J ENDOVASC THER
2006;13(Suppl II):II-52–II-59

◆ CLINICAL INVESTIGATION ◆

Cryoplasty for the Treatment of Femoropopliteal Arterial Disease: Extended Follow-up Results

John R. Laird, MD[1]; Giancarlo Biamino, MD[2]; Thomas McNamara, MD[3]; Dierk Scheinert, MD[2]; Patrick Zetterlund, MD[4]; Elaine Moen, MD[5]; and James D. Joye, DO[6]

[1]Washington Hospital Center, Washington, DC, USA. [2]Universität Leipzig, Herzzentrum, Leipzig, Germany. [3]University of California Los Angeles Medical Center, Los Angeles, California, USA. [4]Salinas Valley Memorial Hospital, Salinas, California, USA. [5]St. Vincent's Hospital, Indianapolis, Indiana, USA. [6]El Camino Hospital, Mountain View, California, USA.

Purpose: To report the findings from a multicenter study of patients treated with cryoplasty who were then followed for an average of >2 years post-treatment.
Methods: Extended clinical follow-up was obtained for 70 patients (45 men; mean age 70.5±8.8 years) who originally received cryoplasty therapy to treat symptoms of intermittent claudication as part of a multicenter investigational device exemption (IDE) study. For all subjects, cryoplasty was used to treat stenoses or occlusions ≤10 cm in the femoropopliteal arteries. The original IDE study protocol enrolled 102 patients with a primary endpoint of target lesion patency at 9 months post-treatment. This collection of additional longer term follow-up data was initiated 2.5 years after the onset of study enrollment.
Results: Extended clinical follow-up ranged from 11 to 41 months (mean 31). The clinical patency rate (freedom from target lesion revascularization) calculated by the Kaplan-Meier method was 83.2% after the original follow-up period of 300 days. After >3 years (1253 days), the clinical patency rate was well maintained at 75.0%.
Conclusions: Long-term data indicate that cryoplasty is a durable therapy, with relatively low long-term restenosis rates compared to other endovascular treatment approaches.
J Endovasc Ther 2006;13(Suppl II):II-52–II-59

Key words: Peripheral arterial disease, endovascular therapy, angioplasty, femoropopliteal segment, restenosis, cryoplasty, intermittent claudication

Endovascular therapy is increasingly employed as the initial approach in treating femoropopliteal arterial disease, having been shown to result in less morbidity and mortality than bypass surgery.[1] Durability, however, has emerged as the Achilles' heel of percutaneous transluminal angioplasty (PTA) and its various adaptations.

Conventional PTA is limited by high rates of dissection, recoil, and restenosis, leading to an excessive need for reintervention.[2] Stenting has reduced the incidence of dissection

This study was supported by CryoVascular Systems, Inc.

Dr. Laird has served on the advisory boards for CryoVascular Systems, Inc. and Boston Scientific. Dr. Biamino is a paid consultant to CryoVascular Systems, Inc. Dr. Zetterlund has stock in Boston Scientific. Dr. Joye is a co-inventor of the PolarCath device and was a shareholder in CryoVascular Systems, Inc. Dr. McNamara serves on the advisory board for Boston Scientific. The other authors have no commercial, proprietary, or financial interest in any products or companies described in this article.

Address for correspondence and reprints: John R. Laird, MD, Cardiovascular Research Institute, Washington Hospital Center, 110 Irving Street NW, Suite 481, Washington, DC 20010 USA. E-mail: *John.R.Laird@medstar.net*

and recoil but has done little to improve restenosis rates.[3–6] While drug-eluting stents have improved outcomes in percutaneous treatment of coronary arteries, such encouraging results have yet to be reproduced in the peripheral vessels. Various endovascular strategies, including laser and excisional atherectomy, have proven beneficial as adjunctive therapies, particularly when used as debulking mechanisms prior to primary treatment, but have not been proven superior to PTA when employed as the initial therapy.[7,8] Clearly, there is a need for a durable minimally invasive approach to treating diseased femoropopliteal vessels.

