Percutaneous Pulmonary Endoarterial Biopsy in an Experimental Model of Pulmonary Hypertension*

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Study objectives: **The aims of this study were: to evaluate the performance of a novel arterial biopsy catheter in obtaining pulmonary endovascular samples in hypertensive dogs; to compare the results of pulmonary endoarterial biopsy in hypertensive vs normotensive dogs; and to assess the histologic changes in the hypertensive model.**

Design and interventions: **Thirty-four dogs (27 with normal pulmonary arterial pressures and seven with pulmonary hypertension) were catheterized through an external jugular vein to obtain endovascular biopsy samples from distal pulmonary arteries 2 to 3 mm in luminal diameter. To induce pulmonary hypertension, seven dogs were given repeated infusions of 0.6- to 0.9-mm ceramic microspheres into the superior vena cava. Endoarterial samples were obtained at pulmonary systolic arterial pressures ranging from 10 to 110 mm Hg.**

Measurements and results: **Sixty-two biopsy catheterization procedures were performed in the 34 dogs. After 12 initial procedures of technique refinement, endoarterial samples were obtained in each of the last 50 procedures (21 in normotensive dogs and 29 in hypertensive dogs). The average number of endovascular biopsy samples retrieved was 7.1 (range, 2 to 12) from a mean of 8.6 (range, 2 to 15) biopsy attempts per catheterization (success rate=83%). The average biopsy piece measured 1.13 mm in length, 0.33 mm in depth, and up to 1.0 mm in width. The biopsy success rates and endoarterial sample sizes were similar in normotensive and hypertensive dogs. Smooth muscle cells and endothelial cells were grown from the biopsy samples. There were no significant procedural complications, except for one self-limited hemorrhage. Histologically, samples obtained from dogs with pulmonary hypertension showed characteristic changes when compared with biopsies from normotensive dogs.**

Conclusion: **This new endoarterial biopsy catheter was safe and effective when used to obtain pulmonary endoarterial samples in dogs with normal and experimentally elevated pulmonary arterial pressures. The quality and quantity of the biopsy samples allowed identification of pathologic changes.** ***(CHEST 1998; 114:241-250)***

Key words: endoarterial biopsy catheter; pulmonary artery; pulmonary hypertension

Pulmonary vascular diseases are poorly understood, difficult to diagnose, and frequently associated with significant morbidity and mortality because of imperfect therapeutic options.[1-8] Part of the limitation in the study, diagnosis, and management of pulmonary arterial diseases has been the inaccessibility of pulmonary vascular tissue for analysis. We tested the safety and efficacy of a novel endoarterial biopsy catheter in obtaining endovascular biopsy samples from distal pulmonary arteries 2 to 3 mm in luminal diameter. Our initial results in normotensive dogs have been published previously.[9] In this study, we describe our entire experience, including a comparison of results in dogs with normotension and chronic pulmonary hypertension induced by repeated infusions of ceramic microspheres into the pulmonary vascular bed.

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Materials and Methods

Study Protocol

The normotensive and hypertensive study protocols were approved by the Animal Subjects Committee of the University of California San Diego, and the procedures followed were in accordance with institutional guidelines. The catheterization procedure for each of the 34 mongrel dogs (weight, 20 to 30 kg) included 10 mg/kg of propofol IV, intubation, ventilation at a rate of 12 to 18 breaths/min, and continuous anesthesia with 1.5 to 2.0% isofluorane. In each dog, a femoral arterial line was placed for monitoring. An 8F thermodilution catheter (Baxter-Edwards; Irvine, Calif) was advanced through an external jugular vein sheath to a branch pulmonary artery, where pressures and cardiac outputs were obtained.

Among the 27 normotensive dogs, procedures in the first 12 constituted our initial experience with and refinement of the biopsy technique. Of the next 15 dogs, seven were included in our previous manuscript[9] and the other eight are included in this study. These 15 dogs underwent a total of 21 biopsy procedures (one biopsy procedure each in 14 dogs, and seven separate biopsy procedures in one dog).

Hypertension Model

To develop the chronic pulmonary hypertension model, 0.6- to 0.9-mm ceramic microspheres were delivered approximately once a month through an 11F sheath placed in the external jugular vein in each of seven dogs. The first two dogs died acutely with pulmonary edema and low (<50 mm Hg) systemic Po_2 levels. Microsphere infusions were given to the other five dogs at each session until the Po_2 in arterial blood gas samples decreased to 60 to 70 mm Hg. Twenty-nine biopsy procedures were performed in these five dogs. Endoarterial biopsy sampling was performed immediately after the microsphere infusions, because that was when the animals developed the highest pulmonary arterial pressures. Biopsies were also performed sequentially as chronic pulmonary hypertension developed in order to observe the chronic endovascular changes. Significant pulmonary hypertension, defined as pulmonary vascular resistance at least twice the normal level, developed 4 to 6 months after the initiation of microsphere infusions. Between catheterization procedures, the dogs were monitored daily with an assessment of vital signs, oral intake, and urine and stool output.

Biopsy Procedure

The biopsy procedure was described previously.[9] The biopsy catheter has an external diameter of 2.5 mm (7.5F) and is composed of two flexible polymeric tubes that slide relative to each other. The inner tube has a stainless steel tip with a beveled opening designed to accommodate arterial tissue. The outer tube terminates in a stainless steel cutting tube. To obtain the biopsy sample, a vacuum is transmitted to the beveled opening of the inner tube, causing a tissue sample to be drawn in. The outer tube is then advanced over the inner tube, thereby severing the tissue sample. In this study, the source of the vacuum was a two-stage rotary vane vacuum pump (Dayton Electrical Mfg Co; Chicago, Ill), which could achieve an ultimate vacuum of 29.9 inches Hg. To obtain pulmonary endoarterial samples reliably, a differential of at least 1.5 inches Hg in vacuum (open compared to closed beveled window) was required.

