very near to real clinic is enabled. Next, we believe that our model is superior because lymphatic ducts and veins of both sides were used. Thus, it is possible to perform the LVA technique multiple times during only a single abdominal operation. Owing to this study, we can now identify lumbar lymphatic ducts and iliolumbar veins for several minutes after the abdominal operation and complete bilateral LVA within a short duration. After creating the anastomosis, a patency test was performed to assess the state of the anastomotic part, as is done during normal microsurgery, and we noted countercurrent blood flow in the LVA area to better judge the success or failure of the procedure. We can now confirm anastomosis patency without using imaging modalities such as indocyanine green (ICG) fluorescent angiography in the case of this model

because the diameters of the lymph duct and vein were comparatively large, with a large amount of lymphatic fluid and venous flow.

Creation of an LVA is a surgical procedure in which anatomically different lymphatic ducts and veins are anastomosed. The anastomotic region can be confirmed using lymphatic duct scintigraphy and ICG postoperatively, but a detailed examination is difficult.[17-21] An experimental animal model of LVA may provide important information about LVA operation other than use as a training model. In this rat experimental model, lymphatic duct is comparatively large and, it seems that end-to-side anastomosis and side-to-side anastomosis are possible other to end-to-end anastmosis. If this animal model is used to compare various anastomosis styles, we could determine the optimal anastomotic style of LVA that could maintain patency for a long term postoperatively.

Conclusion

We report on an LVA model involving the use of the lumbar lymphatic duct and iliolumbar veins of rats. The diameter, nature, and placement of the anastomosis using this model are very similar to that noted during real human surgery. We consider this model a superior training model for LVA surgery and useful in the comparison of anastomotic styles of LVA in the future.

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Figure legends

Figure 1
A stainless steel ruler with calibrations set to 0.05 mm

Figure.2-a
A 5-cm line made for a central abdominal incision

Figure.2-b
We prevented drying of the intra-abdominal organs after the laparotomy by using wet tissues.

Figure.2-c
The left lumbar lymph duct indicated by the white arrow and iliolumbar vein indicated by the black arrow
The diameter of the lymph duct was 0.7 mm, and that of the vein was 0.8 mm.

Figure.2-d
After lymphaticovenular anastomosis, we performed 6 sutures using 11-0 nylon.
Countercurrent blood flow into the lymphatic duct was confirmed.

A Novel Lymphaticovenular Anastomosis Rat Model

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Short Title: A Novel Lymphaticovenular Anastomosis Model

