

Arterial reconstruction with human bioengineered acellular blood vessels in patients with peripheral arterial disease

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ABSTRACT

Objective: Vascular conduit is essential for arterial reconstruction for a number of conditions, including trauma and atherosclerotic occlusive disease. We have developed a tissue-engineered human acellular vessel (HAV) that can be manufactured, stored on site at hospitals, and be immediately available for arterial vascular reconstruction. Although the HAV is acellular when implanted, extensive preclinical and clinical testing has demonstrated that the HAV subsequently repopulates with the recipient's own vascular cells. We report a first-in-man clinical experience using the HAV for arterial reconstruction in patients with symptomatic peripheral arterial disease.

Methods: HAVs were manufactured using human vascular smooth muscle cells grown on a biodegradable scaffold. After the establishment of adequate cell growth and extracellular matrix deposition, the vessels were decellularized to remove human cellular antigens. Manufactured vessels were implanted in 20 patients with symptomatic peripheral arterial disease as above-knee, femoral-to-popliteal arterial bypass conduits. After HAV implantation, all patients were assessed for safety, HAV durability, freedom from conduit infection, and bypass patency for 2 years.

Results: Twenty HAVs were placed in the arterial, above-knee, femoral-to-popliteal position in patients with rest pain (n = 3) or symptomatic claudication (n = 17). All HAVs functioned as intended and had no evidence of structural failure or rejection by the recipient. No acute HAV infections were reported, but three surgical site infections were documented during the study period. Three non-HAV-related deaths were reported. One vessel developed a pseudoaneurysm after suspected iatrogenic injury during a balloon thrombectomy. No amputations of the HAV implanted limb occurred over the 2-year period, and no HAV infections were reported in approximately 34 patient-years of continuous patient follow-up.

Conclusions: Human tissue engineered blood vessels can be manufactured and readily available for peripheral arterial bypass surgery. Early clinical experience with these vessels, in the arterial position, suggest that they are safe, have acceptable patency, a low incidence of infection, and do not require the harvest of autologous vein or any cells from the recipient. Histologic examination of tissue biopsies revealed vascular remodeling and repopulation by host cells. This first-in-man arterial bypass study supports the continued development of human tissue engineered blood vessels for arterial reconstruction, and potential future expansion to clinical indications including vascular trauma and repair of other size-appropriate peripheral arteries. (J Vasc Surg 2020;■:1-12.)

Keywords: Peripheral arterial disease; Arterial reconstruction; Bioengineered blood vessel; HAV

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Author conflict of interest: J.H.L. is CEO of Humacyte, and is a paid employees of Humacyte, including salary and stock options, and serves on the board of directors. R.D.K. and B.T. are paid employees of Humacyte, including salary and stock options. H.L.P. is a paid employee of Humacyte and has stock options. H.L.P. has patents licensed to Humacyte. L.E.N. is a founder and shareholder of Humacyte. L.E.N.'s spouse has equity in Humacyte. L.E.N. serves on the board of directors and has patents that are licensed to Humacyte and produce royalties for L.E.N. L.E.N. has received unrestricted gifts to support her research at Yale. A.P. is a consultant paid by Humacyte. Payment includes salary and stock options. S.C. has been a paid employee and consultant to

Humacyte, and owns shares in the company. N.Z., M.I., S.P., T.Z., W.W., M.G., and J.T. have been paid as investigators in the study. W.W. has been paid a consulting fee. R.S. has no conflicts of interest to disclose.

Presented at the 2017 Vascular Annual Meeting of the Society for Vascular Surgery, San Diego, Calif, May 31-June 3, 2017.

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The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214

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<https://doi.org/10.1016/j.jvs.2019.11.056>

Arterial bypass for peripheral arterial disease (PAD) and vascular trauma has progressed over the past 70 years.¹ Early reports highlighted the use of both autologous vessels and synthetic conduits as options for vascular repair.^{2,3} Although vascular reconstruction has evolved, the need for suitable bypass conduit has remained an important consideration for every surgical case. Many conduit technologies have been developed for vascular bypass, including the harvesting of autologous vessels, as well as polyesters, xenografts, fresh/frozen homografts, and expanded polytetrafluoroethylene. Although each of these technologies has been important in the advancement of modern vascular surgery, each material has specific limitations when used in surgical vascular reconstruction.

Autologous saphenous vein, which is considered the gold standard for vascular repair, requires prolonged operating time and produces harvest site pain, as well as an increased risk of wound infection from the donor site. Furthermore, saphenous vein quality can vary by patient and can be constrained by vein scarring, vascular disease, and varicosities.⁴ Conduits made of synthetic materials suffer from a lack of true biocompatibility and a prolonged risk of infection. Xenografts have elevated rates of early thrombosis and medial calcification,⁵ and cryopreserved veins have a persistent risk of immune recognition.⁶ Human umbilical veins, developed as bypass materials several decades ago, do not show appreciably improved outcomes as compared with synthetic conduits in recent clinical studies.⁷

In an attempt to address the need for immediately available, nonantigenic human vascular material to be used for vascular reconstruction, we have devised a method to grow human vessels *in vitro* using human vascular cells, that are cultured on a biodegradable scaffold. These vessels are then rendered acellular by a decellularization process that gently rinses antigenic cellular material from the vessel, preserving the extracellular matrix proteins and mechanical integrity of the conduit, resulting in a human acellular vessel (HAV).^{8,9}

We have reported the use of these vessels in a phase I/II clinical study using the HAV as arteriovenous access in patients with end-stage renal disease.¹⁰ To evaluate the early clinical usefulness of the HAV as an arterial conduit, we conducted this study in patients with symptomatic occlusion of the superficial femoral artery (SFA). To our knowledge, this phase II study is the first assessment of a completely human bioengineered blood vessel as an arterial bypass conduit in the peripheral circulation.

