A multicenter, phase I evaluation of cryopreserved venous valve allografts for the treatment of chronic deep venous insufficiency

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Purpose: **A phase I feasibility study was conducted to determine whether cryopreserved venous valved segments would remain patent/competent in a short-term period (6 months).**

Methods: **The target group consisted of 10 patients ($C_{4\text{-}6}$, E, A_D, P_R). The exclusion criteria included untreated superficial/perforator venous disease, significant venous or arterial obstruction, hypercoagulability or coagulopathy, and significant preexisting medical conditions. Required preoperative tests were venous duplex, ascending/descending venography, and a physiologic study (eg, APG, blood typing, an ankle/brachial index, and if post-thrombotic, a hypercoagulability work-up). A single-valve transplant was placed below all reflux, aided by anticoagulation with or without a distal arteriovenous fistula. Postoperative assessment included duplex scanning/clinical examination (at 1, 3, and 6 months), descending venogram (at 1 month), and physiologic study (at 1 and 6 months). The primary end point was valve patency/competence, with clinical outcome as a secondary end point. Adverse events were recorded.**

Results: **After eliminating protocol violations, nine patients with superficial femoral (5) or popliteal (4) vein valve transplants were studied. Six-month actuarial results show a patency rate of 67% ± 16% and 78% ± 13%, respectively, a primary and secondary competency rate of 56% ± 17% and 67% ± 16%, respectively, and a 100% patient survival rate. Clinical outcome averaged 1.1, with healing and/or freedom from ulcer recurrence, in six of nine patients. A postoperative risk of seroma formation (3) and cellulitis (1) exists.**

Conclusion: **In patients with few remaining therapeutic options, one can achieve a 6-month assisted patency and competency rate of 78% and 67%, respectively, with an improved clinical outcome. (J Vasc Surg 1999;30:854-66.)**

Moderate to severe chronic venous disease affects more than six million people in the United States, with an estimated 800,000 new cases reported annually.[1,2] More than 75% of these people have some component of deep involvement, and approximately 85% of these people have insufficiency as the major pathophysiologic abnormality.[2] If 50% of patients have primary valvular insufficiency,[3,4] approximately 2 million people (generally postphlebitic) are potential candidates for surgical correction, other than valvuloplasty, of the offending venous insufficiency. However, not all patients may be proper candidates because of either anatomic abnormalities or the ability of conservative treatments to ameliorate the patient's symptoms. In an active venous practice, approximately 10% of postphlebitic patients with venous reflux actually have anatomy amenable to current surgical intervention.[5,6] This would suggest that approximately 200,000 people are potential candidates for aggressive surgical therapy, when warranted.

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Valve transposition operations are possible in less than 5% of all patients considered for surgical intervention.[7,8] Only 60% of the remaining patients have a competent upper-extremity valve available for transplantation.[5] Disregarding that such thin-walled upper-extremity valves may dilate in time, resulting in recurrent incompetence,[8,9] this would still leave approximately 40% of postphlebitic patients (more than 50,000 people) who have chronic venous disease and severe symptoms with no appropriate valve for transplantation. The need for an alternative valve to act as a substitute for the autogenous valve becomes obvious.

Generally, off-the-shelf venous valve substitutes have faired poorly in animal experimentation.[10-14] However, a cross-matched cryopreserved venous valve allograft implanted into a chronic deep venous insufficiency greyhound model aided by a distal arteriovenous fistula (dAVF) has remained patent and improved venous hemodynamics during a 2-month period of study.[15] The use of a cryopreserved superficial femoral vein valve to replace defective autogenous valves in humans is appealing in two respects. These valves are accustomed to the hemodynamic stresses inherent to the lower limb and have a size and thickness equal to the recipient site. Transition to clinical trial seemed reasonable, because further animal study would likely add little to our understanding of the allograft, and cryopreserved tissues are routinely used clinically, eliminating some of the safety questions we might have otherwise faced.[16,17]

MATERIALS AND METHODS

The patients chosen to participate had end-stage chronic deep venous insufficiency and were a very specific group, which necessitated a multi-institutional research team for timely patient accrual. We thought that 10 patients would be sufficient to answer the basic safety and allograft valve function issues being considered. The primary objective was to evaluate the ability of a cryopreserved valve containing venous allograft to remain patent and competent when transplanted as a substitute valve into patients with isolated chronic lower-extremity deep venous insufficiency. These were patients who had essentially no remaining options for correcting the hemodynamic problem causing their severe symptoms (clinical classes 4 to 6).[18] The clinical impact was a secondary end point of this initial safety and feasibility study. Appropriate patients were recruited when they came to the participating surgeons' clinics.

Before patient recruitment, all aspects of this prospective study were discussed and agreed on by the participating investigators. The written protocol, with consent form, was approved by the respective institutional review boards. Patients were fully informed of potential risks, alternative approaches, and the possible benefit of this surgical treatment before studies that would not have been required for a standard autogenous valve transplantation procedure were obtained.

Preoperative assessment

Clinical inclusion criteria. Patients were restricted to those with class 4 or higher signs of chronic deep venous insufficiency[18] that were lifestyle disabling, even with appropriate conservative medical treatment. The etiology was primary valvular incompetence or of a secondary cause, such as the postphlebitic syndrome.

