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Systematic Diagnosis of Lysosomal Storage Diseases in Patients with Hepatosplenomegaly

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Abstract

The clinical detection of hepatomegaly and splenomegaly—abnormal enlargement of the liver and spleen, respectively—requires a comprehensive evaluation, as numerous underlying conditions may be responsible, including metabolic, congestive, neoplastic, infectious, toxic, or inflammatory etiologies. Within metabolic disorders, lysosomal storage diseases (LSDs) are a rare and ultra-rare group, collectively affecting approximately 1 in 5000 live births. These disorders arise from genetic mutations impacting lysosomal enzymes, membrane proteins, or transporters, causing intracellular accumulation of metabolites and subsequent organ dysfunction. Advances in early diagnosis and targeted therapies have improved survival and quality of life for many affected individuals. Consequently, LSDs should be considered in patients presenting with hepatosplenomegaly across all age groups. This review outlines the diagnostic evaluation of hepatosplenomegaly with particular focus on LSDs, highlighting clues from patient history, physical examination, laboratory findings, and imaging. Molecular testing, preferably over biopsy, is emphasized, with enzymatic analysis recommended when possible.

Keywords: Lysosomal storage diseases, Hepatomegaly, Splenomegaly, Organomegaly, Hepatosplenomegaly, Biomarkers

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Background

Hepatomegaly and splenomegaly (HSM) represent abnormal liver and spleen enlargement, respectively, detectable through physical examination or imaging modalities [1,2]. Physical exam often identifies these enlargements, but palpation may miss, underestimate, or overestimate the organ size [2,3]. Therefore, imaging is recommended to confirm organomegaly and characterize features such as cysts, masses, or signs of congestion or infiltration. Magnetic resonance imaging (MRI) and computed tomography (CT) are considered gold-standard techniques for accurate liver and spleen volume assessment; however, limitations including cost, radiation exposure, claustrophobia, and presence of metal implants may restrict their use [4]. In such situations, ultrasound is a practical, widely accessible, and well-tolerated alternative [2,4].

The differential diagnosis for hepatomegaly, splenomegaly, or both is extensive, encompassing metabolic, congestive, neoplastic, infectious, toxic, and inflammatory causes. Evaluation of HSM often requires a detailed workup involving pediatricians, internists, gastroenterologists, or hematologists. Initial laboratory studies may reveal hepatocellular injury, cholestasis, or impaired synthetic liver function [5]. Hence, a structured

and systematic approach reduces unnecessary invasive procedures and accelerates diagnosis.

Among metabolic contributors to HSM are lysosomal storage diseases (LSDs). While individually rare, as a group they occur in roughly 1 in 5000 live births [6]. Most LSDs follow an autosomal recessive inheritance pattern, with exceptions such as mucopolysaccharidosis type II, Fabry disease, and Danon disease, which are X-linked. Lysosomes are organelles critical for degradation of lipids, proteins, and carbohydrates, as well as for signal transduction and ion balance. Dysfunction of lysosomal enzymes, transporters, membrane proteins, or activators results in substrate accumulation and impaired lysosomal function [7].

LSDs exhibit heterogeneous manifestations, affecting multiple organs from fetal life into adulthood, with a wide spectrum of severity. Attenuated or late-onset forms are increasingly recognized in adults, leading to higher referrals to adult metabolic centers worldwide [8]. In adults, screening for Gaucher disease, Niemann-Pick type C (NPC), cholesterol ester storage disease (CESD), and acid sphingomyelinase deficiency (ASMD) is commonly considered.

Multidisciplinary care and disease-specific therapies have markedly improved morbidity, mortality, and quality of life in LSD patients. Nevertheless, early recognition and intervention are critical to achieve these outcomes. Definitive diagnosis also enables family screening and informed genetic counseling [9].

After diagnosis, patients should be referred for comprehensive, disease-specific management. Therapeutic options include enzyme replacement therapy (ERT), which is available for ASMD [10]. Other strategies include substrate reduction therapy [11], while advanced liver disease may necessitate liver transplantation [12]. For NPC, management is largely supportive, with miglustat providing a disease-modifying effect in patients with late-onset neurological involvement [13]. Hematopoietic stem cell transplantation (HSCT) has been trialed in LSDs with neurocognitive involvement, such as Hurler syndrome [14].

This review presents a practical diagnostic framework for HSM, emphasizing LSDs. Clinical clues from history, physical exam, laboratory tests (including biomarkers and urine biochemical assays), and imaging are discussed. Molecular testing, supplemented with enzymatic analysis when available, is recommended as the preferred confirmatory approach, whereas biopsies should be reserved as a last resort.