Figure.1
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Figure.2-a
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Figure.2-b
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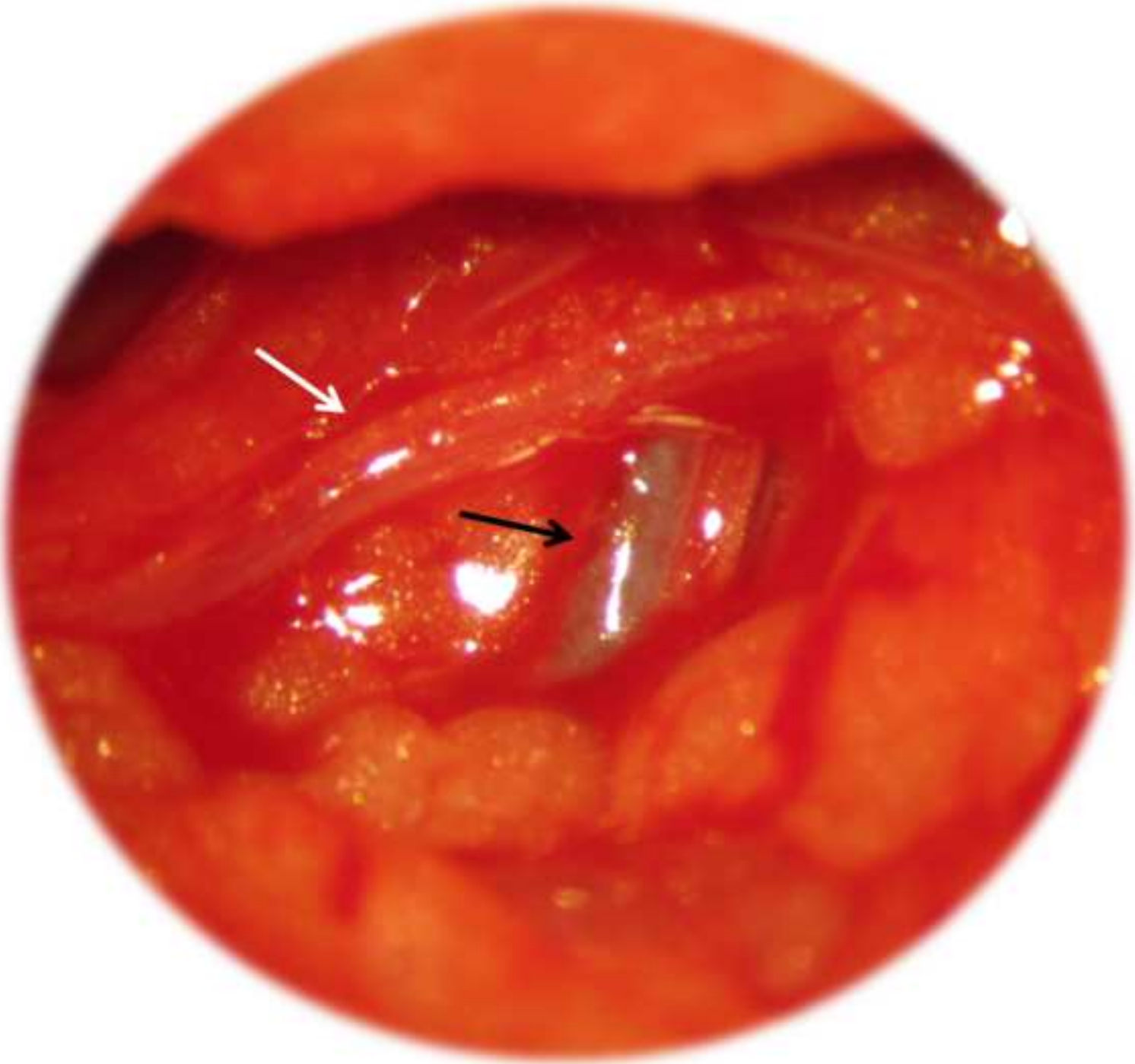

