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Long term effects of enzyme replacement therapy in an Italian cohort of type 3 Gaucher patients

Annalisa Sechi [a,*], Laura Deroma [a], Andrea Dardis [a], Giovanni Ciana [a], Nicole Bertin [a], Daniela Concolino [b], Silvia Linari [c], Chiara Perria [d], Bruno Bembi [a]

[a] Regional Coordinator Centre for Rare Diseases, University Hospital of Udine, Udine, Italy
[b] Department of Paediatrics, University Magna Graecia, Catanzaro, Italy
[c] Regional Reference Centre for Inherited Bleeding Disorders, University Hospital of Florence, Florence, Italy
[d] Section of Childhood and Adolescence Neuropsychiatry, Department Experimental and Clinical Medicine, University of Sassari, Sassari, Italy

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ABSTRACT

Background: The chronic neuropathic form of Gaucher disease (GD3) is characterised by hepatosplenomegaly, anaemia, thrombocytopenia, bone alterations and central neurological involvement. Enzyme replacement therapy (ERT) has been demonstrated to be effective in non neuropathic Gaucher disease, but long term results in patients with GD3 are still limited and contrasting. A possible role of genotype in determining the response to ERT has been hypothesised.

Patients and methods: All patients affected by GD3, treated with ERT, and followed-up in 4 different Italian centres (Udine, Catanzaro, Sassari and Florence) were included. Data on clinical conditions, laboratory values, neurological and neuropsychological examinations, radiological and electrophysiological features were collected retrospectively from clinical records.

Results: Ten patients (6 females, 4 males) with four different genotypes (L444P/L444P, L444P/F231I, P159T/unknown, C.115+1G>A/N188S) were identified. They received ERT infusions from 3 to 21 years. Haematological parameters and organomegaly improved/normalised in all patients. Three patients showed severe progressive skeletal deformities. 6/10 patients were neurologically asymptomatic when they started ERT for systemic symptoms. During the follow-up, 2/6 developed an important central nervous system disease; 2/6 developed mild central symptoms; and 2/6 did not show any neurological symptom after 5, and 20 years of treatment respectively, despite the presence of epileptiform abnormalities at the electroencephalogram. Overall, neurological involvement worsened over time in 6/10 patients, 3 of whom developed progressive myoclonic encephalopathy and died.

Conclusions: ERT improved the systemic manifestations in patients with GD3, but was not able to counteract the progression of neurological symptoms in the long term.

1. Introduction

Gaucher disease (GD; OMIM: 230800, 230900 and 231000) is the most common lysosomal storage disorder, due to a decreased activity of the enzyme glucocerebrosidase, that causes progressive accumulation of the glycolipid glucocerebroside, primarily in cells of the monocyte–macrophage system [1].

Based on the occurrence of neurological involvement, GD is commonly classified into three different types: *chronic non neuronopathic* (type 1), *acute neuronopathic* (type 2), and *chronic neuronopathic* (type 3). However, the clinical manifestations of the disease encompass a continuous spectrum of phenotypes, ranging from rapidly progressive infantile forms to a slowly progressive late-onset disease, without neurological involvement [2].

The conventionally called *chronic neuronopathic form* (GD3) is characterised by systemic manifestations (haematologic complications, liver and spleen enlargement, and skeletal disorders) of different degrees, associated with signs of the central nervous system involvement, including myoclonus, seizures, ataxia, cognitive impairment, and supranuclear gaze palsy [1,3].

The human glucocerebrosidase is encoded by the *GBA* gene (*GBA;* MIM# 606463; GenBank accession no. J03059.1), located on chromosome 1q21. More than 300 mutations in the *GBA* gene have been

Abbreviations: GBA, glucocerebrosidase; GD3, chronic neuronopathic Gaucher disease; ERT, enzyme replacement therapy; MRI, magnetic resonance imaging; CT, computed tomography; EEG, electroencephalogram; BAER, brainstem auditory evoked potentials; Hb, haemoglobin concentration.

* Corresponding author at: Regional Coordinator Centre for Rare Diseases, University Hospital Santa Maria della Misericordia, Udine, Italy, p.zzale SM della Misericordia 15, 33100 Udine, Italy.
E-mail address: sechi.annalisa@aoud.sanita.fvg.it (A. Sechi).

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reported to date, including all kinds of defects such as single base changes, splicing alterations, insertions, partial and total deletions, and gene-pseudogene rearrangements (www.hgmd.org).

Enzyme replacement therapy (ERT) is available for GD since 1991. Before 1994, patients received macrophage-targeted placental glucocerebrosidase (alglucerase, Ceredase®), later replaced by a recombinant enzyme (imiglucerasi, Cerezyme® or velaglucerase alfa, Vpriv®). ERT showed to decrease both mortality and morbidity, being effective against systemic manifestations in GD type 1 in the short and long term [4,5]. Nevertheless, the effectiveness of ERT on neurological involvement is still debated. Only few clinical reports analysed the long term effects of ERT on GD3, providing conflicting results [6–10]. A possible role of genotype in the response to ERT has been hypothesised [6,9].

The aim of this study was to evaluate retrospectively the effects of long term ERT on the systemic and neurological manifestations in an Italian cohort of GD3 patients.

2. Patients and methods

All patients affected by GD3, treated with ERT and being followed up in four different Italian Centres (Udine, Catanzaro, Sassari and Florence) from 1991 to January 2013 were enrolled in a retrospective cohort study. Diagnosis of GD was defined both biochemically (demonstration of a deficient activity of glucocerebrosidase in either peripheral blood leukocytes or cultured skin fibroblasts) and by molecular analysis of *GBA* gene.

