EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

T-box protein 4 mutation causing pulmonary arterial hypertension and lung disease

Arnaud Maurac, Émilie Lardenois, Mélanie Eyries, Maria Rosa Ghigna, Isabelle Petit, David Montani, Anne Guillaumot, Brice Caput, François Chabot, Ari Chaouat

Please cite this article as: Maurac A, Lardenois É, Eyries M, *et al*. *T-box protein 4* mutation causing pulmonary arterial hypertension and lung disease. *Eur Respir J* 2019; in press (https://doi.org/10.1183/13993003.00388-2019).

T-box protein 4 mutation causing pulmonary arterial hypertension and lung disease

Arnaud Maurac (1), Émilie Lardenois (2), Mélanie Eyries (3), Maria Rosa Ghigna (4), Isabelle Petit (5), David Montani (6), Anne Guillaumot (1), Brice Caput (7) François Chabot (1, 8), Ari Chaouat (1, 8)

Affiliations

(1) Pôle des spécialités médicales/département de pneumologie, CHRU de Nancy, rue du Morvan, 54500 Vandœuvre-lès-Nancy, France

(2) Laboratoire d'anatomie et cytologie pathologiques, CHRU de Nancy, rue du Morvan, 54500 Vandœuvre-lès-Nancy, France

(3) Département de génétique, hôpital Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France

(4) Pathology Department, Marie Lannelongue Hospital, INSERM UMR_S 999, Le Plessis-Robinson, France

(5) Département de radiologie, CHRU de Nancy, rue du Morvan, 54500 Vandœuvre-lès-Nancy, France

(6) Univ. Paris–Sud, AP-HP, Centre de Référence de l'Hypertension Pulmonaire, Service de Pneumologie, INSERM UMR_S999, Hôpital de Bicêtre, Le Kremlin Bicêtre, France

(7) Service de chirurgie thoracique, CHRU de Nancy, rue du Morvan, 54500 Vandœuvre-lès-Nancy, France

(8) Université de lorraine, Inserm, U1116 ; 54500 Vandœuvre-lès-Nancy

Corresponding author

Professeur Ari CHAOUAT, Pôle des spécialités médicales/département de pneumologie, CHRU de Nancy, rue du Morvan, 54500 Vandœuvre-lès-Nancy, France

Mail : a.chaouat@chru-nancy.fr

Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease mainly characterized by a widespread obstruction of distal pulmonary arteries (1). Untreated, this disease rapidly leads to right heart failure, then to death (2). Heritable PAH (hPAH) represents about 30% of PAH cases, and this category includes familial forms with or without identified mutations, and sporadic forms carrying a mutation. The first genetic abnormalities discovered in hPAH are *bone morphogenetic protein receptor-2* (*BMPR2*) mutations. Loss of function of this receptor results in proliferation of vascular smooth muscle cells and selection of endothelial cell clones resistant to apoptosis (3). Since, several other mutations have been implicated in PAH. Mutation in *T-box protein 4 (TBX4)* gene lead to an autosomal-dominant disorder called "small patella syndrome" or "coxopodopatellar syndrome", characterized by patellar aplasia and abnormalities of the feet and pelvis (4). Recently, *TBX4* mutation has been reported in childhood-onset PAH (5) and more rarely in adults (6, 7).

We report a case of a *TBX4* mutation carrier presenting PAH but also bronchial and pulmonary parenchymal abnormalities potentially related to this mutation.

Case report

A 34 -year-old woman presented in a tertiary care hospital with a potential diagnosis of severe PAH. She was a former smoker (5 Pack-Year) and had no medical history. The patient had progressive dyspnoea in New York Heart Association (NYHA) functional class III and she experienced right heart failure two months before admission.

