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CASE STUDY

Diffuse interstitial pneumonia and pulmonary hypertension: a novel manifestation of chronic granulomatous disease

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ABSTRACT: The present authors report the case of an adult with chronic granulomatous disease who developed an unusual lung fibrosis associated with severe pulmonary hypertension.

Histological analysis of a lung biopsy showed a diffuse infiltration with pigmented macrophages without granulomas, which particularly involved the pulmonary arterial and venular walls. Clinical and histological findings were suggestive of pulmonary veno-occlusive disease.

Such a clinical association has not been previously described in the literature and might be due to the persistent expression of gp91phox at a very low level.

In conclusion, the present case report illustrates a novel manifestation of chronic granulomatous disease.

KEYWORDS: Chronic granulomatous disease, lung fibrosis, pigmented macrophages, pulmonary hypertension, pulmonary veno-occlusive disease

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency affecting the innate immune system. The disease is caused by mutations in any one of four genes encoding subunits of the superoxide-generating phagocyte NADPH oxidase, resulting in an absence or very low level of enzyme activity. Microbicidal derivatives of superoxide (hydrogen peroxide, hypohalous acids and the hydroxyl radical) are important for killing potential pathogens. Consequently, CGD patients are highly susceptible to severe, and sometimes fatal, bacterial and fungal infections. More than two-thirds of all cases are X-linked recessive and result from defects in the cytochrome b-245 beta polypeptide gene (CYBB), which encodes the gp-91phox subunit.

The lungs are the most common site of infection in CGD, and bacterial and fungal pulmonary infections are responsible for more than half of all fatal events in patients with CGD [1, 2]. The persistence of microorganisms, often within the phagosomal vacuoles of the neutrophils or macrophages, promotes a chronic inflammatory state. In an attempt to close off these sites of infection, granuloma formation occurs and can reach massive proportions [3, 4]. The chronic granulomatous infiltration of lungs and lymph nodes can result in a severe restrictive lung disease in 5–10% of patients [1, 5].

The present authors describe a new clinicopathological entity in an adult patient with CGD, characterised by the massive infiltration of lung structures with vacuolated and pigment-laden macrophages without granulomas or infections, resulting in severe pulmonary hypertension with a prominent veno-occlusive component.

CASE REPORT

A 39-yr-old white French male was evaluated at the present authors' institute for progressive dyspnoea (New York Heart Association functional class II) and dry cough of 2 yrs' duration.

A diagnosis of CGD had been established at the age of 13 yrs in the context of a liver abscess. Since then, the subject had been taking

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prophylactic co-trimoxazole and flucytosine. He had remained free of systemic or respiratory infections, but was operated on twice for perineal Verneuil's disease. Granulocyte function tests showed very low production of reactive oxygen species (ROS) using phorbol myristate acetate (PMA)-induced chemiluminescence and flow cytometric dichlorofluorescein diacetate oxidation assay. The nitroblue tetrazolium (NBT) reduction assay was applied to whole blood and showed no NBT reduction under lipopolysacccharide stimulation; a residual activity of 28% was found in the presence of *Staphylococcus epidermidis*. Western blot analysis showed a very low level of gp91phox and p22phox compared with healthy donor, although both proteins were still detectable (~1–3% of the healthy control). Genetic analysis revealed an X-linked form of CGD with a splicing mutation of the last nucleotide of the CYBB third exon (c.252G>A; fig. 1). The family pedigree showed the same mutation in the patient's brother, who had suffered from tuberculosis, and the mother was heterozygous for the mutation.

The patient had smoked 15 cigarettes·day^{-1} since 20 yrs of age. He was never exposed to appetite suppressants or amphetamines and had no known occupational or domestic exposure. His physical examination revealed bilateral, basal crackles. Laboratory examinations showed no abnormalities.

