Early View

Research letter

CSF2RB mutation related hereditary PAP the “long and winding road” into adulthood

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CSF2RB mutation related hereditary PAP

the “long and winding road” into adulthood

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Genetic analysis pre-lung transplantation diagnosed a case of hereditary pulmonary alveolar proteinosis (PAP) complicated by fibrosis (PF) in adulthood. The need for genetic testing in GM-CSF autoantibody negative and unclassifiable PAP is highlighted.

**Key words:** hereditary pulmonary alveolar proteinosis; pulmonary fibrosis; CSF2RB mutation; lung transplantation; i-GM-CSF; allogeneic hematopoietic stem cell transplantation;
Pulmonary Alveolar Proteinosis (PAP), refers to the inappropriate, intra-alveolar accumulation of surfactant and relates to a multitude of aetiologies (1). The loss of the respiratory reserve due to the alveolar filling may gradually lead to hypoxemic respiratory failure and its consequences although in a minority opportunistic lung infections and pulmonary fibrosis may ensue, modifying outcome (1). Irrespective of the aetiology, patients present in common a “crazy paving pattern” on high-resolution computerized tomography (HRCT) scan of the lungs and a PAS positive staining on bronchoalveolar lavage (BAL) obtained cytospins or on surgical tissue samples. (1) In the majority of cases PAP are autoimmune PAP, caused by the loss of signalling of granulocyte-macrophage colony-stimulating factor (GM-CSF) due to the development of anti-GM-CSF autoantibodies. Secondary or hereditary PAP are rarer. Secondary PAP relates to a multitude of clinical conditions and environmental exposures that may reduce numbers or functionality of alveolar macrophages (1, 2). Hereditary PAP is usually diagnosed in children where loss of signalling of GM-CSF is due to mutations in genes encoding the α or β chain of GM-CSF receptor, CSF2RA or CSF2RB respectively (2-4). Mutations in SFTPB (surfactant protein B), SFTPC (surfactant protein C), ABCA3 (encoding ATP-binding cassette subfamily A member 3) and NKX2-1 (encoding thyroid transcription factor 1 (TTF1)) may relate to the development of a wide range of surfactant accumulation abnormalities corresponding at tissue level to PAP patterns (congenital PAP) occasionally in conjunction with pulmonary fibrosis (1, 2).

Herein we describe the ultra-rare case of a 47-year-old man smoker (90py), referred to the lung transplantation clinic for evaluation on September 2022 in
a context of end-stage fibrotic interstitial lung disease (ILD) remaining oddly unclassifiable over the last 26 years. The history of the patient started in 1996 when 21-years-old, asymptomatic he performed a chest-x-ray disclosing ILD. This led to an extensive work-up documenting in second time (2003) through surgical biopsy, PAP and pulmonary fibrosis (Figure 1a). Treatment with cyclophosphamide was initiated, followed by a combination of azathioprine and corticosteroids for 24 months. On the course of the following years, the patient dropped-out from any medical care and lost all his medical records. In 2012, at 37-years-old, the patient was readmitted to another hospital due to deterioration. Levels anti-GM-CSF antibody were not measured due to technical reasons and the patient was managed with lobar lavage with fiberoptic bronchoscopy with improvement (5). An HRCT performed in 2013 is shown in Figure 1b. He continued to be non-compliant to any regular follow-up for years and in August 2022 (47-years-old) he was re-admitted to a third hospital with life-threatening deterioration and hypoxemic respiratory failure. New HRCT was performed (Figure 1c). On September 2022 the patient was referred to our center for pre-transplant evaluation with the ambiguous diagnosis of combined PAP and pulmonary fibrosis. He was on late WHO-FC III, receiving long-term oxygen therapy (nasal cannula 3 lt/min) and was listed for lung transplantation. However, a month later the patient was intubated after an episode of lung infection leading to refractory hypoxemia and respiratory acidosis. He was transferred to the ICU, to receive V-V ECMO as a bridge for lung transplantation performed 35 days later. After a rather complicated post-transplantation clinical course, due to infections, difficult weaning from mechanical ventilation and acute kidney injury, the patient was successfully discharged home from the ICU,
with low oxygen needs and improved renal function and is alive 11 months after the life-saving intervention of high-emergency lung transplantation.

The histology examination of the explanted lungs confirmed PAP and pulmonary fibrosis (Figure 1d). Due to the early-onset pulmonary fibrosis without any other obvious explanation combined with PAP and in an effort to optimize pre-transplant evaluation, the patient underwent genetic testing for telomere-related gene (TRG) mutations and proved negative. Further evaluation for surfactant and proteinosis related gene mutations was performed and found to be homozygous for the CSF2RB (NM_000395.3) nonsense variation c.631C>T, p.(Arg211*) (Figure 1H).

This variation, which is absent from the genome aggregation database (gnomAD, assessed 08.08.2023) is predicted as pathological according to the Combined Annotation Dependent Depletion score (CADD-score = 36, https://cadd.gs.washington.edu/). It leads to a premature stop codon in the sixth exon out of 14 and is therefore expected to result either in the production of a severely truncated protein or in the absence of protein production through activation of the nonsense-mediated mRNA decay (NMD) pathway. According to the guidelines of the American College of Medical Genetics and Genomics (6) this variation was classified as likely pathogenic (PVS1, PM2).

