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The mucopolysaccharidoses (MPS) are a group of rare progressive genetic disorders of glycosaminoglycan (GAG) metabolism caused by deficiency of enzymes responsible for lysosomal GAG degradation. The accumulation of partially degraded GAG and the resulting disturbance of cellular homeostasis leads to progressive cellular and tissue damage ultimately resulting in multiorgan system involvement.[1] Mucopolysaccharidosis type 1 (MPS I) results from deficiency of the lysosomal enzyme α-L-iduronidase (IDUA) because of pathogenic variants in the *IDUA* gene.[2] MPS I presents clinically as a disease spectrum spanning early onset, progressive severe disease with cognitive impairment (Hurler syndrome), to later onset progressive disease with highly variable and later onset central nervous system (CNS) involvement (attenuated MPS I). Attenuated MPS I encompasses a spectrum previously referred to by the eponyms Hurler-Scheie and Scheie syndromes.[1] Untreated patients with the most severe form of MPS I usually die in the first decade. In contrast, life expectancy for untreated patients with attenuated disease ranges from mortality in the second or third decade to full life expectancy, albeit with considerable morbidity. The birth incidence of MPS I is ~1 in 100 000 live births estimated from a number of population studies.[3]

Although the disease pathophysiology is not well understood, most of the clinical manifestations of MPS I are secondary deleterious effects because of disturbed GAG metabolism. Organs involved include the brain, musculoskeletal system, heart, lungs, and eyes. Many symptoms and complications are difficult or impossible to reverse. Thus, initiation of treatment early in the natural history of disease is thought to be a key factor in achieving optimal outcome.[2] Available disease modifying therapies include hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT) with laronidase.[4] Because early intervention with HSCT has been demonstrated to stabilize neurocognitive function in MPS I,[5-7] it is currently recommended as standard for patients who are predicted to have severe disease.[4,6] ERT with laronidase is used for treating nondirect CNS manifestations of MPS I. Clinical trials and follow-up studies of ERT in MPS I have demonstrated improvements in some somatic manifestations and functional outcomes in patients with attenuated MPS I.[8-13] Laronidase has also been found to be useful in the peritransplant period for patients with severe MPS I.[4,14] The use of ERT in the peritransplant period has been shown to be safe and has led to clinical improvements particularly in patients with significant cardiopulmonary disease before transplantation. In addition, ERT before transplantation has been reported to alleviate symptoms that may have influenced the conditioning regime or candidacy for transplantation of some patients.[14]

Because MPS I is a progressive disorder, the success of both HSCT and ERT depends on early initiation of treatment. Therefore, early identification of patients is critical. Because many of the early disease manifestations represent common childhood symptoms (eg, inguinal/umbilical hernia and recurrent upper respiratory tract infections), diagnosis based on early symptom recognition is challenging and has been met with limited success. Newborn screening (NBS) strategies should be more effective in this regard. MPS I NBS via determination of IDUA activity in dried blood spot (DBS)-derived samples is currently underway in the US[15,16] and in pilot programs in Taiwan,[17] Italy,[18] Austria,[19] and Hungary.[19] The US Department of Health and Human Services recommended uniform screening panel[20] provides the list of core and secondary disorders

CNS	Central nervous system
DBS	Dried blood spot
ERT	Enzyme replacement therapy
GAG	Glycosaminoglycan
HSCT	Hematopoietic stem cell transplantation
IDUA	α-L-iduronidase
MPS	Mucopolysaccharidoses
MPS I	Mucopolysaccharidosis type I
MRI	Magnetic resonance imaging
NBS	Newborn screening
uGAG	Urine GAG

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Table I. Analysis of symptom frequency in patients ≤2 years of age (from Pastores et al[22])

Symptoms/complications	Percentage of patients ≤2 y of age with symptom
Coarse facies	98
Valvular disease	95
Corneal clouding	90
Hepatomegaly	84
Upper airway obstruction → OSA	82
Kyphosis gibbus	75
Joint contractures	72
Hernia	70
Dysostosis multiplex	70
Cognitive impairment	60
Enlarged tongue	60
Splenomegaly	60
Eustachian tube obstruction → otitis media	55
Hip dysplasia	42
Genu valgum	38
Reactive airway disease	37
Scoliosis	35
Carpal tunnel syndrome	25
Pes cavus	18
Glaucoma	10
Heart failure	3
Cor pulmonale	2

OSA, obstructive Sleep Apnea.

that should be included in every NBS program. A proposal for MPS I NBS received a positive recommendation by the US Secretary of Health and Human Services' Advisory Committee on Heritable Disorders in Newborns and Children,[21] and the US Secretary approved the addition of MPS I to the recommended uniform screening panel in February 2016.

Limited Ability to Define Disease Severity is an Inherent Challenge for MPS I NBS

Given the wide clinical spectrum of MPS I and the differences in therapy based on subtype, the precise determination of where a patient fits in the broad spectrum of disease is essential. It is important to note that no currently available biochemical criteria can reliably distinguish phenotypes. Therefore, additional assessments including molecular analysis and skilled clinical assessments are required. Table I summarizes data obtained from the MPS I Registry highlighting the features observed in patients less than 2 years of age. It is important to note that many of the signs and symptoms considered characteristic of MPS I may not necessarily differentiate between attenuated and severe phenotypes, or may not be present in neonates.[23] A summary of the challenges in predicting MPS I disease severity in individuals detected by NBS is summarized in Table II.