The cryoplasty technique was designed to address the primary pitfalls of PTA, i.e., dissection, recoil, and restenosis, by combining the dilation force of balloon angioplasty with the delivery of cold thermal energy to the vessel wall. The saline and radiopaque contrast medium used to inflate the balloon in conventional angioplasty is replaced with nitrous oxide, which both dilates and cools the balloon. The application of this cooling treatment results in a benign modification of the plaque, potentially leading to several benefits: (1) altered plaque response that prevents the formation of large tears deep into the vessel wall, which can occur in conventional angioplasty and result in dissection[9]; (2) reduced vessel wall recoil due to freeze-induced alteration of the elastin fibers of the vessel[10,11]; and (3) smooth muscle cell (SMC) apoptosis (a noninflammatory form of cell death triggered by cold exposure and interstitial ice formation), which is associated with reduced neointima formation and restenosis.[12]

The prospective, nonrandomized, 16-center investigational device exemption (IDE) study of cryoplasty began enrollment in November 2001; the last of the 102 patients was treated in December 2002.[13] Study methodology, lesion characterization, and follow-up terms were chosen in accord with outcome assessment guidelines set by the TransAtlantic Inter-Society Consensus (TASC) on the recommended management of peripheral arterial disease.[14] For this trial, a 9-month endpoint was approved by the Food and Drug Administration as an appropriate follow-up period for treatment in the femoropopliteal segment.

The initial technical success rate ($\leq$30% residual angiographic stenosis and $<$50% residual narrowing by duplex) in femoropopliteal lesions $\leq$10 cm was 94.1%. Stand-alone cryoplasty success (without the need for adjunctive stenting) was achieved in 85.3%. In particular, the strongest immediate attributes of cryoplasty appear to be low rates of major dissection (6.9%) and the need for bailout stenting (8.8%) during the procedure.

Midterm results have been promising as well, with a 9-month clinical patency rate of 82.2%.[13] Only 16 patients required repeat revascularization during the study period. At 9 months, ankle-brachial indices (ABI) averaged 0.88 ± 0.16 (compared to 0.72 ± 0.17 at baseline), and 89% of patients reported improvement in claudication. These findings were consistent with the first clinical cryoplasty experience for femoropopliteal disease, in which an 83% angiographic patency rate was reported at 18 months after treatment.[15]

To better assess the long-term durability of cryoplasty therapy, the investigational sites involved in the original IDE study were invited to participate in continued surveillance of their study subjects. We present here the outcome of this extended follow-up, which started 2.5 years after enrollment began in the randomized trial. Survival free from target lesion revascularization (TLR) in the study cohort over the entire follow-up period was the primary endpoint of the analysis.

METHODS

Patient Selection in the Original Trial

The FDA-approved prospective, nonrandomized, multicenter study included 102 patients with intermittent claudication caused by femoropopliteal arterial disease.[13] To be eligible for the study, patients had Rutherford category 2 or greater intermittent claudication caused by de novo or restenotic (non-stented) lesions in the superficial femoral artery (SFA) or popliteal artery. Lesions up to 10 cm long were eligible for treatment in patients with at least 1 patent runoff vessel to the foot. The lesions were subsequently classified as TASC class A, B, or C stenoses or occlusions. Patients excluded from the study included those

J ENDOVASC THER
2006;13(Suppl II):II-52–II-59

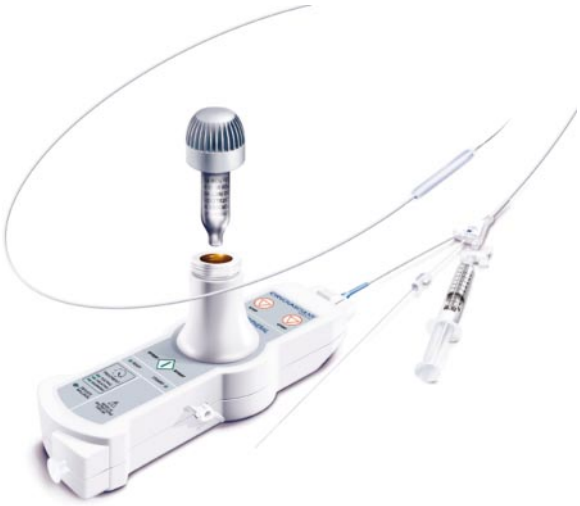

Figure 1 ◆ PolarCath Peripheral Dilatation System.

with recent myocardial infarction or stroke, serum creatinine levels >2.5 mg/dL, rest pain, or ischemic foot ulceration. The patients provided signed consent, and the protocol received institutional review board approval at all enrolling centers.

