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CASE REPORT

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A case of type I Gaucher disease with cardiopulmonary amyloidosis and chitotriosidase deficiency

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Abstract Severe cardiopulmonary amyloidosis developed several months after a total splenectomy in a patient with type 1 Gaucher disease and led within a year to his death at 48 years of age. The autopsy findings were dominated by extensive pulmonary and cardiac amyloid infiltration. No Gaucher cells were found in the lungs. Aside from a glucocerebrosidase deficiency the patient was also deficient in chitotriosidase, an enzyme whose activity is usually greatly increased in the serum of Gaucher patients. Analysis of mutations in the glucocerebrosidase gene revealed heterozygosity for N370S and D409H mutations. The normal amount of glucocerebrosidase was found in the spleen by Western blotting. We suggest that amyloidosis should be considered in the differential diagnosis of severe cardiopulmonary disease in Gaucher patients.

Key words Gaucher disease · Amyloidosis · Chitotriosidase deficiency

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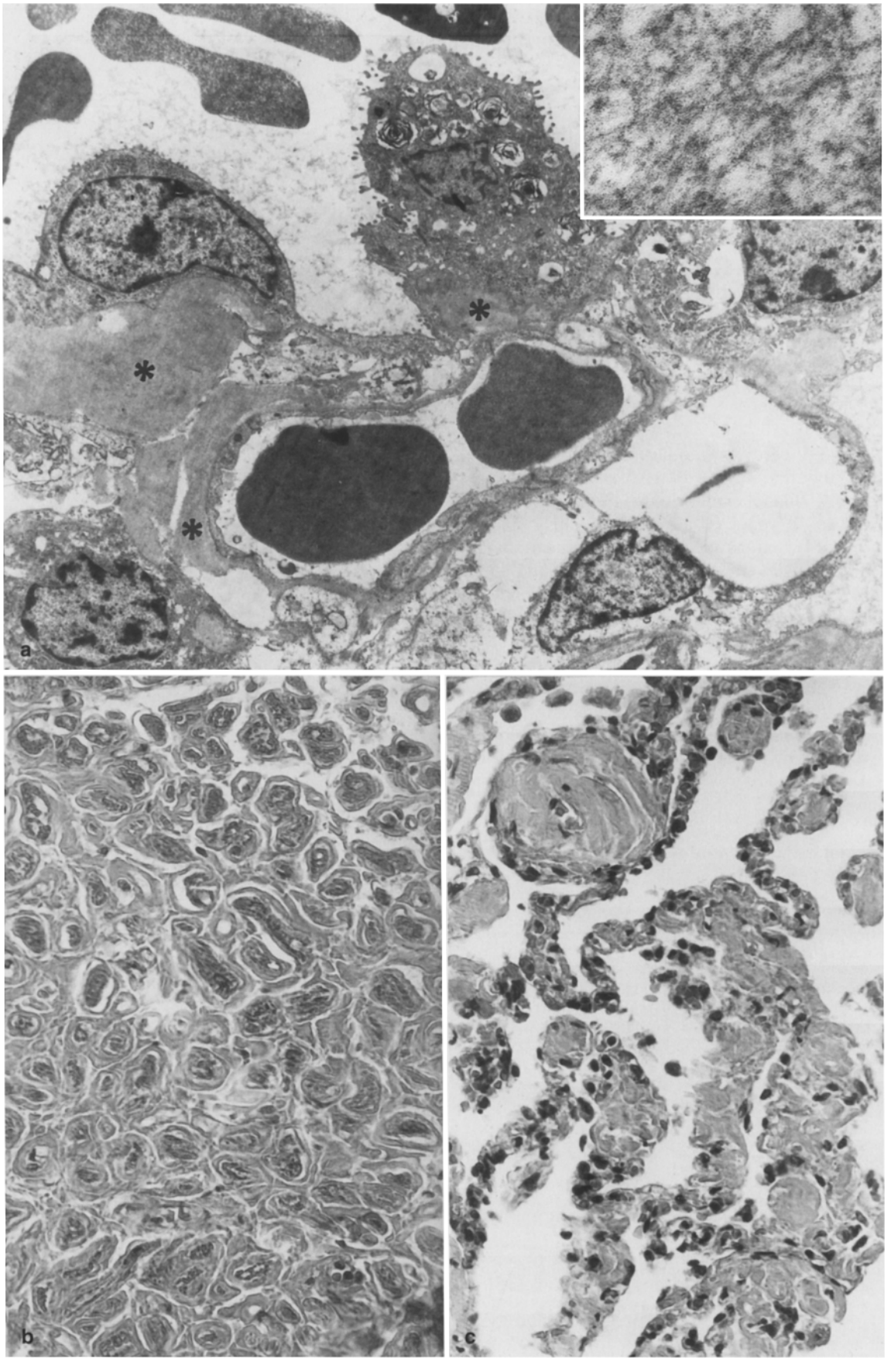
Introduction

