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A clinical study of ultrasonic localization-assisted combined transplantation of a bilateral anterolateral thigh perforator flap for the repair of large-area skin and soft tissue defects of the extremities

C. LIU[1], F. GAO[2], X.-L. LIU[3], G.-Z. WU[4]

[1]Department of Breast Surgery, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China
[2]Department of Clinical Laboratory, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China
[3]Department of Hand and Foot Surgery, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China
[4]Department of Plastic and Reconstructive Microsurgery, China-Japan Union Hospital, Jilin University, Changchun, Jilin, China

Liu and Gao equally contributed to this work

Abstract. – OBJECTIVE: The aim of this study was to investigate the clinical efficacy of the combined transplantation of a bilateral anterolateral thigh perforator (ALTP) flap for the repair of large-area skin and soft tissue defects of the extremities.

PATIENTS AND METHODS: Twelve patients who had received bilateral ALTP flap reconstructions for large-area skin and soft tissue defects of the extremities were retrospectively analyzed. The areas of the skin and soft tissue defects were measured preoperatively (18.0×11.0 - 38.0×15.0 cm^2). The wounds were on the forearm, elbow, upper arm, foot, and lower leg. Color Duplex Sonography (CDS) was used to localize the site where the perforator artery of the bilateral thighs penetrated the deep fascia. The selected area was evaluated according to the number of perforating branches and the range of supply. The flap areas and repairable range were further evaluated according to the number of perforating branches detected during the operation to determine whether to retain the deep fascia. It is important to design and adjust the anastomosis of the vascular pedicle according to the specific situation on transfer of the flap to the recipient site. The donor sites of all the patients in the study were closed in the first stage. The amount of bleeding and the blood supply to the flap after vascular anastomosis were evaluated during the operation. The postoperative survival of the flap and complications, such as bleeding, infection, and arteriovenous crisis, were closely monitored. All patients were followed-up at one, three, and six months after surgery to assess their satisfaction with the appearance of the flap transplantation and the recovery of limb function.

RESULTS: The bilateral ATLP flaps survived successfully in all 12 cases and all donor sites were closed in the first stage. No post-surgery complications, including hematoma, wound dehiscence, and infection, were observed at the donor sites, resulting in high patient satisfaction.

CONCLUSIONS: Combined transplantation of bilateral ALTP flaps can repair large-area skin and soft tissue defects in one stage, which not only reduces the number of operations and hospitalization costs but also reduces the damage to the limbs caused by the cutting of large-area flaps from only one side. The accuracy of the surgery was improved by ultrasound-assisted localization. In summary, combined transplantation of bilateral ALTP is a rational yet effective way to repair large-area skin and soft tissue defects of the extremities.

Key Words:
Anterolateral thigh perforator, Extremities, Soft tissue defect, Color duplex sonography, Microsurgery.

Introduction

Currently, large-area wounds of the extremities caused by high-energy violence are usually clinically repaired by using a pre-expanded flap[1], conjoined flap[2,3], or lobulated flap[4]. However, these

Corresponding Authors: Guangzhi Wu, MD, Ph.D; e-mail: wuguangzhi@jlu.edu.cn
Xilin Liu, MD; e-mail: liuxilin@jlu.edu.cn

