Wall Morphology of the Pulmonary Artery —Intravascular Ultrasound Imaging and Pathological Evaluations—

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Summary: The pathological assessment of the pulmonary vascular bed is essential for the diagnosis and management of congenital heart disease with pulmonary hypertension. To investigate the relationship between intravascular ultrasound (IVUS) findings and the Heath-Edwards (H.E.) grade of human pulmonary arteries, 40 pulmonary arterial segments from 17 autopsies and 5 patients with pulmonary hypertension were evaluated using a high-frequency IVUS imaging system. Histological observations were carried out at the same sites that were examined with IVUS and at the distal pulmonary arterioles of the same branches. The subjects were subdivided into 3 groups—group I: 7 patients with pulmonary hypertension (20 segments); group II: 10 patients without pulmonary hypertension (20 segments); and group III: 5 patients with pulmonary hypertension who were studied in vivo. All 20 segments from group I had a three-layered appearance, and 3 patients with H.E. grade < 3 had an increased thickness of the echolucent zone due to media hypertrophy without an intimal reaction. In contrast, IVUS findings from patients with severe pulmonary hypertension (H.E. grade ≥ 3) showed an increased thickness with a bright inner layer due to intima hyperplasia. Evidence for a plaque-like lesion was defined in one patient with severe pulmonary hypertension. The percentage of wall thickness derived from IVUS was significantly correlated with that determined by histological examination in group I. The pulmonary artery walls in group II had a one-layer appearance. Similar IVUS findings were obtained from the in vivo study regardless of whether pulmonary hypertension was present. These findings indicate that the IVUS may be a unique technique for evaluating the morphology of the pulmonary artery or arteriole in pulmonary hypertension and may provide objective and quantitative evaluation without a lung biopsy.

Key words: intravascular ultrasound imaging — pulmonary hypertension — histopathologic findings — congenital heart disease — lung biopsy

Introduction

Histopathologic assessment of the

pulmonary artery is essential for the diagnosis and management of heart disease with pulmonary hypertension

This study in part was presented at the 66th Scientific Sessions of the American Heart Association in Atlanta, 1993.

(Hislop and Reid, 1973; Friedil et al. 1974; Clarkson et al. 1976; Haworth and Reid, 1977; Haworth et al. 1977; Haworth and Lynne, 1978; Rabinovitch et al. 1980; Yamaki et al. 1980). Heath Edwards (H.E.) grading is a classic method, but the most reliable approach requires lung biopsy tissue to make an accurate diagnosis. Recent advances in the miniaturization of both phased array and mechanical ultrasound devices have

resulted in alternative approaches to vascular imaging and have led to an increasing interest in “invasive” echocardiography (Isner et al. 1990; Valdes-Cruz et al. 1991; Goar et al. 1992; Pandian and Hsu, 1992; Sohn et al. 1994; Sugimura et al. 1994). Intravascular ultrasound (IVUS) imaging may provide in vivo histologic information regarding the structure and composition of the normal arterial wall and superimposed lesion (Gussenhoven

TABLE 1.
Summary of hemodynamic results, intravascular ultrasound findings and Heath-Edwards grading in 17 autopsy cases (7 with pulmonary hypertension and 10 without cardiopulmonary disease) and 5 in vivo cases

