First histological description of pulmonary and vascular abnormalities of pulmonary hypertension associated with *KDR* pathogenic variant

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To the Editor:

Loss of function (LoF) pathogenic variants in the *kinase insert domain receptor* (*KDR*) gene have recently been associated with heritable pulmonary arterial hypertension (PAH) [1–3]. The *KDR* gene encodes the second tyrosine kinase receptor, vascular endothelial growth factor receptor 2 (VEGFR-2) for VEGF-A, that transduces the signal of the vascular endothelial growth factor (VEGF) pathway that is highly expressed in the lungs and necessary for the development of lung vasculature during embryogenesis and for the maintenance of the pulmonary vessels and alveolar structures in adults [4, 5]. Thus, a decrease of VEGF signalling induced by *KDR* LoF variants could lead to PAH, as was described *in vitro* and in animal models [6, 7]. In humans, a specific phenotype seems to be associated with pathogenic *KDR* variants, with severe precapillary pulmonary hypertension (PH), low diffusing capacity for carbon monoxide (D_{LCO}) and possible parenchymal involvement, including emphysema or interstitial lung diseases [1–3]. To date, a total of 10 *KDR* pathogenic variants have been described in PAH patients [1, 2]. However, response to specific PAH therapies and description of parenchymal and pulmonary vascular abnormalities associated with *KDR* pathogenic variant have never been reported.

Herein, we describe the evolution and the pathological assessment of a patient carrying *KDR* pathogenic variants (c.976+2T>C, p.His267Lysfs*37) previously reported in 2020 [1]. The patient was diagnosed with severe PAH at the age of 30 years. Remarkably, he presented with severe hypoxaemia with an arterial oxygen tension (P_{aO_2}) at 61 mmHg on room air, a major decrease in D_{LCO} at 19% of theorical values contrasting with normal pulmonary volumes, and interstitial lung disease including reticulations, septal lines and ground glass opacities associated with mild emphysema and mediastinal enlarged lymph nodes on chest high-resolution computed tomography (CT) [1]. In view of the initial clinical New York Heart Association functional class (NYHA FC) III, 6-min walk distance (6MWD) of 210 m and haemodynamic severity (mean pulmonary artery pressure (mPAP) 48 mmHg, pulmonary artery wedge pressure (PAWP) 3 mmHg, cardiac index (CI) 2 $L \cdot min^{-1} \cdot m^{-2}$ and pulmonary vascular resistance (PVR) 13.6 WU), dual oral combination therapy (endothelin receptor antagonist and phosphodiesterase 5 inhibitor) was initiated. After an initial improvement (NYHA FC II, 6MWD 450 m, PVR 6.2 WU), the patient experienced clinical (NYHA FC III) and haemodynamic (mPAP 60 mmHg, CI 1.6 $L \cdot min^{-1} \cdot m^{-2}$ and PVR 17.7 WU) deterioration. CT scans and pulmonary functional tests were unchanged. The patient was finally transplanted after having been listed on the French high-priority allocation list for lung transplantation. Remarkably during the pre-transplant assessment, diffuse systemic vascular atherothrombosis (abnormally severe for the age in absence of tobacco exposure) was described on Doppler ultrasound preferentially affecting the carotid arteries and coronarography found a significant stenosis of the anterior interventricular coronary that was stented.

Histological examination of explanted lungs (figure 1) on haematoxylin and eosin sections and using an automated chromogenic multiplexed immunohistochemistry assay on the Discovery Ultra automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) with four antibodies targeting CD31, smooth muscle actin, cytokeratin 7 and TTF-1, revealed diffuse parenchymal reorganisation. As seen on the CT scan, presence of subpleural and centrilobular pulmonary emphysema was confirmed in most lung

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This study confirms that KDR loss of function variants are associated with interstitial and pulmonary vascular remodelling, alveoli alterations, mild emphysema and possible vascular systemic involvement https://bit.ly/3SmTrpW