Technique

Baseline evaluation was performed for all patients who met the enrollment criteria, including resting ABI and lower extremity arterial duplex ultrasound. Patients received 325 mg/d of aspirin and 75 mg/d of clopidogrel for 4 days before treatment (or a 300-mg preprocedural loading dose); intravenous heparin or bivalirudin was administered during the procedure. Standard angiography was performed to identify the target lesion and confirm that trial criteria were met.

Cryoplasty was performed with the PolarCath Peripheral Dilatation System (CryoVascular Systems, Inc., Los Gatos, CA, USA), components of which include a catheter, a microprocessor-based inflation unit, and a nitrous oxide cylinder (Fig. 1). Using standard interventional techniques, a 0.035-inch guidewire is advanced through a 7-F sheath to the site of the lesion. The cryoplasty balloon is situated at the lesion, and pressurized liquid nitrous oxide is delivered. As it enters the balloon, the nitrous oxide undergoes phase change to a gas, resulting in balloon expansion. The balloon is inflated in 2-atmosphere increments until the nominal dilation force of 8 atmospheres is achieved. The temperature on the surface of the balloon is reduced to −10°C. The treatment cycle lasts for 20 seconds, after which the balloon is passively warmed and then deflated; thereafter, it is either removed or repositioned.

Post-treatment, patients received 30 days of clopidogrel and were encouraged to take aspirin indefinitely. ABI measurement was performed within 24 hours, and a lower extremity arterial duplex scan was completed within 7 days of treatment. Patients returned for clinical assessment, ABI measurement, and duplex ultrasound at 3 and 9 months.

Endpoints and Statistical Analysis

The primary endpoint of the extended follow-up protocol was target lesion primary patency, defined as the absence of clinically driven repeat revascularization of the target lesion or the need for surgical bypass grafting or amputation due to reocclusion of the target lesion as diagnosed by arteriography or duplex scan. Follow-up information was obtained via office visits, telephone interviews, or review of medical records. The Kaplan-Meier method was employed to calculate survival free from TLR over the entire follow-up period.

RESULTS

Extended follow-up information was ultimately obtained for 70 patients (45 men; mean age 70.5±8.8 years) from the 7 highest-enrolling investigational sites. Of the 80 patients originally enrolled at these 7 sites, 4 died of unrelated causes and the remaining 6 patients either could not be located for follow-up or refused to participate. Follow-up ranged from 11 to 41 months (mean 31) post-treatment.

Comorbidities and baseline lesion characteristics in the initial IDE study cohort and the extended follow-up group were similar. Risk factors in the follow-up group included coronary artery disease (64.3%), hypertension (87.1%), hyperlipidemia (82.9%), and history of smoking (72.9%). Target lesions were located mainly in the SFA (84.3%); 15.7% were

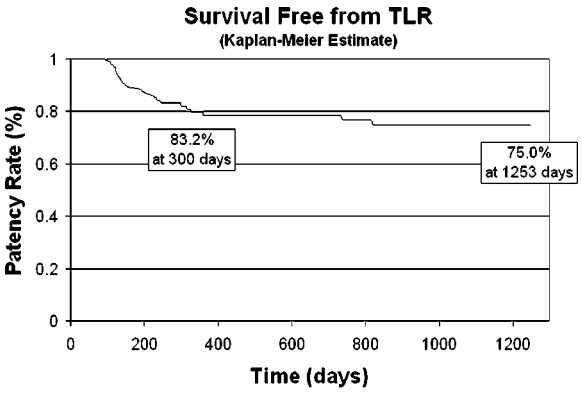

Figure 2 ◆ Survival free from target lesion revascularization in 70 patients followed for >3 years after cryotherapy in the femoropopliteal segment.

in the popliteal artery. The majority of lesions were de novo (84.3%); only 15.7% were restenotic. At baseline, 9 (12.9%) lesions were totally occluded. Target lesion stenoses at baseline ranged from 65% to 100% (mean 87.1%±9.1%), and lesion lengths ranged from 2.0 to 10.0 cm (mean 4.8±2.7) for the extended follow-up group, similar to the 4.7-cm average lesion length in the initial 102-patient study group. The distribution of TASC A, B, and C lesions in the extended follow-up group was also similar to the distribution in the original study group and included a large percentage of complex or TASC C lesions (42.9%). The remainder were TASC A (35.7%) or TASC B (21.4%).