case	age (years)	diagnosis	PA pressure PVRI (mmHg) (unit.m ²)		cause of death	IVUS findings intima media		Heath-Edwards grade
Group I								
1	34	PPH	130/70 (93)	28	heart failure	‡	‡	4
2	27	PPH	120/50 (84)	25	heart failure	‡	‡	3
3	29	VSD	70/30 (50)	16	heart failure	‡	+	3
4	37	ASD	150/66 (94)	33.7	heart failure	‡‡	+	5
5	6 months	p/oTAPVR	100/45 (62)	20	heart failure	‡	+	2
6	15	DORV, p/oFontan			heart failure	+	+	1
7	14 days	TGA			heart failure	+	+	1
Gorup II								
8	3	leukemia			respiratory failure		one-layer	0
9	8	medulloblastoma			renal failure		one-layer	0
10	2	pyruvic acidemia			respiratory failure		one-layer	0
11	3	neuroblastoma			respiratory failure		one-layer	0
12	3	malignant lymphoma			MOF		one-layer	0
13	8	hemophilia			respiratory failure		one-layer	0
14	19	muscular atrophy			respiratory failure		one-layer	0
15	1	leukemia			respiratory failure		one-layer	0
16	1 month	intracranial hemorrhage			respiratory failure		one-layer	0
17	1	encephalo-myopathy			respiratory failure		one-layer	0
Gorup III (in vivo)								
18	5 months	PDA	63/35 (45)	9.2		+	+	2
19	1	VSD,CoA	80/40 (55)	9.3		+	+	1
20	6 months	VSD	70/20 (40)	4.4			one-layer	0
21	22	p/oVSD,PDA, Eisenmenger	115/50 (75)	19.4		‡‡	+	(-)
22	17	VSD, Eisenmenger	130/85 (90)	26.2		‡‡	+	(-)

PA: pulmonary artery; PVRI: pulmonary vascular resistance index; PPH: primary pulmonary hypertension; VSD: ventricular septal defect; ASD: atrial septal defect; TAPVR: total anomalous pulmonary venous return; DORV: double outlet right ventricle; TGA: transposition of the great arteries; p/o: post operation; CoA: coarctation of the aorta; PDA: patent ductus arteriosus; MOF: multiple organ failure

et al. 1989; Mario et al. 1992). However, this method has been adapted primarily for the coronary artery. IVUS findings from human pulmonary arteries were compared with corresponding histologic sections and the relationship between IVUS findings and H.E. grade was examined in this project.

Methods

Patients

The in vitro study utilized 40 segments of pulmonary arteries from 17 patients, between 14 days and 37 years old. In all patients the cardiac anatomy at autopsy was determined and histopathologic findings of the pulmonary artery were evaluated. The patients in the in vitro study were subdivided into 2 groups (Table 1).

Group I: [pulmonary hypertension group] Twenty pulmonary arterial segments from 7 patients with pulmonary hypertension (H.E. grade 1 to 5) were studied. Three patients had congenital heart disease with histopathologic pulmonary hypertension (H.E. grade 1 to 5). Two patients had primary pulmonary hypertension (H.E. grades 3 and 4). Two patients had undergone cardiac surgery.

Group II: [normal pulmonary pressure group] Twenty segments of pulmonary arteries from 10 patients without cardiopulmonary disease were analyzed. All 10 patients were free of cardiac disease at autopsy and had no histopathologic findings of pulmonary hypertension.

Group III: [in vivo study group] This group consisted of 5 patients with pulmonary hypertension (mean pulmonary

arterial pressure > 40 mmHg). Three (cases 18, 19 and 20) had undergone radical operations and lung biopsies, simultaneously. Case 18 was a 5-month-old girl with a patent ductus arteriosus and pulmonary hypertension. Case 19 was a 1-year-old boy with coarctation of the aorta, a ventricular septal defect and pulmonary hypertension. Case 20 was a 6-month-old boy who had a perimembranous ventricular septal defect with pulmonary hypertension. Lung biopsy specimens were not taken in 2 cases (cases 21 and 22). Case 21 was a 22-year-old female with complications of Eisenmenger syndrome due to a ventricular septal defect. Case 22 was a 17-year-old female with a post-operative ventricular septal defect, a patent ductus arteriosus and severe pulmonary hypertension (Eisenmenger syndrome).

