Gaucher disease: Review, epidemiology and modelling of the biological and clinical markers evolution

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Abstract

Gaucher disease (GD) is a rare genetic lysosomal disorder which is sometimes complicated by bone events (BEs) such as avascular necrosis, bone infarct or pathological fracture. It modifies a number of biomarkers (increased chitotriosidase, angiotensin converting enzyme, ferritin and tartrate-resistant acid phosphatase, and decreased platelets levels). Specific treatments are available. In 2009, we established a French Gaucher Disease Registry (FGDR). An epidemiological analysis of all cases of GD in France identified 562 patients, registering 378 with follow-up (including 90 deaths and 46 monoclonal gammopathies) and 283 undergoing follow-up. BE occurred before treatment (130 BE in 67 patients), but also during it (60 BEs in 41 patients), with frequencies at 10 years (95% CI) of 20.3% (14.1%–26.5%) and 19.8% (13.5%–26.1%), respectively after diagnosis and treatment onset. The present work focused particularly on ferritin, showing that other iron-based parameters remained normal (in 72 patients), and it is the first to describe (decreased) glycosylated ferritin in 25 patients with GD. An analysis of 62 patients showed […]

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GAUCHER DISEASE: REVIEW, EPIDEMIOLOGY 
AND MODELLING OF THE BIOLOGICAL AND 
CLINICAL MARKERS EVOLUTION

Thesis submitted to the Faculty of Medicine of 
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GAUCHER DISEASE: REVIEW, EPIDEMIOLOGY AND MODELLING OF THE BIOLOGICAL AND CLINICAL MARKERS EVOLUTION
Summary of the thesis

Gaucher disease: Review, epidemiology and modelling of the biological and clinical markers evolution

Gaucher disease (GD) is a rare genetic lysosomal disorder which is sometimes complicated by bone events (BEs) such as avascular necrosis, bone infarct or pathological fracture. It modifies a number of biomarkers (increased chitotriosidase, angiotensin converting enzyme, ferritin and tartrate-resistant acid phosphatase, and decreased platelets levels). Specific treatments are available.

In 2009, we established a French Gaucher Disease Registry (FGDR). An epidemiological analysis of all cases of GD in France identified 562 patients, registering 378 with follow-up (including 90 deaths and 46 monoclonal gammapathies) and 283 undergoing follow-up. BE occurred before treatment (130 BE in 67 patients), but also during it (60 BEs in 41 patients), with frequencies at 10 years (95% CI) of 20.3% (14.1%–26.5%) and 19.8% (13.5%–26.1%), respectively after diagnosis and treatment onset.

The present work focused particularly on ferritin, showing that other iron-based parameters remained normal (in 72 patients), and it is the first to describe (decreased) glycosylated ferritin in 25 patients with GD.

An analysis of 62 patients showed that BEs occurred before and during treatment; biomarkers were modelled (using mixed models) and the impact of slope and values at treatment onset were tested on BEs.

In 2009, a worldwide shortage in the supply of imiglucerase led to treatment modifications or interruptions for patients with GD type 1. We describe the shortage’s impact on therapeutic management and the disease’s course in French patients.

We report on the first dynamic model of how biomarkers evolve during the course of GD. This model enabled us to estimate that 95% of biomarkers response to enzyme replacement therapy (ERT) was achieved in 2 years. We also found that by using the current treatment, about 65% of patients should experience a normalisation of chitotriosidase and ferritin.

We used a frailty model to analyse the occurrence of repeated BEs in Gaucher patients after treatment initiation.

In the discussion, we developed the use of joint models for analysing repeated longitudinal data (biomarkers) and survival time (BEs) in GD.

Further studies should continue to use biomarker modelling with the patient registry in order to try to predict BE and other complications before treatment.
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PART I: GAUCHER DISEASE (REVIEW)
Abstract Review Gaucher Disease

Gaucher disease (GD, ORPHA355) is a rare autosomal recessive genetic disorder caused by a deficiency in glucocerebrosidase activity, leading to accumulation of its substrate, glucosylceramide, in macrophages which then take on a characteristic appearance and are called Gaucher cells.

Its incidence is around 1/40,000 to 1/50,000 births in the general population, but can reach 1/800 births in the Ashkenazi Jewish population.

Infiltration of the bone marrow, spleen and liver by Gaucher cells is considered the main cause of the cytopenia, splenomegaly, hepatomegaly and bone lesions associated with the disease. Type 1 Gaucher disease, which affects over 90% of patients, is characterized by visceral involvement. Types 2 and 3 GD are characterized by systemic involvement associated with neurological impairment that is very severe in type 2 GD and variable in the type 3 form.

The phenotypic expression of GD varies considerably. Diagnosis is often delayed and GD should be considered in case of splenomegaly with a non-obvious etiology and prior to splenectomy which can worsen the course of the disease. The diagnosis must be confirmed by assay of leukocyte glucocerebrosidase. Mutations in the GBA1 gene must be identified since they may be of prognostic value in some cases.

Genetic counseling should be provided to families of patients to screen for GD or possible heterozygosity for the disease. Screening for possible heterozygosity for the disease in spouses of at-risk subjects is useful in cases of consanguinity or if a patient’s spouse is of Ashkenazi Jewish descent. Prenatal screening is advised for couples who have had a child with type 2 or 3 GD or disease with fetal onset.

Disease-specific treatment consists in intravenous enzyme replacement therapy (imiglucerase, velaglucerase or taliglucerase) whose efficacy is determined by the improvement of clinical signs and laboratory values and biomarkers such as chitotriosidase, CCL18 and glucosylsphingosine. Enzyme replacement therapy has little effect on neurological problems. Orally administered substrate formation inhibitors are also indicated: miglustat is less effective and less well tolerated than enzyme
replacement therapy and eliglustat whose efficacy appears similar to enzyme replacement therapy requires precautions for use. Patients have been found to be predisposed to developing Parkinson’s disease during the course of type 1 GD and the risk of neoplasias associated with the disease is still a subject of discussion.

Key words:

Gaucher disease, lysosomal storage disease, glucocerebrosidase, enzyme replacement therapy, substrate reduction therapy.
Introduction

Lysosomes are subcellular organelles containing enzymes responsible for the physiological degradation of cellular components. Lysosomal storage diseases are a group of very heterogeneous hereditary diseases caused by a genetic mutation affecting the activity of one of the lysosomal enzymes, activator proteins or transport proteins (Figure 1). Substrates are basically sphingolipid metabolites, essential components of cell membranes that have an important effect on the signaling pathways (40). Disease generally results from accumulation of the substrate(s) normally catabolized by the enzyme, leading to cellular dysfunction and clinical abnormalities.

Definition

Gaucher disease (GD, OMIM #230800, ORPHA355) is a lysosomal storage disease. It is the most common of the lysosomal storage diseases (15%) and was first described by Philippe Gaucher in 1882 based on the case of a patient with massive splenomegaly without leukemia. It is a rare, autosomal recessive genetic disease caused by a mutation in the GBA1 gene, located on chromosome 1 (1q21), leading to a decrease in the activity of a lysosomal enzyme, glucocerebrosidase (GCase) (or glucosylceramidase or Glucosylceramide-beta-glucosidase; Enzyme Commission Number: 3.2.1.45), which hydrolyzes glucosylceramide (Gcer) and its deacetylated form glucosylsphingosine into ceramide and glucose (Figure 1). More than 300 GBA1 mutations have been described (53). Gaucher disease may also exceptionally be caused by a deficiency in the activator of GCase, saposin C (116). Three forms of GD have been identified: type 1 is the most common form and typically causes no neurological damage, while types 2 and 3 are characterized by neurological impairment.

The incidence of the disease is around 1/40,000 to 1/50,000 births in the general population, but can reach 1/800 births in the Ashkenazi Jewish population (43, 109).
Figure 1: Lysosomal degradation of selected sphingolipids. The eponyms of individual inherited diseases are given. Enzymes and activator proteins involved (in blue), and enzyme-related diseases (in red) are indicated (58, 100). Sap: saposin. In lysosomal storage diseases, an enzyme deficiency is responsible for the accumulation of its substrate in the cytoplasm of the cell (overload disease). Gaucher disease is caused by a deficiency in glucocerebrosidase (GCase) (or β-glucosidase), which leads to accumulation of glucosylceramide (Gcer). Glucosylceramide forms fibrillar aggregates that accumulate in macrophages resulting the cytoplasm of these cells presenting a characteristic “crumpled tissue paper” appearance. These cells are called Gaucher cells; they infiltrate various organs (bone marrow, spleen, liver) and are responsible for the major signs of the disease.

Adapted from Schulze (100) and Kolter (58).
Pathophysiology

Mutations in the GBA1 gene lead to a decrease in the activity of GCase. The consequences of this deficiency are generally attributed to accumulation of the enzyme’s substrate, Gcer, in macrophages, inducing their transformation into Gaucher cells. Gaucher cells have a characteristic appearance in optical microscopy: they are large cells with eccentric nuclei with condensed chromatin and cytoplasm with a heterogeneous “crumpled tissue paper” appearance. This feature is related to the presence of Gcer aggregates in characteristic twisted, fibrillar arrangements that can be visualized by electron microscopy (62). Gaucher cells mainly infiltrate the bone marrow, spleen and liver, but also other organs and are considered responsible for the disease’s symptoms. Gcer is a complex glycolipid derived from the degradation of the cell membranes of red and white blood cells, which contain large amounts of glycosphingolipids. Gcer accumulation in the bone marrow is considered the first step of bone involvement leading to vascular occlusion and compression, the sources of necrotic complications (76). The pathophysiological mechanisms of neurological involvement remain poorly explained; Gcer turnover in neurons is very low and its accumulation is only significant when the residual activity of the enzyme is very low, that is to say only with certain types of GBA1 mutations (85). Exceptionally, GD may be caused by a mutation of the PSAP gene leading to a deficiency in saposin C without GCase deficiency (116). These patients generally present with neurological damage similar to that of type 3 GD.

Subpopulation of Gaucher cells

Recent results indicate that Gaucher cells do not result from the transformation of just any macrophage cells, but correspond to a distinct M2 subpopulation issued from an alternative differentiation pathway (17). This subpopulation has been described as possessing anti-inflammatory, immunomodulatory and tissue repair properties and includes macrophages that remove abnormal hematopoietic cells, or phagocytose the nuclei of erythroblasts. However, GCase deficiency of the macrophage precursors, or monocytes, is identical in a given patient (11, 12), which suggests that the metabolic consequences depending on cell differentiation in the tissues favor the “Gaucher cell”
phenotype. The plasma cytokine profile shows concurrent activation close to the Gaucher cells (17) of inflammatory M1 macrophages, presumably implicated in the “pseudo-inflammatory” state that was described many years ago and the heterogeneous manifestations of the disease.

Some cytokines, chemokines and other molecules are increased in the plasma (IL-1b, IL-6, IL-8, TNFa (Tumor Necrosis Factor), M-CSF (Macrophage-Colony Stimulating Factor), MIP-1b, IL-18, IL-10, TGFb, CCL-18, chitotriosidase, CD14s, CD163s) and could be implicated in hematological and bone complications (5, 8, 50, 117). Only some of these molecules are expressed by the Gaucher cells themselves. This is the case of chitotriosidase and CCL18 which thus constitute quite specific disease biomarkers (17).

Osteoporosis may be linked to IL-10 which inhibits osteoblast activity, but also to IL-1b, IL-6 and M-CSF, MIP-1a and MIP-1b, which stimulate bone resorption by an increase in osteoclast activity (5, 117).

**Metabolic consequences other than the accumulation of glucosylceramide**

New metabolic consequences were identified in a mouse model by the group of P. Mistry (81) (Figure 2A). Gcer is also the substrate of an alternative pathway in which a ceramidase transforms Gcer into glucosylsphingosine which diffuses into fluids due to its solubility. This pathway is favored in cases of GCase deficiency. In the cytoplasm, glucosylsphingosine is metabolized by a GCase that is active at a neutral pH (GBA2 gene), producing sphingosine and sphingosine-1-phosphate (S1P) (33, 80). Sphingosine could be particularly toxic to the bone; in this model, deletion of GBA2 reverses the “Gaucher disease” phenotype, in particular the effects on the bone. It likely also has an impact on the nervous system as GD-related neurological impairment appears to result from neuronal dysfunction and death due in particular to the accumulation of glucosylsphingosine (52). Glucosylsphingosine, normally not found in the human brain, is detectable in the brains of patients with GD-related neurological lesions despite no Gaucher cells being observed in the nervous system. Thus, glucosylsphingosine could be a biomarker that may be more specific and more sensitive than chitotriosidase or CCL18 (33, 95). Glucosylsphingosine could be a source of S1P influencing the differentiation, migration and survival of several cell types including lymphocytes and macrophages (69). Further studies are required to check if the consequences are exactly the same in humans.
PART I: GAUCHER DISEASE (REVIEW)

Figure 2
Figure 2: The larger and more complex impact of glucocerebrosidase (GCase) deficiency.

Expression of GCase varies from one cell type to another and depending on the tissue. A: In a mouse model, in case of GCase deficiency, glucosylceramide (Gcer) is transformed via an alternative ceramidase pathway into glucosylsphingosine which is degraded by cytoplasmic GCase2 (GBA2 gene), active at a neutral pH, to produce S1P, a very active metabolite (80). B: Protein maturation takes place in the Golgi apparatus; the transport and delivery of GCase to the lysosomes require a particular molecule, LIMP-2, which combined with adapter complexes (AP-1 and AP-3), allows GCase to reach the lysosome where the acidic pH breaks the molecular link (41). C: LIMP-2 is a lysosomal membrane protein (LMP), whose highly glycosylated intra-lysosomal part protects the membrane of the lysosome. LIMP-2 anomalies can induce a phenotype close to type 3 Gaucher disease (GD) (41). D: In GD, mutated GCase presents misfolding that favors its association with molecular chaperones such as Hsp70/90 and Hsp27, which direct it toward the proteasome and thus contribute to the reduction of its activity (41).

Adapted from Mistry for A (80), Gonzalez for B, C and D (41)

Steps in the synthesis of glucocerebrosidase implicated in the deficiency of the enzyme GCase deficiency is not only related to direct impairment of its enzymatic function, but also to abnormalities in the system responsible for transporting and delivering the enzyme to the lysosome or enzyme misfolding during its passage in the endoplasmic reticulum, leading to its degradation by the proteasome (96, 126) (Figure 2 B-D). Unlike the other molecules transported to the lysosome, the transport and delivery of GCase does not depend on the mannose 6-phosphate system, but implicates other molecules such as Lysosomal Integral Membrane Protein 2 [LIMP-2] (91); GCase becomes active after its link with LIMP-2 is broken in the lysosome at an acidic pH. Lysosomal Integral Membrane Protein 2 mutations have been described associated with neurological disorders (13, 99), and could alter the GD phenotype, particularly by transforming it into a type 3 form (120).
Cells other than Gaucher cells are altered
In parallel, it has been demonstrated that the enzyme deficiency could have an impact on many cells including hematopoietic progenitor cells, erythrocytes or mesenchymal cells (11, 21, 61), hepatocytes (110) and the nerve cells of patients with neurological lesions.

Relationship between the GBA1 gene and Parkinson’s disease
Another unexpected consequence was identified based on the observation that patients with type 1 GD were predisposed to Parkinson’s disease. A heterozygous mutation in the GBA gene (even without GD which requires a double mutation) is now considered a risk factor for Parkinson’s disease (4, 23, 98). The prevalence of heterozygous mutations (essentially N370S, but also L444P, 84GG, IVS+1, V394L and R496H) was found to range from 3 to 8% in Caucasian Parkinson’s disease patients (98, 103) and was higher in the Ashkenazi Jewish population, reaching 15% or even 31% (4, 103). Several studies have shown that the accumulation of Gcer promotes the formation of α-synuclein polymers which aggregate to form Lewy bodies in the nerve cells in Parkinson’s disease (Figure 3). Furthermore, these polymers have an inhibitory effect on GCase (70, 84, 128). The most recent studies show that this effect could be modulated by the co-activator of GCase, saposin C, which could partly explain the limited penetrance of this neurological impairment in patients with GD (47, 129).
**Figure 3: Relationship between glucocerebrosidase (G Case) and neurological diseases with Lewy bodies.**

Normally, GCase binds to α-synuclein, facilitating its elimination. Mutated GCase promotes the accumulation of α-synuclein molecules which form oligomers. These oligomers are capable of binding to the GCase molecules and inhibiting them, increasing the enzyme deficiency. These oligomers accumulate in the cytoplasm of nerve cells, forming insoluble aggregates called Lewy bodies. Saposin C could have a modulating effect by its binding to GCase which would maintain its activity.

Adapted from Mazzulli (70), Yap (128, 129), Murphy (84), Gruschus (47) and Cullen (27).
Altered iron metabolism

Iron is stored in the body in ferritin to avoid toxicity to cell components. In GD, ferritin levels are increased in Gaucher cells and the synthesis of hepcidin which inhibits intestinal absorption of iron is increased. Transcription of the hepcidin gene is increased in Gaucher cells by certain cytokines (IL6 and IL1beta); the thus activated macrophages can also induce iron retention by an autocrine mechanism (72) and a decrease in glycosylation capabilities leading to a decrease in glycosylated ferritin (108). Given its increase in parallel to the activity of GD, ferritin could be a useful biomarker.

Clinical presentations

Gaucher disease is characterized by hepatosplenomegaly, cytopenia, sometimes severe bone involvement and, in certain forms, neurological impairment. The variability of the clinical presentations of GD could be explained by a continuum of phenotypes (102). However, four major phenotypic presentations are usually distinguished. They are described below in order of increasing severity:

Type 1 Gaucher disease (ORPHA77259)

Type 1 GD, classically defined by the absence of neurological impairment, is the most common form of the disease (prevalence of 90-95%). The clinical presentation is very variable, ranging from asymptomatic throughout life to severe forms in childhood. The initial symptoms vary considerably and patients can be diagnosed at any age (109). Depending on the studies, the median age of diagnosis is between 10 and 20 years (22, 109). Type 1 GD often affects functional prognosis, but is rarely life threatening. Asthenia is common (50% of patients) and often impacts school life and socio-professional activities. In children, growth retardation and delayed puberty are common (growth <5th percentile in 34% of children) (57). Splenomegaly is observed in more than 90% of patients and is sometimes massive with the spleen weighing up to several kilograms and causing abdominal pain or distension. It may be the only clinical sign and lead to numerous tests being performed if GD is not
considered. It may be complicated by splenic infarction. Rupture of the spleen only occurs exceptionally.

Hepatomegaly is noted in 60 to 80% of patients. The development of fibrosis then cirrhosis is rare (109). Hepatic and splenic infarction may be observed, manifested as acute pseudo-surgical abdominal pain.

A hemorrhagic syndrome which is rarely severe and usually related to thrombocytopenia (60-90% of cases) or, optionally, to coagulation or primary hemostasis disorders or, to a degree, to platelet disorders may be observed at diagnosis. Mucocutaneous bleeding (epistaxis, gingival bleeding, menorrhagia etc.) is common; postoperative hemorrhage or bleeding during birth and spontaneous hematomas have been reported (psoas hematomas, etc.). Anemia, observed in 20-50% of cases, is generally moderate. Leukopenia is rare.

Bone involvement causes acute pain manifested as very painful bone crises, predominantly in the pelvis and lower limbs and more rarely in the upper limbs, and/or chronic pain that should be assessed using a visual analog scale or digital scale. The severity of the pain varies but it may have an impact on functional prognosis. The pathophysiology of bone manifestations is poorly known and justifies the use of common terminology. The painful bone crises are probably associated with ischemic vaso-occlusive phenomena. It seems that they may be reversible and not show up as lesions with medical imaging. However, they most often cause abnormalities referred to as bone infarcts on the long bones (metaphyses, diaphyses) and flat bones, and lesions referred to as avascular necrosis (AVN) on the epiphyses. In addition to the vascular theory explaining the ischemic events (bone infarcts and avascular necrosis), a mechanical theory has also been put forward to explain the spontaneous or trabecular microfractures that are observed (mechanical or spontaneous osteonecrosis), in particular on the femoral head, the femoral condyle and the tibial plateau.

The acutely painful bone crises are more common in children (30% of children with type 1 GD). They usually progress over 7 to 10 days and are associated with local inflammation, mild fever (38°C), polynuclear leukocytosis and a moderate inflammatory syndrome. This picture may mimic osteomyelitis (pseudo-osteomyelitis),
delaying diagnosis (125, 130). Avascular necrosis is observed in 15% of cases, most often on the femoral or humeral heads, more rarely on the femoral condyles or tibial plateaus and exceptionally on the feet (talus, calcaneus), hands and spine (vertebra plana). In the long term, AVN may be complicated by osteoarthritis, often justifying joint replacement surgery. Bone crises are predictive of future bone infarcts and osteonecrosis.

Moderate losses in bone mass (osteopenia) or more severe declines (osteoporosis) increase with age and menopause in normal subjects. Loss in bone mass occurs earlier and is more severe in patients with GD and may cause pathologic fractures (long bones, vertebrae, etc.). Bone mass decline seems to be correlated with other bone and visceral complications (87). Focal lytic lesions that can erode the cortical bone and promote pathologic fractures are sometimes observed (32). Extra-osseous extension of Gaucher cells secondary to the destruction of the cortical bone only occurs exceptionally ((88).

Secondary bone tumors including osteosarcomas, osteoblastomas, etc. have been reported (131).

Bone manifestations may be clinically asymptomatic or lead to chronic pain. When MRI is not available, standard radiographs may be used to objectify bone remodeling disorders with enlargement of the metaphyseal/diaphyseal region of the lower part of the femur referred to as Erlenmeyer flask bone deformity appearing during childhood, the sequelae of osteonecrosis and bone infarction, thinning of the cortical bone, focal lysis, fractures and osteoarthritis. They may also be used to follow up patients after joint replacement surgery. Magnetic resonance imaging (MRI) is the reference examination and is used to objectify bone marrow infiltration (80% of cases) at a very early stage, bone infarction, osteonecrosis and bone lysis. CT scans can be used in some cases to clearly identify cortical lesions and determine the risk of fracture and osteoarthritis. Bone marrow infiltration and Erlenmeyer flask bone deformities do not seem to correlate with the other bone complications (36).

Pulmonary involvement may be related to infiltration of the lungs by Gaucher cells, creating interstitial disease that can lead to pulmonary fibrosis, restrictive lung disease secondary to spinal deformation, or pulmonary arterial hypertension which is more common in splenectomized patients, particularly women, or may be caused by hepatopulmonary syndrome complicating hepatic cirrhosis. Pulmonary involvement is rare in all GD phenotypes.
Renal involvement manifested as proteinuria and hematuria reflects infiltration of the glomeruli by Gaucher cells. Skin involvement is manifested as yellow-brown hyperpigmentation usually on the anterior parts of the tibias and cheeks. Ocular manifestations such as vasculitis or whitish spots (corresponding to glucoceramide deposits) and digestive localizations are only observed exceptionally. Contrary to the conventional definition of type 1 GD, certain neurological manifestations associated with the type 1 phenotype have been described over the past several years. Patients with type 1 GD are at an increased risk of developing Parkinson’s disease (4 to 20 x) often at an early stage and which is resistant to dopamine agonists in some cases (37,38,50,51). The prevalence of generally minimally symptomatic peripheral neuropathies and small fiber neuropathies is 14% and therefore higher than in the general population (14).

**Type 3 Gaucher disease (ORPHA77261)**

Also called juvenile or subacute neurological GD, the type 3 form (5% of cases) combines the visceral manifestations described in type 1 GD with usually oculomotor neurological involvement appearing before the age of 20 years in most cases. Like type 1 GD, type 3 GD phenotypes are very varied, particularly as regards neurological involvement. Some patients present moderate systemic involvement with horizontal gaze ophthalmoplegia as the only neurological symptom while others present more severe forms with varying neurological signs including progressive myoclonus epilepsy (16% of patients), cerebellar ataxia and spasticity (20 to 50% of patients), and dementia in some cases (59, 115). Neurological signs may occur several years after the visceral manifestations, even in treated patients where the patient was initially identified as having type 1 disease. Disease onset is more common in young children with neurological symptoms appearing before the age of 2 years in half the cases (115). Spinal surgery may be required for sometimes severe and progressive kyphosis which may develop through an unknown mechanism despite specific GD treatment. Cardiac involvement with valve calcification and corneal involvement are reported mainly in patients with type 3 GD with the D409D genotype.
Subjects with exceptional saposin C deficiencies almost always present neurological impairment comparable to that observed in type 3 GD (112).

**Type 2 Gaucher disease (ORPHA77260)**

Type 2 GD (<5% of cases), is characterized by early and severe neurological impairment starting in infants aged 3 to 6 months and systemic involvement with hepatosplenomegaly. The triad rigidity of the neck and trunk (opisthotonus), bulbar signs (particularly severe swallowing disorders) and oculomotor paralysis or bilateral fixed strabismus is very evocative of the disease. The signs may be associated with trismus and hypertonia with pyramidal and possibly extrapyramidal rigidity (74).