To perform the biopsy procedure, a long 8F sheath was wedged in a distal, pulmonary artery branch that was 2 to 3 mm in diameter and multiple biopsy samples were obtained. After each biopsy, the catheter was removed, the tissue specimen was placed in formalin for histologic examination or in growth media for cell culture, and the sheath was flushed. After the biopsy sampling, a repeat angiogram was performed to assess the degree of vascular injury, including the presence of thrombus, vascular occlusion, extravasation of contrast material, or aneurysm formation. A follow-up angiogram was performed 1 month after the biopsy procedure.

Pathology

Among the normotensive animals, five dogs were euthanized immediately after one biopsy procedure to study the acute effects of biopsy. Four dogs (two at 2 weeks and two at 8 weeks after the biopsy procedure) underwent repeat angiography, followed by euthanasia and pathologic examination, to study the chronic effects of biopsy. Three hypertensive dogs were euthanized: one immediately after one biopsy (systolic pulmonary artery pressure=100 mm Hg), the second 6 months after one biopsy, and the third 32 months after initiation of microsphere infusions and 10 separate biopsy procedures. Both the normotensive and hypertensive pulmonary arteries were subjected to gross and microscopic pathologic examination.

Gross pathology

The surface regions of the lung from which biopsy samples were obtained were examined for evidence of external injury. The pulmonary arterial tree, from the main pulmonary artery to the small peripheral arteries in all lobes, was dissected and examined. Cross-sections of arteries at the sites of biopsy were obtained and stained with hematoxylin-eosin and with a combination Masson trichrome/modified Verhoeff-van Gieson elastic stain.

Histology

Biopsy samples were immediately fixed in formalin, oriented by a skilled technologist using 4× magnification to ensure placement of the specimens on edge, routinely processed, and embedded in paraffin so that resulting sections showed the longest endothelial surface and deepest extent of tissue. Standard 5-μm sections were stained with hematoxylin-eosin, a combination Masson trichrome/modified Verhoeff-van Gieson elastic stain, or immunoperoxidase stain with antibodies to smooth muscle actin or von Willebrand factor. The endovascular biopsies were evaluated by a pathologist (CAB) familiar with the changes of pulmonary hypertension. The length and depth of each biopsy section were measured on the slide using a calibrated ocular micrometer. The width was estimated by gross examination of the specimens before embedding. Endothelial thickness was quantified by measuring at least one but not more than five areas of well-oriented biopsy pieces. Data are presented as mean±SEM. Immunoperoxidase stains were assessed quantitatively as to the type of cell and pattern of staining, and assessed semiquantitatively as negative, weakly positive, or strongly positive.

Results

Overall

The 34 dogs (27 normotensive, seven hypertensive) underwent 62 catheterizations for pulmonary endoarterial biopsies. The first 12 procedures con-

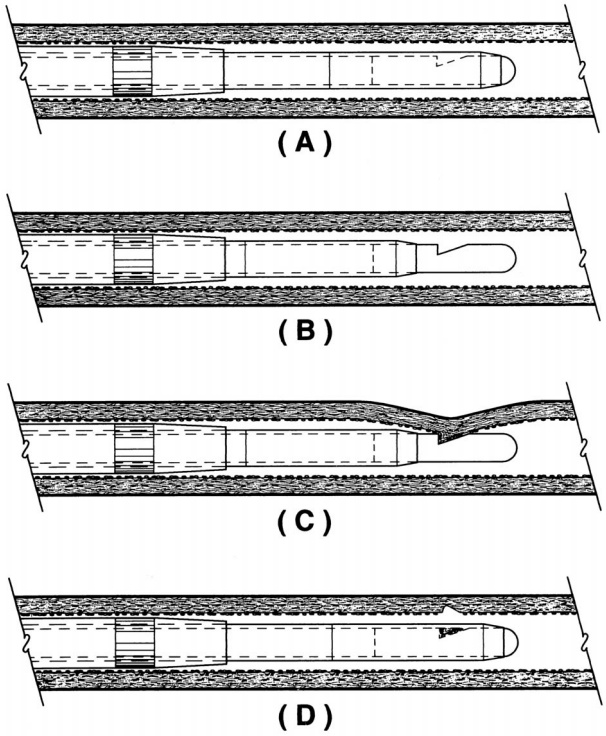

FIGURE 1. Schematic sequence of the biopsy procedure. (*A*) the distal end of the catheter is shown extending from the distal end of the radiopaque-tipped introducer sheath, with the outer tube covering the beveled opening of the inner tube. (*B*) the outer tube is retracted, exposing the inner tube. (*C*) on vacuum transmission through the inner tube, an endoarterial tissue sample is drawn into the beveled opening. (*D*) the outer tube is advanced over the inner tube, severing the tissue sample. (Reprinted with permission from Rothman et al.[9])

stituted our refinement of this new method. At each of the next 50 procedures (21 in normotensive dogs and 29 in hypertensive dogs), endovascular tissue was obtained at each biopsy procedure (Fig 1, 2). The average number of biopsy samples obtained at each catheterization was 7.1 out of a mean of 8.6 attempts (success rate=83%). In 47 procedures, the biopsy samples were composed solely of endoarterial tissue. In three procedures, early in our experience, the biopsy samples were composed predominantly of thrombus mixed with thin layers of endovascular tissue.

Normotensive Dogs

In the 21 biopsy procedures performed on normotensive dogs, the average number of endovascular tissue samples retrieved at each catheterization was 6.9 out of a mean of 7.9 attempts per procedure (success rate=88%).

Pulmonary Hypertension Model

Five dogs received multiple microsphere infusions and underwent several endoarterial biopsy procedures. The average pulmonary vascular resistance (measured in dynes · sec · cm^{-5}) increased from 193 at baseline to 617 after the first microsphere infusion. One month later, the pulmonary vascular resistance decreased to 205, increasing to 645 immediately after the second microsphere infusion. The pulmonary vascular resistance, measured before further microsphere infusions was 543 at 9 months and 665 after 12 months. The highest acute pulmonary vascular resistance was 1,209. Lung perfusion scans showed major areas of hypoperfusion after each microsphere infusion, partial recovery of flow between microsphere infusions, and larger and more persistent perfusion deficits after multiple microsphere infusions.

