Diagnosis of the mucopolysaccharidoses

Thomas J. A. Lehman1, Nicole Miller2, Becky Norquist2, Lisa Underhill3 and Joan Keutzer3

Abstract

The mucopolysaccharidoses (MPSs) often present a diagnostic challenge, particularly for patients who have more slowly progressive disease phenotypes, as early disease manifestations can be subtle or non-specific. However, certain types of bone and joint involvement should always prompt consideration of an MPS diagnosis, such as early joint involvement without classic inflammatory features or erosive bone lesions, claw hand, spinal deformities or dysostosis multiplex. All such patients should be referred to a geneticist or metabolic specialist for diagnostic evaluation. The earlier the diagnosis is made, the better the potential outcome of treatment. Each type of MPS is associated both with deficient activity of a specific lysosomal enzyme that degrades specific glycosaminoglycans (GAGs) and with abnormalities in urinary GAG excretion. MPS patients usually excrete excess GAG in urine and/or have different relative proportions of types of GAG in urine as compared with age-matched normal subjects. Although urinary GAG analyses (both quantitative and qualitative) can suggest the most likely type of MPS, diagnosis must be confirmed by enzyme assay. Multiple assays may be necessary to identify the disease subtype. Correct identification of the MPS type is essential to guide treatment and management decisions.

Key words: Glycosaminoglycan, Laboratory diagnosis, Mucopolysaccharidoses, MPS, Hurler, Hunter, Sanfilippo, Morquio, Maroteaux–Lamy, Sly.

Introduction

The mucopolysaccharidoses (MPSs) are a heterogeneous group of rare inherited disorders caused by deficiency of a lysosomal enzyme that is necessary to break down or catabolize complex carbohydrates known as mucopolysaccharides or glycosaminoglycans (GAGs) [1, 2]. Accumulation of GAGs can cause progressive damage to a broad range of tissues. This damage often manifests as stiff joints, skeletal malformations, retarded growth, pulmonary deficits, and ocular, hepatic, cardiac and sometimes neurological abnormalities [1, 2]. Early and accurate diagnosis of MPS is critical to the provision of appropriate supportive care and, when available, disease-specific treatment—enzyme replacement therapy (ERT) or haematopoietic stem cell transplantation (HSCT) [3–6]. Early recognition and appropriate management profoundly affect patient quality of life and may potentially slow or prevent development of irreversible pathologies [7–12].

Although recent advances have improved treatment for MPS disorders, lack of disease awareness, the non-specific nature of many symptoms and variable clinical presentations frequently impede rapid and accurate diagnosis. For patients with slowly progressing MPS disease phenotypes, the gap between earliest symptoms and diagnosis is often years, sometimes decades [4, 5, 13–15]. Bone and joint symptoms are common early manifestations of MPS, and undiagnosed patients may initially be referred to a rheumatologist. This is especially likely for patients who have more slowly progressive forms of these disorders. A recent survey of 60 adult and paediatric rheumatologists in North America and Europe found that ~80% were unable to recognize symptoms of MPS I and did not know the appropriate diagnostic tests [16]. This led to the development of a diagnostic algorithm for the evaluation of patients with joint contractures (Fig. 1). The most suggestive rheumatological feature is development of joint pain and joint contractures at an early age without concomitant inflammation. Table 1 lists other key clinical features often apparent in a routine physical exam or medical history that should heighten the suspicion of MPS.
Enhanced disease awareness among rheumatologists is paramount to ensure that patients whose bone and joint symptoms are suggestive of MPS are referred to a geneticist or metabolic specialist for a diagnostic evaluation.

**Laboratory testing for an MPS disorder**

An MPS diagnosis is based on laboratory results from urinary GAG analyses and enzyme activity assays. Enzyme activity assays measure enzyme activity in tissue (blood or fibroblasts). Quantitative GAG assays measure overall elevation of GAG as compared with GAG levels expected in age-matched normal subjects. Qualitative GAG assays detect the type of GAG excreted. Determining the correct MPS type is essential to ensure appropriate therapeutic management. The type of MPS cannot be reliably determined on the basis of clinical presentation or test results alone. High clinical suspicion and thorough testing help ensure an accurate diagnosis of MPS (Fig. 2). Table 2 summarizes the diagnostic assays available for each of the MPS disorders as well as clinical
features that can help distinguish one disorder from another. As diagnosis of these disorders can be complex, we recommend that patients suspected of having MPS be referred to a geneticist or metabolic specialist for laboratory testing. Table 3 lists some common diagnostic pitfalls.