METHODS

Production of HAVs. HAVs were 6 mm in diameter and 35 to 42 cm in length. Human vascular smooth muscle cells were derived from deceased organ and tissue donors, meeting eligibility requirements for all relevant communicable diseases. After smooth muscle cell isolation and expansion, cells were seeded onto degradable

ARTICLE HIGHLIGHTS

- **Type of Research:** Prospective, open-label, single-arm treatment, multicenter pilot study
- **Key Findings:** All human acellular vessels functioned as intended and had no evidence of structural failure or rejection by the recipient.
- **Take Home Message:** The human acellular vessel is a promising experimental therapy, and early clinical experience supports the continued development this product for arterial bypass and reconstruction.

polymer scaffolds that were contained within flexible, single-use bioreactors. Developing vessels were subjected to pulsatile cyclic distension and then were decellularized to remove immunogenic cellular antigens while preserving the comparatively nonimmunogenic extracellular matrix constituents. The process to grow the HAVs takes approximately 10 weeks (Fig 1).¹¹⁻¹³

Study design. We performed a prospective, open-label, single treatment arm, multicenter pilot study. The primary objectives of the study were to evaluate the safety of the HAV as an above-knee femoral-to-popliteal bypass graft, and to determine the patency (primary, primary assisted, and secondary) over 24 months. Patients with PAD requiring above-knee peripheral bypass surgery were screened within 14 days of the planned operation. Patients were between the ages of 54 and 79 years (inclusion criteria age 18-80 years) with a projected life expectancy of at least 2 years, and with claudication at a distance of more than 200 m or with ongoing rest pain. All patients had a total occlusion segment of the SFA (Inter-Society Consensus for the Management of Peripheral Arterial Disease II type B [n = 5] and type C lesions [n = 15]¹⁴) with adequate proximal inflow (distal external iliac artery, common femoral artery, or proximal SFA) and distal outflow (SFA or above-knee popliteal artery), with at least two-vessel runoff below the knee to the ankle. Appropriate anatomy was assessed and confirmed preoperatively with conventional or computed tomography angiography. The HAV was used as a conduit for bypass of an arterial occlusive lesion within the SFA, which was not amenable to endovascular therapy, and in which suitable autologous conduit was not available for bypass.

A total of 20 eligible patients were enrolled at 3 clinical sites and received the HAV implant (day 1). Patients were followed for 2 years to assess the safety and efficacy of the HAV in terms of patency, necessary graft interventions, and relief of PAD symptoms of rest pain and claudication. Six follow-up visits were performed in the first year: immediately after surgery (days 5 and 15); at weeks 6, 12, and 26; and at month 12. Two follow-up visits were performed in the second year (months 18 and 24).

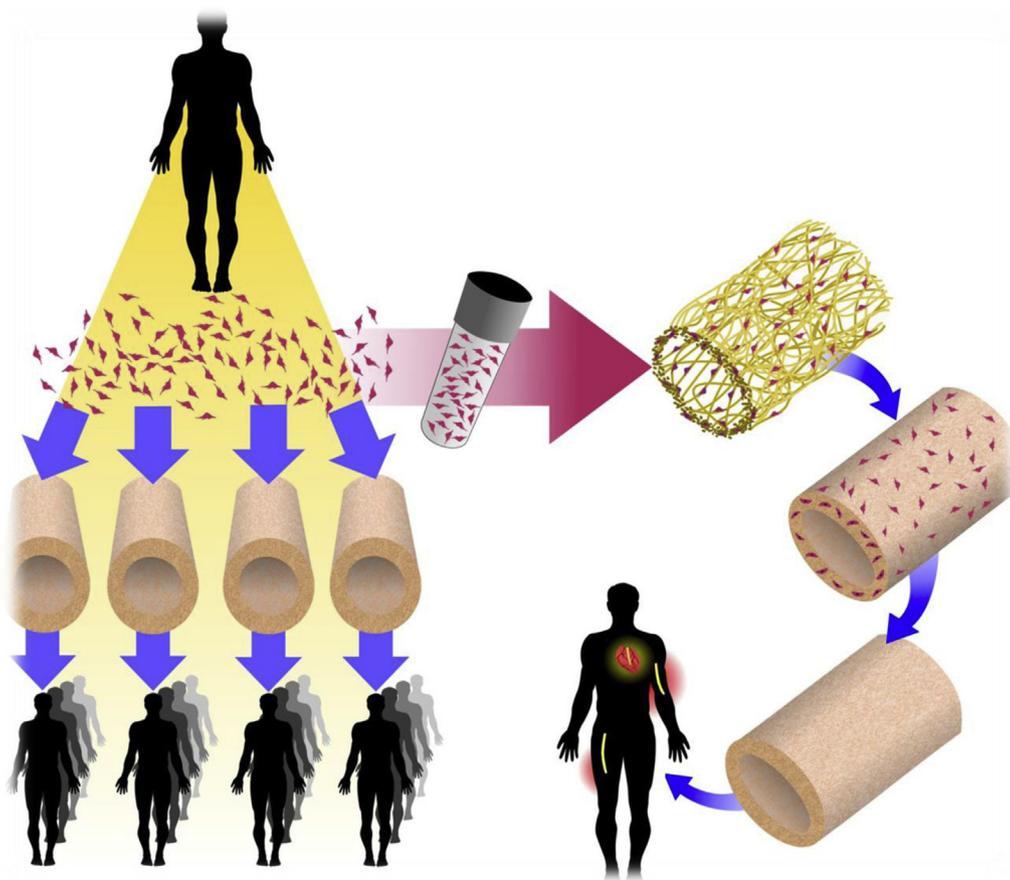


Fig 1. Production of human acellular vessels (HAVs). Donor smooth muscle cells are seeded onto a biodegradable scaffold within a single-use bioreactor. During culture, a cellular bioengineered vessel is grown, which is then decellularized to produce the HAV.

This study was conducted in full conformity with the Declaration of Helsinki (revised 2008), Good Clinical Practice, and the International Council for Harmonisation requirements for Good Clinical Practice. The independent ethics committee of each participating clinical center approved the protocol, and each patient provided written informed consent before enrolment.

Statistical methods. The primary efficacy end points (primary, primary assisted, and secondary patency rates of HAV at month 24) were summarized using Clopper-Pearson two-sided 95% confidence intervals for binomial proportions. Kaplan-Meier analyses were used to evaluate time to loss of patency. The rate and type of graft interventions and other efficacy variables were analyzed descriptively. All safety analyses were descriptive.

Investigational product. The Humacyte HAV is a tissue-engineered HAV composed of human collagen types I and III and other extracellular matrix proteins, including fibronectin and vitronectin.

Procedure. Enrolled patients were implanted with a HAV in the extremity in which they suffered from symptomatic PAD. The proximal and distal target arteries

were surgically exposed in the standard fashion and the HAV delivered between the two incisions using a sheath tunneler. Proximal and distal anastomoses were fashioned in an end-to-side configuration using 5-0 or 6-0 polypropylene sutures (Fig 2). At the conclusion of implantation, HAV patency and distal arterial runoff to the foot were confirmed with a high-quality duplex ultrasound examination or with conventional intraoperative angiography (Fig 3).