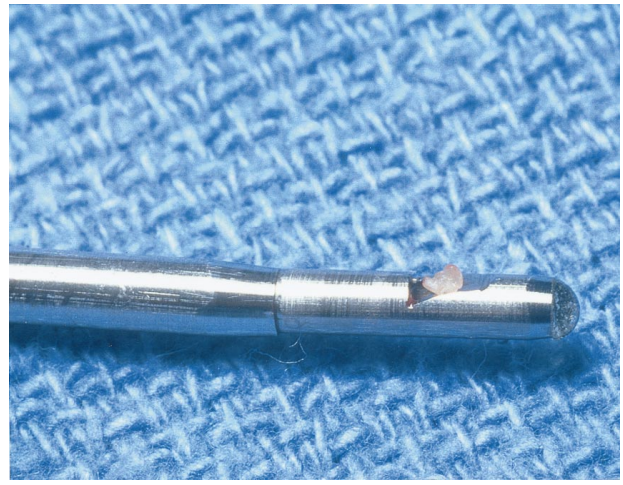

FIGURE 2. Freshly obtained biopsy sample. After the biopsy procedure, removal of the biopsy catheter, and retraction of the outer catheter tube, a pulmonary endovascular sample is maintained in the beveled opening of the inner catheter tube (×6 magnification).

During the 29 biopsy procedures performed on hypertensive dogs, an average of 7.3 endoarterial samples were obtained out of a mean of 9.2 attempts per procedure (success rate=79%). The success rates of the biopsy procedure at various levels of pulmonary arterial pressure are depicted in Table 1. Excluding two biopsy catheterization procedures in

Table 1—*Biopsy Success Rates at Different Systolic Pulmonary Arterial Pressures*

Systolic Pulmonary Arterial Pressure, mm Hg	No. of Attempts	No. of Samples	Success Rate (%)
<25	165	145	88
25-49	138	100	72
50-79	26	25	95
80-110	102	86	84

which there were technical difficulties in obtaining biopsy samples, the success rate in the hypertensive dogs was 86% (198/231).

Clinical Effects of the Biopsy Procedure

Passage of the stiff guidewire and the long Mullins sheath to a peripheral pulmonary artery occasionally resulted in transient mild hypotension or nonsustained ventricular ectopy. Upon removal of the stiff guidewire and the dilator of the long sheath, hemodynamics returned to baseline. Passage of the endoarterial biopsy catheter through the long sheath into a distal pulmonary artery branch, the biopsy procedure itself, and removal of the biopsy catheter inside the long sheath to remove the biopsy sample did not cause hemodynamic or cardiac rhythm changes. Upon completion of the biopsy procedures, the dogs recovered from anesthesia without complications. There were no episodes of sustained cough, tachypnea, or significant respiratory distress immediately after the procedure or during the follow-up period.

Angiography

Pre- and postbiopsy angiograms were performed during every procedure. A total of 90 vessels (46 in normotensive dogs and 44 in hypertensive dogs) had adequate-quality angiograms for analysis. Among the 46 normotensive arteries imaged during 21 procedures, angiograms performed immediately after the biopsy showed no hemorrhage or vascular occlusions. Seven arteries had a thin layer of persistent contrast in the outline of the vessel, which we refer to as vascular cuffing. Among the 44 hypertensive arteries imaged during 29 procedures, postbiopsy angiograms showed vascular cuffing in one, a self-limited hemorrhage in one, and complete occlusion in seven vessels. However, at a follow-up catheterization 1 month later, the vascular cuffing and each of the occlusions had resolved and the hemorrhage was not evident on angiography. Irregularities of vascular contour were observed frequently on angiography immediately after biopsy, but the vascular contour became uniform and no aneurysms were seen at follow-up in either the normotensive or hypertensive dogs. Therefore, the incidence of vascular changes was 15% in normotensive vessels (7 of 46) and 20% in hypertensive vessels (9 of 44). The incidence rates did not differ significantly.

We compared the incidence of postbiopsy vascular changes in single arteries that had undergone more than seven biopsies with the incidence in arteries that had been subjected to seven or fewer biopsies at one catheterization. In the 34 vessels subjected to more than seven biopsies, 11 (32%) showed significant vascular changes; including cuffing in six, hemorrhage in one (after 13 attempts at the same vessel), and occlusion in four. Among the 56 vessels subjected to seven or fewer biopsies, 5 (9%) demonstrated abnormalities; including vascular cuffing in two, occlusion in three, and no hemorrhages ($p<0.001$).

Gross Pathology

At necropsy immediately after the biopsy procedure in five normotensive dogs and one hypertensive dog, the lungs showed no surface evidence of injury or blood in the pleural spaces. Examination of the luminal surface of the arteries subjected to biopsies revealed small indentations or endovascular flaps, separated from 1 to 2 mm to several centimeters. There were no thrombi or aneurysms. Occasionally, the perivascular space demonstrated limited hemorrhage.

Necropsy of four normotensive dogs (two at 2 weeks, two at 2 months) and two hypertensive dogs (one 6 months after biopsy, one 32 months after multiple biopsies) showed no external surface injury, no indentations or vascular flaps on the luminal surface of biopsied vessels, and no thrombi, aneurysms, or perivascular hemorrhage. The hypertensive dog that was sacrificed at 32 months was the one that had the self-limited hemorrhage 12 months earlier. In this animal, there was no evidence of extravascular blood or aneurysm formation at the time of necropsy.

The normotensive dogs that were not euthanized either were subjected to microsphere infusions and became hypertensive, or were subsequently used in separate unrelated experiments by other investigators in the laboratory. The two hypertensive dogs that were not euthanized are still alive, doing well clinically, and being studied for long-term hypertensive changes.

