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β-Galactosidase Deficiency in Colombia: Report of 20 Patients Detected Using Dried Blood Spot Samples

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Abstract

β-Galactosidase (BGal) is the first enzyme involved in the catabolism of sphingolipids. Two pathologies have been directly associated with its deficiency: GM1 gangliosidosis and Morquio B. Morquio B is among the rarest types of mucopolysaccharidosis (MPS). We aim to document the β-galactosidase deficiency in Colombia. We evaluated leukocytes from 1492 healthy Colombian individuals and 923 patients, referred between 2005 and August 2014. Dried blood spot (DBS) samples from the same number of patients were evaluated. β-Galactosidase was measured with 4-methylumbelliferyl-β-D-galactoside. As a control enzyme, the total hexosaminidase activity was also evaluated. We identified 14 patients with GM1 gangliosidosis, 5 patients with Morquio B, and 1 patient with I-cell disease. We could establish a reference value for Bgal in Colombian leukocyte samples. GM1 gangliosidosis is the main pathology associated with a direct deficiency of BGal. The high number of patients found with MPS IVB indicates that there are patients who could be misdiagnosed due to an unawareness of the disease.

Keywords

β-galactosidase, GM1 gangliosidosis, Morquio B, leukocytes, dried blood spot

Introduction

β-Galactosidase (BGal) is a lysosomal enzyme that cleaves the β linkage of galactose from GM1 gangliosides.[1,2] This enzyme is encoded by the GLB1 gene, which is located in 3p21.33 and is composed of 16 exons with 2 splicing sites that give rise to 2 different proteins: BGal and elastin-binding protein (EBP).[2] β-Galactosidase is the first enzyme involved in the catabolism of sphingolipids; although its main substrate is the GM1 ganglioside, it also uses keratan sulfate and other specific oligosaccharides (such as α-L-arabinosides and β-D-fucosides) as alternative substrates.[1]

The versatility of Bgal to degrade different substrates can be explained by its catalytic mechanism that involves the formation of a multi-enzyme complex with 2 more proteins: Neuraminidase (NEU-1) and Protective protein/Cathepsin-A (PPCA).[2,3] Although the coupling with an additional enzyme (N-galactose-6-sulfate-sulfatase) has been suggested, there is still not enough evidence that supports this hypothesis.[3]

The failure in the coupling of this enzymatic complex brings as a consequence several pathologies depending on the mutation and the enzymes involved.[1,3,4] If the deficiency affects the folding or catalytic activity of NEU-1, the patient is diagnosed with sialidosis (OMIM: 256550).[2,5,6] But, if the mutation is located in the gene encoding PPCA, the disease would then be galactosialidosis (OMIM: 2566540).[2,3,6] This is the reason why, when evaluating markers such as urine oligosaccharides to make a diagnostic approach, it is necessary to take in account that the excretion of these compounds needs to be correlated with the evaluation of BGal, NEU-1, and PPCA enzymatic activities in leukocytes.[2]

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Two pathologies have been directly associated with the deficiency of Bgal, named GM1 gangliosidosis (GGM1; OMIM: 230500) and Morquio B disease (or mucopolysaccharidosis[MPS] IV B; OMIM: 253010).[1,4] Although several mutations have been described, some authors suggest that those affecting the catalytic pocket of the enzyme are mainly associated with GGM1, while mutations located in the surface of this enzyme are more frequently found in patients with Morquio B and interfere with the correct enzyme complex assembly.[3,5,7] This hypothesis is consistent with the phenotypical presentation of both of these diseases, since GGM1 phenotype is neurologically devastating and has low life expectancy for children, while in Morquio B, there is no neurological commitment and life expectancy is greater, finding even adults with late diagnoses.[1,8]

Although GGM1 is a pathology characterized by neurological involvement, the clinical manifestations range widely. To better orient the diagnosis, 3 forms of the disease have been described: an infantile form affecting children younger than 2 years old that is characterized by neurodegeneration, developmental regression, hypotonia, splenomegaly, and dysmorphic face; a juvenile form, presented in late childhood and adolescence (between 2 and 6 years old, approximately), with patients usually showing cerebral and cerebellar atrophies; and an adult form, with patients exhibiting in addition some psychotic episodes.[1,2,5,8]

On the other hand, Morquio B disease is a deficiency characterized by bone abnormalities and the accumulation of keratan sulfate. This mucopolysaccharide generates confusion in the diagnosis, hence patients can be wrongly identified as having Morquio A in a urine test. This happens because the same substrate is accumulated in cells but the deficient enzyme is different (N-galactose-6-sulfate-sulfatase).[2,9] Although usually these patients do not show neuronal commitment, Giugliani et al[9] reported a case of 2 siblings with mild mental commitment, thus suggesting that keratan sulfate can also be stored in the nervous system.

