Morphometric Study of Pulmonary Arterial Changes in Pulmonary Langerhans Cell Histiocytosis

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- **Context.**—Pulmonary hypertension (PHT) is a complication of pulmonary Langerhans cell histiocytosis (PLCH); however, the pathogenesis remains largely unknown. Few studies have evaluated histopathologic changes in pulmonary arteries (PAs) of patients with PLCH; systematic quantification of arterial remodeling has yet to be undertaken.

- **Objective.**—To quantify the extent of arterial remodeling among patients with PLCH through morphology and to correlate these results with pertinent clinical parameters.

- **Design.**—Patients with PLCH were identified from institutional files (1995–2015) along with age-, sex-, and smoking status–matched controls. Morphometric analysis of intimal and medial thickness of small to medium PAs was performed in patients with PLCH within PLCH lesions (lesional) and away from PLCH lesions (nonlesional) and controls. Paired measures were compared with Wilcoxon signed rank tests.

- **Results.**—Twenty-five patients with PLCH (14 men: median age, 46 years; interquartile range, 37–55 years) and 25 controls were included. The lesional arteries of patients with PLCH demonstrated thicker PA intima and media than controls ($P < .001$ and $P < .001$, respectively), as did PLCH nonlesional arteries compared to controls ($P < .001$ and $P < .001$, respectively). The PA intima and media were thicker within the PLCH lesions than nonlesional arteries ($P = .02$ and $P = .002$, respectively). Patients with PLCH-related PHT had a worse prognosis than those without PHT ($P = .04$; hazard ratio, 4.5 [1.1, 22.2]). Echocardiography parameters including right atrial size ($P = .007$), estimated right atrial pressure ($P = .01$), and right ventricular systolic pressure ($P = .01$) were inversely associated with survival.

- **Conclusions.**—Our findings suggest that factors other than direct vascular obstruction or inflammatory cell infiltration contribute, at least in part, to the vascular remodeling in PLCH.

(Pulmonary Langerhans cell histiocytosis (PLCH) is an interstitial lung disease (ILD) occurring nearly exclusively in smokers. This disease is usually characterized histopathologically by bronchiolocentric nodules composed of Langerhans cells and mixed inflammatory cells, including eosinophils and occasional neutrophils. Secondary pulmonary hypertension (PHT) is a common cause of morbidity and increased mortality in patients with PLCH and has been reported in up to 92% of these patients. Although PHT is a common complication of PLCH with serious implications, mechanisms behind its development are not fully understood.

Within the broad spectrum of ILDs, PHT frequently results from the interplay of fibrosis-mediated parenchymal and capillary destruction, endothelial dysfunction, hypoxic vasoconstriction, and the production of leukotrienes. These changes are known to correlate with the clinical parameters of decreased oxygen saturations and pulmonary function test (PFT) abnormalities. Similarly, PLCH-related PHT may, in part, result from similar pathologic mechanisms; however, prior studies indicate that unlike other ILDs, PLCH-related PHT does not match the degree of hypoxemia or PFT abnormalities. In addition, the severity of PHT among patients with PLCH often surpasses that of their idiopathic pulmonary fibrosis counterparts. These observations suggest that other disease-related mechanisms may be contributing to the development of PHT among patients with PLCH.

Theorized alternative mechanisms for PLCH-related PHT include the elaboration of cytokines by the Langerhans cells of PLCH, which may exert both localized and potentially widespread vascular remodeling. Direct invasion by immune cells (both Langerhans cells and the associated inflammatory milieu) has also been observed and is thought to influence vascular changes. Destruction of pulmonary parenchyma secondary to cystic changes in PLCH may lead to further, additive insults.

A systematic evaluation of pulmonary arteries and arterioles with comparison to matched controls may assist in delineating the mechanisms contributing to PLCH-related PHT. While semiquantitative measurements of myointimal fibroplasia have been documented in a subset of patients with PLCH, a formal morphometric assess-
ment has not been undertaken. Herein, we present a morphometric study of the pulmonary arteries with unique insights into the vascular changes present in PLCH and correlate these findings with pertinent clinical parameters.