techniques use a single ultra-long, wide flap for transplantation, and the donor site is closed by skin grafting, inevitably increasing damage and the risk of complications. The anterolateral thigh flap is clinically regarded as the main component, and the combined harvesting of perforator flaps in the thoracodorsal[5] and inferior epigastric arteries[6] suggests a novel approach for the repair of large-area wounds of the extremities. Perforator flap surgery was first described by Koshima and Soeda[7] in 1989. This broke through the traditional concept that the deep fascia vascular network was essential for the survival of the flap, increasing the scope of flap transplantation. Currently, perforator flaps are widely used for the repair and reconstruction of various skin and soft tissue defects, and several novel surgical methods[8-12] have been derived and developed. However, a single conventional perforator flap cannot cover large-area skin and soft tissue defects on the extremities effectively, and the use of combined transplantation requires more donor sites for the flap, increasing both the difficulty and risk of the operation. Recently, the flap donor site has received more attention, and reducing the damage at the flap donor site is strongly emphasized by clinicians in literature. Pre-expansion of flap donor sites for ultra-large-area wounds, together with optimization of the flap design and combined flap transplantation have been used[2,3,10] to repair ultra-large-area wound defects and minimize damage at the donor site. As a typical perforator flap, the anterolateral thigh perforator (ALTP) is characterized by a concealed donor site, long vascular pedicle, and large diameter, and has the advantages of causing no damage to vital vascular and neural tissues and not affecting limb function after flap harvesting. Hence, the ALTP has been widely applied[1,3,6,10] in repairing tissue defects in various parts of the trunk and limbs. The anterolateral thigh flap can be flexibly adapted according to the patient's injury and defects and includes the flow-through anterolateral thigh flap, chimeric flap, and lobulated flap. However, the perforators vary in complexity in different populations, with differences between the sexes and ethnic groups. Flap harvesting and wound repair are incredibly challenging. Technological development has led to the wide application of digital devices in clinical examination and diagnosis. Handheld Doppler devices allows preoperative assessment of the most favorable surgical procedures while Color Duplex Sonography (CDS)-assisted localization enhances the localization coincidence rate[13,14]. In this study, a CDS-assisted localization technique combined with a bilateral anterolateral thigh perforator (ALTP) flap was used to repair large-area soft tissue wounds of the extremities. All donor sites were closed in the first stage, thus achieving good clinical efficacy.

Patients and Methods

Clinical Data

Twelve cases who received bilateral ALTP flaps for repairing large-area skin and soft tissue defects of extremities during January 2018 - June 2022 were retrospectively analyzed. Of these, eight were male and four were female. Their ages were 19-60 years old, with an average age of 34 years old. The injuries were caused by traffic accidents in four cases, crush injuries in three, heavy-object injuries in two, and machine strangulation in three cases. After emergency debridement, it was found that the bone, joint, or tendon, and other deep tissues were injured to varying degrees, including five cases of wounds involving the shank and foot, six cases involving the upper arm and forearm, and one case involving the wrist stump, with the defect areas ranging from 18.0×11.0 cm^2 to 38.0 cm×15.0 cm^2. This study was approved by the Ethics Committee of China-Japan Union Hospital. Signed written informed consent was obtained from the participants before the study.

Operation Method

All patients underwent primary emergency debridement after trauma, with fracture reduction and internal fixation performed in patients with fractures, exploratory repair performed in patients with vascular, nerve, and tendon injuries, and replantation of the avulsed skin conducted in patients with skin avulsion. Additionally, vacuum sealing drainage (VSD) was performed on wounds with poor soft tissue conditions and heavy contamination, and microbial culture and drug sensitivity tests were conducted for the determination of suitable antibiotic treatment. The bilateral anterolateral thigh ultra-long perforator flap repair was performed in patients with clean wounds and clear boundaries of skin necrosis at the wound margins. The patient's physical condition was comprehensively evaluated preoperatively, and CDS (SIEMENS S2000, Munich, Germany) was used to localize the site where the large perforator anterolateral thigh penetrated

Figure 1. **A-B**, Large skin and soft tissue defect of the left elbow and upper arm before the operation. **C-D**, The location of the perforator using preoperative color Doppler ultrasonography. **E**, Design of a free anterolateral thigh perforator flap on right thigh (size: 20.0×7.0 cm^2). **F**, Design of a free anterolateral thigh perforator flap on left thigh (size: 14.0×6.0 cm^2). **G**, The right proximal perforating branch coming from the transverse branch of the lateral circumflex femoral artery, and the distal end coming from the descending branch of the lateral circumflex femoral artery. **H**, The perforating branches of the left anterolateral thigh perforator flap coming from the descending branch of the lateral femoral circumflex artery. **I-J**, Successful coverage of the defect was achieved.

through the deep fascia, followed by marking the body surface.

Recipient Site Treatment

The operations were performed under general anesthesia administered both intravenously and by inhalation. During the operation, a heating blanket was used to prevent the patient's temperature from dropping due to the prolonged operation time, blood loss, and fluid infusion. All patients were in a supine position. Routine disinfection was performed on both lower limbs, as well as in the case of upper limbs with skin and soft tissue defects. Arteries and veins with calibers suitable for anastomosis with the flap vessels were explored along the proximal end of the recipient site. Generally, the dorsal artery of the foot, anterior tibial artery, posterior tibial artery, or their branches were selected for the lower leg and foot, and the ulnar artery, radial artery, or their branches were selected for the forearm and hand. The veins accompanying the arteries were selected, and the vessels in the recipient site were marked. Cloth samples corresponding to the size and shape of the recipient site wound were cut and used as templates for flap cutting.