All patients received 2 days of antibiotic prophylaxis started intravenously before surgery and continued intravenously or intramuscularly. Additionally, antithrombotic prophylaxis with unfractionated heparin up to 5000 IU was given intraoperatively and followed by low-molecular-weight heparin at a prophylactic dose, daily, until patients were fully mobilized. Starting on the day after the discontinuation of low-molecular-weight heparin, dual antiplatelet therapy (aspirin 75-300 mg and clopidogrel 75 mg) was initiated and continued until HAV abandonment.

HAV interventions and adverse events were recorded at scheduled all study visits at days 5 and 15; weeks 6, 12, and 26; months 12, 18, and 24. The patency of the HAV was determined based on ultrasound findings, graft

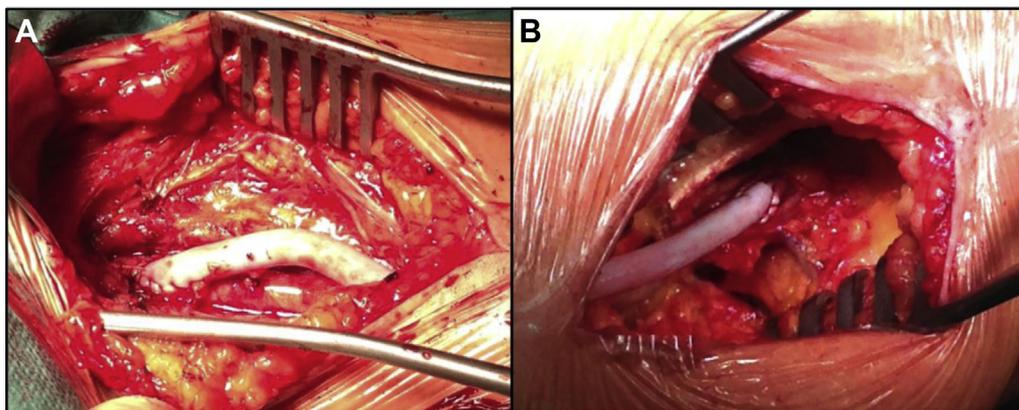


Fig 2. Human acellular vessel (HAV) implantation. Proximal (A) and distal (B) anastomoses of the first HAV implant for arterial bypass performed in the left lower extremity on October 11, 2013 (Szczecin, Poland). Photos courtesy of Humacyte, Inc.

interventions, and clinical examination of the graft site starting at day 1 and continuing through all follow-up visits. PAD symptoms, including claudication distance, pain at rest, and ischemic ulcers, were documented. Resting ankle to brachial indices (ABIs) were assessed at baseline and at follow-up visits between 6 weeks and 24 months. Panel-reactive antibody (PRA; antibodies directed against class I or II human leukocyte antigens) and anti-HAV serum IgG levels at 6 months after HAV implantation were compared with those at baseline. The first vessel in this study was implanted on October 11, 2013, and the final study visit of the last patient enrolled in the study occurred on May 30, 2016.

Immunoassays. PRAs directed against MHC class I and class II antigens were assessed in patient serum samples preoperatively, and at 6 months after implantation, using clinical-grade testing regimens. Anti-HAV IgG antibodies were also assessed at these two time points. To measure levels of anti-HAV IgG antibodies, a customized enzyme-linked immunosorbent assay was used. HAV samples were coated onto multiwell plates, and patient serum samples were incubated. After washing, a monoclonal antibody directed against human IgG was used in a sandwich-type enzyme-linked immunosorbent assay reaction with a colorimetric readout. Pooled human sera not exposed to the HAV were used as a negative control.

Explant histology. In this study, explanted HAV samples were obtained from three patients during an otherwise indicated surgical revision of the HAV. Explanted HAV tissues were fixed in 10% neutral buffered formalin, embedded into paraffin, and sectioned (5 μ m thick) for staining. After deparaffinization and rehydration, routine hematoxylin and eosin (Statlab reagents; Statlab, McKinney, Tex) staining was performed. Immunofluorescence staining was performed using a protocol similar to that previously described.¹⁵ Primary antibodies for human CNN1 (1:50, ab700; Abcam, Cambridge, UK), alpha

smooth muscle actin (1:200, Abcam, ab5694), CD31 (1:300, Abcam, ab32457), and CD34 (1:50, #3569; Cell Signaling Technologies, Danvers, Mass) were incubated on tissue sections overnight at 4°C. Fluorescence conjugated secondary antibodies (A11001 and A11012; Invitrogen, Carlsbad, Calif) were diluted 1:400 and incubated on the tissue sections for 1 hour at room temperature. Slides were mounted with medium containing 4,6-diamidino-2-phenylindole to counterstain the nuclei. Sections were imaged using a Nikon TE2000U microscope equipped with a Photometrics CoolSNAP HQ2 camera (Tucson, Ariz). Image acquisition and processing was done using μ Manager and Fiji software (Image J software).

RESULTS

A single HAV was implanted into 20 patients over the course of a study enrolment period 7 months. All patients were Caucasian and 65% were male, with a median age of 66 years. Demographics are summarized in Table I. Overall, 75% of patients had hypertension, 45% had diabetes, and 25% were current tobacco smokers; additional comorbidities are also summarized in Table I.

The proximal anastomosis was fashioned to the common femoral artery in 17 patients and to the proximal SFA in three patients; the distal anastomosis was fashioned to the distal SFA in 11 patients and to the proximal popliteal artery in nine patients. The median length of arterial occlusion was 20.2 cm (range, 10.1-28.0 cm) as assessed by computed tomography or conventional angiography. All patients had a total segmental occlusion of the SFA with five patients having type B and 15 patients having type C lesions under the Inter-Society Consensus for the Management of Peripheral Arterial Disease II classification system.¹⁴ None involved occlusion of the popliteal artery. The median total HAV length used for the bypass procedure was 28 cm (range, 23-30 cm). The mean follow-up was 20.7 months with a cumulative follow-up of 34.4 patient-years.



Fig 3. Arteriograms. Preoperative arteriogram revealing occlusion of the right superficial femoral artery (SFA), but with suitable proximal target (common femoral artery) and distal target (proximal popliteal artery) for above knee bypass (A). Arteriogram 5 months after implantation with patent HAV bypass (B).