**MATERIALS AND METHODS**

**Patient and Control Cohort**

Surgical pathology and autopsy archives were queried for patients with a diagnosis of PLCH (1995–2015). Cases were reviewed by a thoracic pathologist for diagnostic confirmation. Age-, sex-, and smoking status–matched controls were identified from medical autopsies. Smoking status was defined as whether or not there was a documented history of smoking, regardless of duration. The medical records were reviewed for salient clinical features, including age, sex, smoking history, PFT data, echocardiographic measurements, and right heart catheterization data. The study was approved by the Institutional Review Board (No. 15-003943).

**Histologic Review**

Cases were semiquantitatively evaluated on routine hematoxylin-eosin–stained sections for cellularity of PLCH lesions (“none,” “fibrous-predominant,” “intermediate,” or “florid” based on the composition of 0% cellularity [Figure 1, A], greater than 0% to 10% [Figure 1, B], greater than 10% to 50% [Figure 1, C], and greater than 50% [Figure 1, D], respectively). The degree of vascular invasion by lesional or immune cells was semiquantitatively categorized as “none,” “few,” “moderate,” and “extensive.”

PLCH-related changes of pulmonary parenchyma, including microscopically apparent cystic changes, bronchiolocentric scars and fibrosis, and background smoking-related changes including rarefication of pulmonary parenchyma (emphysematous change) and/or respiratory bronchiolitis, were recorded. Ancillary studies used for diagnosis, including CD1a (clone MTB1, Leica Biosystems, Buffalo Grove, Illinois), S100 (polyclonal, Dako, Santa Clara, California), and langerin (clone 12D6, Leica Biosystems), were recorded. BRAF V600E (clone VE1, Spring Bioscience, Pleasanton, California) immunohistochemistry was performed in a subset of the cases.

**Analysis of Vasculature and PLCH Lesions**

Verhoeoff–Van Gieson stains were obtained for all lesional and control blocks to aid in the evaluation of pulmonary vasculature. Pulmonary arteries and arterioles (collectively referred to hereafter as “arteries,” “artery,” or “arterial”) were measured within the lesion (“lesional”) and away from the lesion (“nonlesional”) in PLCH specimens. Nonlesional arteries were defined as at least 1 microscopic field (using an ×10 objective; ×100 original magnification) away from a PLCH lesion. Arterial measurements from age-, sex-, and smoking status–matched autopsy specimens were defined as “controls.”

Figure 1. Range in lesional cellularity. Cases are graded semiquantitatively for Langerhans cell lesional cellularity as none (A), fibrous-predominant (B), intermediate (C), and florid (D) (hematoxylin-eosin, original magnification ×100).
Two-dimensional measurements of lesional slides were performed by using imaging software (Olympus cellSens Software, Center Valley, Pennsylvania). Each lesion was measured along its long and short axes, with the product of those 2 measurements equaling the area of the lesion. Lesional areas were summed (where applicable) to derive the total PLCH area.

Lesional, nonlesional, and control pulmonary arteries available in cross-section on histologic slides were evaluated with Verhoeff–Van Gieson stain by imaging software. The vessel external diameter (ED) was measured from the external elastic lamina (EEL) to the opposing EEL across the short axis of the vessel (Figure 2, A). Internal diameter (ID) was defined as the distance from internal elastic lamina (IEL) to opposing IEL (Figure 2, B). Medial thickness (M1 and M2) is measured on either side of the vascular lumen from EEL to IEL (Figure 2, C). Intimal thickness (I1 and I2) is likewise measured on either side of the vessel from IEL to vascular lumen (Figure 2, D) (original magnification ×400 [A through D]).