Etiologies of Liver and Spleen Enlargement

Frequently encountered causes

In clinical practice, the evaluation of hepatomegaly and/or splenomegaly (HSM) usually begins with more prevalent conditions unless there is clear evidence pointing toward one or more lysosomal storage diseases (LSDs). Common contributors include metabolic disorders such as Hemochromatosis or Wilson disease; cardiovascular congestion including heart failure or thrombosis; malignancies like leukemia, lymphoma, hepatoblastoma, hepatocellular carcinoma, or metastatic liver tumors; hematologic conditions including thalassemia and sickle cell disease; infections such as cytomegalovirus, toxoplasmosis, or hepatitis viruses; inflammatory disorders like sarcoidosis or systemic lupus erythematosus; toxic insults, e.g., acetaminophen overdose; and infiltrative diseases such as amyloidosis [15, 16].

Lysosomal storage disorders

Patients with LSDs generally exhibit enlarged but non-tender liver and spleen, often with smooth organ contours [17]. However, irregularities in organ shape do not rule out LSDs. Hepatosplenomegaly is more frequently identified in early-onset or severe disease forms. **Table 1** lists LSDs associated with HSM, organized by type along with their causative genes. Conditions involving lysosome-related organelles—such as Chediak–Higashi syndrome or Hermansky–Pudlak syndrome—are excluded here, though they can occasionally result in HSM in the context of hemophagocytic lymphohistiocytosis [18].

Table 1. LSDs with documented hepatosplenomegaly

Category	Disorder	Affected Gene	Hepatosplenomegaly Severity
Sphingolipidoses	Gaucher disease [19]	GBA	++
	Acid Sphingomyelinase deficiency [20]	SMPD1	++
	Saposin C deficiency [21]	PSAP	++
	Farber disease (severe) [22]	ASAH1	(+)
	Farber disease (mild/intermediate) [22]	ASAH1	(+/−)
	GM1 gangliosidosis type 1 [23]	GLB1	++
	GM1 gangliosidosis type 2 [23]	GLB1	(+)
	Sandhoff disease (acute infantile) [24]	HEXB	(+/−)
	GM2 gangliosidosis, AB variant [25]	GM2A	(+/−)
	Multiple sulfatase deficiency [26]	SUMF1	+
MPS	Mucopolysaccharidosis I [27]	IDUA	++
	Mucopolysaccharidosis II [28]	IDS (X-linked)	+

	Mucopolysaccharidosis IIIA, IIIB [29]	SGSH, NAGLU	+
	Mucopolysaccharidosis IIIC, IIID [30]	HGSNAT, GNS	(+/−)
	Mucopolysaccharidosis IVA, IVB [31]	GLB1, GALNS	(+)
	Mucopolysaccharidosis VI [32]	ARSB	(+)
	Mucopolysaccharidosis VII [33]	GUSB	(+)
OLS	Aspartylglucosaminuria [34]	AGA	(+/−)
	Fucosidosis [35]	FUCA1	(+)
	Galactosialidosis [36]	CTSA	++
	Alpha mannosidosis [37]	MAN2B1	+
	Schindler disease type III [38]	NAGA	(+/−)
	Neuraminidase deficiency type I [39]	NEU1	(+/−)
	Neuraminidase deficiency type II [40]	NEU1	+
IMP	Mucolipidosis II alpha/beta [41]	GNPTAB	++
	Mucolipidosis III alpha/beta [41]	GNPTAB	(+)
	Nephropathic Cystinosis [42]	CTNS	+
	Late-onset nephropathic cystinosis [43]	CTNS	(+/−)
	Free sialic acid storage disease [44]	SLC17A5	+
	Niemann-Pick type C [45]	NPC1, NPC2	(+)
Other	Lysosomal acid lipase deficiency (Wolman disease/CESD) [46]	LIPA	++
	Infantile onset Pompe disease [47]	GAA	+

Symbols: ++ = typically present, + = often present, (+) = occasionally present, (+/−) = rarely present. Abbreviations: CESD = cholesterol esterase storage disease; IMP = integral membrane protein defects; MPS = mucopolysaccharidoses; OLS = oligosaccharidoses.

HSM frequently occurs in MPS types I–VII, ASMD, lysosomal acid lipase deficiency (Wolman disease in children, CESD in adults), GM1 gangliosidosis type I, mucolipidosis type II, galactosialidosis, saposin C deficiency, NPC, and Gaucher disease. Less commonly, HSM is observed in aspartylglucosaminuria and Sandhoff disease, while it is typically absent in metachromatic leukodystrophy.