An echocardiography revealed normal left ventricular function, a moderately dilated right ventricle and elevated systolic pulmonary artery pressure (70 mmHg). Ventilation/perfusion lung scan ruled out venous thromboembolism disease. A pulmonary function test was unremarkable with the exception of a moderate to severe decrease in carbon monoxide

diffusion capacity (53% of the predicted value). Arterial blood gases showed a normal oxygen partial pressure (PaO_2) and hypocapnia. Six-minute walk distance was 480 meters with a decrease of oxygen saturation from 98% to 91%. Right-sided heart catheterization (RHC) diagnosed pre-capillary pulmonary hypertension (PH) (mean right atrial pressure, 2 mmHg; pulmonary artery pressure (mean), 59/16 (35) mmHg; pulmonary artery wedge pressure, 6 mmHg; cardiac index, 2.4 L/min/m^2 and pulmonary vascular resistance (PVR) ,7.2 Wood units). Chest CT showed peripheral reticular lines, centrilobular nodules, thin-walled cysts that were prominent in the upper lobes and an appearance resembling diverticula in the trachea and large bronchi. Minimum intensity projection images highlighted a mosaic appearance showing distal airway involvement (figure 1).

Next generation sequencing (NGS)-based targeted sequencing gene panel investigating predisposing genes for PAH found a copy number variant in *TBX4* gene which is the complete deletion of the exon 5 (c.(549+1_550-1)_(702+1_703-1)del). Clinical examination showed shortened 4^{th} and 5^{th} feet arches without a « classic » small patella syndrome, and pelvis X-ray revealed a lack of ossification in both *ischia*.

A diagnosis of heritable PAH was made, and she was subsequently started with an association of an endothelin receptor antagonist and a phosphodiesterase-5 inhibitor.

Due to the development of a ptosis, a change of the voice and a pseudo-nodular thymus with hypermetabolic signs on PET, myasthenia gravis was suspected and a thymectomy was performed. During this surgery a lung biopsy from left upper lobe was performed.

Pathology of the lung showed fibrotic densification incorporating many cholesterol crystals located in the perivascular connective tissue (figure 2). Cholesterol clefts were found both within interstitium and in alveoli and were surrounded by numerous lymphocytes and foamy macrophages, without the formation of granuloma. No fibroblastic foci were noticed in lung

biopsy. Dilated bronchi containing cell debris, macrophages and cholesterol clefts were enclosed by dense collagen bundles. Major vascular remodelling was observed within interstitial fibrosis. Pulmonary arteries were thickened, showing increase of the muscular media and intima. No blood clots were found in pulmonary vessels. No vascular inflammation was found, nor plexogenic arteriopathy.

Outside these foci, the parenchyma appeared emphysematous with thickened bronchiolar wall, characterized by expansion of muscular layer and mild fibrosis.

The patient responded partially to specific-PAH drug therapy. At 4 months of treatment NYHA functional class was unchanged (III), as well as six-minute walk distance (525 meters). Brain-Type Natriuretic Peptide was at 12 pg/ml and PVR decreased by 50% compared to baseline. After thymectomy, pulmonary haemodynamic measurements worsened ~~slightly~~ then remained stable. The last RHC under endothelin receptor antagonist and phosphodiesterase-5 inhibitor was performed 18 months after the diagnosis of PAH and showed a mean pulmonary artery pressure of 39 mmHg, cardiac index of 3.8 l/min/m^2 and PVR of 6.7 Wood units. Compared to the previous evaluation shown above, NYHA functional class and six-minute walk distance were unchanged.

Discussion

The patient reported here had parenchymal pulmonary abnormalities, airway diverticula, small patella syndrome, and PAH. The known role of TBX4 in lung development suggest that bronchial, lung parenchymal and pulmonary vascular abnormalities observed in this patient are at least in part related to *TBX4* mutation.