Chest radiograph showed diffuse mild interstitial infiltrates. High-resolution computed tomography (HRCT) of the chest revealed scattered ground-glass attenuation with discrete superimposed interlobular septal thickening in the lower lobes (fig. 2a).

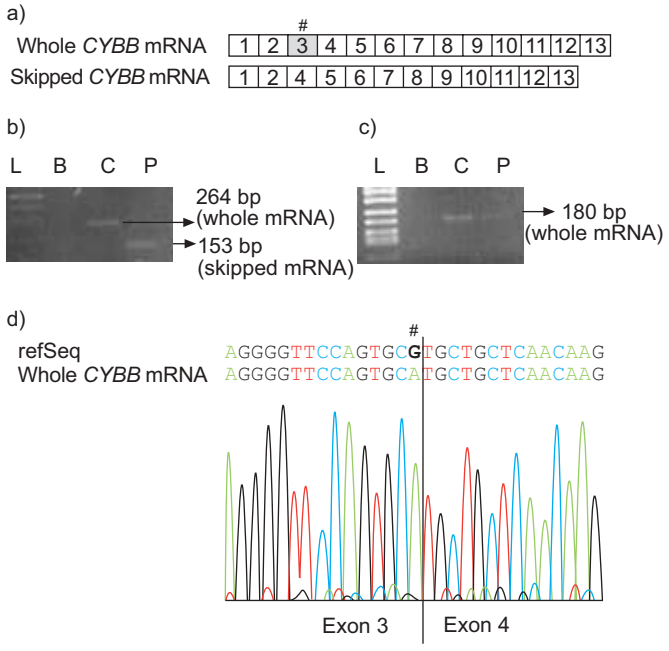

FIGURE 1. Residual expression of whole cytochrome b-245 β polypeptide gene (*CYBB*) mRNA in the chronic granulomatous disease (CGD) patient harbouring the *CYBB* mutation c.252G>A in splice donor site of exon 3 of *CYBB*. a) Schematic representation of mRNAs found in the CGD patient. b) and c) Representative gel pictures showing RT-PCR products for the patient (P) along with the 100 base-pair (bp) ladder (L), the blank (B) and the wild-type control (C) showing the mRNA skipped for b) exon 3 and c) residual whole mRNA. d) Electrophoregram of the whole mRNA of the patient at the exon3–exon4 junction. #: mutation.

Lung function tests demonstrated a mild nonreversible airway obstruction and decreased carbon monoxide diffusing capacity (table 1). An echocardiogram showed normal left heart function but elevated systolic pulmonary artery pressure (40 mmHg). There was no evidence of pulmonary thromboembolism on the ventilation/perfusion lung scan. Right heart catheterisation (table 1) confirmed pre-capillary pulmonary hypertension with a negative response to acute vasodilator challenge with inhaled nitric oxide (10 ppm). There was no evidence of connective tissue disease or HIV infection.

Fibreoptic bronchoscopy was normal. Bronchial biopsies showed no granuloma. Bronchoalveolar lavage (BAL) was performed in the right middle lobe and revealed an increased cellularity (350,000 cells·mL^{-1}), with 62% macrophages, 32% neutrophils and 6% lymphocytes. Most macrophages contained a brown-to-yellow finely granular pigment and optically empty intracytoplasmic vacuoles. Perls' Prussian blue staining highlighted a large number of iron-laden macrophages (60%; fig. 3a) with a Golde score of 120 [6]. No pathogen was identified in either the BAL or the sputum.