Acute outcomes for the 70 follow-up patients were similar to those in the initial patient group; the post-treatment residual stenosis ranged from 0% to 40% in the target lesion (mean 10.7%±10.4%), and 5 (7.1%) lesions required stents. The procedural success rate was 92.9%, and the stand-alone technical success rate was 85.7%.

Utilizing the Kaplan-Meier method, the clinical patency rate (defined as freedom from TLR) was 83.2% after the original follow-up of >9 months (300 days) and 75.0% after 3.4 years (1253 days; Fig. 2).

Case Studies

The first patient was an 81-year-old man with a history of smoking, coronary artery disease, hypertension, and hyperlipidemia who

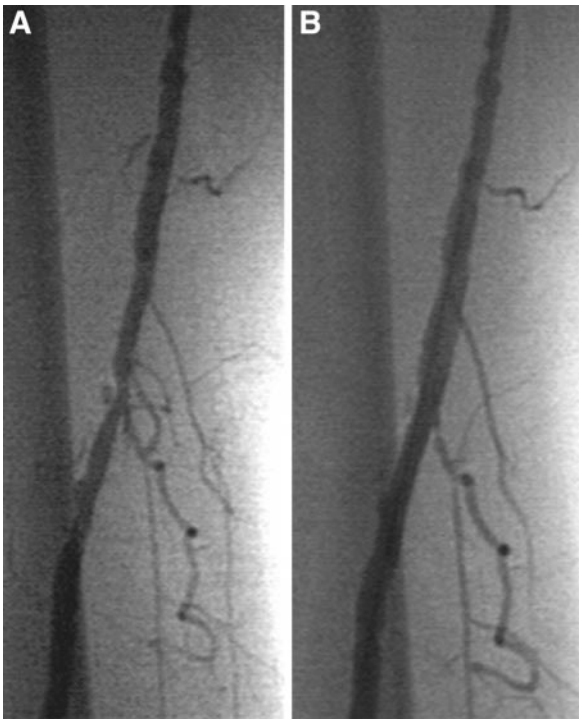

Figure 3 ◆ In the first case (**A**), a 6-cm lesion with up to 85% stenosis in the right SFA was treated with cryotherapy. After treatment (**B**), there was no residual stenosis at the treatment site.

presented with bilateral lower limb claudication. A clinical examination confirmed exercise-induced claudication in both limbs. Angiography revealed a 6-cm lesion with up to 85% stenosis in the right SFA (Fig. 3A). The lesion was dilated with a 5×20-mm PolarCath catheter followed by 2 dilations with a 6×40-mm PolarCath catheter. Acute angiographic results revealed 0% residual stenosis at the treatment site (Fig. 3B) and improved distal runoff. At 3- and 9-month follow-up, the patient reported improvement in claudication. Long-term clinical evaluation performed at 41.1 months after the procedure found a resting ABI of 1.0 in the target limb. No surgical or interventional procedures had been performed in the target vessel since the time of the index procedure. The patient reported improvement in claudication, with exercise limited only by shortness of breath.