Although chest CT and lung pathology showed pulmonary parenchymal abnormalities, we eliminated group 3 pulmonary hypertension. According to current knowledge, several criteria were in favour of group 1 pulmonary hypertension (i.e. a PAH); FEV_1, forced vital

capacity and PaO_2 were within the normal range (8). However, in the light of recent publications showing severe lung development abnormalities in infant, as well as the present case observed in an adult, the position of PH in patients with a *TBX4* mutation in the clinical classification is likely to be reassessed (9, 10).

TBX4 belongs to the T-box family of transcription factors and is characterized by a DNA-binding motif known as the T-domain. *TBX4* is expressed in many tissues including lung and bone (11). In addition to the bone involvement, subjects who are heterozygous for *TBX4* may develop PAH (5, 6). The penetrance of PAH in *TBX4* mutation carrier is largely unknown and such a mutation is an uncommon genetic cause of heritable PAH in adults (7). The long-term response to the specific treatment of PAH in these patients is also unknown.

Diverticula on the trachea and main bronchi visible on chest CT and densification foci of the lung parenchyma with cholesterol crystals on lung biopsy in the patient presented here may be consistent with a lack of airway continuity (12). Two recent publications describing 3 new-borns with a *TBX4* mutation, 2 having a coxopodopatellar syndrome and who developed at birth respiratory failure suggests that our patient's lung abnormalities may be related at least partially to a lung development disorder (9, 13). Alternatively, patients with TBX4 mutations may be more susceptible in environmental exposure such as cigarette smoking. Thus, the association of severe pre-capillary pulmonary hypertension and unusual lung parenchymal disease should lead to suspicion of TBX4 mutation.

It has been shown in vitro that lung development was dependent of transcription factors TBX4 and TBX5 (14). Complex compound inheritance including *TBX4* mutation was found in 10 out of 26 individuals having rare lethal lung hypoplasia (10). Although, chest CT and results of the lung biopsy in our patient do not demonstrate an alteration of lung development we suspect such a process from the known role of TBX4.

Conclusions

We report a case of heritable PAH due to *TBX4* mutation with parenchymal lung abnormalities possibly due to an alteration of the process of lung development. Given that dyspnoea on exertion is a common sign of both PAH and parenchymal lung or bronchial diseases, we suggest a careful lung assessment on chest CT in heritable PAH due to *TBX4* mutation. Conversely, it also seems important from a clinical point of view not to ignore pulmonary vascular disease when pulmonary parenchymal involvement is obvious.

References

1. Peter Dorfmüller. Pathology of pulmonary vascular diseases. *Pulmonary circulation*, 3rd ed. Hodder Arnold; 2011. p. 29–45.

2. Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Respir J* 2015;46:903–975.

3. Soubrier F, Chung WK, Machado R, Grünig E, Aldred M, Geraci M, Loyd JE, Elliott CG, Trembath RC, Newman JH, Humbert M. Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol* 2013;62:D13-21.

4. Bongers EMHF, Duijf PHG, van Beersum SEM, Schoots J, Van Kampen A, Burckhardt A, Hamel BCJ, Losan F, Hoefsloot LH, Yntema HG, Knoers NVAM, van Bokhoven H. Mutations in

the human TBX4 gene cause small patella syndrome. *Am J Hum Genet* 2004;74:1239–1248.

5. Levy M, Eyries M, Szezepanski I, Ladouceur M, Nadaud S, Bonnet D, Soubrier F. Genetic analyses in a cohort of children with pulmonary hypertension. *Eur Respir J* 2016;48:1118–1126.

6. Gräf S, Haimel M, Bleda M, Hadinnapola C, Southgate L, Li W, Hodgson J, Liu B, Salmon RM, Southwood M, Machado RD, Martin JM, Treacy CM, Yates K, Daugherty LC, Shamardina O, Whitehorn D, Holden S, Aldred M, Bogaard HJ, Church C, Coghlan G, Condliffe R, Corris PA, Danesino C, Eyries M, Gall H, Ghio S, Ghofrani H-A, *et al.* Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. *Nat Commun* 2018;9:1416.

