Gaucher disease in children

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GAUCHER DISEASE IN CHILDREN

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Abbreviations

ACE- Angiotensin converting enzyme

ALT- Alanine Aminotransferase

AST- Aspartate Aminotransferase

CBC- Complete blood count

ERT- Enzyme replacement therapy

GD- Gaucher's disease

GERD- Gastroesophageal reflux disease

ICGG- International Collaborative Gaucher Group Registry

LSD- Lysosomal storage disease

MRI- Magnetic resonance imaging

NMDA- N-Methyl-D-aspartic acid

PCT- Pharmacological chaperone therapy

PT-Prothrombin time

PTT- Partial Prothrombin time

RBC- Red blood cells

RER- Rough endoplasmic reticulum

SRT- Substrate reducing therapy

TRAP- Tartrate-resistant acid

WBC- White blood cells

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1. Abstract

Gaucher disease (GD) is the most common lysosomal storage disease worldwide. The incidence of the disease is significantly higher in certain ethnic groups, such as in Ashkenazi Jews. Mutation in the *GBA1* gene results in a decreased catabolic enzymatic activity of the enzyme glucocerebrosidase which leads to the accumulation of glucocerebroside in various organs and their dysfunction. Other genetic mutations at different loci seem to modify the severity of the disease by unclear mechanisms. GD is differentiated into three types. Currently, the common understanding of the disease suggests a spectrum of presentations, ranging from asymptomatic to extremely severe disease rather than three separate entities. Three types of classification of disease are in use, indicating severity and organ system affected in patients.Type 1, known as non-neuropathic, mostly affects the bones, spleen, liver and bone marrow. Type 2 and 3, known as the neuropathic forms of the disease, differing in severity, age of diagnosis, rate of progression and life expectancy. GD2 usually manifests during infancy and has an accelerated progression the results in mortality during 2nd to 4th years of life. Type 3 varies more in presentation and severity. The most common clinical findings in children with GD 1 are hepatosplenomegaly, anemia, and thrombocytopenia. Clinical findings that increase the suspicion for GD require a definitive diagnosis using glucocerebrosidase assay. Before initiation of treatment, a complete baseline assessment is performed to assess severity and to set goals for treatment. The specific treatment for GD in children is enzyme replacement therapy (ERT). This therapy was shown to lower morbidity and complication related to the disease. None of the neuropathic symptoms presenting in GD2 and GD3 seemed to benefit from ERT. These patients will benefit from ERT only in cases of visceral and bone involvement. The follow up of pediatric patients with GD is a lifelong journey that will require regular checks which are essential to assess the efficacy of the treatment and the need for modifications.

2. Sažetak

Gaucherova bolest (GB) je najčešća lizosomska bolest nakupljanja u svijetu. Učestalost ove bolesti je znatno veća u nekim etničkim skupinama kao što su Židovi Aškenazi. Mutacija u genu *GBA1* dovodi do smanjene kataboličke enzimske aktivnosti enzima glukocerebrozidaze koja dovodi do nakupljanja glukocerebrozida u različitim organima i rezultira njihovom disfunkcijom. Mutacije na drugim genskim lokusima, čini se, mijenjaju težinu bolesti putem nejasnih mehanizama. GB se obično dijeli na tri podvrste, ali trenutno razumijevanje bolesti je da se bolest ne sastoji od tri zasebna entiteta, već zapravo ima spektar prezentacija, od asimptomatskih do teških oblika bolesti. Tri podvrste bolesti se još uvijek često koriste kako bi se označilo koji su organski sustavi pogođeni u određenom pacijentu i koliko teško. Tip 1, poznat kao ne-neuropatski, najčešće utječe na kosti, jetru, slezenu i koštanu srž. Tip 2 i 3 poznatiji su kao neuropatski oblici bolesti i međusobno se razlikuju po težini, dobi dijagnoze i očekivanoj duljini životnog vijeka. GB tip 2 se obično dijagnosticira tijekom prve dvije godine života, a smrt se događa od druge do četvrte godine života. Tip 3 ima varijabilniju kliničku sliku. Najčešći klinički nalazi kod djece s GB su hepatosplenomegalija, anemija i trombocitopenija. Klinički nalazi koji povećavaju sumnju na GB će zahtijevati konačnu dijagnozu mjerenjem aktivnosti glukocerebrozidaze. Prije započinjanja liječenja obavlja se cjelovita evaluacija kako bi se odredila ozbiljnost bolesti i kako bi se postavili ciljevi liječenja. GB se u djece liječi nadomjesnom enzimatskom terapijom (NET). Pokazalo se da ova terapija smanjuje morbiditet i komplikacije povezane s GB-om. Niti jedan od neuropatskih simptoma koji su bili prisutni u tipovima 2 i 3 GB nije se poboljšao s NET-om. Bolesnici s ovim tipovima GB imat će koristi od NET-a samo u slučajevima kad su zahvaćene kosti i visceralni organi. Praćenje pedijatrijskih slučajeva s GB-om je cjeloživotno putovanje koje zahtijeva periodične provjere, potrebne kako bi se procijenila učinkovitost liječenja i potreba za izmjenama istog.

3. Introduction

Gaucher's disease (GD) in one of the lysosomal storage diseases (LSD). GD is the commonest of these diseases worldwide. These diseases are a consequence of genetic mutations leading to a lack of or diminished catabolic enzymatic activity, subsequently leading to accumulation of metabolic products in variable organ systems and cells throughout the body. The enzyme involved in the development of GD is glucocerebrosidase (acid β-glucosidase, D-glucosyl-N-acylsphingosine glucohydrolase, or GCase). Latest registry reports indicate that GD becomes clinically symptomatic during the first 20 years of life, with the majority of cases reporting either laboratory or physical clinical findings preceding that. Due to the availability of treatment and management protocols, early diagnosis and treatment initiation will result in decreased morbidity and increase life expectancy in patients. The purpose of this paper is to provide an outline of contemporary data and knowledge about the pathophysiology of the disease, laboratory and clinical findings, current guidelines of management and follow up and to familiarize physicians with future lines of treatment. These tools can hopefully hasten the diagnosis and treatment of GD.

4. Epidemiology

GD annual incidence is approximately 1/40,000 to 1/60,000 in the general population. It should be taken into consideration that the disease incidence is highly dependent on ethnicity and consanguinity, evidenced by the significantly higher incidence of the disease (mostly type GD1) among Ashkenazi Jews in which the occurrence is 1/800 [1,2]. Different types report different symptoms and affected areas and are grouped as follows: Type 1 (formerly known as non-neuropathic) is the most common type and comprises 90-95% of patients. This type mostly affects the bones, liver, spleen and bone marrow. Type 2 and 3 are significantly less common in the general population and are encompassing only 5% of all patients (4% and 1% respectively) [3]. In addition to the Ashkenazi group, the largest single ethnic group represented, a further retrospective analysis performed in Sweden found a higher occurrence of GD3 than the worldwide

representation. The increased prevalence of GD3 in Sweden is a result specific mutation which is more prevalent in the province of Norrbotten in Sweden [4].

5. Genetic mechanism and pathogenesis

As an autosomal recessive disease, GD is a result of reduced activity of the enzyme glucocerebrosidase, which breaks down the glycosphingolipid glucosylcerebroside to glucose and ceramide in the lysosomal compartment. The gene, *GBA1*, which codes for the enzyme, is located on the long arm of chromosome 1 (1q21). More than 300 mutations have been identified so far [5]. The only known mutation that does not involve *GBA1* gene that results in the development of GD (mostly GD 3) is a rare mutation of the protein saposin C. This protein is an activator of glucocerebrosidase enzyme [6]. The identification of these mutated genes was thought to lead to clear phenotype-genotype correlation. Unfortunately, so far, there is only a limited number of specific mutations which provide valuable, accurate information about the predicted phenotype. The most known of these is *N370S* which results in GD type 1. Patients who inherited the *N370S* gene, whether they are homozygous (two alleles of *N370S*) or heterozygous with another mutated gene, will mostly develop type 1 disease. A rare and unique gene mutation is *D409H* mutation [5], resulting in the development of an extremely rare presentation that includes calcification of aortic and mitral valves, corneal opacities and hydrocephalus (Type 3C) [7].