Right lung biopsy was performed under mini-thoracotomy in April 2002. Histological examination of the lung showed a preserved architecture. The alveolar walls were focally thickened by fibrosis, which extended into the interlobular septa, and the subpleural and peribronchovascular tissues. Within this fibrosis, a dense brown-to-yellowish-green pigment was observed, which was partly stained by Perls' Prussian blue. When mixed with haemosiderin, this pigment showed a particular perivascular, subpleural and bronchovascular bundle distribution (fig. 3b). Accumulation of macrophages was seen in the alveolar spaces, with a large number of siderophages present; lipid intracytoplasmic vacuoles (stained by Oil Red O) were also noted (fig. 3c). Periodic acid–Schiff stain and CD1a immunohistochemistry was negative. Discrete lesions of respiratory bronchiolitis were noted, with pigmented macrophages in the lumen of respiratory bronchioles and in surrounding alveolar spaces. Muscular arteries showed concentric hyperplasia of the media (fig. 3d). There was no evidence of plexiform lesions or thrombi. Perivascular infiltrates involved >50% of the vessels observed on the biopsy. In the thickened interlobular septa, veins were difficult to visualise due to the pigment deposition and associated proliferating vascular channels, suggestive of veno-occlusive disease. Occasional lymphoid nodular infiltrates were observed in the subpleural areas. No granulomas, capillaritis, vasculitis or a typical pattern of lung fibrosis were observed. The extensive search for infectious agents, including a PCR with *Tropheryma whippelii*-specific sequences, was negative.

Oral prednisone (0.5 mg·kg^{-1}·day^{-1}) was given for 3 months. With therapy, no clinical, haemodynamic, radiological or respiratory function improvement was observed (table 1). During the next 5 yrs, the patient was lost to follow-up.

In May 2007, the patient was admitted for increasing dyspnoea. Pulmonary function tests showed a decline in lung function, with more severe airways obstruction (table 1).

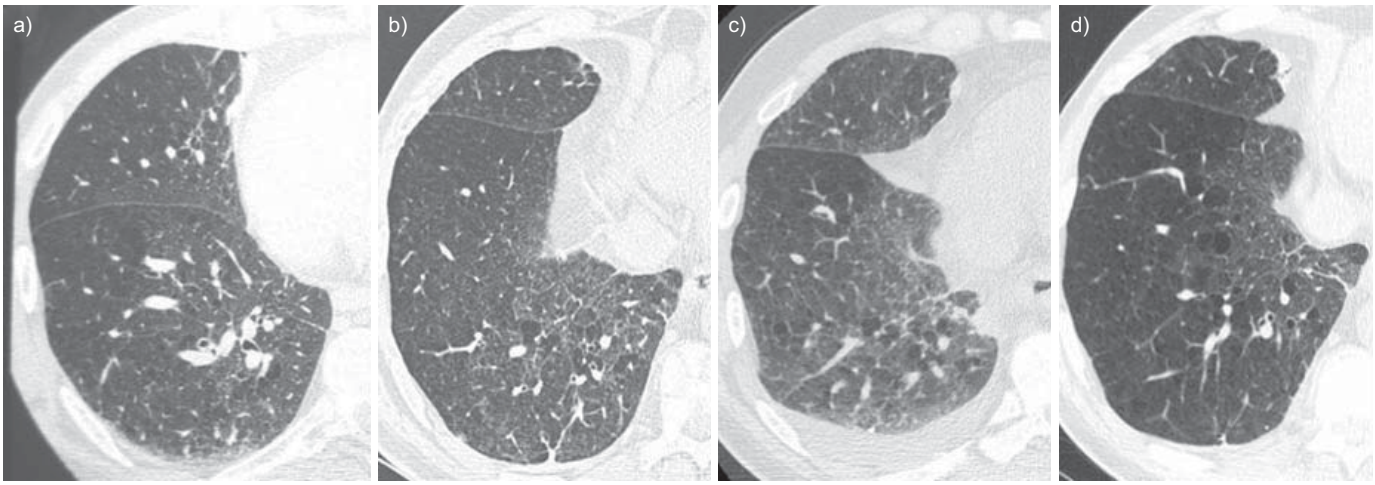

FIGURE 2. a) High-resolution computed tomography of the chest at the time of diagnosis in 2002 shows mild scattered ground-glass attenuation. b) Ground-glass attentuation has increased, with interlobular septal thickening clearly visible in May 2007. c) Computed tomography 24 h after sildenafil initiation shows a right pleural effusion and more extensive ground-glass attenuation, d) both of which decrease rapidly after sildenafil cessation.