%MT = \[
\frac{(M1 + M2)}{ED} 
\times 100
\]

%IT = \[
\frac{(I1 + I2)}{ID} 
\times 100
\]

Figure 2. Lesional, nonlesional, and control pulmonary arteries and arterioles available in cross-section on histologic slides are evaluated with Verhoeff–Van Gieson stain. The vessel external diameter (ED) is defined as the distance between external elastic lamina (EEL) across the short axis of the vessel (A). Internal diameter (ID) is defined as the distance from internal elastic lamina (IEL) to opposing IEL (B). Medial thickness (M1 and M2) is measured on either side of the vascular lumen from EEL to IEL (C). Intimal thickness (I1 and I2) is likewise measured on either side of the vessel from IEL to vascular lumen (D) (original magnification ×400 [A through D]).

The percentage difference was calculated between the matched specimen types for %IT and %MT (PLCH lesion – control, PLCH nonlesion – control, PLCH lesion – PLCH nonlesion).

**Histologic Calculations**

Percentage medial thickness (%MT) was calculated as the sum of each of the measured medial thicknesses (M1 and M2) divided by the external vascular diameter (ED), and subsequently multiplied by 100 [%.MT = [(M1+M2)/ED] × 100]. Percentage intimal thickness (%IT) was similarly calculated as the sum of the measured intimal thicknesses (I1 and I2) divided by the internal vascular diameter (ID) multiplied by 100 [%.IT = [(I1+I2)/ID] × 100]. The percentage difference was calculated between the matched specimen types for %IT and %MT (PLCH lesion – control, PLCH nonlesion – control, PLCH lesion – PLCH nonlesion).
Continuous data were summarized with medians or means and interquartile ranges or ranges, as appropriate. Categorical data were summarized with frequencies and percentages. The percentage difference in %IT and %MT between the specimen types was compared by using Wilcoxon signed rank tests. The remaining clinical features were compared between groups (PLCH cases versus controls, PLCH cases with versus without PHT) by using Kruskal-Wallis tests for continuous or ordinal data, and using Fisher exact tests for categorical data. Associations between continuous variables were assessed with nonparametric Spearman correlations. Associations between clinical parameters and overall survival from time of PLCH diagnosis to death (among PLCH cases only) was performed with Cox proportional hazards regression. Hazard ratios, 95% confidence intervals, and likelihood ratio test P values were reported from the survival models. P values less than .05 were considered statistically significant. All analyses were performed with SAS version 9.4 (SAS Institute Inc, Cary, North Carolina).

RESULTS

Patient Demographics

Twenty-five patients with PLCH and 25 controls were included in the study. Clinical characteristics of all subjects are summarized in Table 1.

| Table 1. Clinical Characteristics of Patients With Pulmonary Langerhans Cell Histiocytosis (PLCH) and Controls |
|---------------------------------------------------------------|
| PLCH, n = 25 | Controls, n = 25 |
| Male, n (%) | 14 (56) | 14 (56) |
| Age at diagnosis, median (interquartile range), y | 46 (37–55) | 47 (36–55) |
| Smoking history, median (interquartile range), pack-years | 27 (20–38)a | 25 (18–40)b |
| No. of patients in NYHA class 0, I, II, III, IV | 1, 11, 2, 6, 1a | 1, 7, 0, 1, 1b |

Abbreviation: NYHA, New York Heart Association.

a Data available for 21 patients with PLCH.

b Data available for 11 controls.

Statistical Analysis

Histologic Features

PLCH lesions ranged in cellularity and histopathologic changes, as illustrated and summarized in Figure 1, A through D, and Table 2. Likewise, there was variability in the degree of vascular invasion by lesional cells and immune cells, as illustrated and summarized (Figure 3, A and B; Table 2). Emphysematous changes were noted in 17 of the 25 controls (68%; 3 severe, 3 moderate, and 11 mild) and respiratory bronchiolitis in 13 controls (52%).
Eleven of 25 PLCH specimens (44%) used immunohistochemical stains to support a histopathologic diagnosis at the time of initial case evaluation. CD1a staining was performed in 10 of 25 cases (40%), S100 in 6 of 25 cases (24%), and langerin in 4 of 25 (16%) cases. All 3 markers were expressed in the Langerhans cells. The results of BRAF V600E immunohistochemistry are summarized in Table 2.