GM1 gangliosidosis has a calculated incidence of 1:100.000 to 200.000 births.[8] Meanwhile, Morquio B is among the rarest types of MPS yet described, showing a variable global incidence that ranges between 1:75.000 (North Ireland) and 1:640.000 (West Australia).[3] For Latin America, some case reports of both diseases are available in the literature, but, except Brazil,[10] no other country has official statistics about their incidence. This aspect is of great importance for the diagnosis of Morquio B in Latin American population, given that another syndrome (Morquio A), with similar clinical aspects and excretion of GAGs, is prevalent in some countries and could lead to a misdiagnosis in patients showing normal enzymatic values when they are analyzed for MPS IVA.

The purpose of the present article is to document the Bgal deficiency in Colombia. Here we identified 14 cases of GGM1, 1 case with I-cell disease, and 5 new cases with Morquio B, 4 of them diagnosed during the past 6 months. The high number of patients found for MPS IVB is related to a higher number of patients referred for high-risk screening of Morquio A, suggesting that the disease could not be as rare as expected and that there are patients being misdiagnosed due to an unawareness of the disease.

Methods

The analysis of Bgal is used in our laboratory with 2 purposes: the first one is to evaluate patients with clinical symptoms suggesting either GGM1 or Morquio B; the second one is to determine the quality of all samples arriving to the laboratory, which means that besides analyzing a specific enzymatic activity of a patient with clinical signs suggesting a lysosomal disorder, we test the activity of both Bgal and total hexosaminidase to determine whether the sample is suitable for analysis (see Figure 1).

Patients

We evaluated leukocytes from 1492 healthy colombian individuals (age: 2 months to 75 years) and 923 patients (mainly from Colombia, >98%) with progressive neurological damage and/or multiple dysostosis (age: 2 months to 50 years) who were referred to our laboratory between 2005 and August 2014 as part of a high-risk screening program either for lysosomal storage diseases or oriented toward Morquio A disease. Their results were used to determine a cutoff value for the detection of patients with decreased BGal activity. For dried blood spot (DBS) samples, we evaluated the same group of patients, but the DBS activity values were compared with their reported reference values.[11]

The control group is defined as apparently healthy and clinically defined individuals. Meanwhile, patients are defined as subjects with clinical findings related to a lysosomal storage disease that came from neurology, pediatrics, and genetic services from different locations in Colombia. Every person who participated in this study signed an informed consent approved by the Ethics committee of the Universidad de Los Andes, and all the procedures followed the regulations stated in the Helsinki Declaration (1993).

Samples

Blood samples were obtained by venipuncture or finger prick according the patient's age and were directly collected on Whatman filter paper grade 903 (Schleicher and Schuell, Dassel, Germany). Then they were dried at room temperature for a period of at least 8 hours and then the samples were stored at 4°C in sealed plastic bags to avoid deterioration caused by humidity. The maximum time between sampling and analytical processing was no longer than 30 days. Samples were sent from different locations in Colombia and transportation times to the laboratory were between 2 and 7 days. The confirmation of deficient enzymatic activities required the isolation of total leukocytes, cellular lysis, and fluorometric evaluation of enzyme

