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Original Research

Optical Coherence Tomography of Pulmonary Arterial Walls in Humans and Pigs (*Sus scrofa domesticus*)

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Pulmonary arterial hypertension (PAH) is a devastating disorder characterized by progressive elevation of the pulmonary pressures that, in the absence of therapy, results in chronic right-heart failure and premature death. The vascular pathology of PAH is characterized by progressive loss of small (diameter, less than 50 μm) peripheral pulmonary arteries along with abnormal medial thickening, neointimal formation, and intraluminal narrowing of the remaining pulmonary arteries. Vascular pathology correlates with disease severity, given that hemodynamic effects and disease outcomes are worse in patients with advanced compared with lower-grade lesions. Novel imaging tools are urgently needed that demonstrate the extent of vascular remodeling in PAH patients during diagnosis and treatment monitoring. Optical coherence tomography (OCT) is a catheter-based intravascular imaging technique used to obtain high-resolution 2D and 3D cross-sectional images of coronary arteries, thus revealing the extent of vascular wall pathology due to diseases such as atherosclerosis and in-stent restenosis; its utility as a diagnostic tool in the assessment of the pulmonary circulation is unknown. Here we show that OCT provides high-definition images that capture the morphology of pulmonary arterial walls in explanted human lungs and during pulmonary arterial catheterization of an adult pig. We conclude that OCT may facilitate the evaluation of patients with PAH by disclosing the degree of wall remodeling present in pulmonary vessels. Future studies are warranted to determine whether this information complements the hemodynamic and functional assessments routinely performed in PAH patients, facilitates treatment selection, and improves estimates of prognosis and outcome.

Abbreviations: OCT, optical coherence tomography; PAC, pulmonary artery catheter; PAH, pulmonary arterial hypertension.

Pulmonary arterial hypertension (PAH) is a devastating disorder characterized by progressive elevation of pulmonary pressures that, when untreated, can lead to chronic right heart failure and death.[14] The vascular pathology of PAH is characterized by neointimal formation, medial thickening, intravascular thrombi and, in severe cases, intravascular clusters of disorganized endothelial cells that give rise to tortuous endovascular channels.[8] Most of the early vascular lesions are found in small (diameter, less than 50 μm) pulmonary arteries. However, as the disease advances, pulmonary arteries (diameter, 50 μm or larger) proximal to these lesions also display evidence of luminal narrowing and medial thickening.[7,8,15] Most patients with PAH are younger than those with chronic systemic vascular disorders (that is, coronary artery disease, peripheral vascular disease, systemic hypertension), whose vascular pathology involves mostly large to medium-sized arteries. However, both patient populations demonstrate various pathologic features, including vascular smooth-cell accumulation, neointimal formation, inflammation, luminal narrowing, and alterations in the composition of the extracellular matrix.[6,17]

The only definite way to diagnose PAH is through right heart catheterization to directly measure the pressure in the pulmonary circulation. Although pulmonary angiography during right heart catheterization cannot be used to diagnose PAH, it provides supportive evidence of PAH by demonstrating significant peripheral small vessel loss and luminal narrowing in the remaining central vessels. Angiography can help clinicians visualize pulmonary vessels in real time, but this diagnostic technique has important limitations. The use of ionized contrast can cause allergic reactions and may trigger acute renal failure due to contrast-induced nephropathy.[26] In addition, pulmonary angiography provides information regarding gross vessel appearance and small vessel perfusion but not about the state of vascular wall remodeling or the extent of luminal narrowing associated with PAH at any stage.[5,16] Therefore, imaging techniques are urgently needed that complement the hemodynamic information obtained via right heart catheterization with a safe and reproducible method to assess vascular wall pathology, thereby allowing clinicians to correlate the clinical evolution of PAH with the progression of vascular pathology.

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The last decade has seen tremendous progress in the development of intravascular imaging modalities that can identify patients at risk for developing complications related to systemic vascular disease and therefore prevent disease-related morbidity and mortality.[4] One such modality is optical coherence tomography (OCT), an imaging technique that uses a thin (diameter, 1.0 mm) wire and near-infrared light to capture micrometer-resolution, 3D images from within optical scattering media (for example, biologic tissue).[1] Superior to other intravascular imaging techniques, OCT is frequently used in patients with coronary artery disease, where it provides high-resolution images of the coronary arterial wall that correlate highly with pathology seen in explanted vessels.[10,11,21] To date, several small studies have demonstrated the application of OCT to the evaluation of vascular remodeling in both idiopathic PAH and chronic thromboembolic PAH.[7,21] However, despite OCT's obvious advantages in the characterization of vascular remodeling in discrete segments of the pulmonary circulation, whether OCT provides anatomic information across the length of the pulmonary artery has not been tested.