### Pulmonary Arteries

A median of 5 arteries (interquartile range [IQR], 3–6.5) was measured within PLCH lesions and a median of 4 arteries (IQR, 3–10) within the nonlesional parenchyma of PLCH cases. Among controls, a median of 9 arteries (IQR, 4–10) was measured.

Results of morphometry of arteries in patients with PLCH and controls are presented in Table 3 and illustrated in Figure 4, A through C. Lesional artery %IT and %MT were significantly greater than those of nonlesional arteries for patients with PLCH (P = .002 and P = .02, respectively) and when compared to arteries in controls (P < .001 and P < .001, respectively). Nonlesional arteries also had significantly greater %IT and %MT than controls (P < .001 and P < .001) (Table 3).

### Clinical Features

All patients with PLCH and controls had a history of smoking. Quantification of smoking pack-years was available for 21 (of 25) patients with PLCH and for 11 (of 25) controls. Clinical characteristics including age, sex, smoking status, and New York Heart Association (NYHA) Functional Classification findings are summarized in Table 1. Chest imaging was abnormal (including nodularity, cystic changes, and/or ground glass opacities) for 24 of the 25 patients with PLCH (96%). Of the 20 controls with chest radiography performed, 10 (50%) had an abnormal result, while for the remaining half the result was reported as normal.

Pulmonary function testing data were available for 19 (of 25) patients with PLCH and 3 (of 25) control patients. Eight patients with PLCH (32%) and 3 controls (12%) had a clinical diagnosis of PHT (none of the controls with a diagnosis of PHT had PFT data available). Of the 3 controls, PFTs were interpreted as normal, nonspecific abnormalities, and moderate obstructive physiology (n = 1, each). Forced expiratory volume in 1 second (FEV₁) (P = 0.04), forced vital capacity (FVC; %) (P = 0.03), exercise oximetry (%) (P = .01), and diffusing capacity of the lungs for carbon monoxide (DLCO; %) (P = 0.02) were lower among PLCH patients with PHT (Table 4). Rest oximetry was relatively preserved in both the PLCH group (data available in 14 of 25 cases) and the control group (data available in 2 of 25). Exercise oximetry was available in a subset of patients (12 patients with PLCH and 2 control patients) (Table 4). Echocardiographic parameters were evaluated for patients with PLCH and controls (Table 5).

### Correlation of Clinical Parameters With Histologic Findings

#### Overall Correlation of Oximetry With PLCH Histologic Parameters.—
Rest oximetry parameters did not correlate with lesional %IT (Spearman correlation, −0.085) and %MT (Spearman correlation, −0.099) or nonlesional %MT (Spearman correlation, −0.075) in patients with PLCH. Likewise, exercise oximetry did not show any correlation with Spearman correlation coefficients of 0.186 and 0.421 for lesional %IT and %MT, respectively, and of 0.525 and −0.082 for nonlesional %IT and %MT, respectively, in these patients. DLCO failed to correlate with lesional %IT and %MT (0.002 and 0.052) and nonlesional %MT (−0.391). However, nonlesional %IT did correlate with rest oximetry (Spearman correlation, 0.764) and DLCO (Spearman correlation, 0.724).

#### Smoking and Histologic Findings.—
Smoking pack-years were not associated with histologic parameters, including lesional arterial measurements (%IT Spearman correlation, −0.128; %IT, 0.108) and nonlesional measurements (%MT, −0.173; %IT, 0.096). Lesional extent (overall size of PLCH lesion) also did not correlate with smoking pack-years (Spearman correlation, 0.062).