Additional genetic conditions

Other inherited disorders may also manifest with HSM. For instance, splenomegaly in sickle cell disease or liver enlargement in alpha-1-antitrypsin deficiency or Beckwith–Wiedemann syndrome [17,48]. Beyond LSDs, additional inborn metabolic disorders (IMDs) implicated in HSM include urea cycle defects, classical galactosemia, glycogen storage diseases, hereditary fructose intolerance, tyrosinemia type I, prolidase deficiency, mitochondrial disorders, fatty acid oxidation defects, congenital disorders of glycosylation, and peroxisomal diseases [15]. Thus, genetic and metabolic etiologies should always be considered in the differential diagnosis for HSM.

Work-Up for Suspected LSDs

The first step in evaluating HSM includes basic labs and imaging to rule out common causes. If LSDs are suspected, referral to a specialist in biochemical genetics or a clinician familiar with LSDs is ideal. However, due to geographical or workforce limitations, initial assessment may rely on primary care or general specialty physicians, who should recognize the typical LSD patterns.

Clinical history and physical exam findings may provide clues for LSD suspicion in the context of HSM [49,50]. If suspicion remains high or common causes are excluded, expedited genetic and metabolic testing is warranted.

Figure 1 presents a diagnostic algorithm for adult HSM. Consanguinity increases the likelihood of IMDs and LSDs, which are often caused by biallelic pathogenic variants [51]. Certain LSDs may be detected prenatally, with non-immune hydrops and HSM visible on prenatal imaging [49]. In children, signs may include developmental delay, intellectual disability, regression, or autism, whereas in adults, psychiatric symptoms, cognitive decline, or early-onset dementia may suggest late-onset LSDs, particularly NPC [50,52].