Lack of Routine Biochemical Markers

The routine laboratory diagnostic IDUA enzyme assay cannot distinguish between the different phenotypes. Although many biomarkers of MPS have been reported, their ability to reliably distinguish MPS I subtypes, particularly in early infancy, has not been demonstrated.[24] Patients with Hurler syndrome, in general, have higher levels of urine GAG (uGAG) than patients with attenuated disease, but the precise threshold level that distinguishes these subtypes, particularly at early disease stages, is unknown. In sophisticated studies using cultured MPS I fibroblasts and evaluating enzyme kinetics, immunoquantification, and in vitro turnover studies, it was possible to correlate between the genotype, the biochemical phenotype, and clinical course.[25] This complex assay requires specific antibodies that make it difficult to reproduce in other laboratories. The analysis of oligosaccharides, derived from heparan sulfate and dermatan sulfate from MPS I fibroblasts and measured by electrospray tandem mass spectrometry, has also been shown to discriminate between patients with and without CNS pathology.[26] These studies require long-term fibroblast cultures and mass spectrometry-based GAG assays. Kingma et al[27] developed an algorithm to predict clinical severity in MPS I based on genotyping and an optimized IDUA assay, however, this assay is not routinely available. In a preliminary proteomic study, the lysosomal protein β-galactosidase was elevated 3.6- to 5.7-fold in severe but not attenuated MPS I,[28] and 1 study has reported that serum heparin-cofactor II-thrombin complex levels can differentiate patients with severe MPS I from patients with attenuated MPS I.[29] Neither of these studies evaluated the utility of these biomarkers in the neonate or early infant, and, thus, their utility for use in NBS is limited.

Heterogeneity of IDUA Variants

Over 200 *IDUA* pathogenic variants have been identified. Because MPS I is an autosomal recessive disorder, both pathogenic alleles are required to be identified to consider using genotype to predict phenotype. An analysis of pathogenic *IDUA* variants in patients with MPS I has shown clear genotype-phenotype correlations.[30] Homozygosity or compound heterozygosity for common nonsense mutations (eg, p.W402X and p.Q70X) predominated among patients with severe disease,

Table II. Challenges in predicting disease severity following positive NBS for MPS I

- No biochemical criteria reliably distinguish MPS I subtypes
- Many signs and symptoms that establish an MPS I diagnosis in older patients do not differentiate between attenuated and severe phenotypes, or are not present in newborns
- In the absence of 2 pathogenic *IDUA* variants previously reported to be associated with defined disease severity, genotype/phenotype correlation is complicated by the existence of private (reported only in single individuals with MPS I) missense mutations that can not be used to predict the phenotype
- IDUA enzyme analysis is complicated by pseudo-deficiency because of:
  - Benign variants
  - Reduced in vitro enzyme activity in clinically unaffected individuals
  - Prevalence in African American population

whereas patients with attenuated disease have at least 1 allele that contains a missense or a splice site variant. Although 62% of patient genotypes included at least 1 nonsense allele, all patients with 2 nonsense alleles, whether homozygous or compound heterozygous, had severe disease. However, not all patients with severe disease had nonsense mutations.

Up to 70% of pathogenic variants are recurrent and, thus, may be helpful in phenotype prediction. Although genotype-phenotype correlations have been established with some common variants, there are individuals and families with unique missense variants that cannot be used to predict the clinical phenotype.[30-32]

IDUA Pseudodeficiency

The presence of *IDUA* pseudodeficiency alleles (benign variants) complicates NBS screening for MPS I. Pseudodeficiency is decreased IDUA enzyme activity when measured using current artificial substrates with no evidence of altered GAG metabolism.[33-35] *IDUA* pseudodeficiency alleles can result in decreased enzyme activity when found in the homozygous state or in the compound heterozygous state with another pseudodeficiency allele, a pathogenic variant, or a variant of unknown significance. The Missouri NBS program reported that of 46 infants with a positive NBS screen, 26 had leukocyte IDUA enzyme activity below normal levels but above levels identified in patients with confirmed severe MPS I.[36] Follow-up testing showed that uGAG levels in these infants were not indicative of severe MPS I, and no patient was homozygous or compound heterozygous for previously reported pathogenic variants. Four purported pseudodeficiency missense *IDUA* alleles were identified (p.A79T, p.H82Q, p.D223N, and p.V322E) of which p.A79T was prevalent in the African American population.[36] The common finding of pseudodeficiency in individuals detected by NBS complicates the interpretation of confirmatory results, however, this is resolved in most cases by DNA sequencing, which is now considered standard-of-care when the diagnosis of MPS I is being considered.