Here, we report the capacity of OCT to obtain both longitudinal and cross-sectional images that provide accurate anatomic information on healthy pulmonary arteries in explanted human lungs and during the pulmonary arterial catheterization of a live adult pig (*Sus scrofa domesticus*).

Materials and Methods

OCT of human explanted lungs. A total of 3 healthy donor lungs were obtained from failed donors via the Pulmonary Hypertension Breakthrough Initiative. The explanted lungs were submerged in ice-cold saline, and blood was cleared by infusing cold saline through a cannula sutured into the hilar pulmonary artery. With the cannula in place, a 7.5-French Swan–Ganz pulmonary artery catheter (PAC) was introduced into the hilar pulmonary artery and advanced under fluoroscopy into the segmental pulmonary arteries. Once wedged, a C7 OCT wire (diameter, 1.0 mm; St Jude Medical, St Paul, MN) was introduced and advanced toward the lung periphery by using contrast injection to guide the wire into open distal arteries. Once the wire reached the distal vessels at its size limit, longitudinal and cross-sectional images were captured and analyzed by using the C7-XR OCT Intravascular Imaging System software (St Jude Medical). Once imaging was completed, dye was injected to mark the site for histologic analysis. Vessel histology by using sections stained with hematoxylin and eosin was performed to validate OCT findings and measurements. A total of 3 independent vessels per lung lobe were evaluated before the OCT wire was removed and the labeled pulmonary arteries were dissected free from surrounding lung tissue.

OCT of porcine pulmonary arteries. All animal studies were performed under an animal protocol (APLAC-26148) approved by the Stanford University Administrative Panel on Laboratory Animal Care and followed the guidelines included in the *AALAS Position Statement for Humane Care and Use of Laboratory Animals* and *Recognition and Alleviation of Pain and Distress in Laboratory Animals*[3]. A single SPF female juvenile Yorkshire pig (*Sus scrofula domesticus*; weight, 55 kg) was obtained from a local commercial swine farm (Pork Power Farms, Turlock, CA) and maintained on free-choice food (product no. 203S, Nature's Match Sow and Pig Complete Feed, Purina Mills, St Louis, IL) and water; the pig was vaccinated and dewormed and was apparently healthy during clinical examination before surgery. The pig was acclimated for at least 24 h before use in the study. Before surgery, the pig was handled carefully to avoid undue excitement, and body temperature was kept constant by means of a circulating-water heating pad (Gaymar T/Pump Heating System, Eickemeyer, Tuttlingen, Germany) and cage heating. The pig fasted overnight before surgery and was euthanized by anesthetic overdose immediately after the procedure.

After the induction of anesthesia with 1% to 4% isoflurane and tiletamine–zolazepam, the pig was connected to a mechanical ventilator. Its vital signs were monitored continuously throughout the entire procedure. By using a femoral approach and under fluoroscopic guidance, a PAC (0.035-in., guide-wire–compatible) was floated first into the right ventricle and then advanced into the pulmonary artery until the PAC was wedged in the lower segmental pulmonary arteries. Once the PAC was located in the pulmonary artery, two 0.014-in. wires were inserted into the guide-wire lumen of the PAC. The PAC was then removed, leaving the 2 guide wires. Next, the PAC was advanced over one of the 0.014-in. guide wires, and the OCT catheter (C7) was advanced over the other 0.014-in. guide wire. The OCT catheter was advanced as far as possible in the targeted pulmonary artery. Once the PAC reached the entrance of targeted pulmonary artery, the balloon was inflated, contrast was injected, and images were recorded. Once the images were obtained, the catheter was left in place, and dye was injected thru the PAC to mark the target pulmonary vessel. Two arteries were studied, one in the right lower lobe and the other in the left lower lobe. The procedure was well tolerated during anesthesia. At the end of the study, the pig was euthanized via anesthetic overdose, the lungs were explanted, and the pulmonary artery containing the tip of the OCT catheter was dissected, fixed, and embedded in paraffin. Histologic sections stained with hematoxylin and eosin were compared generally with corresponding OCT images; precise 1:1 comparison was not possible.