#### Overall Correlation of PFT and Echocardiographic Features With PLCH Parameters.—
FEV₁ and FVC results did not correlate with %IT (Spearman correlation, 0.156 and 0.153, respectively) or %MT (−0.010 and 0.125) in lesional arteries. Lack of association was also noted in nonlesional %MT (FEV₁, −0.131; FVC, −0.165). However, there was a direct correlation between %IT in nonlesional arteries and FEV₁ and FVC (FEV₁ Spearman correlation of 0.893, P < .001; FVC Spearman correlation of 0.825, P < .001).

Echocardiographic features including right ventricular systolic pressure (RVSP) and right atrial pressure did not correlate with histologic parameters, including lesional %MT (Spearman correlation, −0.090 and 0.115, respectively), nonlesional %MT (0.056 and −0.021, respectively), and nonlesional %IT (Spearman correlation, −0.420 and −0.066, respectively). Lesional %IT was not significantly associated with RVSP, but trended in that direction (Spearman correlation, −0.458; P = .09). Lesional %IT was not associated with right atrial pressure (Spearman correlation, −0.347).

#### Extent of PLCH Lesions and Clinical Diagnosis of PHT.—
PLCH patients with a clinical diagnosis of PHT (n = 8) had a median total PLCH area of 74.6 mm² (IQR, 45.4–138.5) as compared to those PLCH patients without a
clinical diagnosis of PHT (median, 45.3 mm²; IQR, 28.1–67.9) \( (P = .13) \). Histologically identified cystic changes were correlated with a clinical diagnosis of PHT \( (P = .04) \) in patients with PLCH.

**Patient Outcome**

PLCH patients with PHT had a worse prognosis than those without PHT \( (P = .04; \text{hazard ratio}, 4.5 [1.1, 22.2]) \). Overall survival was inversely correlated with right atrial size \( (P = .007) \), estimated right atrial pressure \( (P = .01) \), and RVSP \( (P = .01) \).

Overall survival did not correlate with PFT parameters or hypoxia, nor was survival affected by lesional activity for patients with PLCH \( (P = .18) \), BRAF immunoreactivity \( (P = .86) \), or the presence and severity of vascular invasion by immune cells \( (P = .14) \).

There was no significant association of overall survival with nonlesional arterial changes observed on histologic evaluation \( (%IT P = .51; \%MT P = .18) \). Overall survival was worse in cases with a lower lesional artery %IT \( (P = .008; \text{hazard ratio}, 0.91 \text{ for } 1 \text{ percentage point increase } [0.82, 0.98]) \). This relationship was not significant for lesional artery %MT \( (P = .10) \), though the estimated hazard ratio was of the same magnitude and direction \( (0.91) \).

**DISCUSSION**

We observe that pulmonary arteries within active lesions of PLCH are characterized by thicker intima and media than pulmonary arteries that are distant from active lesions. We also show that intima and media of pulmonary arteries within PLCH lesions and distant from PLCH lesions are thicker than age-, sex-, and smoking status–matched controls. To our knowledge, this study is the first to quantitatively document pulmonary arteries in patients with PLCH.

The pathogenesis of pulmonary arterial remodeling within active lesions of PLCH is not well understood. Remodeling of pulmonary arteries may result directly from infiltrates of Langerhans cells and/or immune cells. We observe direct invasion of arteries by Langerhans cells and immune cells in 73% of lesions with greater than 10% lesional cellularity. Our findings confirm previous studies that show similar cellular inflammation of the lesional arterial walls, although previous reports do not comment on the number of patients in which this finding occurs.\(^2,7,11\) However, direct vascular invasion by Langerhans cells and/or immune cells likely does not entirely account for the pulmonary arterial changes present in PLCH. We find that pulmonary arteries distant from the active PLCH lesions also have significantly greater intimal and medial thickness than non-PLCH controls. This finding supports the conclusion that another process integral to vascular remodeling is occurring. The presence of circulating cytokines, elaborated by Langerhans cells and/or their associated inflammatory milieu, may, at least in part, also play a role in the pathogenesis of the arterial changes in PLCH. Certain molecules, including transforming growth factor-\( \beta 1 \), have been shown to increase in patients with PLCH.\(^9\) Moreover, PLCH lesions produce interleukin 1, interleukin 6, and platelet-derived growth factor,\(^10\) the latter of which is increased in the arteries of patients with severe idiopathic pulmonary arterial hypertension compared to control specimens.\(^13\) Adding credence to this concept is recent evidence of the interplay of neoplastic cells and