Methods

An expert group of North American physicians, clinician scientists, and genetic counselors with experience in MPS I diagnosis, treatment, and management, as well as NBS, convened to review state-of-the-art practices for diagnosis of infants with MPS I identified through NBS programs. The expert group invited to this discussion were defined by virtue of their active participation in the development of diagnostics, clinical trials and/or ongoing clinical evaluation, management of patients with MPS, and participation in NBS programs. Current practice and evidence from both published information and personal experience was reviewed at a meeting in Salt Lake City in March of 2015. Literature review was performed by searching PUBMED with key words: Mucopolysaccharidosis I, Hurler syndrome, Hurler-Scheie syndrome, Scheie syndrome, NBS, and lysosomal disorders; the bibliography was provided to attendees before the meeting date. The objective of this review was to develop an algorithm for addressing patient follow-up testing and monitoring given the wide clinical spectrum of MPS I and the different therapeutic recommendations based on disease subtype. Preliminary information on the algorithm developed was presented at the 2015 American Academy of Pediatrics Meeting.[37]

Evaluation, Diagnosis, and Management of Patients Following Positive MPS I NBS

Currently, NBS programs for MPS I begin with analysis of IDUA activity measured directly from the DBS. Available screening assays include fluorometric,[15,18] digital microfluidics,[15] and tandem mass spectrometry-based analyses.[38-41] MPS I screening may be included in multiplex assays that include screens for multiple lysosomal storage disorders in a single DBS sample.[40] If IDUA levels are below established cut-off values, the process outlined in the algorithm in the **Figure** is proposed to confirm and delineate disease severity, and guide appropriate follow-up and treatment. The main concept underlying this algorithm is the critical importance of precisely defining the phenotype of the patient with particular emphasis on ensuring that patients with Hurler syndrome be identified and directed to transplantation programs. The approaches used for enzyme analysis from the NBS card uses artificial substrates and are similar to the methodology that is used for the diagnostic assay albeit with a much smaller sample volume. Because of the latter consideration, a repeat assay using a fresh blood sample is required after a positive NBS. The issue of pseudodeficiency relates to the use of the artificial substrates that are used to measure iduronidase enzyme activity. Both the NBS assays and the diagnostic assay use these artificial substrates and, thus, are not able to distinguish pseudodeficiency from true deficiency. The frequency of pseudodeficiency will be different in different populations; data to date suggests that the frequency is highest within the African American population. At this time, there does not appear to be straightforward means using the initial NBS card to definitively and accurately exclude a diagnosis of MPS I after the positive NBS result. Nevertheless, the seriousness of MPS I and the critical need for early treatment initiation particularly for individuals with Hurler syndrome necessitates the steps that follow.

Confirmatory IDUA enzyme analysis (from fresh blood leukocytes, serum, or plasma) should be performed for all DBS-positive newborn samples, ideally arranged by a geneticist/metabolic disease team. A normal level of enzyme activity in the confirmatory assay is indicative of a false positive DBS assay thus excluding a diagnosis of MPS I. Following a positive confirmatory enzyme assay, all patients should be urgently assessed by a genetic/metabolic disease specialist for further clinical, molecular, and biochemical assessments. Because the turnaround time for the confirmatory enzyme analysis would be approximately 1 week and, if positive, the subsequent step of *IDUA* gene sequencing would have an approximate 3 weeks turnaround time, it is anticipated that screening programs would likely obtain both samples at the initial time of patient recall and not proceed to *IDUA* gene sequencing until IDUA deficiency is confirmed. It is anticipated that advances in se-