A trained cardiologist analyzed the cross-sectional images of the pulmonary arteries. Measures of lumen diameter, lumen area, and wall area were obtained for the smallest and largest segments of the artery that could be imaged reliably; this was done for both the right and left lower lobar arteries imaged. All cross-sectional images analyzed were taken from segments where no side branches were present.

Histologic analysis. Human pulmonary arteries and pig lungs were fixed in 10% formalin for 24 h and then embedded in paraffin, according to standard techniques. Sections were mounted on glass slides and stained with hematoxylin and eosin.

Results

Pulmonary artery wall anatomy in explanted human lungs. In explanted human lung, we were able to advance the OCT catheter distally from the wedged tip of the PAC until it reached pulmonary arteries that were approximately 2 mm in diameter. The OCT catheter could not be advanced beyond this vessel diameter in any of the 3 lobes evaluated. Once the pulmonary artery was cannulated, cross-sectional (Figure 1 A) and longitudinal (Figure 1 B) montages of OCT images were obtained and delineated the distribution of the intimal and medial layers within the pulmonary arterial wall. After imaging, we dissected the pulmonary artery and repeated the OCT imaging ex vivo prior to fixation and paraffin embedding (Figure 1 C). Histologic analysis demonstrated that OCT pro-

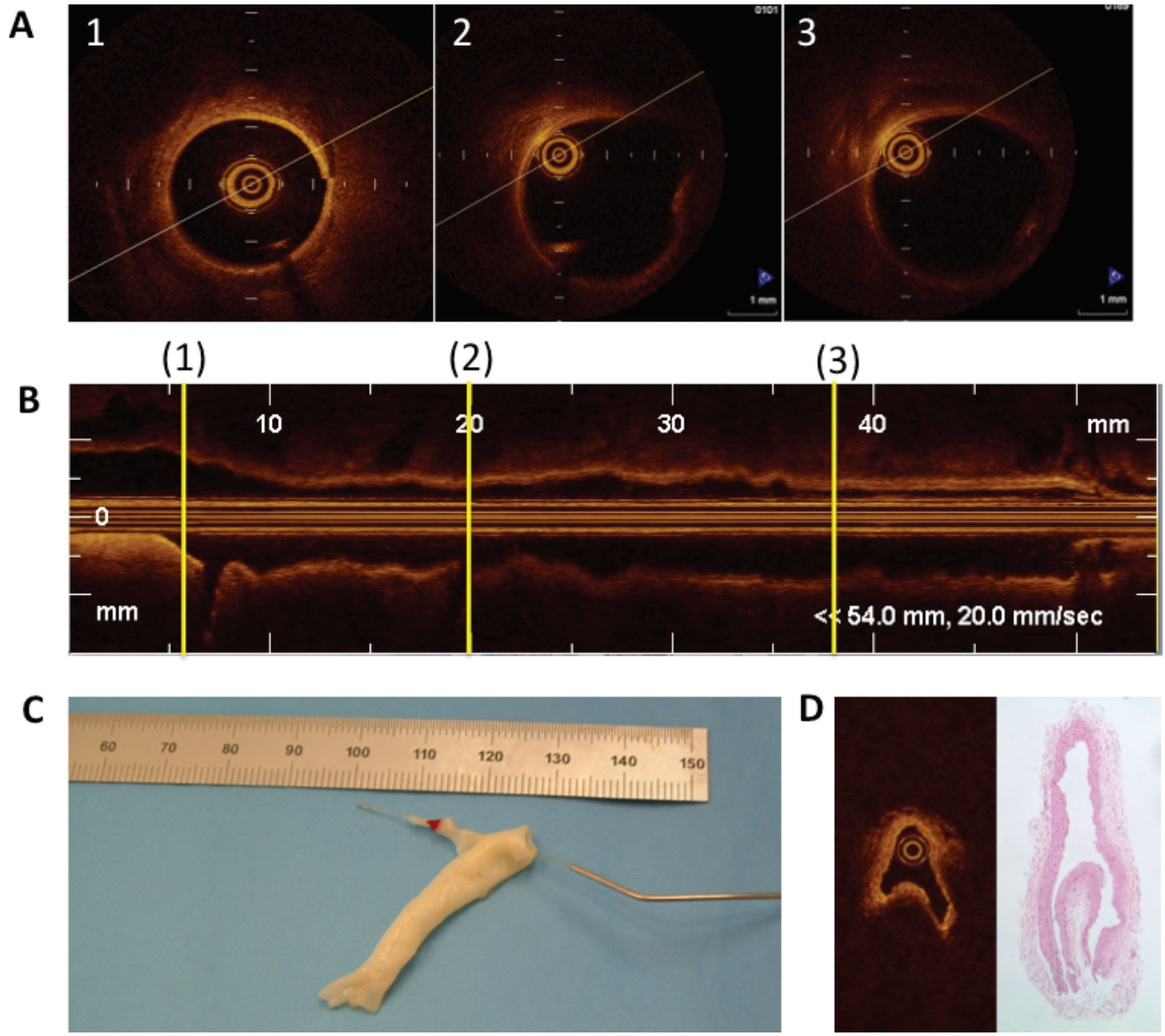

Figure 1. OCT of pulmonary arteries in explanted human right lung and dissected free vessels. (A) Cross-sectional images obtained when OCT catheter was advanced into explanted right lung, (B) representing different segments (1, 2 and 3) along the length of the pulmonary artery. (C) OCT also was performed in pulmonary arteries dissected free from lung tissue and images correlated with (D) histologic sections stained with hematoxylin and eosin.