IVUS imaging

A commercially available IVUS system (Endosonics Corp.) with 3.5 F catheters was used in this study. The catheter has a synthetic aperture with a 64-element array arranged circumferentially and operates at 20 MHz. The ultrasonic real-time scan provides a 360-degree display of a two dimensional image. The imaging of pulmonary arteries was performed at 22 °C in a saline solution (Gussenhoven et al. 1989; Mario et al. 1992). For the in vivo study, the catheter was introduced through a 6 F long sheath (Cordis Corp.) or advanced into the pulmonary artery using a guide wire. The catheter was set as distal as possible. Two observers reviewed, in a blinded fashion, the echo findings of 40 vessel segments recorded on S-VHS video tape (Fig. 1, Site A). The

Fig. 1. Schema of the pulmonary artery. Intravascular ultrasound was performed at site A. The presence or absence of intima and media-adventitia was determined by microscopic analysis (site A) and the pulmonary arteriole was evaluated by Heath-Edwards grading (site B). A histologic section (A') was obtained from a pulmonary artery of a patient with Eisenmenger syndrome due to an atrial septal defect (Case 4) with a corresponding echographic cross section (A). Ultrasound evidence of a plaque-like lesion due to hyperplasia of the intima was observed (arrows). Elastica van Gieson stain. Magnification 20 $\times$. A microphotograph of a pulmonary arteriole with medial atrophy and intimal fibrosis was classified as Heath-Edwards grade 5 (B). Elastica van Gieson stain. Magnification 200 $\times$.

following classification of echodensity (Gussenhoven et al. 1989) was used for the description of the ultrasound image: echolucent, minimal or no reflections (black); intermediate, soft reflections (grey); dense, bright reflections (white);

and hard, bright reflections with shadowing. Ultrasound dimensional measurements were made with an off-line computer analysis of the images. The following ultrasound dimensional measurements were calculated (Fig. 2): external

Fig. 2. A histologic cross section (C) obtained from a pulmonary artery of a patient with primary pulmonary hypertension (Case 1), a corresponding echographic cross section (A) and a schematic (B). A bright thick inner layer, a middle echolucent zone, and an outer bright layer are representative of the three-layered appearance. The microphotograph shows intimal hyperplasia and medial hypertrophy. Elastica van Gieson stain. Magnification $20\times$.

diameter, defined as the measurement across two diameters between the media-adventitia interface (Pigholi et al. 1986; Gussenhoven et al. 1989); and wall thickness, defined as the measured diameter from the leading edge of the intima to the media-adventitia interface (Pigholi et al. 1986; Gussenhoven et al. 1989). Eight sites were measured and

the mean of the 8 readings was used.

Histological analysis

Histological observation was carried out at the same sites that the pulmonary artery was examined with IVUS and at the distal pulmonary arteriole of the same branch. After the ultrasound studies of all 40 segments, the sites in

the arteries were marked with ink. The arteries were processed for routine paraffin embedding. Transverse sections (5 μ m thick) perpendicular to the longitudinal axis of the artery were cut at 1 mm intervals. The histologic sections were stained by the Elastin van Gieson stain technique. The Elastin van Gieson technique was used as counterstaining for elastin fibers, muscle and connective tissue. In addition, sections were stained by the hematoxylin azophloxine technique to delineate connective tissue. The presence or absence of intima and media-adventitia was determined by microscopic analysis (Fig. 1, site A) and distal pulmonary arteries were evaluated by the Heath Edwards grade (Fig. 1, site B) (Heath and Edwards, 1958). The external diameter of an artery was measured at two diameters from the distance between the external elastic laminae (Hislop and Reid, 1973; Haworth and Reid, 1977; Haworth et al. 1977; Haworth and Lynne, 1978). The wall thickness was measured from the external to internal elastic lamina at 8 sites using a Cosmozone (NIKON INC.) and the mean of the 8 readings was used (Hislop and Reid, 1973; Haworth and Reid, 1977; Haworth et al. 1977; Haworth and Lynne, 1978). All histologic evaluations were performed by one observer. The percentage wall thickness was then calculated using both the IVUS findings and the histological data by this formula (Hislop and Reid, 1973; Haworth and Reid, 1977; Haworth et al. 1977; Haworth and Lynne, 1978): percentage of wall thickness = $(2 \times \text{wall thickness} / \text{external diameter}) \times 100$.

