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Early Diagnosis of Gaucher Disease and ASMD in Sardinia: The "Ichnos" Project

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To the editor.

Gaucher disease (GD) and Acid Sphingomyelinase Deficiency (ASMD) are autosomal recessive lysosomal storage disorders (LSDs) caused by biallelic pathogenic variants in *GBA1* and *SMPD1*, respectively. The resulting enzymatic defects lead to progressive accumulation of undegraded sphingolipids within macrophages and parenchymal cells, producing chronic, multisystemic, and often irreversible organ damage.[1,2] Diagnostic delays remain common worldwide and contribute to significant morbidity, impaired quality of life, and increased healthcare burden.[3] Clinical overlap between GD and ASMD, particularly splenomegaly, hepatomegaly, cytopenias, bone involvement, and constitutional symptoms, further complicates early recognition. Dried blood spot (DBS) enzymatic assays provide a practical, first-line tool for screening for these conditions and allow simultaneous measurement of glucocerebrosidase (GCase) and acid sphingomyelinase (ASM) activities.[4,5] When enzymatic results are borderline or discordant with clinical suspicion, molecular testing is required to confirm diagnosis, identify carriers, and facilitate cascade testing within families.[5] Multiple biomarkers, including ferritin, chitotriosidase, and CCL18, are frequently used to support diagnosis and monitor disease activity; more recently, glucosylsphingosine (Lyso-GB1) has emerged as the most specific and sensitive marker for GD, with strong correlation to disease burden and therapeutic response.[6-8] Analogous biomarkers for ASMD include lysosphingomyelin (Lyso-SM) and its derivative Lyso-SM-509.[9]

Within this context, we conducted the Ichnos Project, a multicenter observational initiative designed to evaluate whether a structured, cross-departmental diagnostic approach could enhance early detection of GD and ASMD in Sardinia, a genetically and geographically distinctive region. The project involved major hospitals across the island and incorporated standardized referral criteria and a unified diagnostic algorithm (**Figure 1**).

Figure 1. Diagnostic algorithm for discriminating GD from ASMD. The algorithm describes the approach used for the Ichnos Project's patient enrollment.[1]
Abbreviations: CHD, coronary artery disease; HDL, high-density lipoprotein.

Between April 2022 and May 2023, 196 individuals presenting with clinical findings suggestive of LSDs, including unexplained splenomegaly or hepatomegaly, cytopenias, bone pain, hyperferritinemia, or multisystemic features, were screened across six major hospitals in Sardinia. Participating departments included Hematology, Oncology, Internal Medicine, Pediatrics,

Table 1. Clinical and demographic characteristics of patients enrolled (*n* = 196).

Variable	
Clinical characteristics	
Age, median *years* (IQR)	50.5 (15.7-66)
Male sex, *n* (%)	82 (41.8)
Laboratory parameters	
Hemoglobin, median *g/dL*	12.8 (11.6-13.9)
Leukocyte count, median x10^9/L	5.4 (4.2-7.1)
Platelet count, median x10^9/L	110 (80-225)
AST, median *UI/L* (IQR)	29 (22-38)
ALT, median *UI/L* (IQR)	33 (24-45)
Total cholesterol, median *mg/dL*	162 (145-188)
HDL-c, median *mg/dL*	39 (33-46)
LDL-c, median *mg/dL*	82 (68-120)
Triglycerides, median *mg/dL* (IQR)	156 (118-212)
Enzymatic activity	
GCase activity, median *nmol/h/ml* (IQR)	5.6 (4.4-7.4)
ASM activity, median *µmol/h/L* (IQR)	5.65 (3.5-8.5)
Biomarkers	
Lyso-GB1, median *ng/mL* (IQR)	2.5 (0.5-4.1)
Lyso-SM509, median *ng/mL* (IQR)	0.3 (0.3-0.475)

Abbreviations: ALT, alanine aminotransferase; ASM, acid sphingomyelinase; AST, aspartate aminotransferase; GCase, glucocerebrosidase; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; Lyso-GB1, glucosylsphingosine; IQR, interquartile range.