Gaucher disease (GD) is the most frequent sphingolipidosis. It is caused by β-glucocerebrosidase deficiency, which leads to storage of glucosylceramide predominantly in the cells of macrophage origin. A rare cause of GD is deficiency of the β-glucocerebrosidase activator saposin C. In the most prevalent non-neuronopathic (type 1) form of the disease the usual symptoms include a variable degree of splenomegaly, hepatomegaly, bone involvement, anaemia, thrombocytopoenia or leucopoenia. The clinical expression in type 1 patients ranges from complete lack of symptoms to severe symptoms even in early childhood [3]. It was only recently found that the activity of chitotriosidase, a novel enzyme belonging to the chitinase family, is elevated several hundred-fold in the serum of Gaucher patients. Several Gaucher patients and healthy individuals have been found to be deficient in serum chitotriosidase [8, 13]. Amyloidosis is a rare fatal complication of GD [6, 7] (C.E.M. Hollak, unpublished observation). We present a patient with a type 1 GD, severe cardiopulmonary amyloidosis and chitotriosidase deficiency.

Clinical history

This male patient was born in 1945. Splenomegaly was noted in adolescence, and the diagnosis of GD was made on the basis of bone marrow investigation and later confirmed by β-glucocerebrosidase assay in white blood cells (see "Results"). At 48 years of age the patient underwent splenectomy because of progressive splenomegaly, severe hypersplenism and pancytopoenia. The spleen weighed 5000 g. Histology showed changes typical for GD, with massive red pulp involvement without signs of amyloidosis at histological or ultrastructural level. Several months after the splenectomy progressive cardiomyopathy developed with exertional dyspnoea, hepatomegaly and oedema of the feet. He died of intractable heart failure 10 months after splenectomy.

Electrocardiography showed a left anterior fascicular block. Echocardiography revealed infiltrative cardiomyopathy with thickening of the ventricular walls and severely compromised systolic function. The global ejection fraction was decreased (0.24).


a
b
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The pulmonary studies revealed decreased vital capacity (3.02 l, 62% of the normal value) and forced expiratory volume (2.19 l/s). The diffusion capacity was also markedly decreased (55% of the normal value). Bronchoalveolar lavage showed normal monocytic population without Gaucher cells.

Open lung biopsy showed extensive amyloid infiltration of larger vessels and less intensive infiltration of alveolar septa (Fig. 1c). There was a paucity of PAS-positive alveolar macrophages, which had none of the cytological features of Gaucher cells. Electron microscopy showed amyloid fibrils around alveolar and septal capillary basement membranes (Fig. 1a) but not around those of bronchioli. The larger vessels were extensively infiltrated with amyloid in all the layers. A small sample of adipose tissue noted beneath the parietal pleura displayed pericellular amyloid deposits around adipocytes.

Skeletal X-ray revealed no signs of bone involvement. About 60% of the cells in the bone marrow were typical Gaucher cells, and the rest were normal haematopoetic cells. No myeloma cells were found, and Congo Red staining did not reveal the presence of amyloid.

