Case Report: Pancytopenia as an indicator for lysosomal storage disease (Gaucher's Disease) [version 2; peer review: 1 approved]

mail_outline Alberto Ortega-Rosales

https://orcid.org/0000-0003-0694-7636

Carlos Burneo-Rosales Gilda Romero-Ulloa Gabriela Burneo-Rosales

Abstract

Lysosomal storage disorders are a group of rare hereditary diseases of which Gaucher Disease (GD) is among the most frequent. GD type 1 is the most common form of this condition. Patients with this variant may present with hematologic cytopenias, hepatosplenomegaly, multi-faceted bone involvement, and, late in adult life, with neurological disorders such as Parkinsonism and peripheral neuropathy. Because cases of Gaucher disease are uncommon in Ecuador where information about GD is very limited, we present an illustrative patient with thrombocytopenia, leukopenia and hepatosplenomegaly whose diagnosis was not suspected until performance of a bone marrow biopsy.

Our aim is to emphasize the importance of early recognition of pathology suggestive of GD type1 so that this disease is routinely included in the differential diagnosis in patients with unexplained cytopenias and hepatosplenomegaly. Once suspected, the diagnosis may be established non-invasively by testing for lysosomal glucocerebrosidase (acid-β-glucosidase) deficiency either in dried blood spots (DBS) or in peripheral blood leukocytes and further elucidated by sequencing the GBA1 gene for pathogenic variations (mutations). Timely, early diagnosis should be followed by appropriate treatment (either intravenous enzyme replacement [ERT] or oral substrate synthesis inhibition ["SRT"] directed at preventing irreversible complications and morbidity and improving health-related quality of life.

Keywords

Gaucher disease, lysosomal storage disorders, cytopenia, β-glucocerebrosidase.

Revised Amendments from Version 2 (NJW)

Introduction

Lysosomal storage disorders (LSDs) are rare, generally recessive, hereditary monogenic diseases. Because of total or partial functional loss of specific lysosomal enzymes or their cofactors, un-degraded and sometimes water-insoluble substrates accumulate in cellular lysosomes of various tissues causing multi-organelle and cellular dysfunctions that extend to immediate cellular and extracellular microenvironments. These processes lead to immune and inflammatory pathophysiology that is expressed as signs and symptoms of clinical disease.[1,2] Gaucher disease (GD) is one of the most common LSDs with an estimated incidence of 1:40.000 to 1:86.000 new births in the general world population. There is no known racial or national proclivity for GD with the exception of Jews who trace their last 1,000 year ethnicity to regions of eastern, northern and central Europe referred to in Jewish tradition as *Ashkenaz*. Most Ashkenazi Jews currently reside in Israel, Western Europe, Russia, North America and a number of Latin American countries. The incidence of GD among Jews with four Ashkenazi grandparents is estimated to be 1:450 births although the recognized prevalence may be only

1:850 due to low penetrance genotypes[3]. In 1950, there were approximately 5,000 Jews in Ecuador of whom almost all were immigrants from Germany or eastern Europe. The current Jewish population is about 300 of a total Ecuadorean population of about 17 million[4].

GD is characterized by defective function of lysosomal glucocerebrosidase[5] that leads to accumulation of glucocerebroside (glucosylceramide) in the lysosomes of different cells, but primarily in tissue macrophages resident in bone marrow, spleen and liver.[6] The sphingolipid storage process, that includes minor but pathogenic substrates such as glucosylsphingosine, ultimately leads to pancytopenia, hepatosplenomegaly, bone involvement (including loss of bone mineral, medullary infarction, osteonecrosis, osteolysis and pathologic fractures) and, sometimes neurological alteration early in life[7]. There are three major clinical subtypes, which are classified by the absence or presence and progression of central neurological involvement: type 1 or non-neuropathic form; type 2, the acute neuropathic form of infantile onset; and type 3, the neuronopathic form of juvenile onset[7]. However, there is phenotypic heterogeneity within each sub-category, partly but not totally determined by genotype. In symptomatic patients, GD is usually a progressive disease that, if left untreated, can cause irreversible organ damage, severe morbidity, reduced quality of life and even premature death[8].

