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COMBINED LUNG AND LIVER TRANSPLANTATION FOR SHORT TELOMERE SYNDROME

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INTRODUCTION

Telomeres are DNA-protein structures located at the chromosome ends and terminating with an essential single-stranded 3′-overhang.[1] Their role is to maintain genomic integrity by protecting chromosomes from degradation and illegitimate recombination. Telomeres progressively shorten during cell division because of the inability of DNA polymerase to replicate the 3'-end of chromosomes. Telomerase limits telomere attrition by synthesizing *de novo* telomere sequences at the end of chromosomes.

Despite the action of telomerase, telomere shortening is unavoidable, leading to cell senescence and death by activation of p53-dependent signaling. Mutations in telomere maintenance genes are associated with a variety of diseases, including dyskeratosis congenita, an inherited disorder characterized by bone marrow failure, pulmonary fibrosis chronic liver disease (noncirrhotic portal hypertension or cryptogenic cirrhosis) and an increased risk of cancer. The clinical phenotype of short telomere syndromes as well as age at presentation and disease severity vary among affected individuals within the same pedigree depending on the extent of telomere shortening.

Here, we describe a 52-year-old male patient with short telomere syndrome who underwent combined lung and liver transplantation for terminal pulmonary fibrosis associated with noncirrhotic portal hypertension.

CASE REPORT

The patient first came to medical attention in 1986, at the age of 21 years, because of abnormal liver function tests associated with moderate thrombocytopenia. Liver cirrhosis was suspected but a first liver biopsy was inconclusive, revealing only mild perisinusoidal fibrosis.

The patient was referred to Lausanne University Hospital in January 2017 because of rapidly progressive dyspnea. A diagnosis of pulmonary fibrosis was made, with a restrictive ventilation defect (total lung capacity and forced vital capacity 69% and 58% of the predicted

values, respectively), markedly reduced carbon monoxide diffusion capacity (31% of the predicted value) and rapid progression to oxygen dependence. Orthodeoxia was documented, with arterial oxygen saturation of 94% in the supine position as compared to 78% after 3 minutes in the standing position. A contrast-enhanced echocardiography revealed late left-to-right shunting compatible with concomitant hepatopulmonary syndrome. The alveolar-arterial oxygen gradient was calculated at 50 mmHg.

A new liver workup was performed in the context of lung transplant evaluation, as splenomegaly with thrombocytopenia suggested the presence of portal hypertension. This workup showed normal transaminases (ALT 24 U/l, AST 42 U/l), discrete cholestasis (alkaline phosphatase 113 U/l, γ-GT 93 U/l), normal total bilirubin, an INR of 1.3 and an albumin of 28 g/l (at least partially attributable to malnutrition). Testing for viral hepatitis and autoimmune liver disease were negative. Transjugular liver biopsy revealed a hepatic venous pressure gradient of 12 mmHg and, on histological examination, sinusoidal dilation associated with small-sized central veins, perisinusoidal fibrosis, moderate hepatocyte anisocytosis, glycogen nuclei and significant iron overload (7043 µg per g liver dry weight on quantitative analysis) (Fig. 1A-C). There was no cirrhosis and nodular regenerative hyperplasia was not evident on the biopsy specimen. Facing significant iron accumulation in the absence of a history of blood transfusions, mutations in the *HFE* and other genes associated with hereditary hemochromatosis (*HJV, HAMP, TRF2* and *SLC40A1*) where ruled out.

Hematological assessment was motivated by pancytopenia with macrocytic anemia (hemoglobin 119 g/l, MCV 103 fl), thrombocytopenia (42 G/l) and leucopenia (2.4 G/l including: neutrophils 1.2 G/l, lymphocytes 1.0 G/l, monocytes 0.1 G/l, eosinophils 0.1 G/l and no basophils). The peripheral blood smear demonstrated the presence of dacryocytes and moderate anomalies of neutrophil segmentation. Trephine biopsy revealed a hypocellular marrow, with an estimated cellularity of 35% and mild signs of dyserythropoiesis as well as dysmegakaryopoiesis and no sign of fibrosis. Conventional karyotype was normal and thus a myelodysplasic syndrome could be reasonably excluded. The patient had none

of the classic mucocutaneous findings of dyskeratosis congenita but he also presented severe osteoporosis associated with multiple bone fractures.