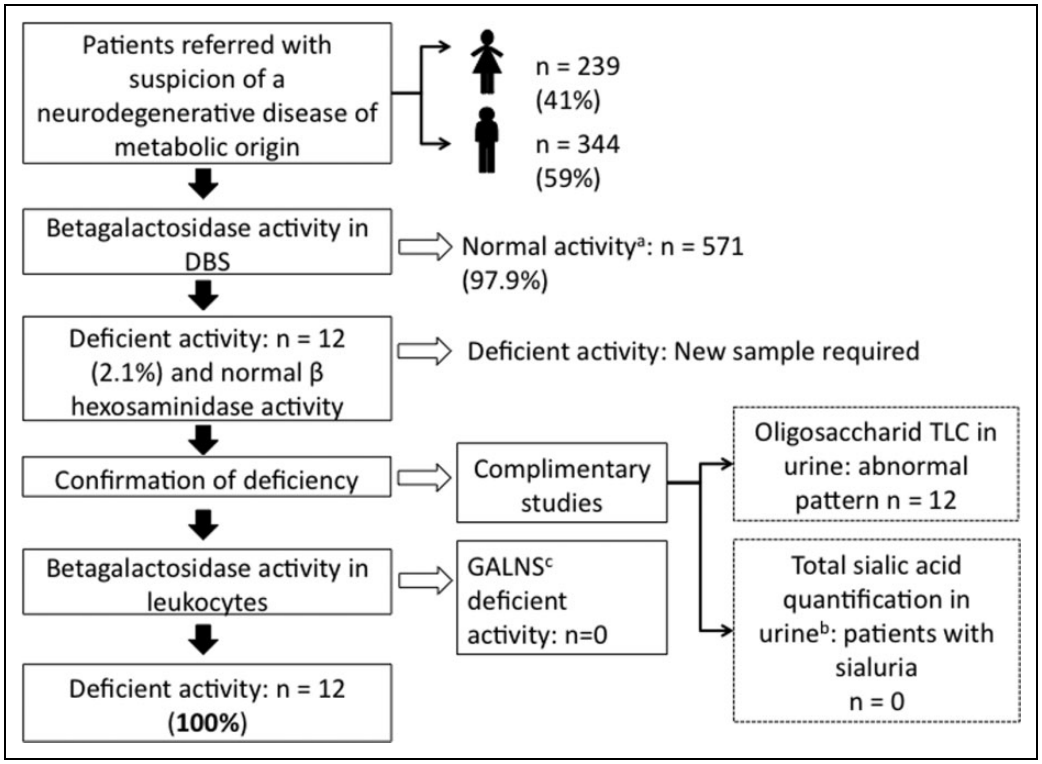

Figure 1. Flowchart illustrating the analysis of samples to determine β-galactosidase deficiency.

activity. These samples were processed according to the method described by Shapira.[12]

Enzymatic Assays

Both methods for evaluation of BGal used the same fluorogenic substrate (4-methylumbelliferyl-β-D-galactoside) and incubation buffer (citrate-phosphate). The DBS samples were analyzed by duplicate according to the method described by Uribe and Giugliani,[11] and the enzymatic activities were expressed as nanomol of substrate hydrolyzed per milliliter of sample per hour (nmol/mL/h). Here milliliters are calculated in accordance to what Uribe and Giugliani[11] described for DBS punches of 1.2 mm. Leukocyte samples were analyzed following the protocol of Shapira[12] by duplicate, and activities are expressed in nanomol of substrate hydrolyzed by milligram of protein per hour (nmol/mg protein/h).

Analyses for total hexosaminidase (TH) activity (control enzyme) were made in both leukocyte and DBS samples using 4-methylumbelliferyl N-acetyl-β-D-glucosaminide as described by Uribe and Giugliani[11] and Shapira,[12] respectively. A 4-methylumbelliferone (4-MU) curve was used for calibration and Sigma Aldrich (St Louis, Missouri) provided all the reagents.

Complimentary studies to evaluate galactosialidosis and sialidosis involved sialic acid quantification and oligosaccharides characterization by thin-layer chromatography of spontaneous urine samples (supplementary Figure 1). These were evaluated strictly following the procedure described by Skoza and Mohos.[13]

Statistical Analysis

Descriptive statistics and graphics were made using R[14] and Prism 5 (GraphPad Software Inc, La Jolla, CA, USA). A Shapiro-Wilk test was used to evaluate normal distribution among control participants, and a Student t test or Mann-Whitney U test was used to determine significant differences between the studied groups. A receiver–operating characteristic (ROC) curve was used to determine a cutoff value in leukocytes samples.

Results

Of all the patients analyzed, we found a total of 14 patients with GGM1, 5 patients with Morquio B, and 1 patient with I-cell disease. Although the patients with GGM1 were diagnosed through the past 9 years, only during the last year, 4 patients with Morquio B disease were identified (Table 1). With the DBS program, adopted in 2005, we detected the first 3 patients with GGM1 in 2007 from Colombia (AF-1) and Peru (AFNC-1 and AFNC-2).