**TABLE 1 Signs and symptoms suggestive of MPS**

<table>
<thead>
<tr>
<th>Common bone and joint features&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Early joint involvement without classic inflammatory features or erosive bone lesions</td>
</tr>
<tr>
<td>Claw hand (see Summers and Ashworth in this supplement [49])</td>
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<tr>
<td>Spinal deformity (subtle or overt gibbus, scoliosis, kyphosis, lordosis) (see Muenzer [50], Morishita and Petty [51] and White [52] in this supplement)</td>
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<tr>
<td>Radiological evidence of dysostosis multiplex (see Muenzer [50], Morishita and Petty [51] and White [52] in this supplement)</td>
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<thead>
<tr>
<th>Other common clinical signs&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Coarsening of facial features over time</td>
</tr>
<tr>
<td>Corneal clouding (can be mild or severe) (see Summers and Ashworth in this supplement [49])</td>
</tr>
<tr>
<td>Short, stiff neck</td>
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<tr>
<td>Frequent respiratory infections, chronic nasal congestion, noisy breathing/snoring</td>
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<tr>
<td>Heart murmur</td>
</tr>
<tr>
<td>History of hernia repair surgery (inguinal and/or umbilical) [42, 43]</td>
</tr>
<tr>
<td>Short stature</td>
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<tr>
<td>Abnormal gait (especially toe walking)</td>
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<tr>
<td>Abdominal protuberance due to liver and spleen enlargement</td>
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</tbody>
</table>

<sup>a</sup>Can be subtle or overt. Absence of any particular sign or symptom does not rule out MPS.

**FIG. 2 MPSs: the path for laboratory testing.** Based on clinical suspicion, a primary care physician or specialist may refer a patient to a geneticist or metabolic specialist who will acquire the patient’s clinical history. Using a reliable laboratory, the geneticist or metabolic specialist should order relevant urine GAG analysis (quantitative and qualitative) and enzyme activity assays. If enzyme activity testing identifies a defect in a lysosomal enzyme, molecular testing should also be performed when feasible. The geneticist or metabolic specialist should review the patient’s medical history and, in collaboration with the laboratory, interpret the laboratory assay results before completing a diagnosis and providing treatment recommendations. Urine GAG analysis can be used to monitor therapy. When a sibling of an MPS patient is identified, the undiagnosed sibling should undergo the same clinical history and laboratory test process. It may also be possible to proceed directly to enzyme activity testing and molecular testing for the undiagnosed sibling. DS: dermatan sulphate; HS: heparan sulphate; KS: keratan sulphate.

**Urine GAG analysis**

In normal subjects, urinary GAG excretion varies with age, higher values being found during the first years of life, followed by a slow and constant decrease thereafter. Qualitatively, ~90% of the GAG content in normal urine consists of chondroitin-4 and -6 sulphate, with the remaining
## Table 2 Diagnosis of MPS disorders