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**Figure 4.** Examples of lesional, nonlesional, and control pulmonary arteries and arterioles. An arteriole within a pulmonary Langerhans cell histiocytosis (PLCH) lesion, showing marked intimal thickening (A). A pulmonary artery in a patient with PLCH, but within unaffected lung, demonstrating intimal thickening without significant medial thickening (B). Pulmonary artery in a control patient (C) (Verhoeff–Van Gieson, original magnifications \( \times 400 \) [A] and \( \times 200 \) [B and C]).
cytokines, proposed to lead to arterial changes akin to PHT in patients with primary lung cancer. While these cytokines and growth factors have been implicated in the pathogenesis of PHT, the extent to which these cytokines directly promote vascular remodeling or incite fibrosis (with secondary vascular response) remains unclear.

Current evidence suggests that cigarette smoke itself is associated with vascular remodeling. Since PLCH is known to occur predominantly among smokers, we controlled for this potential confounder by matching our PLCH patients with age-, sex-, and smoking status–matched controls. Smoking history (pack-years) was similar between the PLCH and control cohorts, thereby suggesting that smoking status is not entirely responsible for the pulmonary arterial changes that appear to occur in the lungs of patients with PLCH.

Chronic hypoxic state is also known to elicit vasoconstriction and subsequent arteriopathy. In our study, DLCO is significantly lower in PLCH patients with a clinical diagnosis of PHT than PLCH patients without PHT, confirming similar results in previous studies. However, our PLCH cohort has relatively normal percentage oxygen saturation both at rest and with exercise (medians of 95.5% and 93.5%, respectively). Furthermore, rest and exercise oximetry parameters do not correlate with lesional and nonlesional %IT and %MT in our study, making the effects of chronic hypoxia a less likely etiology. Therefore, PHT in PLCH might differ from other well-defined mechanisms of PHT within the category of ILD. While there is mechanistic overlap, PLCH patients with PHT generally have PFTs that fail to correlate with PHT severity as they do in other ILD cases.

In contrast to our PFT findings and those previously published by Vassallo and Crausman, Chaowalit did not find a correlation between PFT parameters and PHT severity. However, our PLCH patients with PHT do have a significant difference in PFT parameters, including DLCO, FEV1, FVC, and exercise oxygenation, when compared to those without a diagnosis of PHT. The progression of PLCH lesions results in cystic changes, reflected as an obstructive pattern on PFTs. Our data suggest that parenchymal destruction may at least contribute to the development of PHT in patients with PLCH. Bolstering this theory is the fact that all patients with a clinical diagnosis of PHT in our study have cystic changes on histologic review, and that cystic changes were statistically more likely to be seen in patients with a diagnosis of PHT. Correspondingly, we found that right atrial enlargement, right atrial pressure, and RVSP are all associated with decreased survival. Therefore, the diagnosis of PHT in this patient population is important.