Apnea related to increasingly frequent and lengthy laryngeal spasms occurs after a few months. Psychomotor development is then altered, although some children may still continue acquiring skills. Convulsions that occur later manifest as myoclonic epilepsy that is resistant to antiepileptic drugs. Splenomegaly is almost always present, associated with thrombocytopenia in 60% of cases. Failure to thrive (30% of patients) may be the first warning sign, sometimes associated with cachexia. Lung lesions are sometimes also observed resulting from repeated aspirations and pulmonary infiltration by Gaucher cells. There is no bone involvement in type 2 GD. Death occurs before the second year of life following massive aspiration or prolonged apnea.

**Fetal Gaucher disease (ORPHA85212)**

Fetal GD is the rarest (<1%) and most severe form of the disease. It is detected at the fetal stage by hydrops fetalis, hepatosplenomegaly, ichthyosis, arthrogryposis, facial dysmorphia and fetal thrombocytopenia. Death often occurs in utero or soon after birth (75). The fetal form of the GD must be confirmed by biochemical methods for appropriate genetic counseling and prenatal diagnosis in future pregnancies.
**Laboratory result abnormalities**

*Complete blood count*

Thrombocytopenia of varying degrees is common (90% of cases): <60x10^9/L in 15% of cases; 60-120x10^9/L in 45% of cases and 120-150x10^9/L in 30% of cases. Anemia is less common (36% of cases) and moderate with hemoglobin levels rarely found to be less than 9 g/dL; leukopenia is rare. Cases of GD without thrombocytopenia are observed. These cytopenias are attributed to splenic sequestration and bone marrow infiltration, but a direct impact of the enzyme deficiency on immature hematopoietic cells has been described (11, 49). The blood count may be normal in patients with a history of splenectomy.

*Hemostasis*

Several hemostatic abnormalities have been described in GD including prolonged PT (prothrombin time) and APTT (activated partial thromboplastin time), possibly related to a deficiency in factor X, factor V, thrombin or a more global deficiency, factor XI deficiency, common among Ashkenazi Jews, deficiencies secondary to liver failure (rare in GD), to a deficiency in vitamin K or a to potentially associated genetic deficiency (von Willebrand disease), or even acquired VWD (83). However, the relationship with potential signs of bleeding is not obvious, especially as platelet disorders are fairly common (104).

*Total protein, serum protein electrophoresis and immunofixation*

These examinations must be carried out to screen for polyclonal hypergammaglobulinemia (25-91% of cases) and possible monoclonal gammopathy (1-35% of cases) (29, 46). Specific treatment reduces polyclonal hypergammaglobulinemia, but seems to have a limited effect on monoclonal gammopathy (18).

*Others dosages*

Liver function tests (free and conjugated bilirubin, transaminases, alkaline phosphatase, gamma GT) may be carried out sometimes revealing cholestasis, but rarely cytolysis.
C Reactive Protein (CRP) levels may be high in case of bone crises (bone infarction) or infectious complications (cholecystitis more common in GD).

Assays of serum calcium, serum phosphorus and vitamin D are recommended. Vitamin D deficiency seems to be more common in GD than in the general population and supplementation is highly recommended when the 25(OH)D level is less than 75 nmol/L (54).

Auto-antibodies (antinuclear, anti-phospholipid antibodies) have been found in GD, usually without clinical signs. Antibodies directed against the therapeutic enzyme (imiglucerase) are detected in 14% of cases, but are of no consequence in practice. They are only assayed in case of allergic reaction or loss of (101).

Certain bone remodeling markers can be assayed: in theory, a decrease should be observed in bone formation markers (e.g. osteocalcin), while bone resorption markers (such as ICTP for C-terminal type I collagen telopeptide) should be normal or increased, but the published studies are generally discordant (68).

**Diagnosis of Gaucher disease**

Diagnosis of GD is often delayed by several years relative to the onset of the first clinical and laboratory signs, and this is a recurring problem with rare diseases characterized by progressively worsening symptoms.

Diagnosis is confirmed by assay of GCase activity. Establishing enzyme deficiency is the only sure way to diagnose GD. Typical enzyme activity values in patients with GD vary between 10 and 30% of the normal value. Enzyme activity is assayed in total leukocytes or, better still, in mononuclear cells. Patients can now be screened using blood spots collected on blotting paper, but any potential deficiency should be confirmed using the previous method. More accurate techniques such as flow cytometry can be used to quantify enzyme activity in specific cell populations revealing activities of the order of only 10% of normal activity (11, 12).
Exceptional saposin C deficiency should be tested for in case of normal GCase activity when the clinical picture and biomarkers point to GD and especially when chitotriosidase levels are very high. The diagnosis is made by PSAP gene sequencing.

Myelograms need not be performed to confirm diagnosis of GD but may be performed in cases of isolated thrombocytopenia and/or splenomegaly in which case the Gaucher cells are highly evocative when their appearance is typical. Exceptionally, it may not be possible to use cytology for diagnosis when only few cells are available. It may be difficult to distinguish Gaucher cells from the so-called “pseudo-Gaucher” cells observed in some blood disorders or infectious diseases such as myeloma with histiocytic accumulation of immunoglobulin crystals (24), Waldenstrom’s disease and other lymphomas with monoclonal gammopathy (94), chronic myeloid leukemia or myelodysplasia (106, 127), or atypical mycobacteria (19). A myelogram may be justified during the course of the disease in case of monoclonal gammopathy.

The most common mutations in the GCase gene (GBA1 gene, long arm of chromosome 1 [1q21]) are tested for using a Polymerase Chain Reaction technique, but the gene may also be sequenced if a typical mutation is not found.

Genotyping is essential since it can provide prognostic information through potential genotype-phenotype correlations and it is particularly important in children to determine if they are at risk of developing a neurological form of the disease. Over 300 mutations of the gene have been described, but the five most common are N370S, 84GG, L444P, IVS2 and Rec. The most common mutation is the N370S mutation which is particularly common in the Ashkenazi Jewish population (about 75% of the alleles) and accounts for about 25-30% of the alleles in the Caucasian population. The presence of the N370S mutation (c.1226A> G) in the homozygous or heterozygous state excludes the risk of neurological involvement (type 2 or 3 GD) but does not make it possible to prejudge the severity of bone and visceral involvement. N370S homozygote patients can remain asymptomatic for a long time. Patients homozygous for the L444P (c.1448T> C) mutation are at very high risk of developing neurological impairment. Patients with the D409H mutation in the homozygous state, which is exceptional, present characteristic heart valve damage. Patients with two “zero” mutations, that is to say, responsible for a total absence of enzyme activity (RecNciI, c.84dupG) do not survive beyond the perinatal period (fetal forms incompatible with life).
Prenatal diagnosis of GD can be performed by measuring enzyme activity following chorionic villus sampling at 10-12 weeks of amenorrhea or in cultured amniotic cells at about 16 weeks of amenorrhea. This testing is only offered to couples who have had a child with Type 2 or 3 GD, the only situations where medical termination of pregnancy should be considered. Type 1 GD can be treated effectively so prenatal diagnosis of this form of the disease is not routinely available in France.

**Disease biomarkers**

The oldest biomarkers of GD are tartrate-resistant acid phosphatase (TRAC) and angiotensin converting enzyme (ACE), but their lack of specificity and the availability of newer and more specific biomarkers have rendered them less useful today (2). Currently, the most interesting biomarkers are chitotriosidase, CCL18 and ferritin.

**Chitotriosidase** is produced in large quantities by Gaucher cells and it has been used as a biomarker since 1994 (51). Its levels are often high without treatment, it can be used to monitor treatment efficacy and it is considered to have some prognostic value (118). However, chitotriosidase levels vary considerably among patients initially because of a mutation (24-bp duplication) in the CHIT1 gene that leads to total deficiency (homozygosity for the mutation) in 6% of the general population. Furthermore, a third of the patients present with a heterozygous mutation making it difficult to interpret chitotriosidase concentrations which therefore cannot be used for real between-patient comparisons (20). Increased chitotriosidase levels are also observed to a lesser extent in some diseases such as other lysosomal storage diseases (Niemann-Pick disease for example), sarcoidosis or visceral leishmaniasis.

**CCL18** is a chemokine produced by various types of cells, but particularly macrophages, essentially of the M2 type, and dendritic cells (42); CCL18 promotes the recruitment of Treg lymphocytes through CCR8 (55). Gaucher cells produce CCL18, found at high levels in the plasma. CCL18 may also be increased in chronic inflammatory diseases such as idiopathic pulmonary fibrosis, some cancers and scleroderma and it is generally not a good sign (15, 114). It levels are also increased in
allergic reactions. In GD, plasma levels of CCL18 are 10-50 times higher than those of control patients (16, 17, 31). Its levels vary less than those of chitotriosidase since there is no genetic polymorphism; the kinetics of CCL18 and chitotriosidase are generally similar at treatment induction. It is essential to evaluate its levels when patients present with chitotriosidase deficiencies.

**Ferritin** is increased in most patients (> 85%), while serum iron, transferrin saturation and soluble transferrin receptor concentrations remain normal (73). Iron reserves accumulate preferentially in the liver and bone marrow. Ferritin levels could be predictive of the onset of bone complications (107) and they should therefore be monitored in GD. Blood-letting is theoretically not indicated for hyperferritinemia in GD; patients can be tested for associated hemochromatosis if transferrin saturation is high (105).

**Glucosylsphingosine** is a novel biomarker (see section 2.2.2.) whose sensitivity and specificity are superior to that of chitotriosidase and CCL18 (33, 95). Glucosylsphingosine has recently been demonstrated as very valuable for patient monitoring (39, 78) and has yet to be assessed on a large scale. Assays are recommended at the same rate as the other biomarkers.

**Radiological investigations**

**Abdominal MRI**

Abdominal magnetic resonance imaging (MRI) is the most appropriate examination for assessing liver and spleen dimensions and morphology. Organ volume calculated by MRI is often used in international studies. The spleen sometimes presents nodules suggestive of lymphoma (Fig. 4) corresponding to clusters of Gaucher cells associated with bone infarction-related fibrosis.

When MRI is unavailable or in cases of uncontrollable claustrophobia, abdominal ultrasound may be used instead.

**Standard radiological imaging:**

Standard radiographs are used to detect: Erlenmeyer flask deformity of the femurs with widening of the lower third, accompanied by thinning of the cortical bone which may
appear scalloped (65), avascular necrosis and bone infarct sequela (34% of cases), lytic lesions (18% of cases) that are generally well delineated without peripheral increased bone density, and sequelae of traumatic or pathologic fractures. The initial assessment should include radiological imaging of the pelvis, spine, femurs, tibias and humeri. Radiological imaging need not be systematically renewed thereafter for monitoring purposes except to specifically follow the progression of avascular necrosis to osteoarthritis. The sensitivity of standard radiological imaging for the detection of abnormalities in GD is low (123).
Figure 4: Examples of bone images.
A: Erlenmeyer flask deformity of the lower part of the femur (front X-ray of the knee).
B: Lower extremity of femur modified by complex infarcts (profile X-ray of the knee).
C: Bilateral severe osteonecrotic lesions of the femoral heads (front X-ray of the pelvis).
D: Numerous hyperintense areas corresponding to areas infiltrated by Gaucher cells (bone scintigraphy with Tm99).
E: Bone infarcts of the lower part of the femur (MRI of the knee).
F: Osteonecrotic lesion of the femoral head (MRI of the hip).
G: Diffuse infiltration of the bone marrow by Gaucher cells (MRI of the pelvis).

X-ray and MRI images courtesy of Dr. N. Belmatoug (CRML, Hôpital Beaujon, Clichy).
**Bone MRI**

Bone magnetic resonance imaging (MRI) is the test of choice for evaluating the effects of GD on bone. Bone marrow infiltration is generally predominant at the proximal and distal ends. T1 weighted sequences are recommended to detect and quantify bone marrow infiltration, while T2 weighted sequences are used to detect complications such as avascular necrosis or bone infarction (123). Hypointense signals are generally observed in T1 weighted sequences, reflecting the replacement of normal bone marrow fat by Gaucher cells. Infiltration may be quantified by means of the various scores used in reference centers, such as the Bone Marrow Burden (BMB) score (38, 66). Assessment of bone marrow infiltration is more difficult in children due to the presence of red bone marrow in the long bones. Magnetic resonance imaging is used to assess the extent to which the lesions have spread and whether complications are recent (edema due to recent infarction) or longstanding. Other types of MRI are used for semi-quantitative assessments of bone marrow infiltration (Quantitative Chemical Shift Imaging), but they are not available in all centers (48). Whole-body MRI makes it possible to reduce the examination time, in particular for disease monitoring purposes. The images must be carefully analyzed because additional images are sometimes required for the less visible sites, especially the extremities (hands and feet).

**Bone density**

Bone densitometry is used to objectify osteoporosis which is common in adults or children > 5 years and to calculate lumbar spinal and femoral bone mass. Osteopenia is defined by a Z score > -2 before the age of 50 years and before menopause or a T score between -1 and -2.5 after the age of 50 and after menopause; Osteoporosis is defined by a Z score ≤ -2 before the age of 50 years and before menopause or T score ≤ -2.5 after the age of 50 and after menopause. The severity of the osteopenia might be correlated with genotype, splenomegaly and hepatomegaly (87).

**Osseous scintigraphy**

99mTc bone scintigraphy is sometimes used to locate bone lesions throughout the skeleton (especially the spine, femur, pelvis, tibia) when MRI is not available (77). It enables the detection of clinically asymptomatic lesions or sequelae of bone infarcts in atypical sites (jaws, hands feet), as well as cracks and fractures.
**Others**

Echocardiography is used to screen for pulmonary arterial hypertension (PAH)

**Genetic counseling**

Genetic counseling must be provided during a clinical genetics visit. The purpose of genetic counseling for families of patients with GD, is: 1) to screen ascendants, siblings, and descendants for GD or possible heterozygosity for the disease (making the person a carrier) by tracing a family tree, GCase assays and genotyping (for mutations of the index case if previously characterized) as the enzyme assay alone does not usually allow reliable diagnosis of heterozygotes (carriers); 2) to inform at-risk couples of the probability of transmitting the disease in a homozygous form and the potential clinical consequences of this; screening for possible heterozygosity among spouses of persons at risk is especially useful in case of consanguinity or if the spouse is of Ashkenazi origin.

**Prenatal diagnosis**

Prenatal diagnosis of GD is recommended for couples who have had a child with Type 2 or 3 GD (currently incurable forms) or a form with fetal onset GD. It must be organized as part of a specific genetic counseling visit and requires prior analysis of the family index case and enzyme and/or molecular examinations of the two parents.

Prenatal diagnosis involves: 1) determining the genotype of fetal cells obtained by trophoblast biopsy (10-12 weeks of amenorrhea) or amniocentesis (from 15-16 weeks of amenorrhea) if mutations of the index case were previously identified; 2) assay of GCase activity in trophoblast cells or cultured amniotic cells if the genetic study of the index case was not informative or could not be carried out.

Thus, Gaucher disease should be considered in case of recurrent epistaxis, increased abdominal volume or bone pain. It is important to include the GD in the diagnostic decision tree in case of splenomegaly with a non-obvious etiology, especially if it occurs with thrombocytopenia, hyperferritinemia or polyclonal hypergammaglobulinemia.
Management

Usually specific treatments

All patients require regular monitoring. Specific medication is not justified in all GD patients. Once it has been initiated, treatment must generally be administered for life. There are currently two specific types of treatment for GD: enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). The goal is to treat patients before the onset of complications, the sequelae of which are disabling or not improved by treatment including massive fibrous splenomegaly, osteonecrosis, secondary osteoarthritis, vertebral compression and other fractures, hepatic fibrosis and lung fibrosis.

Enzyme replacement therapy

The principle is to supply the lacking GCase to cells, particularly Gaucher cells. After using an enzyme extracted from human placentas (alg glucerase) in the early 90s, Genzyme SA (imiglucerase, CEREZYME-SANOFI®) developed a recombinant GCase. The enzymes are deglycosylated exposing their mannose residues allowing their uptake by macrophage receptors and their transfer to lysosomes. Imiglucerase is produced by mammalian cells (Chinese Hamster Ovary cells); it obtained marketing authorization (MA) in 1996. Other recombinant enzymes have since been developed: velaglucerase (VPRIV®, Shire, MA in 2010) produced by human fibroblasts and taliglucerase (UPLYSO®, Pfizer) produced by carrot cells, which was available by Temporary Authorization for Use (ATU) during a period of imiglucerase shortage (2009-2011), but did not obtain marketing authorization in France. The differences between imiglucerase and velaglucerase are minimal. Taliglucerase undergoes specific glycosylation, related to its production in plant cells.

These products are administered intravenously at the rate of one infusion every 14 days. The usual starting dose is 60 U/kg/14 days, but it may vary depending on the country. The first administrations should take place in a hospital. Home administration supervised by a healthcare professional may be considered only in adult patients having received several infusions in a hospital without side effects. The dose and rate of the infusions may be adjusted according to the clinical course and biomarkers. Enzyme replacement therapy usually has a rapid effect on clinical symptoms (asthenia, pain) and
laboratory abnormalities (particularly thrombocytopenia); bone marrow infiltration and osteopenia regress gradually with ERT and skeletal events decreases, although ERT does not completely prevent their occurrence. Early treatment with enzyme replacement therapy reduces the risk of AVN (79). Treatment does not completely prevent the occurrence of skeletal-related events (109). Disease control with low doses remains poorly understood but may be related to specific intracellular pharmacokinetics (Berger, 2012 #857). There are currently no criteria for the preferential use of one or other of the enzyme replacement therapies (imiglucerase or velaglucerase) to treat type 1 GD; only imiglucerase has a marketing authorization for type 3 GD. None of the enzyme replacement therapies are indicated for type 2 GD as treatment has no impact on the progression of the rapidly severe neurological effects (74).

Prices of the various enzymes are currently extremely high; for example, in France, the cost of treating a patient of 60 kg at the dose of 60 U/kg every 14 days, is of the order of € 320,000 and € 354,000 per year for imiglucerase and velaglucerase, respectively.

Criteria for enzyme replacement therapy:

- Specific treatment should be considered in type 1 GD if any of the following criteria are present (severe form) (6):
  - Symptomatic thrombocytopenia and/or platelets ≤ 50x10^9/L, ≤ 80x10^9/L in case of pregnancy. Using the platelet count as the sole criterion for specific treatment remains delicate. The only specific recommendation for GD (<60x10^9/L) is not justified by a risk of bleeding, but is derived from the International Registry (6). This threshold does not correspond to the usual risk threshold: 50x10^9/L for general surgery, 80x10^9/L for epidurals and 100x10^9/L for neurosurgical procedures. This probably explains why recommendations vary by country. In the French registry, the median platelet count before treatment was 76x10^9/L. We recommend that patients should not be treated above 80x10^9/L based on this criterion alone. Between 50 and 80x10^9/L, the indication must be defined on a case-by-case basis.
  - Symptomatic anemia and/or hemoglobin ≤ 10 g/dl (excluding other causes).
  - Symptomatic hepatomegaly and/or splenomegaly (pain, abdominal distension).
  - Past or present clinically symptomatic bone involvement: bone crises, osteonecrosis, bone infarction, pathologic fractures.
- Only radiological bone disease: infarcts, osteonecrosis, pathologic fractures, lysis, documented osteoporosis (> -2.5 SD).
- Involvement of other organs (lungs, hepatic fibrosis, heart, kidneys) related to GD (after exclusion of all other etiologies).
- Type 3 GD.
- Children: any GD with one of the above signs or growth retardation or delayed puberty, or an asymptomatic form with a genotype predisposing to a type 3 form.

For any patient not presenting the above criteria, the decision on treatment should be taken on a case-by-case basis following a multidisciplinary discussion.

Safety is generally good. Ten to 15% of patients (with imiglucerase) develop antibodies against the enzyme (not with velaglucerase?), usually without clinical signs. Allergic reactions are rare (<1.5% of patients) and include urticaria, diarrhea, hypotension, or laryngeal discomfort. The risk of allergy seems a little more common with taliglucerase. Pregnancy is not a contraindication to imiglucerase replacement therapy since no fetal malformations were described in pregnant women in whom treatment was continued. Velaglucerase also appears to be well tolerated (35). Furthermore, ERT may be required to control the disease, since it can worsen during pregnancy and to limit thrombocytopenia which can be harmful during pregnancy or childbirth and contraindicates epidural anesthesia.

*Substrate reduction therapy*

The principle of substrate reduction therapy (SRT) is to reduce excess cell Gcer by decreasing its production. Miglustat (Zavesca®, ACTELION) is a Gcer synthetase inhibitor which reduces the synthesis of Gcer in Gaucher cells. It obtained a European marketing authorization in November 2002 for the treatment of mild to moderate Type 1 GD (1). Its efficacy is real on the size of the liver and spleen as well as on the decrease of chitotriosidase levels but its efficacy on hematological parameters is more limited and improvements take longer (improvement of anemia after 24 months, little
improvement of thrombocytopenia). Its efficacy on bone symptoms remains poorly evaluated.

It is administered orally at the recommended dose of 100 mg 3 times daily, but doses may need to be progressively increased at the start of treatment to improve safety. Miglustat can produce side effects (diarrhea, weight loss, tremor of the hands, possible peripheral neuropathy) which generally regress with dose reduction or treatment discontinuation. Diarrhea can be effectively controlled with loperamide and certain dietary measures (limiting the consumption of disaccharides in the form of sugars and milk).

It is a second-line treatment to be used when enzyme replacement therapy is no longer accepted by the patient or cannot be used due to intolerance. Miglustat is strictly contraindicated in pregnancy and contraceptive methods must be used by both male and female patients. To date, miglustat has not been found to have an effect on neurological symptoms, despite that fact that it crosses the blood-brain barrier.

Eliglustat (CERDELGA®, GENZYME/SANOFI), also a substrate inhibitor, was granted a marketing authorization in France in 2015. It is also an orally administered Gcer synthase inhibitor that is more specific and more potent than miglustat. It was evaluated in phase 1, 2 and 3 clinical studies regrouping overall nearly 400 patients whose follow-up results were published after 4 years (26, 63, 64, 82). The studies demonstrated significant efficacy versus placebo, non-inferiority compared to imiglucerase (reference product) over a period of 1 year and satisfactory safety. The four-year extension phase of the phase 2 study also demonstrated that it had an effect on bone (56). This drug is proposed as first-line treatment in patients with type 1 GD. Due to potential drug-drug interactions, its use with CYP2D6 inhibitors calls for special caution depending on the patient's metabolizer status (CYP2D6 genotyping required before any prescription). Eliglustat is indicated for the long-term treatment of adults with type 1 GD who are cytochrome 2D6 poor, intermediate or extensive metabolizers (prior determination). It is not indicated for use in ultra-rapid metabolizers. Adverse effects are uncommon and usually mild and include dizziness and headache in less than 5% of cases. Given that it is metabolized by cytochrome P450, certain drug-drug interactions should be anticipated. Eliglustat is an alternative to enzyme replacement therapy and miglustat for patients who cannot be treated by ERT.
Eliglustat is an alternative to enzyme replacement therapy for non-splenectomized treatment-naïve patients (no treatment for more than 1 year or never treated) not presenting any recent bone events (absence of data). Eliglustat can be offered to replace enzyme replacement therapy for patients stabilized on ERT (favorable non-inferiority study) to reduce the constraints of infusions. Finally, as a precautionary measure, it is preferable to avoid the use of Cerdelga® during pregnancy or in case of pre-existing heart disease (risk of an increase in ECG intervals).

Other specific treatments

Ideally, bone marrow transplantation could “cure” patients with GD (92), but this treatment is no longer offered given its benefit/risk ratio and the current availability of effective and well tolerated therapies.

Gene therapy

Gene therapy involves introducing the GCase gene into hematopoietic cells, which are then injected into patients. A preliminary clinical protocol was used to test the technique in 3 human patients (34). However, the GCase levels were too low for a clinical effect to be achieved. Lentiviral vector gene transfer techniques have been used in mouse models of GD with promising results that, however, are still in the domain of basic research (28).

Molecular chaperones

Molecular chaperones are small molecules that enable proteins to take their specific molecular configuration which determines their functional efficacy. They also protect proteins by preventing inappropriate aggregation and facilitate their passage through the membranes and thus their transport into lysosomes as regards lysosomal enzymes. Molecular chaperones may therefore help the production of functional enzymes and thus restore the intracellular activity of even mutant GCase. The development of this type of treatment for GD is still in the early stages and clinical trial have yet to be conducted, but the strategy is still under consideration (86). The effect is thought to be responsible for the results of pilot studies with ambroxol (67, 71).
Symptomatic treatments

In the era of enzyme replacement therapy, splenectomy should be avoided in GD. Its potential consequences include the usual risks (infectious, thrombosis, neoplasia) (60), but also a risk of worsening of GD (25) due to an increased risk of skeletal-related events, hepatic fibrosis, cirrhosis, hepatic carcinoma and PAH. Splenectomy should be exceptional and considered only in rare cases of non-response to well conducted enzyme replacement therapy with persistent severe cytopenia (usually related to massive nodular and fibrous splenomegaly) or in cases of splenic rupture. The usual recommendations for splenectomy should be followed (pre-vaccination and antibiotic prophylaxis).

The painful bone crises often require immobilization and use of level I and II, or even III analgesics. Temporary immobilization is often required; specific treatment typically reduces the frequency and intensity of these crises (9).