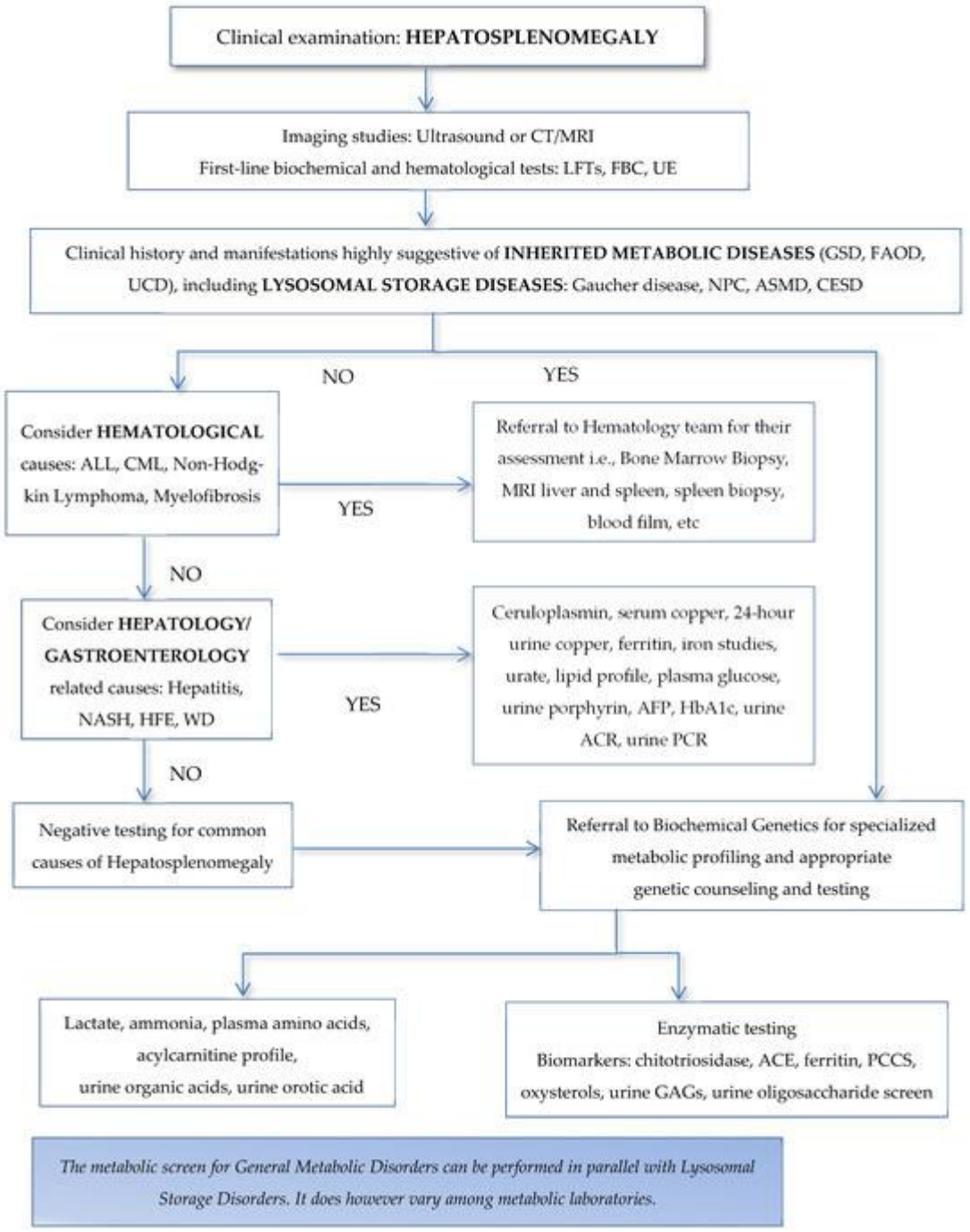

Figure 1. HSM diagnostic workflow

Abbreviations: ACE = angiotensin converting enzyme; AFP = alpha-fetoprotein; ASMD = acid sphingomyelinase deficiency; CESD = cholesterol esterase storage disease; CML = chronic myeloid leukemia; FBC = full blood count; FAOD = fatty acid oxidation disorders; GAGs = glycosaminoglycans; GSD = glycogen storage diseases; HFE = Hemochromatosis; LFTs = liver function tests; PPCS = N-palmitoyl-O-phosphocholineserine; NASH = non-alcoholic steatohepatitis; NPC = Niemann–Pick disease type C; UCD = urea cycle disorders; UE = urea and electrolytes; WD = Wilson disease; ACR = albumin/creatinine ratio; PCR = protein/creatinine ratio.

Clinical features of LSDs

Patients with LSDs may present with distinctive skeletal abnormalities, particularly dysostosis multiplex, commonly observed in MPSs, oligosaccharidoses, and mucolipidoses. A notable diagnostic indicator is coarse facial appearance, which becomes more obvious with age [35,53]. Typical facial characteristics include frontal bossing, flattened nasal bridge, macroglossia, prominent brow ridges, rounded cheeks, thick lips, and widely spaced teeth. Progressive macrocephaly is often reported in children with GM1 gangliosidosis, Sandhoff disease, and

GM2 gangliosidosis caused by GM2 activator deficiency [54].

In attenuated or late-onset forms, classic facial or skeletal signs may be subtle or absent. Additional manifestations may involve the skin: angiokeratomas appear in some oligosaccharidoses [35], and extensive dermal melanocytosis is frequently seen in children with MPSs [55,56], though other cutaneous abnormalities may arise in adults [57].

Farber disease is characterized by a triad of hoarseness, subcutaneous nodules, and joint deformities, with milder forms mimicking juvenile arthritis [22]. Renal involvement is also reported: nephrotic syndrome in Sialidosis and Galactosialidosis, and Fanconi syndrome in cystinosis [58–60].

When hepatosplenomegaly (HSM) is present, an ophthalmologic examination may reveal a cherry-red macula in severe early-onset LSDs, while corneal clouding or cystine crystals can be seen in both children and adults [60–62]. Vertical supranuclear gaze palsy is highly suggestive of NPC, whereas slowed horizontal saccades are characteristic of neuronopathic Gaucher disease [63,64].

Imaging

Imaging studies help distinguish benign from malignant lesions and identify cysts or gaucheromas [16,65]. Some LSDs have disease-specific imaging features, such as adrenal calcifications in Wolman disease [66].

Beyond standard MRI or ultrasound, elastography has proven useful for evaluating liver fibrosis in Gaucher disease [67]. Non-invasive tools like FibroScan allow assessment of liver stiffness and controlled attenuation parameters [68].

A skeletal survey can detect dysostosis multiplex, including: J-shaped sella, thickened diploic spaces, short clavicles, broad ribs, dysplastic vertebrae, scoliosis, tapered pelvis, rounded iliac wings, hip dysplasia, shortened long bones with hypoplastic epiphyses, short/wide metacarpals with thin cortices and proximal pointing, and irregular hypoplastic carpal/tarsal bones. Dysostosis multiplex is hallmark in MPSs, oligosaccharidoses, and mucolipidoses [35,53,57].

Additional skeletal features include bone infarcts, arthropathy, and lytic lesions in Gaucher disease [69], while reduced bone mineral density is common in Gaucher disease and ASMD, particularly in adults [70].

Initial laboratory evaluation

First-line labs aim to detect liver injury, cholestasis, and cirrhosis. Many LSDs with organomegaly show normal liver function tests, so normal labs do not exclude these conditions [20].

