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Pulmonary Capillary Hemangiomatosis: An immunohistochemical Analysis of Vascular Remodeling

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Abstract

Question of study: Pulmonary capillary hemangiomatosis (PCH) is an extremely rare cause of severe pulmonary hypertension. It is characterized histologically by exuberant proliferation of capillaries that often invade alveolar septae, bronchial walls, and pleura. Expression of vascular remodeling markers in PCH is not known.

Materials / patients and methods: Using antibodies directed against vascular remodeling markers known to be abnormally expressed in plexiform lesions of idiopathic pulmonary hypertension, we performed the first detailed immunohistochemical analysis of the lungs in a patient with PCH.

Results: As in plexiform lesions, the PCH lesions have increased expression of markers associated with cellular proliferation and angiogenesis such as vascular endothelial growth factor and MiB-1. In contrast to plexiform lesions, the PCH lesions retain markers of cell growth suppression such as peroxisome proliferator-activated receptor-gamma (PPAR-γ) and caveolin-1.

Answer to question: This study suggests that the aberrant endothelial cells that lead to the characteristic lesions of PCH and idiopathic pulmonary hypertension are distinct.

Key words: alveolar hemorrhage, angiogenesis, cor pulmonale, pulmonary hypertension

Introduction

Pulmonary capillary hemangiomatosis (PCH) is a rare disorder characterized by proliferation of thin-walled capillary channels and venule-sized vessels that can infiltrate the walls of alveoli, airways, blood vessels, and pleura [1]. Rarely, extrapulmonary involvement may occur in the mediastinum, pericardium, thymus, and spleen [2]. In its most severe form, PCH is manifested by relentless pulmonary hypertension (PH) and right heart failure. First described in 1978 [3], only 43 cases have been well described in the English literature [4-10]. We herein report a man with severe pulmonary hypertension and refractory hypoxemia due to PCH who died from massive hemorrhage into the chest cavity ten days after an open lung biopsy. Since PCH may be diagnostically confused with idiopathic PH, we queried whether the angioproliferative lesions of PCH have features in common with the plexiform lesions of idiopathic PH by comparing their qualitative expression of vascular remodeling markers [11].

Case History

An otherwise healthy 34 year-old man noticed abnormal dyspnea on exertion two years prior to presentation to our hospital. He was diagnosed with pulmonary hypertension of unexplained etiology. Six months prior, he was prescribed supplemental oxygen. Six weeks prior to transfer to our institution, he was hospitalized for anasarca and severe hypoxemia. There was no history of recreational or anorectic drug use. Medications on transfer included warfarin, furosemide, digoxin, and risperidone.

On examination, the temperature was 98.9 °F, blood pressure 112/80 mmHg, heart rate 104 beats per min, respiratory rate 22 breaths per min, and oxygen saturation of 94% while breathing 100% oxygen delivered by non-rebreathing mask. Anasarca and clubbed fingers were present. Bilateral basal lung crackles, a loud fixed P_2, and a right-sided S4 gallop were auscultated.

An arterial blood gas obtained while breathing 100% oxygen at an elevation of 5,280 feet revealed a pH of 7.48, $PaCO_2$ 35 mmHg, and PaO_2 62 mmHg. Spirometry and lung volumes were normal but the corrected DL_{CO} was 33% of predicted. A chest radiograph revealed cardiomegaly and enlarged bilateral pulmonary arteries (Fig. 1A). A high-resolution CT (HRCT) scan of the chest showed ground glass opacities, left periaortic and small right hilar lymphadenopathy, and centrilobular nodules (Fig. 1B). An echocardiogram demonstrated a markedly dilated right ventricle with severe tricuspid regurgitation, and a right ventricular peak pressure of 90 mmHg. A moderate poste-

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rior pericardial effusion was present with no tamponade physiology. Right heart catheterization demonstrated a mean pulmonary artery pressure of 56 mmHg, pulmonary capillary wedge pressure of 16 mmHg, and no evidence of a left-to-right intracardiac shunt. Pulmonary angiogram was negative for pulmonary emboli. Serological assays for HIV, anti-nuclear antibody, anti-neutrophil cytoplasmic antibody, Scl-70, anticardiolipin antibody, and hepatitis B and C antibodies were all negative. Bronchoscopy with lavage demonstrated a non-bloody wash, a normal white cell differential, and hemosiderin-laden macrophages. He was treated with furosemide with significant improvement of the anasarca. Warfarin was replaced by intravenous unfractionated heparin on the day of admission. Despite some symptomatic relief, oxygen requirement remained at a FiO_2 of 100%.