Seven patients discontinued the study before the 2-year end point; four after graft occlusion and three who died before study completion. No patients were lost to follow-up. For the three reported deaths, HAVs were known to be functional at the patient's last study

visit. There were no HAV-related deaths. One death was due to cardiopulmonary failure approximately 2 weeks after implantation, one was due to metastatic small cell lung cancer 22 months after implantation, and one death occurred at 19 months after implantation.

Table I. Patient demographics and medical history

Variable	
Demographics	
Male	13 (65)
Female	7 (35)
Age, years	66 (54-79)
Body height, cm	167.5 (154-178)
Body weight, kg	79 (60-99)
Body mass index, kg/m ²	28.0 (22.8-34.3)
Caucasian ethnicity	20 (100)
Medical history	
Hypertension	15 (75)
Diabetes ^a	9 (45)
Current tobacco user	5 (25)
Myocardial infarction	5 (25)
Coronary artery disease	4 (20)
Hyperlipidemia	4 (20)
Arteriosclerosis	3 (15)
Coronary angioplasty	3 (15)
Carotid artery stenosis or restenosis	2 (10)
Values are number (%) or median (range).	
^a Includes type 1 diabetes mellitus and type 2 diabetes mellitus.	

owing to an undetermined cause. None of the events were considered related to the investigational product or the surgical procedure.

During the study, two patients developed rest pain at the 12 months follow-up visit, which resolved after successful intervention on the HAV. One patient reported rest pain and ischemic ulcers at 18 months follow-up visit. These resolved after replacement of the HAV with surgical revision to a more distal site using a synthetic graft.

Patency and interventions. With deaths censored, patency probability rates obtained by Kaplan Meier estimates of 24-month primary, primary assisted, and secondary patency rates were 58%, 58%, and 74%, respectively (Table II; Fig 4). Six of the 20 patients (30%) required at least one intervention on the HAV during the course of the study and in total, nine interventions were performed over the course of the study. These interventions were either a thrombectomy (n = 4), an angioplasty procedure (n = 4), or thrombectomy/angioplasty performed concurrently (n = 1). Most interventions were successful at restoring patency. However, in one patient, the graft patency could not be restored and the HAV was replaced with synthetic bypass graft material to a more distal target. Two patients who had previously undergone successful interventions developed a recurrent thrombosis which was not treated and the HAV was left occluded. Two patients experienced HAV thrombosis with no or minimal symptoms and refused interventions on the HAV.

Ultrasound examinations of the HAVs were performed at days 5 and 15, and at weeks 6, 12, 26, and at months 12, 18, and 24 (Table III). Midvessel inner diameters were recorded for each vessels examined and at each time point. The average midvessel diameter began at 6.0 mm (range, 5.6-6.1 mm) at day 5 and decreased to 5.2 mm by month 24 (range, 2.9-5.8 mm). The largest midvessel diameter recorded was 7.0 mm. These data show that the HAVs were mechanically stable during the follow-up period and did not develop aneurysmal dilatation in any patient (aneurysmal dilatation being defined as >50% increase in diameter, or 9 mm).

The median claudication distance was 50 m at Screening. Six weeks after implantation of the HAV, median claudication distance increased to 1000 m and remained at this level for all patients with a patent HAV. The median resting ABI at screening was 0.64 and, by week 6 post HAV implantation, the median ABI had increased to normal (1.0); at months 3, 12, and 24, the median ABI was reported as 1.0, 0.9, and 0.96, respectively (Fig 5). None of the patients with a patent HAV experienced a decrease in resting ABI.

Complications and infection. Twenty-six HAV-related complications were reported in 10 patients and included thrombosis, anastomotic stenosis, HAV stenosis, pseudoaneurysm, and postprocedural hematoma. The most frequently reported events were HAV thrombosis (7 patients) and anastomotic stenosis (4 patients). One patient with multiple HAV thromboses had rest pain at screening and month 12 follow-up visit. There were a total of 25 procedural (index surgery) related events including lymphocele, local swelling, wound infection, and seroma. There were no HAV-related infections reported during the study and no amputations of the treated extremity were reported. Three surgical site infections were reported; two were superficial wound infections and one infection that developed in association with a postoperative lymphocele. The most common events are listed in Table IV.

One patient developed a pseudoaneurysm that was noted at the 3-month study visit. The pseudoaneurysmal segment was excised and replaced with an interposition segment of expanded polytetrafluoroethylene and the bypass remained patent through the duration of the study. The histologic assessment of this specimen revealed that the HAV had vascular cells within the wall, indicating signs of remodeling with appropriate cell types. A hole in the vessel was apparent at the site of the pseudoaneurysm on both gross and histologic examination. Overall, the histologic assessment showed little to no inflammation, no infection, and no sign of immunologic reaction to the graft. All clinical records that were reviewed suggest that the most likely cause of the resulting pseudoaneurysm was from iatrogenic trauma to the vessel during the passage of an

Table II. Kaplan-Meier analysis: Patency rates

Patency	Month 6			Month 12			Month 18			Month 24		
	No.	Probability, %	95% CI	No.	Probability, %	95% CI	No.	Probability, %	95% CI	No.	Probability, %	95% CI
Primary	15	79	54-92	12	63	38-81	12	63	38-81	4 ^a	58	33-76
Primary assisted	15	79	53-92	12	63	38-80	12	63	38-80	4 ^b	58	33-76
Secondary	17	90	64-97	16	84	59-95	15	79	53-92	6 ^c	74	48-88

CI, Confidence interval for graft patency probability; No., number of patients still at risk.

For months 6, 12, 18, and 24, days 180, 360, 540, and 720 were used (see Fig 4), although the visits of the patients may have taken place at different actual days owing to allowed time windows. Patients who died with the graft still patent were censored at that time point.

^aFive patients completed the study after the last loss of primary patency event at day 559 and before day 720, and were not at risk at day 720.

^bFive patients completed the study after the last loss of primary assisted patency event on day 561 and before day 720, and were not at risk at day 720.

^cSix patients completed the study after the last loss of secondary patency event on day 561 and before day 720, and were not at risk at day 720.