Family history revealed that the father and a brother died of pulmonary fibrosis at the age of 50 and 55 years, respectively, without clinical evidence of chronic liver disease or portal hypertension (Fig. 2). Moreover, a nephew required liver transplantation at the age of 15 years because of hepatopulmonary syndrome associated with noncirrhotic portal hypertension. Of note, the explanted liver of the nephew showed histological findings similar to the ones found in our patient (see below).[2]

The association of pulmonary fibrosis, noncirrhotic portal hypertension, hematologic and bone disease as well as the family history suggested a short telomere syndrome, which was confirmed by flow-fluorescent *in situ* hybridization revealing median lymphocyte and granulocyte telomere lengths below the first percentile of the normal reference range. Whole exome sequencing did not reveal any mutation of known pathogenic significance in the genes involved in telomere maintenance.

Due to rapidly deteriorating lung disease despite treatment with pirfenidone as well as danazol and clinically significant portal hypertension, the patient was listed for combined lung and liver transplantation, which was successfully performed in October 2017. Pathological examination of the explanted liver revealed complex vascular liver disease with obliterative portal venopathy and foci of nodular regenerative hyperplasia together with sinusoidal congestion and cholestasis (Fig. 1D). No portal or hepatic vein obstruction were found. There was no significant fibrosis. The histological picture was indicative of noncirrhotic portal hypertension. Pulmonary alterations consisted mainly in areas of honeycombing associated with fibrotic tissue (i.e. usual interstitial pneumonia), lesions typically found in idiopathic pulmonary fibrosis (Fig. 1E).

Two years after transplantation the patient is well on a triple immunosuppressive treatment regimen with tacrolimus, mycophenolate mofetil and prednisone, with improved dyspnea and normal liver function tests. Hematological parameters have normalized on continued danazol

treatment, except for fluctuating, very moderate normocytic anemia and lymphopenia likely ascribed to continued danazol treatment and the immunosuppressive medication.

The post-transplant period was marked by serious infectious complications from which the patient fortunately recovered. His renal function, which was normal before transplantation, deteriorated to KDIGO stage G3b chronic renal insufficiency (current creatinine value of 117 µmol/l; normal range, 62-106 µmol/l) mainly attributed to calcineurin inhibitor treatment. Regarding the fractural osteoporosis, the patient presented a new left humerus fracture which was treated conservatively.

The patient experienced significant improvement in functional health as assessed by a 6-minutes walking test, with a walking distance of 415 meters without oxygen supplementation and no desaturation after transplantation as compared to a walking distance of 400 meters on 9 liters per minute of oxygen and SpO2 of 81% before transplantation.

DISCUSSION

Short telomere syndromes are very rare.[1,3] Lung manifestations and bone marrow failure are well characterized and recognized. However, the spectrum of liver disease is broad, ranging from diverse vascular lesions associated with nodular regenerative hyperplasia to cryptogenic cirrhosis,[4] and awareness among gastroenterologists and hepatologists remains low. Indeed, in our patient liver function test abnormalities were noted before the occurrence of respiratory symptoms,[5] but the diagnosis of noncirrhotic portal hypertension was established only 30 years later in the context of pulmonary workup.

Our patient presented with rapidly progressive lung disease requiring urgent lung transplantation. The role of lung transplantation in interstitial lung disease is well established. However, liver transplantation for short telomere syndromes has only very rarely been reported. Indeed, to our knowledge this is only the second case of combined lung and liver transplantation, performed after careful risk assessment of this procedure in a 52-year-old patient with associated bone marrow and bone disease.[6]

The decision to perform combined lung and liver transplantation was driven mainly by the increased risk of postoperative liver decompensation linked to significant portal hypertension. Moreover, concomitant hepatopulmonary syndrome represented an additional argument for combined transplantation.

Lung transplant outcomes in patients with pulmonary fibrosis in the setting of telomeropathy were recently compared to those who did not harbor any variants in telomere-related genes.[7] The authors demonstrated that patients with pulmonary fibrosis linked to telomeropathies had a higher risk of chronic lung allograft dysfunction and death. However, our patient did not develop acute or chronic rejection.