Flap Design and Dissociation

Before flap harvesting, the perforator of the flap was detected and marked using CDS. The flap was then designed and the boundary of the flap to be cut was drawn to correspond with the recipient soft tissue defect area and the location of the perforator. During the operation, the outer and upper margins of the flap were cut, followed by cutting the skin, subcutaneous tissue, and deep fascia. The flap was inverted medially under the deep fascia, and the perforator vessel was explored and protected according to the position of the perforator marked before the operation. The fascia lata was cut at the muscle space between the rectus femoris and vastus lateralis, and the descending branch of the lateral femoris circumflex artery was explored within the muscle space. Retrograde dissection of the perforator vessel was performed to the main trunk of the descending branch of the lateral femoris circumflex artery, and the branches along the way were ligated. The medial side of the flap was then cut, and the flap was separated until only the vascular pedicle was connected, while the blood supply of the flap was carefully observed. After ensuring that the perforator was not damaged, the main artery (i.e., the descending branch of the lateral femoris circumflex artery) was cut off and ligated to free the flap completely. The flap should be cut according to the thickness of the patient's subcutaneous fat and should exceed 5-10% of the defect area. The pedicle should be cut based on the length of the blood vessels required by the recipient site. The flap of the opposite limb can be freed, while the bilateral flaps can be cut and freed simultaneously to improve efficiency and reduce the operation time.

Flap Transplantation and Combination

The vascular pedicle was cut off simultaneously on both sides and perfused with normal heparin saline. The anatomical relationship of the flap should be further confirmed after this disconnection. Furthermore, it should be designed according to the actual number of perforating branches and the range of the perforating branches that could supply nourishment. The proximal end of the major artery of the proximal flap was first anastomosed with the proximal extremity of the artery at the recipient site and then anastomosed with the venae comitantes. The distal end of the major artery of the proximal flap was anastomosed with the major artery of the distal flap, and the distal end of the proximal flap venae comitantes was anastomosed with the venae comitantes of the distal flap. Finally, the flap was sutured without tension between the recipient site and the flap.

Closure of Recipient Site

After complete hemostasis, negative pressure drainage was retained at the flap donor site, and the deep fascia, subcutaneous tissue, and skin incision were sutured. Direct suturing was possible in all the patients, with an arc scar left after the wound healing.

Results

In this study, the flaps in all 12 cases survived and all donor sites were closed in one stage. No post-surgery complications (e.g., hematoma, wound dehiscence, and infection) were observed at the flap donor sites. All patients were followed-up postoperatively. The flaps had a soft texture, red color, and good elasticity, showing a good range of motion in patients with a cross-joint flap and no interference with basic activities necessary for daily life. Two patients subsequently complained that the flap was slightly swollen and required flap reconstruction, while the others were satisfied with the flap. The mean follow-up

time was 16.4 months (range = 9-32 months). Most cases showed satisfactory contour, and there was no excessive bulk.

Typical Cases

Case 1

A 58-year-old male patient suffered skin and soft tissue defects of the left upper arm and elbow due to a machine crush injury, with a wound area of about 19×12 cm^2. During the operation, we cut a right ALTP flap with an area of 20×7 cm^2 and a left ALTP flap with an area of 14×6 cm^2. The right anterior thigh flap was placed at the proximal end, and the left anterior thigh flap was placed at the distal end. After separating the brachial artery and vein and their muscle branches in the recipient site, end-to-end anastomosis was performed between the proximal descending branch of the right anterolateral thigh flap and the brachial artery, while the proximal descending branch of the left anterolateral thigh flap was anastomosed with the distal descending branch of the right anterolateral thigh flap, and the transverse branch of the right anterolateral thigh flap was anastomosed with the distal descending branch of the left anterolateral thigh flap. The vein was anastomosed with a venae comitantes of the brachial artery and subcutaneous vein. The donor sites were closed directly. The postoperative course was uneventful (Figure 1).