Clinical exclusion criteria. Exclusion criteria were chosen to safeguard patients and to eliminate conditions that would add bias to the study. Patients were required to be older than 18 years and neither pregnant nor planning further pregnancies. Patients with proximal iliac vein occlusion (outflow disease) were excluded, as were those with primary venous valvular disease who, in the opinion of the operating surgeon, would be better served by valvuloplasty. Patients with superficial or perforator venous insufficiency were not acceptable candidates. Also excluded were patients with significant preexisting co-morbid conditions, including congestive heart failure, peripheral arterial insufficiency, vasculitis, known coagulopathy, contraindications to chronic anticoagulation (heparin or warfarin), morbid obesity (more than 175% of ideal body weight), and active infection with sepsis. Furthermore, patients unwilling or unable to comply with postoperative follow-up visits and examinations were excluded from this study.

Preoperative evaluation. A routine history and physical examination was performed to evaluate and prepare a patient for possible valve transplantation. Specific patient demographics recorded included age, sex, the presence of hypertension (140/90 mm Hg or higher or on medications), diabetes mellitus, hyperlipidemia (history with or without medication), a history of coronary artery disease, the New York Heart Association (NYHA) classification, and a smoking history (past, present, never). Pertinent chronic venous disease demographics included any history of deep venous thrombosis, leg edema, past or present venous ulcers, and the number of ulcer recurrences experienced in the last 5 years. The use of nightly and intermittent leg elevation, compressive support (with degree of compression), and a

history of earlier surgery to correct superficial, perforator, or deep venous insufficiency were recorded.

Preoperative testing. Each of the following tests were performed in all patients. A venous duplex scan evaluated venous occlusive disease, especially earlier deep venous thrombosis (recannalization, collaterals, etc). It was used to assess venous reflux, as per each institution's standards, generally by using a reflux time of more than 0.5 seconds to separate abnormal from normal results.[19] It served as a baseline for postoperative evaluation of allograft patency and competence. It allowed determination of the donor vein diameter, to match that of the recipient vein. An ascending venogram more clearly defined the venous anatomy and also helped determine the optimal recipient bed and diameter of donor allograft. A descending venogram determined the presence and location of venous valves and the degree of venous reflux.[20] Only grade-3 and -4 reflux were considered to be of surgical significance.[20] An ankle-brachial index was obtained to establish the absence of peripheral vascular disease (0.9 or higher was considered acceptable). A blood type was required to allow proper ABO matching. A hypercoagulable work-up was required in all patients with a previous history of venous thrombosis, in an attempt to conform to the exclusion criteria established, and included levels of antithrombin III, activated protein C, protein S, anticardiolipin and antiphospholipid antibody, lupus anticoagulant, prothrombin time, activated partial thromboplastin time, and a platelet count.

One or more of the following venous hemodynamic tests confirmed that reflux was the major disorder in each patient. Tests were repeated when the transplant was successful to determine a meaningful clinical outcome score. The study was not required when the allograft failed. Venous refilling time (VRT) was assessed by means of photoplethysmography. Intravenous pressure measurements were a means of providing a measure of venous filling time (VFT), ambulatory venous pressure (AVP), and VRT.[21] A VRT less than 20 seconds was considered abnormal. A number of venous hemodynamic parameters, including the calf pump, obstruction, and insufficiency, were evaluated by means of air plethysmography. A quantitative measure of overall venous reflux was provided by means of the venous filling index (VFI_{90}), and less than 2 mL/sec was considered to be a normal reading.[22] A significant effect, as evaluated by means of air plethysmography, was considered to be a change by one or more categories (mild, moderate, severe), as defined for each noninvasive laboratory test involved, and was then used in clinical outcome scoring instead of the AVP or VRT.[18]

The CEAP[18] classification was determined by means of the clinical examination and history (clinical grade and etiology) and by means of venography, with support from duplex scanning and plethysmographic or intravenous pressure studies (anatomy and pathophysiology).

Because only a single valve transplant was to be evaluated, the transplant was located below all significant thigh reflux. When the profunda system was competent, the valve was placed in the superficial or popliteal vein. When the profunda system was incompetent, the allograft transplant was placed in the most normal vein (distal superficial femoral or popliteal) below all reflux, as demonstrated by means of descending venography. The diameter of the donor vein was matched to the recipient by measuring the recipient vein diameter with venography, duplex scanning, or both in the most normal-appearing segment determined to lie below all venous reflux.

Operative management

The specific surgical technique was left to the discretion of the surgeon, but the general consensus was to use an interrupted technique with nonabsorbable suture (5-0 to 7-0 prolene). Sufficient recipient vein was removed for optimal fit, and the length of the donor vein was at least three times its diameter to avoid injury to the venous cusps when performing the anastomosis. Post-thaw donor valve competence was confirmed by means of retrograde distention of the vein with a syringe filled with heparin solution, aided by gravity drainage. Furthermore, at the completion of the operation, the clinical strip test was performed. The use of an adjuvant dAVF was left to the discretion of the operating surgeon. Documentation of the site of implantation (superficial femoral vein or popliteal vein), length of donor vein used, and the use of an adjuvant dAVF was required. All patients were given heparin (fractionated or low-molecular weight) before and during implantation, and it was continued until adequate conversion to warfarin anticoagulation was made. Oral anticoagulation was continued for the 6 months of the study protocol.

Cryopreserved allograft. Superficial femoral vein allografts with competent valve(s) were removed from qualified donors by organ procurement organizations and regional tissue banks and sent, with consent, to CryoLife (Kennesaw, Ga).

