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CASE REPORT

Birt-Hogg-Dubé syndrome: metalloproteinase activity and response to doxycycline

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INTRODUCTION

Birt-Hogg-Dubé syndrome (BHDS) is a rare, inherited autosomal-dominant genodermatosis caused by mutations in the *folliculin* gene (FLCN), which is located within the chromosomal band 17p11.2 (1). Patients with BHDS are prone to fibrofolliculomas, trichodiscomas, and acrochordons on the face, neck, and upper trunk; renal tumors; pulmonary cysts; and spontaneous pneumothorax (2-4).

The pathogenesis of lung cyst formation and pneumothorax remains unclear, but studies have shown that *folliculin* is strongly expressed in lung fibroblasts and macrophages. *Folliculin* mutations may alter cytokines and proteases, which are important in maintaining extracellular matrix (ECM) integrity, leading to an inflammatory response and matrix degradation with subsequent remodeling (5,6).

The pulmonary architecture depends on interactions between collagen and elastin fibers in the ECM, which maintain alveoli wall integrity (7). The overexpression of metalloproteinases (MMPs), regardless of whether they are associated with the suppression of tissue inhibitors of metalloproteinases (TIMPs), leads to matrix breakdown, tissue destruction and cystic lesions (7,8).

Because there is currently no treatment for BHDS, we decided to describe our experience treating one BHDS patient with doxycycline, which is an MMP inhibitor (9) that has been previously used to treat cystic lung disease (10).

Case Description

A 44-year-old female non-smoker complained of mild dyspnea upon exertion in 2004 and presented with a spontaneous pneumothorax in 2005. Chest computed tomography (CT) demonstrated bilateral thin-walled cystic lesions (Figure 1). Pulmonary functional tests (PFTs) showed normal carbon monoxide diffusion capacity (DL_{CO} = 81% of the predicted value), lung volume, and expiratory flow rate, but an increased residual volume (RV) and total lung capacity (TLC) ratio (RV/TLC = 0.45) were found, as shown in Table 1. An abdominal CT was also normal. A lung biopsy and pleurodesis by videothoracoscopy were performed, and the patient was diagnosed with lymphangioleiomyomatosis (LAM). She was referred to our institution in 2006 to participate in a doxycycline treatment protocol.

All patients with a diagnosis of LAM who were enrolled in this protocol were submitted to lung function evaluation and ELISA-based quantitative serum and urinary MMP-2 and -9 analysis (R&D Systems; Minneapolis, MN, USA) before and after doxycycline treatment (11).

After six months of receiving doxycycline (100 mg/day), the patient noticed resolution of the exertional dyspnea and improvement in exercise tolerance. After treatment, DL_{CO} and forced vital capacity (FVC) showed increases from 16.8 to 19.7 mL/min/mmHg and 2.87 to 3.12 L, respectively. The forced expiratory volume in the first second (FEV_1), RV and RV/TLC values pre- and post-doxycycline were, respectively, 2.36 and 2.35 L, 2.23 and 1.51 L, and 0.45 and 0.32 (Table 1).

The MMP blockade induced by doxycycline was effective, resulting in a reduction in serum MMP-9 levels from 143 to 36 ng/mL, and urinary levels of MMP-9 became untraceable (55 pg/mL before doxycycline). MMP-2 levels were not detectable before or after treatment.

During follow-up, the patient presented with soft and pedunculated papules on her neck and upper thorax. The biopsy of these lesions was compatible with acrochordons. The patient's family medical history also revealed other cases of cystic lung disease (Figure 2).

The lung biopsy specimen was reviewed in 2007. Histological analysis of the lung tissue revealed several cystic areas with an emphysematous aspect in the lung parenchyma, mostly in the subpleural region. Cyst walls were formed by collapsed alveolar parenchyma or slightly thickened pleural tissue. LAM cells were not observed in the cyst walls, and monoclonal antibody HMB-45 (human melanoma black-45), S100 protein and alpha smooth muscle actin were also not observed (Figure 3).

Based on the family history, the presence of cutaneous lesions (acrochordons), the CT findings and the lung histological review, the diagnosis of BHDS was established.

The positive response to doxycycline treatment, which had never been demonstrated in BHDS, and the association between the loss-of-function mutation in *folliculin* and pulmonary extracellular matrix degradation (6), led us to evaluate MMP behavior in lung tissue.

Immunohistochemical analysis in the patient's lung tissue revealed a large number of MMP-9-positive cells, mostly

No potential conflict of interest was reported.

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Figure 1 - High-resolution chest CT. **A)** A CT scan showing thin-walled, air-filled cystic lesions, including one dominant cyst (6×5 cm). **B)** In addition to the cystic lesions, posterior pleural thickening due to previous pleurodesis is observed on the right side.