vided an accurate image of the intimal and medial layers of the pulmonary artery, with no evidence of vascular trauma (Figure 1 D). However, the fixation process prevented quantitative comparison of measured wall thickness between OCT and histology. Taken together, these findings demonstrate that OCT can provide accurate information regarding pulmonary arterial morphology that correlates with the histologic characteristics of tissue sections.

OCT imaging of porcine pulmonary arteries. We encountered no difficulties during insertion and advancement of the OCT catheter into the distal pulmonary circulation of the right and left lower lung lobes of an adult pig (Figure 2). At the completion of the study, the lungs were removed and sectioned (Figure 3 A) to reveal the imaged pulmonary arteries. Similar to what we found with human tissue, OCT captured detailed longitudinal (Figure 3 B) and cross-sectional (Figure 3 C) images of the pulmonary arteries without causing any overt trauma to or dissection of the vascular tissue. The pig tolerated the procedure well, and post-mortem pathologic examination of both lungs failed to show any evidence of hemorrhage or tissue injury that could be attributed to the OCT catheter.

Figure 4 shows a longitudinal section of the images of the pulmonary artery in the right lower lobe, along with the cross-sectional images taken at the smallest (Figure 4 A), an intermediate (Figure 4 B), and the largest (Figure 4 C) vessel diameter imaged. As expected, in the absence of PAH, the intima of the pulmonary arteries was thin (Table 1); the intimal and medial layers were not sufficiently thick to separate on OCT imaging and measure individually.

Discussion

The goal of the current study was to establish the utility of OCT as an imaging technique for characterizing pulmonary vascular