The results of BGal activity in leukocyte samples allowed us to establish a cutoff value for Colombian population (53.6 nmol/mL/h) with a 95% confidence and 100% sensitivity and specificity, using an ROC curve. The activity range for 1492 control participants was 80.1 to 557 nmol/mL/h (median: 203 nmol/mL/h, mean: 222.36 nmol/mL/h), while the range for patients was 0.47 to 7.84 nmol/mL/h, with a median of 2.88 nmol/mL/h and an average of 2.82 nmol/mL/h (Figure 2). Given that neither the results from patients nor the results from

Table 1. Patients Diagnosed With GLB1 Deficiency.[a]

Year of Remission	Diagnosis	Patient	Age	Sex	Country	Begal DBS, nmol/mL/h	Hexo DBS, nmol/mL/h	Begal LEU, nmol/mL/h	Residual Activity DBS[b]	Residual Activity LEU
2007	GGM-1	AF-1	1.0	M	COL	0.08	523.53	1.56	0.17%	0.70%
2007	GGM-1	AFNC-1	1.0	F	PER	2.30	769.50	2.92	4.90%	1.31%
2007	GGM-1	AFNC-2	1.8	F	PER	0.91	453.80	7.84	1.94%	3.53%
2008	GGM-1	AFNC-3	0.7	F	PER	7.51	161.80	3.18	16.01%	1.43%
2008	GGM-1	AF-2	1.0	F	COL	0.55	282.10	0.47	1.17%	0.21%
2008	GGM-1	AF-3	1.5	M	COL	0.91	999.61	0.66	1.94%	0.30%
2008	GGM-1	AF-4	1.5	M	COL	1.46	673.81	1.35	3.11%	0.61%
2008	GGM-1	AF-5	1.8	M	COL	2.28	755.10	2.22	4.86%	1.00%
2010	GGM-1	AF-6	2.5	F	COL	1.33	303.2	3.17	2.84%	1.43%
2010	GGM-1	AF-7	2.3	F	COL	1.91	244.92	5.55	4.07%	2.50%
2011	GGM-1	AF-8	4.2	F	COL	0.95	330.58	10.79	2.03%	4.85%
2011	GGM-1	AF-9	1.1	F	COL	2.33	724.84	1.97	4.97%	0.89%
2011	GGM-1	AF-10	1.5	M	COL	1.73	576.90	3.49	3.69%	1.57%
2012	GGM-1	AF-12	0.5	M	COL	1.35	684.04	0.05	2.88%	0.02%
2013	I-CELL	AF-13	2.9	M	COL	34.60	1795.80	5.84	73.77%	2.63%
2000	MPS IVB[c]	AF-11	4.0	M	COL	2.10	476.80	27.60	4.48%	12.41%
2014	MPS IVB	AF-14	8.0	F	COL	3.07	527.18	2.71	6.55%	1.22%
2014	MPS IVB	AF-15	0.8	M	COL	2.80	618.13	0.00	5.97%	0.00%
2014	MPS IVB	AF-16	10.9	M	COL	3.85	299.83	2.45	8.21%	1.10%
2014	MPS IVB	AF-17	16.8	M	COL	1.75	294.98	3.61	3.73%	1.62%

Abbreviations: COL, Colombia; DBS, dried blood spot; LEU, leukocytes; GGM-1, gangliosidosis GM1; I-CELL: I-cell disease; MPS IVB, mucopolysaccharidosis type IVB; PER, Peru; AF, Colombian affected patient; AFNC, Non-Colombian affected patient.
[a]All the enzymatic activities are reported as nanomol of substrate hydrolyzed by mililiter of blood per hour. [b]Compared to the reference values established for Colombian population (see Uribe & Giugliani[11]). [c]Patients with normal GALNS (Galactose-6-sulfate-sulfatase) activity, the deficient enzyme in patients with Morquio A.