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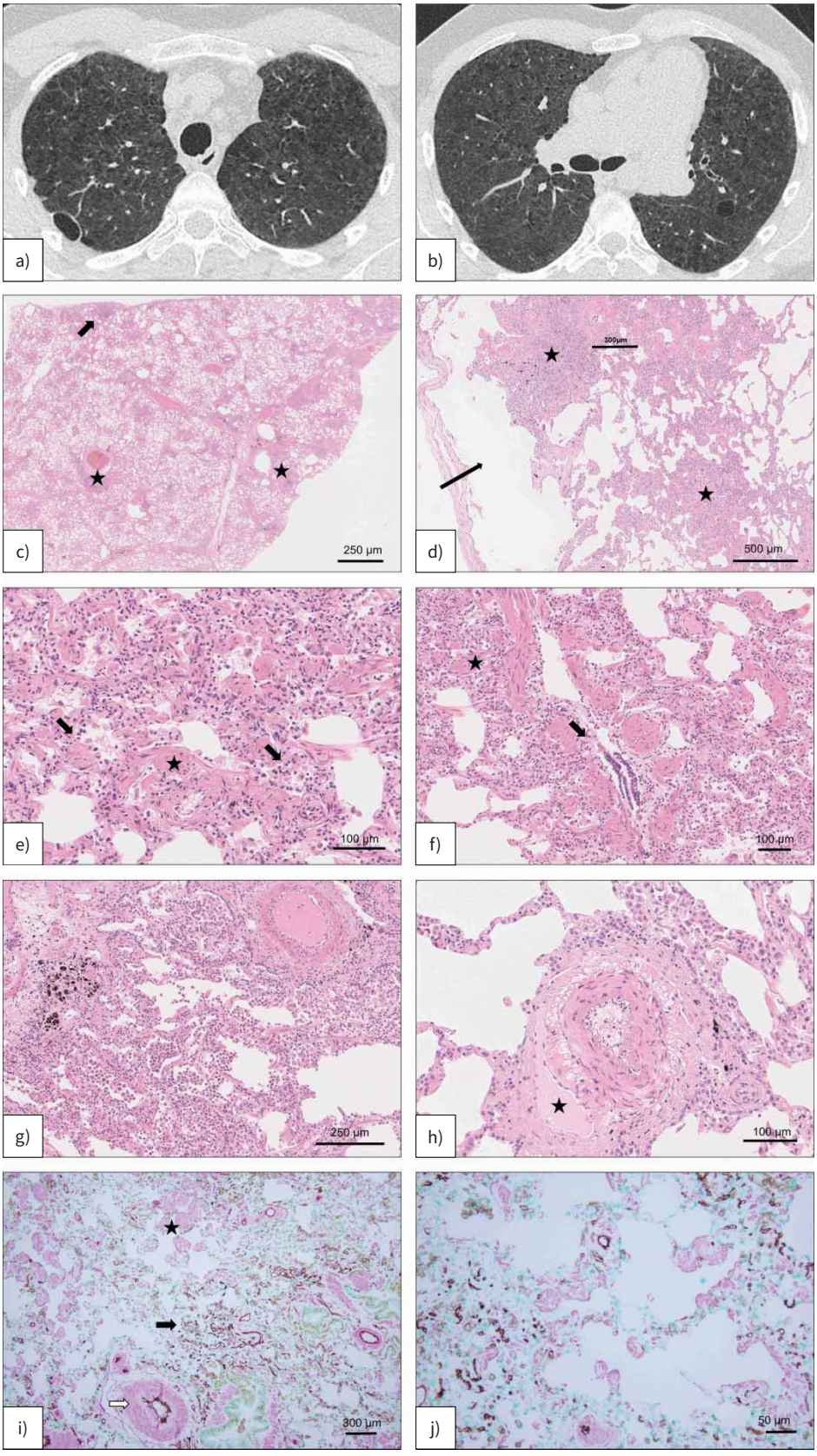

FIGURE 1 High-resolution computed tomography (CT) of the chest (a and b) and lung pathological lesions (c–j) of our patient carrying *kinase insert domain receptor* (*KDR*) pathogenic variant. a) Axial CT section (lung image) obtained at the level of trachea. The presence of subpleural and centrilobular pulmonary emphysema

is noted. b) Axial CT section (lung image) obtained at the level of the tracheal bifurcation showing centrilobular pulmonary emphysema and subpleural mosaic attenuation. c–h) Haematoxylin and eosin (HE) lung sections. c) Overview of a lung parenchymal section. The architecture is altered by a subpleural (arrow) and peri-bronchial interstitial thickening (stars). d) Emphysema with large subpleural bulla (arrow) and interstitial thickening foci (stars). e) Parenchymal interstitial thickening foci containing smooth muscle cells (star). Alveola are dysmorphic: thickening of walls with inflammatory and smooth muscle cells recruitment, often associated with pneumocytes enlargement (arrows). f) The bronchial endings appear altered with discontinuous epithelium (arrow). Hyperplasia of smooth muscle tunic is noted (star). g) Focus of macrophagic alveolitis (alveolar haemorrhage). h) Remodelling of pulmonary artery (<500 µm) with smooth muscle cells hyperplasia. No intimal fibrosis or plexiform lesions are seen. Perivascular lymphatic ectasia is present (star). i and j) Multiplex immunostaining of representative lung samples. i) Smooth muscle actin (pink), CD31 (dark brown), cytokeratin (green) and TTF-1 (dark yellow) labelling enhances the disruption of alveolar capillary network (black arrow), deposition of interstitial smooth muscle (star), pulmonary vasculopathy (white arrow) and lung structural alteration (emphysema). j) similar lesions seen at higher magnification.