All genetic mutations will consequently lead to decreased activity of the enzyme glucocerebrosidase (GCas). The decreased enzymatic activity could be a result of both diminished activity of the enzyme or decreased production of it. Another process that leads to decreased lysosomal enzymatic activity is the defective transportation of the enzyme from the rough endoplasmic reticulum (RER) to its final destination in the lysosome. The abnormal transportation of the enzyme will lead to its aggregation in different cellular compartments (cytoplasm, RER) and premature breakdown by proteasomes [1,8].

Decreased lysosomal enzymatic activity leads to accumulation of the metabolite glucosylcerebroside in lysosomes. RBC and WBC are removed from the circulation by

the macrophages of the mononuclear phagocyte system on regular intervals. These cells contain a significant amount of glycosphingolipids, glucosylcerebroside being among these in their cell membranes. For this reason, macrophages are the cells most significantly affected by this lysosomal enzymatic deficiency. The accumulation of glucosylcerebroside in the lysosomes promotes the transformation of macrophages into Gaucher cells. These cells are described under the light microscope as enlarged cells having "crumpled tissue paper" appearance. Gaucher cells are thought to be the culprit of most pathological manifestations of the disease. These cells usually infiltrate the spleen, liver, and bone. Liver and spleen infiltration will result in hepatosplenomegaly. Bone infiltration by these cells will lead to the skeletal complications [9] (see clinical presentation).

Neurological involvement is significantly less understood since glucosylcerebroside levels are low in the neural system in comparison to other organs involved. It is hypothesized that only cases of extremely low levels of enzymatic activity, such as those observed in type 2 and three diseases, will cause neuronal involvement [10].

Accumulation of glucosylcerebroside in macrophages promotes shifting of this metabolite toward an alternative metabolic path. This path will result in the increased formation of specific metabolites such as glucosylsphingosine. Glucosylsphingosine is undetectable in GD patient without neurological manifestations and unaffected individuals but is elevated in GD patients with neurological disease [11].

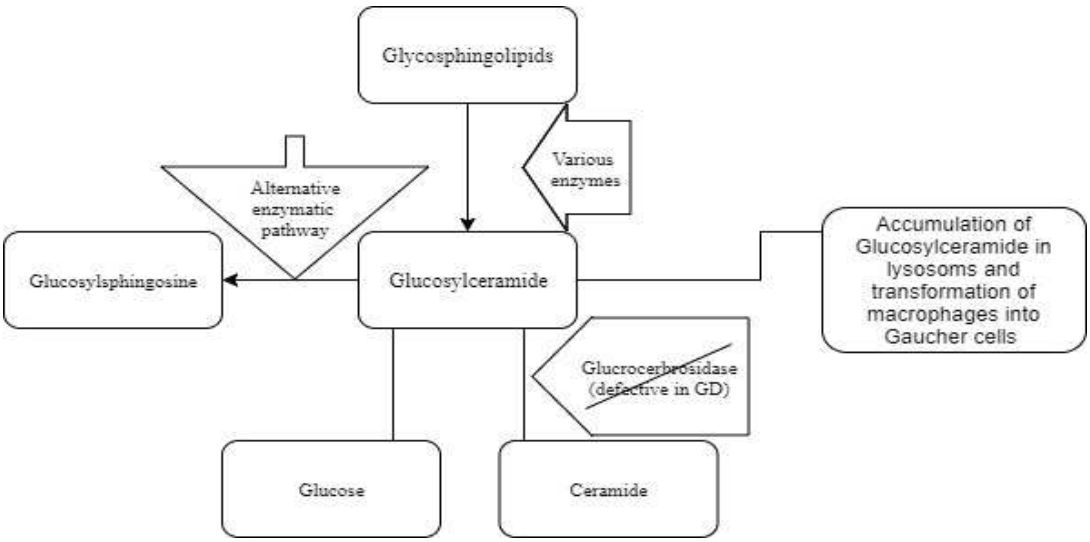


Figure 1: Biochemical pathway leading to accumulation of glucocerebroside and glucosylsphingosine. Modified from Pastores et al. [18].

Furthermore, gene modifiers, genes that can modify or alter gene expression, probably lead to the broad spectrum of phenotypic presentation among patients with the same *GBA1* mutation. A recent study performed on 15 inbred mouse strains that were injected with the same amount of glucocerebrosidase enzyme inhibitor, induced decreased enzymatic activity mimicking GD. The experimental sample showed significant genetic variance in the group. It allowed the researchers to investigate specific gene modifiers that modified the severity of disease presentation. The study resulted in identifying 17 putative modifier genes were identified, among which are *NR2B* and the B subunit of the NMDA Receptor. Treatment with NMDA antagonist prolonged the lifespan. The generalizability of the finding to human subjects is limited and pending on further investigation and clinical studies. [47].

6. Clinical presentations

GD manifests profoundly different clinical presentations, ranging from a severe prenatal presentation which results in hydrops fetalis, to entirely asymptomatic patients. Current

consensus utilizes the three types, despite understanding that they no longer present distinct entities, but rather a spectrum of phenotypes. The tripartite classification is in common usage, illustrating the onset age, neural involvement and the rate of progression.

6.1 GD type 1

GD 1 (non-neuropathic) is the most common type globally [3]. It is distinguished from the other types of the disease by the lack of neurological symptoms. The spectrum of clinical presentation varies extensively, ranging from utterly asymptomatic patient to severely symptomatic patients presenting during early childhood. For many years it was considered a disease that manifests and becomes clinically symptomatic during adulthood. In fact, two-thirds of GD1 patients become clinically symptomatic during childhood and adolescence [12]. Description of the clinical presentation is done according to the most common symptoms and signs presenting from childhood to adolescence. What follows is a brief overview according to most common organ systems.

Splenomegaly is observed in 95% of children diagnosed with the disease before the age of six. This finding is one of the earliest findings a physician should be aware of, and for this reason, it is suggested as the starting point of the algorithm for the diagnosis of GD in children (presented in the diagnosis section) [12]. Splenomegaly may lead to hypersplenism- a condition resulting in decreased numbers of RBC, platelets, and WBC. Spleen volume can reach up to 15 times the average volume in the same age group [13]. Splenomegaly can result in abdominal distention, pain and discomfort, early satiety and gastroesophageal reflux due to its increased size occupying the abdominal cavity leading to compression of adjacent structures. Two rare complications due to enlarged spleen size are splenic infarction (more common) and splenic rupture [14]. Both present as severe abdominal pain which requires immediate intervention [15,16]. The preferred imaging modality for estimating the extension of spleen enlargement is MRI [17]. In settings where MRI is unavailable ultrasonography is used, despite its limitations in accuracy.

Hepatomegaly– is recognized in more than 85% of children diagnosed with GD1 at time of diagnosis. The liver is enlarged to a lesser extent than spleen, usually ranges from 1.5-2.5 times more than average [13]. Hepatomegaly can lead to a similar physical presentation as splenomegaly due to its enlarged size. Hepatic infarction is a rare complication that can present as severe abdominal pain, similarly to splenic infarction and managed in the same manner [15,16].