7. Eyries M, Montani D, Nadaud S, Girerd B, Levy M, Bourdin A, Trésorier R, Chaouat A, Cottin V, Sanfiorenzo C, Prevot G, Reynaud-Gaubert M, Dromer C, Houeijeh A, Nguyen K, Coulet F, Bonnet D, Humbert M, Soubrier F. Widening the landscape of heritable pulmonary hypertension mutations in pediatric and adult cases. *Eur Respir J* 2018;doi:10.1183/13993003.01371-2018.

8. Nathan SD, Barbera JA, Gaine SP, Harari S, Martinez FJ, Olschewski H, Olsson KM, Peacock AJ, Pepke-Zaba J, Provencher S, Weissmann N, Seeger W. Pulmonary hypertension in chronic lung disease and hypoxia. *Eur Respir J* 2019;53:.

9. Suhrie K, Pajor NM, Ahlfeld SK, Dawson DB, Dufendach KR, Kitzmiller JA, Leino D, Lombardo RC, Smolarek TA, Rathbun PA, Whitsett JA, Towe C, Wikenheiser-Brokamp KA. Neonatal Lung Disease Associated with TBX4 Mutations. *J Pediatr* 2018;doi:10.1016/j.jpeds.2018.10.018.

10. Karolak JA, Vincent M, Deutsch G, Gambin T, Cogné B, Pichon O, Vetrini F, Mefford HC, Dines JN, Golden-Grant K, Dipple K, Freed AS, Leppig KA, Dishop M, Mowat D, Bennetts

B, Gifford AJ, Weber MA, Lee AF, Boerkoel CF, Bartell TM, Ward-Melver C, Besnard T, Petit F, Bache I, Tümer Z, Denis-Musquer M, Joubert M, Martinovic J, *et al.* Complex Compound Inheritance of Lethal Lung Developmental Disorders due to Disruption of the TBX-FGF Pathway. *Am J Hum Genet* 2019;doi:10.1016/j.ajhg.2018.12.010.

11. Papaioannou VE. The T-box gene family: emerging roles in development, stem cells and cancer. *Development* 2014;141:3819–3833.

12. Betancourt SL, Martinez-Jimenez S, Rossi SE, Truong MT, Carrillo J, Erasmus JJ. Lipoid pneumonia: spectrum of clinical and radiologic manifestations. *AJR Am J Roentgenol* 2010;194:103–109.

13. Szafranski P, Coban-Akdemir ZH, Rupps R, Grazioli S, Wensley D, Jhangiani SN, Popek E, Lee AF, Lupski JR, Boerkoel CF, Stankiewicz P. Phenotypic expansion of TBX4 mutations to include acinar dysplasia of the lungs. *Am J Med Genet A* 2016;170:2440–2444.

14. Cebra-Thomas JA, Bromer J, Gardner R, Lam GK, Sheipe H, Gilbert SF. T-box gene products are required for mesenchymal induction of epithelial branching in the embryonic mouse lung. *Dev Dyn* 2003;226:82–90.

Legends of the figure

Figure 1

Chest CT

A) Coronal plane at the level of the carina in lung window showing mainly many diverticula next to the trachea and large bronchi and a mosaic pattern of lung attenuation.

B) Axial plane at the upper third of the lung in lung window showing parenchymal destruction resembling emphysema, few diverticula next to the trachea and few nodules in the right upper lobe.

C) Axial minimum intensity projection reconstruction at the lower third of the lung showing parenchymal heterogeneity with mosaic attenuation pattern emphasizing distal airway involvement.