GD is a rare disorder with which many physicians, including hematologists, are unfamiliar. As a worldwide phenomenon, patients are often misdiagnosed or diagnosis significantly delayed despite the availability of accurate and minimally invasive diagnostic tests[9]. In Ecuador, despite extensive annotation of population genetics and genomics[10], very few cases of GD have been reported. Therefore, greater knowledge of the clinical and demographic characteristics of this clinical entity can improve early recognition, reduce the rate of inaccurate or delayed diagnoses for patients with GD, allowing timely treatment aimed at reducing morbidity and preventing irreversible sequelae[11].

Description of the case

A 29-year-old housewife from Loja-Ecuador, of mixed ethnicity, with no relevant family or personal history, was evaluated at the outpatient service of the Isidro Ayora General Hospital, Loja, Ecuador. She presented with a pulsating holocranial headache of slight intensity. Concomitantly, she related a history of heartburn which responded positively to self-medication with omeprazole; additionally, she mentioned she was experiencing progressive loss of weight, fatigue and pain in the lower limbs and hands. Physical examination showed ecchymosis and petechiae scattered throughout the body. The liver and spleen were palpably enlarged. Routine laboratory results are summed up in Table 1.

Table 1. Laboratory results of patient's parameters and their normal range.

Patient's parameters		Normal range
Leukocytes (White blood cell)	3400 mm3	4.500 – 11.000 mm3
Neutrophils	1.9 K/uL	2.0 – 8.0 K/uL

Commented [TW1]: More information here would be valuable:
1.Details of the mixed ethnicity. Mestizo, Native American, any Afro? This is particularly important in light of the N409S/N409S genotype.
2. History of pregnancy and outcomes.
3. More details about family history including parents, grandparents, siblings. Any history of Parkinsonism or cancer in the family? How long is the family living in Loja. Where did they come from?

Patient's parameters		Normal range
Lymphocytes	1.5 K/uL	1.0 – 5.1 K/uL
Red blood cell	4.3 K/uL	4.0 – 11.0 K/uL
Hemoglobin	12.5 g/dL	12.1 – 15.1 g/dL
Hematocrit	38%	36.1% – 44.3%
Platelet	59 K/uL	150 – 400 k/uL
PCR (C-Reactive Protein (CRP))	16.1mg/L	0.0 – 3.0 mg/L
VSG (erythrocyte sedimentation rate (ESR)	33 mm/h	0 to < 20 mm/h
Total bilirubin	3 mg/dL	0.3 – 1.0 mg/dL
BD (Direct bilirubin)	0.48 mg/dL	0.0 – 0.3 mg/dL
BI (Indirect bilirubin)	2.52 mg/dL	0.1 – 0.5 mg/dL

Viral serology tests for hepatitis B, C and human immunodeficiency virus were negative. An ultrasound of the upper abdomen was performed, where there was evidence of splenomegaly and an enlarged liver without other abnormalities [Figure 1]. Hepatosplenomegaly was observed in the CT-Scan in addition to degenerative osteoarthritis of the dorsal spine.

A bone marrow biopsy demonstrated abundant cells between the bone trabeculae, consisting of hematopoietic tissue, with a marked decrease in megakaryocytes of hypolobular nuclei, and myeloid-erythroid relationship conserved. In the inter and paratrabecular spaces, many clusters of large macrophages with small, regular nuclei displaced to the periphery and “crumpled paper” cytoplasm were observed as found in classical Gaucher cells [Figure 2]. Now, suspecting GD, measurement of WBC glucocerebrosidase was performed that showed deficient activity (0.27 µmol/L/h (normal range: 2.3 – 12 nmol/h/mL). Sequential analysis of the GBA1 gene showed the presence of an apparently

Commented [TW2]: As Dr Sidransky had previously asked and suggested, what diagnoses were considered at this point that prompted the performance of the bone marrow biopsy? ITP? How did you explain the elevated total and indirect bilirubin? For example, was hereditary spherocytosis considered? Gilbert’s? Were there any gall stones on either the US or CT scans? The decrease in megakaryocytes is somewhat unusual for GD.

homozygous pathogenic alteration in the GBA1 gene (Nucleotide change: c.1226A>G; Amino acid change: G p. Asn409Ser).