Table I. Specific demographics of patients with venous disease involved in this study

Patient	*Affected side*	*Earlier DVT*	*Earlier venous surgery*	*Ulcer*		*Ulcer size*	*Hemodynamic evaluation*	
				Present	*Past*		*VRT (seconds)*	*VFI_{90} mL/sec*
GC	L	no	yes	no	yes	N/A	12	6.0
GP	R	yes	yes	yes	yes	10 sq cm	4	3.0
FT	L	yes	yes	yes	yes	8 sq cm	10	–
ML	L	yes	yes	yes	yes	2 sq cm	3	1.5
WW	L	yes	yes	yes	yes	4 sq cm	0	26.0
SC	L	yes	no	yes	yes	Entire lower leg	29	5.5
EB	L	no*	no	yes	yes	16 sq cm	–	–
GS	L	yes	yes	no	yes	N/A	1	4.5
JB	L	yes	yes	no	yes	N/A	10	9.4

*Venogram consistent with old DVT (recannalization of superficial femoral vein).
DVT, Deep venous thrombosis; *VRT,* venous refilling time; *VFI_{90},* venous filling index; *N/A,* not applicable.

The vein was aseptically dissected of surrounding fat, and any leaking branches were sutured for hemostasis. The most proximal valve (when multiple valves were present) was tested for competence by means of a hydrostatic pressure device to 125 mm Hg to confirm competence. The vein allograft was then incubated in a tissue culture medium with an antibiotic, placed in a cryoprotectant, and controlled-rate frozen to –135°C or colder. The allograft was shipped at –70°C and thawed within 72 hours for surgical use. Once thawed by a four-step protocol, the tissue handled as any human vein.

Postoperative assessment

Any adverse event was recorded, whether or not it was directly related to the valve transplantation. This included serious medical events, reoperations, the need for allograft explant, and death. Aneurysm formation, cusp degeneration, immunologic reaction, and tears or perforations in the donor allograft, either intraoperatively or postoperatively, were recorded. Wound complications, such as dehiscence, cellulitis, and seroma formation, were documented. Deep venous thrombosis with or without emboli, recurrent venous ulcers after having been healed once, or other problems also required documentation.

Scheduled patient visits were 1, 3, and 6 months after surgery and included a history, physical examination, and a venous duplex study of the valve site. The primary end point of allograft valve patency and competence was determined by means of serial duplex scanning. A descending venogram scheduled at 1 month was used as a means of confirming the duplex findings. Venous hemodynamic studies were obtained at 1 and 6 months when required by continued valve function. The secondary end point of clinical outcome was determined by means of the history, physical examination, and venous hemodynamic study(ies).[18]

Data analysis/statistical methods

Data from all participating centers were entered into a database and pooled for analysis. Recipient demographics and the CEAP classification were defined by means of descriptive statistics. The allograft valve performance and patient survival were evaluated by means of statistical actuarial methods to provide cumulative graft patency (%), overall freedom from valve incompetence (%), and overall patient survival (%). Complications, adverse events, and explant data were summarized.

RESULTS

Eleven patients were recruited for this study. A 63-year-old man was considered non-compliant because he discontinued his anticoagulant therapy shortly after surgery and was found to have an allograft occlusion 50 days postoperatively. The allograft had been placed in the popliteal vein and was documented to be occluded by means of a duplex scan and ascending venography, and the patient was asymptomatic, with a healed ulcer. A patient was recruited to replace him in the study. The other protocol violation involved a 37-year-old man who was found to have a protein S deficiency, only after an occlusion was discovered 86 days after implantation. The valve was in the superficial femoral vein, and the patient was not clinically improved. Because this information was not known until late in the study, a replacement patient was not recruited. The nine remaining patients form the basis for this phase I feasibility study.

Most patients were men (7 of 9), and their average age was 56.8 years (range, 36 to 77 years). The

Table II. CEAP classification of venous insufficiency

	Clinical			*Anatomic*		*Pathophysiologic (deep disease)*	
Patient	*Grade*	*Symptoms*	*Etiologic*	*Deep*	*Other*	*Reflux*	*Obstruction*
GC	5	yes	P	yes	S	yes	
GP	6	yes	S	yes		yes	yes
FT	6	yes	S	yes		yes	yes
ML	6	yes	S	yes		yes	yes
WW	6	yes	S	yes		yes	yes
SC	6	yes	S	yes		yes	yes
EB	6	yes	S	yes	P	yes	
GS	5	yes	S	yes		yes	yes
JB	5	yes	S	yes		yes	

S, Superficial reflex; *P,* perforator reflex.

patients were quite healthy, with the exception of their venous disease. Only one patient had a NYHA class greater than 1 (NYHA class 2); one patient had type 1 diabetes mellitus; and only 22% of patients were hypertensive, smokers, past smokers, and/or had a past history of coronary artery disease.

The patients' venous disease demographics are presented in Table I. In addition, all patients had complaints of edema, skin changes consistent with chronic venous disease, and used leg elevation to alleviate some of the edema and discomfort of the disease. Of those patients with ulcers, four patients had ulcers that had been continually present for the last several years, whereas the other two patients had ulcers that recurred three or more times in the same period. In those patients with a healed ulcer, each patient had experienced three or more recurrences in the previous 5 years. One patient (EB) did not use compressive therapy because of severe discomfort, but all other patients used it at a compression level of 30 mm Hg or greater. Table II further classifies the patients by means of the CEAP method. The patients can be defined, in general, as $C_{5\text{-}6,S}$ E_S A_D P_R with some outliers. The one case of superficial reflux was suggested by means of venographic mild greater-saphenous reflux, which was not considered significant and did not alter venous hemodynamic studies to any degree. The one case of isolated perforator reflux was located in the lower calf, below all deep disease, and appeared mild by means of duplex study. The finding of old deep venous occlusive disease (recannalization, etc) by means of duplex scanning or venography did not translate into hemodynamic abnormalities, but it is included to help define the patients being treated.

