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Research letter

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Confocal LASER endomicroscopy in Niemann–Pick disease type B

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A 41-year-old female was referred to our institution for the recent worsening of a chronic cough and dyspnea that had slowly increased over the past six years. She had never smoked, had no notable medical history and was not under any medication. She had two healthy children and no past family history. In 1999, some first pulmonary function tests (PFT) were performed

in another center to explore her chronic cough, showing a subnormal forced vital capacity (FCV, 2.64L, 95% of predicted) and diffusing capacity for carbon monoxide (D_{LCO}, 90% of predicted). The chest computed tomography (CT) showed an interstitial lung disease (ILD) with interlobular septa thickening in the upper lobes associated with ground glass opacities (**Figure 1A**). No definitive diagnosis was made and the patient was lost to follow-up. Twenty years later, she was referred to our pulmonology department for further investigation of a recent worsening of her cough and a slowly progressive dyspnea. The clinical examination was unremarkable, except for the perception of crackles in the bases. Her saturation was of 97% on room air. The CT scan identified similar findings characterized by mild smooth thickening of the interlobular septa and intralobular lines, ground-glass opacities and cysts mainly in the lower lung zones (**Figure 1B**). No honeycomb, pleural effusion or mediastinal adenomegaly were observed. A prominent hepatosplenomegaly was also noted. The haemoglobin was of 13.3 g/dl, the blood leukocyte count was of $8.4x10^9$/L and the platelet count was of 160 G/L. The serum levels of creatinine and C-reactive protein were 43 µmol/l and 2.7 mg/dL, respectively. The liver enzymes, lipid profile and haemostasis parameters were inconspicuous. The gamma globulins were of 13.8 g/l. The angiotensin converting enzyme was normal at 33 U/l (12-68 *U*/L). Antinuclear (ANAs) antibodies as well as granulocyte-macrophage colony-stimulating factor (GM-CSF) autoantibodies were negative. No proteinuria was detected. The PFT demonstrated a restrictive pattern with a total lung capacity of 3.11 L (TLC, 73% of predicted), and a forced expiratory volume of 2.24 L in one second (FEV_1, 95% of predicted), 2.65 L FCV (96% of predicted) and a 73% of predicted D_{LCO}. The arterial blood gas analysis was normal (pH 7.4, 88 mmHg Pa_{O2} and 41 mmHg Pa_{CO2}). The distance in the six-minute walk test was 450 meters (77% of predicted) with a minimal saturation of 90%. In the right lower lobe, a real-time probe-based Confocal LASER Endomicroscopy (pCLE, Cellvizio®) was performed.–The patterns of distribution of elastin fibers and fibrillar organization in segmental and subsegmental bronchi appeared normal [1]. The underlying elastin structure was not visualized in alveoli. Cystic areas were not imaged. We observed fluorescent intra-alveolar globular complexes of 20-30 µm filling the alveolus,