<table>
<thead>
<tr>
<th>MPS typea</th>
<th>Other names</th>
<th>Qualitative GAG assay: type of GAG excreted</th>
<th>Enzyme activity assay: enzyme defective in disorder</th>
<th>Distinguishing clinical features relative to other MPS disordersa</th>
<th>Caveats for MPS laboratory tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPS I</td>
<td>Hurler, Hurler-Scheie, Scheie</td>
<td>DS, HS</td>
<td>α-L-iduronidase</td>
<td>Children with cognitive impairment rarely have overt behavioural issues [4]</td>
<td>Total urinary GAG is usually elevated, but false negatives can occur, especially with spot testing [16]</td>
</tr>
<tr>
<td>MPS II</td>
<td>Hunter</td>
<td>DS, HS</td>
<td>Iduronate sulphatase</td>
<td>Progressive corneal clouding [2]</td>
<td>Total urinary GAG is usually elevated, but false negatives can occur, especially with spot testing [16]</td>
</tr>
<tr>
<td>MPS III</td>
<td>Sanfilippo, types A-D</td>
<td>HS</td>
<td>A: heparan N-sulphatase, B: α-N-acetylgalactosaminidase, C: acetyl-CoA: α-glucosaminide acetyltransferase, D: N-acetylglucosamine-6-sulphatase</td>
<td>Behavioural/cognitive changes precede overt bone and joint involvement [53]</td>
<td>Corneal clouding not common and usually mild [2, 54]</td>
</tr>
<tr>
<td>MPS IV</td>
<td>Morquío, types A and B</td>
<td>A: KS, CS, B: KS</td>
<td>A: galactose 6-sulphatase, also known as GALNS or N-acetylgalactosamine 6-sulphatase, B: β-galactosidase</td>
<td>Cognition is usually fully preserved, even in severely affected patients [2]</td>
<td>Total GAG level is frequently in the normal range [18], galactose-6-sulphatase activity can be labile when assayed by DBS; additional controls may be necessary to ensure accuracy when diagnosing MPS IVA [39]</td>
</tr>
<tr>
<td>MPS VI</td>
<td>Maroteaux-Lamy</td>
<td>DS</td>
<td>Arylsulphatase B (ASB) or N-acetylgalactosamine 4-sulphatase</td>
<td>Cognition is usually fully preserved, even in severely affected patients [1]</td>
<td>Measure qualitative (DS), quantitative urine GAGs, multiple sulphatases and multiple enzymes; total urinary GAG may be within the normal range in slowly progressing patients [60]</td>
</tr>
<tr>
<td>MPS VII</td>
<td>Sly</td>
<td>DS, HS, CS</td>
<td>β-Glucuronidase</td>
<td>Mild to severe corneal clouding usually develops; may not be an early sign [2, 54, 55]</td>
<td>Hydrops fetalis is common at birth [1, 2]</td>
</tr>
<tr>
<td>MPS IX</td>
<td>Hyaluronidase deficiency</td>
<td>Unknown; four patients described in the literature [56, 57]</td>
<td>Hyaluronidase</td>
<td>No ocular features</td>
<td>Elevated plasma hyaluronan levels</td>
</tr>
</tbody>
</table>

*aNote that clinical features alone should not be used to rule out or rule in a particular type of MPS. DS: dermatan sulphate; HS: heparan sulphate; CS: chondroitin-4 and -6 sulphate; KS: keratan sulphate.*
for the known disease-causing mutations in addition to molecular testing includes looking at specific if appropriate controls.

Heparan sulphate can be present in trace amounts in urine of normal individuals. Lab tests may be routed to non-MPS-specialized lab.

Enzyme activity

The diagnosis of MPS should be confirmed by enzyme activity testing (Table 2). Geneticists and metabolic specialists should also test for related diseases, such as multiple sulphatase deficiency and mucolipidosis, both of which have some clinical features in common with MPS. Enzyme activity typically is measured in leucocytes or cultured fibroblasts (skin biopsy or punch). For many types of MPS, enzyme activity can be measured from a dried blood spot (DBS). DBS-based assays offer considerable practical advantages with respect to sample collection, storage and transport, and multiple enzyme activity tests can be performed on a single DBS. Recent technological advances have made these assays sensitive and specific if appropriate controls are performed. It is recommended that a positive result from a DBS be confirmed by a tissue-based assay. Due to the complexity of these assays, referral to a geneticist or metabolic specialist is essential.

Mutation analysis

Mutation analysis or molecular testing includes looking for the known disease-causing mutations in addition to looking for abnormal sequences in the gene coding for a particular enzyme. Molecular testing has limited utility in initial screening because of the extreme genetic heterogeneity that characterizes all types of MPS. Although several MPS disorders are associated with specific commonly occurring mutations, most families have private mutations [3-5]. Thus the primary use of molecular testing is to confirm a diagnosis of a particular type of MPS or to evaluate family members when the type of MPS and the family mutation is known (Fig. 2). Molecular testing can have prognostic value if the mutations that are identified have been well characterized [21-25].