Bone disease is probably the most debilitating consequence of GD1 and affects the quality of life to the most significant extent. The skeletal involvement is a slow and gradual process. Consequently, the extent of bone disease is found to correlate with the age of the patients (figure 3). Patients who are diagnosed later in life are found to have more clinical and radiological evidence of bone involvement than those diagnosed and treated earlier in life [13]. Bone involvement commonly affects the pelvis and lower limbs [4]. This can result in moderate lower extremity pain in children which on many instances is incorrectly diagnosed as typical growing pain [13]. This continued bone involvement results in radiological findings indicating bone disease in 81% of children at the time of diagnosis [13] (figure 3). MRI is the preferred modality to evaluate bone involvement such as bone infarction, avascular necrosis, marrow infiltration, lytic lesions and new fractures. One specific radiological finding is known as ‘Erlenmeyer flask deformity' refers to the narrowing of the diaphysis and the expansion of the metaphysis (figure 2). These are found in the lower part of the femur. This finding is common in children with GD1 but is not pathognomonic and may be detected in other diseases (Niemann-Pick, sickle cell disease, etc.) [14].

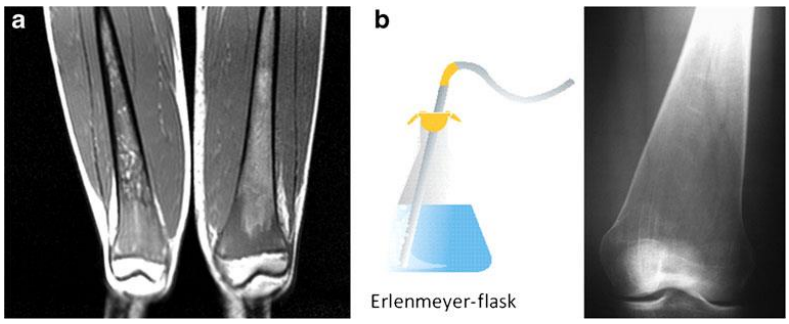


Figure 2: a. MRI showing marrow infiltration. b. X-ray showing Erlenmeyer flask deformity. Modified from Orvisky et al. [10].

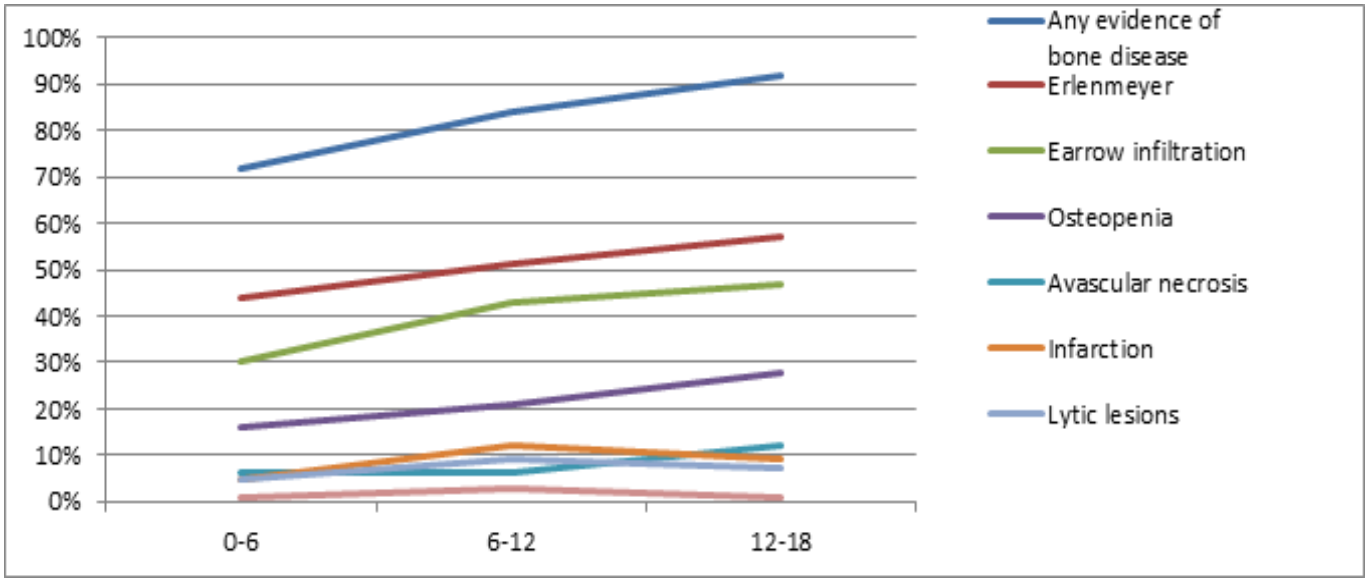


Figure 3: Percentage of children with specific bone findings at the time of diagnosis according to age. Modified from Kaplan et al. [13].

Bone mineral density is usually decreased as a result of the bone disease and can result in pathological bone fracture. Bone crisis is a common presentation of bone involvement in children with GD (30%) [14]. In addition to severe pain, it is accompanied by edema and warmth in the affected area, leukocytosis, and low-grade fever. The mentioned symptoms can last up to 7-10 days. These symptoms can be accompanied by an imaging finding of elevated periosteum, mimicking the presentation of osteomyelitis (pseudo-osteomyelitis) [14,18].

Delayed growth and puberty are of particular interest in regards to the pediatric population. Growth retardation can be divided to severe growth retardation (less than the 5th percentile), and moderate (less than 25th percentile), it is recognized in 34% and 28% of children at the time of diagnosis, respectively [13]. The degree of growth deceleration usually correlates with the severity of the disease. Puberty and sexual maturation are delayed in more than 60% of patients [17]. Both are consequences of the disease itself, shown by the improvement of these symptoms upon initiation of enzyme replacement therapy (ERT). An indirect effect of both growth and puberty delay

is emotional distress experienced by children that should also be addressed by physicians [19].

Cytopenia is a frequent finding in GD1 prior to initiation of treatment. Thrombocytopenia and anemia are a result of multiple factors such as hypersplenism, bone marrow infiltration and the direct effect of accumulated metabolites on hematopoiesis [20, 21]. Approximately 40% of children are found to have a certain degree of anemia or thrombocytopenia at the time of diagnosis. Decreased number of WBC (leukopenia) can also occur but to a lesser extent [13].

Thrombocytopenia manifests as mucosal bleeding that can be observed during teeth brushing. It can additionally present as frequent and prolonged nosebleeds or excessive bleeding following trauma [18]. Bleeding disorders may also be due to platelet aggregation abnormalities and coagulation disorders. As a consequence, PT and PTT might be prolonged [22].

Anemia usually manifests as general fatigue, inability to play for prolonged periods of time or the lack of desire to participate in physical activity.

Leukopenia is seldom severe enough to necessitate any intervention or to have any clear consequences.

Hypergammaglobulinemia in children is commonly due to increased production of polyclonal antibodies. This usually resolves with the initiation of ERT [23].

6.2 GD type 2 (acute neuropathic, infantile)

GD 2 is the most severe form of the disease. It affects only 1% of individuals with GD [14]. It is characterized by visceral and neurological involvement but lack of bone involvement. Initial symptoms present most commonly around 6 months of age and in the vast majority before the age of 2. Visceral signs are similar to the ones described in GD1 and include hepatosplenomegaly and cytopenia. Neurological symptoms include: bulbar signs (stridor, swallowing impairment), hypertonicity (of the neck and trunk

known as opisthotonus, and of the jaw known as trismus). Oculomotor paralysis can also occur. Psychomotor development is substantially limited in these patients. Most patients suffering from GD 2 die by 2-4 years of age most commonly due to apnea and pulmonary complications [24]. Severe presentation of GD2 also known as perinatal or lethal form, presents with several unique findings. Skin findings include collodion skin abnormalities, referring to the bright, shiny and tight appearance of those newborns' skin. The skin of these newborns is usually being shed during the first weeks of life and puts them at risk for dehydration due to fluid loss through the skin. Nonimmune hydrops fetalis could be another presentation of this severe form of the disease and result in death of the fetus or premature delivery [24].