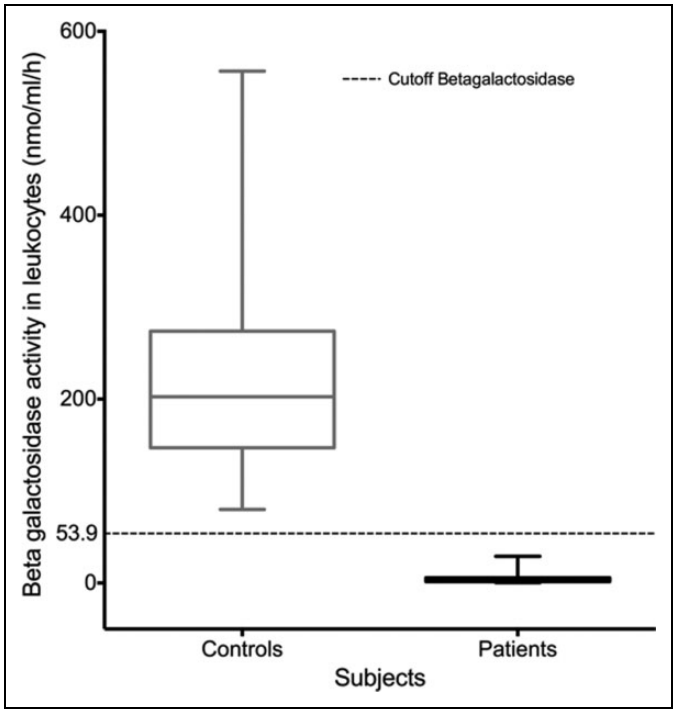

Figure 2. β-Galactosidase activity in leukocyte samples. Midline represents median and whiskers show the minimum–maximum range.

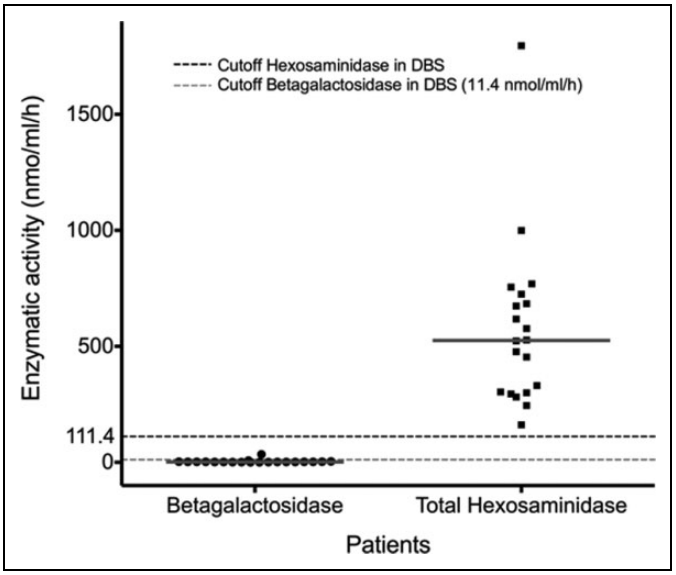

Figure 3. β-Galactosidase and total hexosaminidase activity from patients diagnosed with a GLB1 deficiency. Midline represents median.

controls followed a normal distribution, we used a Mann-Whitney *U* test to compare them. As a result, we found a significant difference between the groups studied, with a *P* value <.0001. We also compared between gender, finding no differences between males and females neither in leukocyte samples (*P* value = .89, Mann-Whitney) nor in DBS (*P* value = .78, Student *t* test).

With regard to the results from DBS in patients, we found decreased activities compared to the reference value reported