- Cholestasis is often present in early-onset NPC
- Liver failure may occur in ASMD and NPC
- Elevated liver enzymes can appear in Farber and Pompe disease
- Rare cirrhosis is reported in Gaucher disease and MPS types I and II [71,72]
- CESD is suspected in patients with hyperlipidemia and mildly elevated liver enzymes [73]
- Wolman disease manifests in childhood with failure to thrive and markedly abnormal labs

Hematologic studies are critical. Anemia, thrombocytopenia, and coagulopathies are frequent in pediatric and attenuated adult Gaucher disease. For example, mild thrombocytopenia (~110,000/μL) may be the only lab abnormality in some adult patients, prompting diagnosis at age 35 (personal observation, KMS). Certain LSDs show vacuolated lymphocytes [74] or azurophilic inclusions [75].

Other metabolic disorders may mimic LSDs:

- Wilson disease, alpha-1-antitrypsin deficiency (cirrhosis)
- Glycogen storage defects, gluconeogenesis defects (hepatomegaly with hypoglycemia/seizures)
- Tyrosinemia type I, Fanconi–Bickel disease (renal involvement)
- Hereditary fructose intolerance (abnormal LFTs)

These are mostly pediatric disorders but should also be considered in adults under catabolic stress (fasting, pregnancy, steroids) [5].

Recommended first-line investigations for HSM:

- Liver function tests: ALT, AST, GGT, ALP, bilirubin, albumin, AFP, total bile acids
- Full blood count, coagulation panel, peripheral smear
- Renal function: urea, creatinine, electrolytes, blood gases
- Lipid profile: triglycerides, cholesterol
- Glucose, lactate, ammonia
- Alpha-1-antitrypsin
- Copper, ceruloplasmin
- Ferritin, iron studies
- Free/total carnitine and acylcarnitine profile
- Plasma/urine amino acids, urine organic acids, urine ketones, urine reducing substances

Second-line tests for LSDs involve:

- Enzymatic assays
- Glycosaminoglycans (GAGs)
- Oligosaccharides
- Other specific or non-specific biomarkers

These are performed in specialized metabolic laboratories, not generally available in standard commercial labs.

Genetic testing

Genetic testing offers a non-invasive approach (using blood, saliva, or buccal swabs) for diagnosing LSDs and other inherited disorders, especially in centers with limited

access to specialized biochemical LSD diagnostics. Pre- and post-test genetic counseling is strongly recommended. Molecular analysis can be conclusive when pathogenic or likely pathogenic variants are identified. However, variants of uncertain significance (VUS) may necessitate additional targeted biochemical tests, such as enzymology or biomarker assessments, which can confirm the diagnosis and potentially reclassify the variant. VUS findings are non-diagnostic and are more common in populations underrepresented in genomic databases. Genetic testing is usually performed alongside or after the biochemical evaluations discussed previously, including urine analysis, enzymatic assays, and biomarker testing.

Sanger sequencing for single-gene testing is now rarely used. Instead, short-read next-generation sequencing (NGS) has become widely accessible and cost-effective, supporting approaches from gene panels to exome or whole-genome sequencing [76]. Gene panels allow simultaneous analysis of multiple genes linked to a specific phenotype or disease group, such as LSDs or hepatosplenomegaly (HSM) [77]. It is crucial to verify that the genes of interest are included in the selected panel and to note the turnaround time (typically 3–4 weeks for commercial labs). For outpatient assessments, gene panels or exome sequencing are generally preferred, whereas rapid exome or genome sequencing can provide results within a week for urgent or critical care situations [78].

Some limitations exist: certain variants may be missed, such as partial exon deletions in short-read NGS panels [79]. Pseudogenes can complicate sequencing of specific genes, including GBA and IDS, relevant to Gaucher disease and MPS II, respectively [76].

Enzymatic analyses

Enzymatic assays performed on plasma, leukocytes, or fibroblasts can often provide a definitive biochemical diagnosis for LSDs. However, these tests are not applicable to disorders affecting integral membrane proteins. Around 60% of LSDs involve deficiency of a specific lysosomal hydrolase, representing 85–90% of diagnosed patients [6]. Laboratories may offer large enzyme panels or more targeted testing, so clinicians should verify that the requested panel covers the suspected disorders.

Enzyme activity is typically measured in plasma or peripheral blood leukocytes, and dried blood spots are increasingly used as an initial sample source [80]. Historically, cultured skin fibroblasts were used, but they are mostly replaced by peripheral blood due to the invasiveness and time required for culture. Nonetheless, fibroblasts remain valuable for atypical cases or specific analyses, such as neuraminidase measurement in sialidosis or galactosialidosis and filipin staining for suspected NPC [81-83].