Left heart function evaluated by echocardiography was normal. Right heart catheterisation showed progression of pulmonary hypertension and moderately elevated pulmonary capillary wedge pressure (P_{pcw}) with a significant gradient of 2.66 kPa between P_{pcw} and diastolic pulmonary artery pressure, indicating that this patient had significant pulmonary vascular disease (table 1). HRCT of the chest revealed an increase of the ground-glass attenuation and septal reticulations (fig. 2b).

Sildenafil (20 mg *t.i.d.*) was started as a specific treatment for pulmonary hypertension. Within 24 h, dyspnoea increased with hypoxaemia, pleural effusion and frank increase in ground-glass attenuation on computed tomography scan, suggestive of pulmonary oedema (fig. 2c). Sildenafil was stopped and furosemide was given, with rapid improvement (fig. 2d).

DISCUSSION

The present case report describes an adult with CGD, with no history of recurrent respiratory infection but with a previously unreported infiltrative lung disease with pulmonary hypertension. Histologically, this interstitial pulmonary disease was characterised by extensive infiltration of pigmented macrophages with lipoid vacuoles in the perivascular interstitium, in the subpleural tissue and in the interlobular septa, with limited inflammation and without histological findings of granuloma or infections.

Phagocytes, such as neutrophils and macrophages, play an essential role in host defences against microbial pathogens. They contain a phagocyte NADPH oxidase enzyme that generates ROS used to kill pathogens. A defect in the NADPH oxidase activity results in CGD, and life-threatening

TABLE 1 Pulmonary lung function tests and haemodynamic parameters at diagnosis and after corticotherapy

	At diagnosis (April 2002)	After 3 months of corticosteroid therapy (August 2002)	At re-admittance (May 2007)
Lung function test			
FEV_1 % pred	76	73	44
VC % pred	94	95	83
FEV_1/VC %	68	63	42
TLC % pred	97	101	106
$D_{L,CO}$ % pred	35	35	27
$D_{L,CO}/V_A$ % pred	39	35	32
P_{a,O_2} mmHg	74	74	57
P_{a,CO_2} mmHg	34	34	37
Right heart catheterisation			
$\bar{P}_{pa}$ (systolic/diastolic) mmHg	37 (55/24)	42 (54/29)	50 (60/38)
P_{pcw} mmHg	12	13	18
CI $L \cdot min^{-1} \cdot m^{-2}$	3.3	3.1	3.3

FEV_1: forced expiratory volume in 1 s; % pred: % predicted; VC: vital capacity; TLC: total lung capacity; $D_{L,CO}$: diffusing capacity of the lung for carbon monoxide; V_A: alvedar volume; P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: arterial carbon dioxide tension; $\bar{P}_{pa}$: mean pulmonary arterial pressure; P_{pcw}: pulmonary capillary wedge pressure. CI: cardiac index. 1 mmHg=0.133 kPa.