Case 2

A 65-year-old male patient suffered a comminuted right calcaneus fracture, together with skin and soft tissue defects of the right foot and distal leg due to injury from a heavy object. Radical debridement led to the exposure of tendons and bones. The lateral side of the right plantar calcaneus, the plantar aponeurosis, and the distal calcaneus tendon of the lower leg were exposed, and the wound area was about 18×13 cm^2. During the operation, the left and right ALTP flaps were designed to repair the Achilles tendon wound, and the plantar and lateral part of the heel, respectively. We cut the right ALTP flap measuring 21×9 cm^2 and the left ALTP flap measuring 9×6 cm^2 and separated the posterior tibial artery and vein of the recipient site. End-to-end anastomosis was performed between the proximal end of the anterolateral descending branch of the left thigh and the posterior tibial artery of the recipient site, while the proximal end of the descending branch of the anterolateral right thigh flap was anastomosed with the distal end of the descending branch of the left perforator flap, and the transverse branch of the anterolateral right thigh flap was anastomosed with the distal end of the descending branch of the anterolateral right thigh flap, and the vein of the flap was anastomosed with the posterior tibial vein of the recipient site. The flap survived with a good blood supply postoperatively (Figure 2).

Discussion

The rapid development of industrial modernization has led to increasing numbers of injuries caused by high-energy damage, usually resulting in large-area soft tissue defects of the extremities. However, ultra-large-area wounds cannot be covered by a single flap, especially in cases with bone and tendon exposure and severe vascular injury, which is clinically challenging. Currently, these injuries are usually repaired using combined flaps[1,3,6,10]. The combination of bilateral anterolateral thigh overlength perforator flaps retains not only the advantage of the increased flap length to cover the width of the wound using the overlength flap lobulation design but also in allowing direct suturing at the donor site[15]. The combination of flaps can greatly increase the repairable area, providing a three-dimensional repair close to the structure and shape of the recipient site for large-area wounds with deep tissue exposure. All the patients in this group had severe large-area skin and soft tissue defects on the limbs. The recipient site was divided into two widths according to the area and shape of the wound and a sample cloth template was prepared in accordance with the dimensions. The flaps were designed in series or parallel depending on the number of defective arteries in the recipient site. The distal end of the major artery of the perforator flap was anastomosed with the distal end of the major artery of the recipient site in patients with defective or damaged arteries that could not supply blood to the recipient site, and blood flow bridging was used to avoid damaging (or reconstruction of) the major artery in the recipient site, which significantly improved ischemia in the distal limb[16,17]. Compared with other combined flaps with similar repair areas, such as the thoracodorsal artery perforator flap and the perforator flap in the inferior epigastric artery, this method has several advantages, namely, that the process of bilateral flap harvesting is essentially similar, and the di-