Histology

The average biopsy sample size measured on the slides from the normotensive specimens was 1.18 ± 0.54 mm (range, 0.23 to 2.3 mm) in length by 0.29 ± 0.23 mm (range, 0.06 to 0.99 mm) in depth. In the hypertensive specimens, the average section size of the biopsy samples was 1.10 ± 0.80 mm (range, 0.1 to 4.0 mm) in length by 0.38 ± 0.29 mm (range, 0.04 to 1.2 mm) in depth ($p=$not significant).

Systematic analysis for the presence of endothelium was performed in biopsy sample sections from 20 normotensive and 25 hypertensive dogs. Clearly identifiable endothelium was observed in tissue samples from 14 of 20 normotensive dogs (70%) and 17 of 25 hypertensive dogs (68%; $p=$not significant).

The biopsy samples from the hypertensive dogs

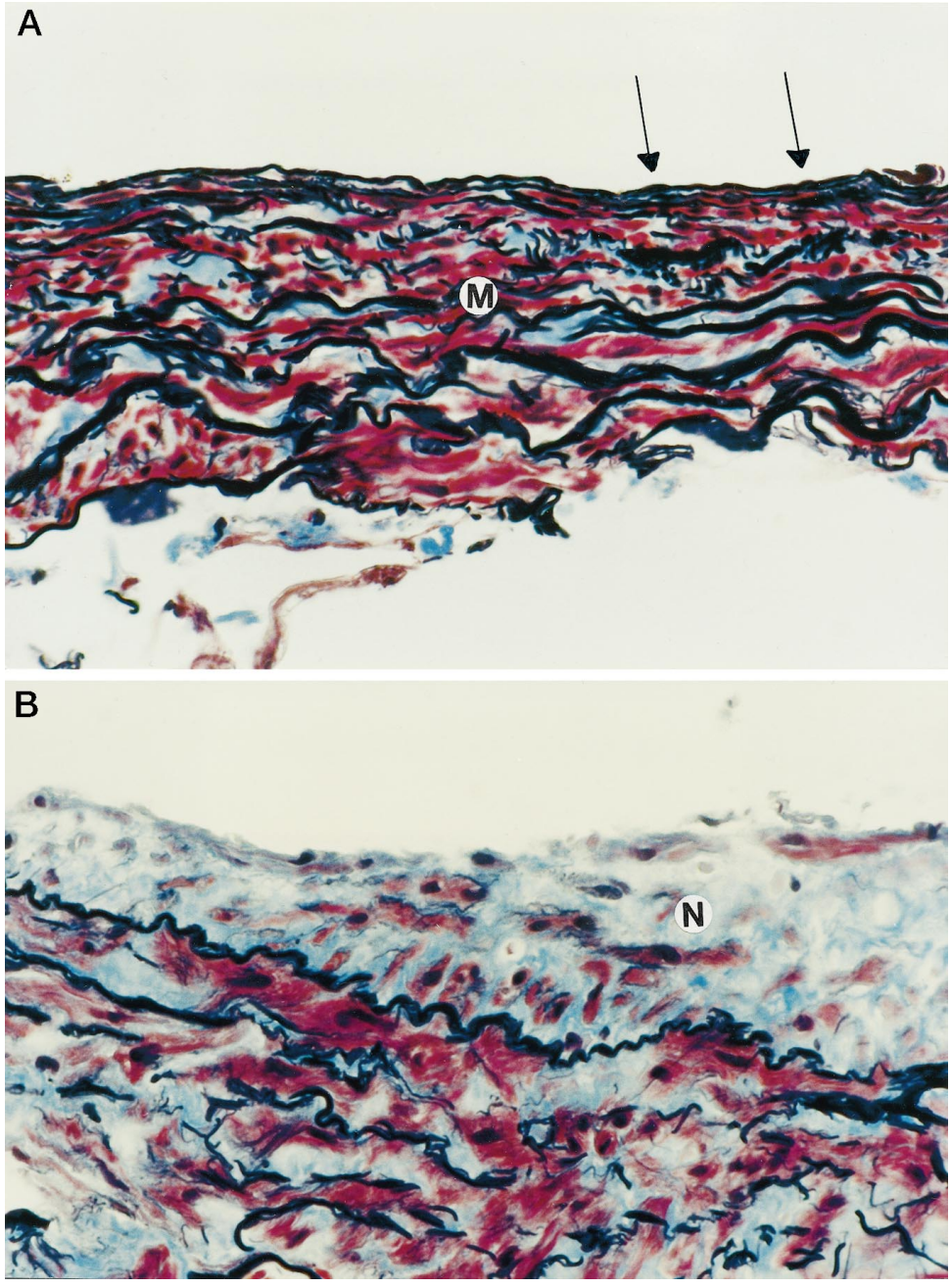

FIGURE 3. Normotensive and hypertensive pulmonary artery cross-sections. (*Top*) biopsy sample from a normotensive pulmonary artery showing normal vascular architecture including endothelial cells (arrows), relatively uniform elastic laminae, and a portion of the media (M). (*Bottom*) biopsy sample from a hypertensive animal showing irregular neointimal (N) thickening composed of collagen and smooth muscle cells (trichrome/elastic stain, photographed at ×400).

showed intimal thickening with a mean distance from luminal surface to internal elastic lamina of 0.030± 0.027 mm (range, 0 to 0.3 mm); normotensive samples had a mean intimal thickness of 0.004±0.006 mm (range, 0 to 0.016 mm; $p<0.0001$). The neointimal layer consisted mainly of hypocellular collagen (Fig 3). Immunoperoxidase stain for smooth muscle actin highlighted occasional smooth muscle cells in this layer (Fig 4, *Top*). Stain with von Willebrand factor revealed a thin layer of endothelial cells on the luminal region of the neointima (Fig 4, *Bottom*). There did not appear to be an increase in the number of endothelial cells in the neointimal layer. The thickness of the neointimal layer increased (Fig 5) as a function of time, the number of microsphere infusions, and the level of pulmonary arterial pressure and pulmonary vascular resistance. As

