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# High bone turnover is associated with lower bone density in Hurler-Scheie and Hunter syndromes treated with enzyme replacement therapy

L. E. Polgreen

A. Petryk

K. Rudser

A. Kaiser

L. M. Scibora

*See next page for additional authors*

spots (DBS) using methanol: acetonitrile:water (80:15:5) and analyzed by HPLC tandem-mass spectrometry with dimethyl psychosine as the internal standard. The coefficient of variation was 8.8%. The relationship between decreases in lyso-GL-1 levels and changes in spleen and liver volumes, hemoglobin, platelet count, and chitotriosidase levels were evaluated. At baseline, all patients with available data showed elevated lyso-GL-1 levels. The median value (942 ng/mL, range: 248-2418) was elevated 165-fold compared to normal controls (5.7 ng/mL, range 3.0-14.8) with no overlap. Following treatment with eliglustat, the median lyso-GL-1 level decreased by 61% (SD: 11.0%) after 1 year, with continued decreases through year 3 and a final median reduction of 83% (SD: 11.8%) after year 4. The decrease in lyso-GL-1 level paralleled the decrease in chitotriosidase level. Preliminary analyses indicate that the decreases in lyso-GL-1 levels correlated with the decreases in spleen and liver volumes and chitotriosidase levels and with the increases in hemoglobin concentration and platelet count over time. Lyso-Gl-1 levels decreased significantly but did not fully normalize in treatment-naïve GD1 patients following 4 years of treatment with eliglustat. The decrease in lyso-GL-1 levels correlated with improvements of the major clinical manifestations of the disease. Lyso-GL-1 may prove to be a more useful marker of treatment response to eliglustat than chitotriosidase since it is in the causal pathway of GD1.

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## Functional outcome measures in pediatric therapeutic intervention: Application and issues in a rare disease

Dawn L. Phillips[a], Beth Leiro[b], [a]*UNC Division of Physical Therapy, Chapel Hill, NC, United States,* [b]*Beth Leiro Pediatrics, Chapel Hill, NC, United States*

A better understanding is needed of the functional outcome measures that are useful for the development of treatments in children with pediatric rare diseases. The literature is often void of descriptions of functional presentation in the disease natural history or in response to intervention strategies and unable to serve as a guide for tool selection. The known disease characteristics, including any heterogeneity in presentation, must first be defined. The International Classification of Functioning Disability and Health (ICF) provides a framework to define the disease-specific body function and structure impairments, activity limitations and participation restrictions. Once the ICF dimensions are populated to reflect the rare disease presentation, areas of anticipated response to intervention can be defined and appropriate measures to capture efficacy can be selected. Key factors must be considered when selecting instruments such as age, functional ability, need for normative data, developmental areas to be assessed such as cognitive, gross or fine motor development, or activities of daily living, and tool responsiveness to change. Several widely used instruments, appropriate for evaluation of functional outcomes in infants and children, will be reviewed focusing primarily on motor outcomes. Issues and best practices regarding application, placement within the ICF model and interpretation of these tools in rare diseases will be discussed. Hypophosphatasia, a rare inborn error of metabolism with a wide spectrum of sequelae that can lead to compromised physical function, cognitive skills and the ability to perform activities of daily living, is used to illustrate practical applications of different instruments. This discussion aims to increase awareness of the important aspects that need to be considered when evaluating pediatric patients with rare disorders and to assist healthcare professionals and researchers in identification of age- and disease-appropriate tools.

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## Beneficial effect of agalsidase beta on long term evolution of patients with Fabry disease and kidney transplant

Luis F. Pineda-Galindo, Leslie Moranchel-García, *Instituto Mexicano del Seguro Social, México, D.F., Mexico*

*Introduction:* Fabry disease (FD), an inherited lysosomal disease in which deficiency of a specific enzyme (α-gal A) leads to tissue accumulation of glycosphyngolipids (predominantly globotriaosylceramide - GL-3), is frequently associated with chronic organ damage. Enzyme replacement therapy (ERT) has proven to benefit organ function and patient survival. Few publications explore the long term effect of ERT on patients subjected to transplant.