GD3 was defined by the presence of chronic neurological signs or symptoms at the time of data collection.

Molecular analysis: Genomic DNA was extracted from peripheral blood leukocytes, cultured fibroblasts and lymphoblasts using QIAamp DNA blood Mini Kit (Qiagen GmbH, Hilden, Germany). *GBA* gene exons and most intronic regions were PCR amplified using primers designed to selectively amplify the gene (GenBank J03059.1) and not the homologous pseudogene (GenBank J03060.1) as previously described by Koprivica et al. [11].

Putative mutations were confirmed by sequencing duplicate PCR products and by the DNA analysis from parents and relatives whenever possible.

Information on clinical condition, laboratory values, and results from diagnostic procedures were collected from clinical records. Data were assessed before therapy start, every 6 months for the first two years and annually afterwards. Clinical assessment included physical, complete standardised neurological, neuropsychological and ophthalmologic evaluations. Laboratory data were focused on haematological parameters (haemoglobin concentration and platelet count). As for the diagnostic procedures, information was gathered on radiological studies (magnetic resonance imaging (MRI) or computed tomography (CT) of the abdomen to detect organ volumes; radiograms or MRI images of the femora and bone density studies to assess bone involvement), and electrophysiological assessment (electroencephalogram (EEG), brainstem auditory evoked potentials (BAER) and saccadic eye movements recorded by means of an infrared bichannel probe using the *limbus tracking* technique). Intellectual impairment was assessed by age appropriated intelligence quotient (IQ) tests. Mental retardation was defined as mild (total IQ score 50–69), moderate (IQ 35–49) or severe (IQ < 35).

Anaemia was defined as a haemoglobin concentration (Hb) <12 g/dL in males and <11 g/dL in females over 12 years of age, whilst younger children were considered anaemic when their Hb was <10.5 g/dL. Thrombocytopenia was defined as a platelet count <120.000/mm^3 [12]. Hepatomegaly and splenomegaly were defined as organ volumes 1.25 times greater than expected, where expected organ volume values in millilitres were estimated using specific formulas (liver volume (mL) = 25 × patient's weight (kg); spleen volume (mL) = 2 × patient's weight (kg)) (Ludwig J 1979). Bone density was considered pathological when Z-score was ≤ −2.0, according to the International Society of Clinical Densitometry guidelines [13].

Data at baseline (T0), and after 5 (T5), 10 (T10) 15 (T15) and 20 years (T20) from ERT start, were considered for analysis.

3. Results

Ten unrelated patients (6 females and 4 males) affected by GD3, and treated with ERT were enrolled. A detailed description of each patient is reported in Table 1.

Four different genotypes were encountered: 5 patients were homozygous for L444P mutation, 3 had a L444P/F213I genotype, and 2 carried rare alleles (P159T/unknown, C.115+1G>A/N188S).

Study patients showed the first symptoms between 6 months and 5 years of age (median: 24 months) and were mostly diagnosed immediately after ($n = 3$) or within a few months ($n = 3$), whilst in three cases diagnosis was made after one year and in one case after 8 years from symptom onset. The median age at first enzyme infusion was about 6 years, most patients starting to be treated more than 2 years after diagnosis. When ERT was started, four patients had already undergone a complete splenectomy, and one a partial resection. Infusions were made every 7 or 14 days, at a dosage ranging from 60 to 240 U/kg/month. Patients who were neurologically asymptomatic at baseline started ERT due to systemic symptoms. ERT was well tolerated in all patients, and no adverse effects were observed. Two patients (pt 2 and pt 3) started an associated substrate reduction therapy with miglustat in 2009, during the shortage of imiglucerase, and were on combined therapy at the end of the follow-up.

Three patients died during the follow-up, due to progressive bulbar palsy and aspiration pneumonia (pt 4, pt 7) and to complications of the haematopoietic stem cell transplantation, performed in an attempt to halt the progressive neurological deterioration (pt 8).

3.1. Systemic involvement

At baseline, anaemia was observed in 3 patients (pt 1, pt 3, pt 4) and thrombocytopenia in three others (pt 1, pt 6, pt 10). Median Hb levels progressively improved after ERT start in all patients, whilst platelet count increased especially during the first 5 years of treatment. All patients showed normal haematological parameters at the end of the follow-up (Fig. 1).

At baseline, hepatomegaly was present in all patients. Splenomegaly, present in all non splenectomised patients, was more pronounced than hepatomegaly. Liver volume decreased after ERT, reaching normal values at the end of the follow-up in all patients. Spleen volume decreased, as well, but at the end of the follow-up all patients still presented a mild splenomegaly. Both liver and spleen volumes showed a greater reduction during the first 5 years of ERT (Fig. 2).

As for bone involvement, two patients (pt 9 and pt 10) showed important vertebral column deformities at baseline, which worsened over time despite treatment and finally led to a restrictive pulmonary insufficiency. Other three patients reported bone pain at baseline, two of them having a history of femoral fracture. After five years of treatment, only one of them (pt 4), suffering from a deformity secondary to the bone fracture, still complained of bone pain. No bone crises or bone fractures were reported during the whole follow-up period.

Radiological abnormalities, including Erlenmeyer flask deformity, lytic lesions, and osteosclerosis, were observed in 4 patients at baseline and remained stable during the follow-up period. Patients 7 and 8, who died during the follow-up, suffered from osteopenia. Bone density was normal in all the other subjects (data not shown).