For suspected multiple sulfatase deficiency (MSD), measuring at least three different sulfatases is recommended, as not all are always deficient, particularly in milder forms [26,84]. Mucolipidoses show high plasma hydrolase activity but reduced fibroblast enzyme levels [41].

A known limitation is pseudodeficiency, where in vitro enzyme activity is low, but the patient does not exhibit typical clinical features. This occurs due to reduced specificity of artificial assay substrates caused by pseudodeficiency variants [41]. In such cases, biomarkers and genetic testing may clarify the diagnosis. Conversely, some patients (e.g., ASMD with SMPD1 p.Q294K) may show normal enzyme activity despite being affected [85].

Enzyme activity can vary between early-onset and adult-onset cases. Severe early-onset LSDs typically show absent or minimal activity, whereas attenuated or late-onset forms exhibit residual activity. Residual enzyme function may be undetectable in leukocytes or dried blood spots, but measurable in fibroblasts. In adults, enzyme levels slightly below the reference range may require additional confirmation using multiple sample types, combined with genetic and biomarker testing, to support a highly suspicious clinical diagnosis.

Biochemical urine testing

Urinary biochemical assessments for LSDs involve glycosaminoglycans (GAGs), oligosaccharides, and sialic acids, especially in the context of hepatosplenomegaly (HSM). A thorough evaluation typically includes quantitative measurement of GAGs, GAG extraction, as well as two-dimensional low-voltage electrophoresis and thin-layer chromatography (TLC) for oligosaccharides and sialic acids. Since laboratories employ different qualitative (1D or 2D electrophoresis) and quantitative methods, clinicians should verify which disorders are screened at their referral facility. For instance, some labs only perform GAG extraction and quantitative analysis if the initial screening is positive (Supplemental Figure S1). Awareness of the lab's testing protocol is therefore critical.

Elevated urinary quantitative GAGs may suggest MPS, although patients with attenuated forms may have normal results. Additionally, GAG excretion decreases with age, so laboratories should interpret results against age-specific reference ranges.

Excess urinary oligosaccharides and sialic acids can be detected by TLC, though testing approaches vary between laboratories. For example, the Willink laboratory routinely performs TLC on all urine samples submitted for suspected MPS due to overlapping clinical features across LSDs. Abnormal findings can suggest alpha-mannosidosis, aspartylglucosaminuria, galactosialidosis, GM1 gangliosidosis, mucolipidosis II/III, neuraminidase deficiency, or free sialic acid storage disease

(Supplemental Figure S2). Like GAGs, the intensity of oligosaccharide and sialic acid abnormalities decreases with age. Abnormal results should always be confirmed with enzymatic assays or targeted gene testing.

Recently, some laboratories have adopted liquid chromatography-tandem mass spectrometry (LC-MS/MS) for GAG and oligosaccharide analysis. Advanced methods, including non-reducing end assays, demonstrate higher sensitivity and specificity than traditional approaches, although they remain expensive and not widely available [86,87].

LSD biomarkers

Current and emerging biomarkers for LSDs are summarized in **Table 2**. Many new biomarkers initially show nearly 100% sensitivity and specificity, but routine usage reveals limitations. Non-specific biomarker elevations reported in the literature are listed in **Table 3**, highlighting the importance of understanding these limitations as biomarker-based diagnostics become more common.

Table 2. Biomarkers for lysosomal storage diseases associated with hepatosplenomegaly