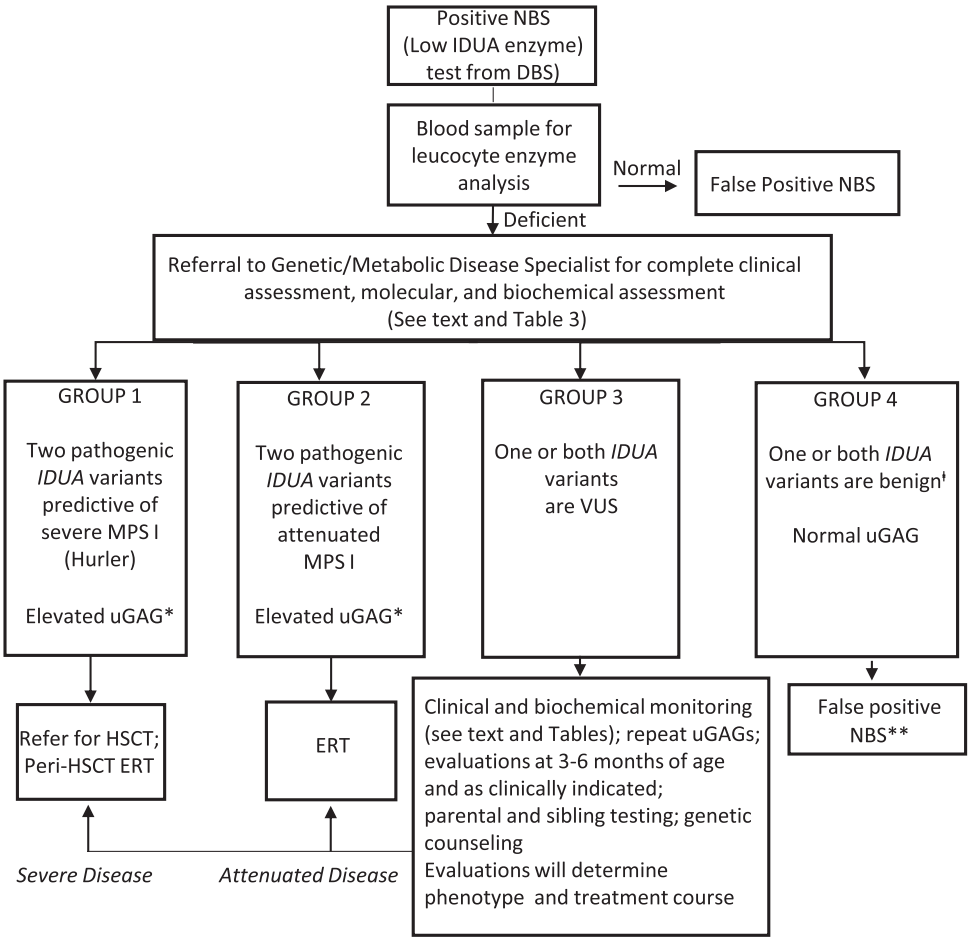

Figure. Proposed decision making algorithm for positive MPS I newborn screen. *VUS*, variant of unknown significance (ie, non-recurrent missense variant).

quencing technologies could result in a more rapid turnaround for this step. Because of the complex nature of the discussion, the initial patient recall should be communicated to the family and organized by a genetics/metabolic team that is well versed in this condition. This would ensure appropriate and supportive discussions take place as well as provide an opportunity for the care team to determine whether more rapid clinical assessment of the newborn is required.

To accurately determine the appropriate management for each patient, molecular, biochemical, and clinical assessments must be integrated into the decision-making process. *IDUA* gene sequencing, biochemical assessment, and a comprehensive clinical evaluation (**Tables I** and **III**) should be performed following confirmation of low/deficient IDUA enzyme activity. Quantitative uGAG determination using dye-binding methodology is currently used by most laboratories. It is noteworthy that quantitative uGAG excretion in young infants can be difficult to interpret. Although uGAG analysis has proven to be useful as an initial screen in symptomatic older individuals, uGAG levels in newborns can be elevated relative to older infants and normative data for infants under the age of 1 month have not been established in most laboratories. At this time, other blood or urine glycosaminoglycan based quantitative methodologies[24] have not been adequately evaluated in the newborn period; as this data becomes available their potential role in NBS will emerge.

The patient's genotype is an important component in decision-making and should be interpreted in context with clinical and biochemical assessments.[30] If the *IDUA* genotype analysis identifies 2 pathogenic alleles known to be or likely to be associated with severe disease in the presence of elevated uGAG levels (group 1 in algorithm), then the patient should be immediately referred for HSCT assessment with consideration of peritransplant ERT because outcomes following HSCT for severe MPS I are closely related to disease burden and, thus, age at transplantation.[5,48] Early diagnosis and treatment is highly correlated with improved cognitive, language, and behavioral outcomes,[48] and may also impact long-term musculoskeletal

Table III. Clinical evaluations/observations helpful for MPS I disease severity assessment

Assessments	Severe MPS I	Attenuated MPS I
Musculoskeletal including radiologic assessment to identify early signs of dysostosis multiplex	Severity of the dysostosis tends to correlate with disease severity; mild dysostosis, particularly of the hips, ribs, and vertebrae can be detected on radiographs at birth. Gibbus deformity an early sign of severe disease	Later onset of skeletal abnormalities[22,42,43]
Cardiac including echocardiogram for signs of heart valve thickening and ECG	Early signs of valvular disease are suggestive of severe MPS I disease; cardiac involvement by echocardiography is observed much earlier than clinical symptoms; mitral valvular disease most common,[44] rare cases of early endocardiofibroelastosis	Cardiac involvement detected at later age than in severe MPS I[45]
Neurologic		
Neurocognitive	Early psychomotor development may be normal; developmental delay is usually obvious by age 18 mo; language delays[22]	In attenuated MPS I intellect may be normal or nearly normal. If intellectual abilities decline, the course is more protracted than in individuals with severe disease.
Brain and spine imaging	Signal alterations, enlarged PVS, and ventriculomegaly are consistent findings, although incidence in the early months of life is unknown[46]	Signal alterations, enlarged PVS, and ventriculomegaly and spinal stenosis are frequent findings, atrophy is less frequent[42]
Hydrocephalus	Communicating high-pressure hydrocephalus is common; increase in intracranial pressure can contribute to rapid cognitive decline[22]	Lower frequency early in attenuated MPS I, but may have insidious onset[22]
Ophthalmological including visual acuity, retinal exam and corneal exam	Early detectable corneal involvement is suggestive of more severe MPS I disease and may be observable by 6-7 wk of age	Onset of corneal clouding at later age (median age 9.1 y) than in severe MPS I[42]
Respiratory including airway and nasal congestion and lung function tests	Frequent upper respiratory-tract infections common before age 1 y; chronic recurrent rhinitis and persistent copious nasal discharge without obvious infection are also common; sleep apnea common[22]	Rhinorrhea and sleep apnea are common;[22] recurrent ear nose and throat symptoms[46]
Observation for facial dysmorphisms	Coarsening of the facial features, caused by storage of GAGs in the soft tissues of the orofacial region and facial bone dysostosis, becomes apparent within the first 2 y[22]	Coarseness of facial features less obvious than in severe MPS I[42,47]
Gastrointestinal including spleen volume, liver volume and observation for hernia	Inguinal or umbilical hernias are common before age 1 y in severe MPS I; hepatosplenomegaly is common[22]	Hernia present in 65% of patients with attenuated MPS I, with median onset 3 y;[42] hepatosplenomegaly variable[22]
Audiometry	Hearing loss is common in severe MPS I and correlates with severity of somatic disease[22]	Moderate to severe hearing loss may develop[22]