*Case reports:* Patient 1 is a 39 year old male in whom FD was confirmed with α-gal A measurement (0.0 μmol/L/hr; reference range: 6.73 ± 2.5 μmol/L/hr) and mutation analysis (c.729 > C). Disease progression led to ESRD and peritoneal dialysis requirement in 2006. He began ERT in 2006 with biweekly agalsidase beta, which led to remission of symptoms. In 2007 he received a cadaveric kidney transplant. At 7 years post-transplant he maintains stable allograft function, remission of Fabry symptomatology and stabilization of cardiac and neurologic tests. Patient 2 is a 41 year old male in whom FD was confirmed with α-gal A measurement (0.1 μmol/L/hr; reference range: 2.0-14.6 μmol/L/hr) and mutation analysis (49 del CGCTT). In 2009 he developed ESRD and required hemodialysis. He began ERT that same year with biweekly agalsidase beta. In March 2011, he received a cadaveric kidney transplant. At 5 years post-transplant he maintains stable allograft function, remission of Fabry disease symptomatology and stabilization of cardiac and neurologic tests.

*Conclusions:* ERT provides long-term clinical benefits, improved quality of life and survival, stabilization of organ function, and favors adequate graft survival in kidney transplant recipients with FD. Although FD is not associated with disease recurrence in kidney allografts, ERT is essential to detain or minimize disease progression in other organs of transplanted patients and to improve patient/graft survival. ERT must be started as early as possible to ensure better outcomes.

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## High bone turnover is associated with lower bone density in Hurler-Scheie and Hunter syndromes treated with enzyme replacement therapy

Lynda E. Polgreen[a], Anna Petryk[b], Kyle Rudser[b], Alex Kaizer[b], Lesley Scibora[c], Bradley S. Miller[b], Chester B. Whitley[b], Ellen Fung[d], [a]*LA BioMed at Harbor-UCLA, Torrance, CA, United States,* [b]*University of Minnesota, Minneapolis, MN, United States,* [c]*St. Thomas University, St. Paul, MN, United States,* [d]*UCSF Benioff Children's Hospital Oakland, Oakland, CA, United States*

We have previously identified deficits in bone mineral density and estimated bone strength in children and adolescents with muco-polysaccharidosis (MPS) types I and II. The goal of this study was to test the association between changes in the markers of bone resorption and formation, and bone deficits. We analyzed peripheral quantitative computed tomography (pQCT) and fasting biomarker data in 35 subjects, mean age 13 ± 4 years, with MPS I or II, participating in a 5-year longitudinal observational cohort study: Hurler syndrome (MPS IH) n = 16; Hurler-Scheie or Scheie syndrome (MPS IA n = 7, Hunter syndrome (MPS II) n = 12. All MPS IH were treated with hematopoietic cell transplantation (HCT); all MPS IA and MPS II were treated with enzyme replacement therapy (ERT) during the entire study. Tibia pQCT outcomes of trabecular bone mineral density (vBMD), cortical vBMD,

periosteal circumference (Peri), and polar section modulus (Zp) were evaluated for association with each of the following biomarkers: markers of bone resorption (urine pyridinolines [PYD] and deoxypyridinolines [DPD]), and formation (bone specific alkaline phosphatase [BAP], osteocalcin [OCN]). Regression analysis was used to estimate associations adjusted for age and tibia length. In the ERT group, increased DPD and PYD were associated with decreased trabecular vBMD (both $p = .04$), and increased OCN with decreased cortical density ($p = .02$). In the HCT group, there were no statistically significant associations, only a trend towards an association of increased DPD with decreased trabecular vBMD ($p = .14$). In summary, our results provide evidence that chronic elevations in bone turnover may contribute to the bone density deficits identified in subjects treated with ERT (MPS IA and MPS II). These data support further investigation into the role of anti-resorptive therapy to prevent bone loss in MPS IA or MPS II treated with ERT. (This project was supported by NIH grants and contracts K23AR057789, U54NS065768, 1UL1RR033183-01, 8UL1TR000114-02).

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## Recommendations for enzyme replacement therapy in classical phenotype of Fabry disease in Latin America

Juan Politei, *Fundación para el Estudio de las Enfermedades Neurometabólicas (FESEN), Quilmes, Argentina*

*Introduction:* Fabry disease is a rare X-linked inherited disorder due to deficient or absent lysosomal α-galactosidase A activity.

*Aim:* To present a recommendation for the initiation of ERT in Fabry patients with classical phenotype, based in the knowledge and expertise of a group of Latinamerican practitioners involved in daily care for Fabry patients.

*Methods:* For the first round, a background document was compiled by the study coordinator with an overview of inclusion criteria applied in other international guidelines. Subsequently, an online questionnaire was set up by the study team to discuss initiation criteria.

*Results:* Forty-eight practitioners responded to the online survey. The criteria were divided in five main sections, with a consensus in all of them. Table 1: all of these findings should be consistent with FD and not fully explained by other pathology.