The biochemical findings were as follows: ESR 30 per hour, total serum protein concentration 69 g/l; serum electrophoresis showed albumin 42.0, α-1-globulin 2.8, α-2-globulin 4.8, β-globulin 7.4, γ-globulin 12.0 g/l. Immunoelectrophoresis showed a small amount of the monoclonal micromolecular protein of the lambda type. Immunocytological study of the circulating white blood cells was normal except for a slightly decreased amount of natural killer (NK) cells. No monoclonal cell population was found. The urine was negative for protein, and glomerular filtration rate was normal (2.3–3.5 ml/s).

Materials and methods

Glucocerebrosidase activity in white blood cells was assayed fluorimetrically [17] with 4-methylumbelliferyl-β-D-glucoside (Sigma). Chitotriosidase activity in plasma was measured fluorimetrically [8] with 4-methylumbelliferyl-β-D-*N*,*N*′,*N*″-triacetylchitotriose (Sigma). Samples of the patient's plasma were taken before and 4 days after splenectomy. Chitotriosidase activity was also assayed in samples from four healthy controls and six β-glucosidase-deficient patients, who were not treated with Ceredase.

For Western blotting, spleens from Gaucher patients and control spleens were homogenised on ice in 4 parts of water using an Ultraturrax homogeniser. Proteins were separated on a 10% SDS-polyacrylamide gel [10]. Following electrophoretic transfer of proteins to nitrocellulose (0.1 μm, Schleicher and Schuell) at 0.9 mA/cm^2 in a semi-dry blotting apparatus in the transfer buffer (48 mmol/l Tris, 39 mmol/l glycine and 20% methanol; [5]), the membrane was blocked with 0.1% Tween-20 (Sigma), 0.5% ovalbumin (Sevac) in phosphate buffered saline (PBS). The membrane was then incubated in the rabbit anti-human glucocerebrosidase antibody 0126 (prepared as described in [19]) diluted with blocking buffer. After washing with 0.1% Tween-20 in PBS, the membrane was incubated in goat anti-rabbit antibody/peroxidase conjugate (GARPO, Biorad) diluted with blocking buffer, washed, and detected by chemiluminescence (ECL kit, Amersham) according to the instructions of the manufacturer. Protein was assayed using a modified Lowry method [11].

Genomic DNA was isolated from peripheral leucocytes using standard methods [2]. Parts of glucocerebrosidase gene were amplified using PCR [14]. PCR products were digested with restriction endonucleases. The following primers and restriction endonucleases were used for detection of mutations: N370S (S (sense primer) 5′-CTTTGCCTTTGTCCTTACCCTCGA, AS (antisense primer) 5′-GTTACGCACCCAATTGGGTCCTCC, XhoI), L444P (S 5′-GGAGGACCCAATTGGGTGCGTAAC, AS 5-GAGGCAC ATCCTTAGAGGAGCTAGGG, NciI). The same PCR product was digested with MspI or StyI for detection of mutations R463C and D409H respectively), 84GG (S 5′-GAATGTCCCAAGCCTT TGA, AS 5-CACTGCCTGAAGTAGATGC, BsaBI), IVS2+1 (S 5′GAATGTCCCAAGCCTTTGA, AS 5′-AGCTGAAGCAAGAG AATCG, HphI), R496H (S 5′-GCTCTGCTGTTGTGGTCGTG, AS 5′-GCCCAGTGCCTCCTTGAGTA, HphI). The fragments were separated on nondenaturing agarose or polyacrylamide gels and stained with ethidium bromide [2].

Exons 9, 10 and 11 of the glucocerebrosidase gene were amplified using primers specific for the glucocerebrosidase gene [4] (sense primer 5′-AACCATGATTCCCTATCTTC, antisense primer 5′-GGTTTTTCTACTCTCATGCA). PCR products were used as a template for asymmetric PCR with the same primers. Single-stranded PCR products purified by precipitation with ammonium acetate [2] were sequenced using the EMBL automated sequencer, AutoRead kit (Pharmacia) and internal labelling with fluorescein-15-dATP or fluorescein-12-dCTP [20].