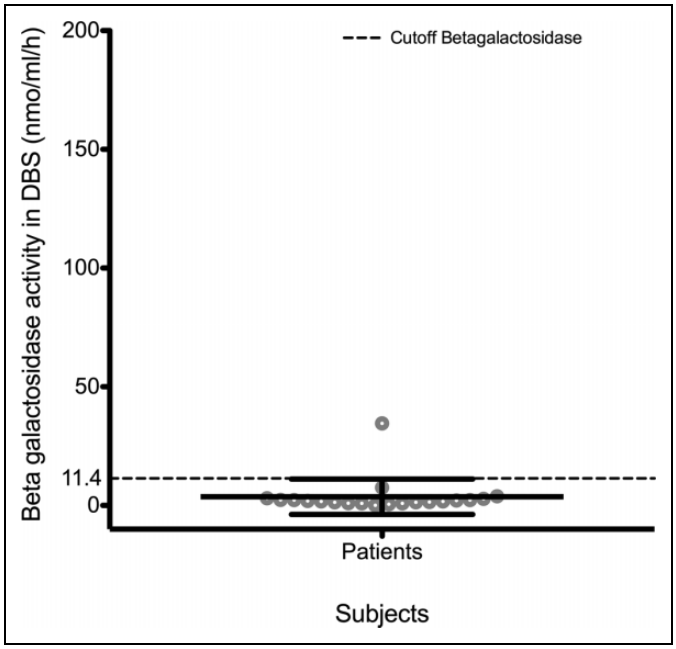

Figure 4. β-Galactosidase activity in dried blood spot (DBS) samples. Midline represents median.

by Uribe and Giugliani.[11] Sample quality was verified through the evaluation of total hexosaminidase activity, finding that all the patients had values comparable to controls.[11] The median of the patients analyzed was 1.74 and the range was 0.08 to 3.85 nmol/mL/h (Figures 3 and 4). From a total of 20 patients showing a decrease in Bgal activity in leukocytes, 14 were diagnosed with GGM1 and 5 with MPS type IVB.

One patient (AF-13, Bgal activity [DBS] = 35.4 nmol/mL/h) showed a marked decrease in leukocytes but a normal Bgal activity in DBS. This result, in addition to an overexpression of TH in DBS, drove us to make further analyses. As a result, the patient showed an increase of sialic acid in urine and a multiple sulfatase overexpression in serum, orienting diagnosis toward I-cell disease (data not shown).

We also determined the percentage of residual activity from all the patients showing a Bgal deficiency, by comparing patients' activities with the mean of the control activities both in DBS and in leukocytes. The results show that all the patients had a decrease of more than 80% (Table 1).

Discussion

β-Galactosidase deficiency is known to be the cause of several rare disorders, due to its cosmopolitan role in the metabolism of gangliosides, oligosaccharides, and mucopolysaccharides. Of these disorders, the most common one is GGM1, a neurodegenerative disease affecting the central nervous system. The clinical symptoms that are regularly associated with this deficiency are hypotonia, developmental delay, and hepatosplenomegaly,[8] which makes the diagnosis more difficult.

In the present study, we found that most patients were diagnosed with GM1 gangliosidosis, making it the most frequent pathology associated with β-galactosidase deficiency in Colombia (n = 14 of 20 patients). Most of these patients were diagnosed during 2008, probably due to a neurodegenerative disease screening made at that time in DBS. Also, most of the residual activities in these kind of samples are below 5%, except 1 Peruvian patient who showed 16.01% of residual activity. It has been reported that the degree of residual activity could be explained by the location or the kind of mutation in the enzyme. For this patient, however, this value, was confirmed in a leukocyte sample, showing a deficient value of 3.18 nmol/mL/h (reference value: 11.4 nmol/mL/h).

When reviewing the literature for reports of patients with BGal deficiency in Latin America, we found that GGM1 is also the main pathology directly associated with a deficiency in BGal activity. For example, in 2007, Cuba reported 11 patients detected after a screening of 851 patients with clinical symptoms suggesting GGM1.[15] During the same year, Santamaría et al[16] reported 20 Argentinian patients (8 of them siblings) affected with different types of GGM1, with the most frequent being the type 1. They also showed 2 Uruguayan patients and a Brazilian one. It is important to know that previously 2 cases of GGM1 were reported in Uruguay[17] and that Brazil has been documenting several patients during the past 22 years, with the first report dating from 1982.[18] All of these publications show that the main type of GGM1 affecting these patients was type 1, which is in accordance with our findings.

Of the remaining 6 patients, 5 were diagnosed with Morquio B. One of the most intriguing aspects when analyzing these data was that 4 of them were detected during the last year, within 3 months. The increase in detected patients can be explained by an increase in the referred samples from patients with suspicion of MPS IVA. Due to some overlapping symptoms between these diseases, it is possible that these 4 patients have initially been thought as patients with MPS IVA, since this last one has been described as a more prevalent disease in Latin American countries.[19-21]

The fact that such a great number of patients were found with a high-risk screening program for MPS IVA suggests that there could be a group of patients with Morquio B who are being misdiagnosed because the disease is considered of too low incidence to look for it. This is why we suggest that when there is clinical suspicion for a Morquio, both Bgal and NAGS activities should be determined in leukocytes.