In the second case, a 52-year-old man with a history of smoking, hypertension, and hyperlipidemia presented with right lower limb

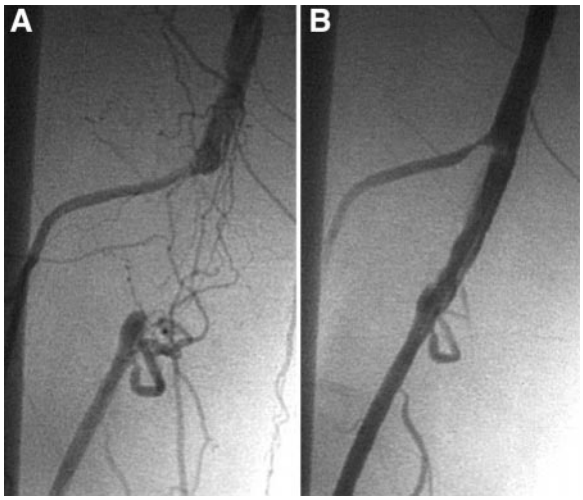

Figure 4 ◆ In the second case example (**A**), a 6-cm-long total occlusion in the right SFA was treated with the PolarCath catheter. Post-treatment angiography (**B**) revealed 20% residual stenosis.

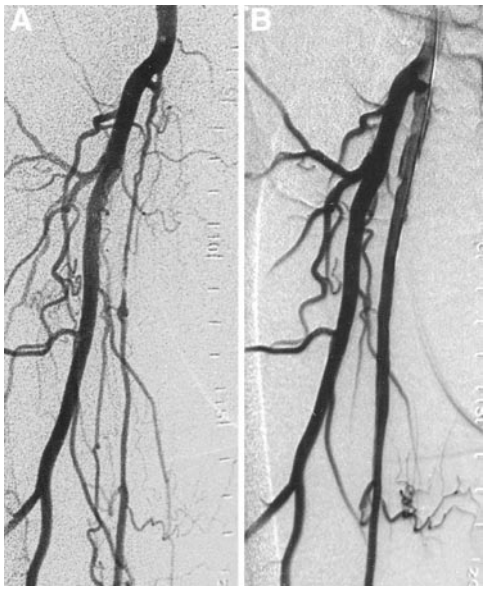

Figure 5 ◆ In the last case (**A**), a 10-cm diffusely diseased arterial segment with areas of 80% stenosis was treated with cryotherapy. Completion angiography (**B**) showed 0% residual stenosis and a type B dissection.

claudication. The resting ABI in the right leg was 0.64. During the index procedure, a 6-cm-long total occlusion was identified in the right SFA (Fig. 4A). The lesion was dilated with 2 inflations of a 5×40-mm PolarCath catheter and 3 inflations of a 6×20-mm PolarCath catheter. Post-treatment angiography (Fig. 4B) revealed 20% residual stenosis with improved distal runoff; a resting ABI of 0.93 was measured prior to discharge. The target limb ABI was 1.1 at 3 months and 1.0 at 9 months post-treatment, and the patient reported complete elimination of claudication. At 29.2 months after the index treatment, the resting ABI was >1.0 in the right leg, and the patient reported maintained improvement in symptoms of claudication. No secondary surgical or interventional procedures had been performed in the target vessel since the time of the initial treatment.

In the final example, a 77-year-old female smoker with a history of coronary artery disease, hypertension, and hyperlipidemia presented with bilateral lower limb claudication. The right leg ABI was 0.51. The clinical exam confirmed claudication in the right leg after 3 minutes of exercise. During the index procedure, a 10-cm diffusely diseased arterial segment with areas of 80% stenosis was identified in the right SFA (Fig. 5A). The lesion was dilated with 3 inflations of a 5×40-mm PolarCath catheter. Post-treatment angiography showed 0% residual stenosis and a type B dissection (Fig. 5B). No further treatment was performed. At 3- and 9-month follow-up, the patient's claudication had resolved. At 26.5 months, the resting ABI was 0.75 in the right leg, and the patient reported continued freedom from claudication. No TLR was reported.

DISCUSSION

Peripheral arterial disease (PAD) is a relentless, progressive disease that affects the entire vasculature, making treatment challenging. Nowhere is this more apparent than in the femoropopliteal vessels, where diffuse disease and calcification are very common, progression to total occlusion is a frequent occurrence, and co-existent disease of the infrapopliteal runoff vessels negatively impacts long-term outcomes following any intervention. PTA results have been suboptimal in this vascular bed, and the long-term results with stents have been disappointing. Femoropopliteal bypass surgery has been considered the

most durable revascularization strategy for femoropopliteal disease, but this procedure is associated with significant morbidity for the patient and may be best reserved as the final revascularization option. As applied to targeted treatment of stenotic or occluded lesions in the femoropopliteal vessels, the optimal treatment strategy would start with the least invasive modality and would seek to delay more invasive measures as long as possible.