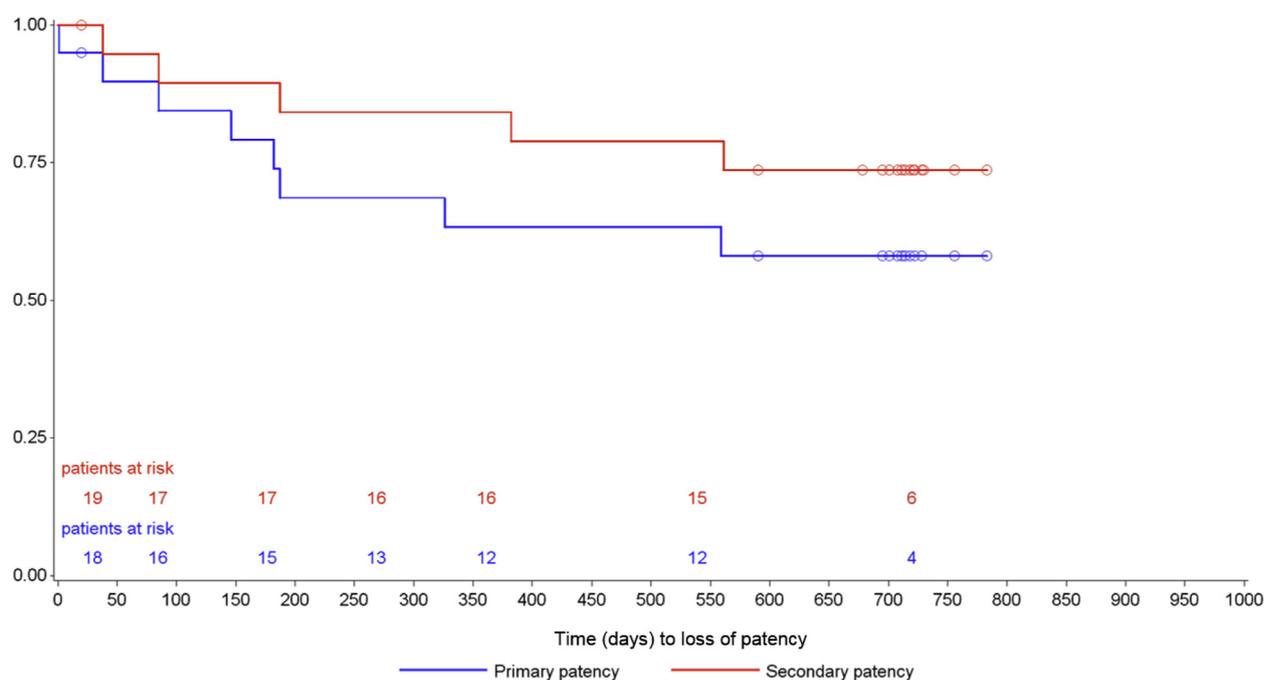


Fig 4. Kaplan-Meier plot. Primary (blue) and Secondary (red) patency overlay. Patients at risk shown for the following key time points: days 30, 90, 180, 270, 360, 540, and 720 (ie, months 1, 3, 6, 9, 12, 18, and 24). Patients who discontinued early or who died with the graft still patent were censored at that time point.

embolectomy catheter in a prior procedure. At the last study visit at 24 months, the HAV remained patent and functional.

Immunologic assessment. There were no increases in PRA class I or class II antibody levels observed from baseline to week 26 postoperatively. Fifteen patients had no PRA class I antibodies detected at either baseline or week 26 time points. Of the remaining five patients who had class I PRAs at baseline, there was no clinically significant increase observed for any patient (mean class I PRA of 16% preoperatively; 18% at week 26; $P = .24$ by Student's paired t -test). All class II PRA values were zero at both time points. There were no significant increases

in anti-HAV IgG levels from baseline in 18 of the 20 patients.

Two patients had increases in anti-HAV IgG levels from baseline that were more than two-fold greater than the baseline values and were considered possibly significant. The first patient experienced no serious adverse events and, at month 18, the HAV had maintained primary patency and the luminal diameter had not changed from its implantation diameter (6 mm). The patient ultimately died of metastatic lung cancer before the 24-month visit, and the HAV had remained patent at the time of death.

The second patient was hospitalized 6 weeks after HAV implantation with an infected lymphocele in the surgical

Table III. Midvessel diameter (mm) assessed by ultrasound examination

Visits	No.	Median	Range
Day 5	20	6.0	5.6-6.1
Day 15	20	6.0	5.2-7.0
Week 6	19	5.8	4.5-6.1
Week 12	18	5.9	4.6-6.4
Week 26	18	5.7	4.6-6.7
Month 12	15	5.4	2.7-6.8
Month 18	15	5.5	2.5-6.6
Month 24	13	5.2	2.9-5.8

No., Number of patients with observations.

wound bed, located close to the distal graft anastomosis. The lymphocele was not in contact with the HAV anastomosis. The lymphocele was evacuated, and, at 8 weeks after implantation, the HAV was patent and the wound was healing. The HAV then occluded sometime between the 11- and the 24-week postimplantation visits without recurrence of ischemic symptoms in the treated leg; therefore, no intervention was done to restore patency. There was no evidence of HAV dilatation on any ultrasound studies and no systemic or local inflammatory response was described.

In vivo vascular remodeling. No infections of the HAV vessel itself were reported during this study. This finding is consistent with the low infection rate observed when the HAV is used for dialysis access, suggesting that the remodeling of the HAV matrix by the recipient patient's own cells may confer resistance to infections, which can be problematic with synthetic grafts.¹⁰ In this study, biopsies of the HAV were obtained from three patients at 13, 50, and 61 weeks after implantation. In all cases, samples of HAV were obtained during a surgical procedure that was clinically indicated for HAV revision or repair. Hematoxylin and eosin staining of explant samples (Fig 6, A1, B1, and C1) show progressive infiltration of the HAV wall with spindle-shaped cells (Fig 6). Immunostaining for smooth muscle markers shows yellow co-staining for smooth muscle alpha actin (red) and calponin (green) in the HAV wall, indicating a smooth muscle phenotype of repopulated cells (Fig 6, A2, B2, and C2). Interestingly, the appearance of CD34⁺ cells in the neoadventitia at the early 13-week time point is accompanied by multiple CD31⁺ microvessel structures, perhaps implying recruitment of CD34⁺ vascular endothelial progenitors to the outer surface of the HAV (Fig 6, A3 and B3). Over time, it appears that the CD34⁺ cells become less frequent, and instead the appearance of robust CD31⁺ microvessels in the outer HAV media are evident at 61 weeks (Fig 6, C3). Although the luminal surfaces of the HAV biopsies did not seem to stain for endothelial markers (Fig 6, A3, B3, and C3 insets), given

that these vessels typically underwent intravascular manipulations/ballooning, it is not surprising that any luminal endothelium might have been stripped.