Some specifics of the allograft operation are provided in Table III. The diameter of the donor allograft was 7 to 12 mm, and the length averaged 4.6 cm (range, 3 to 7 cm). One competent venous valve was documented in each segment, but in two cases, the surgeon felt the valve was moderately incompetent after thawing and performed a bench valvuloplasty. The optimal site of implant was the popliteal vein in four cases and the superficial femoral vein in five cases. In two cases, an adjuvant dAVF was constructed to improve venous flow. A large diameter (0.5 cm) dAVF was constructed simultaneously with and just distal to the allograft transplant in one case with a planned ligation in 6 weeks. The other was constructed at the time of an allograft salvage operation at a distant location (posterior tibial artery to vein) and allowed to function indefinitely. This salvage operation included thrombectomy, valvuloplasty, and saphenopopliteal bypass grafting to correct an obstruction that became evident at a location of a failed autogenous venous valve transplant. Operative complications were observed in four cases. There were two early occlusions, one of which was corrected surgically with the use of a dAVF and is the only case involving secondary patency. The other case of thrombosis was associated with a seroma that involved the transplant site, as documented at the time of seroma drainage. There were two other cases of seroma formation not involving the allograft, and in one of these cases, there was an associated cellulitis. One seroma required surgical drainage, whereas antibiotic treatment of the cellulitis was all that was required in the other case.

Table IV provides data regarding the primary end point (allograft patency and valve competence) and the secondary end point (clinical outcome). Included in Table IV are the follow-up studies at 6 months to determine the valve's effect on lower-limb hemodynamics. All patients survived the study without systemic sequelae. The life-table primary and secondary cumulative graft patency rates are

Table III. The surgical specifics of the cryopreserved allograft transplantation

	Allograft				*Implant*		*30-day complications*		
	Diameter	*Length*	*Blood type*				*Thrombosis*	*Cellulitis*	*Seroma*
Patient	*(mm)*	*(cm)*	*Donor*	*Recipient*	*Site*	*Adjuvant*	*(days)*		
GC	9-9	4	O	O	Pop	dAVF†	20‡		
GP	11-11	4	A	A	Pop				
FT	7-12	3	A	A	Pop	dAVF			
ML	9-10	4	A	A	SFV*			✓	✓
WW	9-12	7	A	A	SFV				
SC	9-10	4	O	B	SFV*				✓§
EB	9-11	3	O	O	Pop				
GS	8-9	5	O	O	SFV				
JB	8-9	7	O	O	SFV		29		✓‖

*Valvuloplasty of allograft performed.
†Only after thrombectomy and to improve 2-degree patency.
‡Reopened surgically.
§Seroma drained; did not involve allograft.
‖Seroma drained; did involve allograft.
Pop, Popliteal; *SFV,* superficial femoral vein; *dAVF,* distal arteriovenous fistula.

Table IV. Clinical outcomes after cryopreserved allograft venous valve transplantation

	3 months			*6 months*					
	Allograft			*Allograft*		*Hemodynamics*			
Patient	*Patent*	*Competent*	*Ulcer healed*	*Patent*	*Competent*	*VRT (seconds)*	*VFI_{90} (mL/sec)*	*Clinical outcome*	*Ulcer healed*
GC	yes*	yes*	N/A	yes*	yes*	–	5.1	+2	N/A
GP	yes	yes	yes	yes	yes	–	–	0‡	no§
FT	yes	yes	50%	yes†	no	7	–	0	no
ML	yes	yes	yes	yes	yes	–	–	0	no§
WW	yes	yes	yes	no	no	–	–	+1	yes
SC	yes	yes	70%	yes	yes	40	0.9	+2	85%
EB	no	no	yes	no	no	–	–	+1	yes
GS	yes	yes	N/A	yes	yes	5	1.4	+2	N/A
JB	no	no	N/A	no	no	14	2.6	+2	N/A

*Secondary.
†Stenotic.
‡Progressive arterial disease.
§Recurrent ulcer.

shown in Fig 1. Fig 2 provides primary and secondary freedom from valve incompetence results. The only allograft thrombosis occurring after the 30-day perioperative period was associated first, at 2 months, with a tibial deep venous thrombosis after the patient sustained a traumatic metatarsal fracture. The allograft was open and competent at 3 months. After a urologic consult for hematuria, the patient was instructed to discontinue his anticoagulants, and within 2 months, the allograft occluded. Two recurrent ulcers occurred in patients with a duplex-confirmed patent and competent valve. In one case, progressive arterial disease resulted in very poor distal perfusion (40 mm Hg) at 4 months of follow-up, and an ulcer recurred. Balloon angioplasty of the superficial femoral and popliteal arterial stenoses improved the pressure to 90 mm Hg, but at 6 months, the ulcer was still not healed. All remaining patients were instructed on and, except for one patient (patient refusal), were using elevation and compression stocking support after the operation. The compression stocking support averaged 30 mm Hg, whereas three patients were using Circard compression.