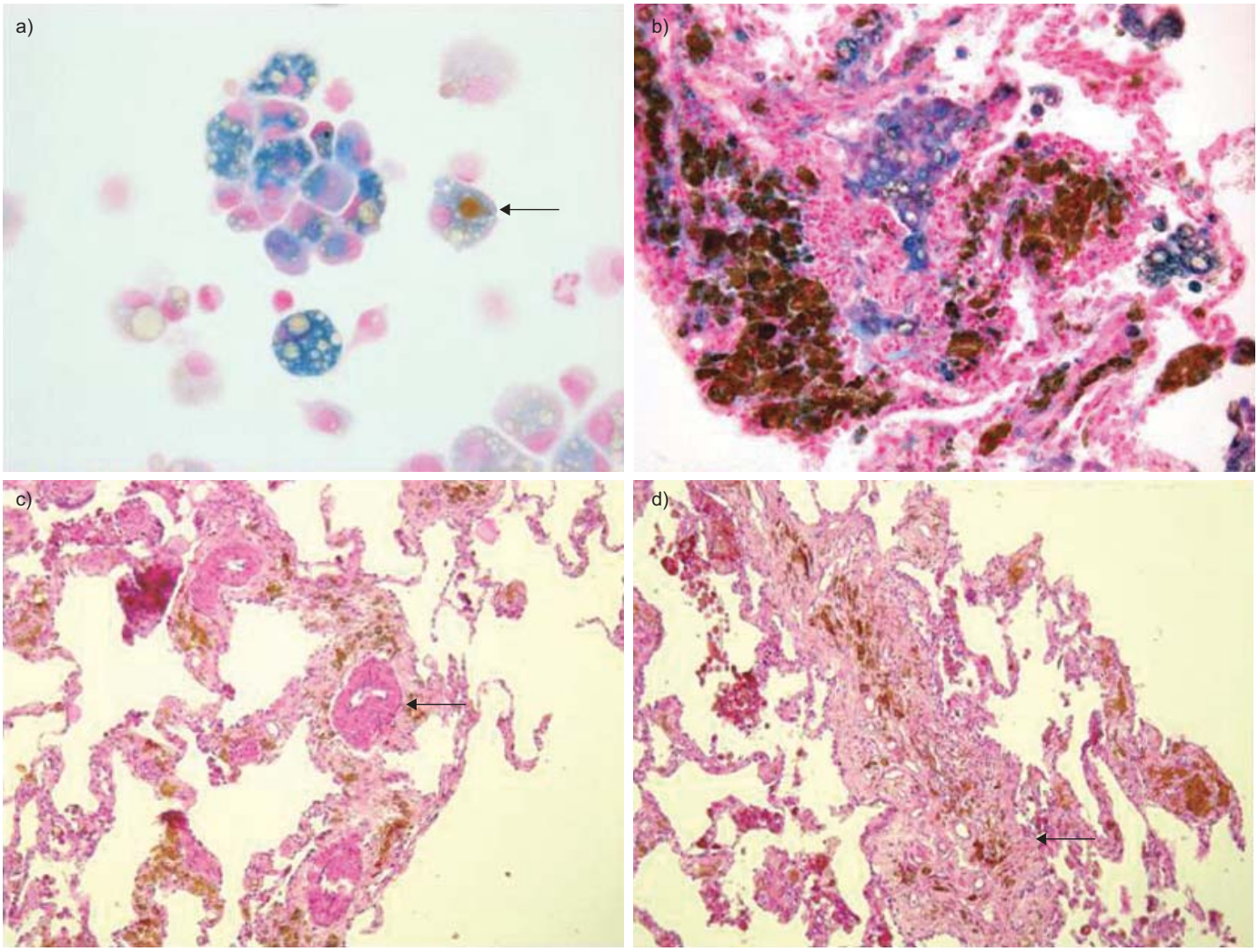

FIGURE 3. a) Bronchoalveolar lavage specimen stained with Perls' Prussian blue showing intracytoplasmic haemosiderin and lipid vacuoles, as well as brown pigment (arrow). b) Histological sections of the lung stained using Perls' Prussian blue showing brown pigment and haemosiderin deposition in the pleura and subpleural interstitial alveolar walls and alveolar macrophages containing intracytoplasmic haemosiderin and lipid vacuoles. c) Muscular arteries showing thickening by hyperplasia of the media (arrow), and d) thick fibrotic and pigmented interlobular septa where veins with vascular channel proliferations cannot be seen, suggesting veno-occlusive disease (arrow).