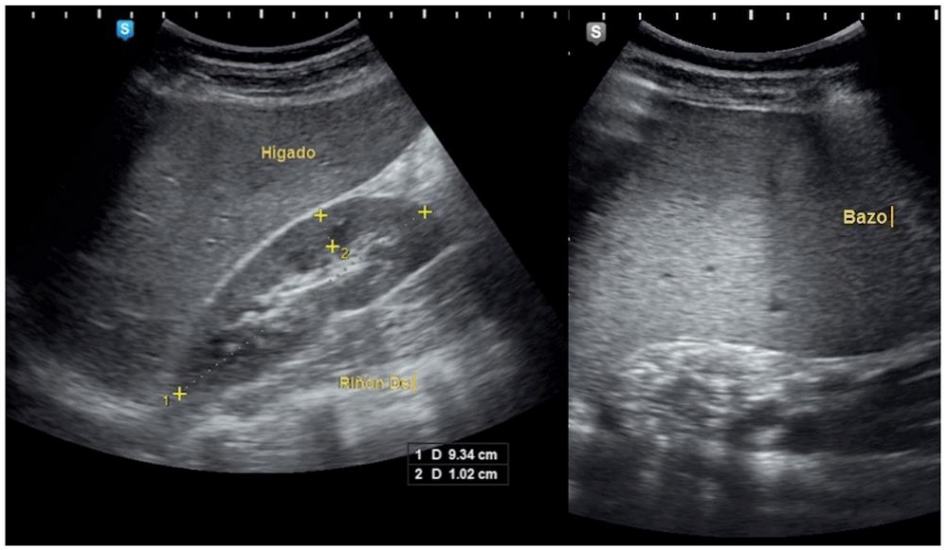

Figure 1. Upper abdomen ultrasonography where hepatosplenomegaly is observed.

Download as a PowerPoint slideDownload as a PowerPoint slide

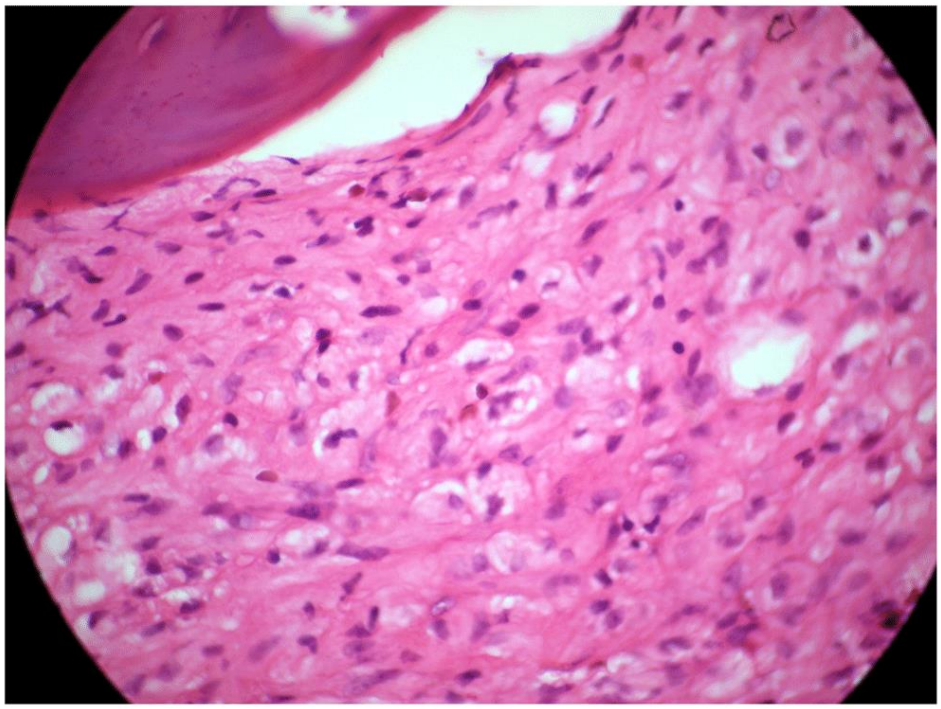

Figure 2. Bone marrow biopsy: Cuts show bony trabeculae, including extensive medullary replacement by histiocytic reticulum-like cells, with eosinophilic, pale cytoplasm and eccentric nuclei, some cells have a vacuolated cytoplasm with folded appearance on crumpled paper.