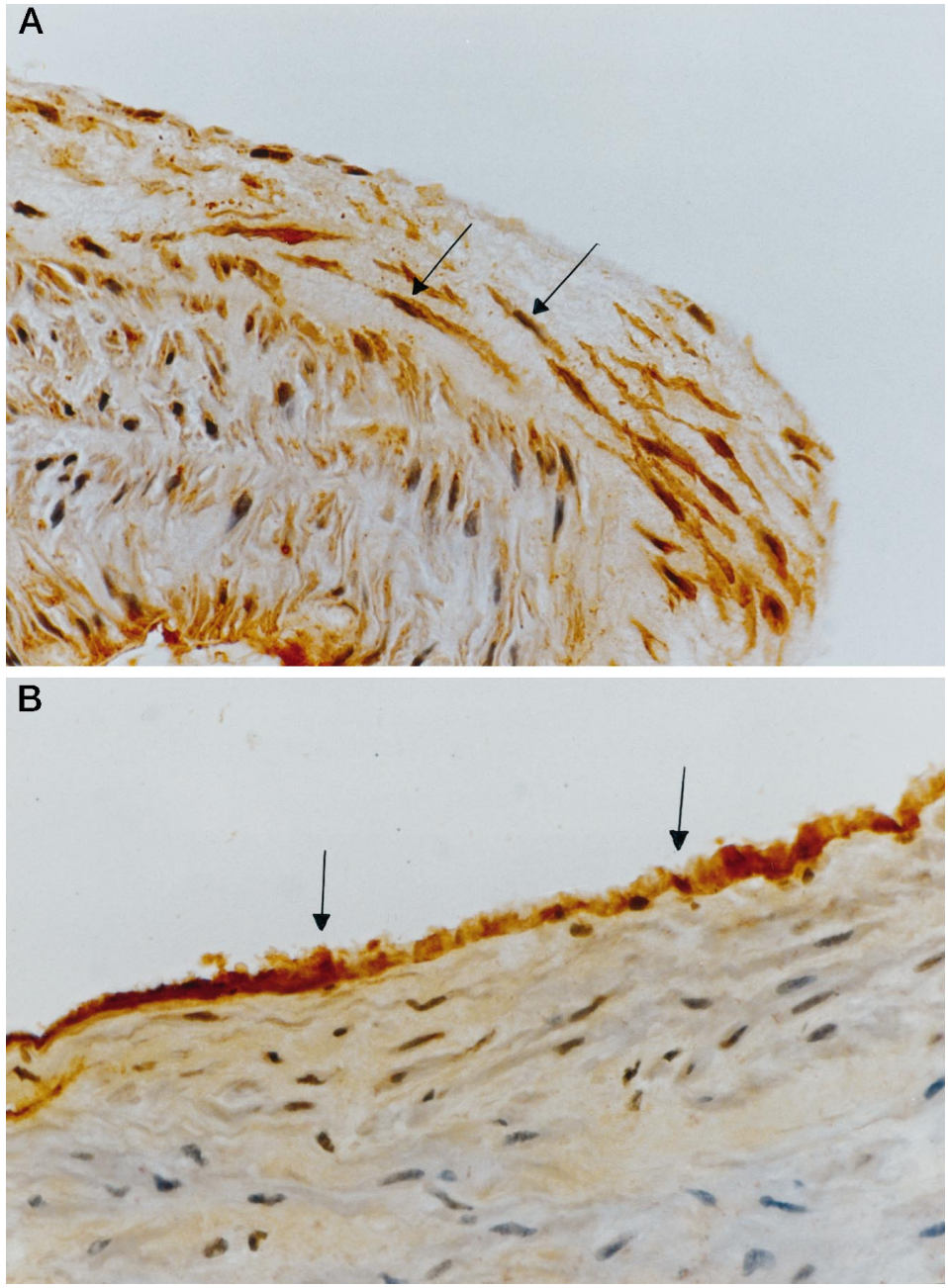

FIGURE 4. Smooth muscle actin and factor VIII stains of hypertensive pulmonary arterial cross-sections. (*Top*) immunoperoxidase stain for smooth muscle actin identifies smooth muscle cells (arrows) in the neointimal layer. (*Bottom*) immunoperoxidase stain for factor VIII identifies the endothelial cell layer (arrows) on the luminal aspect of the thickened neointima (immunoperoxidase stain with diaminobenzidine chromogen, photographed at ×200).

the dogs developed progressive pulmonary hypertension, biopsy samples showed increasing myxoid degeneration of the extracellular matrix between the elastic lamellae and smooth muscle cells of the media and in the neointima (Fig 6, *Top*). In several hypertensive samples, there was fragmentation of the elastic lamellae (Fig 6, *Bottom*).

DISCUSSION

The results of this study demonstrate the efficacy and safety of a novel arterial biopsy catheter in obtaining pulmonary endovascular wall samples in normotensive and pulmonary hypertensive dogs. In the first 12 procedures, we refined the long sheath method and

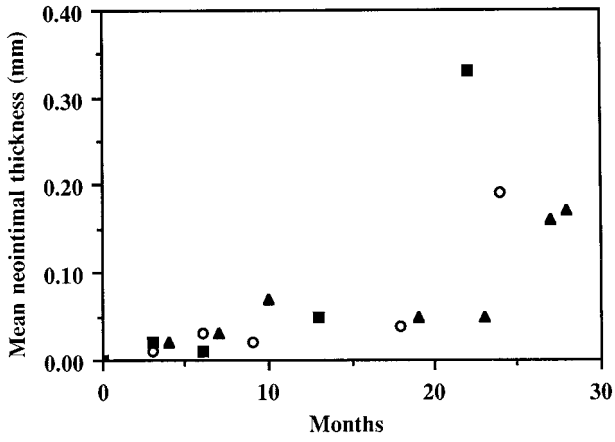

FIGURE 5. Neointimal thickness measured on the endoarterial biopsy sample cross-sections as a function of time after initiation of repeated microsphere infusions. Triangle, circle, and square each represent a different dog (three dogs total).

other technical determinants for successful biopsy. During the last 50 catheterizations, endoarterial tissue samples were obtained in every procedure. In both normotensive and hypertensive dogs, approximately five of every six attempts resulted in retrieval of endovascular tissue. This success rate is similar to that obtained when performing endomyocardial biopsies in patients (A.R., personal experience).