Using this approach of a ''phased revascularization'' or a step-wise treatment strategy, angioplasty or cryoplasty would qualify as the least invasive approaches in the continuum of treatment that includes stenting, bypass surgery, and amputation. Based on the outcomes of this study and prior studies, the advantages of cryoplasty over conventional PTA include lower dissection rates, less frequent bailout stent placement, and improved long-term vessel patency. Stenting may be used provisionally to address dissection and recoil but may not be desirable as a first-line therapy for a number of reasons. Due to the compression, torsion, and flexion forces that the femoropopliteal arteries are subject to, stents can fracture, potentially leading to aggressive restenosis.[16,17] Furthermore, treatment of instent restenosis is difficult, and the placement of stents in the femoropopliteal arteries can complicate future bypass surgery.

Failing interventional therapies, limbs would subsequently be treated with surgical bypass or amputation. In addition to higher morbidity and mortality rates than minimally invasive therapy, bypass surgery is also problematic because most PAD patients also have coronary artery disease, and graft vessels often must be reserved for potential coronary bypass. Moreover, many PAD patients are unsuitable surgical candidates. Ultimately, the progression of PAD leads to critical limb ischemia and finally amputation.

Numerous strategies have been developed to create a durable minimally invasive approach. Many percutaneous treatments that initially appeared promising have proven disappointing once mid and long-term follow-up was completed. Cryoplasty has been shown to produce excellent acute and midterm results, and the extended follow-up results presented here confirm that this approach delivers a long-term benefit in the maintenance of vessel patency.

Cryoplasty is differentiated from other interventional methods by its unique combination of biological and mechanical effects on the vessel wall, including an alteration in the plaque response to dilation, a reduction in elastic recoil post-dilation, and the induction of SMC apoptosis. During the cryoplasty procedure, freezing induces nonuniform volume changes in liquid and solid elements in the plaque and tissue, resulting in the accumulation of thermal stresses.[18] These internal stresses are believed to cause microfractures in the plaque, thereby weakening it and allowing more uniform dilation.[19,20] Additionally, during conventional PTA, the difference in the distensibility or elasticity of plaque compared to adjacent normal arterial tissue causes high tensile stresses to occur at junctions between plaque and the normal wall.[21,22] This frequently causes dissections involving extensive medial tears and separation of plaque from the underlying artery wall, with acute obstruction of the vessel.[23] Freezing produces more homogenous mechanical properties within the plaque and tissue, resulting in more uniform distribution of stress during dilation.[24] These effects are believed to reduce the frequency and severity of dissection during cryoplasty. Dissection is one of the major acute failure modes of PTA, contributing to a high rate of provisional stent placement. Published reports also indicate that severe dissection may initiate an aggressive restenosis response.[25,26]

Elastic recoil represents another acute failure mode of conventional PTA, particularly in vessels with high elastin content.[27] Prior work has established that ice formation in arterial tissue produces an acute alteration in elastin fibers, described histologically as fragmentation of elastin fibers and uncoiling of elastic layers.[10,11] These changes are believed to reduce vessel wall elasticity and elastic recoil following cryoplasty. By reducing both dissection and elastic recoil, cryoplasty reduces the need for provisional stenting.

Another effect of cryoplasty is the induction of SMC apoptosis. During the cryoplasty treatment, ice nucleates first in the tissue adjacent to the balloon and grows radially out-

J ENDOVASC THER
2006;13(Suppl II):II-52–II-59

ward. Because ice does not incorporate solutes, as the ice field grows, it ejects solutes into the remaining unfrozen fraction, creating a hypertonic environment.[28] The SMCs in the frozen region therefore undergo osmotic dehydration, followed by rehydration upon thawing.[28] This exposure to dehydration and rehydration causes sublethal damage to the cells and initiates the apoptotic sequence.[29–32] Prior research has proven that cold treatment triggers the genetic signal for apoptosis in arterial SMCs.[12] It has been postulated that elevated apoptosis of arterial SMCs may yield a reduction in neointima formation and restenosis.[33]