associated with a high intra-alveolar cellularity (**Figure 1C**). A bronchoscopy, macroscopically normal, revealed on bronchoalveolar lavage (BAL) an alveolitis (750 cells/mm^3) mainly composed of macrophages (81%) with foamy cytoplasm's (**Figure 1D**), also with 15% lymphocytes and 4% neutrophils. The BAL cultures of were all negative. Foamy macrophages in the BAL fluid were intensely positive for Periodic Acid Schiff (PAS) while no extracellular PAS stained material was detected, and lightly positive with Oil-Red'O. A transbronchial lung biopsy in the right lower lobe revealed alveolar spaces filled by numerous foamy macrophages (**Figure 1E**). The alveolar septa was not distorted. No granuloma was observed. A decreased acid sphingomyelinase activity in the blood leukocytes was observed (patient 0.15 vs controls 1.07 nmol/h/mg). Sanger sequencing of the *SMPD1* gene identified a homozygous deletion (c.1829_1831delGCC) in exon 6, chromosome 11, which deletes Arg610, ultimately establishing the diagnosis of Niemann-Pick type B disease (NPB) [2]. We report here a progressive pulmonary involvement due to NPB over 20 years. NPB is a lysosomal storage disorder resulting from deficient acid sphingomyelinase activity [3] caused by a mutation in the *SMPD1* (sphingomyelin phosphodiesterase 1) gene [3]. A progressive cell infiltration by foam in reticular tissues results in dysfunctions of major organ systems such as the liver, spleen, bone marrow or lungs [4]. The main differential diagnoses for the pulmonary involvement include other storage diseases, alveolar proteinosis, sarcoidosis, amyloidosis, alveolar microlithiasis, nonspecific and desquamative interstitial pneumonia and the Erdheim-Chester disease [5]. The clinical presentation is usually non-specific, including moderate cough, chronic dyspnea and recurrent upper respiratory tract infections [6]. Biological abnormalities are mainly characterized by thrombocytopenia and dyslipidemia [4]. Diagnosis is strongly suggested by a decreased acid sphingomyelinase activity in blood leukocytes, and ultimately confirmed by the detection of a *SMPD1* mutation [7]. A decrease in D_{LCO} is often reported, but the extent and the pattern of interstitial abnormalities are not necessarily well correlated with other lung function parameters [5]. An original aspect of this case is the slowly progressive worsening, over an extended 20-year period, with the appearance of cysts, as sometimes described [8]. The correlation between the histopathological and imaging findings can likely

be explained: *i)* the interlobular septal thickening is caused by an accumulation of foamy histiocytes in alveolar septas and sometimes subpleural spaces; *ii)* ground glass attenuations are due to partial filling of alveoli by alveolar macrophages; *iii)* the development of cysts can be explained by the migration of storage cells into the bronchiolar lumen, leading to air-trapping and airspace enlargement, as proposed by Baldi et al [8]. Another original point of this case is the very first pCLE description for NPB disease. A pCLE procedure uses a contrast mini-probe with a LASER directed into the alveolar space, generating real-time moving images of intra-alveolar content ("alveoloscopy") in a gray-scale video sequence. Currently, the role played by pCLE in the diagnostic approach of ILDs remains limited [9]. Associating the pCLE fluorescence signal and ex-vivo microscopy could be of interest to establish correlations for further studies. More accurate semiology analyses and correlations with CT in ILDs are strongly needed. French protocol Microsemio-PI (NCT02961335) is ongoing in order to define the semiology of ILDs in pCLE. At the one-year follow-up of our patient, lung exchanges had gradually decreased with a 55% D_{LCO} in 2020, compared to 201 (73%) and 1999 (90%). The interstitial infiltrates increased and more cystic lesions were observed. The patient did not meet the inclusion criteria for an ongoing trial evaluating the efficacy of enzyme replacement therapy with olipudase alfa (NCT0200469), a promising approach as suggested by limited series. A regression of ground glass opacities and reticular lesions has been observed in five patients, translating into an improvement of the D_{LCO} increasing from 53.2% to 67.1% in 30 months [10]. The most significant changes were observed in the most severely affected patients. The outcomes of a phase 2/3 randomized controlled trial (NCT02004691) that included 36 participants are expected. A lung transplantation can be considered in cases of chronic respiratory failure [11, 12]. In conclusion, we report a progressive pulmonary involvement of NPB disease with a 20-year follow-up, as well as the very first pCLE images of this lysosomal storage disease. This minimally invasive approach, at the crossroads between imaging and histology, may represent a valuable tool in the differential diagnosis of ILDs, including NPB.