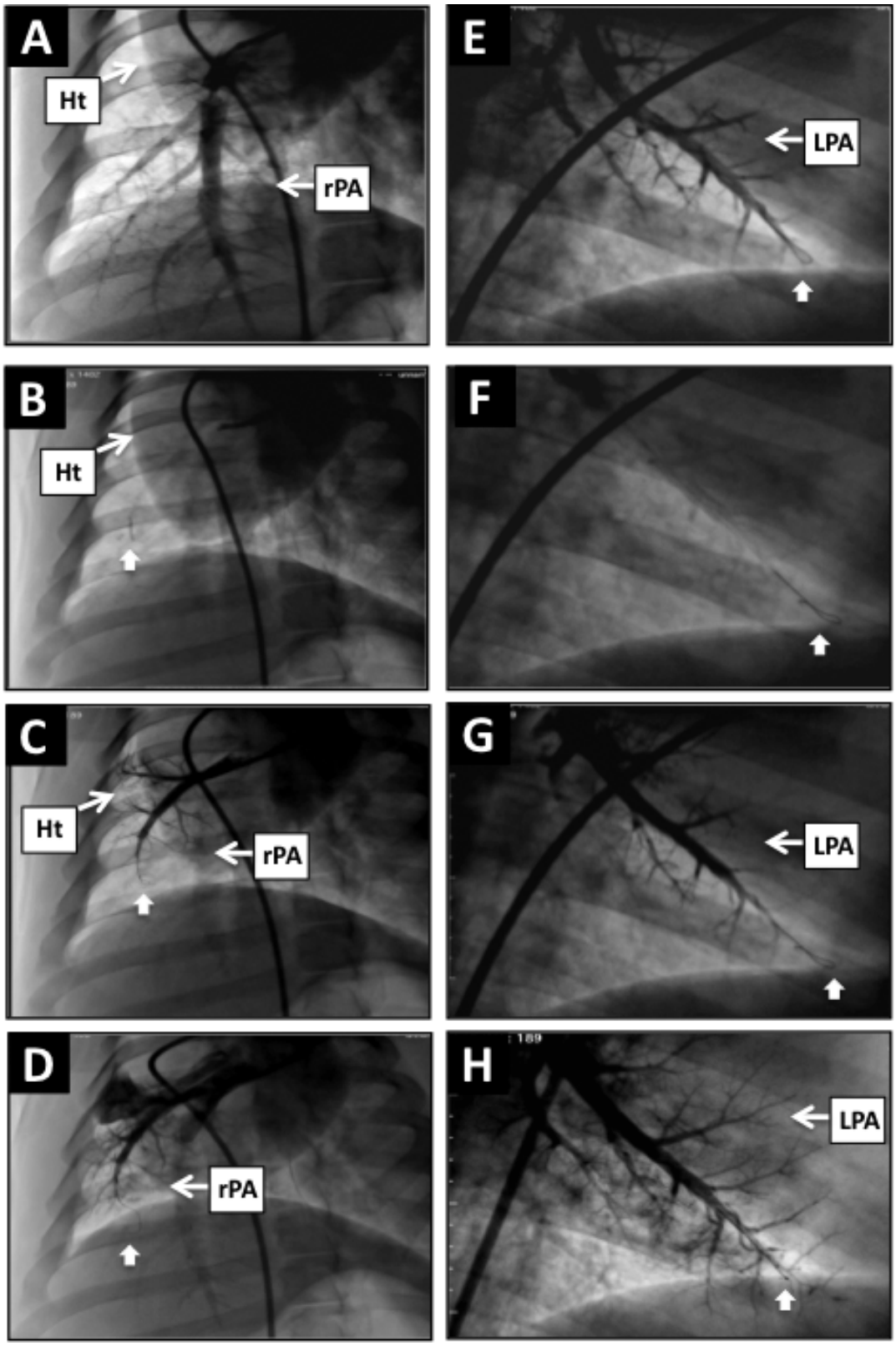

Figure 2. OCT performed during the catheterization of the right and left lung in an adult pig. Arrows indicate the positions of the OCT catheter in pulmonary arteries of the (A–D) right and (E–H) left lung. Angiograms of the right pulmonary artery (RPA) were taken by using a right lateral angle (RLA), whereas those of the left pulmonary artery (LPA) were captured by using a left lateral angle (LLA). The heart (Ht) is labeled as an anatomic landmark.