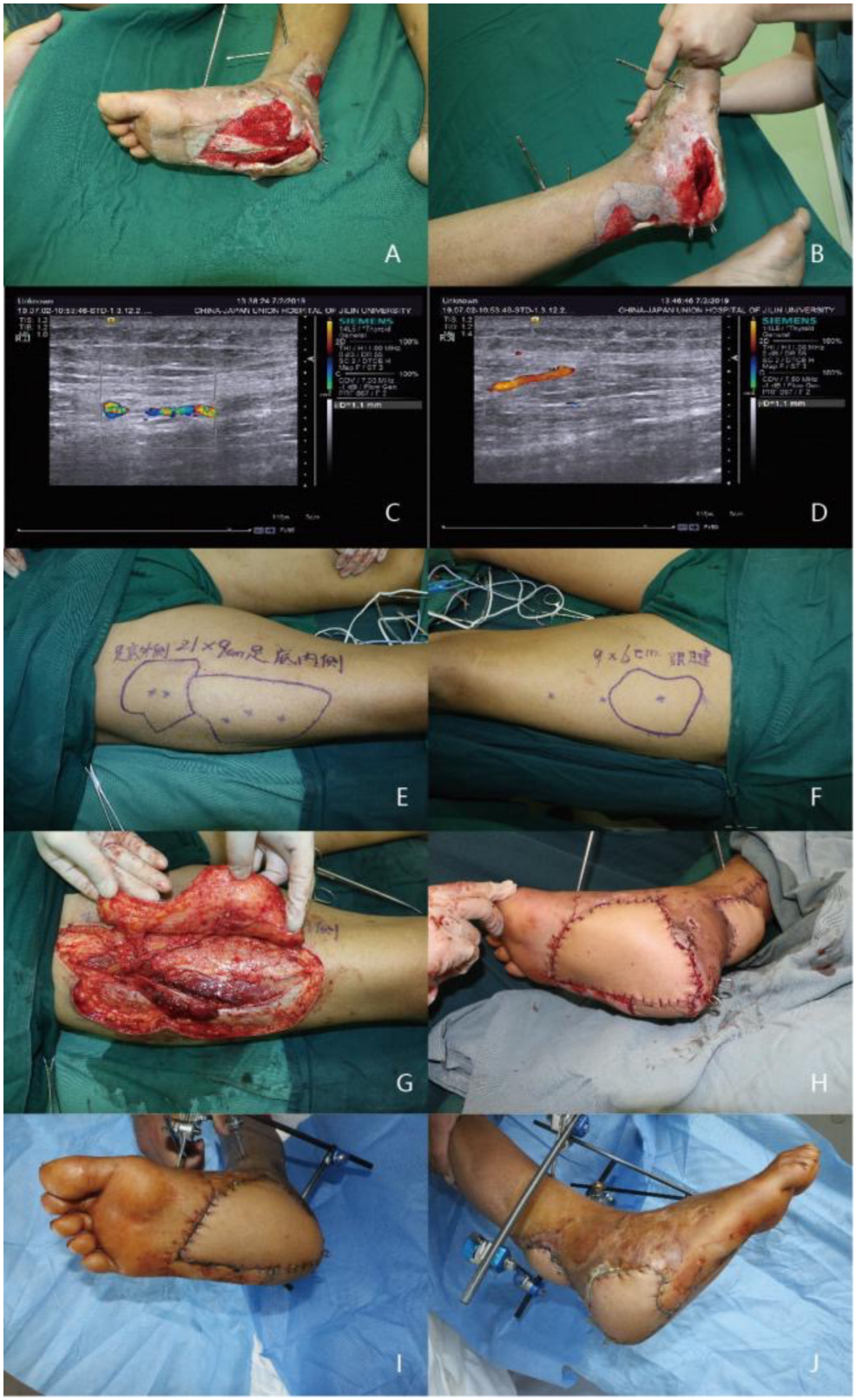

Figure 2. **A-B**, Large area of skin and soft tissue defect of right foot. **C-D**, The location of perforator using color doppler ultrasonography preoperatively. **E**, Design of a free anterolateral thigh perforator flap on right thigh (size: 21.0×9.0 cm^2). **F**, Design of a free anterolateral thigh perforator flap on left thigh (size: 9.0×6.0 cm^2). **G**, Harvest of an anterolateral thigh perforator flap based on the descending branch of the lateral circumflex femoral artery. H, Successful coverage of the defect was achieved. **I-J**, Postoperative view after two weeks.

mensions of the bilateral perforator are not vastly different from the length of the vascular pedicle, thus allowing easy anastomosis. There are several source arteries and perforating branches available in the anterolateral thigh region, among which the descending branch of the lateral circumflex femoral artery is long and has many branches, and its diameter is similar to that of the adjacent perforator or source artery. There are significant advantages when internal pressurization is performed to enhance the blood supply of the flap. The flap combination is flexible. The ALTP flap is designed to fold two flaps into a single wide flap for the repair of wounds that exceed the direct suture width of the donor site. The use of the bilateral flaps increases this advantage and reduces damage to the donor site[18-20]. The wound areas requiring repair in this study were large, with the largest being 38×15 cm^2, and the donor site could be directly sutured, reducing complications, and providing a reliable blood supply. The microsurgical reconstruction of large-area and complicated soft tissue defects of the extremities is challenging. The use of perforator flaps for the reconstruction of injuries to the extremities is growing. Surgeons[21,22] aim to select the ideal dissociated perforator flap to meet the individual requirements for the successful repair of soft tissue defects of the extremities. For large-area defects, the flaps available are the perforator flap in the inferior epigastric artery, the ALTP flap, the perforator flap in the superficial circumflex iliac artery, and the gluteal artery perforator flap. The repair of wounds to the extremities requires dexterity to obtain an aesthetically pleasing result. The perforator flap in the inferior epigastric artery is taken from the abdomen and the thickness of the flap is significantly greater than that of other perforator flaps, which affects not only the appearance but also the functionality of the extremities, especially the hands. Nevertheless, the perforator flap in the inferior epigastric artery has unique advantages for the wounds in the arm, thigh, and lower leg that can be covered and thus require a relatively lower degree of dexterity. For dead space or muscle tissue defects accompanying wounds in the extremities, the ALTP flap and its derivatives can treat most wounds and soft tissue defects of the extremities, while the perforator flap in a superficial circumflex iliac artery and the radial perforator flap of the forearm are characterized by their small size and thinness. After being cut, the donor site recovers quickly with little visible damage, and these flaps are suitable for patients with shallow wounds covering small areas. Trauma to the forearms or lower legs accompanied by soft tissue loss or even dead space must be covered by flaps composed of a large number of tissues (e.g., involving muscle and bone flaps to repair defects and prevent infection), and the ALTP flap is the most suitable for such injuries[16].