Metabolic Disorder Category	Condition	Definitive Diagnostic Markers*	Routine Screening Indicators	Investigational Biomarkers
Sphingolipidoses				
	Gaucher disease [88-92]	Glucosylsphingosine (lyso-Gb1) ***	CCL18/PARC, glucosyl-cholesterol, Chitotriosidase ****	
	Combined saposin deficiency [93-96]	Glucosylsphingosine (lyso-Gb1) ***, Galactosylsphingosine (psychosine), Globotriaosylsphingosine (lyso-Gb3)	Chitotriosidase ****	
	Acid sphingomyelinase deficiency [97-100]	Plasma PPCS (↑↑–↑↑↑↑) with SPC (↑↑↑)	Plasma oxysterols (cholestane-3β-5α-6β-triol, 7-ketocholesterol), Chitotriosidase ****	
	Farber disease [101]		Chitotriosidase ****	C26:0 ceramide
	GM1 gangliosidosis [94,102]	UO		Lyso-GM1, SUOL
	Sandhoff disease [94,102,103]		Urinary DS trace amounts *****	Lyso-GM2, GM2 ganglioside, SUOL
	Multiple sulfatase deficiency [26,105-106]		Urinary DS, HS, KS	Urinary/plasma sulfatides, SUOGL
Mucopolysaccharidoses				
	MPS I [102,105,106]		Urinary DS, HS	SUOGL
	MPS II [102,105,106]		Urinary DS, HS	SUOGL
	MPS III [102,105,106]		Urinary HS	SUOGL
	MPS IV [102,105,106]	Urinary KS		SUOGL
	MPS VI [102,105,106]		Urinary DS	SUOGL
	MPS VII [102,105,106]		Urinary DS, HS	SUOGL
Oligosaccharidoses				
	Aspartylglucosaminuria [102]		Urinary aspartylglucosamine (ninhydrin detection), UO, urinary bound sialic acid	SUOL
	Fucosidosis [102]			SUOL
	Galactosialidosis [102]		US, UO	SUOL
	Alpha mannosidosis [102]		UO	SUOL
	Schindler disease type III [102]			SUOL
	Neuraminidase deficiency [102]		US, UO	SUOL
Membrane Protein Defects				
	Mucolipidosis II [102]	Lysosomal enzyme activities in plasma (↑↑)	Urinary DS trace amount *****, US *****	SUOL
	Mucolipidosis III [102]	Lysosomal enzyme activities in plasma (↑↑)	Urinary DS trace amount *****, US *****	SUOL
	Cystinosis [60]	Leukocyte cystine	Generalized aminoaciduria (Fanconi syndrome)	

	Infantile free sialic acid storage disease [102]		Urinary free sialic acid (N-acetylneuraminic acid)	SUOL
	Niemann-Pick disease type C [88,93,94,97-99, 107-116]	Plasma PPCS (↑–↑↑↑) with SPC (N-↑)	Plasma oxysterols (cholestane-3β,5α,6β-triol, 7-ketocholesterol), Glucosyl-cholesterol, Chitotriosidase ****	N-(3β,5α,6β-trihydroxy-cholan-24-oyl) glycine; Urinary sulphate-conjugated cholesterol metabolites (bile acids)
Miscellaneous				
	Lysosomal acid lipase deficiency [107,108]		↑↑↑ Plasma oxysterols (cholestane-3β,5α,6β-triol, 7-ketocholesterol), Chitotriosidase ****	
	Pompe disease [117]		UO (tetra) *****	Urinary glucose tetrasaccharide (Glc4)

Chito: chitotriosidase; DS: dermatan sulfate; HS: heparan sulfate; KS: keratan sulfate; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MPS: mucopolysaccharidosis; PPCS: N-palmitoyl-O-phosphocholineserine (previously lyso-sphingomyelin-509); SPC: sphingosylphosphorylcholine (alternative name: lyso-sphingomyelin); SUOGL: specific urinary oligosaccharide GAG fragments detected via LC-MS/MS; SUOL: specific urinary oligosaccharides detected via LC-MS/MS; UO: urinary oligosaccharides; US: bound sialic acid in urine.

* In enzyme deficiency disorders, the most reliable diagnosis comes from directly measuring the activity of the defective enzyme. Biomarker tests rarely reach full specificity; Table 3 summarizes other conditions or factors that may elevate levels.

** Thin-layer chromatography (TLC) for urinary oligosaccharides or sialic acids has lower sensitivity and accuracy compared with modern LC-MS/MS assays. Newer biomarker tests may identify conditions that TLC misses, so clinicians must know the capabilities and limits of the lab used.

*** Often expressed as total hexosyl-sphingosine, combining glucosyl- and galactosyl- derivatives.

**** Around 4–6% of the population has reduced clinical sensitivity because of chitotriosidase deficiency. Levels in Gaucher disease are usually very high (↑↑–↑↑↑↑), though outcomes vary with CHIT1 genotype [14]. In other LSDs, chitotriosidase is a less dependable marker. Since it indicates macrophage activation, it cannot be used alone for LSD screening.

***** Detected biomarkers can fluctuate depending on the testing technique. For instance, small amounts of dermatan sulfate may appear in mucolipidosis II/III or Sandhoff disease, but not universally. Elevation symbols: ↑ = slight increase, ↑↑–↑↑↑ = moderate, ↑↑↑↑ = strong.