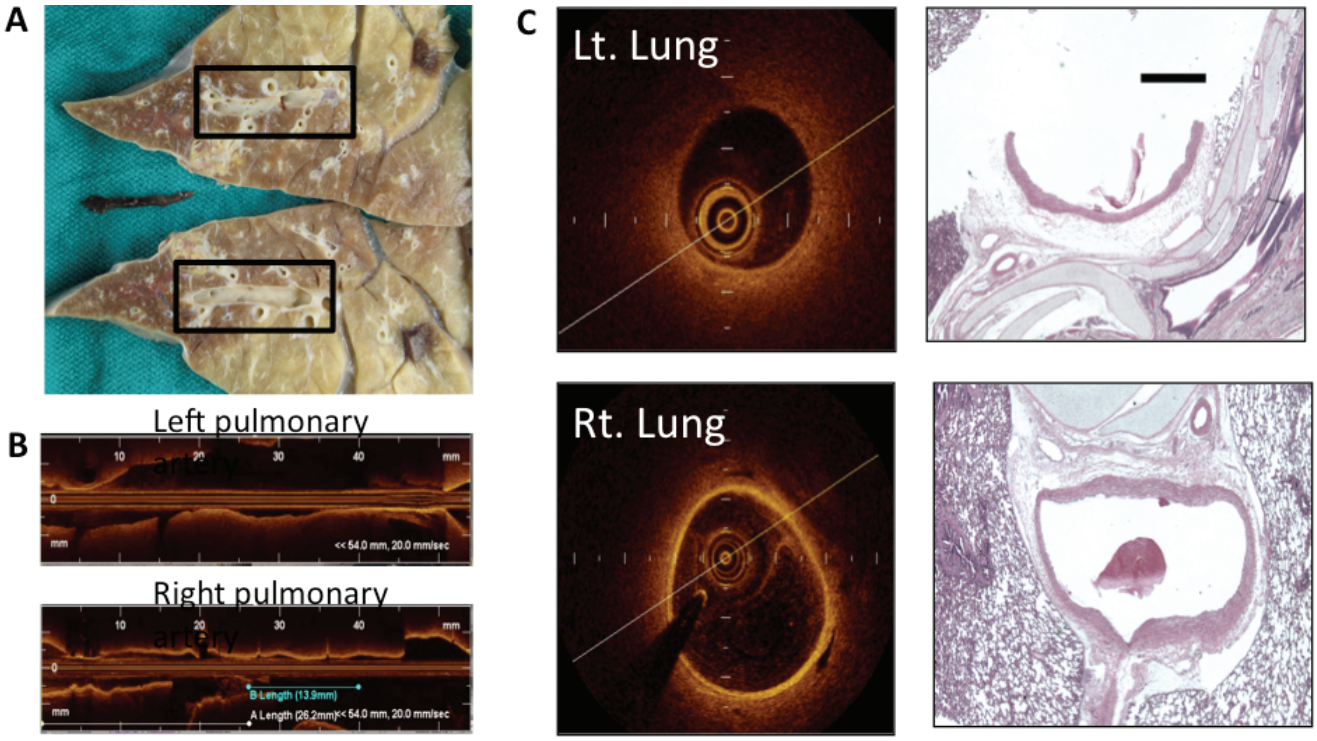

Figure 3. Correlation between OCT images and histologic sections of a pulmonary artery from an adult pig. (A) Sagittal sections of right pig lung showing medium-sized pulmonary arteries evaluated by OCT. (B) Longitudinal and (C) cross-sectional OCT images correlate with histologic sections obtained from pig pulmonary arteries.

remodeling in ex vivo human pulmonary arteries and in animal models that share pulmonary anatomic details with humans. The ability to visualize and quantify the severity of vascular remodeling in the pulmonary arteries could improve our understanding of cardiopulmonary interactions in PAH by allowing us to characterize the extent and distribution of vascular pathology within pulmonary vessels and how these features contribute to the severity of right ventricular impairment. Therefore OCT may enable clinicians to distinguish PAH due to pulmonary vascular disease from mimics such as passive pulmonary venous hypertension due to elevated left atrial pressures. In addition, OCT may supplement the diagnostic yield of right heart hemodynamics by providing important structural information.

To date, most pathologic studies using human and animal samples are based on sectioning a piece of tissue and performing histology via specific staining techniques, such as hematoxylin and eosin, Movat, and others. Unfortunately, this approach limits our ability to appreciate the extent and heterogeneity of morphologic changes along the entire vessel length, resulting in a biased view of disease severity. An advantage of OCT is that it can help capture anatomic information on contiguous segments, thus providing a more complete morphologic picture of the pulmonary artery. This advantage is relevant to future clinical applications given that OCT facilitates the visual inspection and quantification of the vascular remodeling of affected pulmonary vessels. To date, several small studies have shown that OCT can be used to gain detailed anatomic information of pulmonary vascular morphology in patients with PAH[9,12] or chronic thromboembolic PAH,[22] suggesting that routinely using OCT in the evaluation of patients undergoing right heart catheterization might facilitate decisions regarding prognosis and therapy.

The application of OCT to the respiratory system has shown great promise in furthering our understanding of key processes such as the dynamics of the mucociliary escalator,[13,24] quantification of the severity of bronchial wall fibrosis in asthma,[24] and characterization of the alveolar structure[18] in animal models. An advantage of using OCT to study the pulmonary arteries in both ex vivo lungs and animal models is that this methodology captures information regarding the extent and influence of vascular remodeling on local patterns of blood flow and provides an anatomic correlate to measures of arterial stiffness and capacitance.

