Int Surg 2013;**98:**170–174
DOI: 10.9738/INTSURG-D-12-00035.1

Storage of Allogeneic Vascular Grafts: Experience From a High-Volume Liver Transplant Institute

Cemalettin Aydin, Volkan Ince, Emrah Otan, Sami Akbulut, Cemalettin Koc, Cuneyt Kayaalp, Sezai Yilmaz

Liver Transplant Institute, Inonu University Faculty of Medicine, Malatya, Turkey

Allogeneic vascular grafts are often required for vascular reconstruction during living donor liver transplantation. Such grafts are obtained prior to use, making storage conditions a critical issue for maintaining the integrity of the tissue to ensure a successful transplantation. This study describes an optimized storage protocol currently in use at a high-volume liver transplant center. Twenty-nine allogeneic vascular graft tissues obtained during cardiovascular surgery or from cadaveric donors were stored respectively in sterile 50 mL of Ringer lactate solution, without any preservation solutions or antimicrobials, at −22°C for a maximum of 3 months. Prior to use in vascular reconstruction, grafts were thawed in 0.9% NaCl solution at 37°C, and 1 × 0.5-cm^2 tissue samples were collected for microbial culturing and viral serology. ABO compatibility was not performed for any patients receiving vascular grafts. During this prospective study, all 29 allogeneic vascular grafts were used for back-table vascular reconstruction in living donor liver transplantation procedures. A total of 16 grafts were from the saphenous vein, 10 were from the iliac vein, and 3 were from the iliac artery. Bacterial growth was not detected in any tissue samples taken from the stored grafts. No vascular graft-related complications occurred during the 5 months of follow-up. The successful vascular reconstructions achieved with all 29 study grafts demonstrate that the simple, inexpensive storage method described herein is feasible and safe. Randomized, controlled studies should be carried out to further optimize and standardize the technique.

Key words: Liver transplantation – Allogeneic vascular grafts – Homologous grafts – Storage protocol

Reprint requests: Sami Akbulut, MD, Liver Transplant Institute, Inonu University Faculty of Medicine, 44280 Malatya, Turkey. Tel.: +90 422 3410660; Fax: +90 422 3410036; E-mail: akbulutsami@gmail.com

The first successful liver transplantation was performed by Starzl *et al* in 1967. Since then, the procedure has become the gold standard treatment for liver diseases that have progressed to end-stage liver failure.[1] In North American and European countries, most transplanted livers are obtained from cadaveric donors. In Turkey and some Asian countries, most liver grafts are from living donors.[2] In most cadaveric transplant procedures, vascular reconstruction is uncomplicated, because the structure of the donated liver has been preserved. On the other hand, vascular reconstruction in living donor grafts can be challenging if vascular grafts are needed.

Grafts used for vascular reconstruction may be autogeneic, allogeneic, or manufactured from synthetic materials, such as polytetrafluoroethylene. Allogeneic grafts must be obtained prior to any transplantation procedure in which they are used. Thus, for active liver transplantation centers, having a continuous source of allogeneic grafts depends on effective storage methods. The key challenges to this procedure include ensuring that grafts remain free of endothelial injury, protecting the viability or patency of the graft, and maintaining absolute sterility. In this report, we describe an allogeneic vascular graft storage method that was developed in our high-volume liver transplant center and has proven to be successful.

Materials and Methods

Vascular reconstruction models

From 2002 to mid-2012, 875 liver transplantations were performed at the Inonu University School of Medicine's Liver Transplant Institute (Malatya, Turkey). Of these, 77% were living donor liver transplantations, and the remaining 23% were cadaveric. Several vascular reconstruction models were used during these procedures, including iliac artery, iliac vein, and saphenous vein grafts (Fig. 1A–1C). In some cases, inferior vena cava and abdominal aorta grafts were used. To obtain optimal venous drainage of the transplanted liver, all V5, V8, and short hepatic veins having a diameter $\geq$5 mm were integrated to a common large opening model. In cases with impaired hepatic artery flow or intimal dissection, arterial reconstruction was performed using allogeneic vascular grafts. Allogeneic vascular grafts were also used for portal vein reconstruction (Fig. 1D).