When a patient has been diagnosed with a specific type of MPS and his/her mutation is known, the information can be used for carrier testing and prenatal testing of siblings. With regard to carrier testing, it is important to note that except for MPS II (Hunter), the MPSs are inherited in an autosomal recessive fashion, so carriers have a very small likelihood of having children affected with MPS unless the union is consanguineous.

Biomarkers

Currently there are no established biomarkers for any of the MPS types. Urinary GAG levels usually decrease with treatment [7, 11, 26, 27], but are not an ideal biomarker. Other biomarkers under investigation include heparin cofactor II-thrombin complex measured in serum [28, 29], the ratio of dermatan to chondroitin sulphate in urine [29, 30], and dipeptidyl peptidase IV in plasma [31]. An ideal biomarker would be specific to a particular type or types of MPS, would help to differentiate more severe from less severe disease phenotypes, would respond to treatment and would be easily detected and quantified.

Screening for MPS disorders in newborns and at-risk populations

As early diagnosis and early treatment are helpful to the patient and family, implementation of screening programs can improve opportunities for early detection and intervention. In general, screening falls into two categories: newborn screening (which can be performed as a DBS-based assay that screens for a panel of diseases) [32-36] and screening of populations considered at risk of MPS because of specific signs or symptoms.

Newborn screening offers the hope of disease identification and treatment initiation before development of irreversible disease manifestations. Several of the MPSs are not clinically obvious at birth, but they are progressive, debilitating and often life threatening. MPSs for which treatment is available are excellent candidates for newborn screening because early intervention may have a significant impact on disease outcome [36]. The American Council of Medical Genetics recently published guidelines for newborn screening of nine lysosomal storage disorders, including MPS I, II and VI [37].

The potential impact of early, pre-symptomatic intervention has been demonstrated in case studies of MPS I, II and VI sibling pairs in which the younger sibling was...
diagnosed at birth, started on enzyme replacement therapy in infancy and had a much more favourable clinical outcome than the older sibling who began treatment at a later age after symptoms became evident [8, 10, 12].

Symptom-based screening programmes among high-risk populations may help identify MPS patients who have more slowly progressive forms of these diseases. Possible symptom targets include corneal clouding, joint manifestations, surgical history [42, 43] and CTS (nearly ubiquitous among children with MPS and very uncommon in the general paediatric population [44–48]). In this regard, routine screening of children presenting at an early age with hand dysfunction without anatomic lesions may be useful.

**Standardization of assays**

Since MPS diagnostic testing and screening are not currently standardized, open communication between laboratories and physicians is necessary to facilitate the interpretation of specific diagnostic tests and results. Along with clinical signs and symptoms, these diagnostic tests will help ultimately to provide an accurate diagnosis of MPS. We recommend that laboratories implement programs to self-monitor testing proficiency (e.g. for urinary GAG and enzyme activity assays), such as those offered by the European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM; www.erndim.unibas.ch) and the College of American Pathologists (CAP; http://www.cap.org/apps/cap.portal).

**Conclusions**

An MPS diagnosis should be considered for all patients who present with joint symptoms without signs of inflammation, especially in concert with other suggestive clinical signs, such as corneal clouding, frequent respiratory infections and gastrointestinal complaints, heart murmur, short stature, abnormal gait or history of hernia repair. Such patients should be referred promptly to a geneticist or metabolic specialist, as early and accurate diagnosis can maximize therapeutic outcome. High clinical suspicion informed by specific laboratory diagnostic results from multiple samples across multiple tests ensure an accurate diagnosis of MPS.

**Rheumatology key messages**

- Promptly refer all patients with suspected MPS to a geneticist or metabolic specialist.
- Laboratory diagnosis of MPS is based on urinary GAG analyses and enzyme activity tests.
- High clinical suspicion and results from multiple samples and tests inform the diagnosis of MPS.

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**Supplement**

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