DISCUSSION

The patients' general demographics are a reflection of the rather stringent exclusion criteria established for this protocol. The patients were generally middle-aged men with few risk factors for atherosclerosis. These criteria were established to minimize the effect of systemic conditions (heart failure, coagulopathy, sepsis, lower-extremity arterial disease) on mortality, healing, and valve function. The desire

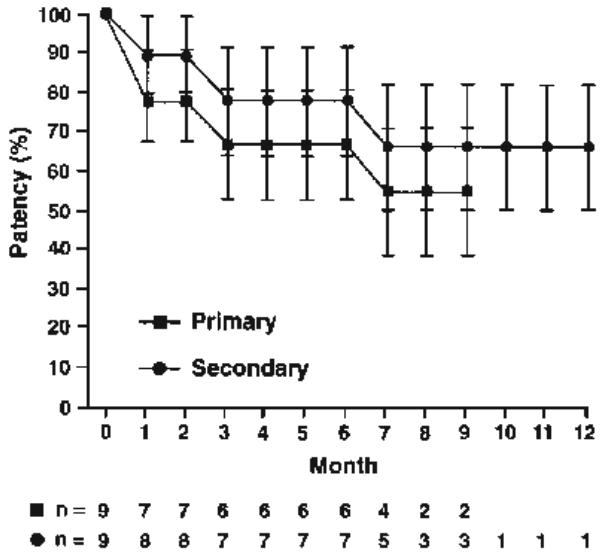

Fig 1. Actuarial primary and secondary patency rates for cryopreserved venous valve allografts transplanted as valve substitutes into the lower extremity venous system of patients with severe isolated deep venous insufficiency. Note that error bars are greater than 10% for all intervals.

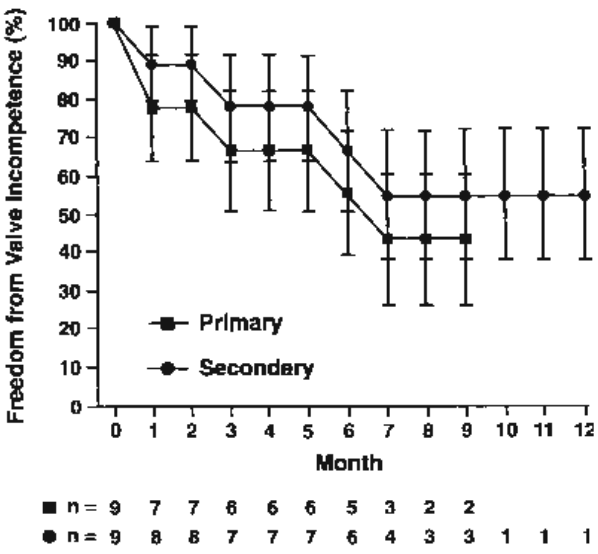

Fig 2. Actuarial primary and secondary competency rates for cryopreserved venous valve allografts transplanted into the lower extremity of patients with isolated and severely symptomatic deep venous insufficiency. Note that error bars are greater than 10% for all intervals.

was to determine how the cryopreserved venous valve transplant would perform in a rather challenging venous system, while eliminating extraneous variables.

The criteria for inclusion defines a severely disabled patient population and translated practically into all participants having or having had recalcitrant venous ulcerations ($C_{5\text{-}6,S}$). The underlying cause was generally the postphlebitic syndrome (89%), which was also reflected by most patients having areas of old scarring (narrowing, recannalization, etc) in the affected venous system, as assessed by means of duplex imaging and ascending venography (67%). Such patients were chosen to participate as long as the supporting diagnostic studies (duplex, descending venography, plethysmethographic studies, etc) confirmed venous insufficiency to be the major problem. Such a damaged venous conduit would pose practical surgical dilemmas, but such patients were considered acceptable candidates because they represent those patients most in need of this type of venous transplant. Earlier reports of venous valve transplantation for the treatment of chronic deep venous insufficiency have been criticized because more than one component (deep, superficial, perforator) of the lower-extremity venous system was defective, making it difficult to discern whether operative repair of one or the other was most important in the ultimate outcome. To eliminate multiple variables as a concern, we eliminated patients with iliac venous occlusion on the side that was considered for transplantation (outflow disease) and patients with major distal occlusive disease. Similarly, superficial disease, perforator disease, or both could not exist or must have been treated before inclusion in this study. Furthermore, the goal of this study was to evaluate only one cryopreserved valve in each patient. The decision was made, therefore, to transplant only one valve below all reflux. Certainly, the popliteal location above or below the knee is acceptable and is championed by some authors,[6,23-25] but a superficial femoral vein location would be acceptable when the profunda system is competent.[5,26-28] Profunda insufficiency can result in recurrent symptoms when superficial femoral vein incompetence is corrected alone.[5,27,28] With these established criteria, the results in this manuscript specifically reflect the effect of a single cryopreserved venous valve transplant for the surgical treatment of isolated deep venous insufficiency. In 78% of these patients, previous surgery was required to establish deep venous insufficiency as the remaining critical hemodynamic problem, as confirmed by means of an abnormal VRT, VFI_{90}, or both.

Even with these exclusion criteria in place to limit variability, other factors were not controlled, so

patient acquisition of this very select group (C_{4-6}, E, A_0, P_R) was possible. Numerous hospitals and various investigators with the liberty to decide on operative technique, particular anticoagulant use (fractionated or low-molecular weight heparin), or the use of a dAVF were all acceptable as study participant variables. Although these factors do add variability to the study, none were considered by the design committee to be of critical importance. A good operation performed by a skilled surgeon using accepted diagnostic, surgical, and postoperative anticoagulation (within the confines of other study parameters) was of prime importance. However, this variability is present and must be considered when reviewing the results obtained.

The observation that two cryopreserved venous valves required bench valvuloplasty just before transplantation is of concern. The presence of a competent valve is confirmed immediately before the cryopreservation process by means of gross inspection and hemodynamic testing. Thermal fracture can occur with careless handling during the cryopreservation process, transit, or at the time of thawing. Cryopreservation also affects smooth muscle and connective tissue function by approximately 50%, but venous compliance and elastic modulus, although somewhat diminished, are generally preserved.[29,30] It is conceivable that this less-severe effect of cryopreservative could result in laxity of the valve cusps or dilation of the vein wall resulting in valvular insufficiency. However, the valves were repaired and transplanted leaving no direct histologic data. The functional results have been acceptable, with both valves patent and competent at 6 months of follow-up. This would suggest that post-thaw valvuloplasty of cryopreserved venous valves is a viable option when such a situation is encountered.