Use of bisphosphonates is controversial in Gaucher disease because the pathophysiology of bone mass decline remains poorly understood. It appears to be the consequence of either an osteoclast or osteoblast disorder or, more likely, of an osteoblast-osteoclast coupling disorder. Specific therapy remains the best treatment for GD-related osteopenia and osteoporosis. Bisphosphonates are nonetheless often indicated in cases of persistent osteoporosis especially in postmenopausal women. They are contraindicated in women wanting to have children (25).

Orthopedic surgery may be required for bone complications including osteonecrosis and pathologic fractures. Except in the context of an emergency, it is preferable to operate the patient after correction of laboratory parameters, particularly thrombocytopenia.

Liver transplantation may be proposed for the rare patients presenting with severe liver disease progressing to fibrosis and liver failure.
Psychological support should be offered routinely and patients can be oriented to associations.

**Monitoring**

Patient monitoring involves, in addition to regular clinical monitoring, monitoring of laboratory parameters and radiological monitoring (Tables 1 & 2). The monitoring of patients with no indication for treatment, with mild disease, is different to that of patients requiring treatment. Enzyme replacement therapy improves hematologic abnormalities and quality of life within a few months (10). Biomarker levels (chitotriosidase, CCL18 and ferritin) decrease relatively quickly with ERT, prior to the normalization of platelet and hemoglobin levels (121). Hepatosplenomegaly decreases more slowly, usually over a period of two years. Improvement of bone abnormalities is usually observed after 2-4 years of treatment, but some abnormalities remain irreversible (hepatic or splenic fibrosis, osteonecrosis and bone infarction sequelae, etc.). A significant proportion of patients show improvements but without normalization of their cytopenia or organomegaly (124).

Patients with Type 3 GD require neurological monitoring in addition to the monitoring proposed for type 1 GD.

Pediatric patients are monitored more frequently: a clinical examination and full battery of laboratory tests must be carried out every six months while imaging is used as a function of disease progression.

**Prognosis**

Currently, available treatments make it possible to reduce cytopenias and organomegalies and significantly decrease bone manifestations, considerably improving the quality of life of patients.

However, outcomes may be unfavorable due to aggressive, irreversible and disabling bone disease despite specific treatment, the onset of Parkinson's disease, or the
occurrence of a blood disease or cancer (HCC), whose relative risk appears higher in GD. Hypergammaglobulinemia and the appearance of monoclonal Ig are two factors which promote the emergence of myeloma whose incidence appears increased in GD, with a relative risk estimated to be at least 5.9 (95% CI: 2.8-10.8) (7, 30, 97, 111). Progressive neurological deterioration in patients with Type 3 GD, for which ERT is unfortunately ineffective, impacts the prognosis of these patients. The outcome is always fatal for patients with type 2 GD.
## Table 1: Monitoring of adult patients with type 1 GD with no indication for treatment

<table>
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<tr>
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<th>Diagnosis, Initial assessment</th>
<th>D0-M3</th>
<th>M3</th>
<th>M6</th>
<th>M12</th>
<th>Year 2</th>
<th>Year 3</th>
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<td>If indication during the course of the disease (monoclonal Ig)</td>
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<tr>
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<td>MRI or ultrasound scan of the spleen, liver(^8)</td>
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\(^1\)The calendar is for patients with stabilized disease (objectives reached), it can be changed depending on the course of the disease

\(^2\)The assay of glucocerebrosidase is used to make a diagnosis; in some very rare cases of normal Glc and signs of GD with no other etiology, a deficiency in saposin C should be considered

\(^3\)May be performed based on the initial clinical examination and laboratory results and can reveal Gaucher cells, but cannot be used to confirm the diagnosis

\(^4\)In case of severe or symptomatic thrombocytopenia at diagnosis

\(^5\)In case of anomalies, carry out a serum immunofixation test

\(^6\)In case of monoclonal Ig

\(^7\)Mandatory in case of chitotriosidase deficiency (6% of the population)

\(^8\)Preferably MRI, and failing that, abdominal ultrasound

\(^9\)Particularly in splenectomized patients, in case of pregnancy or clinical signs

\(\star\)Liver function tests (AST, ALT, GGT, ALP), renal function tests (urinary electrolytes, urea, creatinine using the Cockcroft equation), serum phosphorus and calcium levels, blood sugar levels
### Table 2: Monitoring of adult patients with type 1 GD receiving treatment

<table>
<thead>
<tr>
<th>Procedure</th>
<th>1st year</th>
<th>2nd year</th>
<th>3rd year</th>
<th>Xth year</th>
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<tr>
<td><strong>Diagnosis, Initial assessment</strong></td>
<td>D0-M3</td>
<td>M3</td>
<td>M6</td>
<td>M12</td>
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<tr>
<td><strong>Clinical examination</strong></td>
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<td><strong>Glucocerebrosidase</strong></td>
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<td>Genotype</td>
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<td>Myelogram</td>
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<td>Complete blood count</td>
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<td>Hemostasis assessment</td>
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<tr>
<td>Biochemistry tests*</td>
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<td>Serum protein electrophoresis -&gt; serum immunofixation</td>
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<td>Ig quantification*</td>
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<td>Light chain/k ratio</td>
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<td>Ferritin</td>
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<td>Radiological assessment:</td>
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<td>MRI or ultrasound scan of the spleen, liver</td>
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1. The calendar is for patients with stabilized disease (objectives reached), it can be changed depending on the course of the disease.
2. The assay of glucocerebrosidase is used to make a diagnosis; in some very rare cases of normal Glc and signs of GD with no other etiology, a deficiency in saposin C should be considered.
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5. In case of anomalies, carry out a serum immunofixation test.
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*The calendar is for patients with stabilized disease (objectives reached), it can be changed depending on the course of the disease.*
Conclusions

While it is the most common of the lysosomal storage diseases, GD remains a rare disease and most cases present a gradual onset phenotype, which is why diagnosis is often delayed. It is important to include GD in the diagnostic decision tree in case of splenomegaly, to avoid potentially harmful splenectomy.

Significant new insights in pathophysiology show that GCase deficiency has a much larger impact than only the transformation of macrophages into Gaucher cells. These elements open up new paths for the development of innovative therapeutic strategies. Thus, hopefully, drugs that can modify the neurological phenotype will eventually be developed. It is likely that more complex molecular studies will ultimately contribute to the customization of patient management.

Therapeutic advances in recent years, including the development of new enzymes and a new substrate inhibitor represent significant progress, but research efforts need to be maintained with all relevant stakeholders.

Patients with GD including asymptomatic patients must be monitored regularly to detect any complication of the disease.
PART II: THE EPIDEMIOLOGY OF GAUCHER DISEASE AND MODELLING ITS BIOLOGICAL AND CLINICAL MARKERS BOTH WITH AND WITHOUT TREATMENT.
Research objectives and work plans

This second section of the thesis focuses on a part of our work: modelling of BEs and biomarkers in Gaucher disease (GD) patients of the French registry. Until recently, the epidemiological data on GD were dominated by studies using the International Collaborative Gaucher Group (ICCG) Gaucher Registry, which was sponsored by the pharmaceutical industry (22, 45). However, it did not live up to its users’ expectations, notably regarding incidence, prevalence and the analysis of bone events (BEs) during treatment. BEs remain the major complication of GD and they are reason enough to justify the treatment initiation. Unfortunately, this treatment is not always able to ensure they will not occur again. Currently, neither the longitudinal analysis of biomarkers, nor the use of published severity scores, allows us to predict BEs and few studies have even addressed this topic. The indications for treatment and dose management are still quite approximate and are no help in guiding effective treatment.

The present work’s objectives were to establish the French Gaucher Disease Registry, describe the pathology’s epidemiology in France and examine how best to identify predictors of BEs by modelling the disease’s biomarkers throughout its course.

The first section describes how the French registry for GD was established and the disease’s epidemiology in France.

The second section describes how the biomarkers of GD were modelled in patients undergoing treatment for the disease. It goes on to analyse the risks of developing a first BE while undergoing treatment, by studying how biomarkers have an impact on that risk.

Using a frailty model, section three demonstrates the value of modelling the occurrence of BEs in a way that can analyse repeat events.

Finally, the discussion section goes on to explain the value of a mixed model that is able to jointly analyse the longitudinal data on biomarkers and the occurrence of BEs over time, in a non-published work.
The French Gaucher Disease Registry: a description of its epidemiology in France.

Establishment and approval of the registry.

In 2004, and within the framework of France’s Committee for the Evaluation of Gaucher Disease Treatments (CEGT) and its Reference Centre for Lysosomal Diseases (RCLD), the author and Dr Nadia Belmatoug began working to establish the French Gaucher Disease Registry (FGDR). In 2008, following a call to tender by France’s National Registry Committee – Rare Diseases (CNR-MR) – coordinated by its Institute of Health and Medical Research (INSERM) and its Institute for Public Health Surveillance (InVS) – they presented a dossier requesting authorisation to create an exhaustive and officially approved registry. The CNR-MR visited the site from where the registry would be managed and then approved the FGDR in 2009. Indeed, it had already received authorisation from the French Data Protection Authority (CNIL) in 2004, and this was renewed in 2014. The research necessary to build the registry was approved by the Paris Nord ethics committee.

Features of the GD registry

The registry includes all the patients diagnosed with GD because their level of glucocerebrosidase was < 30%. The registry covers all the patients diagnosed in France since 1980, including its overseas departments and territories. Oral or written consent was requested from each living patient before their data were uploaded to the registry.

Justification for a registry

For a registry to be useful, it must be an exhaustive record of all the cases within a given geographic area. Until now, there was no way of knowing the number of patients diagnosed and suffering from GD in France, nor any means of following their clinical course, either with or without treatment. The registry now enables epidemiological monitoring and follow-up. A number of rare complications can develop as the disease evolves.
(Parkinsonian syndromes, macroglobulemias, lymphoid haemopathies or even solid cancers) and only a complete register can help to identify them. In addition, close monitoring of innovative therapies for GD is vital, and the new register enables this.

The exceptional nature of GD means that randomised prospective studies are extremely difficult to carry out effectively; the registry’s exhaustiveness enables researchers to carry out pertinent retrospective analyses with a minimum of bias.

Projects for developing other European GD registries are currently being discussed. France must be in a position to take part by already having an effective registry in place, one that it may even be able to subsequently export to other countries.

The registry’s objectives

Data from the FGDR have enabled a better understanding of the heterogeneity in current patient management practices across the numerous hospitals treating sufferers. The registry also fulfills a pedagogical role as a valuable reminder of the principal elements which must be considered in patient monitoring. It allows France’s health authorities to discuss the relevance of different specific treatments in order to harmonise patient management. It is a base from which to develop and refine recommendations for patient management, facilitate the introduction of public health programmes, explore the feasibility and development of new therapeutic techniques and develop specific new lines of research. The CEGT is the registry’s scientific advisory panel and can accept diverse research projects.

An analysis of the disease’s natural development without treatment is extremely valuable in order to be able to compare how that development evolves with treatment, and thus how best to determine indications for that treatment. Only a registry can supply this kind of information.

A number of research objectives have been developed using the data in the FGDR. These include: an epidemiological description of the population suffering from GD in France; an analysis of genotype-phenotype relationships; the detection of factors predictive of complications and the identification of biomarkers that allow the disease’s progression to be monitored either with or without treatment; the optimisation of therapeutic techniques
(optimal doses or intervals) and appropriate pharmacoeconomic studies; an analysis of the patient management practices in different sites across the country in order to harmonise them; the selection of patients with whom to test new therapeutic options; proposals for studies on pregnancy and GD, Parkinson’s disease and GD, monoclonal peaks and GD, GD type 3, paediatric GD, and so on.

Methods of recording cases of GD and the information gathered

Identifying cases of GD

There are a number of different sources of information on new cases of GD and together they help to ensure the registry’s exhaustiveness: certified diagnostic laboratories monitor all the cases diagnosed in France (this source is almost complete because the country’s three main laboratories verify all its diagnostic tests); the network of Reference Centres and Competence Centres, notably within the CEGT; all the clinical departments dealing with one or more patients suffering from GD; and health insurance companies. A number of sources usually overlap and validate individual cases.

The information gathered

Dr Jérôme Stirnemann and Dr Nadia Belmatoug are currently in charge of the registry, with Dr Stirnemann responsible for scientific affairs and Dr Belmatoug responsible for administrative ones. Data entry is carried out by three clinical research assistants who travel to each reference or competence centre in order to retrieve their respective data. Each new case of GD is verified by contacting the relevant diagnostic laboratory. Because the number of patients is small, the search for duplicate entries is done manually. It requires the first three letters of each patient’s family name at birth, his given name and his date of birth. The data entry form also includes diagnostic data and data on the disease’s progression.

The development of a specific computerised database

The database was created specifically for GD. It is accessible online and allows users anywhere to input data from a patient’s medical record into a form. It was developed in
collaboration with Dalil Hamroun of the INSERM Clinical Research Centre at the University of Montpellier, France, thanks to a charitable financial contribution from *Vaincre les Maladies Lysosomales*, a charity set up to combat lysosomal diseases. The database will be able to evolve and scale-up to the GD registry’s needs. It is hosted on a secure site at Montpellier’s university hospital centre.

After data entry by a clinical research assistant, physicians using the system can either input the changing information on the progression of the disease (sometimes even directly during a consultation) or send a report including the digitised biological data to the RCLD so that they are input there. Data is systematically monitored by a clinical research assistant or physician from the RCLD.
PART II: EPIDEMIOLOGY AND MODELLING

Summary of the Epidemiology of Gaucher Disease in France (109).

The first article about the FGDR was published in 2012 and summarised the characteristics of the 562 known GD patients as at 31 December 2010.

After having had the FGDR officially approved, we created an innovative online platform and input all the retrospective data on French patients suffering from GD. We subsequently exploited the data, as at 31 December 2010, to carry out the first epidemiological study of GD in France.

Analysis of the registry enabled the identification of three particular groups of patients: a) the entire group of patients recorded (562 patients); b) patients whose treatment was monitored and documented (378 patients); c) patients who had undergone some follow-up monitoring in the preceding two years (283 patients, of whom 36 had received no treatment and 247 had). Descriptions of the principal complications and pathologies associated with GD were also made – as well as their treatments – notably BEs, monoclonal peaks, Parkinsonian syndromes and death.

This description of the epidemiology of GD in France allowed an estimation of the pathology’s incidence (15 patients/year [from 9 to 32] have been diagnosed in France since 1980), but also the prevalence of certain conditions associated with it, such as Parkinson’s disease (14 cases among 378 patients monitored, 3.7%) and monoclonal gammopathies (46 cases, 12.2%). The existence of BEs – both without and with treatment for GD – was confirmed. The probability of a first BE at 10 years was 20.3% (14.1%–26.5%) and 19.8% (13.5%–26.1%), without and with treatment, respectively after diagnosis and treatment onset. A number of factors that increased the risk of BEs under treatment for GD were identified: a BE before treatment; a delay between diagnosis and the start of treatment > 2 years; splenectomy; and age at diagnosis of < 15 years old. A multivariate analysis using the Cox proportional hazards model only succeeded in identifying one co-variable that might increase the incidence of a BE during treatment, and that was a BE before treatment (HR, 2.6 (95% CI, 1.3–5.2); \( p = 0.006 \)).
The French Gaucher’s disease registry: clinical characteristics, complications and treatment of 562 patients

Jérôme Stirnemann\textsuperscript{1,2,4,25*}, Marie Vigan\textsuperscript{1,2}, Dall Hamroun\textsuperscript{5}, Djazia Heraoui\textsuperscript{36}, Linda Rossi-Semerano\textsuperscript{7}, Marc G Berger\textsuperscript{6}, Christian Rose\textsuperscript{9}, Fabrice Camou\textsuperscript{10}, Christine de Roux-Serratrice\textsuperscript{11}, Bernard Grosbois\textsuperscript{12}, Pierre Kaminsky\textsuperscript{13}, Alain Robert\textsuperscript{14}, Catherine Caillaud\textsuperscript{3,15}, Roselyne Froissart\textsuperscript{16}, Thierry Levade\textsuperscript{17}, Agathe Masseau\textsuperscript{18}, Cyrill Mignot\textsuperscript{1,19,20}, Frédéric Sédé\textsuperscript{19}, Dries Dobbelrae\textsuperscript{22}, Marie T Vanier\textsuperscript{23}, Vassili Valayanopoulos\textsuperscript{24}, Olivier Fain\textsuperscript{4}, Bruno Fantin\textsuperscript{22}, Thierry Billette de Villemeur\textsuperscript{2,20}, France Mentré\textsuperscript{3,2} and Nadia Belmatoug\textsuperscript{26}

Abstract

Background: Clinical features, complications and treatments of Gaucher’s disease (GD), a rare autosomal-recessive disorder due to a confirmed lysosomal enzyme (glucocerebrosidase) deficiency, are described.

Methods: All patients with known GD living in France, with ≥1 consultations (1980-2010), were included in the French GD registry, yielding the following 4 groups: the entire cohort; with clinical description; and its subgroups: patients with ≥1 follow-up visits, to investigate complications; recently followed (2009–2010) patients; and patients treated during 2009–2010, to examine complications before and during treatment. Data are expressed as medians (range) for continuous variables and numbers (%) for categorical variables.

Results: Among the 562 registry patients, 265 (46.9%) were females; 454 (80.0%) had type 1, 122 (21.1%) type 2, 37 (6.9%) perinatal–lethal type and 21 (3.9%) type 3. Median ages at first GD symptoms and diagnosis, respectively, were 15 (0–77) and 22 (0–84) years for all types. The first symptom diagnosing GD was splenomegaly and/or thrombocytopenia (37.6% and 26.3%, respectively). Bone-marrow aspiration and/or biopsy yielded the diagnosis for 54.7% of the patients, with enzyme deficiency confirming GD for all patients. Birth incidence rate was estimated at 1/50,000 and prevalence at 1/136,000. For the 378 followed patients, median follow-up was 162 (0–676) years. Major clinical complications were bone events (BE; avascular necrosis, bone infarct or pathological fracture) for 109 patients, splenectomy for 104, and Parkinson’s disease for 14; 38 patients died (neurological complications for 15 type-2 and 3 type-3 patients, GD complications for 11 type-1 and another disease for 9 type-1 patients). Forty-six had monosymptomatic gammopathy. Among 283 recently followed patients, 36 were untreated and 247 had been treated during 2009–2010; 216 patients received treatment in December 2010 (126 with imiglucerase, 45 velaglucerase, 24 taliglucerase, 21 miglustat). BE occurred before (130 in 67 patients) and under treatment (60 in 41 patients) with respective estimated frequencies (95% CI) of first BE at 10 years of 20.3% (14.1%–26.5%) and 19.8% (13.5%–26.1%).

Conclusion: This registry enabled the epidemiological description of GD in France and showed that BE occur even during treatment.

Keywords: French Gaucher’s Disease Registry, Bone events, Enzyme-replacement therapy
Background
Gaucher’s disease (GD), a rare autosomal-recessive disorder with an approximate prevalence of 1/75,000 live births worldwide, is due to the deficiency of a lysosomal enzyme (glucocerebrosidase, glucocerebrosidase or glucosidase-β acid (EC 3.2.1.45)) [1] or, more rarely, its activator (saposin C) [23]. GD diagnosis is based on deficient glucocerebrosidase activity in peripheral leukocytes or cultured skin fibroblasts. Genotyping can sometimes provide prognostic information [4]. This lysosomal storage disease is characterized by liver and spleen enlargement, and bone manifestations (Erlenmeyer-flask deformity, osteoporosis, lytic lesions, pathological and vertebral compression fractures, bone infarcts and avascular necroses leading to degenerative arthropathy) [1,5].

Based on the neurological signs, 3 clinical phenotypes are recognized: type 1, the classic form usually defined by the absence of central nervous system impairment, although an association between type-1 GD and Parkinsonism has been described [6]; types 2 and 3, both rare and severe, have neurological involvement [7]; and the perinatal–lethal GD type, with perinatal onset and death before 3 months of age [8]. GD signs usually appear after a symptom-free period, except in rare cases of fetal onset [9]. Thrombocytopenia and anemia are common, and several biomarkers (chitotriosidase, ferritin, angiotensin-converting enzyme [ACE] and tartrate-resistant acid phosphatase [TRAP]) are elevated during GD evolution [10–17].

Enzyme-replacement therapy (ERT; alglucerase [Ceredase®, Genzyme Corporation, available since 1991] [18], followed by imiglucerase [Cerezyme®, Genzyme Corporation, available since 1996], velaglucerase [Vpriv®, Shire, available since 2010] [19], and taliglucerase [Uplyso®, Pfizer, only authorized for temporary use] [20]), is the reference treatment. Substrate-reduction therapy (SRT), namely miglustat (Zavesca®, Actelion, available since 2002) [21], is indicated for moderate GD when ERT is unsuitable. In June 2009, an acute imiglucerase shortage occurred because of viral contamination (Vesivirus 2117) of cell cultures and other production problems [22]. Since then, that ERT has been in short supply, which was further aggravated in August 2009.

Genzyme Corporation developed an international registry [23] and several countries, e.g., Spain [24,25], Brazil [26] or Japan [27], identified GD cohorts and established exhaustive national registries. While the international registry conducted many important ancillary studies [28-34], its non-exhaustive cohort did not address public health issues in terms of incidence, prevalence and monitoring of care of GD patients. Since 2004, France has created referral centers dedicated to the clinical management of rare diseases, and assigned them several objectives, e.g., improving overall patient clinical care and professional practices, and collecting epidemiological data. In this context, a Referral Center for LysoSomal Diseases (RCLD) was established and a national GD-patient registry was created, in 2009, as a means to examine and meet some of those goals.

The main aims of this study were to describe the epidemiological profile of GD patients in France: GD demographic, clinical, biological and genetic features; complications in patients with follow-up (2009–2010); and treatments for those with recent (2009–2010) follow-up based on data collected since 1980 and available in the French Gaucher Disease Registry (FGDR).

Patients and methods
Registry design
The FGDR developed and maintains a designated RCLD since 2009. Its Evaluation of Gaucher Disease–Treatment Committee (EGDTC) is a national scientific committee to monitor and optimize GD management in France. The French Data-Protection Commission’s (CNIL) approval of the FGDR required oral or written informed consent from patients or their parents. Data from patients who did not consent were not entered. The FGDR was finally certified in 2009 by the French Institute for Public Health Surveillance (InVS) and the French National Institute of Health and Medical Research (INSERM). All GD patients living in France and having ≥1 consultations (i.e., hospitalization or outpatient consultation with a GD specialist) since 1980 were included. For all patients, GD was diagnosed by demonstration of deficient glucocerebrosidase activity in leukocytes [35] or cultured skin fibroblasts. Exhaustive identification of cases was achieved through 3 sources. Only 3 diagnostic laboratories (all included in EGDTC) are accredited in France and, therefore, identify all patients with an enzymatic assay for GD. Based on our Reference Center’s expertise, the French national health insurance (Rare Disease Committee with EGDTC members) validates each GD diagnosis and authorizes coverage for its treatment. Once experts have validated the case, they can ask the treating physician to include the patient in our registry. The indications for treatment are well established to maximize efficacy and avoid unnecessary health insurance expenditures. Each treating physician contacted allowed access to the medical data entered in the FGDR, which is certified by InVS and INSERM.

Each patient’s data were also collected by RCLD physicians or clinical research assistants. The FGDR director controlled data quality. Dr D. Hamroun developed the original Internet software for the FGDR, using 4D Dimension language from 4D (www.4D.com). Data were collected retrospectively between 2009 and 2010, and as of 2011, all data have been recorded prospectively.
A standardized case-report form was used to collect the following information: initial data (age at diagnosis, sex, history related or unrelated to GD, symptoms leading to diagnosis and first symptoms, first diagnostic exam, phenotype, genotype and affected family members); clinical information during the first consultation, at diagnosis and throughout follow-up; body mass index expressed according to the World Health Organization classification; organomegaly (liver and/or spleen, ultrasound measurement of the largest diameter); biological findings initially and throughout follow-up (hemoglobin level, platelet count, leukocyte count, chitotriosidase, ferritin, ACE, TRAP, gammaglobulin (with respective normal values of >12 g/dL, >150×10^9/mm^3, >4×10^9/mm^3, <100 nmol/mL/h, <250 ng/L, <45 IU/L, <7 IU/L and <3 g/L). Plasma chitotriosidase activity was determined using the fluorescent substrate 4-methylumbelliferylβ-D-N,N′,N′-triacylcitolcosirole [10]; ACE, TRAP, ferritin and other markers were measured in the appropriate local laboratories. Bone findings (X-rays, magnetic resonance imaging and, for some patients, scintigraphy and dual-energy X-ray absorptiometry) were recorded during follow-up, with identification of intercurrent events, particularly bone complications. Bone events (BE) were defined clinically, using the bone indications for treatment recommended by the French National Health Authority [36]: avascular necrosis of an epiphysis, bone infarct, pathological and/or vertebral compression fracture(s). Each BE caused a clinical manifestation and was confirmed radiologically. Bone pain alone was not considered a BE without radiological confirmation. Acute bone pain defined a bone crisis. Bone crisis was included in BE only when a bone infarct was identified. Any event, GD-related or not, occurring during follow-up and monitoring of GD-specific therapy was also recorded.