DISCUSSION

The optimal conduit for peripheral arterial bypass has yet to be established¹⁶ and typical processes to use autologous venous conduit are associated with morbidity, increased health care use, and costs secondary to wound complications.¹⁷⁻¹⁹ Wound complications and infection from venous harvest sites occur in as many as 25% of cases using autologous vein.^{17,18} Synthetic alternatives provide additional options when autologous conduit is not available, but these grafts pose a disadvantage to host tissue in terms of patency, biocompatibility, infection, and durability.^{20,21} The increasing incidence of atherosclerotic vascular disease and the short-comings of native autologous vein and synthetic materials only highlight the need for a better vessel replacement option.

The HAV, when used as a conduit for dialysis access, withstands repeated cannulation over periods of more than 1 year without aneurysm or structural degradation and does not require a prolonged time for maturation.¹⁰ There is a low infection rate observed when the HAV was used as a conduit for dialysis access or a vascular bypass for patients with PAD. The potential advantages of the HAV may include lower complication rates and decreased infections, leading to better long-term graft survival. Early clinical observations in this study require additional testing in larger, prospective clinical trials.

The overall goal of regenerative therapies is to repair or replace damaged tissue with new therapies that biologically mimic the failing tissue. Our prior report on the use of human bioengineered blood vessels for hemodialysis access demonstrated the feasibility and the potential benefits of using regenerative therapies for vascular replacement.¹⁰ In that study, 60 HAVs were cannulated with large-bore dialysis needles three times per week. There were no negative immune reactions to the HAV, no reports of vessel degeneration or unexpected conduit failures, a low incidence of overall infection, and favorable enduring patency. Moreover, HAV tissue samples explanted from that study population revealed substantial host recellularization that transformed the once acellular HAV into the patient's own living blood vessel.¹⁰ A bioengineered arterial replacement, like the HAV, that behaves like native tissue within the host could address an unmet medical need in the future. This pilot study of 20 patients is the first-in-man experience with a human bioengineered blood vessel as a conduit for peripheral arterial bypass.

In this trial and in our previously reported hemodialysis access trial, there was no clinical, ultrasound, or angiographic evidence of structural degradation or true aneurysm formation. In the patient in this study who

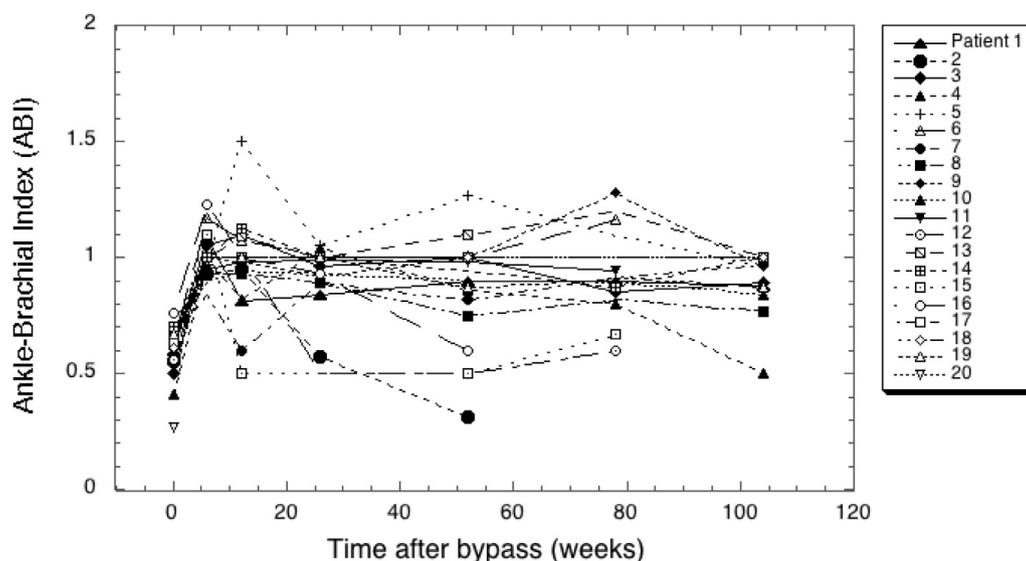


Fig 5. Ankle-brachial indices (ABIs) over time. The ABI values for each patient in the study at the following key time points: weeks 6, 12, and 26 and months 12, 18, and 24. Patient 2, 4, 12, 14, 15, and 16 had graft complications and patient 20 died, with no data recorded.

Table IV. Human acellular vessel (HAV) complications and surgical site infections

Complication/infection	All patients (N = 20)	
	No. of events	No. (%) of patients
Total HAV complications	26	10 (50)
HAV thrombosis	15	7 (35)
Anastomotic stenosis	6	4 (20)
HAV stenosis	2	2 (10)
HAV pseudoaneurysm	2	1 (5)
Postprocedural hematoma	1	1 (5)
Total surgical site infection	3	3 (15)
Postoperative wound infection	2	2 (10)
Infected lymphocele	1	1 (5)

developed the pseudoaneurysm, clinical and histologic information suggests that the initial defect was most likely caused by iatrogenic injury during the early postoperative period.

There was no evidence of immune rejection of the HAV, as detected from clinical explants or from the 6-month serologic assessments. There was no increase in PRA class I or class II levels observed from baseline to week 26. Two patients experienced an increase in anti-HAV IgG that was not associated with any adverse clinical events, such as dilatation or aneurysm formation, nor did this seem to alter the structural integrity of the HAV during the study period. In contrast, cryopreserved human veins are subject to immunologic complications,

and these reactions can result in structural instability and aneurysmal degeneration.⁶

Tissue samples were obtained from implanted HAVs of three patients; specimens were collected at the time of surgical reexposure for open thrombectomy or technical revisions at 13, 50, and 61 weeks in three different patients. Immunostaining techniques revealed positive markers for smooth muscle cells (eg, smooth muscle actin/calponin) and endothelial cells (CD31), and together, these markers are suggestive of positive vascular remodeling. Grossly, there is an appearance that over time, the HAV structurally develops into a vessel with arterial attributes as is demonstrated in Fig 7, where the medial wall of the vessel demonstrates bleeding when transected (Supplementary Video, online only).