Since some of the physical, radiographic and lung function features were atypical for idiopathic PH (e.g., clubbing, ground glass abnormalities, centrilobular nodules, extremely low DL_{CO}), an open biopsy of the left lung was performed after the heparin was discontinued for 12 hrs. He tolerated the procedure well and more than 48 hours post-operatively, subcutaneous enoxaparin 1 mg/kg twice daily was begun for thromboembolic prophylaxis. His condition remained tenuous but unchanged until 10 days post-operatively when he developed a sudden increase in respiratory distress and decline in oxygen saturation. A large left-sided pleural effusion was found on chest radiograph and thoracentesis revealed gross blood. The enoxaparin was discontinued, four units of fresh frozen plasma were administered, and a thoracostomy tube was inserted with evacuation of 1.2 liters of blood. The bleeding ceased and his dyspnea and oxygenation improved. Six hours later, massive hemorrhage recurred from the left pleural space, followed by profound hypotension, cardiorespiratory arrest, and failed resuscitation.

Gross findings at autopsy were remarkable for clotted blood within the left pleural space, clot adherent to the lung biopsy site, and multiple hemorrhagic foci in the lung parenchyma measuring 0.3 to 0.5 cm in diameter that extended to the pleura. Histopathology of both the lung biopsy and post-mortem lung were notable for nodular proliferation of thin-walled, congested capillaries distributed in an interstitial and peribronchiolar distribution (Fig. 2A). These lesions were characterized by multiple layers of capillary channels expanding the alveolar septae, invading bronchiolar walls, and extending to the pleural surfaces (Fig. 2A and 2B). While the biopsy sutures were grossly intact at autopsy, microscopic examination of the post-mortem lungs revealed excessive capillary proliferation at the biopsy site (Fig. 2C).

Materials and Methods

Using formalin-fixed, paraffin-embedded lungs samples from this PCH case and an explanted lung tissue from an individual with idiopathic PH, we performed immunohistochemistry with the following antibodies as previously described: CD31 (Dako, Carpenteria, CA), MiB-1 (Dako), [12], vascular endothelial growth factor (VEGF, Santa Cruz Biotec, Santa Cruz, CA) [13], peroxisome proliferator-activated receptor-gamma (PPAR-γ, GenWay, Columbia, MD) [14], caveolin-1 (clone 2297, gift from Dr. Michael Kasper), prostacyclin synthase (PGI_2-S, gift from Dr. David Dewitt) [15], and latency-associated nuclear antigen-1 (LANA-1) of herpes human virus-8 (HHV-8) encoded by ORF73 (Advanced Biotechnoloies, San Diego, CA) [16].

Results of Immunohistochemistry

The vascular nature of the abnormal PCH lesions was confirmed by immunostaining for the endothelial cell marker CD31 (Fig. 2D). No plexiform lesions were found.

MiB-1 and VEGF-1 are markers of angiogenic proliferation. MiB-1 antibody is specific for Ki-67, a protein present only in growth phases of the cell cycle. "MiB-1 index" quantifies the percentage of cells in a tumor that are actively proliferating and has prognostic value in various cancers [17]. As shown in Figure 3A and 3B, MiB-1 is increased in both the capillary tufts of PCH and plexiform lesions of idiopathic PH [12]. In an effort to determine what may be driving the capillary proliferation in PCH, we immunostained for VEGF-1, a potent growth factor for endothelial cells and known to be abnormally elevated in plexiform lesions [13]. As shown in Figure 3C and 3D, capillary tufts of PCH and plexiform lesions were both associated with increased VEGF-1 expression.

The histologic features of PCH include spindle cells, slit-like vascular spaces, and hemosiderin, similar to Kaposi's sarcoma [18]. Since HHV-8 has recently been associated with idiopathic PH [16], the PCH lung tissue was immunostained for LANA-1 of HHV-8. As shown in Figure 3E, LANA-1 was not detected in the nuclei of the cells in the PCH lung. In contrast, nuclear immunostaining for LANA-1 is present in a patient with idiopathic PH (Fig. 3F).