ECG, electrocardiography; *PVS*, perivascular spaces.

disease.[49] Although the current recommendation for a patient diagnosed with Hurler syndrome is to offer early transplantation, there are no data available that precisely delineates the most favorable time to initiate transplantation. Thus, the exact timing of transplantation after a NBS based diagnosis of severe MPS I will require the transplant center to take into account transplant risks as well as risks of disease progression. The collection of outcome data for patients with MPS I diagnosed by NBS will be critical in providing future insights into this important question. If attenuated disease is predicted by the variants identified (group 2 in algorithm), then the patient should be considered for initiation of ERT. Early initiation of ERT is believed to improve clinical outcomes, possibly by preventing irreversible changes that result from GAG accumulation and secondary pathogenic cascade activation.[50,51] This concept is supported by the observations that lysosomal GAG storage in MPS occurs prenatally,[52,53] outcomes are improved with very early treatment in animal models,[54,55] and early vs later treatment improves outcomes in sibling case studies.[47,56,57]

The proposed algorithm (**Figure**) uses *IDUA* sequencing as a critical component in decision-making. The 2 relatively straightforward situations where genotype and biochemical profiles are predictive of severe (group 1) or attenuated disease (group 2) should lead to prompt treatment initiation. Conversely, there are situations that necessitate clinical and biochemical monitoring in the first 6 months of life to ensure appropriate treatment consideration. In cases where there is not clear genotype-phenotype correlation and disease severity cannot be predicted because of the presence of variants of unknown significance (group 3 of the algorithm), the physical examinations, clinical evaluations, and biochemical assessments may help in predicting the disease course. A detailed physical examination should include assessment for facial dysmorphisms, corneal clouding, joint range of motion, hernias (both inguinal and umbilical), murmurs, liver and spleen enlargement, and scoliosis/kyphosis. The clinical assessments (**Table III**) should include skeletal radiographs, echocardiography, neurocognitive evaluation, ophthalmologic examination, and consideration of brain magnetic resonance imaging (MRI). These evaluations can assist in the identification of patients with severe MPS I because findings of dysostosis multiplex, gibbus deformity, and significant detectable corneal and cardiac valve involvement, in addition to respiratory findings in a newborn infant of 6-7 weeks of age are consistent with a severe disease phenotype. Although the sensitivity of brain MRI to distinguish disease severity early in natural history of disease is uncertain, brain MRI may be helpful in the early classification of patients as early white matter changes would be indicative of Hurler syndrome. Because brain MRI requires sedation, this should be considered when indicated by other findings of the full clinical assessment. Early neurosensory hearing loss as opposed to conductive hearing loss may indicate possible Hurler syndrome. In cases where symptoms are not apparent in early infancy or

the symptoms do not clearly indicate severe disease, frequent follow-up is necessary to more confidently predict disease severity and, thus, the most appropriate treatment course.

In cases where the disease severity cannot be predicted (group 3), clinical and biochemical follow-up is important in establishing the appropriate treatment plan. Assessments should be conducted at 3 and 6 months of age at a minimum, and for longer duration depending on clinical need. Parental and sibling testing may provide insights into the potential phenotype associated with these alleles. For example, if an older sibling had identical findings, this would provide insight into disease severity. Most patients with severe MPS I will show signs of clinical disease by 6 months of age. There is concern that commencement of ERT early in group 3 patients could potentially mask the clinical signs that would lead to distinction between whether a patient would be designated as Hurler or attenuated. For the most part, this will not be difficult for families to accept provided a clear rationale is provided to them. The reality is that group 3 should represent a small group of patients who should have minimal symptoms of disease. If at this young age the patient had significant disease symptoms, the clinical team would likely correctly classify the patient as Hurler and, thus, process accordingly. For patients who cannot be conclusively classified with severe MPS I, or when evaluations indicate attenuated disease, ERT should be started at least by 6 months of age, with the caveat that the characteristic features that may lead to classifying the patient as Hurler syndrome may be masked if ERT is initiated too early, thus, resulting in delay in HSCT initiation.

If a patient has low confirmatory enzyme levels with normal uGAG and normal clinical assessment in the presence of 1 or 2 pseudodeficiency genes, the NBS is assumed to be a false positive (group 4 of the algorithm). Follow-up in 6 months to a year may be considered to confirm that the decreased enzyme activity is benign as well as to alleviate parental concerns. Confirmation of a single pathogenic mutation would be indicative of carrier status, however, and should prompt appropriate genetic counseling of other family members.