Statistical analysis

The percentage of arterial wall thickness calculated from the IVUS findings were compared with that calculated from the histopathological findings by linear regression analysis. Differences with a *p* value < 0.05 were considered significant.

Results

All 20 pulmonary artery segments from the group I patients had a three layered appearance when viewed with a phased array 20 MHz IVUS catheter. Figure 2, illustrates the IVUS imaging (A) and schema (B) of a pulmonary artery from a patient with primary pulmonary hypertension. A bright thick inner layer, a middle echolucent zone, and an outer bright layer are representative of the three-layer appearance. Histologic examination of this segment of the pulmonary artery (Fig. 2C) revealed intimal hyperplasia and medial hypertrophy. Thirteen segments from 4 patients with severe pulmonary hypertension (H.E. grade 3 to 5) had ultrasound evidence of an increased thickness in the bright inner layer due to intimal hyperplasia and had histological evidence for hyperplasia of the intima (Figs 1 and 2). Four segments from patient 4 (Eisenmenger syndrome due to an atrial septal defect: Fig. 1B, H.E. grade 5) displayed ultrasound evidence of plaque-like lesions (Fig. 1A) due to hyperplasia of the intima (Fig. 1A'). Seven segments from the 3 patients with mild pulmonary hypertension (H.E. grade 1 to 2) had ultrasound evidence of a thickened echolucent zone due to

Fig. 3. A histologic section (B) obtained from a pulmonary artery of a patient with a post operative total anomalous venous return (Case 5) and a corresponding echographic cross section (A). The media hypertrophy appeared as a bright thick inner layer, a middle echolucent zone and an outer bright layer. Elastica van Gieson stain. Magnification 20 $\times$. A microphotograph of a pulmonary arteriole with hypertrophy of the media and without an intimal reaction was classified as Heath-Edwards grade 2 (C). Elastica van Gieson stain. Magnification 200 $\times$.

medial hypertrophy and had histological evidence of hypertrophy of the media with no intimal reaction (Fig. 3). A significant correlation was observed between the percentage of wall thickness of all the segments from group I measured by IVUS and that measured from histo-

logical examination ($y=1.05x+1.91$; $r=0.89$, $p<0.001$ $n=20$). All 20 pulmonary arterial walls (Group II) produced soft echoes which did not allow ultrasonic distinction between the intima, media and adventitia (Fig. 4). The in vivo study of two pulmonary arteries (Cases 18 and

Fig. 4. A microphotograph of a histologic section (B) and the corresponding intravascular ultrasound cross section obtained from the pulmonary artery of a patient without pulmonary hypertension (Case 8). The intima, media and adventitia cannot be distinguished with ultrasound. Elastica van Gieson stain. Magnification $20\times$.

Fig. 5. (In vivo) An intravascular ultrasound image from the pulmonary arteries of a patient with mild pulmonary hypertension (Case 18; Heath-Edwards grade 2) demonstrated a three-layered appearance.

Fig. 6. (In vivo) An intravascular ultrasound image from the pulmonary arteries of a patient with pulmonary hypertension (Case 20; Heath-Edwards grade 0) had a one-layer appearance.

Fig. 7. (In vivo) An intravascular ultrasound image from the pulmonary arteries of a patient with severe pulmonary hypertension (Case 21) demonstrated a plaque-like lesion.