This report regards “the long and winding road” to the adulthood of a childhood ILD (chILD) survivor affected from hPAP related to CSF2RB homozygous likely pathogenic variation c.631C>T p.(Arg211*) combined to pulmonary fibrosis, still surviving at the age of 47 years-old after lung transplantation.
Hereditary PAP related to CSF2RB mutations represents one of the rarest forms of the disease so far reported in three children: the first patient was homozygous for the c.812C>T, p.(Ser271Leu) variation, for the two remaining patients the information about the variation was not available (3, 7, 8). Survival into adulthood has been described only once in a 36-year-old female patient carrier of the c.631del, p.(Arg211Glufs*54) variation at homozygous state with combined PAP and pulmonary fibrosis who also received bilateral lung transplantation surviving 4 years. Autopsy confirmed recurrence of both PAP and fibrosis in the donor lungs (9, 10).

Recurrence of PAP represents an additional challenge in the management of combined hPAP and pulmonary fibrosis in patients undergoing lung transplantation and relates to the replacement of donor alveolar macrophages by the defective (CSF2RB mutated) recipient monocyte-bone-marrow-origin alveolar macrophages (11). Allogeneic hematopoietic stem cell transplantation has been attempted once in an 18 years old lung transplanted patient for end-stage hPAP related to CSF2RA mutation and pulmonary fibrosis, diagnosed at the age of 35 months (12, 13); eventually this option should also be taken into consideration in this case. Speculations about the late onset of hereditary CSF2RB-mutations-related PAP include potential activation of multiple intracellular signaling pathways by GM-CSF binding to the GSF2RA alone (9). However scientific documentation through functional studies for such a rare disease is still missing and therefore no recommendation for the use of inhaled-GM-CSF can be made. On the contrary whole lung lavage might be attempted in case of PAP reappearance, so far not detected in our patient one-year post-lung transplantation (11, 14). Pulmonary transplantation of human induced
pluripotent stem cell-derived macrophages might represent an alternative option, although its interference with the rejection process is unknown (15).

The eventual recurrence of fibrosis in the donor lungs is another concern in the future management of our patient. There is no established pathophysiological mechanism linking fibrogenesis and hereditary or autoimmune PAP; several mechanisms have been postulated including surfactant homeostasis dysregulation and GM-CSF deficiency (16). Antifibrotic treatment might be attempted based on the limited evidence for the management of progressive pulmonary fibrotic diseases overall. It seems though that the development of pulmonary fibrosis in that case although it occurs rarely represents an event in the natural history of the disease heralding the end of the “winding road” (17).

In this patient, an earlier diagnosis of hereditary PAP might have contributed to an optimal application of precision medicine and hopefully a better outcome. This calls for genetic testing in all GM-CSF autoantibody negative and unclassifiable PAP patients.
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Authors contributions: SAP conceived of the study, had major contribution at the analysis and interpretation of data and wrote the manuscript; CL performed the genetic analysis regarding the SRG mutations, studied the CSF2RB pathogenic variation, had major contribution at the analysis and interpretation of data and wrote part of the manuscript; AF performed the pathological examination of all biopsy samples, had major contribution at the analysis and interpretation of data and wrote part of the manuscript; LK performed the pathological analysis of the explants and had substantial contribution at the analysis and interpretation of data; IT and FF had major contribution at the lung transplantation of the patient, had substantial contribution at the analysis and interpretation of data and wrote part of the manuscript; IED had major
contribution in the management of the patient, collection, analysis and interpretation of data; MPD performed the review of radiological images, contributed substantially at the critical interpretation of data and wrote part of the manuscript; FK, MK, LK, ZD, AG had substantial contribution in the management of the patient during the last 25 years of his life and in the collection and critical interpretation of data; CK performed the genetic analysis regarding the TRG mutations and had substantial contribution in the analysis and interpretation of data; RB, NN, MG had major contribution in the analysis and interpretation of data and revised critically this work for important intellectual content; EDM conceived of the study, had major contribution to the acquisition, the analysis and interpretation of data, supervised the accuracy and integrity of any part of the work, coordinated the study team and wrote the final version of the manuscript with SAP; all authors read and approved of the final version of the submitted publication.

Figure legends:
Figure 1: a) Histological features of the lung VATS biopsy (2004, at the age of 29 years old, 18 years prior to transplantation) showing PAP, and chronic lymphocytic inflammation associated with interstitial cholesterol clefts granulomas and cystically dilated subpleural alveolar spaces with collagenous fibrosis; b) Chest CT scan performed in 2013 at the age of 38 years old shows extensive patchy distributed, ground glass opacities and nodular consolidations with some subpleural sparing (arrow). A few cysts are present but there are no signs of distortion; pure crazy paving pattern is not disclosed probably due to the hereditary nature of the development of the disease and the very long
evolution in this patient; c) Nine years later, at the age of 47 years old, ground-glass-opacities and consolidations have dramatically decreased, replaced by numerous clustered cysts; d) Histological features of the explanted lungs showing mixed PAP and UIP patterns with honeycombing and fibrosis on the explanted lung; note the clear demarcation between the PAP areas and UIP areas with minimal overlap. (Hematoxylin and eosin stain)