Download as a PowerPoint slideDownload as a PowerPoint slide

Unfortunately, enzymatic replacement or substrate reduction therapy are not available at our hospital. At the time of writing the patient is regularly monitored until ERT can be obtained. The patient remains in good general condition, without worsening signs of symptoms. It should be mentioned that although she continues to have thrombocytopenia, leukopenia and hepatosplenomegaly, her condition is expected to remit once the enzymatic treatment has been initiated[12].

Commented [TW3]: Now that the diagnosis of GD1 is confirmed, was any other testing done, especially of the bones (x-rays, MRI, DEXA?) Were any biomarker measurements made such as plasma chitotriosidase? As this is primarily an educational report, it is important to indicate whether recommended pre-treatment assessments were conducted within the capacity of the healthcare available.

Discussion

This clinical case is representative of many clinical, biochemical and genetic characteristics of GD type 1[7,13], a disorder that is characterized by variability in its signs, symptoms, severity and progression[14]. The most common signs and symptoms are: splenomegaly, hepatomegaly, radiological bone disease, thrombocytopenia, anemia], and bone pain [7,15].

GD is caused by mutations in the GBA1 gene, located on chromosome 1 (1q21), leading to markedly decreased activity of the lysosomal enzyme, β-glucocerebrosidase, which hydrolyzes the glycolipid

glucocerebroside into ceramide and glucose[16]. Our patient had sufficiently decreased glucocerebrosidase activity to develop classical manifestations of GD1. However, the identified genotype (N409S/N409S) was unexpected as this DNA variant is most often found among Ashkenazi Jews and is uncommon in the non-Jewish Latin American populations. For example, although the N409S allele was the most prevalent mutation found among 25 GD1 patients from Colombia (which borders Ecuador and which has a current Jewish population of approximately 13,000 of whom many are Ashkenazim who immigrated after World War 2), only two (8%) were homozygous for this variant, one Mestizo and one of unknown ethnicity[17]. Although comparable data for Ecuador are not published, it is therefore improbable that these very rare N409S/N409S GD1 patients in Columbia and Ecuador (ours being the index case) are associated with the 20th century Ashkenazi immigrants.

Rather, it is more likely that the descent of these patients can be traced to the time of the original Spanish conquests in Latin America. Haplotype analysis suggests that the original N409S allele arose in non-Jewish Europeans, was subsequently introduced into the Iberian Jewish (Sephardi) population sometime in the middle ages pre-dating the appearance of N409S in the Ashkenazi Jews[18]. Due to internal and external prohibition of intermarriage and sporadic bottleneck events, genetic diversity decreased in the Iberian Jewish population and N409S frequency increased. The prevalence of N409S homozygous GD1 patients in the modern Spanish Gaucher Registry population (N=178) is 14.6%[19] and likely reflects the eventual integration of large numbers of Iberian Jews who converted to Catholicism (conversos) into the general Spanish population. However, the inexorable persecution the Holy Office of the Inquisition caused great peril to the New Christians. Many illegally emigrated to the Spanish-Portuguese new world colonies where, for a time, the power of the Inquisition was less pronounced. It is likely that the N409S allele was introduced to Latin America by these Spanish-Portuguese emigres of the 16th century, some of whom eventually settled in Loja and its vicinity in what is now modern Ecuador[20-22]. It is conceivable that our patient is a descendant of converso settlers of Loja. This hypothesis could be further examined by testing for the presence of a GBA1 intronic polymorphism, 5470G→A that is present only in N409S alleles of Iberian origin[23].