Table 1 - Pulmonary function tests performed before and during doxycycline treatment and 18 months after doxycycline interruption.

Pulmonary function variables	Before doxy	During doxy		After doxy
		3 months	6 months	18 months
FVC - L (% pred)	2.87 (82)	3.03 (83)	3.12 (85)	2.33 (70)
FEV1 - L (% pred)	2.36 (87)	2.34 (79)	2.35 (80)	2.21 (80)
FEV1/ FVC	0.82	0.77	0.76	0.95
TLC - L (% pred)	4.87 (94)	x	4.74 (93)	x
RV/TLC (% pred)	0.45 (137)	x	0.32 (97)	x
DLco - mL/min/mmHg (% pred)	16.84 (81)	x	19.7 (80)	x

FVC: forced vital capacity; FEV_1: forced expiratory volume in the first second; TLC: total lung capacity; RV: residual volume; D_{LCO}: carbon monoxide diffusion capacity.

macrophages and neutrophils, in the cyst wall, whereas the adjacent lung parenchyma presented scattered MMP-9-positive cells along the alveolar walls (figure 3). MMP-2 staining showed scattered positive inflammatory cells in the lung parenchyma with no specific cyst staining.

During the subsequent months, the patient developed gastric intolerance symptoms, leading to the interruption of doxycycline therapy. Spirometry performed 18 months after doxycycline interruption revealed a decrease in lung volume and expiratory flow rates (Table 1) with worsening of the pulmonary symptoms.

DISCUSSION

In a non-smoking female who develops spontaneous pneumothorax and presents with cysts in the thoracic high resolution CT, LAM is a strong diagnostic possibility. However, other cystic lung disorders, such as BHDS, may have the same presentation.

In the past several years, lung cyst pathogenesis has been widely studied in pulmonary cystic diseases, such as LAM, Langerhans cell histiocytosis (LCH) and cystic lung light chain deposition disease (CL-LCDD). MMPs appear to have an important role in the pathogenesis of cyst formation (8,12-14). These proteins belong to a family of proteolytic enzymes and are classified according to their specific substrates into gelatinases (MMP-2 and -9), interstitial collagenases (MMP-1, -8 and -13) and stromelysins (MMP-3, -7 and -10). These enzymes are mainly responsible for ECM remodeling, but they also affect cell migration, angiogenesis and pulmonary immunity (7,15,16).

In LAM, lung cyst development is affected by MMP-2 and -9 upregulation in LAM cells (abnormal smooth muscle-like cells) rather than in vascular and bronchiolar smooth muscle cell (14,17). High serum MMP-9 levels are also present in the blood of patients with LAM (17). Zhe et al. described the downregulation of TIMPs, especially TIMP-3, followed by MMP-2 and -14 overexpression, in LAM cells (18).

LCH is characterized by irregularly dilated alveolar spaces and degraded elastic fibers that are surrounded by granulomatous lesions. An immunohistochemical study showed MMP-2 expression in the epithelial basement membranes of these damaged areas and collagen type IV impairment (12,19).

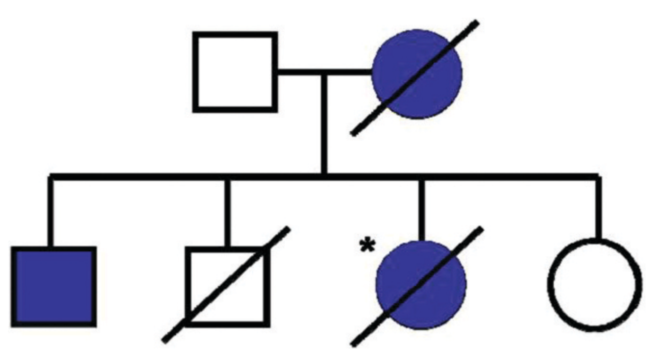

Figure 2 - Pedigree of the family. Square = male member, circle = female member; solid symbol = pneumothorax, open symbol = no pneumothorax; slash through symbol = cystic lesions on lung CT; * our patient.