6.3 GD type 3 (juvenile or subacute or chronic)

GD3 can present with bone involvement, similar to GD1, and with neurological involvement, similar to GD2, along with visceral involvement. Age of presentation is highly variable, most commonly during childhood but might also happen in infancy or adulthood. It usually follows a slow progressive course in comparison to the rapidly progressive course of type 2. Patients usually survive to 3rd and 4^{th} decades of life [18]. Type 3 has wide range of presentation. On one side of the spectrum, patients can suffer from an isolated neurological finding such as horizontal ophthalmoplegia, whereas on the other side of the spectrum patients can suffer from more severe symptoms such as debilitating hypertonicity or frequent seizures. Neurological symptoms which are commonly observed in GD3 patients are myoclonic epilepsy, cerebellar ataxia and spasticity [18,25, 26]. GD patients can initially be considered to have GD1 type due to the lack or mild presentations of neurological symptoms. Therefore it is essential to perform focused periodic neurological examinations to patients presenting solely with visceral and bone involvement, in order to assess any changes that will lead to reclassification. The cardiovascular (3C) form is an extremely rare presentation of GD. It mostly presents during childhood in individuals which are homozygous for the *D409H* mutated allele. Common manifestations are mitral and aortic valve calcifications, splenomegaly, corneal opacities, supranuclear ophthalmoplegia and hydrocephalus [18].

7. Diagnosis

In many cases the diagnosis of GD is made years after initial symptoms which in type 1 usually start during childhood and adolescence [13]. Due to the availability of treatment and with intention of prevention of irreversible consequences, timely diagnosis is of paramount importance. For this reason it is essential for physicians to diagnose a child with the disease as quickly as possible in order to prevent future irreversible consequences of the diseases. In the following section important diagnostic tests will be described as well as a proposed diagnosis algorithm in children [13].

Upon completion of the initial workup of a susceptible patient, further inquiry into detailed clinical and family history is necessitated. Susceptible ethnic groups are an addition factor for differential diagnosis. The most important of these ethnic groups are Ashkenazi Jews. The clinical history should include all signs and symptoms previously described (see clinical presentation section). Signs for abdominal distention or pain, early satiety, loss of weight and fatigue. Any history of bleeding or bruising should be investigated while focusing on mucosal bleeding, such as bleeding during teeth brushing and epistaxis. Growth deceleration or failure to thrive are of particular interest in the pediatric population and could be one of the initial presenting symptoms in a slowly progressing disease. Musculoskeletal examination should be performed. This includes different radiological modalities such as x-rays, MRI or ultrasound. Neurological examination is critical in order to identify patients with GD2 and GD3. Blood work is an essential part of the initial workup and can demonstrate a decreased number of platelets, moderate anemia and on rare occasion leucopenia.

7.1 Definitive diagnosis modalities

Glucocerebrosidase activity essay

The diagnosis of GD, regardless of the type, is made by proving a decreased activity of the glucocerebrosidase enzyme. This is done by a fluorometric assay using the substrate 4-methylumbelliferyl-β-D-glucopyranoside to measure enzyme activity. The assay is done in different cells such as leukocytes, mononuclear or cultured fibroblasts

from a skin biopsy. Enzymatic activity in affected individuals is usually 0-15% in comparison to non-affected individuals [18].

Genetic testing

Genetic testing is used as an additional confirmatory test to glucocerebrosidase activity assay, for carrier screening and to a limited extent to correlate phenotype with genotype. A child diagnosed with the disease using glucocerebrosidase enzyme assay is commonly referred to genetic analysis test, which will determine the genotype of the child. An identified genotype is used for screening other relatives that could be carriers or still asymptomatic. The recognition of a specific genotype could provide information about the course of the disease. As described earlier the mutation *N370S* will result in GD1 in contrast to the *L444P* genotype which will mostly result in a more severe neurological disease [1,5].

Specific techniques are used to detect a genetic mutation in the *GBA1* gene. The genetic analysis usually follows three steps of progression. The initial technique used is targeted analysis for pathogenic variants in a proband. This detects the most common variants which can change in prevalence among different populations. In Ashkenazi Jews, four variants are found in 90% of diagnosed patients. In comparison, these four genes are contributing only to 60% of GD patients in the general population [18]. In cases in which the mutation cannot be detected by the targeted analysis test, the subsequent step will be the utilization of the sequence analysis method. This technique will sequence the entire *GBA1* gene and will identify small genetic deletions/insertions and missense, nonsense, and splice site variants. Approximately 99% of mutations will be detected by sequence analysis. Rarely sequence analysis is not able to detect small deletions and duplications in the gene, and on that rarity, it leads to the utilization of deletion/duplication analysis [18] (figure 4).

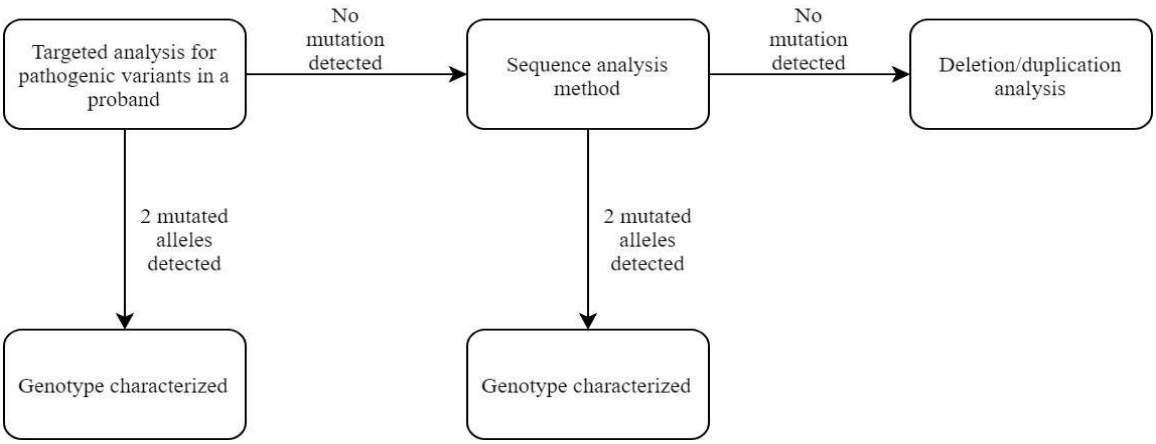


Figure 4: Suggested algorithm for genetic diagnosis of GD. Modified from Pastores et al. [18].

7.2 Bone marrow aspiration

Bone marrow aspiration and its examination are not mandatory or essential for the diagnosis of GD. In most cases, it is performed due to the low number of platelets and RBC, or hepatosplenomegaly that can raise suspicion for hematological or malignant diseases. The bone marrow examination could provide valuable information when Gaucher cells are identified on microscopic examination. It is critical to acknowledge that neither the presence or the absence of these cells confirms or excludes the diagnosis of GD by itself. "Look-alike " pseudo-Gaucher cells are known to be found in other diseases such as hematologic malignancies [27,28], sickle cell disease [29] and different mycobacterial infections [30]. Due to the close resemblance of pseudo-Gaucher to Gaucher cell, determination of clear differentiation is done with extra caution [31].

7.3 Biomarkers in the diagnosis and follow up

Several biomarkers are elevated in patients with GD. These are used to lead the algorithm towards glucocerebrosidase enzyme assay that will confirm the diagnosis(see diagnosis algorithm, Figure 5), or follow the response to treatment and predict prognosis.