The variety of causes and resultant complexity of injuries to the extremities pose a huge challenge to surgical departments. Typical soft tissue defects of the extremities are irregularly shaped and are often accompanied by multiple adjacent soft tissue defects. These wounds are usually accompanied by the exposure of tendons, nerves, and bones, and free skin graft transplantation is challenging. The lobulated perforator flap provides sufficient flap area for the repair of large-area skin defects. The length at the donor site is converted to the width of the recipient site, thus the width at the donor site is not excessive. The donor area is closed in one stage, without skin transplantation at the donor site. Qing et al[15] applied Computed Tomography Angiography (CTA) to locate the perforator and design a lobulated perforator flap, with good postoperative results. The lobulated perforator flap provides two independent lobate flaps for the reconstruction of complicated wounds while minimizing morbidity at the donor site. This method expands the indications of the ALTP flap for the reconstruction of all types of soft tissue defects. Perforator flaps in the posterior interosseous and superficial circumflex iliac arteries are two types of perforator flaps with short vascular pedicles. Due to their short vascular pedicles, their clinical application is limited, and they are mostly used in specific clinical cases. The ALTP flap is the most commonly used clinically. If the flap is cut from the outer third of the line from the anterior superior iliac spine to the lateral edge of the patella, a 20-cm vascular pedicle can be separated[23]. However, if the flap is cut from the inner third of the line from the anterior superior iliac spine to the lateral edge of the patella, the length may be less than 8 cm. Meanwhile, if the perforator is separated 5 cm near the midpoint of the line from the anterior superior iliac spine to the lateral edge of the patella, the length of the vascular pedicle is about 10 cm[24]. When the perforator is separated at the midpoint of the line from the anterior superior iliac spine to the lateral edge of the patella, the length of the vascular pedicle is about 13.2 cm. The ALTP flap advantage is the length of the vascular pedicle. Before the operation, the appropriate perforator lengths should

be selected according to the imaging positioning, and the perforator should then be separated at the specific operation site. It is unnecessary to explore and delay the operation process blindly, even if the length of the vascular pedicle does not meet the requirements during the operation. Compared with the large-area flap, including the latissimus dorsi flap, the combined flap has the advantage of not only treating large-area tissue defects, but also covering irregular or dead-space wounds by changing the combined shape or carrying muscle flaps. Jia et al[25] applied the combined ALTP flap and anteromedial thigh perforator flap to the reconstruction of large-area soft tissue defects of the forearm and lower leg while Kadota et al[26] used a left perforator flap in the inferior epigastric artery and bilateral ALTP flap to repair a case with a large soft-tissue defect caused by fatal necrotizing fasciitis. D'Arpa et al[27] applied bilateral free ALTP flaps for the repair of a damage caused by a large-area soft-tissue resection due to bilateral axillary purulent eczema, and Qing et al[28] reported the repair of a large-area complicated wound of the upper limb using a chain combination of the bilateral ALTP flap. The chain double- or triple-leaf combined bilateral ALTP flap was specifically designed before the operation through an analysis of the wounds and imaging localization of the shape and number of the perforating branches. The ALTP flap was originally designed to cover the wound, focusing on improving the survival of the flap at the recipient site and reducing the post-surgical complications at the recipient sites. There are also concerns about the closure of the donor site in one stage. Patients may develop scar contracture, donor site infection, and other complications if the donor site cannot be closed in one stage. In the actual operation, if the width of the wound at the recipient site was large, the length of the flap could be converted into the width to cover the wound, allowing the closure of the flap donor site in one stage. However, if there is significant tension when closing the donor site, the suturing should not be forced, and delayed closure in a second stage should be considered. The combined nature of transplantation of the ALTP flap allows different strategies for combination. Muscle flaps can be used to fill dead space in patients with defects in soft tissues and bones. Additionally, when there are defects in major blood vessels, the combined flap can also be applied to reconstruct blood vessels to reduce the damage to the vessels in the recipient site. In this study, lobulation was performed in five patients to accommodate the wound shape when designing the flap. The advantage of the anterolateral thigh flap is that it allows flexible application for the effective repair of soft tissue defects, which not only solves the issue of coverage these defects but also extends the application of the combined ALTP flap transplantation. If the bilateral flaps are cut simultaneously during the operation, the distal and proximal flaps can be connected, and the vascular pedicle can be anastomosed after cutting the bilateral vascular pedicle and separation of the flaps. If the bilateral flaps are cut and separated in turn, cutting the vascular pedicle should be performed last and on the bilateral side simultaneously to reduce the duration of ischemia in the flaps. All flaps were observed to survive without bleeding, infection, arteriovenous crisis, or other complications.