Study objectives and design

This study originated from concerns about the optimum storage conditions for allogeneic vascular grafts and the risks of microbiologic contamination. The origin, characteristics, and storage methods of 29 allogeneic grafts used in back-table vascular reconstruction during living donor liver transplantations that had been performed between May 1 and 30, 2012, were analyzed prospectively. All of the vascular grafts were cultured for microbial contamination. In addition, the occurrence of allogeneic graft–related complications was monitored.

Allogeneic grafts

Allogeneic vascular grafts used in this series were obtained from either saphenous veins resected during variceal surgery, or from iliac arteries, iliac veins, abdominal aortae, and infrahepatic inferior vena cava of cadaveric donors during harvesting procedures. All of the grafts were stored in sterile containers filled with 50 mL of Ringer lactate solution and without antibacterial agents or preservation solutions (*e.g.*, histidine-tryptophan-ketoglutarate solution, University of Wisconsin solution, Euro-Collins solution). Each container was packaged using double-sterile surgical gloves and was kept in a deep freeze at –22°C for a maximum of 3 months. Before use in reconstruction, grafts were thawed in 0.9% NaCl solution at 37°C. ABO compatibility was not performed for any recipients before applying the vascular grafts.

Microbial culture

For each graft applied to a recipient, a 1×0.5-cm^2 tissue sample was collected for microbial culturing methods with Sabouraud agar, blood agar, eosin methylene blue agar, and chocolate agar. Additional samples were processed for Gram staining. All sample tissues were delivered to the microbiology laboratory within 2 hours of thawing. The cultures were incubated at 36.5°C for 24 hours. Cultures with no growth after 24 hours were incubated for an additional 24 h and rechecked. Donor medical history and viral marker profiles were assayed to rule out hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) infection.

Results

The study analyzed data from 29 recipients (age range, 12–64 years; 22 male and 7 female recipients) and 29 donors (10 male and 19 female donors). The demographic and clinical characteristics of the recipients and donors are summarized in Table 1.