Chitotriosidase is an enzyme produced in excessive amounts by Gaucher cells. It has been utilized as a biomarker for over two decades [32]. This biomarker is mostly used to follow the response to treatment. Chitotriosidase can also be used to a lesser extent as a prognostic factor, whereby high activity correlates with a worse prognosis. The main disadvantage of chitotriosidase is that approximately 6% of patients with GD can also have coexistent mutations in the enzyme's gene, which in turn can lead to a complete (homozygous) or diminished (heterozygous) levels of it. Therefore, in these patients using this biomarker can be misleading. Chitotriosidase can also be elevated in other lysosomal storage diseases. These render the results and make them less reliable [33].

CCL18 is a chemokine formed by different cells, most important being dendritic cells and macrophages [34]. Also, Gaucher cells can also produce high levels of this chemokine. Thus it can be found in high levels in patients' plasma. The levels of CCL18 could be elevated in different diseases which result in chronic inflammation, as well as in allergic reactions [35]. High levels are usually indicative of worse prognosis [14].

Glucosylsphingosine is a relatively new biomarker detected which is considered to be superior to the previous two due to its reliability, specificity, and sensitivity but is still not widely used [18].

Elevated levels of ***ferritin*** are typically found in patients with GD (>85%) [36].

Two additional biomarkers which are less commonly used today due to the availability of better biomarkers are ***tartrate-resistant acid phosphatase*** (TRAP) and ***angiotensin-converting enzyme*** (ACE). Both are frequently elevated in GD Patients [37].

7.4 Screening of GD

In some countries, glucocerebrosidase activity essay is being performed as part of national screening programs for detection of metabolic disease in newborns using a dried blood spot technique. This leads to early diagnosis and follow up of patients and early treatment initiation. This will consequently prevent the development of unnecessary complications [18].

7.5 Diagnostic algorithm

The most current and comprehensive diagnostic algorithm is the 2014 report by Italian experts, based on the International Collaborative Gaucher Group Registry (ICGG). Delete the sentence the algorithm is intended [39].

99% of children with GD under the age of 6 are found to have splenomegaly to a certain extent at the time of diagnosis and is the sole sign in 50% of those patients [39]. For that reason, it was chosen to be the starting point of the algorithm. Following detection of an enlarged spleen that could be a result of other diseases as well, a noninvasive, available and rapid blood test is the next step in the algorithm. Thrombocytopenia and anemia are found in 50% and 41% respectively at the time of diagnosis in children [39]. Next step is dependent on whether thrombocytopenia or anemia was detected in blood tests. If any of these was found, or if there is no other acceptable etiology for the enlarged spleen, the algorithm guides to tests which are more specific for GD. Blood Tests are evaluating levels of biomarkers such as TRAP, ACE, ferritin, CCL18, chitotriosidase, and glucosylsphingosine. An x-ray will also be performed during this step, evaluating for the presence of Erlenmeyer flask deformity which is found in 49% of children at the time of diagnosis [39]. Growth deceleration evaluation and assessment of ophthalmological abnormalities are being completed simultaneously. If none of the following criteria was detected bone marrow aspiration is being performed to evaluate for hematological, hemato-oncological and communicable diseases etiologies. Examination of bone marrow aspiration might detect Gaucher or pseudo-Gaucher cells. Finding of any of these cells and its evaluation should be done with respect to other findings on bone marrow aspiration. If any of the following criteria described is being detected, the risk of GD is significantly high and necessitates a confirmatory test of glucocerebrosidase activity essay [12] (figure 5).

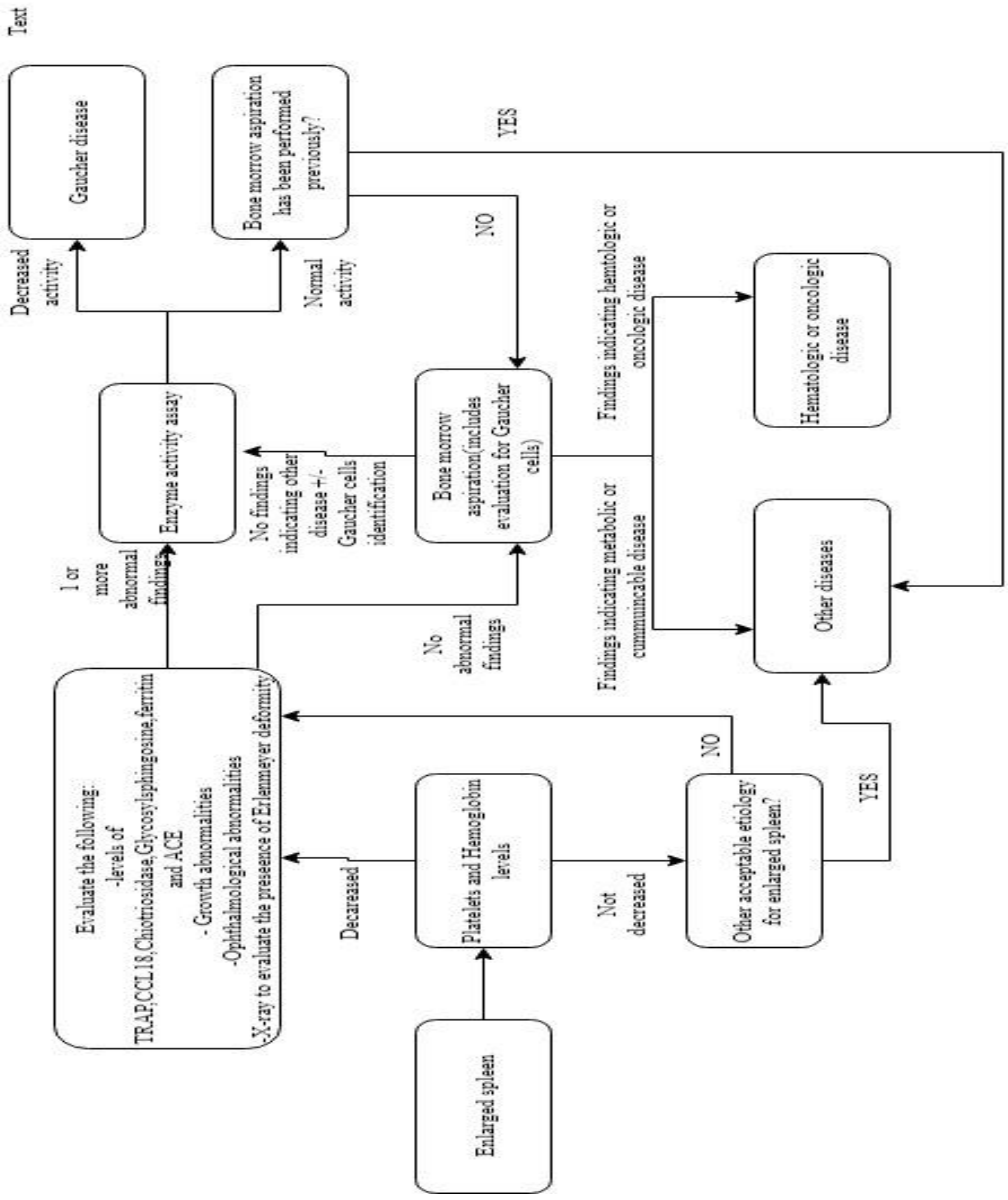


Figure 5: Diagnostic algorithm. Modified from Di Rocco et al [12].

8. Treatment and management

Management of a child diagnosed with GD starts instantaneously after diagnosis is made and confirmed by appropriate modalities. It follows a standard timeline that starts with diagnosis and followed by establishing a baseline of common parameters which will be closely monitored during treatment (figure 6). The next step after establishing

baseline parameters will be the initiation of treatment (mostly ERT, bone marrow transplantation is reserved for selected cases) in eligible patients. Subsequently, a close follow up is required to adjust treatment to individually treat symptomatic complications of the disease (bone crisis, anemia, etc.).