In two cases, a distal arteriovenous fistula was constructed. In both cases, the venous valve segment remained patent for the 6-month follow-up period. In the case of a 0.5-cm-diameter simultaneous dAVF with ligation in 6 weeks, the valve was found to be patent and competent by means of duplex scanning and descending venography 1 month after ligation. The VRT was 24 seconds. However, at the 6-month evaluation period, a patent vein remained, but one valve was scarred and no longer moved on duplex imaging. The VRT reverted to 7 seconds. The other fistula was peripherally placed and allowed to function indefinitely, and the valve was patent and competent at 6 months. The large-diameter, closely positioned fistula may have caused some local changes not evident in early evaluation, but which caused a delayed valve cusp scarring. Past experiences would not substantiate a detrimental effect of even a large flow fistula on proximal venous valves.[15,31] Whether a more peripheral fistula with lower but continuous flow may be more clinically applicable or whether a dAVF is beneficial at all for early patency cannot be answered by this study.

A postoperative seroma occurred in 33% of our patients. This complication has been reported by Raju in only five of 107 limbs.[5] The role of lymphatic compensation for the malfunctioning venous system in the management of peripheral edema has been documented by means of a dramatic increase in lymphatic flow in patients early in the disease, with signs of later damage.[32] In either case, any surgical lymphatic disruption without adequate ligation could result in the formation of a seroma. This problem occurred only when operating on the superficial femoral vein, a situation akin to its presentation after arterial bypass grafting operations in the same area.[33] Luckily, the presence of a seroma did not necessarily translate into valve thrombosis. A conscious effort to ligate lymphatic channels should be made to prevent this surgical complication.

The operation is safe, resulting in no mortality or limb- or life-threatening surgical or medical complications. Although deep venous thrombosis occurred in the three protocol patients who experienced a valve occlusion and in the two protocol violations, no patient experienced extensive deep venous thrombosis or signs of pulmonary emboli. Anticoagulant therapy probably explains this result in those patients who were treated with it, whereas a protracted slow occlusion of the valve with adequate time for collateral development might explain this result in the other two patients.

The primary goal of this study was to evaluate the ability of cryopreserved venous valves to remain patent and competent in this patient population. Is a primary patency rate of 66.7% and freedom from valve incompetence of 55.6% at 6 months an acceptable result in this patient population? As described earlier, these patients have severe symptomatic deep venous disease, with no remaining therapeutic options for symptom relief. Two patients had even failed an earlier autograft venous valve transplant. Because there are no other clinical series of allograft venous valve transplantation available for comparison, the standard becomes upper-extremity venous valve autografts to the lower-limb insufficient venous system. The difficulty with such a comparison is that direct evidence of the valve status is not

always reported. Direct duplex evaluation of the valve transplant was available in the Tufts series; all valves were patent and competent at 2 years.[23] Only six of 11 patients in the Atlanta series had competency confirmed by means of descending venography at 6 or more months after transplantation, and all valves were found to be competent.[24] Thirty-one of 71 valve transplants were venographically evaluated by Taheri et al,[6] with one occluded and two incompetent valves found, for a failure rate of 9.7%, but the length of follow-up is unclear. Raju's series evaluated 44 of 54 axillary vein valve transplants with serial duplex scanning follow-up, and he reported a 6-month valve competency rate of approximately 50%.[26] His series is slightly different from the others in that a prosthetic sleeve was placed around the valve transplant to prevent later dilation. The Sweden experience reported a 25% axillary vein valve transplant failure rate (two occlusions, one incompetence) in 12 limbs at 6 months, with eight of 11 valves failing within 6 to 48 months.[27] Finally, Kistner and associates report that two of two valve transplants were either competent or had only minor reflux after 4 years or longer of follow-up.[28] These results certainly run the gamut. As with most instances of vascular surgical repair in low-flow systems, autografts generally perform most favorably. However, this review of the literature would suggest that the early results of a cryopreserved venous valve, as reported in this study, would make it an acceptable alternative when no autologous tissue is available.

There are several theoretical reasons to help explain why cryopreserved venous valve transplants might fail. Two failed early with adequate anticoagulation, which would suggest a technical error. However, the one case operatively approached for secondary patency demonstrated thrombus in the cusps and on the vein wall, but no misplaced sutures nor technical narrowing. The clot was mechanically removed, a dAVF was constructed and aggressive anticoagulation was undertaken, and the valve functioned throughout the remainder of the study. The other two valve failures presented as an occlusion within a few months of discontinuing anticoagulation in one case and as a single valve cusp thickening with loss of movement in the other. As shown clinically when using venous allografts in the arterial system, rejection may be a concern.[34] Cytotoxic T-cells could result in valve-cusp or vein-wall damage, endothelial cells could be destroyed, and/or a humoral immune response could activate the complement cascade.[34] Certainly, endothelial cells are often lost in the first 10 days after allograft transplantation into the vascular system of experimental animals, with reendothelialization occurring within the next 6 months.[35,36] However, not all allografts are so challenged by significant rejection, leading one to conclude that local injury, hypercoagulability, or stasis may still be the causative factor or factors in some cases.[34] Duplex evaluation of valves in the absence of or before failure demonstrated cusps that were thin and rapidly responsive to hemodynamic change. In fact, in the single case of valve incompetence in the otherwise patent allograft, the remaining valve cusp appeared grossly normal by means of duplex evaluation. This would make one think that whatever process is ongoing must be subclinical until such time as an extenuating event intervenes (eg, discontinuation of anticoagulants). Interestingly, one of the protocol-violation patients also occluded, but only after discontinuing anticoagulant therapy. Whatever the underlying causes, long-term anticoagulation appears useful for continued valve function and aids in the prevention of extensive deep venous thrombosis if the allograft fails. The issue of rejection is a valid concern, but our data can shed no further light on this topic. Furthermore, because only one valve failed in the absence of allograft occlusion, it would appear that allograft valves generally remain competent when patency is maintained. Also, the allograft being studied was processed, frozen, and thawed by only one protocol. The rate of freezing/thawing, culture media used, use of specific cryopreservatives, and other factors may affect results observed with the use of cryopreserved tissue,[35,36] and the data presented cannot be extrapolated to all cryopreserved allografts.