The average size of the biopsy specimens did not differ significantly between the normotensive and hypertensive dogs. When compared to endomyocardial biopsy, transbronchial biopsy, or gastrointestinal biopsy, the average pulmonary endovascular sample size obtained in this study was within the range of sample sizes handled routinely by clinical histology laboratories (C.A.B., personal experience).

As a result of the catheter and beveled opening design, the biopsy samples generally stayed in the beveled window, with preserved orientation and integrity. The quality of the endovascular specimens allowed identification of pathologic changes in the hypertensive dogs. The most striking findings were neointimal thickening and myxoid changes. The thickness of the neointimal layer appeared to increase progressively with incremental infusions of microspheres and elevation in baseline pulmonary vascular resistance. In the early stages of the development of pulmonary hypertension, the neointima had a thin layer of endothelial cells on the luminal surface, but was composed predominantly of hypocellular collagen and fibroblasts. In later stages, increased numbers of smooth muscle cells were observed, presumably as a result of migration from the medial layer to the intimal layer. Some samples contained multiple thin undulating layers of apparent elastin fibers in the neointima.

In the medial layer, the salient finding was the myxoid change, which became more prominent with increased pulmonary arterial pressure and resistance. Myxoid change or degeneration is a term used by pathologists to describe the gray-blue appearance acquired by tissue when there is excess extracellular mucopolysaccharide or proteoglycan ground substance. In connective tissue and vessel walls, the presence of myxoid material is considered a degenerative change. The disruption and irregularity of elastic lamellae may have been secondary to fibrosis or degenerative processes associated with remodeling of the medial layer. In early stages of pulmonary hypertension, sample orientation during specimen processing was critical to identifying the neointimal thickening, but in later stages of hypertension, the neointimal thickening, myxoid degeneration, and elastic fiber deposition were easily identifiable in virtually all specimens, regardless of orientation.

The endoarterial wall changes observed in the biopsy samples were representative of the vascular wall changes seen on necropsy examination of cross sections of whole vessels. The vascular pathologic abnormalities in this microsphere infusion model correlate with stages 1 to 3 of the Heath-Edwards classification[10] and have also been described in pulmonary vascular disease secondary to chronic hypoxia,[11] congenital cardiac lesions,[12-16] toxic oil syndrome,[17] granulomatous and collagen vascular diseases,[11] thromboembolic disease,[18] and primary pulmonary hypertension.[15,19-21]

The various classification schemes for arterial changes in pulmonary hypertension[10,22] are based on reviews of sections of lung tissue and vessels *in situ*. These extensive descriptions detail changes in both large and small vessels and the surrounding parenchyma. The large vessels demonstrate characteristic, reproducible changes and are an appropriate target for biopsy, as demonstrated by our study in which relatively large vessels (2 to 3 mm in luminal diameter) were sampled. We also have extensive experience in reviewing large artery endarterectomy specimens (C.A.B., personal experience) and note similarities between the human samples and the experimental animal pulmonary arteries.

We reported previously that vascular cells obtained with this catheter could be cultured.[9] Smooth muscle cells grew quickly, were easily passaged, and were stained uniformly with anti-smooth muscle actin and myosin antibodies. In endothelial growth media, endothelial cells were identified in approximately 15% of growth attempts.[9] Primary cultures were composed of a mixture of endothelial cell islands and fibroblasts. However, after the first subculture, or if primary culture cells were allowed to continue replicating, the endothelial cells were out-