Currently, the most common method used clinically for the preoperative localization of the anterolateral thigh flap is CDS[13,14], which has the advantages of high accuracy, high resolution of small perforating branches, and the ability to distinguish small blood vessels with diameters > 0.1 mm[29] and is also non-invasive. However, the success of the technology relies heavily on the experience of the operators. In addition, the CDS probe has a narrow local detection range, and the anterolateral thigh flap requires an extensive preoperative scope. Therefore, inexperienced operators can easily miss the perforating branch during its localization, resulting in a high false-negative rate and a relatively long detection time. In this study, preoperative CDS was applied to locate the perforator of the anterolateral thigh free flap. However, the interpretation of CDS imaging is highly subjective, and ultrasound physicians vary considerably in their experience with color ultrasound. Thus, to improve the objectivity and increase the accuracy of localization, Computed Tomography Angiography (CTA) is frequently used to locate the perforating branches. The positioning technology of CTA results in high blood vessel contrast and can accurately display the vascular configuration. The vascular routes and the perforating site of the perforating branch can be determined on the sectional images with high repeatability. However, there are often challenges in the imaging of smaller perforators at the distal end of the descending branch of the lateral circumflex femoral artery[30]. Dynamic Infrared Thermography (DIRT) is a non-invasive detection method that is cheap, simple, portable, and intuitive[31,32]. However, the accuracy of DIRT in locating the flap perforators is relatively poor[33]. This technolo-

gy not only can reduce the impact of the surrounding environment changes on the monitoring results but can also eliminate some false-positive hot spots during the perforator localization[30]. The hot spots shown on the thermogram provide only indirect indications of the presence of perforators in particular areas, and they are vulnerable to the influences of both ambient and self-core temperatures. Additionally, the presence of surrounding dressings and light can affect the results[31]. The thickness of the flap fat layer also affects the position accuracy. Both CTA and Magnetic Resonance Angiography (MRA) have several limitations in the location of perforator vessels. CTA cannot display the perforator vessels with diameters < 2 mm, nor is able to accurately locate the skin entry points of the perforator vessels and mark the body surface. Cina et al[34] found that the internal diameters of perforator arteries measured by CTA were inconsistent with those observed during the operation. Keys et al[35] believed that it was not possible to design perforator flaps based solely on preoperative CTA data. The advantage of digital flap design is that the perforator vessels in the donor site are identified with three-dimensional digital information, and the perforator arteries in the donor site are selected before the operation to avoid temporary changes to the surgical plan due to vessel variations or loss.

Conclusions

The combined transplantation of bilateral ALTP flaps did not affect the survival rate of the flaps. The clinical practice and postoperative observations of this study showed that the use of bilateral ALTP flaps was effective for the repair of large-area skin defects of the extremities and is thus worthy of clinical application. The combined transplantation of bilateral ALTP flaps for the repair of large-area wounds was designed according to the size and shape of the wounds using preoperative ultrasonic localization of the perforator vessels and resulting in excellent clinical repair.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contributions

XLL and GZW conceived the review, FH acquired data; XLL, CL and GZW participated in the process of writing and reviewing the manuscript. All authors contributed to the conception and revision of the manuscript and approved its submission.

Informed Consent

Signed written informed consent was obtained from the participants before the study.

Ethics Approval

This study was approved by the Ethics Committee of China-Japan Union Hospital.

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