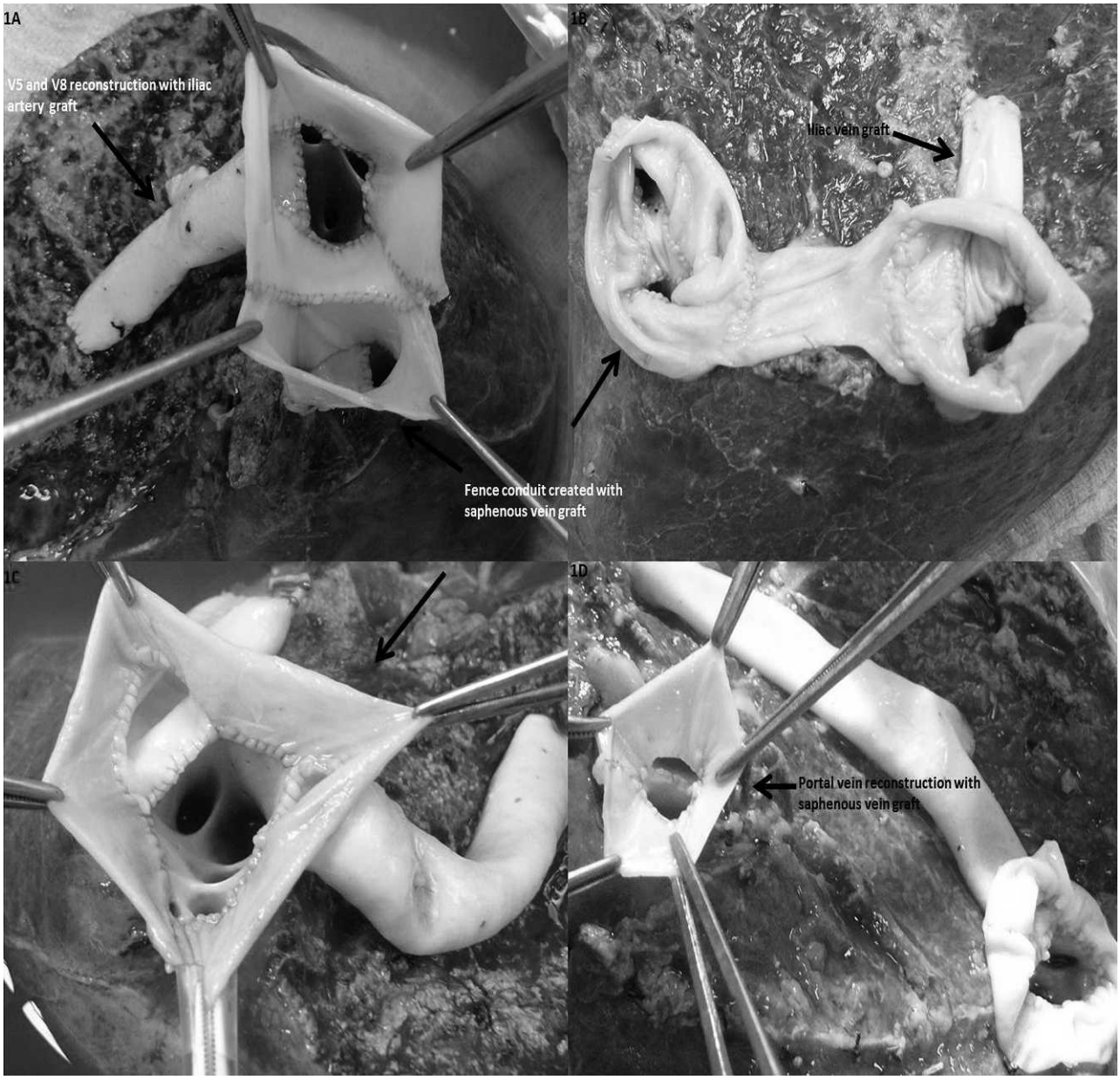

Fig. 1 (A–C) Hepatic vascular reconstruction including allogeneic vascular grafts: V5, V8,and short hepatic veins are extended to the right hepatic vein to create a "common large opening" drainage model using iliac artery and iliac vein grafts. (D) Following the transplantation procedure, a fence conduit was generated to provide a wide opening to accommodate a saphenous vein graft. The right anterior and posterior portal veins were combined to form a single opening using the saphenous vein graft.

Right lobe hepatectomy was performed in 25 of the living donors, and left lobe hepatectomy was performed in the remaining 4. All 29 vascular grafts were applied as venous reconstructions on the operating room back-table. Among the allogeneic grafts, 16 were from the saphenous vein, 10 were from the iliac vein, and 3 were from the iliac artery. The saphenous veins grafts were obtained during variceal surgery. The iliac artery and vein grafts were obtained from cadaveric donors. Vascular grafts preserved with the simple storage technique described above were used within an average of 40 days (range, 15–85 days). All of the tissue samples obtained from stored vascular grafts were free of bacterial contamination. All of the vascular graft donors were seronegative for HBV, HCV, and HIV.

Table 1 Demographic and clinical features of both organ recipient and donor patients