In contrast to MiB-1 and VEGF-1, there is decreased expression of PPAR-γ, caveolin-1, and PGI_2-S in the plexiform lesions of idiopathic PH. PPAR-γ belongs to a family of ligand-activated transcription factors which may function in tumor suppression. It has been noted to be decreased or absent in a population of cells within the plexiform lesion [14]. Figures 4A and 4B demonstrate that PPAR-γ expression is present in PCH capillaries whereas it is not detected in most of the endothelial cells within the plexiform lesions, respectively. Caveolin-1 serves a dual and somewhat opposing role in angiogenesis [19]. It negatively regulates endothelial cell proliferation and cell cycle progression and yet is necessary for differentiation and tubule formation. Caveolin-1 is highly expressed in the capillary tufts of PCH (Fig. 4C) while it is absent in many endothelial cells within the plexiform lesion (Fig. 4D). PGI_2-S is an enzyme that catalyzes the production prostacyclin, a potent vasodilator and suppressor of platelet adhesion and cell growth. In the endothelial cells of both PCH capillary lesions and plexiform lesions of idiopathic PH, PGI_2-S was found to be decreased (Fig. 4E and 4F, respectively) [20].

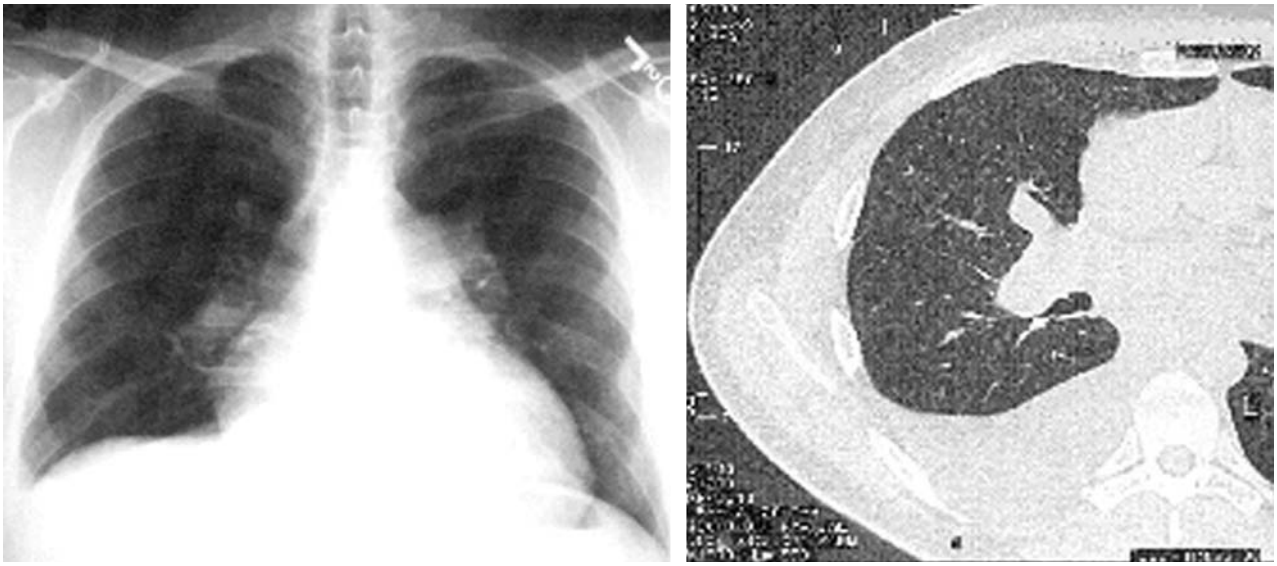

Fig. 1. Chest radiograph (A) revealed cardiomegaly and enlarged bilateral pulmonary arteries. In (B), a high resolution CT of the chest demonstrated ground glass appearance, increased septal thickening, and diffuse fine nodules.