During the past few years, the reports for Bgal deficiency from Latin American countries, except Brazil, are scarce. Some other reports, like one from Argentina[16] and another from Cuba,[22] show the worldwide documented low incidence of the disease (just 1 case in Cuba during the past 20 years). However, brazilian studies had demonstrated that the incidence of the mutation might not be as low as expected, since their results show a genetic penetrance of the mutation as high as 1:58.[10]

Brazil is also the country that reports more cases of Morquio B in the region, documenting 4 patients between 2004 and

2011. Given that we report the same number of patients in less than a year, we consider that this disease should be taken more into account when patients with neurodegenerative disorders associated with skeletal displasias are being diagnosed. Another aspect to take into account is that several mutations affecting GLB1 gene have shown intermediate phenotypes that make difficult to discern between GGM1 and Morquio B disease.[9,21,23-25]

Also, it is important to accomplish that some patients with Morquio B have been diagnosed in the adulthood,[21,26] indicating that the late detection of this disease could leave several patients without diagnosis. Actually, in this study, we found that 4 of our 5 patients were identified at an old age (more than 3 years old) and their initial diagnosis was not of a GLB1 deficiency, since they were thought to have Morquio A disease due to the skeletal abnormalities.

We also found 1 patient with suspicion of GGM1, who initially showed a decrease in BGal activity in leukocytes but when analyzed in DBS showed a normal activity for this enzyme. The patient also showed an overexpression of TH activity in DBS, while in leukocytes the activity of this enzyme was comparable to control values, discarding that the low activities were due to a defective sample. The absence of correlation between DBS and leukocyte results suggested a disorder in the transportation of the enzymes to the lysosome instead of an enzymatic deficiency.

Since DBS samples are heterogeneous, it is possible that the activity of Bgal corresponded to an extracellular activity, indicating that the enzyme was not properly targeted to the lysosome and that the differences between results in leukocytes and DBS could be explained by this activity. To confirm this hypothesis, we analyzed some biomarkers such as sialic acid and mucopolysaccharides in urine, and multiple sulfatases (arylsulfatase A, B, and iduronate sulfatase) in serum. The results showed a general excretion of the biomarkers analyzed (suggesting a defect on neuraminidase and sialidase) and an overexpression of sulfatases in serum, indicating that there was a general deficiency of several lysosomal enzymes within the cell (sulfatases, neuraminidase, sialidase, and β-galactosidase), directing the diagnosis toward I-cell disease.

Although I-cell disease is not a defect of a lysosomal enzyme, it is classified between these disorders because the failure in targeting multiple lysosomal hydrolases.[27] Due to the fatality of the disease caused by the global involvement and multiple enzyme deficiency, it is very rare to find patients with this disease. In Colombia, this patient represents the first report for the disease, but further molecular analyses are being carried out to completely define this patient.

In spite of the feasibility of DBS for the screening of patients, the variations here shown suggest that, at least for Bgal, this kind of sample cannot be used as a diagnostic test, but instead it should be used as a diagnostic approach, and a second enzymatic evaluation in leukocytes must be made for a definitive diagnosis. In a retrospective study, Chamoles et al[28] showed that DBS samples are useful for the diagnostic approach of patients for whom it is not easy to make a diagnosis, due to the facility of taking an old sample and testing it again. Here we could illustrate how this kind of sample is also useful for the detection of patients through a high-risk screening program, making the diagnosis accessible to more patients and the sampling easier to the health care workers.

In conclusion, the present study allowed us to identify 14 patients with GGM1, 5 patients with Morquio B disease, and 1 patient with I-cell disease. We could also establish a reference value for Bgal activity in leukocyte samples of Colombian population and to determine that, as is reported in other Latin American countries, GGM1 is the main papthology associated with a direct deficiency of BGal. However, the amount of cases with Morquio B warns about the necessity to include it as a diagnostic possibility for children showing either Morquio A phenotypes or a delay in development associated with GGM1 but without cognitive involvement.

Finally, we suggest the use of DBS samples as a tool for the diagnosis of rare diseases, such as the ones discussed in this article, especially in low-income countries, where the cost can be diminished and the test could be available to a wider population.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

The online data supplements are available at http://iem.sagepub.com/supplemental.

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