For histology and immunohistochemistry tissues were fixed in 10% formaldehyde. Routine histology was followed by histochemical techniques including PAS and Congo Red stainings with or without permanganate preoxidation. For immunohistochemistry the sections were deparaffinised with xylene and blocked with 5% fetal calf serum in PBS. In selected cases pretreatment with saturated lead thiocyanate (10 s in a microwave oven) or pretreatment with trypsin was used. The sections were then incubated with a battery of primary antibodies as per the manufacturers' instructions. The following antibodies were used: rabbit anti-prealbumin and anti-ubiquitin, mouse anti-kappa and anti-lambda Ig chains (Dakopatts), anti-Ig heavy chains (Sevac), anti-amyloid A protein (Sevac). The anti-chitotriosidase antibody (K 259) was prepared as previously described [8] and tested in dilutions ranging from 1:100 to 1:500. The sections were then washed with PBS, incubated with appropriate secondary and tertiary antibodies conjugated with peroxidase (Sevac) and developed using 1 mmol/l 3,3-diaminobenzidine (Sigma) in PBS.

Results

Cardiomegaly and extensive pulmonary amyloid infiltration dominated the autopsy findings, with chronic congestion, organ induration and generalised oedema also found. The heart (500 g) was dilated with wall hypertrophy of both chambers (right ventricle 5 mm, left ventricle 15–20 mm). The muscle was macroscopically pale. The coronary arteries were free of atherosclerosis, and the coronary sinus was rigid and widely open. The heart valves were almost smooth, with the usual glistening surface lost. The parietal endocardium was unremarkable. Histology showed extensive amyloid infiltration of both ventricular and atrial muscles. The fibres were embedded in the amyloid mass (Fig. 1b) displaying a variety of degenerative changes, including lipofuscin deposition. Many of them showed signs of hypertrophy. Some of the amyloid deposits were nodular. Moderate amyloid deposition (diffuse and nodular) was found in the parietal endocardium, in the heart valves, throughout the vessel wall of arteries and veins (intra- and extramurally) and in the wall of the aorta and pulmonary artery. The coronary sinus was infiltrated by amyloid. Epicardial adipocytes were ensheathed by amyloid.

Fig. 1 **a** Electron micrograph of the lung amyloid deposits localised in the capillary and alveolar basement membranes (*). ×3500. *Insert* amyloid fibrils. ×104 000. **b** Massive amyloid deposits around cardiocytes and **c** lung amyloid deposition showing vascular and septal alveolar involvement in a less affected side. HE staining, **b, c** ×320

The lung findings resembled those seen in the biopsy specimen. The absence of Gaucher cells was confirmed in a great many samples. Massive amyloid infiltration was found in the vessels of larger calibres. Extensive amyloid infiltration was present in the muscular layers of the gut and in the vessels generally. A minimal amount of amyloid was seen in the hepatic lobules and in the adrenal cortex. The bone marrow was free of amyloid. The kidneys were not available for examination, but the absence of overt proteinuria suggests minimal involvement. Gaucher cells were found in the lymph nodes and in the liver lobules. Extensive sheets of Gaucher cells were seen in the femoral bone marrow.

The amyloid displayed strong typical green dichroism with Congo Red stain, which resisted permanganate preoxidation. Electron microscopy showed the typical fibrillary pattern. However, all the immunohistochemical stainings, which could be carried out only in paraffin sections, were negative (prealbumin, kappa and lambda Ig chains, Ig heavy chains, amyloid A protein). It should be stressed that the IgG epitopes followed were positive in the dispersed plasmocellular population in the samples. There was a strong focal linear positivity with anti-ubiquitin antibody, which was seen only around part of the external perimeter of the heart muscle fibres.

β-Glucosidase activity in white blood cells was decreased (2.8 nmol mg^{-1} h^{-1}; controls 8–18 nmol mg^{-1} h^{-1}). Western blotting showed normal amounts of β-glucocerebrosidase in the spleen. Screening for frequent mutations of the glucocerebrosidase gene revealed heterozygosity for N370S and D409H mutations, which was confirmed by direct sequencing of genomic PCR products.