The secondary end point evaluates clinical impact and, as with most series involving venous valvular repair or transplantation, is not as conclusive as one would have planned. Except for one case of allograft thrombosis, all venous ulcers, in the six patients who had them, had healed (n = 4) or were healing (n = 2) at 3 months, with a competent valve in place. All patients had fewer complaints of swelling and pain. By 6 months, the clinical results were more difficult to interpret. Three allografts had occluded, but the patients were symptomatically improved. In those cases (n = 2) where ulcers had been present, healing had occurred, whereas in the remaining case, a dramatic improvement in VFI_{90} (9.4 to 2.6 mL/sec post-transplant) was observed. The clinical outcome score for the first two patients was a +1, because no hemodynamic studies were available for awarding a higher score. These results are consistent with those

of other authors, who have observed that ligation may prevent venous reflux with improved clinical results, as long as an incompetent collateral channel is not present.[37,38] Recurrent reflux in the absence of occlusion was observed in a single patient; the result was reversion to the presurgery status of major reflux and venous ulceration. The other five patients had a competent valve in place, as proven by means of duplex study. Two patients had significant clinical improvement with normalization of at least one parameter indicative of venous reflux (VRT, VFI_{90}, or both). One patient had clinical improvement, but only modest improvement in the VFI_{90}. Recurrent ulceration had been a problem in this patient, but did not resurface during this study. Two patients had competent valves, but recurrent ulceration. One case might be explained by worsening arterial disease (ankle pressure of 40 mm Hg) in the only patient in this series who had diabetes mellitus. Partial correction of the arterial diseases with percutaneous angioplasty (ankle pressure 90 mm Hg) at 5 months of follow-up had not yet resulted in ulcer healing. The other patient did not return for venous hemodynamic study, and, therefore, no further comment can be made. Such a finding of recurrent or continued ulceration in the face of a competent valve, although uncommon, has been reported by other authors.[26,39] Consistent with other reported series, hemodynamic parameters of venous reflux are often not corrected completely by a single valve transplant, even when it is placed below all apparent reflux.[5,27,28,39] Collectively, however, these results suggest that, devoid of extenuating circumstances, a patent and competent valve translates into an improved clinical result, especially when venous hemodynamics are improved. Recurrent incompetence results in recurrent symptoms similar to the preoperative state. However, allograft occlusion not resulting in other areas of thrombosis and occurring below all venous reflux may initially result in an improvement of the patient's clinical condition. Recurrent symptoms caused by collateral dilation and reflux[37] were not observed during the short duration of this study.

In conclusion, this study evaluated the use of a single cryopreserved ABO blood type-matched valved venous allograft, placed to correct isolated deep venous insufficiency in patients with recalcitrant venous ulceration. The primary aim of this 6-month feasibility study was to determine allograft patency and competence, with a secondary evaluation of clinical outcome. Six-month actuarial results documented an approximately 70% patency rate, approximately 60% competency rate, and 100% patient survival. Overall, these results are less impressive than the best reported results of axillary vein transplantation, suggesting that the most appropriate use of the cryopreserved allograft valve is in patients with no autograft option. The clinical outcome was generally improvement, with freedom from ulcer recurrence in six of nine patients. Long-term anticoagulation appears to be helpful in maintaining valve function and preventing extensive thrombosis when allograft failure occurs. Valve patency and competence is desirable for optimal results, but, in early evaluation, isolated allograft thrombosis may prevent reflux, resulting in clinical improvement. Recurrent reflux results in recurrent symptoms.

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DISCUSSION

Dr Alan B. Lumsden (Atlanta, Ga). Dr Dalsing and his colleagues have presented a stimulating and somewhat controversial study. The enormous clinical need for an effective valve replacement and the practical appeal of cryopreserved superficial femoral vein valves cannot be disputed. Valves could potentially be available off-the-shelf. We can get various lengths of superficial femoral vein, variable numbers of valves; size matching can be achieved, and as you have heard, the clinical testing that is required to obtain these grafts is ABO typing. There is essentially no earlier human data available, so this is an important report. Indeed, the only earlier venous data on cryopreserved femoral veins also comes from Dr Dalsing's laboratory, from his greyhound model, which was followed up for 2 months, showing patency. There is significantly more data available on the use of this kind of conduit in the arterial literature, in which cryopreserved saphenous vein has been widely used, but it has been used very much as a niche conduit, usually in a situation in which we are presented with limb threat, in which there is no alternate conduit available, and in which long-term patency generally is not expected, nor has it been demonstrated. We all accept that, in general, the superficial saphenous vein bypass grafts have limited longevity.