**Study design**

This investigation was undertaken to describe and analyze clinical, biological, radiological and therapeutic data recorded in the FGDR for all patients from diagnosis until 31 December 2010, the closing date. The local Institutional Review Board of Northern Paris Hospitals, Paris–Diderot University, AP–HP (Ethics Committee) reviewed and approved the research project.

To simplify the description, we defined 4 groups: the entire cohort and its subgroups. Data from the entire cohort of patients entered in the FGDR described, when available, diagnosis characteristics for these patients, the GD-incidence rate (defined as total number of cases diagnosed between 1980 and 2010 divided by the total French population during the same period), birth incidence rate (defined as the total number of cases diagnosed between 1980 and 2010, divided by the total number of live births during the same period) and prevalence were estimated for the French population. For patients with ≥1 follow-up visits in addition to the initial assessment form, we investigated their GD complications (splenectomy, Parkinson’s disease (PD), monoclonal gammopathy (MG), BE, first treatment and deaths). Recently followed patients had consulted in 2009–2010: a map showing the locations of hospitals monitoring them was drawn. For patients seen and treated in 2009–2010, BE were analyzed, before and under (ERT and/or SRT (ERT/SRT)). Clinical, biological and radiological monitoring of these recently treated patients was also investigated. Specific GD ERT (imiglucerase, velaglucerase, taliglucerase, miglulstat) was studied, particularly during the period of imiglucerase shortage (June 2009–December 2010). Patients were distinguished according to their age on 1 June 2009 (<15 or >15 years) for the description of the shortage that began at that time. That age was chosen because it defines the limit between adult medicine and pediatrics.

**Statistical analyses**

All statistical analyses were computed with SAS software (version 9.2; SAS Institute Inc; Cary, NC). Data are expressed as medians (range) or interquartile range [IQR; Q1;Q3] for continuous variables and numbers (%) for categorical variables. Because this was a retrospective study, some data were missing, particularly at the onset of follow-up (during the diagnosis phase) or at treatment onset. Given the demonstrated relatively stable clinical and laboratory parameters of untreated patients after GD diagnosis [37], the biological data during the next 2 years changed only minimally and were considered similar to those at diagnosis. Likewise, data for the previous 2 years under ERT/SRT were stable compared to those at treatment onset. Under treatment data were the last values before the end of therapy or at the closing date. When ERT/SRT was interrupted for <6 months, patients were always considered to be on treatment.

Non-parametric tests were used to compare categorical variables (Fisher’s exact test) across patient subgroups. A two-sided p<0.05 was considered significant. For recently treated patients, time to first BE was estimated with the Kaplan–Meier method for 2 periods: diagnosis to treatment onset (before ERT) and first ERT to closing date (under ERT), with only the first BE occurring during each period being considered. Data were censored when no BE occurred prior to ERT start for the first analysis, and until the closing date or treatment discontinuation for the second. We aimed to study a risk effect of BE. First, the impact of splenectomy, time to treatment onset (< or ≥2 years after diagnosis, based on Mistry et al.’s demonstration of lower BE risk after the latter [38]) or age at diagnosis (< or ≥15 years) on BE
occurrence was tested using the log-rank test; second a Cox model was used to derive a predictive model. When several univariate model covariates were significant, a multivariate Cox model with backward selection was used to retain only significant ones. For ME under ERT, the impact of ME occurrence before treatment was also tested.

Results
Entire registry cohort
The FGDR contained 562 patients with confirmed GJ, living in France and having ≥1 consultations between 1980 and 2011. Patient characteristics at diagnosis are reported in Table 1. The male/female ratio was 1.0151 (49.6% female), while median age at first symptoms was 15 (0–77) years. During the 31 years (1980–2010), 474 patients were diagnosed in France, with an annual median of 15 (9–32) patients. The incidence rate was estimated at 0.26 patients/10^6 person-years and birth incidence at 1/136,000. The most frequent first symptom leading to diagnosis (available for 232 patients) was splenomegaly and/or thrombocytopenia. Although others were found, including anemia in 14, 14 splenectomies, 10 severe hemorrhages, 6 neurological symptoms, 3 avascular necroses, 1 vertebral collapse and 1 bone infarct. Although the most common diagnostic test yielding the diagnosis was bone marrow aspiration, all patients’ diagnoses were confirmed by enzymatic assay. Genotypes were determined for 261 patients, with 203 having homo- (39 patients) or heterozygous p.N370S mutations (164 patients, including 41 with p.L444P/p.N370S mutations). Genotype distributions differed significantly (p<0.001): L444P/L444P or L444P/other were found in 14 (6.4%) phenotype-1, 13 (100%) phenotype-2 and 12 (100%) phenotype-3 patients, while the genotypes N370S/L444P N370S/other or N370S/N370S were identified in 203 (93.6%) phenotype-1, no phenotype-2 and no phenotype-3 patients. Other mutations found (associated or not with p.N370S or p.L444P) were: p.R48W, p.C16W, p.G193R, p.K303I, p.W312S, p.G377S, p.W393R, p.V394L, p.D410H, p.M411G, p.A466P, p.R463C, p.1416, 1417delAG and p.RocNeil. Patients were predominantly (84.7%) type 1. Among 161 patients with an affected family member, all but 3 (1 mother, 1 uncle, 1 cousin) were siblings. Ninety-seven patients died: 37 perinatal deaths (28 fetuses and 9 newborns ≤3 months old) and 60 later (33 type 1, 22 type 2 and 5 type 3).

Followed patients
A total of 378 patients, predominantly type 1, had ≥1 follow-up visits after their initial evaluations. The median follow-up duration was 16.2 (0.1–67.6) years. Their characteristics at diagnosis are reported in Table 1. During follow-up, 225 complained of chronic bone pain or clinical bone crisis, 231 had splenomegaly and 163 had hepatomegaly at least once.

Splenectomy
Surgery was performed on 104 patients: 17 before diagnosis, 46 at diagnosis and 41 >1 year postdiagnosis. Among the 41 patients splenectomized after diagnosis, 27 had the surgery before 1991 and only 14 thereafter. Among the 14 splenectomies after 1991, indications were: 2 for refractory idiopathic thrombocytopenic purpura, 2 for splenic rupture, 2 for major hypersplenism, 2 for severe splenic infarct and 2 for splenic hematoma but 4 had wrong or unknown indications. Splenectomy was more frequent in type 1 (29.3%) than type 3 patients (2, 13.3%) (p=0.009). Median age at splenectomy was 24.6 (3–76) years. Only 3 patients were taking ERT/SRT when splenectomy was performed for splenic complications.

ME
One hundred and nine patients experienced ME during follow-up, with a median of 1 (1–8) ME per patient and a total of 223 BE: 89 avascular necroses, 40 bone fractures, 67 pathological fractures and 27 vertebral collapses. Sites were known for 188 BE: 56 avascular necroses affected the femur, 8 the humerus, 5 the tibia and 7 other bones (astragal, iliac crest, calcaneus), with 13 unknown sites; 15 bone infarcts involved the femur, 5 the tibia, 5 the iliac crest and 2 other bones, with 13 sites unknown; 11 pathological fractures concerned the wrists, 9 the ribs, 8 the femur, 8 the humerus and 22 other bones, with 9 localizations unknown. Type-1 patients had 107 (30.7%) ME, type-3 patients had 2 (13.3%), while type-2 patients had no ME (p=0.009). Median age at first BE was 34.1 (0–75.9) years. The first BE occurred in 88 patients without treatment and in 22 under ERT/SRT.

MG
Forty-six patients, all type 1, developed MG during follow-up. Median age at MG diagnosis was 48.2 (21.3–81) years. The median GD-diagnosis-to-MG-diagnosis interval was 1.3 (0–47.5) years. MG was not significantly more frequent in any given genotype (p=0.37): 1 (4%) p. N370S/p.N370S, 15 (60%) p.N370S/other, 6 (24%) p.N370S/p.L444P, 1 (4%) p.L444P/other and 2 (8%) in other/other; 21 patients with MG had no genotype determination. Comparing the p.L444P allele to the others, no significant difference was found (p=1). Thirteen patients were on ERT/SRT when MG was diagnosed (but no pretreatment immunosuppression was available), 23 were treated thereafter and 10 had never been treated at closing date, despite MG.
### Table 1: Baseline characteristics of the FGDR cohort, and subgroups with any follow-up, recent follow-up or treatment

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>Entire cohort (n=562)</th>
<th>Followed patients (n=378)</th>
<th>Recently seen patients (n=283)</th>
<th>Recently treated patients (n=247)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>265 (46.0)</td>
<td>182 (48.1)</td>
<td>144 (50.9)</td>
<td>121 (49)</td>
</tr>
<tr>
<td>Male</td>
<td>297 (54.0)</td>
<td>196 (51.9)</td>
<td>139 (49.1)</td>
<td>126 (51)</td>
</tr>
<tr>
<td><strong>Age, years, median (range) [IQR]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First symptoms (%)</td>
<td>238 (median: 15, range: 0-77)</td>
<td>227 (median: 15, range: 0-77)</td>
<td>182 (median: 15, range: 0-62)</td>
<td>162 (median: 15, range: 0-62)</td>
</tr>
<tr>
<td>Diagnosis (without fetuses)</td>
<td>534 (median: 22, range: 0-88)</td>
<td>378 (median: 213, range: 0-68)</td>
<td>283 (median: 226, range: 0-69)</td>
<td>247 (median: 221, range: 0-67)</td>
</tr>
<tr>
<td>Patients diagnosed before 1991, n (%)</td>
<td>562 (46.4)</td>
<td>378 (47.9)</td>
<td>283 (46.3)</td>
<td>247 (49.4)</td>
</tr>
<tr>
<td>Patients ≤15 years old at diagnosis, n (%)</td>
<td>562 (45.6)</td>
<td>378 (43.9)</td>
<td>283 (40.0)</td>
<td>247 (50.6)</td>
</tr>
<tr>
<td>1ste symptom-to-diagnosis interval, years, median (range)</td>
<td>238 (median: 1, range: 0-36)</td>
<td>227 (median: 1, range: 0-56)</td>
<td>182 (median: 1, range: 0-56)</td>
<td>162 (median: 1, range: 0-56)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>163 (70.2)</td>
<td>155 (71.8)</td>
<td>120 (71)</td>
<td>109 (83.9)</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>51 (22)</td>
<td>46 (21.3)</td>
<td>35 (20.7)</td>
<td>33 (11.8)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>114 (49.1)</td>
<td>107 (49.5)</td>
<td>88 (52.1)</td>
<td>76 (27.1)</td>
</tr>
<tr>
<td>Bone crisis</td>
<td>8 (3.4)</td>
<td>8 (3.7)</td>
<td>6 (3.6)</td>
<td>6 (2.1)</td>
</tr>
<tr>
<td>Chronic bone pain</td>
<td>16 (6.9)</td>
<td>15 (5.9)</td>
<td>13 (7.7)</td>
<td>13 (4.6)</td>
</tr>
<tr>
<td>Other</td>
<td>82 (35.3)</td>
<td>73 (33.8)</td>
<td>53 (31.4)</td>
<td>43 (15.5)</td>
</tr>
<tr>
<td>Test diagnosing CD, n (%)</td>
<td>245</td>
<td>233</td>
<td>189</td>
<td>162</td>
</tr>
<tr>
<td>Enzyme assay</td>
<td>61 (24.9)</td>
<td>53 (22.7)</td>
<td>48 (25.4)</td>
<td>36 (22.2)</td>
</tr>
<tr>
<td>GBA-gene sequencing</td>
<td>1 (0.4)</td>
<td>1 (0.4)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Bone marrow aspiration</td>
<td>118 (48.2)</td>
<td>115 (42.4)</td>
<td>93 (49.2)</td>
<td>83 (31.3)</td>
</tr>
<tr>
<td>Bone marrow biopsy</td>
<td>16 (6.5)</td>
<td>16 (5.9)</td>
<td>13 (6.9)</td>
<td>13 (6.8)</td>
</tr>
<tr>
<td>Bone biopsy</td>
<td>5 (2.0)</td>
<td>5 (2.1)</td>
<td>5 (2.6)</td>
<td>5 (2.1)</td>
</tr>
<tr>
<td>Hepatic biopsy</td>
<td>9 (3.7)</td>
<td>8 (3.4)</td>
<td>6 (3.2)</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>Spleen histology</td>
<td>33 (13.5)</td>
<td>33 (14.2)</td>
<td>21 (11.1)</td>
<td>18 (11.1)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (0.8)</td>
<td>2 (0.9)</td>
<td>2 (1.1)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td><strong>Type, n (%)</strong></td>
<td>536</td>
<td>378</td>
<td>283</td>
<td>247</td>
</tr>
<tr>
<td>1</td>
<td>454 (84.7)</td>
<td>340 (92.0)</td>
<td>274 (96.8)</td>
<td>239 (96.8)</td>
</tr>
<tr>
<td>2</td>
<td>61 (11.4)</td>
<td>15 (4)</td>
<td>1 (0.4)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>3</td>
<td>21 (3.9)</td>
<td>15 (4)</td>
<td>8 (2.8)</td>
<td>8 (3.2)</td>
</tr>
<tr>
<td><strong>Genotype, n (%)</strong></td>
<td>261</td>
<td>229</td>
<td>172</td>
<td>155</td>
</tr>
<tr>
<td>p.A870S/p.A1370S</td>
<td>39 (15.0)</td>
<td>34 (14.8)</td>
<td>28 (16.3)</td>
<td>24 (15.5)</td>
</tr>
<tr>
<td>p.N707S/p.L444P</td>
<td>41 (15.7)</td>
<td>37 (16.2)</td>
<td>31 (18.0)</td>
<td>27 (17.4)</td>
</tr>
<tr>
<td>p.L444P/p.L444P</td>
<td>17 (6.5)</td>
<td>11 (4.8)</td>
<td>4 (2.3)</td>
<td>4 (2.6)</td>
</tr>
<tr>
<td>p.A870S/other</td>
<td>123 (47.1)</td>
<td>114 (48.8)</td>
<td>86 (50.0)</td>
<td>77 (49.7)</td>
</tr>
<tr>
<td>p.L444P/Other</td>
<td>24 (9.2)</td>
<td>17 (7.4)</td>
<td>9 (5.2)</td>
<td>9 (5.8)</td>
</tr>
<tr>
<td>Other/other</td>
<td>17 (6.5)</td>
<td>16 (7)</td>
<td>14 (8.2)</td>
<td>14 (9)</td>
</tr>
<tr>
<td>Affected family, n</td>
<td>562</td>
<td>378</td>
<td>130</td>
<td>108</td>
</tr>
</tbody>
</table>

Note that recent refers to 2009–2010, i.e., the last 2 years. GBA glucosidase β acid.

*No represents the number of patients with available information.

†Several symptoms for each patient.

‡All patients had their definitive diagnoses confirmed by enzymatic assay.
PD
Neurologist-confirmed PD was diagnosed in 14 patients, all had type-1 GDs; 6 had dementia. Median age at PD diagnosis was 60.4 (38.2–77.1) years. The median age at GD diagnosis of patients who developed PD was 46.2 (4.4–70.7) years. PD was significantly more frequent in patients with the p.L444P/p.N370S mutation versus others (p<0.0001): PD was diagnosed in 1 (2.9%) p.N370S/p.N370S patient, 2 (1.7%) p.N370S/other and 5 (13.5%) p.N370S/p.L444P, 6 PD patients had no genotype determination. Five patients were on ERT/SRT at PD diagnosis, 7 were treated thereafter and 2 had never been treated at closing date, despite PD. Treatment (imiglucerase or miglustat) did not have any apparent effect on PD signs. Two patients received miglustat and both stopped it after 1 year because of PD progression or dementia.

Treatments
Among the 378 patients with follow-up, 298 (78.8%) received treatment. The first ERT was alglucerase for 62 (20.8%) patients, imiglucerase for 224 (75.2%), miglustat for 7 (2.3%), velaglucerase for 4 (1.3%) and taliglucerase in 1 (0.3%). The median ERT dose (known for 228 patients) was 120 (30–284) IU/kg/month, with 204 patients receiving ≥120 IU/kg/month. Sixty-two patients were given miglustat during follow-up, with a median treatment duration of 0.7 (0.1–6.7) years. Ten patients had an indwelling catheter and 85 received ≥1 ERT administrations at home. The median diagnosis-to-first treatment interval was 9.1 (0–61.4) years for all 298 patients, but only 1.4 (0–16) years for the 145 patients diagnosed after 1991.

Deaths
Thirty-eight patients died during follow-up: type 2 was fatal for all 15 patients compared to 20 (5.7%) type 1 or 3 (20%) type 3 (p<0.001). Age at death was known for 28 patients: median of 8.4 (0.3–83.4) years for all deceased, but 64.5 (38.4–83.4) years for 13 type-1 patients, 1 (0.3–2.2) year for the 13 type-2 patients and 8.4 (7.2–9.6) years for the 2 type-3 patients. Type-2 and –3 patients died of neurological impairment, whereas 11 type-1 patients' deaths were attributed to: 2 lymphomas, 3 PD, 1 myeloma, 1 osteosarcoma, 2 with anemia or thrombocytopenia complications (1 each with myocardial infarct with anemia or pancytopenia) and 2 had pulmonary hypertension. Mortality was significantly more frequent (p<0.0001) for patients whose genotypes carried the p.L444P mutation; most of them had neurological impairment, as reported above: 1 (2.9%) with p.N370S/p.N370S, 3 (2.6%) p.N370S/other, 4 (10.8%) p.N370S/p.L444P, 5 (31.2%) p.L444P/other and 6 (54.5%) p.L444P/p.L444P; 17 deceased patients had no genotype determination. Fifteen patients were on ERT/SRT when they died, while all others had never been treated (including all type-2 patients).

Recently followed patients
Among followed patients, 95 had no known data during 2009–2010. Median time since the last hospital consultation for these patients was 10.7 (2.4–31.6) years. Thus, 283 patients had consulted during 2009–2010: 36 had never been treated and 247 patients had received ERT/SRT during that period. Their characteristics at diagnosis are reported in Table 1.

Hospital locations of followed patients
Figure 1 shows where the hospitals caring for the 283 patients with recent follow-up are located. Ninety-five patients were followed in Paris, including 73 (76.8%) in the RCLD. Follow-up was done in an internal medicine department for 152 (54%) patients, hematology for 65 (23%), pediatrics for 30 (11%) and other departments (7 gastroenterology, 6 neurology, 10 rheumatology, 13 others) for 36.

Patients without treatment
Thirty-six patients had never been treated: median diagnosis-to-closing-date interval was 7.8 (0.4–39.9) years, for 35 type-1 patients and 1 with type 2; 14 patients had familial GD (all siblings) and 3 patients, who developed BE (2 avascular necroses, 1 pathological fracture and 1 vertebral collapse) during follow-up, refused treatment.

Recently seen and treated patients
During 2009–2010, 247 patients (239 type 1 and 8 type 3) received treatment, with median follow-up at 19.3 (0.2–66.2) years. Among these recently treated patients, all 5 type-1 patients died at a median age of 65.8 (56.8–83.4) years. Table 2 provides clinical, biological and bone data at diagnosis, treatment onset and closing date. Clinical findings, biological values and bone findings tended to improve under ERT/SRT.

During follow-up, 190 BE (73 avascular necroses, 36 bone infarcts, 58 pathological fractures, 23 vertebral compressions) occurred in 86 patients, with a median of 1.5 (1–8) BE/patient. Figure 2 shows Kaplan–Meier estimates of the time to the first BE for the 247 recently seen and treated patients, between diagnosis and ERT/SRT onset (9.2 years of follow-up), and between the latter and the closing date (7.8 years of follow-up). Treatment at the time of BE was imiglucerase for 56 patients, alglucerase for 5 and miglustat for 4. The median imiglucerase/alglucerase dose when BE occurred in 52 patients was 120 (43.5–240) IU/kg/month; 42 patients had doses ≥120 IU/kg/month. The probabilities (95% confidence interval (CI)) of BE occurring by 10 years
before and during treatment were estimated at 20.3% (14.1%–26.1%) and 19.8% (13.5%–26.1%), respectively. Before treatment, 67 patients developed 128 BE: 35 patients with 1 BE, 17 with 2 BE and 15 with ≥3 BE; whereas under treatment, 41 developed 62 BE: 28 patients with 1 BE, 8 with 2 BE and 5 with ≥3 BE, including 22 patients with BE before and under ERT/SRT.

The probabilities (95% CI) of BE occurring by 10 years before ERT/SRT were 11.5% (3.1%–19.8%) versus 24.9% (16.6%–33.2%) for age at diagnosis ≤15 years and >15 years, respectively (p = 0.047). No other covariates were found to influence BE occurrence before ERT/SRT. During treatment, the probabilities (95% CI) of experiencing BE by 10 years were: 11.8% (5.9%–17.6%) and 35.9% (22.2%–49.5%) for patients without or with BE (Figure 3) before ERT/SRT, respectively, with an HR of 9.8 (5.9–16.3) (p < 0.001); 29.1% (16.1%–42.1%) versus 14.7% (8.3%–21.1%) with and without splenectomy, respectively, with an HR of 2.1 (1.5–2.9) (p = 0.005); 34.9% (29.8%–40.1%) vs 50.3% (46.1%–54.4%) for age at diagnosis ≤15 years and >15 years, respectively, with an HR of 0.7 (0.5–0.98) (p = 0.04); and 22.3% (15.1%–29.5%) versus 8.1% (6.8%–17.5%) for diagnosis-to-treatment interval >2 and ≤2 years, respectively, with an HR of 0.5 (0.3–0.9) (p = 0.01). Age at diagnosis >15 years was not significantly associated with BE under treatment (p = 0.54). In the multivariate analysis with backward elimination, BE before treatment was the only significant risk factor retained.