Discussion

The algorithm developed by this working group should provide guidance for interpreting NBS results for MPS I and a framework for the management of infants with this disease who are diagnosed in the newborn period. To assess the effectiveness of NBS for MPS I as well as to address practical issues related to which clinical features at what age and which biomarkers are most helpful in providing clinical delineation of patients, a coordinated effort for data collection as well as the potential of establishment of a sample repository for biomarker evaluation and discovery should be considered. The group acknowledges that they represent a small number of individuals who would be considered as MPS experts but feel that this guidance document will aid in providing the background necessary for physicians to understand the complexity of NBS for MPS I. Although the initial confirmation of a positive MPS I newborn screen can be initiated by a wide range of providers, it is recommended that geneticists/metabolic disease specialists be notified of the positive screen and be closely involved in the interpretation and organization of the confirmatory analysis. This will ensure that families receive accurate and timely information regarding this complex disorder. In addition to direct management of the identified case, genetic counseling should be provided to all families for ongoing support related to NBS applications, disease education, reproductive counseling for the parents, identification of at-risk older siblings, developing a testing plan for subsequent siblings, and discussion about carrier testing for at risk family members. ■

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References

1. Muenzer J. Overview of the mucopolysaccharidoses. Rheumatology 2011;50:v4-12.
2. Muenzer J, Wraith JE, Clarke LA. Mucopolysaccharidosis I: management and treatment guidelines. Pediatrics 2009;123:19-29.
3. Moore D, Connock MJ, Wraith E, Lavery C. The prevalence of and survival in Mucopolysaccharidosis I: Hurler, Hurler-Scheie and Scheie syndromes in the UK. Orphanet J Rare Dis 2008;3:24.
4. de Ru MH, Boelens JJ, Das AM, Jones SA, van der Lee JH, Mahlaoui N, et al. Enzyme replacement therapy and/or hematopoietic stem cell transplantation at diagnosis in patients with mucopolysaccharidosis type I: results of a European consensus procedure. Orphanet J Rare Dis 2011;6:55-62.
5. Aldenhoven M, Wynn RF, Orchard PJ, O'Meara A, Veys P, Fischer A, et al. Long-term outcome of Hurler syndrome patients after hematopoietic cell transplantation: an international multicenter study. Blood 2015;125:2164-72.
6. Kunin-Batson AS, Shapiro EG, Rudser KD, Lavery CA, Bjoraker KJ, Jones SA, et al. Long-term cognitive and functional outcomes in children with mucopolysaccharidosis (MPS)-IH (Hurler Syndrome) treated with hematopoietic cell transplantation. JIMD Rep 2016;29:95-102.
7. Aldenhoven M, Jones SA, Bonney D, Borrill RE, Coussons M, Mercer J, et al. Hematopoietic cell transplantation for mucopolysaccharidosis patients is safe and effective: results after implementation of international guidelines. Biol Blood Marrow Transplant 2015;21:1106-9.
8. Kakkis ED, Muenzer J, Tiller GE, Waber L, Belmont J, Passage M, et al. Enzyme-replacement therapy in mucopolysaccharidosis I. N Engl J Med 2001;344:182-8.
9. Sifuentes M, Doroshow R, Hoft R, Mason G, Walot I, Diament M, et al. A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years. Mol Genet Metab 2007;90:171-80.
10. Wraith JE. The first 5 years of clinical experience with laronidase enzyme replacement therapy for mucopolysaccharidosis I. Expert Opin Pharmacother 2005;6:489-506.
11. Wraith JE, Clarke LA, Beck M, Kolodny EH, Pastores GM, Muenzer J, et al. Enzyme replacement therapy for mucopolysaccharidosis I: a randomized, double-blinded, placebo-controlled, multinational study of recombinant human alpha-L-iduronidase (laronidase). J Pediatr 2004;144:581-8.
12. Clarke LA, Wraith JE, Beck M, Kolodny EH, Pastores GM, Muenzer J, et al. Long-term efficacy and safety of laronidase in the treatment of mucopolysaccharidosis I. Pediatrics 2009;123:229-40. doi:10.1542/peds.2007-3847.
13. Shapiro EG, Nestrasil I, Rudser K, Delaney K, Kovac V, Ahmed A, et al. Neurocognition across the spectrum of mucopolysaccharidosis type I: age, severity, and treatment. Mol Genet Metab 2015;116:61-8.