One important limitation of OCT is that the currently available catheters only accommodate vessels with diameters larger than 2 mm. However, although most of the pathology associated with PAH is thought to occur in small vessels (that is, less than 50 µm in diameter),[19] it is well established that structural remodeling can occur in more proximal, larger arteries, further increasing pulmonary artery resistance by increasing stiffness and impedance within the pulmonary circulation.[23] Therefore, morphologic characterization of these larger vessels by OCT may yield useful information concerning the role of the remodeling of intermediate and large vessels in the pathophysiology of PAH.

In addition, the changes due to PAH do not occur uniformly throughout the lung, and it is unknown as yet whether the OCT assessment of a single pulmonary artery is representative of the underlying disease state. We were unable to individually measure the thickness of the intimal and medial layers in normal porcine lung, but it is highly likely that the development of medial hypertrophy and intimal hyperplasia will enable quantitation of the intimal and medial layers in swine lungs with PAH, as has been shown previously in human populations.[9,22] The ability of OCT

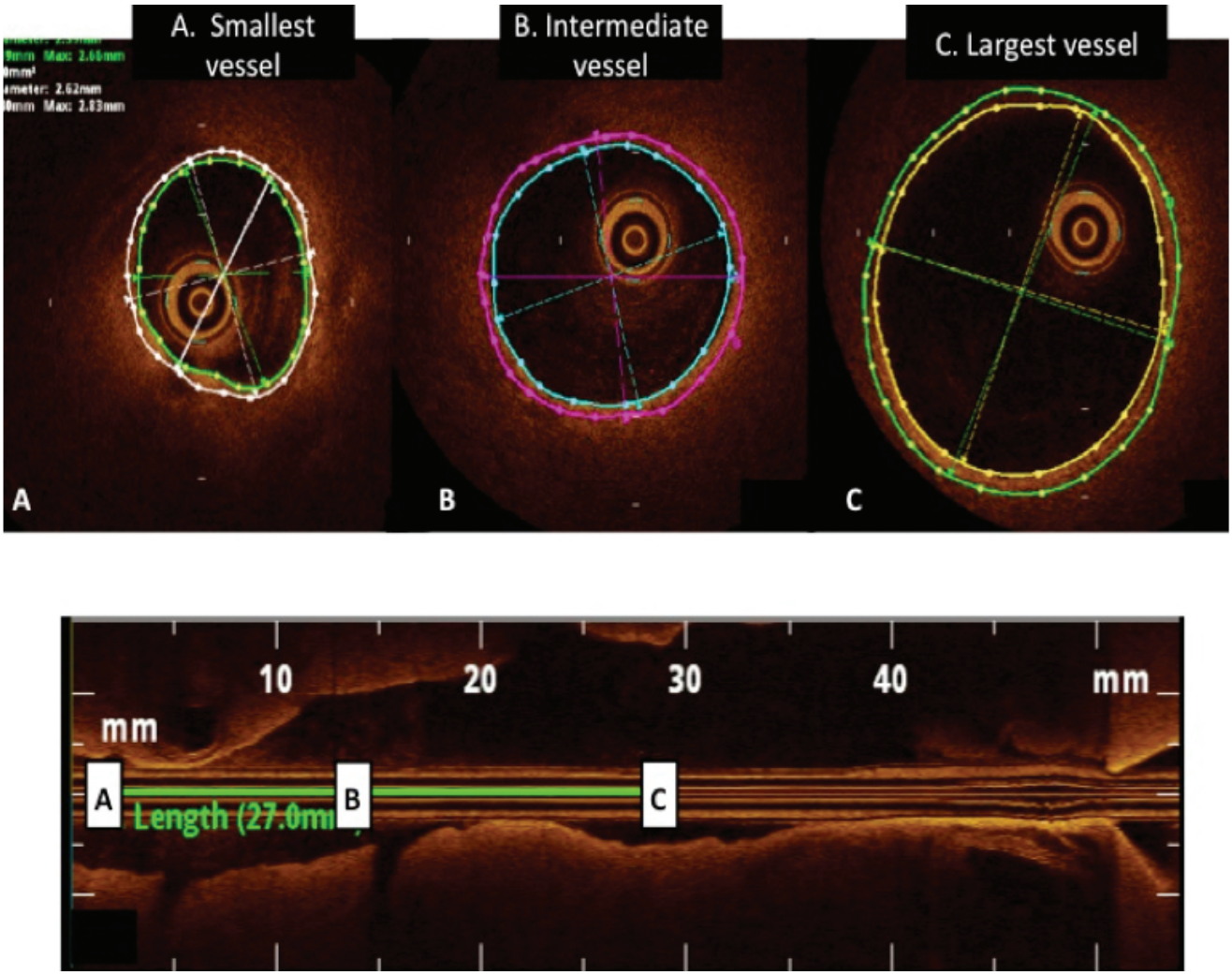

Figure 4. Cross-sectional images obtained from the pulmonary artery of the right lower lobe an adult pig, with sample tracing of the lumen and wall areas and lumen diameters.

Table 1. Cross-sectional measurements of pulmonary arteries measured by OCT

				Diameter (mm)	
		Lumen area (mm^2)	Wall area (mm^2)	Maximal	Minimal
Right lower lobe	Smallest artery	1.58	1.04	1.62	1.23
	Largest artery	14.07	2.68	5.30	3.55
Left lower lobe	Smallest artery	4.53	0.87	2.66	2.19
	Largest artery	12.69	2.29	4.29	3.80

to distinguish the intima from the media in remodeled porcine pulmonary arteries requires validation in a porcine PAH model.

We chose to use pigs in our study in light of our experience in using this model for both hemodynamic and imaging studies of the systemic and pulmonary circulations. In addition, several reports have described the use of pig models to study PAH pathophysiology, making swine an appropriate model animal in which to test the feasibility of OCT.[21] The present study does not include any human or animal model of PAH because we felt it important to obtain an accurate baseline impression of normal pulmonary arteries in both of these models, as well as to demonstrate the safety of the procedure prior to expanding our studies to include animals with PAH. Given the success of the current study, we are now poised to use OCT to characterize pulmonary vascular remodeling in swine models of congenital PAH and chronic thromboembolic PAH.[20,25]