Table 3 reports ERT/SRT prescribed according to the supply-shortage dates for the 247 recently treated patients. The first treatment prescribed was imiglucerase for 185 patients, alglucerase for 50, miglustat for 7, velaglucerase for 4 patients and taliglucerase for 1. The median diagnosis-to-treatment interval was 92 (0.0–47) years for all patients, but only 1.5 (0.0–16) years for patients diagnosed after 1991. The median treatment duration was 7.8 (0–18.3) years. During the supply shortages (June–August 2009), 106 patients discontinued their treatment, 9 switched to miglustat and 46
### Table 2 GD clinical, biological and imaging characteristics at specific times for 247 recently treated (2009–2010) patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>At diagnosis</th>
<th>No.</th>
<th>At ERT/SRT onset</th>
<th>No.</th>
<th>At closing date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years since diagnosis, median (range)</td>
<td>247</td>
<td>–</td>
<td>247</td>
<td>92 (0–47)</td>
<td>151 (77–177)</td>
<td>247</td>
</tr>
<tr>
<td>Age, years, median (range)</td>
<td>247</td>
<td>22.2 (0.5–67.5)</td>
<td>247</td>
<td>16 (1–79)</td>
<td>209 (4–62)</td>
<td>247</td>
</tr>
<tr>
<td>Clinical involvement, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigmentation</td>
<td>184</td>
<td>10 (5.4)</td>
<td>186</td>
<td>14 (7.5)</td>
<td>167</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>184</td>
<td>84 (45.7)</td>
<td>186</td>
<td>106 (57)</td>
<td>167</td>
<td>44 (26.3)</td>
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<tr>
<td>Abdominal pain</td>
<td>184</td>
<td>29 (15.8)</td>
<td>186</td>
<td>43 (23.1)</td>
<td>167</td>
<td>8 (4.8)</td>
</tr>
<tr>
<td>Chronic bone pain</td>
<td>184</td>
<td>70 (38.0)</td>
<td>186</td>
<td>79 (42.5)</td>
<td>167</td>
<td>41 (24.6)</td>
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<tr>
<td>Bone crisis</td>
<td>184</td>
<td>25 (13.6)</td>
<td>186</td>
<td>46 (24.7)</td>
<td>167</td>
<td>12 (7.2)</td>
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<tr>
<td>Breeding</td>
<td>184</td>
<td>37 (20.4)</td>
<td>186</td>
<td>58 (31.2)</td>
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<td>12 (7.2)</td>
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<tr>
<td>Neurological sign</td>
<td>184</td>
<td>7 (3.8)</td>
<td>186</td>
<td>14 (7.5)</td>
<td>167</td>
<td>9 (5.4)</td>
</tr>
<tr>
<td>Other</td>
<td>184</td>
<td>11 (6)</td>
<td>186</td>
<td>10 (5.4)</td>
<td>167</td>
<td>42 (25.1)</td>
</tr>
<tr>
<td>Body mass index, kg/m², median (range)</td>
<td>49</td>
<td>16.6 (13.6–28.1)</td>
<td>78</td>
<td>20.3 (13.6–28.1)</td>
<td>53</td>
<td>22.2 (14.6–34.4)</td>
</tr>
<tr>
<td>Underweight, n (%)</td>
<td>31</td>
<td>62.3</td>
<td>31</td>
<td>39.7</td>
<td>7</td>
<td>13.2</td>
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<tr>
<td>Normal, n (%)</td>
<td>14</td>
<td>25.6</td>
<td>41</td>
<td>52.8</td>
<td>35</td>
<td>66.0</td>
</tr>
<tr>
<td>Overweight/obese, n (%)</td>
<td>4</td>
<td>8.1</td>
<td>5</td>
<td>7.7</td>
<td>11</td>
<td>20.8</td>
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<tr>
<td>Liver and spleen*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Splenectomy, n (%)</td>
<td>247</td>
<td>41 (16.6)</td>
<td>247</td>
<td>63 (25.5)</td>
<td>247</td>
<td>65 (26.3)</td>
</tr>
<tr>
<td>Splenomegaly, n (%)</td>
<td>176</td>
<td>14 (8.3)</td>
<td>129</td>
<td>124 (66.1)</td>
<td>76</td>
<td>42 (5.3)</td>
</tr>
<tr>
<td>Splenic US, median (range) of largest diameter, cm</td>
<td>54</td>
<td>15.8 (10–33)</td>
<td>86</td>
<td>18.9 (10–41)</td>
<td>44</td>
<td>13.6 (6–24)</td>
</tr>
<tr>
<td>Hepatomegaly, n (%)</td>
<td>146</td>
<td>116 (79.3)</td>
<td>140</td>
<td>118 (64.3)</td>
<td>90</td>
<td>40 (44.4)</td>
</tr>
<tr>
<td>Liver US, (median (range) of largest diameter, cm</td>
<td>23</td>
<td>15 (8–22)</td>
<td>81</td>
<td>17.6 (8.4–37)</td>
<td>34</td>
<td>15 (9–22)</td>
</tr>
<tr>
<td><strong>Biological parameter, median (range)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>140</td>
<td>11.5 (5.3–18.9)</td>
<td>169</td>
<td>11.7 (5.4–17)</td>
<td>188</td>
<td>13.2 (8–16.4)</td>
</tr>
<tr>
<td>Leukocytes (x10³/mm³)</td>
<td>126</td>
<td>49 (0.6–15.4)</td>
<td>153</td>
<td>48 (0.5–24)</td>
<td>102</td>
<td>57 (2.1–14.1)</td>
</tr>
<tr>
<td>Platelet count (x10³/mm³)</td>
<td>161</td>
<td>81 (20–420)</td>
<td>185</td>
<td>80 (18–449)</td>
<td>187</td>
<td>160 (18–553)</td>
</tr>
<tr>
<td>Platelets (x10³/mm³) without splenectomy</td>
<td>127</td>
<td>80 (20–240)</td>
<td>137</td>
<td>72 (18–196)</td>
<td>144</td>
<td>139 (18–304)</td>
</tr>
<tr>
<td>Chitotriosidase (nmol/mL/h)</td>
<td>43</td>
<td>8000 (239–47500)</td>
<td>71</td>
<td>9000 (360–65500)</td>
<td>106</td>
<td>992 (19–53400)</td>
</tr>
<tr>
<td>TRAP (U/L)</td>
<td>5</td>
<td>7.1 (1.1–28)</td>
<td>29</td>
<td>10 (4–38)</td>
<td>24</td>
<td>4.5 (1–18.8)</td>
</tr>
<tr>
<td>ACE (U/L)</td>
<td>17</td>
<td>183 (93–1000)</td>
<td>51</td>
<td>190 (6.4–450)</td>
<td>48</td>
<td>575 (12–380)</td>
</tr>
<tr>
<td>Ferritin (ng/L)</td>
<td>36</td>
<td>500 (40–9000)</td>
<td>74</td>
<td>621 (63–3200)</td>
<td>72</td>
<td>337 (43–2200)</td>
</tr>
<tr>
<td>Gammaglobulin (g/L)</td>
<td>14</td>
<td>15.8 (9–28.7)</td>
<td>44</td>
<td>15 (6.6–36)</td>
<td>36</td>
<td>12 (5.4–19.8)</td>
</tr>
<tr>
<td><strong>Imaging of bone lesions</strong>, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erlenmeyer flask</td>
<td>43</td>
<td>9 (20.9)</td>
<td>61</td>
<td>17 (27.9)</td>
<td>50</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>43</td>
<td>6 (14)</td>
<td>61</td>
<td>15 (24.6)</td>
<td>50</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Cortical erosion</td>
<td>43</td>
<td>3 (7)</td>
<td>61</td>
<td>3 (49)</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Lytic lesion</td>
<td>43</td>
<td>4 (9.3)</td>
<td>61</td>
<td>5 (82)</td>
<td>50</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Avascular necrosis sequelae</td>
<td>43</td>
<td>6 (14)</td>
<td>61</td>
<td>11 (18.0)</td>
<td>50</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Infarct sequelae</td>
<td>43</td>
<td>6 (14)</td>
<td>61</td>
<td>8 (13.1)</td>
<td>50</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Fracture sequelae</td>
<td>43</td>
<td>0</td>
<td>61</td>
<td>2 (33)</td>
<td>50</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Infiltration on MRI</td>
<td>40</td>
<td>31 (77.5)</td>
<td>72</td>
<td>53 (73.6)</td>
<td>80</td>
<td>40 (60)</td>
</tr>
<tr>
<td><strong>Tc-Hyperfixation</strong></td>
<td>31</td>
<td>19 (61.3)</td>
<td>56</td>
<td>42 (75)</td>
<td>41</td>
<td>50 (73.2)</td>
</tr>
<tr>
<td><strong>Tc-Hyperfixation</strong></td>
<td>31</td>
<td>5 (16.1)</td>
<td>56</td>
<td>5 (89)</td>
<td>41</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2 GD clinical, biological and imaging characteristics at specific times for 247 recently treated (2009–2010) patients (Continued)

<table>
<thead>
<tr>
<th>Bone densitometry, median (range)</th>
<th>T-score neck</th>
<th>10</th>
<th>-0.6 (-2.1 to 1.1)</th>
<th>27</th>
<th>-1.4 (-4.2 to 1.4)</th>
<th>28</th>
<th>-0.6 (-2.9 to 4.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-score lumbar</td>
<td>8</td>
<td>-1.5 (-2.8 to -0.5)</td>
<td>22</td>
<td>-1.8 (-4.2 to 0.8)</td>
<td>29</td>
<td>-0.9 (-3.0 to 6.2)</td>
<td></td>
</tr>
<tr>
<td>Z-score neck</td>
<td>10</td>
<td>-0.8 (-2.1 to 1)</td>
<td>20</td>
<td>-0.8 (-2.1 to 1.9)</td>
<td>26</td>
<td>-0.5 (-2.6 to 4.4)</td>
<td></td>
</tr>
<tr>
<td>Z-score lumbar</td>
<td>7</td>
<td>-1.9 (-3.3 to 0.3)</td>
<td>15</td>
<td>-1.1 (-3.1 to 0.5)</td>
<td>24</td>
<td>-0.1 (-3.0 to 7.1)</td>
<td></td>
</tr>
</tbody>
</table>

US ultrasound, MRI magnetic resonance imaging.

*Data from 31 patients were used at diagnosis and at ERT/SRT onset.
†Splenomegaly in non-phenylactonized patients.

Figure 2 Time to the first bone event (BE) in 247 GD patients receiving ERT/SRT. The dashed lines represent the curve’s 95% CI; the estimated probability of BE occurrence after 10 years is reported on the y-axis. (A) Between diagnosis and first treatment during the first 30 years of follow-up. (B) Between first treatment and end of treatment or closing date during 15 years of follow-up. No. at risk represents the number of patients followed at the indicated time; No. with BE represents the number of patients with a BE.
reduced their imiglucerase doses. Two patients received a combination of miglustat and imiglucerase, which was continued during the shortage (counted only under imiglucerase in Table 3).

**Discussion**

To date, no other publication has analyzed the comprehensive data entered in the FGDR for 562 patients, minus 3 who refused to participate and 97 who died, leaving 465 patients (among 65.8 million inhabitants), yielding prevalence of 1/140,000 inhabitants in France, a number that is probably underestimated. Concerning the entire cohort, although type 1 predominated (85%), types 2 and type 3 represented 4% each, along with 37 (6.9%) perinatal–lethal type. Moreover, the type-2 incidence was the same as that of type 3 but its prevalence was low because of its associated early mortality. The recent publication on the exhaustive Spanish registry [26] reported data similar to ours, with 88.3% type 1, 6.7% type 2 and 5% type 3. Our birth incidence (1/50,000) was higher than previously reported for the GD frequency in non-Jewish populations from EU countries [25,39,40], with a prevalence (1/136,000), close to that of the Spanish registry (1/149,000) [26]. Bone-marrow aspiration (or biopsy) remained the most common laboratory test (57%) providing the GD

**Figure 3 Impact of BE before treatment on BE occurrence under ERT/SRT for 247 treated GD patients.** The solid bold grey line represents patients without BE before treatment; the solid bold black line represents the times to first BE. Dashed lines represent the 95% CI of those curves; the estimated probability of BE occurrence after 10 years reported on the y-axis.
diagnosis. It is usually the first-line analysis when thrombocytopenia is associated (or not) with splenomegaly and there is no reason to think of immune thrombocytopenia purpura. It is not mandatory and should not be done if the GD diagnosis has been established by enzymatic assay or is already strongly suspected (e.g., possible family history). Rarely, bone marrow aspiration was considered "normal" but another sample contained the characteristic GD cells.

Fourteen (3.7%) of our 378 followed patients had PD, reaching a prevalence comparable to that reported by Bultron et al. [41]. MG and polyclonal gamma globulinemia occur frequently in GD [42-45]. Among the 378 followed patients, 46 (12.2%) had MG, a rate within the previously reported range (1% [42] to 35% [45]), and median gamma globulinemia at ERT/SRT onset in recently treated patients was 21.7 g/L. Usually, MG is unaffected by ERT [43,44]. However, for patients whose MG was diagnosed under treatment, no pretreatment evaluation was available, and MG had probably been present at treatment onset.

Before 1991, splenectomy was the only available treatment, but, since then, it should not have been performed (albeit with exceptions) as a GD treatment. However, it has been used sometimes as a diagnostic tool when splenomegaly and thrombocytopenia coexisted, but should not longer be. Fourteen splenectomies were done after 1991 and after GD diagnosis, usually for patients with splenic complications (splenic infarcts, spleen rupture or large fibrous splenomegaly not amenable to ERT) or a mistaken indication.

BE are the most serious GD complications. They are usually prevented by ERT/SRT, with substantial attenuation of bone pain, bone crises and bone-mineral density [46], although the BE decrease is difficult to evaluate without randomized placebo-controlled trials. In addition, the definition of BE is not homogeneous across studies. Apparently, ERT/SRT does not prevent all BE, as indicated by the estimated respective probabilities of BE occurring by 10 years and during treatment of 20.3% and 19.8%. It is likely that patients on ERT/SRT would probably have had more complications had they not been treated. Furthermore, we showed that BE before treatment increased the risk of BE under ERT/SRT and was the only factor retained in our multivariate analysis. Note that, as reported by Mistry et al. [38], our univariate analyses also found splenectomy and treatment >2 years after GD diagnosis to increase that risk, while sex and age at diagnosis ≤15 years were associated with increased risk of BE before but not under ERT/SRT. Thus, BE persist as a problem that is not fully resolved by treatment. The continuing challenges remain how to identify patients at risk before and under ERT/SRT, and then to decide whether or not these patients would benefit from earlier treatment onset and/or dose intensification.

In summary, the FGDR strong points are its comprehensiveness, independence, accreditation and/or certification by the various health authorities and cooperation generated among the different French centers. This registry also had to manage the immiglucerase shortage, when more severe GD and children were accorded priority treatment. The FGDR also enabled, during that shortage, nationwide management of the ERT/SRT stock and selection of those patients most in need of therapy (velaglucerase and taliglucerase). In France, GD-patient management is organized so that patients receive treatment near their homes, which improves their quality of life. Even though monitoring is not centralized, the FGDR identification and tracking of patients should contribute to improving their specific care management.

**Abbreviations**

ACAT: Acid 

AIDS: Acquired immune deficiency syndrome

ADD: Attention deficit disorder

ADPKD: Autosomal dominant polycystic kidney disease

AGE: Acute gastrointestinal

AGE: Acute general emergency

AGE: Acute gynecological emergency

AGE: Acute general emergency

AGE: Acute gastrointestinal

AGE: Acute general emergency

AGE: Acute gastrointestinal

AGE: Acute general emergency

AGE: Acute gastrointestinal

AGE: Acute general emergency

AGE: Acute gastrointestinal

AGE: Acute general emergency

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal

AGE: Acute gastrointestinal
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Stirnemann et al. Orphanet Journal of Rare Diseases 2012, 7:77
http://www.ojrd.com/content/7/1/77

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References


In order to complete the article presented above with precise data on the affected population, Table 3 presents the overall characteristics of GD data for France, as at 20 October 2015, and as it will be presented in a communication to the WORLDSymposium on Lysosomal diseases (San Diego, 2016).

Table 3: Characteristics of GD in 2015.

<table>
<thead>
<tr>
<th>Overall Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire cohort, n</td>
<td>616</td>
</tr>
<tr>
<td>Deaths</td>
<td>114</td>
</tr>
<tr>
<td>Patients alive (2015)</td>
<td>502</td>
</tr>
<tr>
<td>Patients &lt; 18 years old, n (%)</td>
<td>57 (10.75%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>261 (52%)</td>
</tr>
<tr>
<td>Current median age (years) [min, max]</td>
<td>47.6 [3.4, 96.9]</td>
</tr>
<tr>
<td>Age of first symptoms (years)</td>
<td>15</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>22.7</td>
</tr>
<tr>
<td>Affected family, n</td>
<td>147</td>
</tr>
<tr>
<td>Phenotype 1, n (%)</td>
<td>434 (96%)</td>
</tr>
<tr>
<td>Phenotype 2, n (%)</td>
<td>1 (0.2 %)</td>
</tr>
<tr>
<td>Phenotype 3, n (%)</td>
<td>17 (3.8%)</td>
</tr>
<tr>
<td>Phenotype unknown, n</td>
<td>50</td>
</tr>
<tr>
<td>Patients with splenomegaly during follow-up, n</td>
<td>287</td>
</tr>
<tr>
<td>Patients with hepatomegaly during follow-up, n</td>
<td>274</td>
</tr>
<tr>
<td>Bone event during follow-up, n</td>
<td>133</td>
</tr>
<tr>
<td>Patients undergoing treatment</td>
<td>280</td>
</tr>
<tr>
<td>Patients treated using imiglucerase</td>
<td>187</td>
</tr>
<tr>
<td>Patients treated using miglustat</td>
<td>13</td>
</tr>
<tr>
<td>Patients treated using velaglucerase</td>
<td>62</td>
</tr>
<tr>
<td>Patients treated using taliglucerase</td>
<td>12</td>
</tr>
<tr>
<td>Patients treated using eliglustat</td>
<td>6</td>
</tr>
<tr>
<td>Patients untreated</td>
<td>207</td>
</tr>
<tr>
<td>Patients with discontinued treatment</td>
<td>15</td>
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</tbody>
</table>
Summary of first article on modelling biomarkers in Gaucher disease using nonlinear mixed-effects models (107)

Our first article on modelling biomarkers analysed the occurrence of BEs. However, it only took into account the first BE and its date of occurrence in relation to both the diagnosis of GD for patients without treatment and treatment initiation for patients under treatment. The incidence of BEs was represented using survival curves. Subsequently, the Cox model was used to test the impact of covariates on the incidence of BEs. The association between biomarkers (notably via values at the start of treatment and the slope variations determined using mixed models for each patient) and the incidence of BEs were then tested.

In order to do this, we analysed a single-centre cohort of 73 patients from the Reference Centre for Lysosomal Disease (RCLD). Of these, 62 patients were treated using imiglucerase (enzyme replacement therapy). Incidences of BEs over time were analysed before and during treatment. Biomarkers of GD (chitotriosidase, TRAP, ACE, ferritin and platelets) were modelled before and then during treatment, using mixed models. Other covariates were also analysed at the same time (age at diagnosis, splenectomy, sex, genotype, date of diagnosis) in order to test the potential impact of each covariate to changes in each biomarker and on the incidence of BEs. An analysis of the impact of these covariates on biomarkers before treatment was not carried out because there were too few data.

Firstly, these analyses demonstrated that the occurrence of BEs could happen before and during treatment: this had never before been described in the literature. Among the covariates tested, several had an influence on the biomarkers, but only an age of diagnosis below 15 years old significantly increased the risk of a BE. The analysis of the impact of biomarkers during treatment showed that high levels of ferritin and low levels of platelets at the start of treatment increased the risk of occurrence of BEs during treatment.

These preliminary data showed the advantages of using a mixed-model method in an attempt to resolve a clinical problem: how to recognise patients with a high risk of developing a bone complication who might benefit from a more intensive treatment. The
study data suggested that ferritin and platelets were useful predictors of BEs, thus providing leads for other potential lines of research. The analysis of ferritin levels and the parameters associated with it should continue. The biomarker modelling techniques developed in this study should be used on a larger data set in order to evaluate their predictive strength. This would also help to evaluate the extent to which knowledge about biomarkers and their changing levels helps to predict the occurrence of BEs. It could thus help in the development of more individualised treatments.
Bone events and evolution of biologic markers in Gaucher disease before and during treatment

Jérôme Stimemann1*, Nadia Belmatoug3, Corine Vincent3, Olivier Fain1, Bruno Fantin3, France Mentre2

Abstract

Introduction: Known biomarkers of Gaucher-disease activity are platelets, chitotriosidase, angiotensin-converting enzyme (ACE), tartrate-resistant acid phosphatase (TRAP) and ferritin. The aim of this study was to retrospectively evaluate the frequency of bone events (BE) and biomarker changes during two periods: diagnosis to first enzyme-replacement therapy (ERT) and the latter to the closing date.

Methods: BE of 62 treated patients, among the 73-patient cohort followed at Beaujon Hospital, Clichy, France, were described with Kaplan-Meier curves, and linear-mixed models were used to analyze their biomarker changes and the influence of several covariates (splenectomy, diagnosis year, genotype, age at diagnosis and sex).

Results: BE occurred before (54 events in 21 patients), but also during ERT (12 events in 10 patients), with respective frequencies (95% confidence interval) at 10 years of 22.4% (13.3 to 36.3) and 20.0% (10.2 to 36.9). Biomarker slope changes before and during ERT differed significantly for platelets (+190/mm3/year and 7.035/mm3/year, respectively; P < 0.0001) and ferritin (+9% and -14%, P < 0.0001). High ferritin levels and low platelet counts at ERT onset were significantly associated with BE during ERT (P = 0.019 and 0.039, respectively). Covariates significantly influenced biomarker changes (baseline and/or slope): spleenectomy affected platelets (baseline and changes); TRAP changes and chitotriosidase changes; diagnosis date influenced ACE and TRAP baseline values; and genotype influenced chitotriosidase baseline and changes.

Conclusions: Platelet counts and ferritin levels and their slope changes at ERT onset seem to predict BE during treatment. Biomarker baseline values and changes are dependent on several covariates.

Introduction

Gaucher disease (GD), a rare autosomal-recessive disorder with an approximate prevalence of 1/75,000 live births worldwide, is due to the deficiency of a lysosomal enzyme (glucocerebrosidase, glucosylceramidase or β-glycosidase acid (EC 3.2.1.45)) [1] or, rarely, its activator (saposin C) [2,3]. This lysosomal storage disease is characterized by liver and spleen enlargement, and severe bone complications [1]. Based on the neurological signs, three clinical phenotypes are recognized: type 1, the classic form, affects 95% of the patients and is usually defined by the absence of central nervous system impairment; types 2 and 3 are rare and severe, due to neurological involvement [4].

Type 1 GD has bone complications that can alter the functional prognosis: abnormal bone deformity, such as widening of the femur metaphysis (Erlenmeyer flask), osteopenia, osteoporosis, lytic lesions and pathologic and vertebral compression fractures. Bone infarcts are manifested by acute painful bone crises, and avascular necroses lead to degenerative arthropathy that may require replacement by prosthesis [5]. Thrombopenia and anemia are common. Liver enzymes may be slightly elevated and cholestasis may be present. GD diagnosis is confirmed by the detection of low glucocerebrosidase activity, usually less than 30% of the normal value in peripheral leukocytes. Genotyping can sometimes provide prognosis information [6].

Enzyme-replacement therapy (ERT), alglucerase then imiglucerase, available since 1991, is the reference treatment. Substrate-reduction therapy (miglustat) has been available since 2002, and is indicated for moderate GD when ERT is unsuitable. These treatments are extremely
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Written consent was obtained from each patient. The local Institutional Review Board of Northern Paris Hospitals, Paris-Diderot University, AP-HP (Ethics Committee) reviewed and approved the research project.

A standardized case-report form was used to collect the following information at each visit: initial data (age, sex, history related or unrelated to GD, initial symptoms and their year of onset, test confirming the diagnosis, phenotype, genotype, unknown genetic mutations); clinical information during the first consultation, at diagnosis and throughout follow-up; organomegaly (liver and/or spleen), usually measured using diagnostic ultrasonography (largest diameter); biological findings initially and throughout follow-up. Bone findings (X-rays, magnetic resonance imaging and, for some patients, scintigraphy and dual-energy X-ray absorptiometry) were recorded during follow-up, with identification of intercurrent events, particularly bone complications.

BE were defined as clinical events using the bone indications for treatment recommended by the French National Health Authority [27]: avascular necrosis of an epiphysis, bone infarct, pathological and/or vertebral compression fractures. Each BE had a clinical manifestation and radiological confirmation. Bone pain alone was not considered a BE without radiological confirmation.

Monitoring of GD-specific ERT and combined therapies (analgesics and bisphosphonates) were noted.

GD diagnosis was confirmed by low glucocerebrosidase activity in leukocytes [28] for all patients. Chitotriosidase activity in plasma samples was determined using the fluorescent substrate 4-methyl umbelliferyl β-d-N,N’,N”-(MU)-triacetylcysteine [9]; ACE, TRAP, ferritin and other measurements were made in the appropriate local laboratories. Because this study was retrospective, some data were missing, particularly at the beginning of follow-up (during the diagnosis phase). When missing, the baseline value at ERT onset was replaced with the last known value during the two previous years. When the chitotriosidase concentration was undetectable (patients with homozygous chitotriosidase deficiency), this biomarker was not retested [29].

Statistical analysis

All statistical analyses were performed with SAS software (version 9.1; SAS Institute Inc, Cary, North Carolina, USA). The significance level was set at $P < 0.05$.

First, we described BE frequency using Kaplan-Meier probability-of-BE curves to determine the time to the first BE for treated patients between diagnosis and their first treatment (before ERT), and between first ERT and the closing date (during ERT). Only the first BE occurring during each period was considered. Data were

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Materials and methods

 Patients and data collected

The Referral Center for Lysosomal Diseases (RCLD) is specialized in GD follow-up. A designated French national GD registry was developed and is maintained by the RCLD. Although patients are treated and followed in hospitals near their homes, they are registered with the RCLD, which is available to assist their physicians. However, a cohort of patients is followed and treated in the RCLD. All patients with known GD entered in the RCLD registry, followed on-site in the Department of Internal Medicine, Beaujon Hospital (Clichy, France), and receiving ERT were included. Clinical, biological and radiological data were recorded for all patients from diagnosis until 1 May 2007, the closing date. Data were collected retrospectively for two periods before and during ERT.

---

Expensive, ERT appears to be ineffective against the onset of neurological disorders in type 2 [4,7]. To our knowledge, no complete analysis of bone complications occurring under ERT [8]. However, no data are available on the main bone events (BE; avascular necrosis, bone infarcts, pathological fractures) occurring during ERT.

Several biomarkers (chitotriosidase, ferritin, angiotensin-converting enzyme (ACE) and tartrate-resistant acid phosphatase (TRAP)) are elevated during GD evolution [9-16]. Their concentrations rise with disease progression and generally decrease during ERT [17]. At present, it is not possible to make any formal recommendations concerning the use of any specific marker for patient monitoring [18]. Moreover, it is not known if biomarker levels at diagnosis can predict GD prognosis of treated and untreated patients, and which patients will respond, or not, to therapy [19]. Despite the lack of official guidelines, ACE, TRAP and chitotriosidase are used to monitor GD follow-up [20].

Several studies on chronic diseases used biomarker modeling to describe their evolution: human immunodeficiency virus infection [21], Parkinson disease [22] and diabetes mellitus [23]. Concerning GD patients, most published studies are descriptions of small cohorts (median number of patients 29; range, 18 to 48) [9,10,17,19,24,25] and only one study modeled hemoglobin and platelet levels and splenic volume under ERT [26].

Therefore, this study was undertaken to analyze BE frequencies occurring during the periods before and during ERT in our cohort of GD patients and to model the progression of their biological marker levels or slope changes.

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censored if no BE occurred before the patient started ERT for the first analysis and until the closing date for the second analysis. The LIFETEST procedure was used.

Second, we analyzed the changes of the five GD biomarkers (platelets, chitotriosidase, ferritin, ECA, TRAP) using linear-mixed models for repeated measures with the MIXED procedure. Because of their minimal variations during ERT, hemoglobin levels were not included in this model. The MIXED procedure is a generalization of a standard linear regression, which allows modeling of the parameter changes for each individual over time and takes into account the intrasubject association. Biomarker changes over time could have one of two shapes: either a linear increase or an exponential decrease. For the latter, logarithmic transformation was used in the model. Models of platelet changes used only the counts of nonsplenectomized patients. Two categories were created and analyzed: before and during ERT, regardless of the dose, with analysis of patients receiving full-dose ERT as a subcategory. Before-and-during ERT slopes were compared using the Wald test.