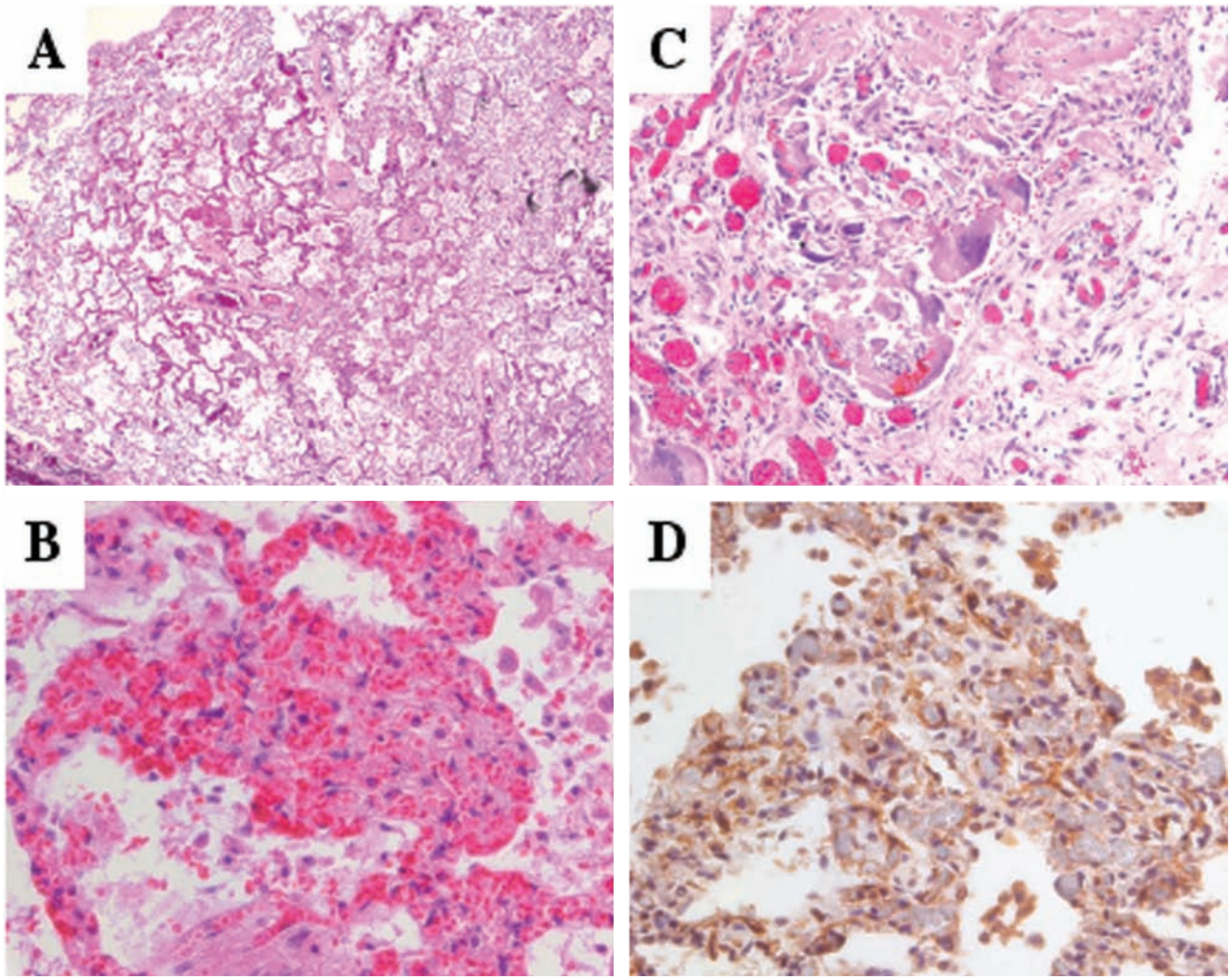

Fig. 2. (A) Histopathology of the lungs revealed proliferating sheets of capillaries with invasion of the arteries, veins, airways, and pleura (H&E, 40X). (B) A higher power view of the capillary tufts of PCH (H&E, 400X). (C) Increased proliferation of capillaries along with granulation tissue at previous biopsy site on autopsy (H&E, 200X). (D) CD31 stain revealed the nature of the capillary tufts (400X).