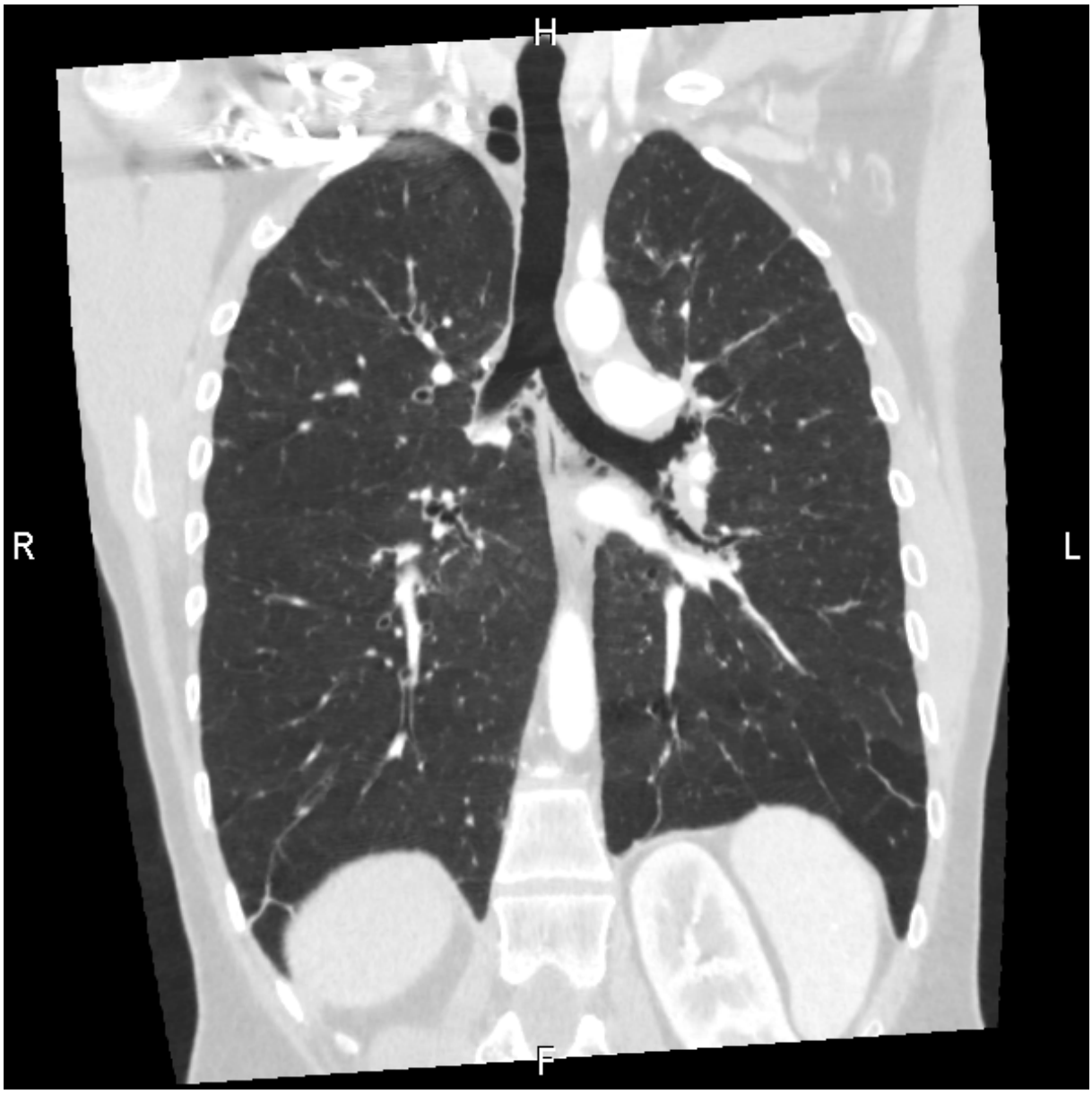
Figure 2

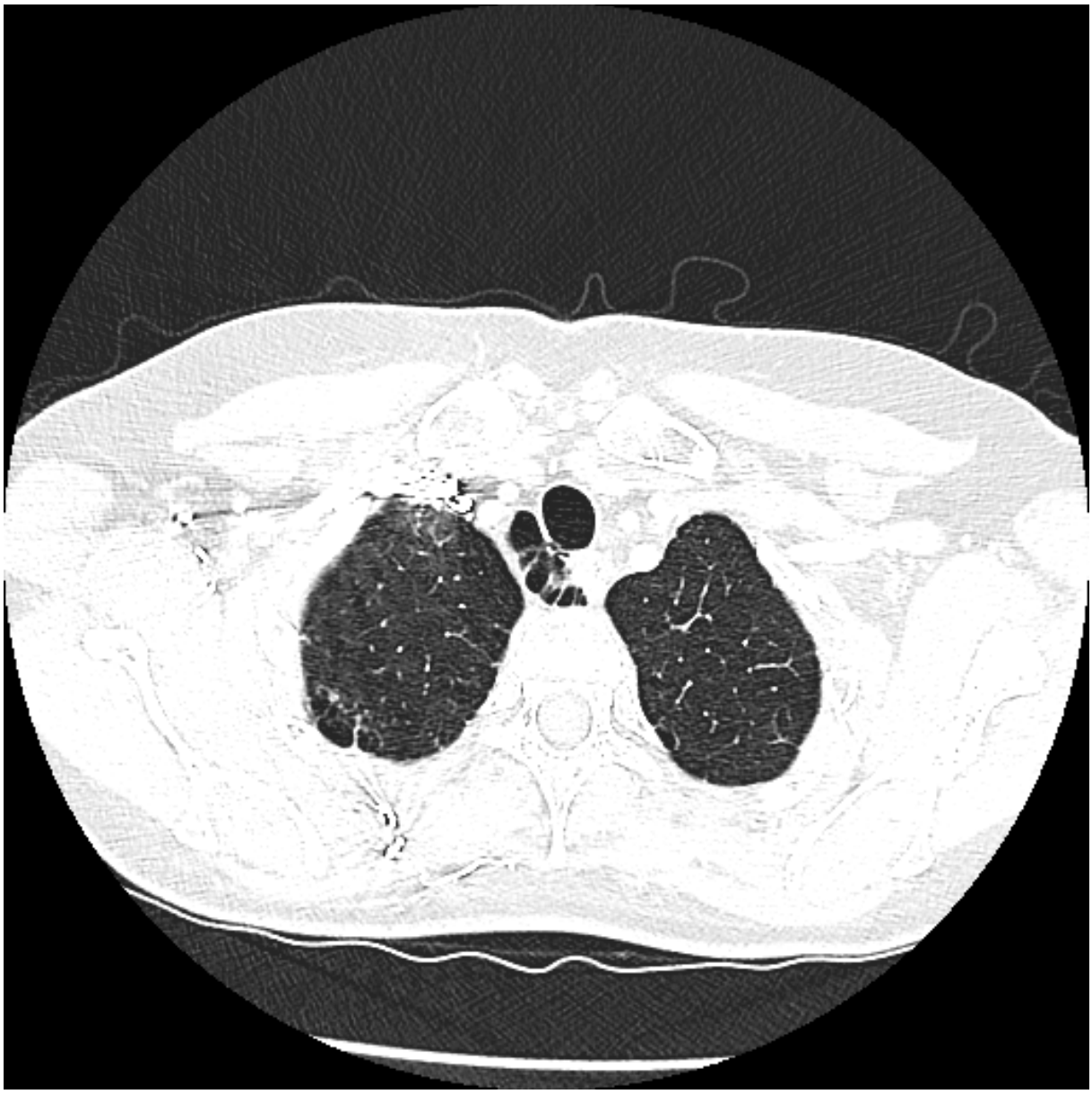
Pathology of biopsy specimen of the upper lobe of the left lung