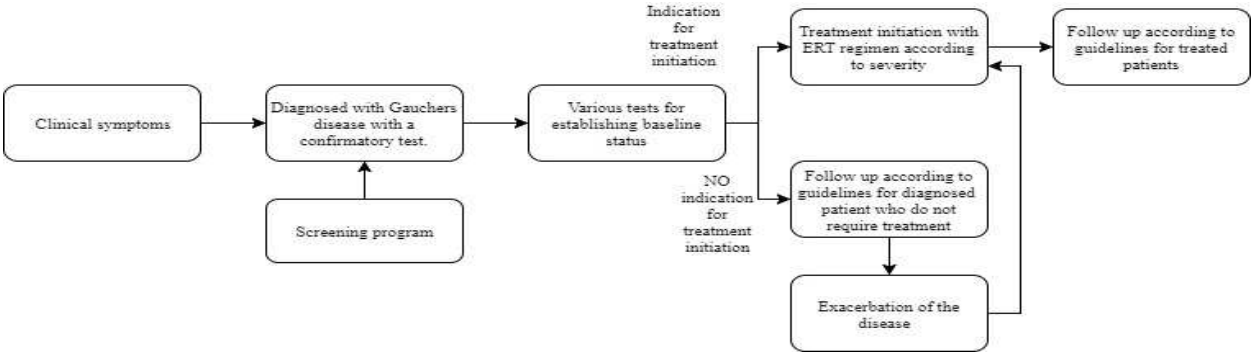


Figure 6: Suggested timeline in the management of children with GD.

Extensive and elaborated clinical work in combination with disease-oriented physical examination are essential after diagnosis has been established by enzyme assay or genetic testing. The findings can define the goals of treatment.

Blood work

Initial blood work is performed to evaluate the state of the patient. This should include full blood count (CBC), hepatic (AST, ALT) and renal function tests, iron, ferritin, vitamin D, vitamin B12 and specific biomarkers. Important biomarkers that should be checked are chitotriosidase, CCL18, glucosylsphingosine, TRAP and ACE [12,14]. All biomarkers should be measured to assess treatment options and its efficacy. Successful treatment will lead to a decrease in their levels [5]. An increased level of polyclonal immunoglobulins is a common finding in children and should be assessed as well. In addition, coagulation functions that include PT and PTT should be evaluated.

Visceral assessment

This includes assessment of liver and spleen sizes. These are assessed preferentially by MRI. In settings where MRI in unavailable, ultrasound can be used although it is not

as accurate. MRI requires the patient to stay still for a considerable period. This can present a challenge in the pediatric population. The use of sedatives should be considered in those cases [18].

Bone

MRI of the spine and femur should be performed to assess bone involvement and infiltration. Each of the two types of MRI images (T1 and T2) are utilized to recognize and grade different bone pathologies. T1 weighted sequence is utilized to recognize and assess bone marrow infiltration. Assessment of bone marrow infiltration in children is significantly challenging. This is due to the presence of red bone marrow which does not exist in adults. As a result, MRI findings can be misleading if not interpreted by a skilled and experienced radiologist. T2 sequence images are done to identify bone infarctions. Bone density is appraised using total body DEXA (dual energy x-ray absorption). Wrist bones X-ray could be used in children to determine their bone age and asses growth delay [14].

Neurological

Neurological examination of any child diagnosed GD is required. As noted earlier, the severity of neurological involvement could be utterly different. Children initially diagnosed with GD1 could suffer from GD3 since their neurological signs were not recognized or did not exist initially. More specifically, ophthalmological abnormalities can be the initial or the sole neurological abnormality in GD3. Children diagnosed with a neuropathic form of GD disease may require additional and more advanced neurological tests such as peripheral hearing measurement, brain MRI, EEG, and brainstem evoked responses [17].

8.1 Application of treatment

Treatment can be divided into specific treatment aimed to decrease the accumulation of glucosylcerebroside and to non-specific, aimed to provide symptomatic treatment of disease complications and manifestations.

Specific treatment includes enzyme replacement therapy, SRT (substrate reducing therapy, not currently approved for the use in children) and bone marrow transplantation. Enzyme replacement therapy (ERT) has revolutionized the treatment for GD1 patients but at the same time did not improve or affect the neurological outcome of patients suffering from neurological pathologies such as in GD2 and GD3. Currently, many screening programs for the early detection of lysosomal storage diseases and other inherited metabolic disease are established. Therefore, more asymptomatic children are diagnosed at earlier ages. Currently, even though ERT is available in most developed countries, it is still relatively expensive. Due to the existence of asymptomatic and barely symptomatic patients, indications for initiation of ERT have been suggested (see table 1) [12].

Each of the following findings indicates initiation of ERT in an <u>asymptomatic child</u>.
Diagnosed during first 20 years of life.
Hemoglobin lower than 4.96 mmol/L
Platelets number lower than 60,000 cells /ml
WBC count lower than 3000 cells /ml
Any symptomatic bone disease such as bone crisis or pain
Any radiological or clinical evidence of bone disease even if the patient is asymptomatic
Growth delay and diminished growth velocity

Pubertal onset delay
Sibling with severe disease already treated with ERT
Genotype associated with severe disease
Height lower than 2 SD (below the 5th percentile)
Decreased bone mineral density, Z score lower than 2
Spleen or liver volume bigger than double the normal liver and spleen size

*A patient that fulfills one or more of the described indications is eligible to ERT.

Table 1. Indications for ERT initiation in symptomatic children. Modified from Kaplan et al [12].

The goal of specific treatment is to prevent and improve the patients' symptoms. Specific goals have been described to guide treatment. These therapeutic goals are intended to provide doctors with tools for assessment of treatment efficiency and success (figure 7).

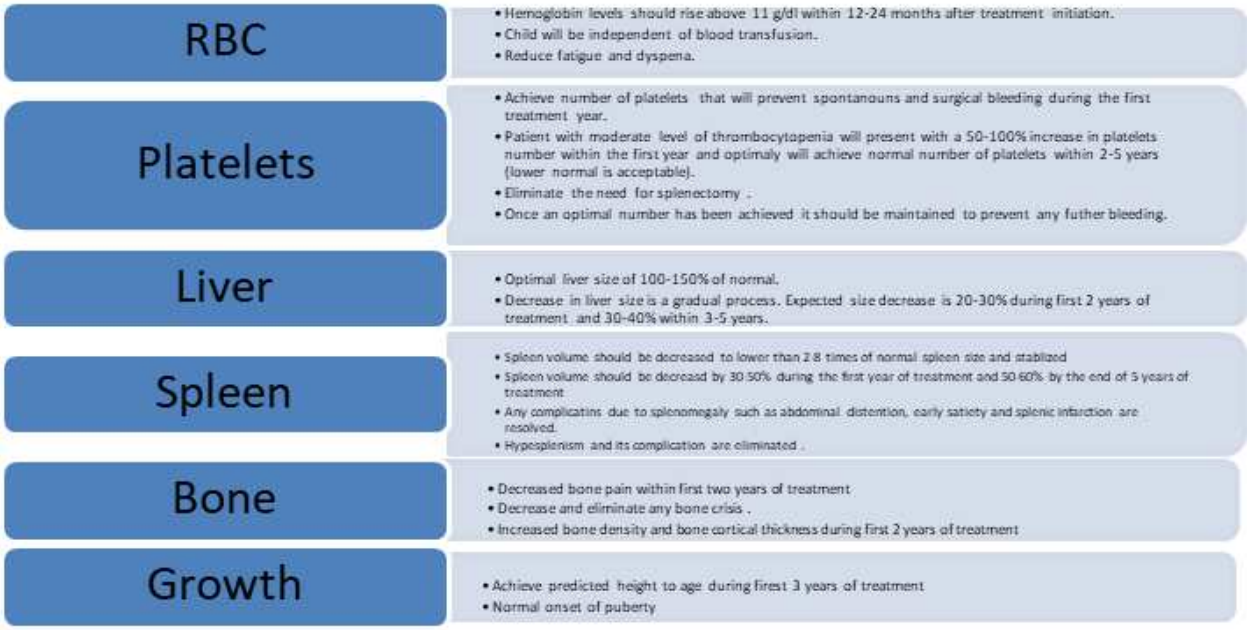


Figure 7. Therapeutic goals. Modified from Pastores et al. [18].