Third, we analyzed the effects of five covariates on BE: splenectomy, diagnosis year (before 1991 or after 1991, the year ERT became available), genotype (N370S/N370S or others), age at diagnosis (before 15 or after 15 years old) and sex. The impact of each covariate on the time to the first BE was tested using the log-rank test. A Cox model, used to estimate hazard ratios (HR) and 95% confidence intervals (CI), was applied to patients before and during ERT. Influence of age at treatment onset on BE occurrence under ERT was tested with a Cox model.

Fourth, the covariates were tested in mixed models. These analyses were only applied to the patients under ERT because of insufficient data on the patients before ERT. Backward selection of the covariates entered into the model was applied to examine associations between a biomarker and the different covariates. For the before-ERT and during-ERT analysis periods, individual baseline and slope values estimated with the linear-mixed models with no covariate for each patient and for each biomarker were extracted. These values were entered into a Cox model, to evaluate the relationship between BE and biomarker changes using the PHREG procedure, and are expressed as HR and 95% CI.

A biomarker effect on BE occurrence before ERT was not analyzed because early information on biomarkers was very sparse.

**Results**

**Cohort**

Seventy-three patients were followed, between 1933 and 1 May 2007, for a median duration of follow-up of 21 (range, 0 to 67) years after diagnosis. Only 62 patients received ERT with a median total duration of follow-up from diagnosis of 23.5 (range, 2 to 67) years and median duration of follow-up under treatment of 6 (range, 0 to 15) years. Only these 62 patients were included in the analysis.

The patients’ characteristics at diagnosis are reported in Table 1. This mostly female cohort had a median age of 14 years at diagnosis, but their first symptoms had started at the median age of eight years. Bone-marrow aspiration or biopsy led to GD diagnosis for 32 (51%) patients, and spleen histology was used for 13%. The diagnosis was confirmed for all patients by determining glucocerebrosidase activity. Only one patient died of GD-associated pulmonary hypertension during follow-up.

All but four patients had phenotype 1 GD. Twenty-eight patients had familial GD affecting siblings for 27 patients and an uncle for one patient. The genotype

| Table 1 Description at diagnosis of the 62 Gaucher-disease patients receiving enzyme-replacement therapy |
|--------------------------------------------------|-----------------|
| Baseline characteristic | Value |
| Sex, n (%) |  |
| Female | 36 (59) |
| Male | 26 (43) |
| Age, years, median (range) |  |
| First symptoms | 8 (0 to 37) |
| Diagnosis | 14 (1 to 48) |
| Patients diagnosed before 1991, n (%) | 47 (76%) |
| Patients <15 years old at diagnosis, n (%) | 34 (55%) |
| First symptoms to diagnosis interval, years, median (range) | 1 (0 to 36) |
| Test leading to diagnosis, n (%) |  |
| Enzyme assay | 7 (11) |
| Enzyme-gene sequencing | 1 (2) |
| Myelogram | 26 (40) |
| Bone-marrow biopsy | 4 (6) |
| Bone biopsy | 2 (3) |
| Hepatic biopsy | 2 (3) |
| Spleen histology | 8 (13) |
| Other | 1 (2) |
| Unknown | 11 (18) |
| Phenotype, n (%) |  |
| 1 | 58 (93) |
| 3 | 4 (7) |
| Genotype, n (%) |  |
| N370S/N370S | 9 (14) |
| N370S/L444P | 12 (19) |
| Other | 27 (44) |
| Unknown | 14 (23) |
| Familial disorder, n (%) |  |
| Yes | 28 (45) |
| No | 23 (37) |
| Unknown | 11 (18) |
PART II: EPIDEMIOLOGY AND MODELLING

was known for more than 90% of the patients, including nine N370S/N370S, 12 N370S/L444P and two L444P/L444P.

The first ERT prescribed was alglucerase for 18 (29%) patients and imiglucerase for 44 (71%); ERT was started at a median of 14 (range, 0 to 61) years after diagnosis; median age (range) at ERT onset was 31.6 (4.4 to 65.9) years. The respective median ages (range) at treatment onset for patients with and without BE were 29.5 (15.6 to 51.1) years and 32 (4.4 to 65.9) years. Cox analysis results showed no influence of age at treatment onset on BE occurrence during ERT (HR = 1.017 (95% CI: 0.975 to 1.061), P = 0.42). All patients taking alglucerase were switched to imiglucerase in November 1996. For alglucerase or imiglucerase, the initial dose was 120 U/kg/month (full dose) for 55 patients, with lower doses for the others: median 90 (range, 30 to 90) U/kg/month. Four patients switched from imiglucerase to miglulast. Twenty-eight of the 55 patients receiving full doses had their doses reduced after a median of 2.9 (range, 0.1 to 12.2) years of ERT.

Table 2 shows clinical, biological and bone data at different times: diagnosis, ERT onset and closing date. However, median times to ERT onset differed when GD had been diagnosed before 1991 or after 1991 (respectively 18 and 7 years; P < 0.05). Median ages at ERT onset and the closing date were 33.3 and 39.8 years, respectively.

During ERT, clinical abnormality rates decreased (except for neurological involvement) and the biological data improved overall during ERT (increased hemoglobin, leukocyte and platelet levels; decreased chitotriosidase, ACE, TRAP, ferritin and gammaglobulin levels). The number of patients with splenomegaly and/or hepatomegaly tended to decline during ERT, and most bone lesions other than BE tended to regress (Table 2).

Overall, 21 (34%) patients were splenectomized: 5 before diagnosis, 16 between diagnosis and ERT onset; none were splenectomized after starting ERT. The median age at splenectomy was 18.3 (range, 1.6 to 49.6) years.

BE characteristics
Kaplan-Meier curves of the time to the first BE in the 62 treated patients, between diagnosis and ERT onset (30 years of follow-up), and between the latter and the closing date (15 years of follow-up) are shown in Figure 1a and 1b, respectively. Before diagnosis, eight patients had already suffered at least one BE. After diagnosis, but before starting ERT, 21 patients had had at least one BE, for a total of 54 BE and a median of two (range, 1 to 8) BE per patient. Ten patients had at least one BE during ERT for a total of 12 BE. The 54 BE before ERT onset were (n (%)): 28 (52%) avascular necroses (with 12 prosthetic replacements), 7 (13%) bone infarcts (with only symptomatic therapy), 12 (22%) pathologic fractures (5 requiring surgical intervention) and 7 (13%) vertebral compression fractures (with symptomatic therapy). Moreover, 23 complaints of bone pain were not corroborated by imaging (hence not included in BE). The 12 BE that occurred under ERT were (n (%)): three (25%) avascular necroses (none with prosthetic replacement), four (33%) bone infarcts with clinical bone crises (with only symptomatic therapy) and five (42%) pathologic fractures (none requiring surgery). Twenty-one complaints of simple bone pain without imaging confirmation during ERT were not included in BE. For nine of the 12 BE, patients received full-dose ERT (120 U/kg/month). Only one patient experienced BE during the first year of ERT (pathological fracture), and 5 of the 10 patients experienced BE between Years 1 and 5 of ERT (two pathological fractures, one avascular necrosis and two bone infarcts).

We determined the probability of a BE occurring by 10 years (95% CI) before and during ERT: 22.4% (13.3% to 36.3%) and 20.0% (10.2% to 36.9%), respectively. Respective mean times (95% CI) to the first BE were 27.6 (21.5 to 33.7) and 12.0 (10.7 to 13.3) years. For four of the 21 (19.0%) patients with at least one BE before ERT, the BE occurred before and during ERT, whereas six of the remaining 41 (14.6%) patients developed BE only during ERT, but had never done so before.

Biomarker evolution before and during ERT
Results of analyses of biomarker changes using linear-mixed models are reported in Table 3. Platelet counts in nonsplenectomized patients were stable before ERT (+190 platelets/µL/year), while chitotriosidase and TRAP decreased slightly, and ferritin and ACE increased slightly. During ERT, platelet counts increased (+7,035 platelets/µL/year), while all other biomarkers declined. Slopes before and during ERT differed significantly (P = 0.0001) only for platelets and ferritin. For patients given full-dose ERT, platelet counts increased slightly faster, ferritin and TRAP decreased faster, but chitotriosidase and ACE declined more slowly. When only patients with full-dose ERT were analyzed, their biomarker-slope variations were comparable to those of the other patients.

Impact of covariates and biomarkers on developing BE
Covariate (splenectomy, date of diagnosis, genotype, age at diagnosis and sex) impact on BE before and during ERT was examined. Before ERT, significantly more BE occurred in patients diagnosed with GD after 15 years of age (HR, 2.6 (95% CI 1.0 to 6.7); P = 0.048), but no
Table 2 Clinical, biological and imaging characteristics of Gaucher disease precisely known at each time

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>At diagnosis</th>
<th>No.</th>
<th>At ERT onset</th>
<th>No.</th>
<th>At closing date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years since diagnosis, median (range)</td>
<td>0</td>
<td></td>
<td>14  (0 to 61)</td>
<td></td>
<td>23.5 (2 to 67)</td>
<td></td>
</tr>
<tr>
<td>Clinical involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigmentation</td>
<td>35</td>
<td>6%</td>
<td>35</td>
<td>20%</td>
<td>29</td>
<td>3%</td>
</tr>
<tr>
<td>Asthenia</td>
<td>45</td>
<td>42%</td>
<td>52</td>
<td>60%</td>
<td>53</td>
<td>26%</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>45</td>
<td>29%</td>
<td>50</td>
<td>34%</td>
<td>54</td>
<td>6%</td>
</tr>
<tr>
<td>Chronic bone pain</td>
<td>1</td>
<td>0%</td>
<td>52</td>
<td>53%</td>
<td>55</td>
<td>45%</td>
</tr>
<tr>
<td>Bone crisis</td>
<td>38</td>
<td>24%</td>
<td>45</td>
<td>49%</td>
<td>51</td>
<td>12%</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>44</td>
<td>52%</td>
<td>53</td>
<td>43%</td>
<td>53</td>
<td>9%</td>
</tr>
<tr>
<td>Lung</td>
<td>41</td>
<td>2%</td>
<td>49</td>
<td>0%</td>
<td>52</td>
<td>6%</td>
</tr>
<tr>
<td>Neurological</td>
<td>40</td>
<td>5%</td>
<td>45</td>
<td>7%</td>
<td>41</td>
<td>15%</td>
</tr>
<tr>
<td>Other</td>
<td>28</td>
<td>4%</td>
<td>32</td>
<td>6%</td>
<td>19</td>
<td>0%</td>
</tr>
<tr>
<td>Splenectomy, n</td>
<td>62</td>
<td>5</td>
<td>52</td>
<td>21</td>
<td>52</td>
<td>21</td>
</tr>
<tr>
<td>Organomegaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>48</td>
<td>85%</td>
<td>47</td>
<td>89%</td>
<td>39</td>
<td>54%</td>
</tr>
<tr>
<td>Liver US (median, range), cm</td>
<td>23</td>
<td>165 (13 to 25)</td>
<td>28</td>
<td>19 (13 to 80)</td>
<td>9</td>
<td>14 (11.8 to 19)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>40</td>
<td>100%</td>
<td>41</td>
<td>95%</td>
<td>30</td>
<td>67%</td>
</tr>
<tr>
<td>Splenic US (median, range), cm</td>
<td>34</td>
<td>18.75 (9.5 to 30)</td>
<td>26</td>
<td>19.4 (6.5 to 31.5)</td>
<td>11</td>
<td>15.2 (9 to 22)</td>
</tr>
<tr>
<td>Biological parameter, median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15</td>
<td>11.4 (7.9 to 14.1)</td>
<td>55</td>
<td>12 (8.3 to 15.1)</td>
<td>58</td>
<td>13.8 (7.3 to 16.2)</td>
</tr>
<tr>
<td>Leukocyte (×10³/μL)</td>
<td>15</td>
<td>4,200 (2,070 to 12,400)</td>
<td>54</td>
<td>4,200 (1,180 to 21,600)</td>
<td>57</td>
<td>6,130 (830 to 11,500)</td>
</tr>
<tr>
<td>Platelet count (×10³/μL)</td>
<td>57</td>
<td>88 (6 to 360)</td>
<td>53</td>
<td>87 (30 to 449)</td>
<td>58</td>
<td>163.5 (37 to 473)</td>
</tr>
<tr>
<td>Chitotriosidase (nmol/mg/min)*</td>
<td>28</td>
<td>9.01 (7.0 to 77.5)</td>
<td>27</td>
<td>9,700 (180 to 77,500)</td>
<td>53</td>
<td>112.3 (8 to 148.9)</td>
</tr>
<tr>
<td>TRAP (U/L)</td>
<td>23</td>
<td>11 (1 to 7)</td>
<td>15</td>
<td>9.6 (1 to 24.5)</td>
<td>36</td>
<td>3.75 (2 to 48)</td>
</tr>
<tr>
<td>ACE (U/L)</td>
<td>28</td>
<td>259.5 (1 to 650)</td>
<td>21</td>
<td>220 (1 to 650)</td>
<td>46</td>
<td>51 (69 to 240)</td>
</tr>
<tr>
<td>Ferritin (ng/L)</td>
<td>38</td>
<td>682.5 (68 to 3,230)</td>
<td>28</td>
<td>721.5 (120 to 3,230)</td>
<td>47</td>
<td>167 (15 to 1,731)</td>
</tr>
<tr>
<td>Gammaglobulin (g/L)</td>
<td>2</td>
<td>17.6 (16.5 to 19)</td>
<td>31</td>
<td>15.8 (7.2 to 25)</td>
<td>47</td>
<td>12.7 (6.5 to 23.6)</td>
</tr>
<tr>
<td>Imaging of bone disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erlenmeyer flask</td>
<td>23</td>
<td>52%</td>
<td>22</td>
<td>64%</td>
<td>14</td>
<td>36%</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>23</td>
<td>57%</td>
<td>18</td>
<td>56%</td>
<td>17</td>
<td>47%</td>
</tr>
<tr>
<td>Cortical</td>
<td>19</td>
<td>32%</td>
<td>13</td>
<td>23%</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Lytic lesion</td>
<td>19</td>
<td>26%</td>
<td>14</td>
<td>21%</td>
<td>15</td>
<td>33%</td>
</tr>
<tr>
<td>Avascular necrosis sequelus</td>
<td>27</td>
<td>37%</td>
<td>17</td>
<td>29%</td>
<td>13</td>
<td>23%</td>
</tr>
<tr>
<td>Infarct sequelae</td>
<td>22</td>
<td>32%</td>
<td>15</td>
<td>40%</td>
<td>12</td>
<td>25%</td>
</tr>
<tr>
<td>Fracture sequelae</td>
<td>18</td>
<td>17%</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>27%</td>
</tr>
<tr>
<td>Infiltration on MRI</td>
<td>25</td>
<td>80%</td>
<td>33</td>
<td>91%</td>
<td>31</td>
<td>81%</td>
</tr>
<tr>
<td><strong>99m</strong>Tc-Hyperfixation</td>
<td>25</td>
<td>84%</td>
<td>29</td>
<td>90%</td>
<td>25</td>
<td>88%</td>
</tr>
<tr>
<td><strong>99m</strong>Tc-Hypostixation</td>
<td>4</td>
<td>50%</td>
<td>5</td>
<td>20%</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Bone densitometry, median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-score neck</td>
<td>3</td>
<td>-2.1 (-2.2 to -1.1)</td>
<td>10</td>
<td>-1 (-2.2 to 1.4)</td>
<td>22</td>
<td>0 (-2.8 to 4.5)</td>
</tr>
<tr>
<td>T-score lumbar</td>
<td>13</td>
<td>0 (-3.1 to 1)</td>
<td>10</td>
<td>-1.9 (-4 to 0.8)</td>
<td>22</td>
<td>-0.9 (-3.6 to 1.6)</td>
</tr>
<tr>
<td>Z-score neck</td>
<td>7</td>
<td>-8 (-10.0 to -8)</td>
<td>7</td>
<td>-0.7 (-2.5 to 1.9)</td>
<td>15</td>
<td>-0.5 (-6.2 to 4.4)</td>
</tr>
<tr>
<td>Z-score lumbar</td>
<td>2</td>
<td>-2.4 (-3.1 to -1.8)</td>
<td>8</td>
<td>-1.3 (-5.1 to 0.5)</td>
<td>15</td>
<td>-0.5 (-5.1 to -2.1)</td>
</tr>
</tbody>
</table>

No. is the number of patients with available information. ACE, angiotensin-converting enzyme; ERT, enzyme-replacement therapy; MRI, magnetic resonance imaging; TRAP, tartrate-resistant acid phosphatase; US, ultrasonography. *In addition, four patients had undetectable chitotriosidase activity (null allele) and are not included for statistical analysis of chitotriosidase.

significant differences were found for the other covariates, and none had an effect during ERT.

Estimated individual slopes of the biomarkers had no significant influence on developing BE before or during ERT but, at diagnosis, ferritin concentration (HR, 1.18 (95% CI, 1.03 to 1.35); \( P = 0.019 \)) and platelet count \( (HR, 0.69 (95\% CI, 0.49 to 0.98); \( P = 0.039 \)) increased the risk of BE during ERT in this univariate model. Risk of BE increased with high ferritin levels and low platelet levels.

For patients under ERT, a multivariate regression model including age at diagnosis and ferritin and
platelet levels found only the baseline platelet count ($P = 0.032$) to have a significant impact.

**Influence of covariates on biomarker changes during ERT**

Table 4 reports the effects of covariates on biomarker values during ERT, including the coefficients of variation of interindividual variability for baseline levels and slopes for each biomarker. These coefficients were particularly high for the platelet count, ferritin and ACE slopes. However, only the ferritin and platelet slopes differed significantly between before and during ERT (Table 3).
Table 3 Changes of the slopes* of the Gaucher-disease biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>No./no.</th>
<th>Before ERT</th>
<th>No./no.</th>
<th>During ERT</th>
<th>No./no.</th>
<th>Full-dose ERT</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (no spleenectomy)*</td>
<td>9/70</td>
<td>190/mm³/y</td>
<td>38/480</td>
<td>7.035/mm³/y</td>
<td>33/220</td>
<td>10.231/mm³/y</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chitotriosidase</td>
<td>10/23</td>
<td>-1.1%/y</td>
<td>52/226</td>
<td>-1.7%/y</td>
<td>37/134</td>
<td>-1.4%/y</td>
<td>0.14</td>
</tr>
<tr>
<td>TRAP</td>
<td>7/14</td>
<td>-0.7%/y</td>
<td>36/214</td>
<td>-4%/y</td>
<td>24/109</td>
<td>-6.4%/y</td>
<td>0.78</td>
</tr>
<tr>
<td>ACE</td>
<td>9/33</td>
<td>0.1%/y</td>
<td>46/263</td>
<td>-5%/y</td>
<td>31/114</td>
<td>-4%/y</td>
<td>0.88</td>
</tr>
<tr>
<td>Ferritin</td>
<td>9/45</td>
<td>4%/y</td>
<td>47/338</td>
<td>-1.4%/y</td>
<td>37/166</td>
<td>-16%/y</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Slopes were estimated by linear-mixed models before enzyme-replacement therapy (ERT), during ERT and for the patient subgroup receiving full-dose ERT.

Figure 2 illustrates the influence of covariates on biomarker changes under ERT. Figure 2a shows the influence of splenectomy on the platelet change. Values at diagnosis and progression slopes differed between splenectomized and nonsplenectomized groups. Splenectomized patients’ platelet counts rose slightly under ERT, with no significant slope change, while nonsplenectomized patients’ counts increased significantly from baseline under ERT but returned to normal after 6.5 years of treatment. Two covariates affected the chitotriosidase decrease under ERT: splenectomy influenced the slope decline and genotype influenced the baseline level (Figure 2b). Patients with the genotype N370S/N370S had significantly higher baseline levels, which decreased more steeply than those of patients with another genotype. This impact could reflect the fact that, patients with that genotype, which corresponds to one of the less severe forms of GD, started ERT later (median, 23 years) than the other patients (median, 16 years) (nonsignificant, NS). Chitotriosidase levels decreased, without reaching a normal level over a median of six (range, 0 to 15) years of follow-up.

The ferritin model did not identify any covariate as significantly influencing this marker’s progression (Figure 2c). The mean prediction curve for ferritin levels decreased and became the normal after three years.

Table 4 Baseline levels and slope changes* of the biomarkers during enzyme-replacement therapy: impact of covariates

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Covariate(s)</th>
<th>N</th>
<th>Baseline</th>
<th>P</th>
<th>CV</th>
<th>Slope of % decrease</th>
<th>P</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>No spleenectomy</td>
<td>38</td>
<td>103,850 (mm³)</td>
<td>55.5%</td>
<td>8.085 (mm³/yr)</td>
<td>80.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleenectomy</td>
<td>20</td>
<td>303,380 (mm³)</td>
<td>-8.82 (mm³/yr)</td>
<td>0.0007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect of spleenectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitotriosidase</td>
<td>No spleenectomy/N370S</td>
<td>6</td>
<td>14,721 nmol/mL/h</td>
<td>9.8%</td>
<td>-3.8% (yr)</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No spleenectomy-other genotype</td>
<td>22</td>
<td>3,581 nmol/mL/h</td>
<td>-1.4% (yr)</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleenectomy-N370S</td>
<td>2</td>
<td>2,823 nmol/mL/h</td>
<td>-2.7% (yr)</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleenectomy-other genotype</td>
<td>11</td>
<td>2,015 nmol/mL/h</td>
<td>-2% (yr)</td>
<td>0.06</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Effect of spleenectomy</td>
<td></td>
<td></td>
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<td></td>
<td>Effect of genotype</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td></td>
<td>47</td>
<td>382 ng/L</td>
<td>15.4%</td>
<td>-14% (yr)</td>
<td>83.6%</td>
<td></td>
<td></td>
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<tr>
<td>ACE</td>
<td>Diagnosis before 1991</td>
<td>35</td>
<td>90.3 IU/L</td>
<td>25.5%</td>
<td>-7% (yr)</td>
<td>19.0%</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Diagnosis in 1991 and after</td>
<td>11</td>
<td>31.7 IU/L</td>
<td>4% (yr)</td>
<td>0.02</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Effect of diagnosis data</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>TRAP*</td>
<td>No spleenectomy-diagnosis &gt;15 yr</td>
<td>10</td>
<td>4.2 (IU/L)</td>
<td>3.6%</td>
<td>-4% (yr)</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No spleenectomy-diagnosis 15 yr</td>
<td>13</td>
<td>6.6 (IU/L)</td>
<td>-9% (yr)</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleenectomy-diagnosis &gt;15 yr</td>
<td>5</td>
<td>3.5 (IU/L)</td>
<td>-3% (yr)</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleenectomy-diagnosis &lt;15 yr</td>
<td>8</td>
<td>5.4 (IU/L)</td>
<td>-2% (yr)</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect of spleenectomy</td>
<td></td>
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<td></td>
<td>Effect of age at diagnosis</td>
<td></td>
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</table>

*Estimated with linear-mixed models under treatment. *Coefficient of variation of interpatient variability. When a log-linear model was used, the evolution is expressed as the percent change of the slope (all markers except platelets). ND, not determined.
Figure 2 Biomarker changes under enzyme-replacement therapy (ERT): impact of covariates. (a) platelet level, (b) chitotriosidase, (c) fentanyl, (d) angiotensin-converting enzyme (ACE), and (e) trypsin-resistant alkaline phosphatase (TRAP). Different covariates were tested to identify those impacting on biomarker evolution (baseline or variation slope); splenectomy, diagnosis year (before 1991 or after 1991), the year ERT became available, genotype (N370X/N370X or others), age at diagnosis (before 15 or after 15 years old), and sex. Solid grey horizontal lines correspond to the normal biomarker value (platelet level >150,000/μL; chitotriosidase <100 nmoL/mL; fentanyl <250 ng/L; ACE <45 IU/L; TRAP <7 U/L). When a covariate had a significant impact on the baseline value or the variation slope, the different curves are shown.

Date of diagnosis significantly affected baseline ACE levels (Figure 2d). Patients diagnosed before 1991 had much higher ACE levels (90.3 versus 31.7 U/L), but their progression slopes did not differ significantly.

Splenectomy and age at diagnosis affected TRAP levels, with the former influencing the decrease rate (slope) and the latter the baseline concentration (Figure 2e). TRAP levels in splenectomized patients decreased more slowly and had started higher, but the difference was not significant. Patients diagnosed before the age of 15 years had the highest baseline TRAP levels but their diagnosis-to-ERT interval was 17 years, as opposed to...
11.5 years for those diagnosed after 15 years (NS). Although that difference was not significant, more substrate could have accumulated in the patients who were diagnosed before the age of 15, because their time preceding ERT onset had been much longer.