We have also used this conduit as an arteriovenous access graft and would confirm the ease of handling of the superficial femoral vein and the suturability reported by Dr Dalsing. But again, this is being done in a very special situation, in which the long-term patency is really not anticipated and in which graft failure is unlikely to be of

significant clinical consequence. Neither of these circumstances essentially exists when we use superficial femoral vein as a venous conduit. Usually, the disease for which it is being used is not immediately limb threatening, and failure could result in either limb threat or exacerbation of limb threat, or could even be life threatening.

One persistent question that hovers over the use of cryopreserved vascular tissue is that of patient sensitization. Why do these grafts fail, and what is the role of the cytotoxic T cells that are generated? It has been clearly demonstrated, including in the use of saphenous veins, that endothelial cells persist and that this can elaborate an immune reaction, so that even if we can potentially control the up-front problems of thrombosis, the long-term sequelae of potential sensitization of these patients are largely unknown. As Dr Dalsing said, the objective here—this is really a phase 1 study—is to determine safety and feasibility and to determine if cryopreserved venous valve segments will remain patent or competent in the short term. It is obvious that this is a very highly selected group of patients, with no superficial or perforator incompetence and no significant venous obstruction. It's the ideal model for testing whether this valve has efficacy.

They initially enrolled 11 patients in this trial, but two patients were subsequently excluded from analysis, one because he was noncompliant with the Coumadin and the other because he had a protein S deficiency. These are the sort of day-to-day problems we have to deal with in patients in whom we would be using a graft such as this. The authors' final analysis is based only on nine patients. They have demonstrated that the procedure was associated in this study with an approximate 44% complication rate, looking at the number of seromas and cellulitis, and that does not include thrombosis of the conduit as a major complication. Primary patency, again in nine patients, not the overall 11 patients, was only 67% at 6 months, and primary valve competency was approximately 56%. As Dr Dalsing intimated, it was safe. None of these patients sustained a pulmonary embolism. It is somewhat surprising that there were only six patients who actually had measurable ulcers, and three of those six patients did have some recurrence or non-healing of their ulcers despite having a functional valve demonstrated. They were both patent and competent, and I think that deserves some speculation.

In summary, despite this well-conducted study with premiere venous investigators, we are left with a series of data that we are not entirely sure how to use. I'm not entirely sure what to deduce or what we should do in the next step. I am trying to figure out what we should do with this data and what the potential implications are for moving to a phase 2 study, so I address the following questions to Dr Dalsing.

How were the conduits selected? You intimated that you tried to match the size of the recipient with the donor vein. How did you actually measure the diameter of the recipient vein? Was this based on the duplex measurements or the venographic measurements? Given the not-insignificant thrombosis rate, would you change either the operative technique or the anticoagulation regimen that you used? And could you comment on the extent of thrombosis that occurred when the device failed? Given that all cryopreserved vascular conduits have limited durability, how do we really manage to justify the use of such a graft in a condition that is really not limb threatening and in which graft failure may exacerbate the underlying problem for which it has been applied? If we do go to a phase 2 protocol, how would you refine the patient-selection criteria or any of the techniques that were involved in the study? Finally, do you think that immunosuppression, which has been intimated in some of the arterial data, has a potential role in prolonging conduit patency?

I would like to thank Dr Dalsing for providing the manuscript. It is excellently written. I think that the study has been very well done and poses many questions that need to be addressed. I would like to thank the Society for the privilege of discussing this paper.

Dr Michael C. Dalsing. Thank you, Al. I will try to go through the questions in order.

The way that we determined the proper size-match was to use venographic data, with a 20% diameter decrease, because of the method in which venograms are performed, or by means of a direct match with duplex scanning. So, we actually tried to end up with the same diameter match.

About what I would do to possibly improve the results: I have always had the impression, based on experimental work, that you might need to use a temporary arteriovenous fistula, but I don't have sufficient data in this paper to tell you if that is true. Long-term anticoagulation is mandatory, based on these nine patients plus some data we have about non-protocol grafts that have failed. Once anticoagulation is discontinued, the allografts generally thrombose within a few months.

When the device does fail, the extent of clotting is generally only at the graft site, which probably explains the low risk of pulmonary emboli and the lack of worsening symptoms after failure. In fact, in our experience, graft occlusion generally resulted in improved symptoms rather than symptom exacerbation.

How would we revise the protocol if we went to a phase 2 study? I think that the only time one would consider using this type of material would be in the end-stage patient who really has lifestyle-disabling problems, and I think that is the type of patient we tried to capture in this study. In that respect, I probably wouldn't change the protocol for a phase 2 study. I would look for those patients who have no other options. I think in some cases we look at venous disease as an all-or-none phenomenon when it comes to treatment. In fact, in those patients who have postphlebitic syndrome, I think we see the same problems that we have observed with arterial surgery. We generally palliate, using some type of autogenous reconstruction that will fail eventually. Having run out of autogenous tissue, we use an alternative graft. Chronic venous insufficiency does not usually result in limb loss or death. However, for the walking wounded with chronically draining ulcers, any help is often viewed as a godsend. Once autogenous valve

repair has run its course, a cryopreserved valve may help the patient to function for another few years. In our experience, failure of the graft with occlusion is not symptomatically a problem, and so, even with failure, a few more years of symptomatic relief can be expected.

Rejection may certainly be a factor in the failure of these grafts, if the arterial graft literature is applicable. Immunosuppression, if ever useful, must be at a degree that would not hinder venus ulcer healing, but would protect the allograft. Probably some type of Cytotoxic T-cell immunosuppressive agent will be required. At this time, I have no data to suggest how this factor may play out in clinical practice.

Thank you.