bacterial and fungal infections. The subject of the present case report had very low, but still detectable, expression of gp91phox, as well as residual mRNA expression normally absent in X-linked CGD. This c.252G>A mutation targets the canonical splice donor site and results in exon 3 skipping. This mutation and the resulting exon 3 skipping were first documented in 1996 [7], and have also been described recently, albeit with a false nomenclature of c.262G>A [8]. Interestingly, the study by BRUNNER *et al.* [8] also report residual NADPH oxidase activity explained by a few mRNA molecules that are still spliced correctly. The present authors confirmed by RT-PCR that their patient presented with at least both types of mRNA: mRNA of *CYBB* with exon 3 skipping (fig. 1b) and a minor fraction of the whole mRNA with the exon 3 carrying the G>A mutation (figs 1c and d).

Of X-linked CGD cases, <10% have a reduced gp91phox protein level with measurable amounts of ROS [9, 10]. This residual activity could explain the lack of severe infection in the present patient, since it appears that having even a small proportion of phagocytes that are NADPH-positive can reduce the risk of serious infection [2, 11, 12].

Detailed reporting of lung histology findings in CGD is infrequent. Classical histopathological features of CGD include active chronic inflammation with neutrophilic infiltration, abscess or granuloma formation [3, 4]. In the present patient, the lack of recurrent lung infection and the residual production of reactive oxygen metabolites probably explained the lack of neutrophilic infiltration and granuloma formation. In the present case, the dominant feature of this interstitial lung disease consisted of an extensive accumulation of pigmented macrophages in the interstitial spaces with cytoplasmic lipid vacuoles, which does not fall into the classical pattern of respiratory bronchiolitis with interstitial lung disease. In a series of 32 surgical lung biopsies from CGD patients, macrophages with yellow-brown pigment were observed in 13% of the specimens, either in areas of fibrosis in small groups or in the interstitium, but never in the alveolar space [3]. An extensive accumulation by pigmented

macrophage is documented in only one case in the literature [4]. In CGD, pigmented histiocytes are often observed in other tissues, such as obstructive lesions of the gastrointestinal tract (57%) and reticuloendothelial organs [4, 13]. They can also be found in skin lesions [14]. The pigmented material is thought to be a ceroid component, or a lipofuschin-like compound, and appears to be composed of lysosomal structures that represent the inadequate digestion of intracellular debris and may be related to the inability of the intra-alveolar macrophage to generate sufficient amounts of oxygen metabolites [4, 15].

The present patient presented progressive pulmonary hypertension that was not explained by hypoxaemia, or cardiac or thromboembolic disease, and did not improve with corticosteroids. Pulmonary hypertension has not been described previously in CGD. Lung histology demonstrated diffuse vasculopathy, involving both arteriolar and venular structures. Infiltration of vascular walls by pigmented macrophages was prominent and probably contributed to chronic pulmonary vascular injury leading to pulmonary hypertension. Evidence of siderophages on BAL, pulmonary oedema induced by sildenafil treatment and lung histology support a significant contribution of venular involvement in pulmonary hypertension and suggest a pulmonary veno-occlusive disease [16]. A diagnosis of pulmonary veno-occlusive disease was further supported by the analysis of the computed tomography of the chest showing ground-glass attenuation and septal reticulations, as well as vasodilator-induced pulmonary oedema [17–19].

The present authors suspect that the *CYBB* gene mutation does not completely explain the clinical observations of their patient, as his nonsmoking brother was asymptomatic with normal lung function tests and HRCT of the chest, despite carrying the same mutation. One could hypothesise that tobacco-smoke exposure might have promoted the occurrence of the disease. The current authors propose that dysregulated macrophages function is linked to chronic granulomatous disease, and stimulation by tobacco-smoke exposure has contributed to the progressive development of parenchymal and vascular disease in the present patient. This would be very similar to what is described in other in-born errors of macrophage metabolism, such as Hermansky–Pudlak syndrome (with lung fibrosis development) [20] or Gaucher's disease (with possible pulmonary hypertension development) [21].

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