Splenectomy eliminates platelet anomalies and displaces lysosomal overload. Nonsplenectomized patients seemed to have higher, but not significantly different, lysosomal biomarker levels at diagnosis compared to splenectomized patients, respectively: chitotriosidase 19,219 versus 2,534 nmol/mL/h (NS); TRAP 12.1 versus 8.0 (NS). In contrast, macrophage biomarker levels were higher in splenectomized than nonsplenectomized patients, respectively: ferritin 1,301 versus 634 ng/L ($P = 0.06$); ACE $251$ versus $202$ IU/L (NS). Generally, for splenectomized patients, when the starting value was lower, the slope was less steep.

Some covariates were significantly associated: patients diagnosed before 1991 were more often male ($P = 0.04$) and had more frequently been splenectomized ($P = 0.03$).

**Discussion**

Probability curves of time to BE (Figure 1) before and during ERT for GD patients showed that bone complications could occur without but also under ERT (about 20% at 10 years). According to the literature, bone crises almost disappeared after two years of ERT [8,30], but the risk of avascular necrosis was estimated to be 13.8/1,000 person-years under ERT in a recent publication [31]. Our findings demonstrated that BE can arise even after many years of ERT, specifically avascular necrosis (three patients) and bone infarcts (four patients). However, no patient had prothrombin replacement after ERT compared to 12 before. BE after ERT seemed to be less serious, as no surgery was required. In addition, simple bone pain occurring under ERT without being confirmed by imaging (21 events) was not considered a BE. Hence, ERT does not eliminate all bone symptoms. Nine BE were documented under full-dose ERT (120 U/kg/month of alglucerase or imiglucerase). We tried to determine the impact of diagnosis year (before or after 1991) on BE occurrence and biomarker evolution: no effect was found on BE occurrence ($P = 0.11$ and 0.42, respectively, for before and after ERT). Although this covariate significantly affected only baseline ACE levels (see Figure 2d), it had no influence on the other biomarkers, for example, platelets and ferritin. No impact of the date of diagnosis on baseline ferritin value or platelet count was found, as shown in Figure 2a, c and in Table 4.

We hypothesize that bone infiltration accumulating over the years before starting ERT could explain bone complications despite treatment. Unfortunately, our analysis does not allow us to confirm or refute that postulate. In a recent article, the risk of avascular necrosis while on ERT seemed lower for patients who had begun treatment within two years of diagnosis compared to those who started it after more than and equal to two years [31]. Moreover, patients were certainly more closely monitored during than before ERT and it is possible that some BE before ERT might not have been diagnosed. A largest study with systematic bone-density data will be considered to compare the bone densitometries of patients with pathological fractures versus those of patients without such fractures during evolution.

Because the platelet level did not rise in splenectomized patients on ERT, it can be concluded that hypoplasia is the main cause of thrombocytopenia. However, 14 (61%) out of 21 splenectomized patients, had at least one platelet count <150,000/mm$^3$ after splenectomy. Therefore, bone-marrow insufficiency seems to explain part of the thrombocytopenia observed over time.

The effect of individual estimated-biomarker values at diagnosis or their slope on BE occurrence under ERT was significant for baseline platelet and ferritin levels in our univariate model, with high ferritin and low platelets at ERT onset being significantly associated with BE during ERT, but only the baseline platelet count was retained in a multivariate model. Nor did the estimated individual slopes of the biomarkers have a significant impact on BE. However, our modeling method seemed to be able to identify predictive biomarkers. Effects of other biomarkers was not significant in our analyses but platelet and ferritin data were available for more patients at diagnosis (57 and 38, respectively) compared to the others (28, 29 and 28, for chitotriosidase, TRAP and ACE, respectively), which could have decreased the power of statistical analysis and partially explain these observations. According to the literature, no biomarkers were able to predict BE occurrence and, other than platelets, ferritin seemed to be the only biomarker affecting BE [19]. The only study using a mixed model for GD [26] found dose-response relationships for ERT, but had not considered biomarkers (chitotriosidase, TRAP, ferritin, ACE), taking into account only the main hematological (hemoglobin and platelets) and visceral manifestations; no prediction of BE occurrence was proposed.

Interpatient biomarker changes and therapeutic responses varied widely (Table 3). TRAP baseline was higher for the group diagnosed before 15 years than those diagnosed later, which seems to support that the TRAP level could differ as a function of age [32]. The respective median ages for the groups of patients diagnosed before and after 15 years were 21 and 40 years, which partially explains these findings. The ACE level was affected by the year of diagnosis, with patients
diagnosed after 1991 having lower baseline levels than those diagnosed before 1991. ERT availability since 1991 could have limited the ACE rise. For some biomarkers, not all patients had the same evolution under ERT; for example, platelet counts rose in 97% of treated patients, while chitosanidase decreased in 100% and ferritin in 96%, but ACE levels in only 78%.

Conclusions

BE can occur in GD even after many years of ERT. Initial ferritin and platelet levels seemed to be able to predict BE occurrence during ERT. To achieve our final objective, to predict BE based on initial biomarker values and their evolution, large cohort studies are needed.

Abbreviations

ACE: angiotensin converting enzyme; BE: bone events; CI: confidence interval; ERT: enzyme replacement therapy; GD: Gaucher disease; HR: hazard ratio; NS: nonsignificant; RCT: Randomized Controlled Trials; TRAP: tetratone-resistant acid phosphatase.

Acknowledgements

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Authors’ contributions

FM, JS, CV, NB, BF and OF designed the research protocol. JS, NB, BF and OF were involved in treating patients and collecting data. JS, OF and FM controlled the accuracy of collected data and conducted the statistical analyses. JS, FM and CV wrote the draft of the paper, which was then corrected and approved by all authors.

Competing interests

Paris-Diderot University received a grant from Genzyme France. JS and NB were reimbursed for congress expenses by Genzyme. Beaujon Hospital received a grant from Shire.

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A description of repeated bone events: the frailty model (122).

The analytical methods used to describe bone events are derived from survival data and only consider the occurrence of a single event. In this paper, we represented repeated BEs in GD, using a frailty model (a repeated time-to-event parametric model).

To validate the use of MONOLIX© (MOdèles NOn LInéaires à effet miXtes, a powerful piece of free software especially for use in nonlinear mixed models, http://www.lixoft.eu) for our future analyses, we carried out a simulation study to evaluate the Stochastic Approximation Expectation Maximization (SAEM) algorithm. SAEM was implemented within MONOLIX for the analysis of repeated events within a framework of parametric frailty models with normal distributions.

We analysed 233 patients of the registry: 28% had already had a BE under treatment and 26% had had a splenectomy. Next, we tested the impact of a BE before treatment (p-value = 0.01) and splenectomy before treatment (p-value = 0.02) on the occurrence of a BE under treatment. The covariable of the impact of a BE before treatment was tested because it was the only significant covariable revealed by the multivariate analysis in the study on the occurrence of a first BE while under treatment.
Research Article

Evaluation of Estimation Methods and Power of Tests of Discrete Covariates in Repeated Time-to-Event Parametric Models: Application to Gaucher Patients Treated by Imiglucerase

Marie Vigan, 1,2,5 Jérôme Stirnemann, 3,4 and France Mentré 4,2

Received 27 November 2013; accepted 21 January 2014; published online 26 February 2014

Abstract. Analysis of repeated time-to-event data is increasingly performed in pharmacoepidemiology using parametric frailty models. The aims of this simulation study were (1) to assess estimation performance of Stochastic Approximation Expectation Maximization (SAEM) algorithm in MONOLIX, Adaptive Gaussian Quadrature (AGQ), and Laplace algorithm in PROC NLMIXED of SAS and (2) to evaluate properties of test of a dichotomous covariate on occurrence of events. The simulation setting is inspired from an analysis of occurrence of bone events after the initiation of treatment by imiglucerase in patients with Gaucher Disease (GD). We simulated repeated events with an exponential model and various drop-out rates: low, medium, and high. Several values of baseline hazard model, variability, number of subject, and effect of covariate were studied. For each scenario, 100 datasets were simulated for estimation performance and 500 for test performance. We evaluated estimation performance through relative bias and relative root mean square error (RAME). We studied properties of Wald and likelihood ratio test (LRT). We used these methods to analyze occurrence of bone events in patients with GD after starting an enzyme replacement therapy. SAEM with three chains and AGQ algorithms provided good estimates of parameters much better than SAEM with one chain and Laplace which often provided poor estimates. Despite a small number of repeated events, SAEM with three chains and AGQ gave small biases and RAME. Type I errors were closed to 5%, and power varied as expected for SAEM with three chains and AGQ. Probability of having at least one event under treatment was 19.1%.

KEY WORDS: AGQ; Gaucher patients; imiglucerase; repeated time-to-event; SAEM.

INTRODUCTION

During evaluation of treatments, one clinical outcome can be the repeated occurrence of the same event. Traditional statistical analyses do not handle these repeated time-to-event (RTTE) data, and often only analysis of the first event is performed in various medical settings.

Nonlinear mixed effect models are the main statistical tool in pharmacoepidemiology (1). RTTE models were first introduced in pharmacoepidemiology by Cox et al. (2) using frailty models. Frailty models (3–6) handle heterogeneity at the individual level, with both fixed and random effect terms (7) and were recently described by Goveindaraju et al. in a tutorial (8). Frailty model are one possible extension to the Cox model (9). In the semi-parametric time-to-event frailty model, the baseline hazard function is unspecified, and the proportionality of the covariate effects on the hazard is assumed. In parametric frailty models, the baseline hazard function is defined as a parametric baseline hazard function. The advantage of a full parametric model is that the clinical trial simulation can be performed. Various methods of estimation for parametric frailty models exist: the likelihood estimation using the Newton-Raphson algorithm, the expectation-maximization (EM) algorithm (10), the penalized likelihood method (11–13), or Bayesian method (14). Liu et al. (15) proposed a novel adaptive Gaussian quadrature (AGQ) which is implemented in PROC NLMIXED of SAS. Some R packages can also fit frailty model such as Survival, FrailtyPack, or Pur, but the main disadvantage is their inability to provide standard errors for the estimate of the random effect variance. Algorithms implemented in R are penalized partial likelihood, maximum likelihood, EM with Markov Chain Monte Carlo (MCMC) algorithms, while in SAS are AGQ or EM algorithms.

Some of the software tools used for nonlinear mixed effect models in pharmacoepidemiology can also be used for parametric frailty models, such as MONOLIX or NONMEM (16). MONOLIX was the first proposed software using Stochastic Approximation Expectation Maximization (SAEM) (17). It has been evaluated by simulation for discrete data (18) and count data (19), but not yet for RTTE.
data. NONMEM implements various algorithms, as the Laplace method and, more recently, SAEM, which can be used for RTTE data. In a previously published simulation study, Karlsson et al. (20) compared EM with importance sampling (IS), Laplace, and SAEM in NONMEM v7. They used an exponential baseline hazard model and a follow-up of 12 days for all patients so did not have any dropout. They found that EM with IS and SAEM are better than Laplace when there are few events; otherwise, they perform equally well. In that simulation, they evaluated parameter estimation but did not evaluate power of the tests to detect impact of discrete covariate in RTTE. In another work, Hirsch and Wienke (21) compared different softwares for gamma and log-normal frailty models and described advantages and limits of each. The different algorithms compared are penalized partial likelihood, EM, and MCMC in R or SAS softwares. In the present study, we evaluated two different software tools and three algorithms MONOLIX with the algorithm SAEM, PROC NLMIXED with the AGO, and Laplace algorithms.

Gaucher disease (GD) is a rare (1/100,000 births), autosomal recessive disease characterized by an enzymatic deficit of glucocerebrosidase (22). Clinical endpoints of this disease are bone events (BEs: avascular necrosis of an epiphysis, bone infarct, pathological and/or vertebral compression fracture), splenectomy, and neurological symptoms. The current treatment of Gaucher patients is an enzymatic replacement therapy (ERT) by imiglucerase. Several biomarkers (chitotriosidase, ferritin, α-glucosidase, and tartrate-resistant acid phosphatase) are elevated during GD evolution. Their concentrations rise with disease progression and decrease during ERT (23). One main clinical endpoint for evaluation of treatment should be the reduction of repeated occurrence of BE. This needs long-term follow-up data after treatment initiation and a large number of patients. We created the French GD Registry (FGDR) in 2009; it is a complete registry of the disease with long-term follow-up. We have already studied the occurrence of the first BE after ERT initiation and the effect of several covariates. Splenectomy and having a BE before ERT were the two significant covariates found with the traditional log-rank test (24). Here we are interested in the effect of treatment on the occurrence of repeated BEs.

The first objective of the present work was to assess by simulation the estimation performance of the SAEM algorithm in MONOLIX and AGO and Laplace algorithms in PROC NLMIXED of SAS by simulation with a variation in dropout rate, the number of subjects, and the variability. We also evaluated the power to detect tests of the impact of a binary covariate on the occurrence of events. The second objective was to apply those algorithms to evaluate the occurrence of repeated BE after treatment initiation by imiglucerase in patients from the FGDR.

MODELS AND NOTATIONS

For the ith (i = 1, ..., N) patient, let \( T_i \) be the time of the \( j \)th (j = 1, ..., \( n_i - 1 \)) event. \( T_{i0} \) is the right censoring time, \( T_{ij} < T_{ij} \) (j = 1, ..., \( n_i - 1 \)), and we defined the censoring indicators as \( \delta_j = 1_{T_{ij} \leq T_{i0}} \). The hazard function can be expressed with the following frailty model:

\[
\lambda_i(t) = \lambda_0(t) \exp(b_i + \beta Z_i)
\]

where \( \lambda_0(t) \) is the baseline hazard function, \( Z_i \) the covariate vectors associated with the vector of regression \( \beta \), and \( b_i \) is the individual random effect, which is assumed to follow a normal distribution with mean 0 and variance equal to \( \sigma^2 \). Under the parametric approach, the baseline hazard is defined as a parametric function. A common model considered in the literature is the Weibull distribution, \( \lambda_0(t) = \alpha t^{\alpha - 1} \), which reduces to an exponential distribution when \( \alpha = 1 \).

The vector of parameter estimated \( \hat{\theta} \) is composed of the fixed effects and the variance of the random effects. The log-likelihood for this model is

\[
L(\theta) = \sum_{i=1}^n \log \left( \prod_{j=1}^{n_i} \left[ \lambda_0(t) \right] \exp \left[ -\int_0^{T_j} \lambda_0(t) dt \right] \right) p(b_i) db_i
\]

where \( p(b_i) \) is the probability density function of \( b_i \), a normal distribution with mean 0 and variance \( \sigma^2 \). Because this expression has no closed form, several specific algorithms as described in the Introduction were developed to perform maximum likelihood estimation. To test the impact of covariate, we can use the Wald test, defined by

\[
W = \hat{\beta}^T \text{Var}^{-1} \hat{\beta}
\]

where \( \hat{\beta} \) is the estimator of the vector of parameter \( \beta \), and \( \text{Var}^{-1} \) is the estimation variance matrix for the vector of parameter \( \beta \). The statistic \( W \) is compared with the critical value of a \( \chi^2 \) with \( p \) degree of freedom where \( p \) is the dimension of the vector of parameter \( \beta \). We can also use the Likelihood Ratio Test (LRT). We estimated the log-likelihood in the model without covariate (i.e., \( \beta = 0 \), \( b_i \) independent and with covariate (i.e., \( \beta \) estimated), \( L_{\text{cov}} \). The test statistic

\[
A = -2(L_{\text{nocov}} - L_{\text{cov}})
\]

is compared with the critical value of a \( \chi^2 \) with \( p \) degree of freedom.

SIMULATION STUDY

Evaluation of Estimation Performance

The standard simulation scenario was composed of \( N = 200 \) patients, an exponential baseline hazard function \( \lambda = 2 \times 10^{-3} \) month\(^{-1} \), a standard deviation (sd) of random effect \( \sigma = 1 \) and no covariate. We chose an exponential distribution (10,20,25), a rather simple model, as it is the first evaluation of MONOLIX on RTTE. This choice was based on the real data where events correspond to BEs, with a risk that does not increase over time of study. Furthermore, in these data, Weibull distribution was not significantly better than exponential distribution (e.g., see Results on real data).

The study design observed a maximum follow-up time of 144 months. We defined three levels of dropout, with no dropout
corresponding to the end of follow-up. The time of censoring was simulated from an exponential model of parameter \( \gamma \), with \( \gamma = 5 \times 10^{-3} \) month\(^{-1} \), for low dropout, and \( \gamma = 1 \times 10^{-2} \) month\(^{-1} \), for high dropout.

We varied the number of patients, \( N = 100, 200, \) or \( 400 \); the value of the hazard function, \( \lambda = 2 \times 10^{-3} \) or \( 4 \times 10^{-3} \); and the sd of the random effect, \( \omega = 0.5, 1, \) or \( 2 \). The variation of \( \lambda \) and \( \omega \) was only performed for the high dropout condition. \( K = 100 \) datasets were simulated in each case. \( \lambda \) and \( \omega \) were estimated by maximum likelihood in each dataset for each algorithm (SAEM, AGQ, and Laplace) for each scenario.

To assess the statistical properties of an estimator, we computed the relative estimation error on each dataset \( k \), \( \text{REE}_k \), as follows:

\[
\text{REE}_k = \frac{\theta_{\text{est}} - \theta_{\text{true}}}{\theta_{\text{true}}} \times 100
\]

where \( \theta_{\text{est}} \) is the estimated parameter value in the \( k \)-th \((k = 1, \ldots, K)\) dataset, and \( \theta_{\text{true}} \) is the true parameter value used in the simulation. Each \( \text{REE}_k \) was expressed in percent. We plotted the box plot of the \( \text{REE}_k \) with the 10% and 90% percentiles. Then, we computed the relative bias (RB) and the relative root mean square error (RRMSE) values from the \( \text{REE}_k \) as follows:

\[
\text{RB} = \sum_{k=1}^{K} \text{REE}_k
\]

\[
\text{RRMSE} = \left( \frac{1}{K} \sum_{k=1}^{K} \text{REE}_k^2 \right)^{\frac{1}{2}}.
\]

Evaluation of Test Performance

We evaluated the power of the test to detect the impact of a dichotomous covariate on the occurrence of events. The standard scenario was defined as before, \( N = 200 \) patients, an exponential baseline hazard function \( \lambda = 2 \times 10^{-3} \) month\(^{-1} \), and a sd of random effect \( \omega = 1 \). We defined a dichotomous covariate, \( Z = 0 \) in group A and \( Z = 1 \) in group B, with 100 patients in each group. We varied the value of the effect of the covariate, \( \exp(\beta) = 1 \) (no effect), 1.5, 2, or 3 and the variability of the random effect \( \omega = 1 \) or 2. \( K = 500 \) datasets were simulated in each scenario. We performed two tests: Wald test and LRT. For the Wald test, in the model with covariates, we estimated \( \beta \) and its standard error (se). For the LRT, we estimated the log-likelihood in the model with covariate and in the model without covariate (i.e., \( \beta = 0 \)).
PART II: EPIDEMIOLOGY AND MODELLING

Table I. Percent of Patients with No, One, or More than One Event for the Three Levels of Dropout in the Simulation Study for the Standard Scenario ($\lambda=2 \times 10^{-3}$ and $\omega=1$)

<table>
<thead>
<tr>
<th>% of patients</th>
<th>0 event</th>
<th>1 event</th>
<th>More than one event</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>70</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Low</td>
<td>77</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>High</td>
<td>82</td>
<td>13</td>
<td>5</td>
</tr>
</tbody>
</table>

computed the number of significant datasets ($p$ value < 0.05).
For each estimation method (SAEM and AGQ), each scenario and each test, we estimated the type I error for the simulations under $H_0$, i.e., $\exp(\beta)=1$, and the power for the simulations under $H_1$, i.e., $\exp(\beta)=1.5, 2, or 3$.

To evaluate the gain in studying all the events, we also tested the impact of the covariate on the occurrence of the first event in one hand by a log-rank test and in the other hand by a Wald test and LRT using a standard exponential model.

Software and Algorithms

We estimated the parameter vector $\theta$ with MONOLIX and PROC NLMIXED of SAS. The algorithms used are SAEM with one and three chains in MONOLIX, AGQ, and Laplace in PROC NLMIXED. The SAEM algorithm (17,26) belongs to the standard EM algorithm which is characterized by exactly likelihood maximization. In the E step, the expectation of the log-likelihood is calculated, and in the M step, new parameters maximizing the likelihood are computed given the likelihood expected in E step. The process is iterative until a stable parameter value is obtained. In SAEM, the E step is divided into (1) a simulation of individual parameters using a MCMC algorithm, (2) followed by a stochastic approximation of the expected likelihood. In the general case, AGQ is a numerical approximation to the integral over the whole support of the likelihood using $\rho$ quadrature points. Laplace (27,28) technique is the simplest AGQ procedure based on the evaluation of the function in one well-chosen quadrature point per random effect. The SE is estimated by a stochastic approach in MONOLIX and is computed from the final Hessian matrix in PROC NLMIXED. Log-likelihood is estimated using importance sampling integration method in MONOLIX and AGQ in PROC NLMIXED. Optimization technique use in PROC NLMIXED is Newton-Raphson algorithm. The analysis of the first event by an exponential model was performed with PROC LIFEREG of SAS and the log-rank test by the PROC LIFETEST of SAS. The estimations were performed using MONOLIX v.4.0, and SAS v.9.3 (SAS Institute, Cary, NC). The specifications of starting values for parameters are the true value used for simulation. Estimation algorithms were utilized with the default settings in the two software tools. Changes from these defaults are listed below. In MONOLIX, standard errors were calculated by stochastic approximation; to estimate the population parameters, we did not use initial simulated annealing; the number of chains was specified as one or three chains, respectively. In SAS, the numbers of quadrature points were specified as 1 or 5 for Laplace and AGQ algorithm, respectively.

The datasets were simulated with R v.2.13 (29).

Results

Figure 1 shows a spaghetti plot of one simulated dataset for the standard scenario with no, low, or high dropout. The number of events per patient is small, even the case for no dropout. Figure 1 shows that the probability of having at least one event

Fig. 2. Box plot of the relative errors (in percent) for three levels of dropout with $\lambda=2 \times 10^{-3}$ (for $\lambda$ (left) and $\omega$ (right)) and $\omega=1$ and for various values of the number of patients (N: 100, 200, and 400) for SAEM with one Markov chain (black), SAEM with three Markov chains (white), AGQ (light gray), and Laplace (dark gray)
Methods and Tests of Covariates in RITE Models

at 10 years is 27.5% for no and low dropout and 28.6% for high dropout. For low or high dropout, few patients have repeated events (Table I). Twenty-one percent of patients have no event for no dropout and around 15% for low and high dropout. Only 9% of patients have more than one event for no dropout, 7% for low dropout, and 5% for high dropout.

For the standard scenario, box plots of REE are plotted for λ and ω in Fig. 2, while RB and RRMSE are given in Table II for the three levels of dropout and for different values of the parameter number of subjects. REE was greater for Laplace, mostly negative for λ and positive for ω, RB and RRMSE were greater for Laplace algorithm, with parameters poorly estimated and a systematic bias, even with 400 patients. SAEM with one Markov chain gave also large REE, especially for ω. RB and RRMSE were greater than SAEM with three Markov chains. Because SAEM with one chain and Laplace algorithm had poor estimation properties in the following, we considered only SAEM with three chains and AGO algorithms with five quadrature points.

Results are very close between SAEM with three chains and AGO, and both algorithms provide good estimates of the parameters λ and ω. For SAEM with three chains and AGO, RB on λ is low (−2% to 2%), and RB on ω is slightly negative (−2% to −2%); they decrease when N increases for SAEM and AGO. RRMSE are reasonable and decrease as N increases (<30% with 200 patients and <22% with 400) for SAEM with three chains and AGO. When N = 100 patients, bias appears in the estimates of λ and ω (Fig. 2). Table III presents REE, RB, and RRMSE for the scenario for high dropout and different values of λ and ω. SAEM with three chains and AGO provide good estimates of the parameters. When λ increases, RRMSE decrease, and when ω increases, its RRMSE decreases. When ω is small, there is bias in the estimates of both parameters, especially ω.

We then evaluated the properties of the test for the SAEM with three chains and AGO algorithm. Figure 3 shows a spaghetti plot for one dataset of the standard scenario, exp(β) = 1 for group A and exp(β) = 2 for group B with high dropout. As expected, there are more events in group B than in group A. In group B, 18% have at least one event and 13% more than one event, compared with 13% and 5% in group A, respectively. Figure 3 shows that the probability of having at least one event at 10 years is higher in group B, 51.6%, than in group A, 44.9.

Figure 4 shows the type 1 error and the power for the standard scenario and different values of ω and β. The tests have adequate properties for both SAEM with three chains and AGO; the Wald test and the LRT perform equally well. Type 1 errors are close to 5%. Powers vary as expected—increasing when β increases and decreasing when ω increases. Figure 5 shows the power of detection of the impact of a covariate using only the first event. Analysis of all the events is more powerful than the log-rank test on the first event.