Rheumatology, Gastroenterology, Orthopedics, Transfusion Medicine, Pathology, Hepatology, Neuropsychiatry, and Rare Disease Units. DBS assays measured GCase and ASM activity. Pathological values were defined as ≤2.5 nmol/h/mL for GCase activity and ≤1.7 µmol/h/L for ASM. Individuals with decreased enzyme activity or highly suggestive clinical features underwent molecular analysis for *GBA1* or *SMPD1*. Quantification of Lyso-GB1 or Lyso-SM509 was performed based on one or more of the following criteria: (i) reduced GCase or ASM enzymatic activity below the normal range; (ii) detection of at least one pathogenic or likely pathogenic genetic variant; (iii) familial relationship with a genetically confirmed case; or (iv) the presence of clinical manifestations highly suggestive of GD or ASMD, particularly skeletal involvement. Written informed consent was obtained from all participants (or their legal guardians for minors) in accordance with institutional and national ethical regulations and the principles of the Declaration of Helsinki. Statistical analysis was performed with R Core Team (2021). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Continuous variables were summarized as median and interquartile range (IQR) due to non-normal distribution, while categorical variables were expressed as absolute frequencies and percentages.

The median age of enrolled patients was 50.5 years (IQR: 15.7-66), with 41.8% male sex (**Table 1**). Most referrals originated from hematology units (45%), followed by Internal Medicine (24%) and Pediatrics (20%) (**Figure 2**). As shown in **Figure 3**, hematologic abnormalities, hepatosplenic involvement, and skeletal manifestations represented the most frequent reasons for referral. At enrollment, anemia and thrombocytopenia were reported in 38 (19.4%) and 29 (14.8%) of screened subjects, respectively. Moreover, splenomegaly was present in 23.5% of individuals and hepatomegaly in 19.4%. Bone involvement affected 27.6% of patients, while hyperferritinemia was reported in 31.6%.

In the analyzed cohort, the median GCase activity was 5.6 nmol/h/mL (IQR: 4.4–7.4). Overall, 10 out of 196 patients (10/196, 5.1%; 95% CI, 2.5–9.3%) presented reduced GCase activity. ASM activity was measured in 168 out of the 196 enrolled patients. The median ASM activity for the analyzed cohort was 5.65 µmol/h/L (IQR: 3.5–8.5). The overall prevalence of pathological ASM activity was 1.2% (2/168; 95% CI, 0.1-4.2%), with two patients exhibiting pathological values of 1.6 and 1.4 µmol/h/L, respectively. Among 57 patients evaluated, the median Lyso-GB1 value was 2.5 ng/mL (IQR: 0.5-4.1), with only one patient exhibiting a markedly elevated Lyso-GB1 concentration of 495 ng/mL. Median Lyso-SM509 values were 0.3 ng/mL (IQR: 0.3-0.475).

Molecular genetic testing was performed in 57 of the 196 enrolled patients (29.1%). Among these, *GBA1* gene analysis was primarily conducted in 34 individuals who exhibited GCase activity ≤ 3.6 nmol/h/mL. Genetic analysis was also conducted in relatives of patients with double mutation and in 10 patients who presented particularly suspicious clinical signs (such as skeletal involvement, splenomegaly, or a family history of Parkinson's disease), or unsuitability of the DBS sample for enzymatic testing. Overall, 2 of 57 patients tested (3.5%) carried biallelic pathogenic or likely pathogenic *GBA1* variants. Patient KE142 harbored two pathogenic variants: *NM_000157.4(GBA1):c.508C>T (p.Arg170Cys)*, located in the exon 5; and the *NM_000157.4(GBA1):c.1226A>G (p.Asn409Ser)* located in the exon 9 (**Supplemental Figure S1**). Patient KB874 also carried two heterozygous variants in exon 10 of *GBA1*: *NM_000157.4(GBA1):c.1483G>C (p.Ala495Pro)* and *NM_000157.4(GBA1):c.1497G>C (p.Val499Val)*. The GCase enzymatic activity of 3.2 nmol/h/mL, and Lyso-GB1 concentration of 6.7 ng/mL were within reference limits, supporting the variants, excluding compound heterozygosity. Four additional patients were heterozygous carriers of *GBA1*