samples, predominantly in the upper lobes. Significant interstitial thickening, predominantly at bronchiolar terminations and in the peripheral lung (subpleural location) was observed. Hypertrophy of interstitial and peribronchial mature smooth muscle cells as well as foci of bronchiolar inflammation were also readily observed. Alveoli appeared altered, with increase of the wall thickness, pneumocyte hyperplasia and desquamation. Interestingly, disruption of the alveolar capillary network was observed. The histopathological traits of the arterial vasculopathy consisted of an increase of the media smooth muscle with mild or no intimal thickening and lack of plexiform lesions (usually observed in PAH). No severe venular lesions, similar to those observed in pulmonary veno-occlusive disease, were present in this case.

These new data help us to better understand the pathophysiological mechanisms of *KDR* LoF leading to PAH. First, it confirms the importance of VEGF signalling for the development of the lung vasculature and the maintenance of pulmonary vascular patency. Pathological lesions in our patient seem to be similar to lesions that were described in experimental models [8, 9]. Indeed, inhibition of VEGF in neonatal or in adult rats reduced alveolarization, leading to enlargement of air spaces and decreased vascular density. In our patient, similar lesions were present with emphysema and interstitial and pulmonary vascular remodelling. It is very interesting that distal parenchymal reorganisation potentially related to small airway remodelling seems to be predominant in this case and could be in part secondary to lung development abnormalities. Furthermore, our patient had a history of mild asthma in the childhood. The VEGF pathway is involved in the formation and integrity of the capillary alveolar barrier due to the crosstalk between epithelial cells, which produce VEGF, and endothelial cells, that express VEGFR-2 [10]. Alveoli alterations could explain the very low D_{LCO} in our patient, and we can suggest that reduced D_{LCO} in association with CT scan parenchymal abnormalities could be an early marker of the development of *KDR* LoF associated disease.

It is noteworthy that the histological lesions described in our patient are different from those seen in typical PAH, such as in *BMPR2* pathogenic variant carriers, suggesting that PAH associated with *KDR* LoF variants is a distinct entity. In addition, despite the fact that reduced D_{LCO} is also observed in biallelic *EIF2AK4* pathogenic variant carriers, no severe venular lesions are present in our patient. Moreover, the poor and transient response to specific vasodilator PAH therapy may be explained by the parenchymal involvement and alveoli alterations, as usually observed in PH due to chronic lung disease (group 3 of the international classification) [11]. Regarding these new data, one may hypothesise that pre-capillary PH in patients carrying *KDR* pathogenic variants could be a consequence of developmental disorders of the lungs and should be classified in group 3 of the international classification. However, the adult onset and the incomplete penetrance of PAH in these patients may suggest that a second hit is required to trigger accelerated pulmonary vascular remodelling in a predisposed dysfunctional pulmonary circulation [1].

Of note, despite a low cardiovascular risk, our patient presented significant atherothrombosis in systemic vasculature and coronary artery disease. No systemic vascular malformation was observed. Nevertheless, it was associated with high cholesterol levels: low density lipoprotein cholesterol was 2.59 $g \cdot L^{-1}$. These data are compatible with VEGF pathway disorders, as we know that VEGF-A is involved in lipid metabolism and in atherosclerosis development and progression [12]. Swietlik *et al*. [2] have reported an increased incidence of systemic hypertension in PAH patients harbouring *KDR* pathogenic variants as compared to other PAH patients, which was not observed in our patient.

In conclusion, our data confirm that *KDR* LoF variants are associated with interstitial and pulmonary vascular remodelling, alveoli alterations, mild emphysema and possible vascular systemic involvement. PH in these patients would be closer to PH due to chronic respiratory disease than to PAH. Physicians should be aware of this rare phenotype/genotype relationship and, by extension, screening of pathogenic variants in development genes (*KDR* or *TBX4*) should be discussed in patients with severe PH associated with atypical lung parenchyma abnormalities on CT scans [13]. Further translational and functional studies are needed to better understand the pathophysiological mechanisms of PAH associated with *KDR* LoF variants.

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