14. Ghosh A, Miller W, Orchard PJ, Jones SA, Mercer J, Church HJ, et al. Enzyme replacement therapy prior to haematopoietic stem cell transplantation in Mucopolysaccharidosis Type I: 10 year combined experience of 2 centres. Mol Genet Metab 2016;117:373-7.
15. Hopkins PV, Campbell C, Klug T, Rogers S, Raburn-Miller J, Kiesling J. Lysosomal storage disorder screening implementation: findings from the first six months of full population pilot testing in Missouri. J Pediatr 2015;166:172-7.
16. Scott CR, Elliott S, Buroker N, Thomas LI, Keutzer J, Glass M, et al. Identification of infants at risk for developing Fabry, Pompe, or mucopolysaccharidosis-I from newborn blood spots by tandem mass spectrometry. J Pediatr 2013;163:498-503.
17. Lin S-P, Lin H-Y, Wang T-J, Chang C-Y, Lin C-H, Huang S-F, et al. A pilot newborn screening program for Mucopolysaccharidosis type I in Taiwan. Orphanet J Rare Dis 2013;8:147-54.
18. Paciotti S, Persichetti E, Pagliardini S, Deganuto M, Rosano C, Balducci C, et al. First pilot newborn screening for four lysosomal storage diseases in an Italian region: identification and analysis of a putative causative mutation in the GBA gene. Clin Chim Acta 2012;413:1827-31.
19. Matern D, Gavrilov D, Oglesbee D, Raymond K, Rinaldo P, Tortorelli S. Newborn screening for lysosomal storage disorders. Semin Perinatol 2015;39:206-16.
20. US Department of Health and Human Services. Advisory committee on heritable disorders in newborns and children. http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpanel/index.html. Accessed November 26, 2016.
21. US Department of Health and Human Services Health Resources and Services Administration. Nominate a condition. http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/nominatecondition/reviews/nominatedconditions.pdf. Accessed November 26, 2016.
22. Pastores GM, Arn P, Beck M, Clarke JTR, Guffon N, Kaplan P, et al. The MPS I registry: design, methodology, and early findings of a global disease registry for monitoring patients with Mucopolysaccharidosis Type I. Mol Genet Metab 2007;91:37-47.
23. de Ru MH, Teunissen QG, van der Lee JH, Beck M, Bodamer OA, Clarke LA, et al. Capturing phenotypic heterogeneity in MPS I: results of an international consensus procedure. Orphanet J Rare Dis 2012;7:22-30.
24. Clarke LA, Winchester B, Giugliani R, Tylki-Szymanska A, Amartino H. Biomarkers for the mucopolysaccharidoses: discovery and clinical utility. Mol Genet Metab 2012;106:395-402.
25. Bunge S, Clements PR, Byers S, Kleijer WJ, Brooks DA, Hopwood JJ. Genotype-phenotype correlations in mucopolysaccharidosis type I using enzyme kinetics, immunoquantification and in vitro turnover studies. Biochim Biophys Acta 1998;1407:249-56.
26. Fuller M, Brooks DA, Evangelista M, Hein LK, Hopwood JJ, Meikle PJ. Prediction of neuropathology in mucopolysaccharidosis I patients. Mol Genet Metab 2005;84:18-24.
27. Kingma SD, Langereis EJ, De Klerk CM, Zoetekouw L, Wagemans T, Ijlst L, et al. An algorithm to predict phenotypic severity in mucopolysaccharidosis type I in the first month of life. Orphanet J Rare Dis 2013;8:99-109.
28. Heywood WE, Camuzeaux S, Doykov I, Patel N, Preece R-L, Footitt E, et al. Proteomic discovery and development of a multiplexed targeted MRM-LC-MS/MS assay for urine biomarkers of extracellular matrix disruptioninMucopolysaccharidosesI,II,andVI.AnalChem2015;87:12238-44.
29. Randall DR, Colobong KE, Hemmelgarn H, Sinclair GB, Hetty E, Thomas A, et al. Heparin cofactor II-thrombin complex: a biomarker of MPS disease. Mol Genet Metab 2008;94:456-61.
30. Terlato NJ, Cox GF. Can mucopolysaccharidosis type I disease severity be predicted based on a patient's genotype? A comprehensive review of the literature. Genet Med 2003;5:286-94.
31. Li P, Wood T, Thompson JN. Diversity of mutations and distribution of single nucleotide polymorphic alleles in the human alpha-L-iduronidase (IDUA) gene. Genet Med 2002;4:420-6.
32. Beesley CE, Meaney CA, Greenland G, Adams V, Vellodi A, Young EP, et al. Mutational analysis of 85 mucopolysaccharidosis type I families: frequency of known mutations, identification of 17 novel mutations and in vitro expression of missense mutations. Hum Genet 2001;109:503-11.
33. Thomas G. "Pseudodeficiencies" of lysosomal hydrolases. Am J Hum Genet 1994;54:934-40.
34. Whitley CB, Gorlin RJ, Krivit W. A nonpathologic allele (IW) for low alpha-L-iduronidase enzyme activity vis-a-vis prenatal diagnosis of Hurler syndrome. Am J Med Genet 1987;28:233-43.
35. Aronovich EL, Pan D, Whitley CB. Molecular genetic defect underlying alpha-L-iduronidase pseudodeficiency. Am J Hum Genet 1996;58:75-85.
36. Pollard L, Braddoc S, Christensen K, Boylan D, Heese B. Diagnostic follow-up of 47 infants with a positive newborn screen for Hurler syndrome: identification of four recurrent IDUA sequence changes that significantly reduce enzyme activity. In: APHL meeting, Anaheim, CA. Proceedings of the 2014 APHl newborn screening and genetic testing symposium, Anaheim, CA, October 27-30, 2014. 2014. http://www.aphl.org/conferences/Documents/Follow-up-2.pdf. Accessed November 26, 2016.
37. Atherton A, Burton B, Day-Salvatore D, Clarke L, Kaplan P, Leslie N, et al. Guidelines for the diagnosis and management of infants with MPS I identified through newborn screening. 2015. https://aap.confex.com/aap/2015/webprogrampress/Paper31844.html. Accessed November 26, 2016.
38. Ombrone D, Malvagia S, Funghini S, Giocaliere E, Bona Della ML, Forni G, et al. Screening of lysosomal storage disorders: application of the online trapping-and-cleanup liquid chromatography/mass spectrometry method for mucopolysaccharidosis I. Eur J Mass Spectrom (Chichester) 2013;19:497-503.
39. Chennamaneni NK, Kumar AB, Barcenas M, Spacil Z, Scott CR, Turecek F, et al. Improved reagents for newborn screening of mucopolysaccharidosis types I, II, and VI by tandem mass spectrometry. Anal Chem 2014;86:4508-14.
40. Gucciardi A, Legnini E, Di Gangi IM, Corbetta C, Tomanin R, Scarpa M, et al. A column-switching HPLC-MS/MS method for mucopolysaccharidosis type I analysis in a multiplex assay for the simultaneous newborn screening of six lysosomal storage disorders. Biomed Chromatogr 2014;28:1131-9.
41. Tomatsu S, Kubaski F, Sawamoto K, Mason RW, Yasuda E, Shimada T, et al. Newborn screening and diagnosis of mucopolysaccharidoses: application of tandem mass spectrometry. Nihon Masu Sukuriningu Gakkai Shi 2014;24:19-37.
42. Thomas JA, Beck M, Clarke JTR, Cox GF. Childhood onset of Scheie syndrome, the attenuated form of mucopolysaccharidosis I. J Inherit Metab Dis 2010;33:421-7.
43. Vijay S, Wraith JE. Clinical presentation and follow-up of patients with the attenuated phenotype of mucopolysaccharidosis type I. Acta Paediatr 2005;94:872-7.
44. Clarke L, Heppner J. Mucopolysaccharidosis type I. In: Pagon RA, Adam MP, Ardinger HH, eds. Gene reviews [Internet]. Seattle (WA): University of Washington, Seattle; 2015. http://www.ncbi.nlm.nih.gov/books/NBK1162/?report=printable. Accessed November 26, 2016.
45. Neufeld E, Muenzer J. The mucopolysaccharidoses. New York: McGraw Hill; 2001.
46. Zafeiriou DI, Batzios SP. Brain and spinal MR imaging findings in mucopolysaccharidoses: a review. AJNR Am J Neuroradiol 2013;34:5-13.
47. Gabrielli O, Clarke LA, Ficcadenti A, Santoro L, Zampini L, Volpi N, et al. 12 year follow up of enzyme-replacement therapy in two siblings with attenuated mucopolysaccharidosis I: the important role of early treatment. BMC Med Genet 2016;17:19-25.
48. Poe MD, Chagnon SL, Escolar ML. Early treatment is associated with improved cognition in Hurler syndrome. Ann Neurol 2014;76:747-53.
49. Yasuda E, Mackenzie W, Ruhnke K, Shimada T, Mason RW, Zustin J, et al. Molecular Genetics and Metabolism Report Long-term follow-up of post hematopoietic stem cell transplantation for Hurler syndrome: clinical, biochemical, and pathological improvements. Mol Genet Metab Rep 2015;2:65-76.
50. Clarke LA. Pathogenesis of skeletal and connective tissue involvement in the mucopolysaccharidoses: glycosaminoglycan storage is merely the instigator. Rheumatology (Oxford) 2011;50:13-8.
51. Muenzer J. Early initiation of enzyme replacement therapy for the mucopolysaccharidoses. Mol Genet Metab 2014;111:63-72.
52. Martin JJ, Ceuterick C. Prenatal pathology in mucopolysaccharidoses: a comparison with postnatal cases. Clin Neuropathol 1983;2:122-7.