Previous case series reported favorable outcomes of combined lung and liver transplantation, especially in patients with cystic fibrosis, with median patient ages between 25 and 35 years.[8] With an age of 52 years, our patient was at the upper end of the age range reported in previous series.

Also of concern in the transplant evaluation of this patient was the increased risk of cancer in patients with short telomere syndromes as well as the hematologic manifestations, both of which may be accentuated by immunosuppressive treatment. Pancytopenia resolved after transplantation, suggesting that it was mainly related to hypersplenism in the setting of noncirrhotic portal hypertension but it is also possible that danazol had a beneficial effect.

The use of danazol was found to increase telomere length in patients with telomere diseases with a significant gain in telomere length at 24 months.[9] In our patient, danazol was introduced in the pretransplant period and maintained until this date without evidence of elevation in liver function tests, muscle cramps, edema or lipid abnormalities. Furthermore, no interaction has been observed with the current immunosuppressive therapy. Indeed, transplantation only corrects telomere length in the transplanted organs, i.e. in our patient's case lung and liver. To minimize other potential high morbidity complications as medullary aplasia or osteoporosis deterioration, we chose to maintain this treatment.

We conclude that short telomere syndromes may comprise a wide range of clinical presentations. In patients with severe pulmonary fibrosis and noncirrhotic portal hypertension, combined lung and liver transplantation can be life-saving.

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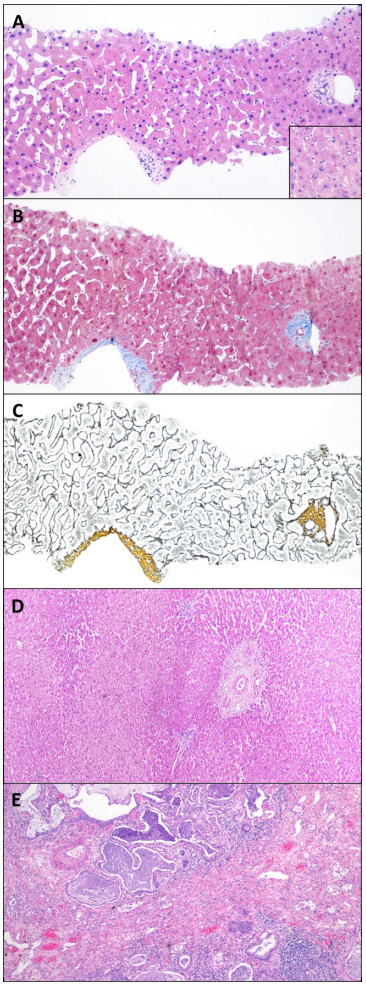
Legends to Figures

Figure 1. Pathological features. (A) Liver biopsy showed areas of sinusoidal dilatation with areas of hepatocyte atrophy, with portal tract displaying maintained bile ducts as well as vascular structures and no inflammation (H&E stain, 10x magnification). Note the hepatocyte anisocytosis and glycogen nuclei (inset). **(B)** No portal fibrosis was observed in the liver biopsy (Masson's trichrome stain, 10x magnification). **(C)** The reticulin meshwork was globally preserved in the liver biopsy (reticulin stain, 10x magnification). **(D)** Similarly to the liver biopsy, the explanted liver featured diffuse sinusoidal dilatation. Some areas displaying nodular regenerative hyperplasia were identified, together with sclerosis of some portal veins (H&E stain, 4x magnification). **(E)** Explanted lung lesions were characterized by a dense interstitial fibrosis delimitating cystic spaces lined by bronchiolar epithelium, suggestive of a usual interstitial pneumonia (H&E stain, 4x magnification).

Figure 2. Family tree. The black arrow denotes our patient. A diagonal line indicates a deceased family member. The number 4 in the circle corresponds to the number of sisters of our patient without any particular medical history.



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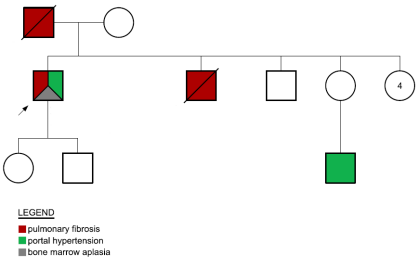

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