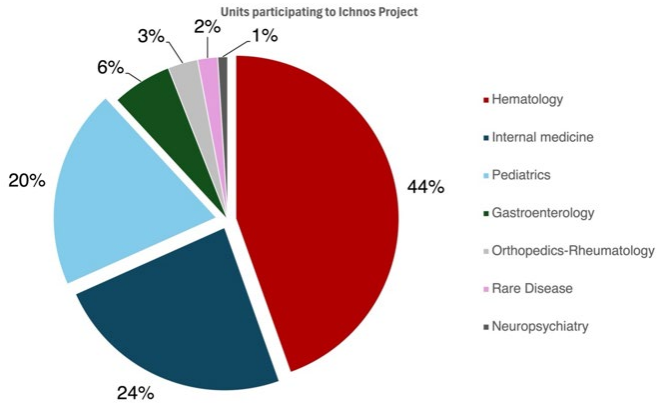

Figure 2. Units participating in the Ichnos Project. Patients recruited by each Unit are reported as a percentage of the total.

variants. Two were first-degree relatives (father and sister) of patient KE142, both carrying the *NM_000157.4(GBA1):c.1226A>G, p.(Asn409Ser)* variant. Another unrelated patient carried the same heterozygous variant. The fourth patient harbored *NM_000157.4(GBA1):c.349G>A, p.(Val117Met)*, located in exon 4. Overall, one confirmed case of GD was identified among the 196 screened individuals, corresponding to a diagnostic rate of 10% (1/10) among subjects with low enzymatic activity and an overall prevalence of 0.51% (95% CI, 0.01–2.83%). Finally, *SMPD1* analysis was performed in 10 patients with ASM activity ≤ 2.5 µmol/h/L, including four who simultaneously exhibited GCase activity ≤ 3.6 No pathogenic variants were identified in the *SMPD1* coding region in any of these cases.

This multicenter study represents the first coordinated effort to implement an integrated diagnostic strategy for GD and ASMD in Sardinia, a geographically isolated region with a relatively homogeneous genetic background. The initiative successfully involved multiple clinical departments, demonstrating the importance of education and collaboration among physicians. Integrating biochemical screening into clinical practice can reduce unnecessary investigations, guide appropriate genetic testing, and shorten the "diagnostic odyssey" experienced by many patients with GD.[10]

The proportion of patients in our cohort with organomegaly, hyperferritinemia, or metabolic abnormalities aligns with previously reported "red flag" profiles for GD in non-specific clinical settings.[11,12] Our findings also support the central role of DBS enzymatic assays integrated with a second-tier biomarker. This combined approach enabled simultaneous assessment of GCase and ASM activity in all enrolled patients, reduced pre-analytical limitations, and facilitated rapid, structured triage toward confirmatory biomarker testing and targeted sequencing. In our cohort, 5.1% of participants exhibited subnormal GCase activity, markedly lower than the 17.3% reported by Motta et al.[12] in a phenotype-enriched population selected for splenomegaly and/or thrombocytopenia. Likewise, the overall GD detection rate of 0.51% observed in the Ichnos Project is substantially lower than the 3.3% prevalence reported by Motta et al.[12] This discrepancy is expected and most likely reflects the broader, multispecialty referral pattern of our study, which inherently lowers the pre-test probability of GD and reduces the overall diagnostic yield.

We acknowledge the limitations of this study. First, the relatively small sample size and the identification of only a single confirmed case do not allow for generalizability of the findings. Second, enzyme activity was measured on DBS, which, despite its practicality, may introduce pre-analytical variability and borderline results in leukopenic patients. Third, not all patients with reduced enzymatic activity underwent complete genetic confirmation, potentially leading to an underestimation