### Table 4. Results of Pulmonary Function Testing

| Pulmonary Function Test Parameter | Control Without Clinical Diagnosis of PHT | PLCH Without Clinical Diagnosis of PHT | PLCH With Clinical Diagnosis of PHT | Controls: n | PLCH with PHT: n | P Value: PLCH With Versus Without PHT Diagnosis |
|----------------------------------|-------------------------------------------|----------------------------------------|-----------------------------------|-------------|-----------------|-----------------------------------------------|
| Clinical diagnosis of PHT, n (%) | 3 (12) | 8 (32) | 8 (32) | n/a | n/a | .11 |
| FEV1, %, median (IQR) | 41, 58, 94 (51–97) | 88 (68–99) | 51 (39–73) | .04 |
| FVC, % predicted, median (IQR) | 62, 92, 104 (64–105) | 96 (89–106) | 71 (40–91) | .03 |
| FEV1/FVC ratio | 30.2, 76.4, 82.2 (60–81) | 78 (67–81) | 60 (55–81) | .11 |
| PFT interpretation | | | | | | |
| Normal | 1 | 6 | 6 | 0 | n/a |
| Nonspecific | 1 | 0 | 0 | 0 | n/a |
| Obstructive physiology | | | | | | |
| Mild | 0 | 6 | 3 | 3 | n/a |
| Moderate | 1 | 2 | 0 | 2 | n/a |
| Severe | 0 | 2 | 1 | 1 | n/a |
| Restrictive physiology | | | | | | |
| Mild | 0 | 2 | 2 | 0 | n/a |
| Moderate | 0 | 0 | 0 | 0 | n/a |
| Severe | 0 | 1 | 0 | 1 | n/a |
| Oximetry at rest, % saturation, median (IQR) | 91, 95 (93–98) | 96 (95–98) | 95 (93–96) | .50 |
| Exercise oximetry, % saturation, median (IQR) | 88, 95 (91–97) | 96 (94–98) | 91 (87–92) | .01 |
| DLCO, % predicted, median (IQR) | 23, 56, 66 (36–73) | 71 (43–79) | 40 (10–46) | .02 |

Abbreviations: DLCO, diffusing capacity of the lungs for carbon monoxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range; n/a, not applicable; PFT, pulmonary function test; PHT, pulmonary hypertension; PLCH, pulmonary Langerhans cell histiocytosis.

* Three control patients had PFT. None of these patients had a diagnosis of PHT; however, 3 other control patients (not included in the table owing to lack of PFT data) did have a diagnosis of PHT.

* Controls: n = 3 (raw data values listed); PLCH: n = 19; PLCH without PHT: n = 12; PLCH with PHT: n = 7.

* Controls: n = 2 (raw data values listed); PLCH: n = 14; PLCH without PHT: n = 9; PLCH with PHT: n = 5.

* Controls: n = 2 (raw data values listed); PLCH: n = 7; PLCH without PHT: n = 7; PLCH with PHT: n = 5.

* Controls: n = 3 (raw data values listed); PLCH: n = 17; PLCH without PHT: n = 11; PLCH with PHT: n = 6.

* Denotes significant P value. For controls in the overall cohort with pulmonary hypertension, individual values are listed in place of the median and IQR given the low number of applicable cases (n = 3).
graphic parameters demonstrated marked abnormalities in the subset of PLCH patients with PHT, making it an effective, noninvasive screening tool. Notably, we found the subset of PLCH patients with PHT, making it an enigmatic. Further studies would be required to confirm that this finding is not an aberrant statistic and to draw definitive conclusions from these data.

**Limitations**

Despite being one of the largest studies to investigate vascular changes in PLCH, the overall study population is still relatively small. Larger, possibly multi-institutional, studies are needed to expand our knowledge on vascular changes in PLCH. Furthermore, while patients with PLCH are usually “treated” by smoking cessation, some patients require steroids, immunosuppressive therapy, or even lung transplant. Our outcome data are not adjusted for these different treatment options owing to too few patients in each category for meaningful statistical analysis. Instead our study focuses on objective measurements of clinical and morphologic features of PLCH.

Given that PLCH specimens were derived from surgical cases, minimal uninvolved lung parenchyma was available for analysis because of a desire to preserve as much normal parenchyma as possible at the time of surgery. The authors were unable to find precedence regarding an accepted definition of “uninvolved” tissue. For this reason, uninvolved lung tissue was defined before the study as a single ×10 objective distance (=100 magnification) from the nearest PLCH lesion. While not an evidence-based distance, this definition provided a standardized approach to classifying lesional tissue from nonlesional parenchyma.