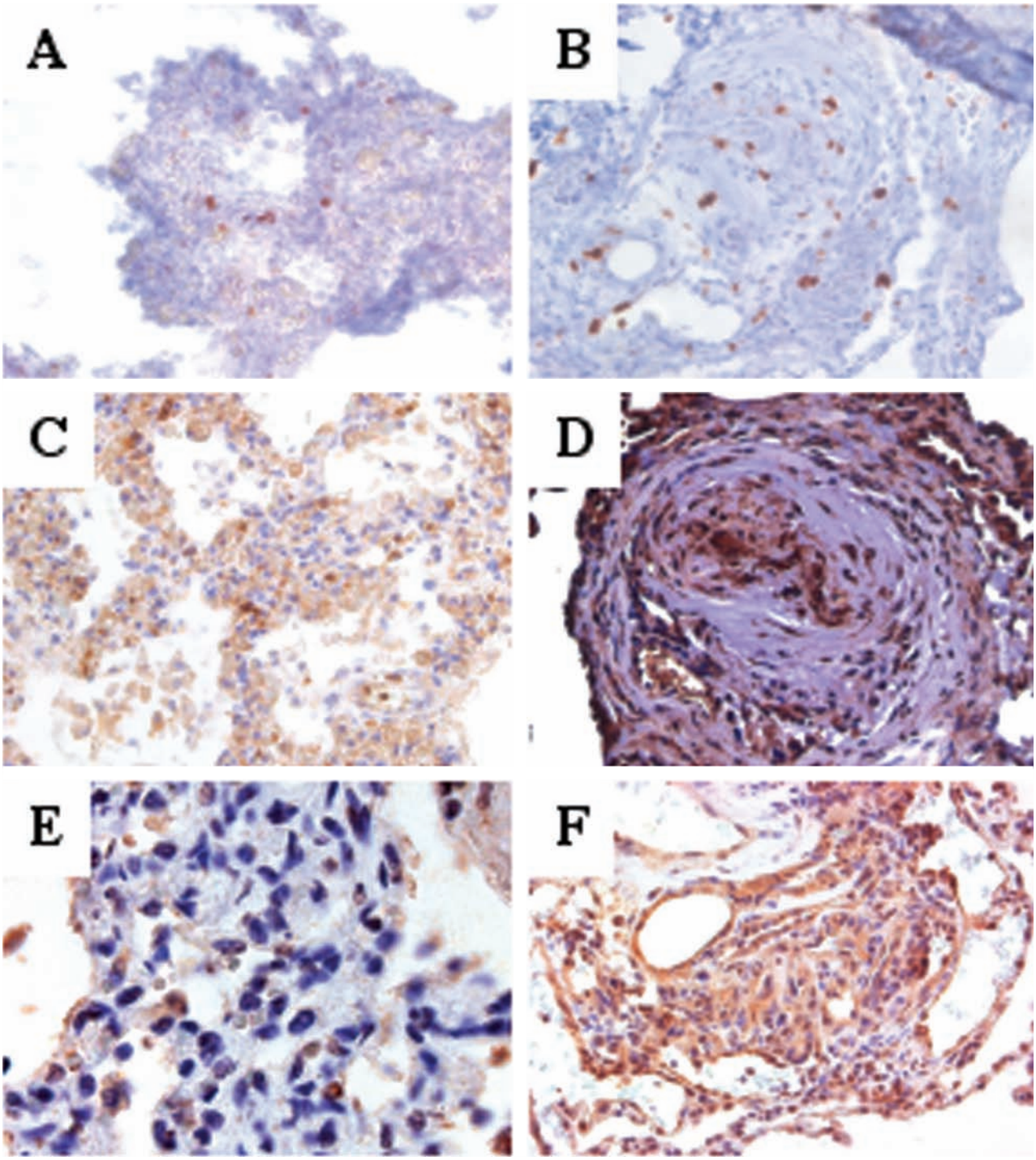

Fig. 3. Immunohistochemical analysis of cellular markers known to be increased in PPH. MiB-1 staining is increased in both (A) PCH and (B) idiopathic PH. VEGF expression appears upregulated in both (C) PCH and (D) idiopathic PH. LANA-1, a reliable marker for lytic and latent HHV-8 infection, is not detected in the nuclei of PCH endothelial cells (E) but is present in endothelial nuclei of the plexiform lesions of idiopathic PH (F). Original magnification for all are 400X.