Although some researchers currently use pigs and human explants, most work involving animal models in the field of PAH is done with rodents, thus begging the question of whether OCT can be applied in that model system. We previously applied OCT to the

study of carotid stents in mice[3] and demonstrated the ability of this modality to capture differences in neointimal formation and medial thickening associated with stent-mediated vascular injury, but we have not tested OCT in the pulmonary arteries of rats and mice. Again, the main limitation associated with performing OCT in rodents is the size of the available catheters (1.0 mm), which severely limits the use of OCT in the distal pulmonary circulation. Catheters currently available for clinical use are 1.0 mm in diameter, but smaller (0.5 mm) catheters are available that could potentially be used to cannulate the pulmonary circulation of rodents. However, regardless of the ability of OCT to access the distal pulmonary arteries, visualization of the wall morphology of the main pulmonary artery trunk and large branches likely will provide useful information for groups that use rodent models to study the effects of pulmonary artery stiffening and remodeling in large arteries on right ventricular afterload.[23]

The equipment and techniques used to perform OCT are undergoing active development. Novel OCT systems that do not require vessel occlusion are becoming available and may simplify OCT imaging of the pulmonary arteries in the future. In addition to supplementing the collected information regarding hemodynamics in the diagnosis of PAH, advanced OCT may eventually become a useful screening tool for high-risk populations, such as patients with systemic sclerosis. Conceivably OCT might detect vascular remodeling even before the pulmonary pressures reach the defined threshold for PAH diagnosis. In addition, using OCT to monitor vessel wall changes over time may be a useful means of assessing response to PAH therapy.

In conclusion, we propose that OCT is a potentially useful research tool that can provide relevant information regarding the burden that pulmonary vascular remodeling in the large and medium sized vessel plays in the cardiopulmonary dynamics of PAH. As we move toward the application of this technology in the clinical setting, we envision that OCT data might complement the hemodynamic and functional assessments that are performed routinely in all patients with PAH, and OCT could help clinicians to track the effect of therapies on the severity of vascular remodeling in any given patient. To that end, we believe that a prospective study that applies OCT in a large clinical cohort is required to answer these questions and establish whether this technology has a role in the current standard of care of PAH patients.

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