The mounting evidence suggests that cryoplasty is an appropriate first step on the interventional treatment path, combining the low morbidity of PTA with significantly improved durability. As such, cryoplasty may be able to provide many PAD patients with longer term benefit, preventing or delaying treatment with stents, bypass surgery, and amputation. This evidence suggests that cryoplasty may open up new treatment options for patients who typically are medically managed until their symptoms progress to late stages, by which time fewer treatment options are available. As a durable minimally invasive treatment, cryoplasty can be offered to patients sooner, potentially delaying or preventing progression to chronic limb ischemia and amputation. Furthermore, cryoplasty is a no-harm therapy that can be repeated, yet leaves open the possibility of future treatment with other therapies should restenosis occur.

Study Limitations

The long-term surveillance of study subjects was limited by the lack of duplex ultrasound evaluations or core laboratory oversight as included in the original IDE study. The assessment of freedom from TLR provides a clinical endpoint that demonstrates the ultimate success of cryoplasty in delaying or preventing additional treatment. This endpoint has its limitations, however, and it may not accurately reflect vessel patency at the treatment site as would otherwise be assessed by duplex ultrasound. The Kaplan-Meier method, which allows continuous adjustment of sample size to calculate survival estimations, was selected as the most appropriate statistical method to assess TLR rates since the length of follow-up varied for subjects in the long-term surveillance group. The TLR endpoint is based on reporting of an important hard clinical event that provides good representation of the status of the patient and the durability of the cryoplasty result.

Conclusion

These long-term data demonstrate that cryoplasty provides lasting results in the often-challenging treatment of PAD of the femoropopliteal arteries. The durability and low incidence of morbidity indicate that cryoplasty should be considered early in the treatment spectrum, potentially preventing or delaying stenting, bypass surgery, and amputation. Ultimately, the goal in treating atherosclerotic femoropopliteal vessels is the patient's improved health and quality of life, and freedom from target lesion revascularization is a primary step in ensuring that result.

REFERENCES

1. Hunink MG, Wong JB, Donaldson MC, et al. Revascularization for femoropopliteal disease. A decision and cost-effectiveness analysis. *JAMA.* 1995;274:165–171.
2. Kandarpa K, Becker GJ, Hunink MG, et al. Transcatheter interventions for the treatment of peripheral atherosclerotic lesions: part I. *J Vasc Interv Radiol.* 2001;12:683–695.
3. Hayerizadeh BF, Zeller T, Krankenberg H, et al. Superficial femoral artery stenting using nitinol stents–a German multicenter experience. *J Invasive Cardiol.* 2004;16:12A–14A.
4. Mewissen MW. Self-expanding nitinol stents in the femoropopliteal segment: technique and mid-term results. *Tech Vasc Interv Radiol.* 2004; 7:2–5.
5. Muradin GS, Bosch JL, Stijnen T, et al. Balloon dilation and stent implantation for treatment of femoropopliteal arterial disease: meta-analysis. *Radiology.* 2001;221:137–145.
6. Do DD, Triller J, Walpoth BH, et al. A comparison study of self-expandable stents versus balloon angioplasty alone in femoropopliteal artery occlusions. *Cardiovasc Intervent Radiol.* 1992;15:306–312.
7. Tielbeek AV, Vroegindeweij D, Buth J, et al.