Glucocerebrosidase deficiency causes accumulation of glucosylceramide in macrophages, transforming them to Gaucher cells that under optical microscopy are conspicuous as enlarged cells with eccentric nuclei and condensed chromatin, and cytoplasm with an appearance as “crumpled paper or napkin”[24]. These cells were abundant in the bone marrow biopsy specimen obtained from our patient. The presence of apparent Gaucher cells (pseudo-Gaucher cells) has also been described in the absence of GD in patients with various hematologic malignancies, hemolytic anemias such as thalassemia, and mycobacterial infections [25]. Thus, the Gaucher cell is not pathognomonic, and only demonstration of absent or reduced glucocerebrosidase activity is the gold standard for the diagnosis of all GD variants.

The usual signs and symptoms associated with GD1 are shared many other more common illnesses including chronic liver disease, hematologic malignancies, and, in relevant parts of the world with infectious diseases such as malaria, leishmaniasis, and mycobacterioses[26]. Failure to include GD in the differential diagnosis of a patient with classic manifestations can lead to unnecessary invasive diagnostic procedures, misdiagnosis resulting in inappropriate treatment such as splenectomy, and to delayed initiation of effective therapy causing largely preventable irreversible complications and permanent disabilities especially from skeletal disease. Therefore, it is essential to continue to disseminate knowledge about GD including its worldwide prevalence, methodologies for diagnosis, and current and evolving guidelines for management and therapy.

Conclusion

Despite its relative rarity, GD should always be considered in the differential diagnosis of patients with unexplained pancytopenia and/or hepatosplenomegaly. The ease of obtaining and transporting stable dried blood spot samples for diagnosis makes it feasible to detect GD and many other rare LSDs even in remote and medically deprived regions of the world. A high index of suspicion together with enzymatic and genetic testing are essential for an early specific diagnosis followed by the administration of timely treatment, thus minimizing the risk for irreversible complications and chronic morbidity that detract from health-related quality of life and even shorten life expectancy.

Consent

Written informed consent for publication of their clinical details and clinical images was obtained from the patient.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

References

1. Schultz ML, Tecedor L, Chang M, et al.: Clarifying lysosomal storage diseases. Trends Neurosci. 2011; 34(8): 401–410. PubMed Abstract | Publisher Full Text | Free Full Text

2. Rigante D, Cipolla C, Basile U, et al.: Overview of immune abnormalities in lysosomal storage disorders. Immunol Lett. 2017; 188: 79–85. PubMed Abstract | Publisher Full Text

3. Linari S, Castaman G: Clinical manifestations and management of Gaucher disease. Clin Cases Miner Bone Metab. 2015; 12(2): 157–164. PubMed Abstract | Publisher Full Text | Free Full Text

4. Wikipedia: The current Jewish population of Ecuador. Accessed 12/14/19.

5. Brady RO, Kanfer JN, Bradley RM, Shapiro D. Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease. J Clin Invest. 1966 Jul;45(7):1112-1115.

6. Dandana A, Ben Khelifa S, Chahed H, et al.: Gaucher Disease: Clinical, Biological and Therapeutic Aspects. Pathobiology. 2016; 83(1): 13–23. PubMed Abstract | Publisher Full Text

7. Stirnemann J, Belmatoug N, Camou F, et al.: A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. Int J Mol Sci. 2017; 18(2): pii: E441. PubMed Abstract | Publisher Full Text | Free Full Text

8. Nalysnyk L, Rotella P, Simeone JC, et al.: Gaucher disease epidemiology and natural history: a comprehensive review of the literature. Hematology. 2017; 22(2): 65–73. PubMed Abstract | Publisher Full Text

9. Mistry PK, Sadan S, Yang R, Yee J, Yang M. Consequences of diagnostic delays in type 1 Gaucher disease: the need for greater awareness among hematologists-oncologists and an opportunity for early diagnosis and intervention. Am J Hematol. 2007 Aug;82(8):697-701.