8.1.1 Types of specific treatment

Enzyme replacement therapy

Enzyme replacement therapy is the standard of care for patients with GD 1 and GD 3 with visceral and bone involvement. The goal of treatment is to overcome and compensate for the deficient activity of glucocerebrosidase enzyme by supplementing it artificially. Three ERT drugs are available and differ according to the cell type used for their production. All enzymes are chemically altered to expose their mannose residue. This alteration promotes their uptake by macrophages. Imiglucerase was the first ERT drug commercially available (1991) and is produced from Chinese hamster ovary cells. The other ERT currently available are Velaglucerase Alfa which is produced from human fibroblast-like cell lines, and Taliglucerase Alfa produced from a carrot cell line [14]. All ERTs are administered intravenously, and their dosing and regimen vary among different countries [18]. The current recommendation for severely affected children is a dose of 60 U/kg on a biweekly basis [7]. Moderately affected children should receive 30 U/kg on a biweekly basis. The treatment regimen can be changed in case the response is insufficient or if the response is too rapid to current treatment. A rapid response can indicate that a regimen with lower dosing can provide sufficient results. Most current regimens use doses of 20-120 U/kg dependent on the clinical response of the patient [12, 17].

ERT treatment in children commonly results in significant increase in platelets number, improvement of anemia, and in a decreased liver and spleen size. The most significant changes occur during the first year of starting treatment and continue to improve in the following seven years [13]. Children with GD who start treatment early in childhood do not experience delayed puberty compared to those who start treatment in late childhood [19]. Improvement in growth retardation was also evident during the first eight years of treatment with a median height improvement of 1.9 Z score unites [38]. ERT results in an increase of bone mass, cortical bone thickening, decrease in episodes of bone crisis [38], diminished bone pain [39] and decrease in the incidence of bone fractures [38]. All these beneficial effects on the skeletal system improve the quality of life and prevent future skeletal complications such as osteoarthritis, joint abnormalities, and

compression fractures. Levels of most biomarkers decrease after starting therapy [40, 41]. Levels of immunoglobulins and ferritin usually normalize as well after treatment [42].

Side effects of ERT are generally well tolerated. An immune-mediated hypersensitivity reaction is the most common side effect. It presents as urticaria, gastrointestinal discomfort, and laryngeal irritations. These reactions are diminished in intensity by slowing down the rate of infusion or by prophylactic treatment with corticosteroids or antihistamines. [14]

Bone marrow transplantation

The goal of bone marrow transplantation is to replace the cells lacking glucocerebroside enzyme with healthy cells from a healthy donor. Bone marrow transplantation has high morbidity and mortality rates. As a result of the high effectiveness of ERT and the risks involved in bone marrow transplantation, it is no longer considered an appropriate treatment for the majority of patients with GD. This procedure might still be considered in specific patients such as those who do not respond to ERT treatment. Since ERT cannot cross the blood-brain barrier, theoretically this procedure can overcome this obstacle and improve the neurological symptoms of patients with GD. There is no current evidence showing that bone marrow transplantation offers any significant improvement of neurological symptoms in GD2 and 3 [43].

Substrate reduction therapy

Substrate reducing therapy is aimed to decrease the synthesis of glucocerebroside and by that preventing its accumulation. It acts by inhibiting the enzyme glucosylcerebroside synthase. It offers the benefit of ease of use since it is taken orally. Thus it increases compliance rate. This drug is currently approved only for the treatment of mild to moderate GD1 in adults; hence it is discussed only briefly [24].

8.1.2 Supportive care, non-specific treatment

Non-specific treatment refers to any treatment that is intended to treat the patients' symptoms and not the etiology of GD. The treatment of common symptoms and complications that were discussed in the clinical presentation section will be presented.

The skeletal system is significantly affected in children with GD disease, both the bone marrow and the bone itself. Bone crisis occurs commonly in children with GD rather in adults. The treatment for these episodes is typically with analgesics and oral prednisolone [44].

ERT treatment typically results in an increase of hemoglobin number, platelets number and rapid decrease in spleen size [9]. In selected cases, when there is an insufficient response to treatment, blood transfusions can be considered to alleviate symptoms such as fatigue and bleeding, due to anemia and thrombocytopenia, respectively. Splenectomy used to be an optional treatment strategy aimed to solve the hematological abnormalities caused by hypersplenism, such as anemia and thrombocytopenia. Currently, this therapy is rarely utilized due to the effectiveness of ERT [6].

Respiratory complications are the most common cause of death in patients with GD2. The most common of these are laryngeal spasm and apnea [6,15]. Maintaining a patent airway in these patients using tracheostomy will prolong patients' lives. These children typically have a low quality of life, and the question of whether to perform tracheostomy or not is usually left to parents or legal guardians [45].

Treating the neurological symptoms of patients with GD2 and GD3 is explicitly challenging, and does not follow standard guidelines. Patients suffering from spasticity and irritability can be treated with benzodiazepines. Parents should be familiarized with common side effects of benzodiazepines which include urinary retention, increased saliva production, constipation, general weakness and respiratory depression. Respiratory depression can further worsen respiratory abnormalities in these patients. Seizures are managed according to the specific seizure type. Phenobarbital is one of the older drugs for the treatment of epilepsy. It is commonly used for seizure control in

infants. Other drugs that are being used are phenytoin, levetiracetam, valproate. These might have significant side effects that should be considered and monitored during treatment [46].

8.2 Future treatments: overview

Gene therapy

Introducing a normal GBA1 gene into hematopoietic stem cells *in vitro*, followed by delivering them into GD patients was thought to treat the disease and at the same time decrease the morbidity and mortality involved in bone marrow transplantation. So far the clinical results of this treatment were unsatisfying [14,18].