Comparison of balloon angioplasty and Simpson atherectomy for lesions in the femoropopliteal artery: angiographic and clinical results of a prospective randomized trial. *J Vasc Interv Radiol.* 1996;7:837–844.
8. Steinkamp HJ, Wissgott C, Rademaker J, et al. Short (1–10 cm) superficial femoral artery occlusions: results of treatment with excimer laser angioplasty. *Cardiovasc Intervent Radiol.* 2002;25:388–396.
9. Zorger N, Manke C, Lenhart M. Peripheral arterial balloon angioplasty: effect of short versus long balloon inflation times on the morphologic results. *J Vasc Interv Radiol.* 2002;13: 355–359.
10. Gage A, Fazekas G, Riley E. Freezing injury to large blood vessels in dogs. *Surgery.* 1967;61: 748–754.
11. Mandeville AF, McCabe BF. Some observations on the cryobiology of blood vessels. *Laryngoscope.* 1967;77:1328–1350.
12. Tatsutani KN, Joye JD, Virmani R, et al. In vitro evaluation of vascular endothelial and smooth muscle cell survival and apoptosis in response to hypothermia and freezing. *Cryo Letters.* 2005;26:55–64.
13. Laird J, Jaff MR, Biamino G, et al. Cryoplasty for the treatment of femoropopliteal arterial disease: results of a prospective, multicenter registry. *J Vasc Interv Radiol.* 2005;16:1067–1073.
14. Dormandy JA, Rutherford RB. Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Consensus (TASC). *J Vasc Surg.* 2000;31:S1–S296.
15. Fava M, Loyola S, Polydorou A, et al. Cryoplasty for femoropopliteal arterial disease: late angiographic results of initial human experience. *J Vasc Interv Radiol.* 2004;15:1239–1243.
16. Kröger K, Santosa F, Goyen M. Biomechanical incompatibility of popliteal stent placement. *J Endovasc Ther.* 2004;11:686–694.
17. Scheinert D, Scheinert S, Sax J, et al. Prevalence and clinical impact of stent fractures after femoropopliteal stenting. *J Am Coll Cardiol.* 2005;45:312–315.
18. Shi X, Datta AK, Mukherjee Y. Thermal stresses from large volumetric expansion during freezing of biomaterials. *J Biomech Eng.* 1998;120: 720–726.
19. Rabin Y, Olson P, Taylor MJ, et al. Gross damage accumulation in frozen rabbit liver due to mechanical stress at cryogenic temperatures. *Cryobiology.* 1997;34:394–405.
20. Rabin Y, Taylor MJ, Wolmark N. Thermal expansion measurements of frozen biological tissues at cryogenic temperatures. *J Biomech Eng.* 1998;120:259–266.
21. Giannattasio C, Failla M, Emanuelli G, et al. Local effects of atherosclerotic plaque on arterial distensibility. *Hypertension.* 2001;38:1177–1180.
22. Richardson PD, Davies MJ, Born GV. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet.* 1989;2:941–944.
23. Fitzgerald PJ, Ports TA, Yock PG. Contribution of localized calcium deposits to dissection after angioplasty. *Circulation.* 1992;86:64–70.
24. Zhang A, Cheng S, Lei D, et al. Experimental study of mechanical behavior of frozen arteries at low temperatures. *Cryo Letters.* 2002;23: 389–396.
25. Nobuyoshi M, Kimura T, Ohishi H, et al. Restenosis after percutaneous transluminal coronary angioplasty: pathologic observation in 20 patients. *J Am Coll Cardiol.* 1991;17:433–439.
26. Ip JH, Fuster V, Badimon L, et al. Syndromes of accelerated atherosclerosis: role of vascular injury and smooth muscle cell proliferation. *J Am Coll Cardiol.* 1990;15:1667–1687.
27. Gardiner GA, Bonn J, Sullivan KL. Quantification of elastic recoil after balloon angioplasty in the iliac arteries. *J Vasc Interv Radiol.* 2001; 12:1389–1393.
28. Mazur P. Cryobiology: the freezing of biological systems. *Science.* 1970;168:939–949.
29. Baust JM. Molecular mechanisms of cellular demise associated with cryopreservation failure. *Cell Preserv Technol.* 2002;1:17–32.
30. Hanai A, Yang W, Ravikumar TS. Induction of apoptosis in human colon carcinoma cells HT29 by sublethal cryo-injury: mediation by cytochrome C release. *Int J Cancer.* 2001;93:526–533.
31. Hollister WR, Mathew AJ, Baust JG, et al. Effects of freezing on cell viability and mechanisms of cell death in a human prostate cancer cell line. *Mol Urol.* 1998;2:13–18.
32. Nagle WA, Soloff BL, Moss AJ, et al. Cultured Chinese hamster cells undergo apoptosis after exposure to cold but nonfreezing temperatures. *Cryobiology.* 1990;27:439–451.
33. Isner JM, Kearney M, Bortman S, et al. Apoptosis in human atherosclerosis and restenosis. *Circulation.* 1995;91:2703–2711.