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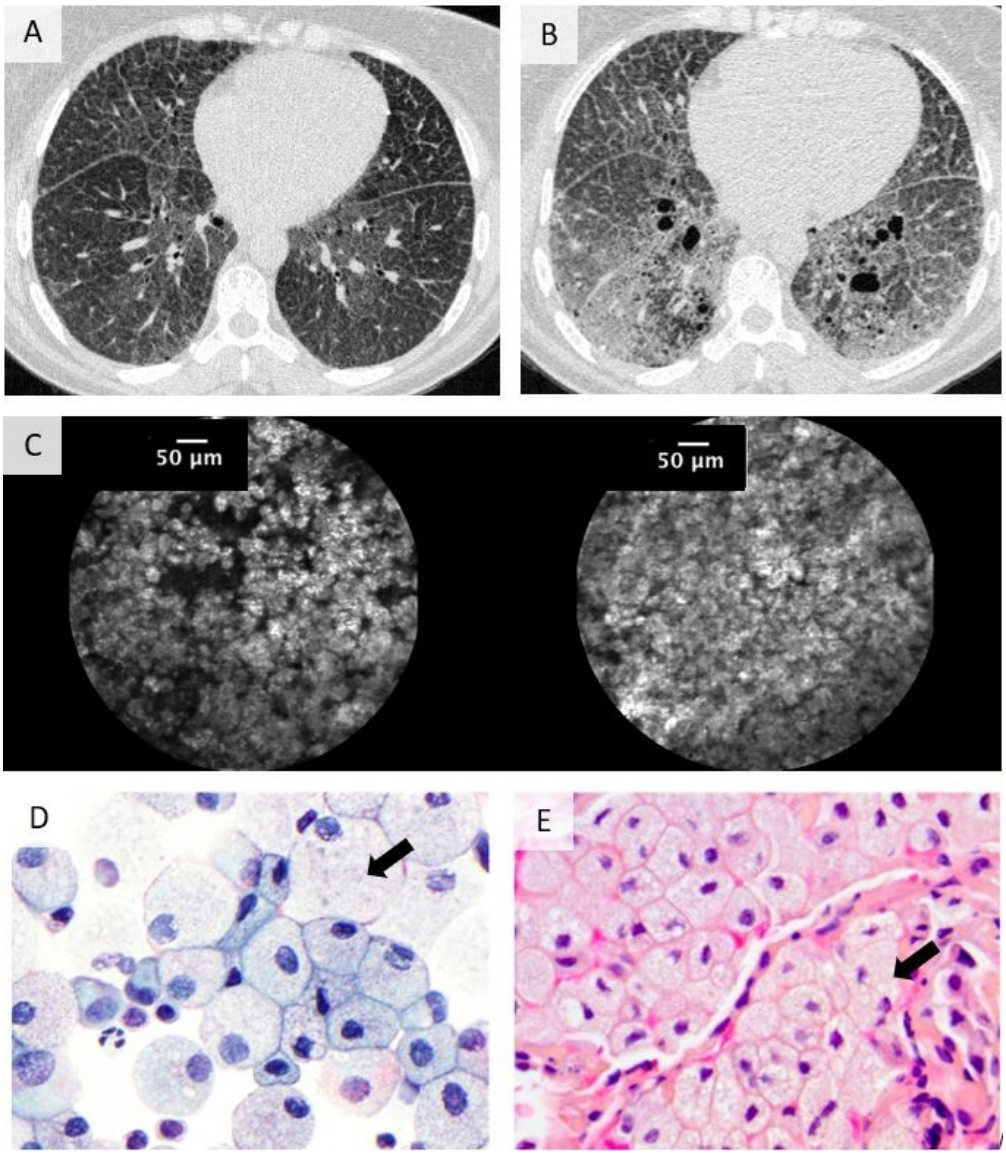

(Panel A and B) Pulmonary transversal window images of chest CT scan evolution between 1999 (A) versus 2020 (B) showing ILD with interlobular septal thickening, ground-glass density in the upper lobes and cysts appearance with basilar predominance. (Panel C) real-time pCLE showing intra-alveolar content with fluorescent globular complexes of 20-30 µm filling the alveolus. (Panel D) BAL Fluid (Papanicolaou stain, x400) revealed an alveolitis (750 cells/mm3) composed of macrophages (81%) with foamy cytoplasm's (black arrows. (Panel E) Transbronchial lung biopsy (x64) in the right lower lobe showing foamy cell with a distended cytoplasm (black arrows) and a small nucleus.