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## Lysosphingolipids in dried blood spots as biomarkers for lysosomal diseases

Giulia Polo[a], Thilini B. Kolamunnage[a], Alessandro P. Burlina[b], Francesca Furlan[a], Andrea Celato[a], Monica Del Rizzo[a], Laura Giordano[a], Alberto B. Burlina[a], [a]*University Hospital of Padua, padova, Italy,* [b]*St. Bassiano Hospital, Bassano del Grappa, Italy*

*Introduction:* Lysosphingolipids, the N-deacetilated forms of sphingolipids, have been identified as potential biomarkers of several sphingolipidoses, such as Gaucher, Fabry, Niemann-Pick diseases and GM1 and GM2 gangliosidosis. To date, different methods have been developed to measure various lysosphingolipids in plasma. Here we present a new LC-MS/MS assay for a simultaneous quantification of lysosphingolipids (Lyso-GL1, Lyso-GL3, Lyso-GM1, Lyso-GM2, Lyso-SM and Lyso-SM-509) in dried blood spot (DBS).

*Materials and Methods:* DBS of healthy controls and patients affected by sphingolipidosis (Fabry, Gaucher and Niemann-Pick A/B diseases) were collected and stored at -20 ° C before analysis. Lysosphingolipids were extracted from 3 mm DBS with a mixture of MeOH:ACN:H2O (80:15:5, v/v) containing internal standard (Lyso-GL1 from plant). Chromatographic separation was performed using a C18 column with a gradient of water with 0.1% formic acid and ACN with 0.1% formic acid in a total run time of 4 minutes. Compounds were detected in positive ion mode ESI-MS/MS by multiple reaction monitoring (MRM). An external calibration curve was used to quantify concentrations of analytes.

*Results:* The method was validated to demonstrate specificity, linearity, lowest limit of quantification, accuracy and precision. The intra-day and inter-day coefficients of variation (CV%) were <15% for all metabolites. Reference ranges were determined in paediatric and adult populations. Elevated levels of lysosphingolipids were identified in Gaucher disease (Lyso-GL1), Fabry disease (Lyso-GL3) and Niemann-Pick A/B disease (LysoSM and Lyso-SM-509).

*Conclusions:* Our LC-MS/MS method allows a rapid quantification of lysosphingolipids for the diagnosis of patients with sphingolipidosis. This assay could also be used as a second-tier test in newborn screening of Fabry, Gaucher and Niemann-Pick A/B diseases.

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## High level of oxysterols in neonatal cholestasis: A pitfall in analysis of biochemical markers for Niemann-Pick disease type C

Giulia Polo[a], Alessandro P. Burlina[b], Francesca Furlan[a], Thilini Kolamunnage[a], Laura Giordano[a], Martina Zaninotto[a], Alberto B. Burlina[a], [a]University Hospital of Padua, Padova, Italy, [b]*St. Bassiano Hospital, Bassano del Grappa, Italy*

*Background:* Niemann-Pick disease type C (NPC) is a rare lipid storage disorder characterised by progressive neurological deterioration. Diagnosing NPC is challenging as clinical signs and symptoms are variable and non-specific. Two oxysterols, cholestane-3β,5α,6β-triol (triol) and 7-ketocholesterol (7KC), have been proposed as biomarkers for aiding diagnosis of NPC. This study evaluated the use of triol and 7KC as biomarkers in cholestatic neonates with suspected NPC.

*Methods:* Plasma triol and 7KC were analysed as dimethylglycine esters using an LC-MS/MS assay in selected neonates with severe cholestasis and suspected NPC ($n = 7$), adults with cholestasis ($n = 15$), patients with confirmed NPC (positive controls; $n = 11$ [1 child and 10 adults]), healthy subjects (negative controls; $n = 40$ [20 children and 20 adults]), and cholestatic adults (comparative reference; $n = 15$). The LC-MS/MS method was subjected to a number of tests for accuracy and consistency.

*Results:* Triol and 7KC levels were substantially and significantly increased in NPC positive patients compared with healthy controls ($p < 0.001$). However, positive results (markedly increased levels of both oxysterols) were identified in 6/7 (86%) neonates with cholestasis. Genetic testing confirmed NPC only in one neonate who had increased triol and 7KC, and increased oxysterol levels among neonates with no identified NPC gene mutations were considered likely due to biliary atresia.

*Conclusions:* While the potential of oxysterols as NPC biomarkers has been well evaluated in older patient populations (without cholestasis), our data suggest that cholestasis might represent a pitfall in oxysterol measurements intended to aid diagnosis of NPC in affected patients.

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