Primary patency was 63% at 12 months and 58% at 24 months, and secondary patency was 84% at 12 months and 74% at 24 months. The initial results from this small phase II study reflect positively on the potential for the HAV to provide long-term bypass durability.

There were no cases of direct HAV infection in 34.4 patient-years of follow-up; three surgical site infections were reported (two postoperative wound infections and one infected lymphocele). In the patient with the infected lymphocele, a lymphatic cyst developed at the surgical exposure site (close to the distal graft anastomosis) that subsequently became infected and required patient hospitalization, surgical drainage, and antibiotics. However, neither the infected lymphocele nor the postoperative wound infections progressed to subsequent HAV infection. Similar to our previous trial in hemodialysis access, there was a very low rate of overall infection in this study (0.09 per patient-year, or three cases in 34.4

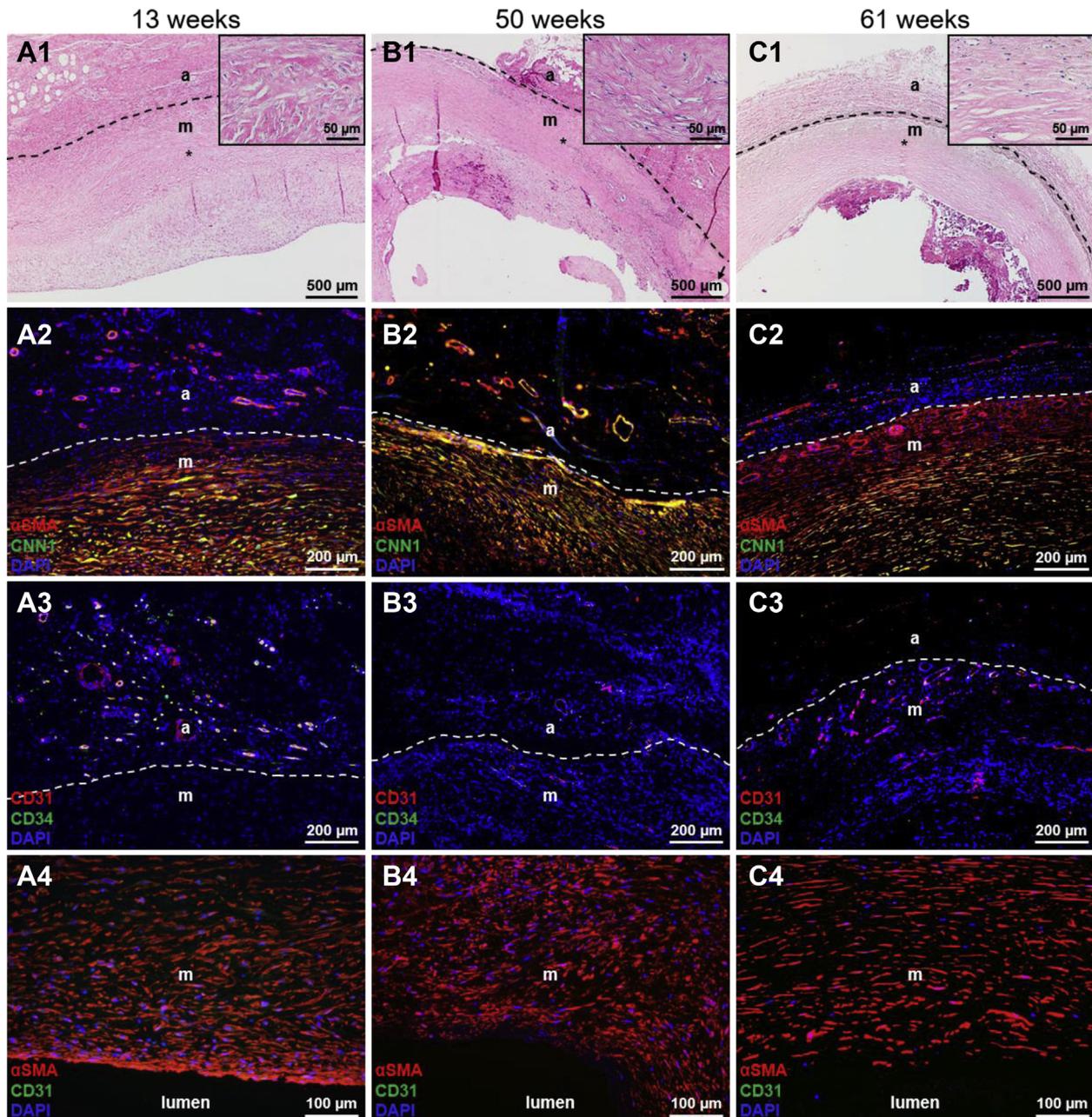


Fig 6. Histologic evaluation of explanted human acellular vessels (HAVs) at 13 (**A**), 50 (**B**), and 61 (**C**) weeks after implantation. Sections of explanted HAV tissue samples stained with hematoxylin and eosin (**A1**, **B1**, **C1**) show development of neoadventitial (a) and medial (m) layers. High magnification inset taken from region denoted with an asterisk (*). The anastomotic suture hole in **B1** is identified with black arrow. Immunofluorescence staining of alpha smooth muscle actin (α SMA, red) and calponin (CNN1, green) reveal myogenic host recellularization (**A2**, **B2**, **C2**). Coexpression (yellow overlay) of CNN1 with α SMA indicative of maturation of smooth muscle cells. Microvasculature within neoadventitia express endothelial marker CD31 (red; **A3**, **B3**, **C3**), and expression of the early endothelial marker CD34 (green) is predominantly observed at 13 weeks after implantation (**A3**). All three explanted samples lacked substantial presence of CD31⁺ endothelial cells on lumen (**A4**, **B4**, **C4**). Nuclei (blue) were counterstained with 4,6-diamidino-2-phenylindole.

patient-years), none of which led to the abandonment or explantation of the HAV.

Rehospitalization for wound complications after peripheral bypass is common, and occurs approximately 10% to 20% of the time, leading to increased morbidity, mortality, limb loss, patient dissatisfaction, and cost to

the health care system.²²⁻²⁴ Rates of wound complications and infection after lower extremity bypass are similar between prosthetic and autologous conduit and range between 4.5% and 6.5%.^{20,22} Lymphocele formation and other wound complications after lower extremity bypass are common in this patient population.