Figure.2-d
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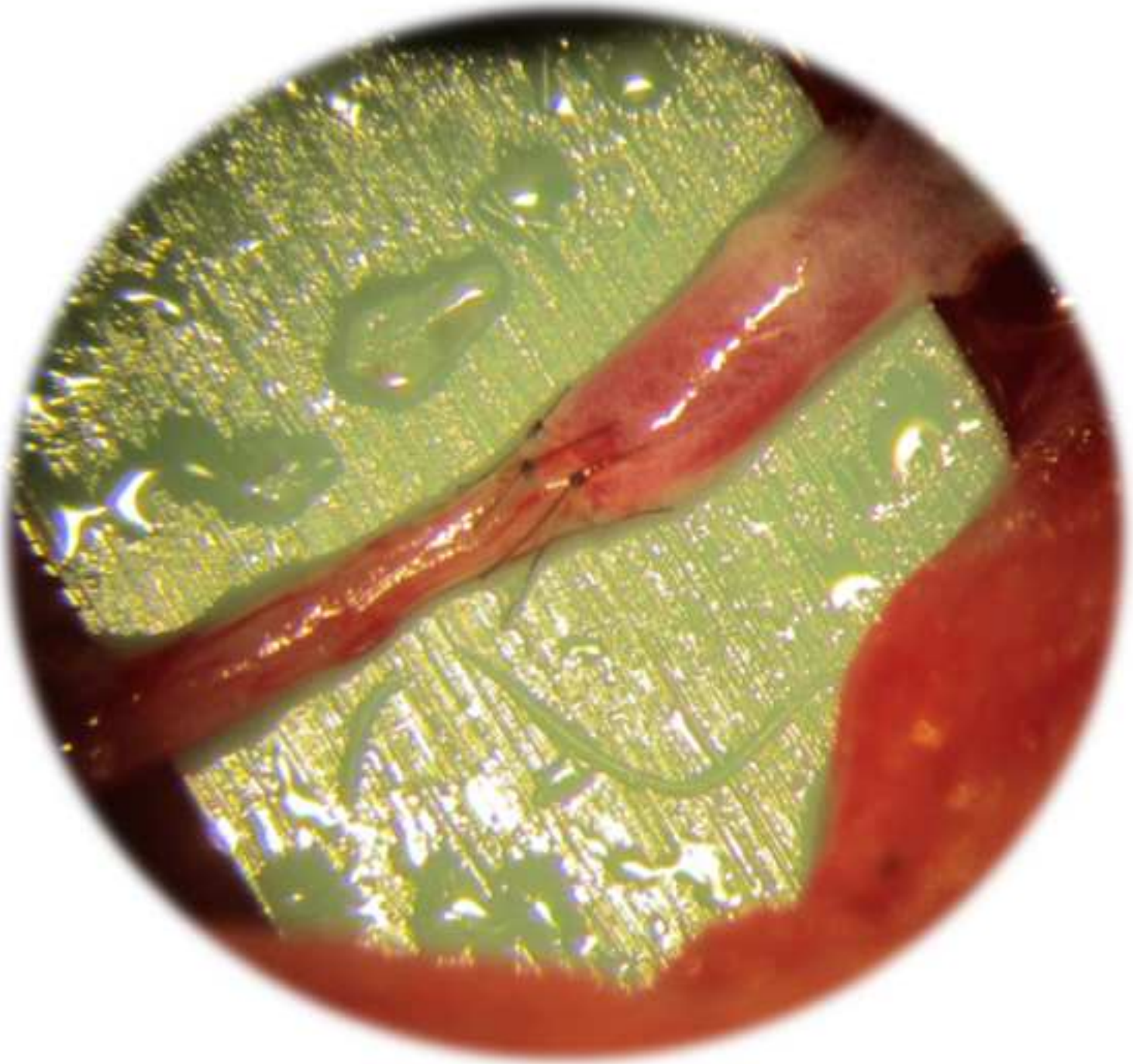

Table.1 Measurements in diameters of lumbar lymph ducts and iliolumbar veins

No.	Body Wight (g)	Rt. LLD (mm)	Rt. ILV (mm)	Lt. LLD (mm)	Lt. ILV (mm)	LVA Time (min)	Anastmosis Patency (Rt/Lt)
1	476	0.6	0.8	0.6	0.8	45	○/○
2	498	0.5	0.8	0.7	0.8	43	○/○
3	539	0.6	0.9	0.6	0.9	48	○/○
4	457	0.6	0.7	0.5	0.8	39	○/○
5	488	0.6	0.8	0.6	0.8	41	○/○
6	501	0.7	0.9	0.7	0.8	43	○/○
7	456	0.5	0.7	0.6	0.7	40	○/○
8	522	0.7	0.8	0.6	0.9	39	○/○
9	488	0.6	0.8	0.6	0.8	43	○/○
10	494	0.6	0.8	0.7	0.8	41	○/○
mean	492	0.60	0.80	0.62	0.81	42.2	

Rt: right
Lt: left
LLD : lumbar lymph duct
ILV: iliolumbar vein
LVA: lymphaticovenular anastomosis
AA: Just after anastomosis
1W: 1 week later

Table.2

Measurements in diameters of lymph ducts and veins about 8 LVA cases

No.	Lymph duct 1 (mm)	Lymph duct 2 (mm)	Lymph duct 3 (mm)	Lymph duct 4 (mm)	Vein 1 (mm)	Vein 2 (mm)	Vein 3 (mm)	Vein 4 (mm)
1	0.6	0.6	0.6	0.7	0.8	0.9	1.0	1.0
2	0.6	0.6	0.8		0.8	0.8	0.8	
3	0.4	0.5	0.6		0.5	0.5	0.8	
4	0.6	0.6	0.8		0.8	1.0	1.0	
5	0.4	0.4	0.5	0.5	0.6	0.6	0.6	1.0
6	0.3	0.5	0.6	0.8	0.6	0.7	0.9	1.0
7	0.3	0.5	0.8	0.8	0.4	0.7	1.0	1.0
8	0.3	0.5	0.6		0.5	0.5	0.6	
mean	0.58				0.76			