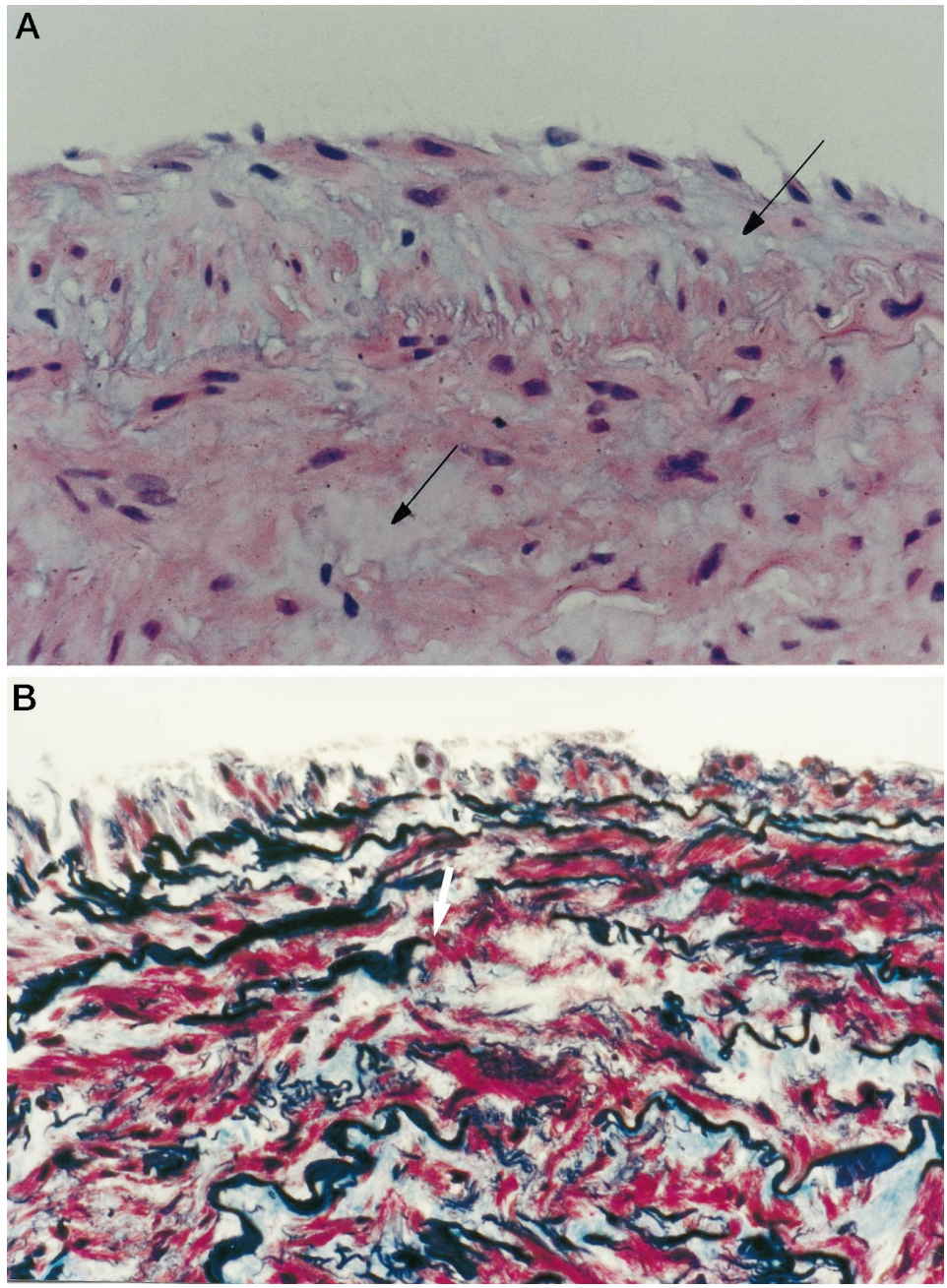

FIGURE 6. Myxoid changes and disorganized elastic lamellae. Biopsies from hypertensive animals showed (*top*) myxoid degeneration in both the medial and neointimal layers (arrows) (hematoxylin-eosin stain, photographed at ×400), and *bottom* fragmentation of the elastic lamellae in the media (arrow) (trichrome/elastic stain, photographed at ×400).

competed and overgrown by the fibroblasts. Nonetheless, endovascular cells obtained by biopsy can be cultured, and proper isolation techniques could result in uniform single cell type populations.

The complication rate associated with the use of the endoarterial biopsy catheter in this study was low. None of the complications was life-threatening. A total of 430 biopsy attempts were performed in the last 50 catheterization procedures. No dog required fluid or blood resuscitation, pressor support, or changes in ventilation as a result of the biopsy attempts. Perivascular cuffing, or persistence of contrast material outlining the vessel on postbiopsy angiography, was observed in eight of 90 biopsied vessels (9%). In every instance, this pattern resolved on repeat angiography at follow-up 1 month later. We surmise that this cuffing pattern represents perivascular, self-limited bleeding, which we de-

tected occasionally in the subadventitia of cross-sections of arteries examined at necropsy immediately after the biopsy procedure. While the endovascular injury during the biopsy may create an intramural tract or space for hemorrhage, forceful sheath manipulation may also contribute, as evidenced by one instance of vascular cuffing observed on a prebiopsy angiogram in one dog. A self-limited hemorrhage forming a saccular hematoma occurred in one of 90 biopsied vessels (1%). This single artery had been subjected to 13 biopsy attempts. No hemorrhage or aneurysm formation in the biopsied vessel was evident 1 month later on follow-up angiography or 12 months later at the time of necropsy.

Seven arteries were completely occluded immediately after biopsy. However, each of these vessels was patent on follow-up angiography 1 month later, suggesting the presence of either spasm or transient thrombosis. Since thrombus was not observed at necropsy in any of the biopsied arteries that were examined acutely after the procedure, transient spasm appears more likely. No aneurysms were observed acutely or on follow-up. However, late aneurysms may still occur, as has been observed following interventions in other arteries, such as balloon angioplasty of coarctation of the aorta, but we estimate this risk to be low with pulmonary endoarterial biopsy.

The incidence of complications (vascular cuffing, transient occlusion, or hemorrhage) was similar for normotensive and hypertensive dogs. However, single vessels that were subjected to multiple biopsies were more likely to develop these complications than vessels that were biopsied more conservatively. In this series, vascular changes occurred in 32% of single vessels biopsied more than seven times, compared with 9% of vessels biopsied seven or fewer times. Therefore, our current recommendation is to perform a maximum of three or four biopsies in any individual vessel.

Irregularities of vascular contour were frequent on postbiopsy angiography. These irregularities likely resulted from a combination of the biopsy's direct endovascular effects and partial vascular spasm. Vessels with irregular contours immediately after biopsy consistently had smooth angiographic contours at follow-up 1 month later. It is possible that angioscopy at the time of biopsy and follow-up may help visualize the biopsy sites and vessels and shed light on the nature of the vascular changes.

This study has several limitations. One limitation is the artificial nature of the microsphere model of pulmonary hypertension, which may complicate extrapolation to pulmonary vascular diseases in humans. The microsphere model also makes it more difficult to obtain biopsies because many of the more ideal vessels for biopsy are frequently occluded with microspheres. While the endoarterial biopsy catheter was associated with few complications in dogs, its safety in humans is not assured. However, the similarity between canines and humans in pulmonary vascular anatomy and histology suggests the possibility of obtaining comparable results in patients. The fluoroscopic and angiographic images in our animal laboratory were suboptimal, potentially underestimating subtle vascular wall changes postbiopsy. However, the clinical evolution, follow-up angiographic findings, and postmortem observations were reassuring. The size of the biopsy catheter and introducer sheath (currently 8F) limits use of the procedure to larger animals or patients weighing more than 10 kg. The optimal vessel diameter to be biopsied is approximately the size of the introducer sheath. However, modifications in technique and design should make the procedure applicable to smaller subjects and a larger range of vessel sizes.