19) showed a three-layered appearance (Fig. 5), and that of another (Case 20) showed one layer without distinction between the intima, media and adventitia (Fig. 6). The lung tissue from a patient with coarctation of the aorta and a ventricular septal defect with pulmonary hypertension had medial hypertrophy on histologic examination (Case 19). The lung tissue from a patient with a patent ductus arteriosus (Case 18) with pulmonary hypertension showed medial hypertrophy with circumferential intimal proliferation. Lung tissue from a patient with a ventricular septal defect (Case 20) and pulmonary hypertension had a normal structure without medial hypertrophy and intimal proliferation. Two patients with severe pulmonary hypertension (Cases 21 and 22) had ultrasound evidence of plaque-like lesions (Fig. 7).

Discussion

Considering the findings obtained in the in vitro study, we compared the IVUS findings in human pulmonary arteries and the corresponding histologic sections, and the relationship between the IVUS findings and the Heath Edwards grade. The in vivo study essentially supported the in vitro observations.

Diagnostic accuracy

A comparison of the IVUS images and the corresponding histological cross sections showed that the characteristics of the echo images of hypertensive lesions are related to the histological composition of the lesions. A previous report described that the IVUS image of the pulmonary artery without pulmonary hypertension had a one-layer appearance (Gussenhoven et al. 1989; Mintz et al. 1992; Porter et al. 1993). Accurate distinction was possible between the IVUS findings of the pulmonary arteries with and without pulmonary hypertension in the present study. In group I, the IVUS images had a three-layered appearance due to hypertrophy of the media or hyperplasia of the intima or both. However, the IVUS characteristics of the intima, media and adventitia were comparable in group II. This observation is in accordance with that of Haworth, who suggested that with increasing pulmonary hypertension, vessels of all sizes tended to progressively increase in wall thickness (Haworth and Reid, 1977; Haworth et al. 1977; Haworth and Lynne, 1978).

Relationship between IVUS findings and Heath Edwards grades

A clear difference between mild pulmonary hypertension (H.E. grades 1 and 2) and severe pulmonary hypertension (H.E. grades 3, 4 and 5) was observed with IVUS findings involving the presence or absence of intimal hyperplasia. However, the pulmonary hypertensive changes could not be quantified into six grades (Heath and Edwards, 1958) using IVUS images, because IVUS could not detect plexiform lesions, local dilatation lesions and pulmonary hemosiderosis (Heath and Edwards, 1958). These changes occurred only in small pulmonary arteries ($<300\mu\text{m}$) (Heath and Edwards, 1958; Naeye, 1966; Wagenvoort et al. 1968; Haworth and Reid, 1977; Haworth et al. 1977; Haworth and Lynne, 1978; Yamaki and Horiuchi, 1979; Rabinovitch et al. 1980; Yamaki et al. 1980) into which the IVUS catheter could not be inserted.

Limitation of this study

The IVUS findings could not be compared with the hemodynamic studies. Six cases in group I were examined in a hemodynamic study, however, these studies were not performed simultaneously (from 1 month to 6 years before autopsy), thus there may have been a change in the severity of the pulmonary hypertension by the time of the IVUS study. One patient (transposition of the great arteries; TGA) and all patients in group II were not included in the hemodynamic study. In this study, 16 arteries, except the one case (TGA), were fixed with formalin. Intimal studies indicated that formalin had a major influence on the echographic characteristics (Pigholi

et al. 1986; Gussenhoven et al. 1989). However, the findings obtained with fresh material were not different from those obtained in the corresponding segment fixed with formalin. It was difficult to perform a lung biopsy at the site of the IVUS images. The IVUS catheter size was larger than the size of the pulmonary artery, as classified by the Heath Edwards grade.

Possible clinical application

Further developments in IVUS imaging could provide clinically important information about pulmonary hypertension. Smaller (equal in size to a doppler guide wire) and higher frequency IVUS catheters may enhance the diagnostic ability of IVUS to the point that it is equal to that of lung biopsy in future histological diagnosis.

Conclusions

Intravascular ultrasound imaging helps to directly visualize the pulmonary vascular system in patients with pulmonary hypertension.

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