Discussion

Severe PCH is characterized by progressive dyspnea, hypoxemia, and relentless pulmonary hypertension but hemoptysis and hemothorax may occur as a result of capillary invasion [4]. Occult intra-alveolar hemorrhage was present in our patient as evinced by the presence of hemosiderin-laden macrophages in the bronchoalveolar lavage fluid. Two physical signs, both present in our patient, are more commonly associated with PCH than idiopathic PH: crackles on lung auscultation and digital clubbing [21, 22]. PCH is nearly always fatal in adults with a median survival of about three years from the first clinical presentation [4]. Hypoxemia and right heart failure are the most common causes of death. The prognosis is better in children,

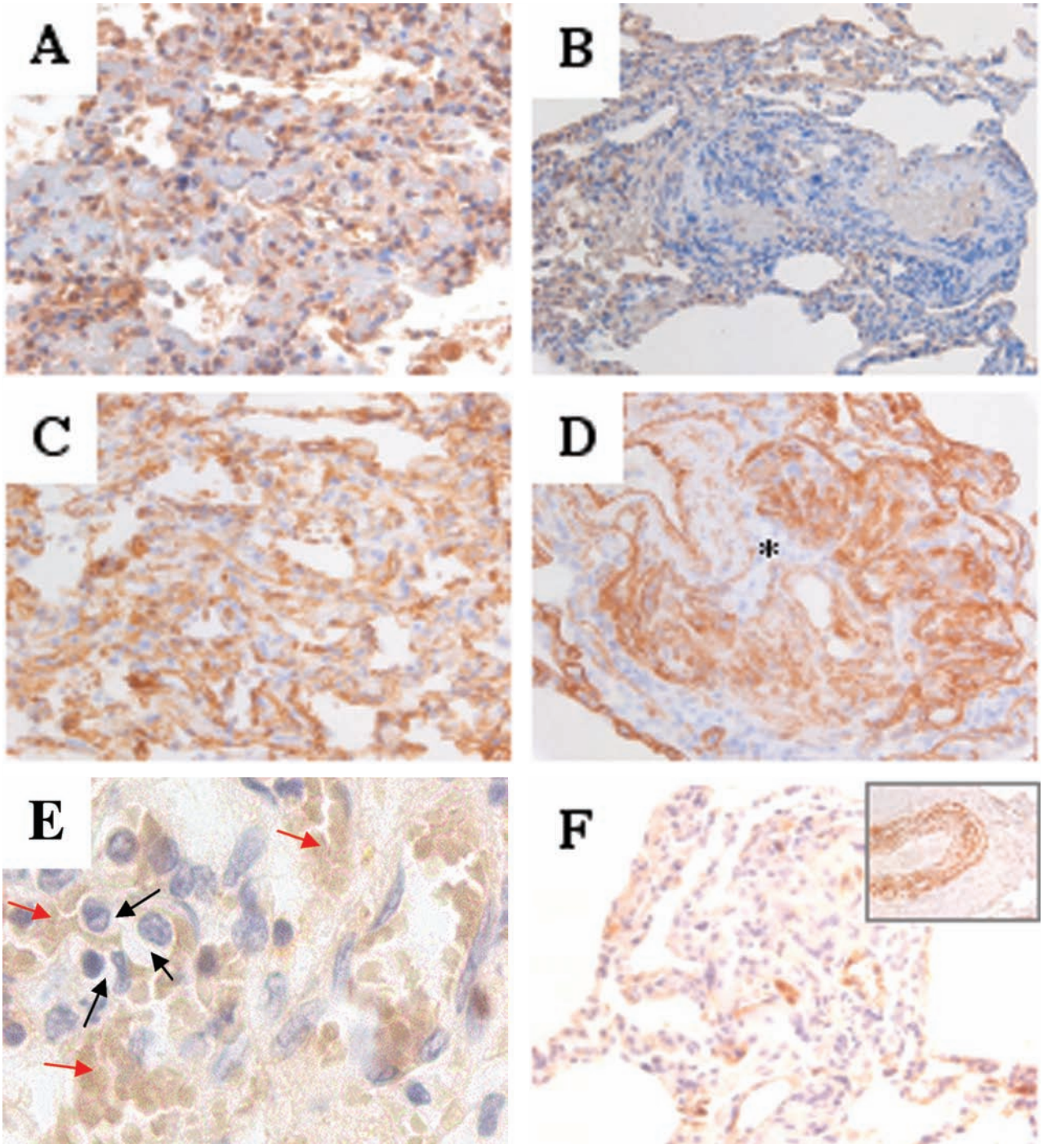

Fig. 4. Immunohistochemical analysis of cellular markers known to be decreased in the plexiform lesions of PPH. PPAR-γ is constitutively present in the PCH lesions (A) but its expression is mostly lost in certain endothelial cells of the plexiform lesions (B). Caveolin-1 remains expressed in the endothelial cells of the capillary tufts of PCH (C) but is decreased in the central part (*) of the plexiform lesions (D). PGI_2-S expression is decreased in both PCH (E) and plexiform lesions of idiopathic PH (F). Endothelial cells in the PCH sample are marked with black arrows and do not stain with the PGI_2-S antibody as opposed to the non-specific staining due the massive amounts of red blood cells engulfing the lesions (red arrows). Inset of F shows that PGI_2-S is present in the endothelial and smooth muscle cells comprising an artery of idiopathic PH. Original magnification for B is 200X; the rest are 400X.

who more often present with milder disease characterized by hemoptysis or lung infiltrates rather than pulmonary hypertension [22].

Imaging and pulmonary function studies may help differentiate PCH from idiopathic PH in individuals with unexplained pulmonary hypertension. Features on HRCT scan that are more indicative of PCH include ground glass opacities, interlobular septal thickening, nodularity, and mediastinal lymph node enlargement [6]. Distinguishing PCH from other causes of post-capillary PH such as pulmonary veno-occlusive disease (PVOD) requires a surgical lung biopsy. This

procedural risk must be weighed carefully in the presence of severe pulmonary hypertension [23]. Our patient died as a result of massive hemorrhage into the pleural space more than a week after the procedure. Due to the delayed bleed, we posit that the source of his fatal hemorrhage was secondary to the exuberant proliferation of anomalous capillaries that infiltrated the healing biopsy site rather than blood vessels incised at surgery (Fig. 2C).