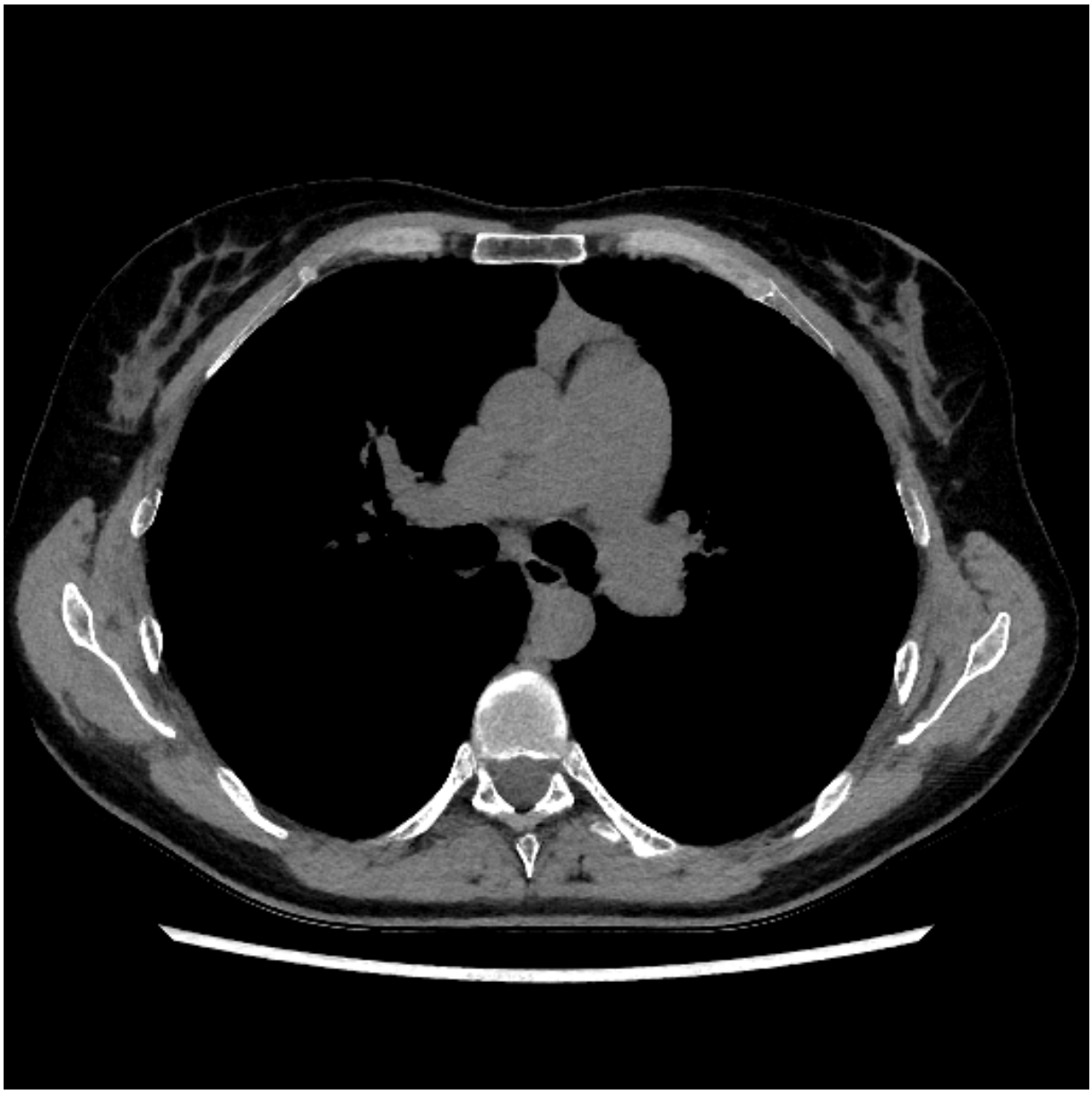
A) Fibrotic densification of the lung parenchyma, with accumulation of endogenous cholesterol clefts central and inflammatory reaction. A marked remodelled pulmonary artery is noticed within fibrosis.