Plasma chitotriosidase activity was decreased (1 and 1.5 nmol ml^{-1} h^{-1} before and after splenectomy, respectively; controls 15.3–35.9 nmol ml^{-1} h^{-1}). This finding is in contrast with the gross elevation of plasma chitotriosidase activity in β-glucocerebrosidase-deficient patients (19 712–53 253 nmol ml^{-1} h^{-1}). Chitotriosidase was immunodetected in the paraffin sections of the spleen biopsy samples. Antibody K 259, which was raised against native chitotriosidase, showed a mosaic pattern in Gaucher cells, encompassing a range from entirely negative cells to strongly positive ones. In splenic sections from two other type I patients, Gaucher cells stained strongly and uniformly with K 259 antibody. Serum chitotriosidase activity was not measured in these patients.

Discussion

Amyloidosis is rarely associated with GD [6, 7] (C.E.M. Hollak, unpublished observation]. A plausible pathogenetic link between these two disorders is represented by immunoglobulin abnormalities. Ig light chains are a well-known building stone of immunoamyloid, which affects the cardiovascular system predominantly and which is associated with monoclonal gammapathy [21]. GD is frequently associated with immunoglobulin abnormalities and lymphoproliferative diseases, including multiple myeloma, both of which appear to be more frequent in Gaucher patients than in the general population [3]. GD is therefore considered to be a disorder in which the immune system is chronically stimulated [16].

In one GD patient with amyloidosis [6] there was an IgM gammopathy; the amyloid, however, was not examined immunahistologically. The second case was not accompanied by serum Ig abnormalities, but lambda chains were detected in the amyloid mass [7]. Although there was a small amount of monoclonal protein of the lambda type in the serum of our patient, immunohistology did not confirm the presence of either light or heavy Ig chains in the amyloid deposits. A possible explanation may be the general unsuitability of paraffin-embedded samples for immunohistochemical studies of amyloid [18]. The presence of the serum monoclonal immunoglobulin fraction itself does not allow speculation about its possible role in the development of amyloidosis, as monoclonal immunoglobulin spikes are found in the serum of a high proportion of GD patients over 50 years of age [12, 15].

Whatever the cause of amyloid deposition in GD, its pattern seems to be similar in our case and the published cases (see above), with severe involvement of the myocardium, large vessels and lungs. Amyloid deposition is not related to the presence of Gaucher cells.

The patient of Hanash et al. [7] was splenectomised 10 years before the amyloidosis was found, and the other two patients were not splenectomised [6], (C.E.M. Hollak, unpublished observation). Splenectomy probably does not play a significant part in the pathogenesis of amyloidosis. Treatment with Ceredase did not prevent progression of amyloidosis in one case (C.E.M. Hollak, unpublished observation).

Mutation D409H has been found only in non-Jewish patients, with a prevalence of 5%. Homozygosity for this mutation has been found in three Arab families with oculomotor apraxia and valvular heart disease [9]. The genotype of the published GD patients was not investigated [6, 7].

Chitotriosidase is secreted from cultivated macrophages, and the elevation of its serum activity – like the elevation of serum hexosaminidase, acid phosphatase and angiotensin-converting enzyme activities in GD – is probably a consequence of macrophage activation [8]. The mosaic staining of splenic Gaucher cells with anti-chitotriosidase antibody in our patient is unexplained – it might suggest synthesis of an enzymatically inactive or unstable enzyme in some phases of Gaucher cell development. The physiological function of chitotriosidase is unknown, and so far no disease state has been associated with its deficiency [13] (J.M.F.G. Aerts, unpublished results]. However, chitotriosidase deficiency may be one of the epigenetic factors influencing the phenotypic manifestation of GD [1].

In conclusion, amyloidosis must be considered a possible, albeit rare, fatal cardiopulmonary complication of

GD. Without amyloidosis the current list of cardiopulmonary affections in GD [3] is incomplete. Any hyaline material deposited in tissues in GD should always be examined for the presence of amyloid.

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