There are no specific treatments for PCH. Anecdotally, corticosteroids and cyclophosphamide are not effective [24]. Treatments with interferon-alpha [22] or doxycycline [7] have been associated with disease resolution but the cases involved had normal arterial blood gases and no clinical evidence of pulmonary hypertension. The use of epoprostenol or other vasodilators in patients with PCH or PVOD may precipitate pulmonary edema and be fatal. A review of 37 patients revealed that anticoagulation was used in the majority [4]; however, given the propensity of PCH patients to bleed, we believe that the use of anticoagulation is contraindicated, especially in the post-surgical setting. Severely ill patients should be considered for lung transplantation.

The etiology of PCH is unknown although case reports have associated PCH with *Mycoplasma pneumoniae* infection [22], autoimmune disease [8], and following lung transplantation [25]. PCH has rarely occurred in related individuals [26].

Our immunohistochemical staining sheds new light on the process of dysregulated angiogenesis in this very rare disorder. Increased expression of MiB-1 and VEGF confirms the rapid proliferation of the aberrant capillaries. As opposed to plexiform lesions, the endothelial cells of PCH lesions retain marker expression indicative of cell growth suppression. In particular, the tumor suppressor proteins PPAR-γ and caveolin-1 were expressed in PCH and absent in idiopathic PH. The continued expression of caveolin-1 reveals an important phenotypic distinction between the endothelial cells of PCH and idiopathic PH. PCH lesions are characterized by profuse but grossly normal appearing capillaries. Tubule formation is a caveolin-1 dependent process. In contrast, loss of caveolin-1 expression in the endothelial cells of plexiform lesions reflects aberrant vasculogenesis; i.e. they do not form tubular structures. Interestingly, the lungs of caveolin-1 knockout mice have markedly thickened septae with multilayered, disorganized tissue with expression of markers consistent with non-differentiated endothelial cells [27].

Based on immunohistochemistry of the lungs of a patient with PCH, it is unlikely that the vascular pathobiology of idiopathic PH is shared by PCH. Despite its rarity, PCH should be considered in the differential diagnosis of pulmonary hypertension especially if parenchymal pulmonary abnormalities are detected on exam or imaging. Biopsy is inherently risky in these individuals. Vasodilatory therapy is contraindicated and anticoagulation should be avoided.

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