Potential future uses of this endoarterial catheter include study of the histologic and immunohistochemical changes that accompany a variety of vascular diseases. This biopsy technique, combined with new cellular and molecular tools, may identify subtle changes unique to certain pathologic processes. For example, there is increasing evidence that endothelial dysfunction plays a key role in the pathogenesis of pulmonary hypertension.[23-27] The ability to procure endothelial cells with this catheter provides the opportunity for further study, including levels of vasoactive mediators such as prostacyclin, nitric oxide, endothelin-1, thromboxane-A_2, and potassium channels using patch clamp techniques. In addition, serial biopsies can be obtained with various therapeutic interventions, such as continuous IV prostacyclin or inhaled nitric oxide, in attempts to further our understanding of the mechanism of action of these agents in remodeling the pulmonary vascular bed. Biopsies could be obtained from asymptomatic carriers in families with familial primary pulmonary hypertension to potentially give insights into the early pathogenesis of the disease. This technique may also aid in the diagnosis and modification of therapy for lung transplant rejection. In future attempts to transfer genes to the vascular wall, this method may be used to confirm genetic modification in endovascular cells and to evaluate the histologic results of these therapies. While the biopsy catheter in this study was used to obtain samples from pulmonary arteries, the method can also be modified to obtain biopsy samples from a variety of systemic arteries.

In summary, this novel endoarterial biopsy catheter was safe and effective in obtaining pulmonary endovascular samples in dogs with pulmonary hyper-

tension. The biopsy results and complications were similar to those obtained in normotensive dogs. The specimens obtained at biopsy were diagnostic of vascular wall changes. This catheter should be useful in the future study, diagnosis, and management of human pulmonary vascular diseases.

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References

1 Rubin LJ. Primary pulmonary hypertension. N Engl J Med 1997; 336:111-17

2 D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension: results from a national prospective registry. Ann Intern Med 1991; 115:343-49

3 Glanville AR, Burke CM, Theodore J, et al. Primary pulmonary hypertension: length of survival in patients referred for heart-lung transplantation. Chest 1987; 91:675-81

4 Rich S, Dantzker DR, Ayres SM, et al. Primary pulmonary hypertension: a national prospective study. Ann Intern Med 1987; 107:216-23

5 Rich S, Chomka E, Hasara L, et al. The prevalence of pulmonary hypertension in the United States: adult population estimates obtained from measurements of chest roentgenograms from the NHANES II survey. Chest 1989; 96: 236-41

6 Rich S, Brundage BH. Pulmonary hypertension: a cellular basis for understanding the pathophysiology and treatment. J Am Coll Cardiol 1989; 14:545-50

7 Perkin RM, Anas NG. Pulmonary hypertension in pediatric patients. J Pediatr 1984; 105:511-22

8 Sandoval J, Bauerle O, Gomez A, et al. Primary pulmonary hypertension in children: clinical characterization and survival. J Am Coll Cardiol 1995; 25:466-74

9 Rothman A, Mann DM, House MT, et al. Transvenous procurement of pulmonary artery smooth muscle and endothelial cells using a novel endoarterial biopsy catheter in a canine model. J Am Coll Cardiol 1996; 27:218-24

10 Heath D, Edwards JE. The pathology of hypertensive pulmonary vascular disease: a description of six grades of structural changes in the pulmonary arteries with special reference to congenital cardiac septal defects. Circulation 1958; 18: 533-47

11 Rabinovitch M. Pathophysiology of pulmonary hypertension. In: Emmanouilides GC, Riemenschneider TA, Allen HD, et al, eds. Heart disease in infants, children and adolescents. 5th ed. Baltimore: Williams & Wilkins, 1995; 1659-95

12 Hoffman JIE, Rudolph AM, Heymann MA. Pulmonary vascular disease with congenital heart lesions: pathologic features and causes. Circulation 1981; 64:873-77

13 Newfeld EA, Paul MH, Muster AJ, et al. Pulmonary vascular disease in complete transposition of the great arteries: a study of 200 patients. Am J Cardiol 1974; 34:75-82

14 Haworth SG. Pulmonary vascular disease in secundum atrial septal defect in childhood. Am J Cardiol 1983; 51:265-72

15 Smith P, Heath D, Yacoub M, et al. The ultrastructure of plexogenic pulmonary arteriopathy. J Pathol 1990; 160:111-21

16 Lakier JB, Stanger P, Heymann MA, et al. Early onset of pulmonary vascular obstruction in patients with aortopulmonary transposition and intact ventricular septum. Circulation 1975; 51:875-80

17 Gomez-Sanchez MA, Saenz De La Calzada C, et al. Clinical and pathologic manifestations of pulmonary vascular disease in the toxic oil syndrome. J Am Coll Cardiol 1991; 18:1539-45

18 Anderson EG, Simon G, Reid L. Primary and thromboembolic pulmonary hypertension: a quantitative pathological study. J Pathol 1973; 110:273-93

19 Pietra GG, Edwards WD, Kay JM, et al. Histopathology of primary pulmonary hypertension: a qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. Circulation 1989; 80:1198-1206

20 Palevsky HI, Schloo BL, Pietra GG, et al. Primary pulmonary hypertension: vascular structure, morphometry, and responsiveness to vasodilator agents. Circulation 1989; 80:1207-21

21 Thilenius OG, Nadas AS, Jockin H. Primary pulmonary vascular obstruction in children. Pediatrics 1965; 36:75-87

22 Rabinovitch M, Haworth SG, Castaneda AR, et al. Lung biopsy in congenital heart disease: a morphometric approach to pulmonary vascular disease. Circulation 1978; 58:1107-21

23 Loscalzo J. Endothelial dysfunction in pulmonary hypertension. N Engl J Med 1992; 327:117-19

24 Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. N Engl J Med 1995; 333:214-21

25 Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 1992; 327:70-75

26 Adatia I, Barrow SE, Stratton PD, et al. Thromboxane A_2 and prostacyclin biosynthesis in children and adolescents with pulmonary vascular disease. Circulation 1993; 88(Part 1): 2117-22

27 Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. N Engl J Med 1993; 328:1732-39