53. Baldo G, Matte U, Artigalás O, Schwartz IV, Burin MG, Ribeiro E, et al. Placenta analysis of prenatally diagnosed patients reveals early GAG storage in mucopolysaccharidoses II and VI. Mol Genet Metab 2011;103:197-8.
54. Baldo G, Mayer FQ, Martinelli BZ, de Carvalho TG, Meyer FS, de Oliveira PG, et al. Enzyme replacement therapy started at birth improves outcome in difficult-to-treat organs in mucopolysaccharidosis I mice. Mol Genet Metab 2013;109:33-40.
55. Dierenfeld AD, McEntee MF, Vogler CA, Vite CH, Chen AH, Passage M, et al. Replacing the enzyme alpha-L-iduronidase at birth ameliorates symptoms in the brain and periphery of dogs with mucopolysaccharidosis type I. Sci Transl Med 2010;2:60ra89.
56. Al-Sannaa NA, Bay L, Barbouth DS, Benhayoun Y, Goizet C, Guelbert N, et al. Early treatment with laronidase improves clinical outcomes in patients with attenuated MPS I: a retrospective case series analysis of nine sibships. Orphanet J Rare Dis 2015;10:131-9.
57. Gabrielli O, Clarke LA, Bruni S, Coppa GV. Enzyme-replacement therapy in a 5-month-old boy with attenuated presymptomatic MPS I: 5-year follow-up. Pediatrics 2009;125:e183-7.