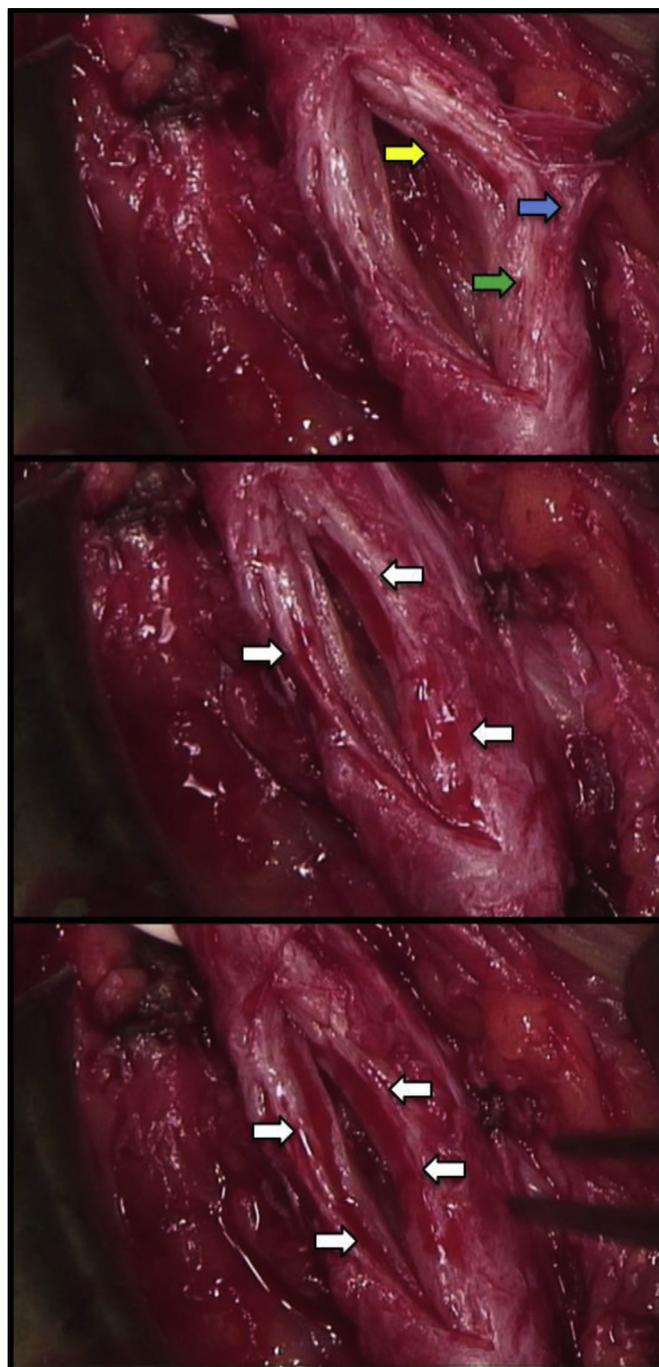


Fig 7. Intraoperative photo. Taken during open surgical intervention 12 months after the initial implant. The human acellular vessel (HAV) has developed appearance of an intima (*yellow arrow*), media (*green arrow*), and adventitia (*blue arrow*). Bleeding from the HAV edges (*white arrows*) serves as evidence that the HAV has undergone neovascularization and developed a microvascular network. (Images courtesy of Jakub Turek and Norbert Zapotoczny, Wrocław, Poland.)

Because this was a phase II clinical trial, the study was limited by the small sample size, and it was not adequately powered to assess efficacy. Furthermore, the study population was entirely European, Caucasian,

and predominantly male. Despite these limitations, we have demonstrated the feasibility of using a bioengineered vessel in a high pressure arterial circuit to treat PAD. There were no evidence of vessel rejection or spontaneous vessel degradation, and the HAV functioned as intended as a vascular bypass conduit.

CONCLUSIONS

In this phase II study, the HAV functioned as intended as a peripheral arterial bypass conduit. The vessel successfully restored in-line blood flow to the distal extremity and foot, and in doing so led to objective and subjective improvements in patients suffering from chronic limb ischemia secondary to PAD. All patients shared the initial benefit of symptomatic relief from rest pain and claudication and the majority of the cohort sustained improvement in ABI from baseline. There were no amputations (minor or major) of the index limb during the 24-month study period. There is histologic evidence that the HAV remodels with host cells as early as 3 months. Patency rates are within the ranges of patency rates of synthetic and autologous venous grafts presented in the literature. The results support the continued development of human tissue engineered blood vessels for vascular reconstruction.

AUTHOR CONTRIBUTIONS

Conception and design: SG, RK, LN, AP, HP, BT, JL
 Analysis and interpretation: SG, RK, LN, AP, HP, BT, JL
 Data collection: PG, MG, MI, SP, RS, JT, WW, NZ, TZ
 Writing the article: PG, SG, MG, MI, AK, RK, LN, AP, HP, SP, RS, BT, JT, WW, NZ, TZ, JL
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 Final approval of the article: PG, SG, MG, MI, AK, RK, LN, AP, HP, SP, RS, BT, JT, WW, NZ, TZ, JL
 Statistical analysis: Not applicable
 Obtained funding: Not applicable
 Overall responsibility: JL

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Submitted May 29, 2019; accepted Nov 23, 2019.

Additional material for this article may be found online at www.jvascsurg.org.

DISCUSSION

Dr Ahmad Alsheekh (*Brooklyn, NY*). I would like to thank you for this interesting study and I would like to ask about these grafts. Is it tolerable to post-implantation interventions like stenting and ballooning after implanting these grafts?

Dr Jeffrey H. Lawson. So, we now have a real-world experience in a little more than 200 human acellular vessels implanted. These function as human vascular tissues, so they've now been put through the paces of regular clinical care. Initially, we babied them and didn't do a lot, but now they've been angioplastied, they've been stented, they've been revised, and they can tolerate all

that. They can tolerate thrombectomy. I did the initial thrombectomies open and we've done now percutaneous thrombectomy.

The only thing we've learned is, which is a little atypical, you know, we tend to oversize balloons in a lot of interventions. In this case, if you oversize a balloon, if it's a 6mm conduit, you can usually go 6 mm for an angioplasty in the conduit itself, 7 mm is okay at the anastomosis; but if you go bigger, you can actually disrupt the vessel. So we've had a couple of vessels that were terminally disrupted by basically a 9 mm balloon that can rip the vessel.