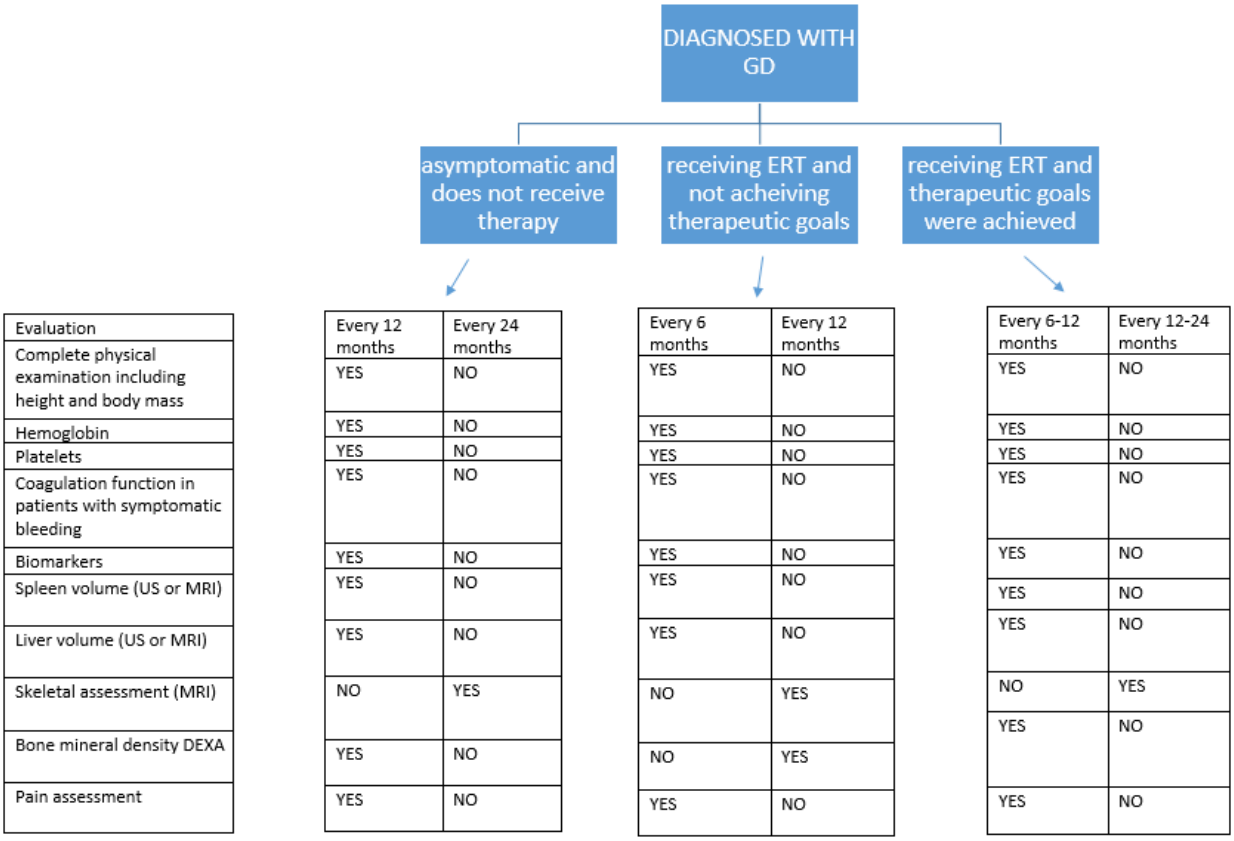
Pharmacological chaperone therapy

Chaperones are small molecules intended to promote proper trafficking, folding, and function of other molecules. Non Mutated proteins produced by the ER are normally recognized by endogenous chaperones, that bind to the protein and promote its proper folding and conformation. This in turn prevents aggregation of the protein in the ER and ensures proper trafficking of the protein to its goal destination (lysosomes) [14]. Abnormal/mutated proteins are not identified by endogenous chaperones leading to their aggregation in the ER and eventually to their degradation. This results in decreased enzymatic activity. Certain mutated proteins still possess residual catabolic activity. The goal of pharmacological chaperone therapy is to provide exogenous chaperone molecules that will bind to the mutated proteins that still possess residual catabolic activity. This in turn will promote their trafficking to lysosomes and will improve the enzymatic activity in patients. The relatively small size of chaperone molecules can aid in the passage of the blood-brain barrier and might provide benefit in the treatment of the neurological symptoms of GD2 and GD3 patients. Ambroxol, originally a secretolytic therapy, was identified by FDA (Food and Drug Administration) as a potential chaperone of the glucocerebrosidase enzyme. It is the most investigated and tested PCT for GD [49]. Treatment of GD with ambroxol was tested in multiple trials, none of which is a randomized control trial with a large amount GD patients. In studies that were performed on cultured fibroblasts from GD patients, the results had shown an

increase in the enzymatic activity levels of glucocerebrosidase after treatment with ambroxol [50,51]. A trial performed by treating healthy non-human primates with a dose of 100 mg/day of ambroxol had shown an increase in the levels of glucocerebrosidase activity in their brains [52]. Treatment trials on people are extremely challenging due to low prevalence of the disease. A trial performed in Israel on 12 patients with GD1 who were not treated with ERT, treated these patients with a dose of 75 mg/day for 6 months had shown positive results, where no patient exhibited exacerbation of the disease and 3 patients exhibited an improvement of their disease. One of the most significant trials that was performed in Japan on five patients with GD3 with neurological symptoms including myoclonus, seizure and pupillary reflex abnormalities. These patients were treated with high dose of ambroxol 25 mg/kg/day. High dose therapy was based on the fact that none of the prior trials had shown any significant toxicity of ambroxol. Patients responded to treatment and had a significant improvement of all 3 symptoms mentioned previously. Levels of glucosylsphingosine levels in cerebrospinal fluid were decreased in comparison to values measured before treatment initiation, indicating significant passage of the drug through the blood-brain barrier [53]. Treatment efficacy with ambroxol in all trails was found to differ according to the specific mutations affecting the patients. Certain mutations were found to be more responsive to treatment than others [50, 51]. Dosing regimen will need to be further investigated even though there is no evidence of severe toxicities with the use of ambroxol that will limit the dosing regimen.

8.3 Follow-up

Follow-up of GD patients is a crucial aspect of their management. A proper well organized follow up will allow the treating physician to gain valuable information about the effectiveness of treatment and will allow him/her to tailor the treatment to individual patients (The current follow up guidelines are presented in figure 8).



Evaluation	Every 12 months	Every 24 months	Every 6 months	Every 12 months	Every 6-12 months	Every 12-24 months
Complete physical examination including height and body mass	YES	NO	YES	NO	YES	NO
Hemoglobin	YES	NO	YES	NO	YES	NO
Platelets	YES	NO	YES	NO	YES	NO
Coagulation function in patients with symptomatic bleeding	YES	NO	YES	NO	YES	NO
Biomarkers	YES	NO	YES	NO	YES	NO
Spleen volume (US or MRI)	YES	NO	YES	NO	YES	NO
Liver volume (US or MRI)	YES	NO	YES	NO	YES	NO
Skeletal assessment (MRI)	NO	YES	NO	YES	NO	YES
Bone mineral density DEXA	YES	NO	NO	YES	YES	NO
Pain assessment	YES	NO	YES	NO	YES	NO

*Significant exacerbation or dose modification should be followed with complete physical examination including height and body mass, measurement of hemoglobin, platelets, PT, PTT, and biomarkers**.**

Figure 8: Follow-up strategy. Modified from Kaplan et al [17].

9. Discussion

Our ability to diagnose GD early in the course of the disease or even earlier with the help of national screening programs, combined with the available ERT treatment, allows us to provide adequate treatment. The advancement in the understanding of the genetic factors underlying disease presentation including gene modifiers will hopefully encourage further investigation of this aspect. This may allow us to understand the pathophysiology of different GD types and to establish a more complete genotype, including identifying different gene modifiers. This, in turn, will help us achieve a better genotype-phenotype correlation. Furthermore, this may offer new targets for therapy in patients [48]. Even though significant advancement and improvement in the management of patients were documented during the last two decades, this is mostly isolated to GD1. We are still not able to provide children suffering from type 2 and 3 with appropriate and effective therapy. Different approaches were tried including ERT, gene therapy, and bone marrow transplantation. All seemed to be ineffective. Since management of GD1 is well established and accepted, the next step will hopefully be to provide GD2 and 3 with treatment that will provide a significant improvement in patients' life expectancy and quality.

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12. Biography

Ori Shoham is a soon to be a medical doctor who studies in the final sixth year in the medical program at the University of Zagreb School of Medicine.

Ori was born in Tel Aviv, Israel, and was raised in Rishon L'zion. During high school, he was a member of the Israeli national handball team. After completing his 3-year mandatory military service in Israel, decided to become a medical doctor. Upon completing his 6th year of the medical studies program in Croatia, passing all his exams with outstanding results, he is ready to begin a new chapter in his medical future.