APPLICATION TO TREATMENT OF GAUCHER PATIENTS

Material and Methods

We used frailty model to analyze data from the FGDR (24). More precisely, we studied the occurrence of repeated
Table III. Relative Bias (RB) and Relative Mean Square Error (RMSE) (in Percent) for High Dropout and N=200, for Various Values of λ (2×10⁻³ and 4×10⁻³) and α (0.5, 1, and 2) for SAEM with Three Chains and AGQ Algorithms

<table>
<thead>
<tr>
<th>Parameter</th>
<th>λ</th>
<th>ω</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAEM 3 chains</td>
<td>AGQ</td>
<td>SAEM 3 chains</td>
<td>AGQ</td>
<td>SAEM 3 chains</td>
</tr>
<tr>
<td>λ</td>
<td>2×10⁻³</td>
<td>RB</td>
<td>9.8</td>
<td>-0.9</td>
<td>-1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RMSE</td>
<td>19.9</td>
<td>19.0</td>
<td>27.5</td>
</tr>
<tr>
<td></td>
<td>4×10⁻³</td>
<td>RB</td>
<td>-5.2</td>
<td>-0.3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RMSE</td>
<td>16.0</td>
<td>17.0</td>
<td>19.9</td>
</tr>
<tr>
<td>ω</td>
<td>2×10⁻³</td>
<td>RB</td>
<td>-51.3</td>
<td>-33.8</td>
<td>-9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RMSE</td>
<td>69.6</td>
<td>74.8</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td>4×10⁻³</td>
<td>RB</td>
<td>-32.7</td>
<td>-15.0</td>
<td>-4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RMSE</td>
<td>62.0</td>
<td>56.2</td>
<td>22.3</td>
</tr>
</tbody>
</table>

BEs in Gaucher patients, since the initiation of treatment (time=0) by imiglucerase monotherapy. Data were censored at the end of therapy, if it was interrupted for more than 6 months or at the closing date. Several patients presenting repeated BEs, a frailty model with an exponential, or a Weibull distribution were tested.

We estimated the parameters λ, α, and ω. We compared the two distributions, exponential and Weibull, with the Wald test and the LRT; i.e., α=1 or not. Then, we evaluated, separately, the impact of two covariates on the occurrence of the BEs—the presence of at least one BE before the initiation of treatment and splenectomy as found in the

Fig. 3. Top: Spaghetti plot of the number of events versus time for high dropout for one simulated dataset of the standard scenario for groups A and B. Circle corresponds to event and plus sign to censor; data of a patient are connected by step functions. Bottom: Kaplan-Meier estimate of the cumulative distribution function for the first event for high dropout for one simulated dataset of the standard scenario. The dashed lines correspond to 95% pointwise confidence intervals.
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Analysis of the first event (24). We next used a multivariate model with backward selection. We estimated \( \exp(\beta) \), its standard error, and the \( p \) value by the Wald test and the LRT for each covariate. Considering the results of the simulation study, data were analyzed using SAEM with three chains and AGQ algorithm.

Results

Among the 185 GD patients treated with imiglucerase, the median (range) follow-up was 6 years (0–13). Figure 6 shows the spaghetti plot of BEs of the treated patients in FGD as a function of time. Twenty-six patients had BEs with a total number of 36 BEs and a maximum of 4 BEs in one patient. Fifty percent of the patients were females. The median age at first symptoms was 18 years (0–61), and the median age at diagnosis was 23 years (0–67). The median age at initiation of treatment was 37 years (1–76). Figure 6 shows that the probability of having at least one event at 10 years is 19.1%.

Table IV shows the values of the estimated parameters for SAEM with three chains and AGQ algorithm and for exponential and Weibull distribution. The results were close for the two algorithms, \( \lambda = 1.4 \times 10^{-5} \) (se=0.5 \( \times 10^{-5} \)) month\(^{-1} \) and \( \alpha = 1.1 \) (se=1.1). For both estimation methods, LRT showed that Weibull distribution is not significantly different than exponential distribution. We obtained the same conclusion with the Wald test, and parameter \( \alpha \) was not significantly different from 1.

Figure 6 shows the Kaplan–Meier estimate of the cumulative distribution function for the first event and the estimate predicted by exponential and Weibull distribution with SAEM algorithm with three chains. Model prediction by SAEM with three chains is close to the Kaplan–Meier curve, in the 95% confidence intervals.

The two covariates tested were the presence of BEs before the initiation of treatment and a splenectomy before treatment. As the exponential distribution is adequately fitted the data, the covariates are tested on this model. Among the 185 patients, 48 had BEs before treatment, and 44 had splenectomy before treatment. The two covariates were significant in the univariate model with the LRT. The result obtained, with SAEM with three chains, for BEs before treatment is \( \exp(\beta) = 3 \) (se=1.2), \( p = 0.01 \), and that for splenectomy is \( \exp(\beta) = 1.9 \) (se=0.6), \( p = 0.02 \). In the multivariate analysis, the BE occurrence before treatment was the only risk factor, and final estimates are given in Table IV. The risk of events was increased three-fold during treatment in patients who already had an event before treatment.

DISCUSSION

We can conclude from this simulation study that SAEM algorithm with three Markov chains (in MONOLIX) and AGQ algorithm with five quadrature points (in PROC NL MIXED of SAS) give rather similar results in the different

![SAEM and AGQ graphs](image)

Fig. 4. Type I error and power of the Wald test (plus sign) and LRT (asterisk) for the three levels of dropout (the dotted lines indicate no dropout, the dashed lines low dropout, and the solid lines high dropout) for \( \omega = 1 \) (top) and \( \omega = 2 \) (bottom) and for SAEM with three Markov chains (left) and AGQ (right). The hatched region represent the 95% prediction interval for the 5% type I error.
scenarios evaluated. The Laplace method gives worse results and, in some scenarios, had very poor estimation properties similar to the results obtained by Karlsson et al. (20) in NONMEM. Similarly, SAEM with one Markov chain gave poor results. Therefore, we did not use the SAEM with one chain nor Laplace method for the evaluation test performance and the analysis of real data. This is the first simulation study with MONOLIX (SAEM) for parameters estimation in RTTE models. The performance estimations are good with unbiased estimates, despite the small number of repeated events in the simulation. Some problems occurred with SAEM and AGQ algorithm when there is a small number of subjects or a small variability.

When the number of subjects increases, the precision of estimation increases. The increased proportion of censored patients is the driving force of events. When higher dropout rate is simulated, fewer events are observed. As expected, RRMSE increases when dropout increases. Datasets with N=400 patients and high dropout rate, or with N=200 patients and no dropout, lead to the same event rate, 21% of patients with at least one event. These two cases gave similar estimation performance (Table II).

The Wald test and the LRT have adequate properties and give similar results for the test of a binary covariate. Notably, the type I error of 5% is correct. We also performed an additional simulation to evaluate the power of Wald test for dataset with 400 patients and high dropout rate with SAEM with three Markov chains. We found a power of 31.4% very close to the power of 31.2% (Fig. 4) found with 200 patients and no dropout. Those results clearly illustrate that high dropout leads also to loss of power and decreased estimation performance as expected. For the assessment of the first event with a parametric model, the results are close to those for all the events due to the small number of patients with more than one event. The power with the good parametric model for the first event is greater than the power of the log-rank test for the first event.

Fig. 5. Type I error for the first event and power of the Wald test (plus sign), LRT (asterisk) and log-rank (circle) for the three levels of dropout (the dotted lines indicate no dropout, the dashed lines low dropout, and the solid lines high dropout) for α=1 (top) and α=2 (bottom). The hatched region represents the 95% prediction interval for the 5% type I error.

Fig. 6. Top: Spaghetti plot of the number of bone events as a function of time since the initiation of treatment for treated patients in French Gaucher Disease Registry. Circle corresponds to event and plus sign to censored; data of a patient are connected by step functions. Bottom: Kaplan–Meier estimate (black) of the cumulative distribution function for the first event for treated patients in French Gaucher Disease Registry and model prediction of cumulative distribution function by SAEM with three Markov chains with exponential distribution (gray solid lines) and Weibull distribution (gray dashed lines). The black dashed lines correspond to 95% pointwise confidence intervals for the Kaplan–Meier estimate.
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Table IV. Estimation of the Model Parameters for Bone Events of Treated Patients in French Gaucher Disease Registry for Model Without and with the Covariate, Bone Events Before Treatment and for Two Types of Distribution, and Weibull and Exponential for SAEM with Three Chains and AGQ Algorithms

<table>
<thead>
<tr>
<th>Model</th>
<th>Covariate</th>
<th>Parameter</th>
<th>SAEM 3 chains</th>
<th>AGQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weibull No</td>
<td>λ month⁻¹</td>
<td>4.9 × 10⁻⁵</td>
<td>5.1 × 10⁻⁴</td>
<td>2.3 × 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>(sc)</td>
<td>4.8 (10⁻⁴)</td>
<td>4.8 (10⁻⁴)</td>
<td>1.3 (10⁻⁴)</td>
</tr>
<tr>
<td></td>
<td>(α)</td>
<td>1.2 (0.2)</td>
<td>1.3 (0.3)</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td></td>
<td>(ω)</td>
<td>0.9 (0.3)</td>
<td>0.3 (0.2)</td>
<td>0.3 (0.2)</td>
</tr>
<tr>
<td></td>
<td>−2 log L</td>
<td>540.9</td>
<td>509.9</td>
<td>509.9</td>
</tr>
<tr>
<td>Exponential No</td>
<td>λ month⁻¹</td>
<td>1.5 × 10⁻³</td>
<td>1.2 × 10⁻³</td>
<td>2.5 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>(sc)</td>
<td>6.0 (10⁻⁷)</td>
<td>5.0 (10⁻⁷)</td>
<td>2.5 (10⁻⁷)</td>
</tr>
<tr>
<td></td>
<td>(α)</td>
<td>1.2 (0.2)</td>
<td>1.2 (0.2)</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td></td>
<td>−2 log L</td>
<td>542.8</td>
<td>512.6</td>
<td>512.6</td>
</tr>
<tr>
<td>Exponential Yes</td>
<td>λ month⁻¹</td>
<td>1.1 × 10⁻³</td>
<td>1.0 × 10⁻³</td>
<td>1.0 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>(sc)</td>
<td>0.1 (10⁻⁷)</td>
<td>0.4 (10⁻⁷)</td>
<td>0.4 (10⁻⁷)</td>
</tr>
<tr>
<td></td>
<td>(β)</td>
<td>1.1 (0.4)</td>
<td>1.1 (0.4)</td>
<td>1.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>(ω)</td>
<td>0.9 (0.3)</td>
<td>1.0 (0.3)</td>
<td>1.0 (0.3)</td>
</tr>
<tr>
<td></td>
<td>−2 log L</td>
<td>536.1</td>
<td>486.4</td>
<td>486.4</td>
</tr>
</tbody>
</table>

The runtime with this model was fast with both software programs, MONOLIX and SAS. Laplace algorithm (i.e., AGQ with one quadratic point) or AGQ with three quadratic points did not give adequate results, and five points were needed. Also, SAEM with three Markov chains was needed.

In the analysis of BEs in Gaucher patients after the treatment initiation by irigzareza in the FGDR, the two methods SAEM and AGQ gave similar results. Our results confirmed that a BE before ERT increases the risk of a BE during ERT (24). For the next step, we should analyze the complete follow-up of patients, taking into account the initiation of ERT as a time-dependent covariate to test the effect of ERT on the change in BEs occurrence.

We performed the simulation with the simplest exponential distribution, but further analysis should be performed with a more complex one, like the Weibull distribution with more parameters or, also, other parametrisations. On the real data, Weibull distribution was not better than exponential distribution, and using the other usual parametrisation gave similar results (not shown). Further evaluations of models with covariates are also needed. First, the evaluation of a binary covariate that changes over time should be performed. Then, we should also study the impact of a continuous covariate changing or not over time (30).

This study shows that SAEM with three Markov chains in MONOLIX and AGQ in PROC NLMIXED are good estimation methods for analysis of RTTE with parametric frailty models and give similar results. However, SAEM with one Markov chain in MONOLIX and Laplace method is often biased and should not be used.

REFERENCES

Discussion: preliminary data on the contribution of joint models

We propose, in this preliminary non-published work, to develop a better joint model, capable of analysing the impact of longitudinal biomarker data on repeated BEs occurring over time.

Firstly, the BEs that occurred during treatment were analysed using a parametric frailty model which allowed an examination of repeated occurrences (survival analysis is only capable of analysing initial BEs). Next, the biomarkers, notably chitotriosidase, were analysed using a nonlinear mixed-effects model (an exponential model). Finally, the link between chitotriosidase and the occurrence of BEs was evaluated (joint models). The parameters were estimated with the help of the SAEM algorithm running on MONOLIX software (lixoft).

An analysis was made of the FGDR data for the 241 patients treated using ERT with alglucerase or imiglucerase. Among them, 46 patients had at least one BE during treatment, with a total of 65 BEs. Data on chitotriosidase concentrations were available for 169 patients, with a median of 4.5 observations [1–22] per patient. The median duration of monitoring during treatment was 7.2 years [0.1–21.6]. Chitotriosidase was modelled under treatment and several covariables were tested: the occurrence of a BE before treatment was associated with twice the concentration of chitotriosidase at treatment initiation (Figure 5). The occurrence of BEs was modelled using a frailty model that was able to integrate the repeated BEs endured by certain patients: the occurrence of a BE before treatment increased the risk of another occurrence during treatment (Figure 6). The change in chitotriosidase concentration during treatment showed an equilibrium rate of 10% of the initial concentration, with a normalisation half-life of 0.6 years. The link between the concentration of chitotriosidase and the occurrence of BEs was evaluated, as were the effects of several covariables (Table 4).

The occurrence of BEs during treatment remain difficult to predict, but joint models may help to address this issue by generalising, for example, the analysis of several biomarkers at once, and these types of analyses will help physicians fine-tune treatment doses for individual patients.
Figure 5: Graph A represents chitotriosidase concentration rates of FGDR patients undergoing treatment, either with (blue) or without (red) the occurrence of other bone events before enzyme replacement therapy (ERT). Graph B represents modelled chitotriosidase concentrations, with and without the occurrence of a BE before treatment. The occurrence of a BE before treatment was associated with twice the concentration of chitotriosidase at treatment initiation. Concentration chitotriosidase is in nmol/h.L; time in years.
Figure 6: Graph A represents occurrences of repeated bone events during treatment, analysed using a frailty model, either with (blue) or without (red) BEs before ERT. Graph B represents the hazard function of bone events either with or without a BE before treatment. The occurrence of a BE before treatment is associated with a tripling of the risk of experiencing a BE during treatment. Tim is in year.
<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Estimates</th>
<th>RSE (%)</th>
<th>P-value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0 \text{ (nmol/h.L)}$</td>
<td>$6.5 \times 10^3$</td>
<td>16</td>
<td></td>
<td>Original chitotriosidase concentration</td>
</tr>
<tr>
<td>$\exp(\gamma_{\text{evtavt}})$</td>
<td>1.8</td>
<td>43</td>
<td>0.02</td>
<td>Chitotriosidase concentration for patients with a prior BE: 2 x greater</td>
</tr>
<tr>
<td>$r$</td>
<td>0.09</td>
<td>22</td>
<td></td>
<td>Amplitude: equilibrium value (nmol/h.L): $0.6 \times 10^3$</td>
</tr>
<tr>
<td>$\kappa \text{ (année$^{-1}$)}$</td>
<td>1.3</td>
<td>31</td>
<td></td>
<td>Annual normalisation rate for chitotriosidase; Half-life response: 0.5 years</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>0.05</td>
<td>32</td>
<td></td>
<td>Instantaneous risk of a BE</td>
</tr>
<tr>
<td>$\exp(\gamma_{\text{evtavt}})$</td>
<td>3.2</td>
<td>26</td>
<td>0.0001</td>
<td>Risk of a BE for patients who had one before treatment (3 x greater)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.1</td>
<td>82</td>
<td></td>
<td>Link between BE and log of chitotriosidase concentration; Increase of 5 x chitotriosidase rate increased risk 1.2 times</td>
</tr>
</tbody>
</table>

**Table 4:** Description of the parameters of the joint model, chitotriosidase and bone events, including the occurrence of a BE before treatment in the model. A BE before treatment is associated with double the concentration of chitotriosidase at the start of treatment and a tripling of the risk of a BE during treatment. A five-fold increase in the concentration of chitotriosidase multiplied the risk of a BE by 1.2.
Conclusions/Perspectives

The establishment of the French Gaucher Disease Registry has enabled an accurate description of the disease’s incidence in France and the first analysis of the entire cohort of patients suffering from it.

After having described the epidemiology of GD in France (109), we demonstrated the value of modelling the disease’s biomarkers in patients undergoing treatment in a first article reproduced above (107).

To date, the biomarkers analysed for monitoring GD do not enable physicians to predict BEs (3) – major complications that they seek to prevent with appropriate treatment. GD does have a number of biomarkers (chitotriosidase, ferritin, angiotensin-converting enzyme/ACE, tartrate-resistant acid phosphatase/TRAP) which seem to reflect both the disease’s activity and tissue infiltration. As such, these biomarkers should be used as prognostic indicators, but this is not currently possible. In an effort to better predict BEs, we decided to model GD’s biomarkers and study their associations with those bone events.

The linear or the nonlinear mixed-effects models are the most appropriate methods with which to study the evolution of a biomarker over time. These models are usually used in pharmacokinetics to model the concentrations of drugs in each patient when numerous parameters must be taken into account. Mixed-effect models allow a biomarker’s starting value and the variation in its slope to be determined for each patient. This can be done even when there are missing data points, which makes the approach particularly appropriate for retrospective studies where missing homogeneous data is common. Nonlinear models are also widely used for modelling decreasing viral loads, or in cancerology, agronomy, genetics and neuroscience. A nonlinear model is one in which the function that links the model’s structural parameters to the process is nonlinear with respect to the parameters. The mixed-effects model is an extension of this, which takes into account the variability associated with individuals. This approach allows us to estimate the characteristics of a population, i.e. the mean and the variability between subjects (inter-subject variability), and the variability of the parameters affecting a subject over time (intra-subject variability),
even in cases where the individual data is scattered. The parameters of this model are split into fixed and random components. The fixed-effect component is identical for all individuals and represents a population effect. The random component is specific to each individual in the population and represents the variability associated with each subject.

In another article (121), we developed a pathophysiological model of the changing levels of biomarkers during treatment. This article, not presented in this thesis, aimed to develop a model of GD’s biomarkers based on its pathophysiology, describing the changes occurring in its biomarkers in response to treatment and analysing the influence of multiple covariables on those responses. Using a simplified exponential pathophysiological model (a nonlinear mixed-effects model/NLMEM), we analysed changes in blood ferritin, chitotriosidase, haemoglobin and platelets in FGDR patients receiving imiglucerase/alglucerase treatment (233 patients). We identified three significant parameters for our model: the initial concentration, the amplitude variation and the rate of normalisation of the biomarkers. We then modelled the four biomarkers together and tested the impact of multiple covariables. Combined modelling allowed us to build a profile of the changing levels of biomarkers in patients for whom there were few biomarker data and to test associations between their changing levels. Using the Bayesian information criterion (BIC), we also compared our exponential model against the Emax model used by Grabowski et al. (44). A comparison between the exponential model and the Emax model gave a gain in BIC for ferritin, chitotriosidase and platelets exponential models, and a loss for haemoglobin. A few covariables had an effect on the changing levels of biomarkers: age, sex and splenectomy. We found a model that was able to analyse the four biomarkers simultaneously, with the same normalisation speed with treatment.

The analysis of survival data is used to study the time elapsed between an initial time point and the occurrence of the event of interest. The Cox model is a semi-parametric model of proportional hazards. It is the model of reference for any analysis of survival data in the presence of a single event. The analysis of recurring events is generating ever more interest and has been the subject of numerous publications in recent years. One of the main reasons for this is that recurring events are found in so many domains, such as cancer relapses or repeated hospitalisations. The analytical methods used for these types of events are derived from those used for survival data. The Cox model only considers the occurrence of a single
PART II: EPIDEMIOLOGY AND MODELLING

Event. Extensions to the Cox model were developed to allow an analysis of recurrent events. Vaupel et al. (119) introduced a random effect to the risk function in Cox’s model so as to consider the heterogeneity of the data due to unobserved factors of influence and the dependence between the times of different events. This random effect, also called frailty, acts multiplicatively on the risk function. Thus, any individual who has experienced a succession of events will be considered frailer. Another notion is that of the dependence between events. This implies that the occurrence of past events has an influence on the occurrence of future events and is represented by frailty model. We represented repeated BEs in GD, using a frailty model (122).

It is still not currently possible to predict the occurrence of a bone event in GD. The only data available are concentrations of BEs at different time points in the disease’s course. The existence of a link between changing levels of biomarkers and the occurrence of events would, however, enable their prediction and could eventually lead to treatment adjustments. In order to test that link, a joint model was used to analyse the association between the concentrations of ferritin, chitotriosidase, the haemoglobin level, the number of platelets and the repeated occurrence of BEs. This was the sub-model of longitudinal markers (121) and the model of repeated occurrences (122) we published.

In our discussion, we described the final objective of this series of studies – testing the impact of modelled biomarkers on the occurrence of bone events in GD in joint models. Once all the analyses are complete, GD biomarkers will be able to be used more effectively for estimating the risk of a bone event and identifying which patients require more intensive treatment.

Survival models are inappropriate for time-dependent covariables, such as GD biomarkers. Indeed, the biomarkers’ principle characteristic is that they are internal covariables: the occurrence of an event has an impact on the value of the biomarker. Further, the biomarkers are measured with a margin of error, whether those errors are due to the measurement instrument or to the different biological variations caused by the subject himself. Finally, biomarker samples are only collected on patients’ visits to their physicians, which means that their levels are not known continuously but only at discrete time points. To overcome these problems, it becomes necessary to build joint models of the longitudinal biomarker.
data and those at the time of the event. Joint models have previously been developed in order to mitigate the different problems caused by separately modelling a biomarker longitudinally and the occurrence of an event (37), a situation where the persistence of bias was often demonstrated. Numerous authors have focused on joint models, including Tsiatis and Davidian (113), who gave an overview of them. They showed that a simultaneous estimation of the parameters of a mixed linear model of the changes in a longitudinal variable and of a survival model of the waiting time until the occurrence of an event, produced non-biased estimates. These models offer an interesting framework for: 1. evaluating the risk of an event occurring and its association with covariables including the biomarker’s longitudinal data, but without the bias caused by missing data on the times at which events occurred and without measurement errors (89); 2. directly exploring the association between longitudinal processes and the times of events (90); and 3. predicting the risk of occurrence of a particular event as a function of the changing levels of a biomarker (93). A joint model is made up of two linked sub-models, one modelling the longitudinal process and the other the time it takes for an event to occur, together with the assumptions and additional specifications that allow us to build a comprehensive representation of the joint distribution of the observed data.

Based on the models developed to date, we now envisage an analysis of the risk of GD complications without treatment in order to determine which patients should receive a precise indication of treatment (those with a high risk of complications) and which should simply be monitored closely. Given the annual price of treatment for a GD patient and the constraints imposed by lifelong treatment, this would prove to be a significant step forward, both economic and therapeutic.

Another extension of this work will be to apply its model to the framework of analysis of a clinical study whose final participants have just been enrolled. A flow cytometry technique has been developed (12) that enables the dosage of residual intra-monocyte enzyme (glucocerebrosidase). In a preliminary study of eight patients treated using imiglucerase, intra-monocyte enzyme activity was measured before each perfusion for a total of six months. The persistence of enzymatic activity 14 days after the perfusion was examined and seemed to be correlated with the seriousness of the disease. The current prospective clinical research protocol (Prof. Marc Berger, Biological Haematology Unit, Estaing–
Clermont-Ferrand university hospital) includes 42 patients. Its main objective is to observe the intra-monocytic kinetics of imiglucerase as a function of the dose and regularity of the perfusions given to treated patients. Using the model developed, it will also examine the correlation between intra-monocytic activity in imiglucerase and the biomarkers and the clinical and biological changes in patients.

We have applied new statistical methods to an analysis of the data in the French Gaucher Disease Registry – namely frailty, nonlinear mixed-effects and joint models. Future research will nevertheless be necessary in order to improve these techniques and expand their use in clinical practice. Modelling the changing levels of biomarkers during treatment is a fine illustration of the usefulness of nonlinear mixed-effects models and how they improve our understanding of pathophysiological mechanisms. Future studies are planned to continue this line of research, with the goal of better understanding changing levels of biomarkers and their links with the occurrence of bone events. Nonlinear mixed-effects models and joint models are particularly appropriate tools for the analysis of longitudinal data and the repeated events seen in Gaucher disease; they can be (and are) applied to numerous pathologies and clinical problems.
List of abbreviations

ACE: Angiotensin-Converting Enzyme
AVN: Avascular Necrosis
BE: Bone events
CEGT: Committee for the Evaluation of Gaucher Disease Treatments
CNR-MR: French National Registry Committee – Rare Diseases
EMA: European Medicines Agency
ERT: Enzyme Replacement Therapy
FGDR: French Gaucher Disease Registry
GCase: glucocerebrosidase
Gcer: glucosylceramide
GD: Gaucher Disease
InVs: French Institute for Public Health Surveillance
INSERM: French National Institute of Health and Medical Research
NCIL: National Commission on Informatics and Liberty
NLMDEM: nonlinear mixed-effects model.
PNDS: Diagnosis and Treatment National Guidelines (Protocole National de Diagnostic et de soins)
RCLD: Reference Center for Lysosomal Diseases
SRT: Substrate Reduction Therapy
TRAP: Tartrate-Resistant Acid Phosphatase
Reference