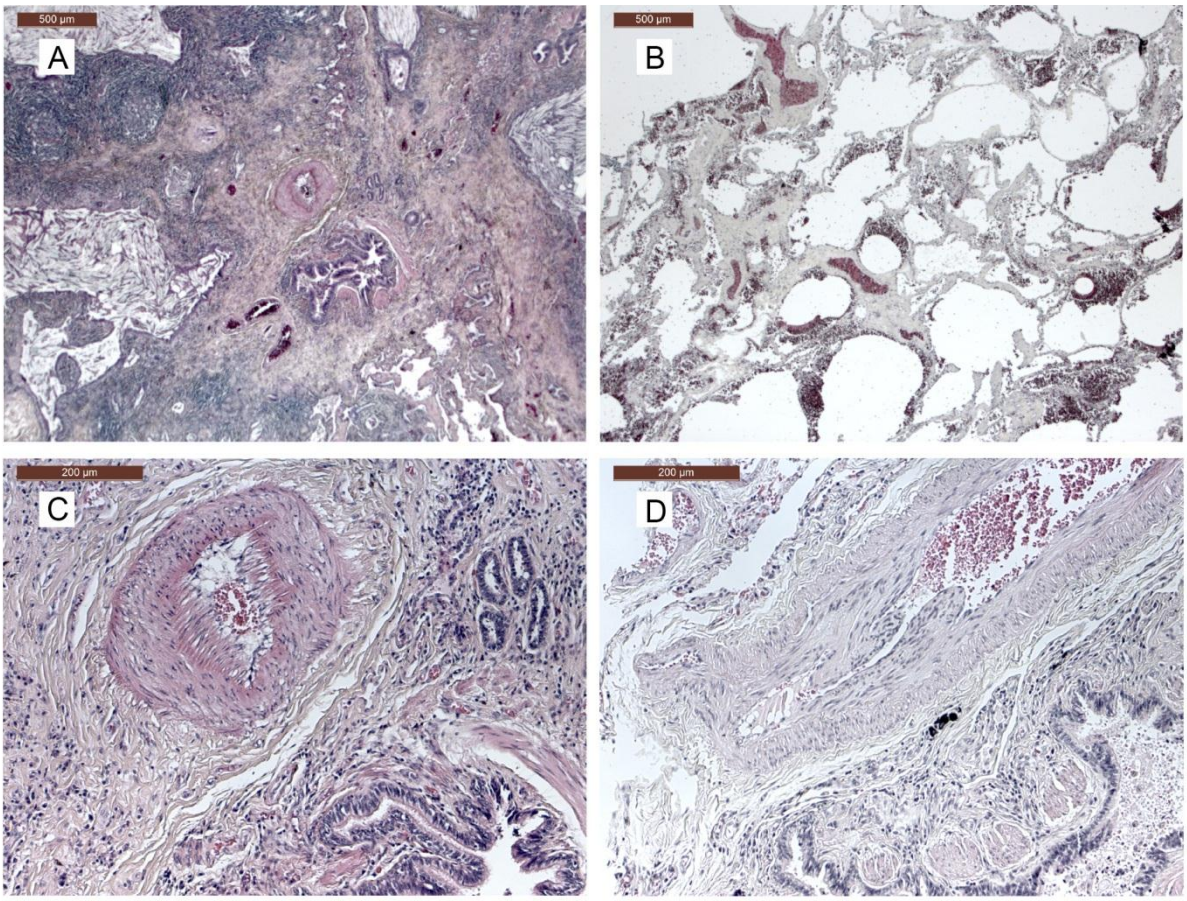
B) Altered lung parenchyma: emphysema-like lesions next to fibro-inflammatory foci

C) and D) Pulmonary artery remodelling is characterized by the increase of both muscular media and intima. There is neither plexiform lesion nor thrombosis.


H
R
L
F






500 µm
A
500 µm
B
200 µm
C
200 µm
D