Characteristic	Recipients (n = 29)	Donors (n = 29)
Age, y, ±SEM (range)	43.1 ± 15.3 (12–64)	28 ± 8.2 (17–49)
Sex, No.		
Male	22	10
Female	7	19
BMI, kg/m^2, ± SEM (range)	24.6 ± 4.25 (15.8–35.8)	23.95 ± 2.7 (19.7–31.5)
Child score, No.		
A	3	
B	10	
C	16	
MELD score, ±SEM (range)	18.0 ± 7.45 (7–37)	
Etiology, No.		
HBV	7	
HBV + HCC	3	
HCV	3	
HBV + HBV + HCC	1	
Cryptogenic	9	
Budd-Chiari	2	
Wilson disease	1	
Autoimmune hepatitis	1	
Primary biliary cirrhosis	1	
Hemangioendothelioma	1	
Donor-recipient relationship, No.		
Daughter		6
Son		5
Cousin		3
Mother		1
Sister		5
Husband		1
Wife		1
Aunt		1
Other		6
Graft weight, g, ±SEM		769.4 ± 155.8
Current status, No.		
Alive	20	29
Deceased	9	0
Cause of death, No.		
Sepsis	3	
Cardiac failure	2	
Respiratory failure	2	
Hepatic artery thrombosis	1	
Intra-abdominal hemorrhage	1	

BMI, body mass index; MELD, Model for End-Stage Liver Disease.

No allogeneic vascular graft–related complications, including graft patency (100%), occurred in any recipient during the 5 months of follow-up.

Discussion

Living donor liver transplantation is an alternative treatment option in countries without an adequate availability of cadaveric livers.[3] It should be noted that cadaveric grafts have advantages over grafts from living donors that are important both surgically and for postoperative graft function. Being able to harvest the retrohepatic inferior vena cava, the portal vein extending as far as inferior mesenteric vein, and the hepatic artery together with the celiac trunk, along with the donor liver, simplifies the ability to establish vascular anastomoses during transplantation. Living donor grafts, on the other hand, can be technically difficult if the hepatic vein, portal vein, and hepatic artery segments present in the transplanted liver are relatively short. In such cases, vascular reconstruction may require several vascular grafts, especially in cases with intimal dissection of the recipient hepatic artery. Reconstruction of hepatic outflow may also require several vascular grafts.

Homologous (allogeneic), autologous, and artificial (synthetic) grafts may all be used for vascular reconstruction. Recipient left portal, paraumbilical, and saphenous vein autografts have also been successfully applied.[4] The most frequent allogeneic vascular grafts are saphenous and iliac veins, iliac arteries, inferior vena cava, and aortae obtained from cadaveric donors. Some transplant centers use homologous grafts harvested during variceal surgery. Synthetic vascular grafts, most often those made from polytetrafluoroethylene, are generally used when suitable allogeneic grafts are not available, and they are often used for reconstruction of V5, V8, and the middle hepatic vein.[5–7]

A consensus exists on reuse of synthetic grafts following resterilization by various techniques. However, optimal preservation techniques and storage time for allogeneic vascular grafts are controversial. Hwang *et al*[5] successfully used cryopreserved allogeneic vascular grafts that had been stored for a mean of 3 months (range, 4 days to 15 months) for hepatic vein reconstruction.[5] In our institution, allogeneic vascular grafts are used for reconstruction of hepatic outflow at nearly every back-table stage. The grafts are stored as described above, with a maximum storage period of 3 months.

Graft storage using various antimicrobial and preservation solutions is becoming more common in the field; cryopreservation, cold storage, and glutaraldehyde treatment methods have also been improved recently.[8] A graft storage technique that uses slow freezing, with the temperature falling from +1°C to –40°C, has also been reported.[9] Cryopreservation using liquid nitrogen and dimethylsulfoxide keeps tissues at a temperature be-

tween –120°C and –190°C. Cold storage techniques keep grafts immersed in saline solutions at +4°C. The objective of all of these storage methods is to maintain the allogeneic vascular graft without inducing damage, immunologic changes, or changes in graft aperture, while also maintaining sterility, for as long a time as possible. The storage protocol described herein does not include any preservation solutions or antimicrobial agents. Microbiologic analysis revealed no sign of contamination prior to graft use, and 5 months of follow-up did not reveal any graft-related infections or problems related to vascular graft patency.