While these data provide novel insights into the possible mechanisms of PHT in patients with PLCH, hitherto undocumented, further studies to evaluate the identified etiologies are needed. Specifically, investigations into the role of circulating cytokines on vascular remodeling in PLCH and the mechanistic consequences of vascular invasion by Langerhans cells and associated inflammation would prove integral to our understanding of PHT in these patients.

**CONCLUSIONS**

Herein we report a quantitative discussion on arterial changes in patients with PLCH compared to age-, sex-, and smoking status–matched controls. Using morphometry techniques we find that arterial media and intima are substantially thicker in lesional arteries than in nonlesional pulmonary arteries in patients with PLCH. We furthermore find nonlesional arteries to be significantly thicker than those of matched controls. Although our findings do not correlate with other clinical metrics defining PHT severity, the results of our study provide unique insight into the potential pathogenesis of PHT in patients with PLCH.

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**Table 5. Echocardiographic Parameters**

| Echocardiographic Parameter | PLCH Overall | PLCH Without Clinical PHT | PLCH With Clinical PHT | Controls Overall | PLCH PHT Versus Non-PHT Control | PLCH PHT Versus Non-PHT Control | PLCH PHT Versus Non-PHT Control |
|----------------------------|-------------|--------------------------|------------------------|----------------|-------------------------------|--------------------------------|--------------------------------|
| Right atrial enlargement, n/N (%) | 8/15 (53) | 2/7 (29) | 6/8 (75) | 5/13 (39) | .48 | .13 | P > .99 | .18 |
| Right atrial pressure, median (IQR), mm Hg | 5 (5–14) | 5 (5–5) | 12 (5–18) | 5 (5–20) | .68 | .06 | .15 | .61 |
| Right ventricular enlargement, n/N (%) | 13 (81) | 8 (100) | 5 (63) | 9 (56) | .25 | .2 | .05 | P > .99 |
| Normal | 0 (0) | - | - | 4 (25) |
| Mild | 1 (6) | - | 1 (12) | 2 (13) |
| Moderate | 2 (13) | - | 2 (25) | 1 (6) |
| Severe | 30 (23–60) | 24 (23–29) | 60 (48–67) | 41 (32–60) | .22 | .004 | .002 | .24 |

Abbreviations: IQR, interquartile range; n/N, number with specified parameter/number evaluated; PHT, pulmonary hypertension; PLCH, pulmonary Langerhans cell histiocytosis.

* P values for right atrial pressure and right ventricular systolic pressure by Kruskal-Wallis test; size by Fisher exact test.

**Table 6. Echocardiographic Parameters**

| Parameter | PLCH Overall | PLCH Without Clinical PHT | PLCH With Clinical PHT | Controls Overall | PLCH PHT Versus Non-PHT Control | PLCH PHT Versus Non-PHT Control | PLCH PHT Versus Non-PHT Control |
|-----------|-------------|--------------------------|------------------------|----------------|-------------------------------|--------------------------------|--------------------------------|
| Right atrial enlargement, n/N (%) | 8/15 (53) | 2/7 (29) | 6/8 (75) | 5/13 (39) | .48 | .13 | P > .99 | .18 |
| Right atrial pressure, median (IQR), mm Hg | 5 (5–14) | 5 (5–5) | 12 (5–18) | 5 (5–20) | .68 | .06 | .15 | .61 |
| Right ventricular enlargement, n/N (%) | 13 (81) | 8 (100) | 5 (63) | 9 (56) | .25 | .2 | .05 | P > .99 |
| Normal | 0 (0) | - | - | 4 (25) |
| Mild | 1 (6) | - | 1 (12) | 2 (13) |
| Moderate | 2 (13) | - | 2 (25) | 1 (6) |
| Severe | 30 (23–60) | 24 (23–29) | 60 (48–67) | 41 (32–60) | .22 | .004 | .002 | .24 |
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