10. Paz-y-Miño C, Guillen Sacoto MJ, Leone PE. Genetics and genomic medicine in Ecuador. Mol Genet Genomic Med. 2016 Jan; 4(1): 9–17.

11. Charrow J, Dulisse B, Grabowski GA, et al.: The effect of enzyme replacement therapy on bone crisis and bone pain in patients with type 1 Gaucher disease. Clin Genet. 2007; 71(3): 205–11. PubMed Abstract | Publisher Full Text

12. Mistry PK, Lopez G, Schiffmann R, Barton NW, Weinreb NJ, Sidransky E. Gaucher disease: Progress and ongoing challenges. Mol Genet Metab. 2017 Jan - Feb;120(1-2):8-21.

13. Giraldo P, Pérez-López J, Núñez R, et al.: Patients with type 1 Gaucher disease in Spain: A cross-sectional evaluation of health status. Blood Cells Mol Dis. 2016; 56(1): 23–30. PubMed Abstract | Publisher Full Text

14. Bohra V, Nair V: Gaucher's disease. Indian J Endocrinol Metab. 2011; 15(3): 182–186. PubMed Abstract | Publisher Full Text | Free Full Text

15. Kaplan P, Andersson HC, Kacena KA, et al.: The clinical and demographic characteristics of nonneuronopathic Gaucher disease in 887 children at diagnosis. Arch Pediatr Adolesc Med. 2006; 160(6): 603–608. PubMed Abstract | Publisher Full Text

16. Hruska KS, LaMarca ME, Scott CR, et al.: Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). Hum Mutat. 2008; 29(5): 567–583. PubMed Abstract | Publisher Full Text

17. Pomponio RJ, Cabrera-Salazar MA, Echeverria OY, G. Miller G, Barrera LA. Gaucher disease in Colombia: Mutation identification and comparison to other Hispanic populations. Molecular Genetics and Metabolism 86 (2005) 466–472.

18. Rodríguez-Marí A, Díaz-Font A, Chabás A, Pastores GM, Grinberg D, Vilageliu L. New insights into the origin of the Gaucher disease-causing mutation N370S: extended haplotype analysis using the 5GC3.2, 5470 G/A, and ITG6.2 polymorphisms.Blood Cells Mol Dis. 2001 Sep-Oct;27(5):950-959.

19. Alfonso P, Aznarez S, Giralt M, Pocovi M, Giraldo P; Spanish Gaucher's Disease Registry. Mutation analysis and genotype/phenotype relationships of Gaucher disease patients in Spain.

J Hum Genet. 2007;52(5):391-396.

20. Chacon-Duque JC, Adhikari K, Fuentes-Guajardo M et al. Latin Americans show wide-spread Converso ancestry and imprint of local Native ancestry on physical appearance. Nat Commun. 2018 Dec 19;9(1):5388. DOI: 10.1038/s41467-018-07748-z.

21. History of the Jews in Ecuador. Wikipedia. Accessed December 15, 2019.

22. Ricardo Ordóñez Chiriboga. La herencia sefardita en la provincia de Loja. 2005: Casa de la Cultura Ecuatoriana.

23. Rodríguez-Marí A1, Díaz-Font A, Chabás A, Pastores GM, Grinberg D, Vilageliu L. New insights into the origin of the Gaucher disease-causing mutation N370S: extended haplotype analysis using the 5GC3.2, 5470 G/A, and ITG6.2 polymorphisms. Blood Cells Mol Dis. 2001 Sep-Oct;27(5):950-9.

24. Chen M, Wang J: Gaucher disease: review of the literature. Arch Pathol Lab Med. 2008; 132(5): 851–853. PubMed Abstract

25. Ash Image Bank: Waldenström macroglobulinemia with pseudo-Gaucher cells. Blood. 2010; 116(18): 3388. PubMed Abstract | Publisher Full Text

26. Thomas AS, Mehta AB, Hughes DA: Diagnosing Gaucher disease: an on-going need for increased awareness amongst haematologists. Blood Cells Mol Dis. 2013; 50(3): 212–7. PubMed Abstract | Publisher Full Text