Most previous studies evaluated storage effects on the viability of graft tissue, patency rates, and endothelial injury. To our knowledge, this is the first report to include results from testing for bacterial contamination in stored vascular grafts. Second, the storage method described herein has not been reported previously, is simple and inexpensive, and requires only simple reagents, microbiologic techniques, and a readily available laboratory freezer for storing the graft tissues. In summary, the freezing storage procedure involves 2 regular sterile, disposable surgical gloves, 1 regular sterile, disposable specimen storage container, and 1 label for each vascular graft tissue obtained from the donor. Therefore, the biggest cost of this storage method may involve the space and energy required in the hospital freezers.

The risk of viral transmission is another potential challenge for safely transplanting vascular grafts. In a recent article from the Centers for Disease Control and Prevention (CDC), organ transplantation centers are discouraged from storing vascular grafts obtained from donors who were positive for hepatitis B surface antigen (HbsAg) or anti-HCV antibodies, or had detectable HBV or HCV sequence by nucleic acid test.[10] Furthermore, the CDC reported that grafts obtained from seropositive donors should be stored in separate freezers from grafts obtained from seronegative donors. In addition, to further prevent technical mistakes, for both living and cadaveric sources, the donor's features and viral marker results should be labeled on the storage containers. Our hospital employs such strict storage methods, which likely played a beneficial role in our study recipients avoiding viral transmission, as well as the more than 700 living donor liver transplantations that have been performed at our hospital over time.

For the series of vascular reconstruction grafts stored using this protocol and described in this study, the results demonstrate that allogeneic vascular grafts can be stored without need of expensive materials and complex methods, and without risk of bacterial contamination. However, future larger-scale prospective randomized studies should be carried out to confirm our findings and further optimize the simplified system.

References

1. Tanaka K, Yamada T. Living donor liver transplantation in Japan and Kyoto University: what can we learn? *J Hepatol* 2005;**42**(1):25–28
2. Brown RS Jr, Russo MW, Lai M, Shiffman ML, Richardson MC, Everhart JE *et al*. A survey of liver transplantation from living adult donors in the United States. *N Engl J Med* 2003;**348**(9): 818–825
3. Marcos A, Fisher RA, Ham JM, Olzinski AT, Shiffman ML, Sanyal AJ, Luketic VA, Sterling RK, Olbrisch ME, Posner MP. Selection and outcome of living donors for adult to adult right lobe transplantation. *Transplantation* 2000;**69**(11):2410–2415
4. Hwang S, Lee SG, Ahn CS, Park KM, Kim KH, Moon DB *et al*. Cryopreserved iliac artery is indispensable interposition graft material for middle hepatic vein reconstruction of right liver grafts. *Liver Transpl* 2005;**11**(6):644–649
5. Hwang S, Jung DH, Ha TY, Ahn CS, Moon DB, Kim KH *et al*. Usability of ringed polytetrafluoroethylene grafts for middle hepatic vein reconstruction during living donor liver transplantation. *Liver Transpl* 2012;**18**(8):955–965
6. Hwang S, Lee SG, Park KM, Kim KH, Ahn CS, Moon DB *et al*. Quilt venoplasty using recipient saphenous vein graft for reconstruction of multiple short hepatic veins in right liver grafts. *Liver Transpl* 2005;**11**(1):104–107
7. Yi NJ, Suh KS, Lee HW, Cho EH, Shin WY, Cho JY *et al*. An artificial vascular graft is a useful interpositional material for drainage of the right anterior section in living donor liver transplantation. *Liver Transpl* 2007;**13**(8):1159–1167
8. Fahner PJ, Idu MM, van Gulik TM, Legemate DA. Systematic review of preservation methods and clinical outcome of infrainguinal vascular allografts. *J Vasc Surg* 2006;**44**(3):518–524
9. Hwang S, Ha TY, Ahn CS, Moon DB, Kim KH, Song GW *et al*. Reconstruction of inferior right hepatic veins in living donor liver transplantation using right liver grafts. *Liver Transpl* 2012; **18**(2):238–247
10. Centers for Disease Control and Prevention. Potential transmission of viral hepatitis through use of stored blood vessels as conduits in organ transplantation–Pennsylvania, 2009. *Am J Transplant* 2011;**11**(4):863–865