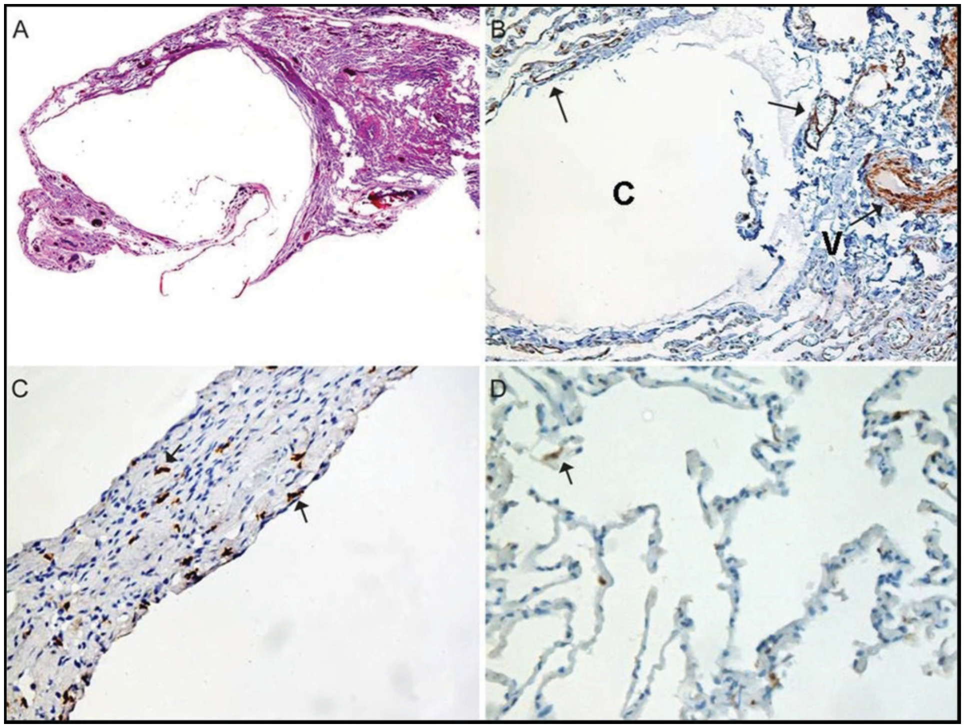

Figure 3 - Histopathological analysis of cystic lung lesions. **A)** A large subpleural cyst within the lung parenchyma. The cyst formed by the collapse of the alveolar walls with a connective tissue lining (H&E staining; 40x magnification). **B)** Details of a smaller cyst stained with an anti-alpha smooth muscle actin antibody. There are no positive LAM cells in the cyst wall. Adjacent blood vessel walls express actin. V = vessel, C = cyst (400x magnification). **C)** Details of a subpleural cyst wall with a large number of MMP-9-positive cells, mostly macrophages and neutrophils, in the cyst wall (400x magnification). **D)** Normal lung parenchyma adjacent to the cyst wall, showing scattered MMP-9-positive cells (arrow) in the alveolar walls (400x magnification).

Pulmonary involvement is especially rare in light chain deposition disease (LCDD), which is a systemic disorder characterized by diffuse monoclonal nonamyloid light chain deposits. There are two types of lung impairment: nodular and CL-LCDD. In CL-LCDD, giant macrophagic cells located around light chain deposits express MMP-2, -9, and -14, which can degrade the elastic network (8,20).

BHDS, an inherited and rare disorder caused by an FLCN mutation, presents with dermatologic and pulmonary involvement and usually manifests during the third or fourth decade of life (2,3). Patients with BHDS have an increased risk of renal cell carcinoma, colorectal neoplasia (3,21) and parotid oncocytomas (22). Abnormal pleuropulmonary findings include lung cysts, pleural blebs and spontaneous pneumothorax (4). Histopathological studies are very limited, especially studies investigating cystic lung pathogenesis. Butnor and Guinee (23) described nonspecific features in the lung biopsies of two BHDS patients, showing intraparenchymal air-filled spaces surrounded by normal parenchyma or a thin fibrous wall. Lung tissue adjacent to the cysts appeared normal. In our patient, we observed subpleural cysts within the lung parenchyma, formed by collapsed alveolar walls with a connective tissue lining.

In contrast to the descriptions of other cystic lung diseases, where a histopathological substrate was present during pulmonary cystic growth, we found lesions resembling emphysema surrounded by normal lung tissue in this BHDS case.

MMP behavior has not been previously described in BHDS. In our pulmonary immunohistochemical analysis, MMP-9 was expressed in a large number of cells, mostly macrophages and neutrophils, located in the cyst wall. However, the lung parenchyma adjacent to the cyst wall presented only a few scattered MMP-9-positive cells. These findings suggest that MMP-9, as in other lung cystic disorders, may be implicated in BHDS cyst development.

Based on the improvement in pulmonary function and the decrease in urinary and serum MMP-9 levels observed in our patient, doxycycline, a known inhibitor of tissue MMPs, may represent a promising therapy for BHDS.

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AUTHOR CONTRIBUTIONS

Pimenta SP diagnosed the patient, wrote the case presentation and contributed to the literature review. Baldi BG contributed to the literature review. Kairalla RA diagnosed the patient and contributed to the presentation of the case. Nascimento ECT and Mauad T contributed to the histopathological diagnosis. Carvalho CRR coordinated the manuscript and was responsible for the final review. All authors read and approved the final manuscript.

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