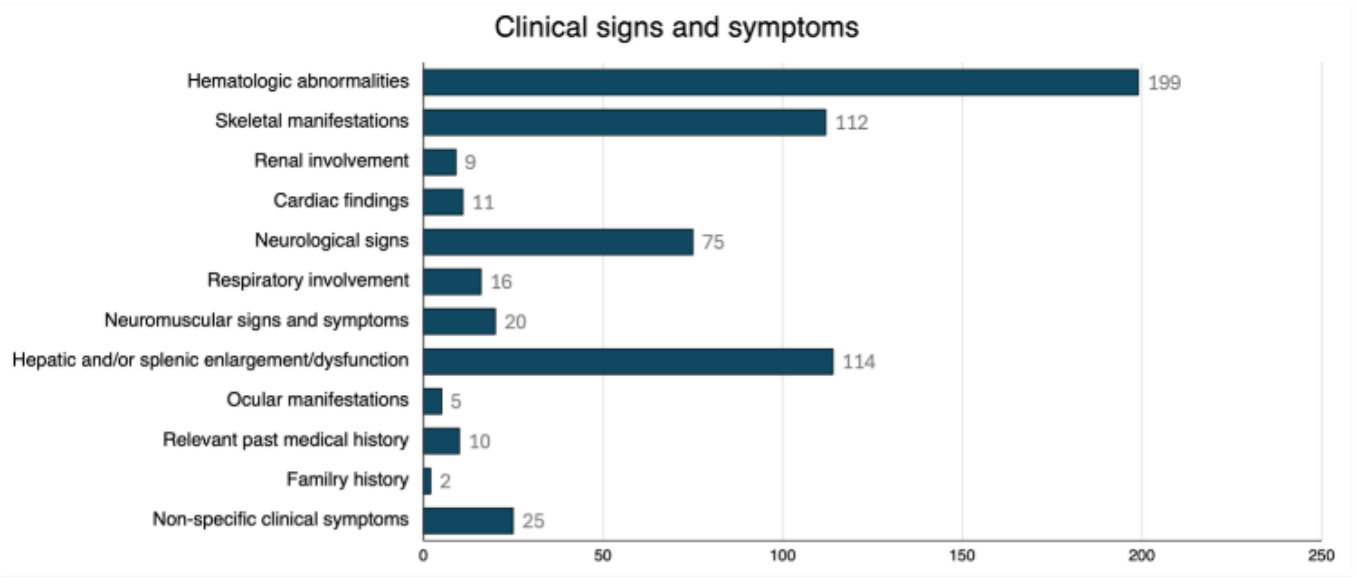

Figure 3. Referral triggers based on clinical, hematologic, and organ-specific findings. Because individual patients may present with multiple manifestations, counts reflect non-exclusive categories.

of disease frequency. However, the Ichnos Project provides an instructive example of the logistical and epidemiological barriers to screening rare disorders in small populations. It also demonstrates that even identifying a single affected individual carries substantial public health significance when the disease is underdiagnosed and treatable. Future efforts should prioritize continued education of frontline clinicians, refinement of biomarker thresholds to improve pre-test probability, and the establishment of centralized diagnostic hubs that can efficiently integrate biochemical, genetic, and clinical data. This network-based model could serve as a scalable framework for early detection of other rare metabolic diseases within a similar healthcare system.

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References:

1. Arslan N, Coker M, Gokcay GF, Kiykim E, Onenli Mungan HN, Ezgu F. Expert opinion on patient journey, diagnosis and clinical monitoring in acid sphingomyelinase deficiency in Turkey: a pediatric metabolic disease specialist's perspective. Front Pediatr. 2023; 11:1113422, https://doi.org/10.3389/fped.2023.1113422 PMid:37435168 PMCid:PMC10330960
2. Giacomarra M, Colomba P, Francofonte D, Zora M, Caocci G, Diomede D, Giuffrida G, Fiori L, Montanari C, Sapuppo A, Scortechini AR, Vitturi N, Duro G, Zizzo C. Gaucher Disease or Acid Sphingomyelinase Deficiency? The Importance of Differential Diagnosis. J Clin Med. 2024; 13:1487, https://doi.org/10.3390/jcm13051487 PMid:38592326 PMCid:PMC10932152
3. 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; 82:697-701, https://doi.org/10.1002/ajh.20908 PMid:17492645
4. Oliva P, Schwarz M, Mechtler TP, Sansen S, Keutzer J, Prusa AR, Streubel B, Kasper DC. Importance to include differential diagnostics for acid sphingomyelinase deficiency (ASMD) in patients suspected to have to Gaucher disease. Mol Genet Metab. 2023; 139:107563, https://doi.org/10.1016/j.ymgme.2023.107563 PMid:37086570
5. Dardis A, Michelakakis H, Rozenfeld P, Fumic K, Wagner J, Pavan E, Fuller M, Revel-Vilk S, Hughes D, Cox T, Aerts J; International Working Group of Gaucher Disease (IWGGD). Patient centered guidelines for the laboratory diagnosis of Gaucher disease type 1. Orphanet J Rare Dis. 2022; 17:442, https://doi.org/10.1186/s13023-022-02573-6 PMid:36544230 PMCid:PMC9768924
6. Marano M, Zizzo C, Malaguti MC, Bacchin R, Cavallieri F, De Micco R, Spagnolo F, Bentivoglio AR, Schirinzi T, Bovenzi R, Ramat S, Erro R, Sorrentino C, Sucapane P, Pilotto A, Lupini A, Magliozzi A, Di Vico I, Carecchio M, Bonato G, Cilia R, Colucci F, Tamma F, Caputo E, Mostile G, Arabia G, Modugno N, Zibetti M, Ceravolo MG, Tambasco N, Cossu G, Valzania F, Manganotti P, Di Lazzaro V, Zappia M, Fabbrini G, Tinazzi M, Tessitore A, Duro G, Di Fonzo A. Increased glucosylsphingosine levels and Gaucher disease in GBA1-associated Parkinson's disease. Parkinsonism Relat Disord. 2024; 124:107023, https://doi.org/10.1016/j.parkreldis.2024.107023 PMid:38843618
7. Giuffrida G, Markovic U, Condorelli A, Calafiore V, Nicolosi D, Calagna M, Grasso S, Ragusa MTV, Gentile J, Napolitano M. Glucosylsphingosine (Lyso-Gb1) as a reliable biomarker in Gaucher disease: a narrative review. Orphanet J Rare Dis. 2023; 18:27, https://doi.org/10.1186/s13023-023-02623-7 PMid:36782327 PMCid:PMC9926807

8. Revel-Vilk S, Fuller M, Zimran A. Value of Glucosylsphingosine (Lyso-Gb1) as a Biomarker in Gaucher Disease: A Systematic Literature Review. Int J Mol Sci. 2020; 21:7159, https://doi.org/10.3390/ijms21197159 PMid:32998334 PMCid:PMC7584006
9. Kubaski F, Burlina A, Pereira D, Silva C, Herbst ZM, Trapp FB, Michelin-Tirelli K, Lopes FF, Burin MG, Brusius-Facchin AC, Netto ABO, Poletto E, Bernardes TM, Carvalho GS, Sorte NB, Ferreira FN, Perin N, Clivati MR, de Santana MTS, Lobos SFG, Leão EKEA, Coutinho MP, Pinos PV, Santos MLSF, Penatti DA, Lourenço CM, Polo G, Giugliani R. Quantification of lysosphingomyelin and lysosphingomyelin-509 for the screening of acid sphingomyelinase deficiency. Orphanet J Rare Dis. 2022; 17:407, https://doi.org/10.1186/s13023-022-02560-x PMid:36348386 PMCid:PMC9641838
10. Nishimura S, Ma C, Sidransky E, Ryan E. Obstacles to Early Diagnosis of Gaucher Disease. Ther Clin Risk Manag. 2025;21:93-101. https://doi.org/10.2147/TCRM.S388266 PMid:39882275 PMCid:PMC11776414
11. Marchi G, Nascimbeni F, Motta I, et al. Hyperferritinemia and diagnosis of type 1 Gaucher disease. Am J Hematol. 2020;95(5):570-576. https://doi.org/10.1002/ajh.25752 PMid:32031266
12. Motta I, Consonni D, Stroppiano M, Benedetto C, Cassinerio E, Tappino B, Ranalli P, Borin L, Facchini L, Patriarca A, Barcellini W, Lanza F, Filocamo M, Cappellini MD; Splenomegaly Gaucher group. Predicting the probability of Gaucher disease in subjects with splenomegaly and thrombocytopenia. Sci Rep. 2021;11(1):2594 https://doi.org/10.1038/s41598-021-82296-z PMid:33510429 PMCid:PMC7843616