Table 3. Reported Non-Specific Elevations of LSD Biomarkers in the Literature

Analyte	Main Clinical Application	Sources of False Positives
C-triol (cholestane-3β,5α,6β) [107,108,115,116,118,119]	Diagnosis of NPC	Carriers of NPC, cases of ASMD/LALD/INCL/Gaucher/CTX, liver bile stasis, elevated cholesterol levels, poor specimen preparation (cholesterol oxidation artifact)
7-Ketocholesterol [108,115,116,120]	Identifies NPC	NPC carriers, ASMD/LALD/INCL/Gaucher/CTX, biliary obstruction, high cholesterol, inadequate sample storage (cholesterol oxidation artifact)
Lyso-SM-509 (PPCS) [93,94,97,98,110-112,121]	Confirms NPC *	Elevations seen in ASMD/LALD/Gaucher/CDGs
Sphingosylphosphorylcholine (SPC) [93,94,121,122]	Detects ASMD *	Rises noted in NPC/Gaucher/peroxisome disorders
Combined hexosylsphingosines [89,90,93,94,121,122]	Specific for Gaucher *	Increases in Krabbe/NPC/Fabry
Lyso-Gb1 [89,90,93,94,121,122]	Gaucher confirmation *	Found elevated in NPC/Fabry
Glc4 in urine [117,123-125]	Pompe identification	GSD-III/VI/IX, DMD, muscle injury, gestation, malignancies
ASG in urine [126,127]	Aspartylglucosaminuria	NGLY1 defect
Chitotriosidase enzyme **	Supports enzyme confirmation	Multiple conditions
Unbound sialic acid (urine) [128]	Sialic acid storage/sialuria	DM, HUS, kidney impairment
Bound sialic acid (urine) [128]	Galactosialidosis/sialidosis	DM, HUS, kidney impairment

Abbreviations: ASMD = acid sphingomyelinase deficiency; CDGs = congenital disorders of glycosylation; CTX = cerebrotendinous xanthomatosis; DMD = Duchenne muscular dystrophy; GSD = glycogen storage disease; HUS = hemolytic uremic syndrome; INCL = infantile neuronal ceroid lipofuscinosis; LALD = lysosomal acid lipase deficiency; NPC = Niemann–Pick disease type C; PPCS = N-palmitoyl-O-phosphocholineserine.

* Using a combined panel of lyso-sphingolipids enhances diagnostic accuracy. For instance, ASMD and NPC can be distinguished based on the levels of PPCS and the relative rise in lyso-sphingomyelin. A large elevation of hexosyl-sphingosine helps differentiate Gaucher disease from NPC. In addition, Krabbe disease is separated from Gaucher disease by chromatographic distinction of glucosyl- versus galactosyl-(psychosine) isomers.

** Chitotriosidase reflects macrophage activity and general inflammatory responses rather than being disease-specific. Elevated chitotriosidase is observed in multiple LSDs as well as in various unrelated conditions, so it should not be used as a standalone diagnostic test. A complete list of all conditions with raised chitotriosidase is beyond the focus of this summary.

The role of biomarkers in diagnosing lysosomal storage disorders (LSDs) is expanding rapidly, driven by the need for large-scale screening (e.g., in newborns) and to overcome particular diagnostic limitations. Biomarkers have proven especially useful in cases where conventional enzyme testing is difficult or unreliable, such as in Niemann-Pick type C (NPC) or cystinosis. They may also serve as complementary tools to enzyme assays. For example, when leukocyte arylsulfatase A levels are inconclusive, sulfatide measurements can help

differentiate true metachromatic leukodystrophy from pseudodeficiencies.

Using panels of biomarkers, chosen based on clinical relevance and diagnostic precision, can improve accuracy compared with single-marker testing. For instance, the combination of N-palmitoyl-O-phosphocholineserine (PPCS), sphingosylphosphorylcholine (SPC), and hexosyl-sphingosine allows distinction among NPC, ASMD, and Gaucher disease. Confirmatory enzyme testing is often required to clarify a biomarker pattern, such as measuring lysosomal acid lipase when plasma oxysterol or PPCS levels are elevated. Biomarkers can also assist in interpreting variants of uncertain significance when integrated with genetic, biochemical, and clinical information.

Pathology

Histopathological studies require invasive tissue sampling, so biopsies are usually reserved for cases where molecular or enzymatic tests are unavailable or inconclusive. The type of tissue sampled depends on the suspected LSD. Skin biopsies provide fibroblasts that can be cultured to measure enzyme activity or biomarkers and assess substrate accumulation in associated structures such as glands, erector pili muscles, and nerve endings. For example, filipin staining patterns indicate NPC, and fibroblast sialic acid levels are elevated in free sialic acid storage disease. Subcutaneous nodules may reveal characteristic Farber bodies.

Liver and muscle biopsies carry greater procedural risks but can provide useful diagnostic clues. A PAS-positive muscle biopsy points toward Pompe disease. In lysosomal acid lipase deficiency, liver specimens may show “sea-blue” histiocytes, vacuolated Kupffer cells, and cholesterol crystals. ASMD often presents with foamy cells and fibrosis, while Gaucher disease may show Gaucher cells in the liver, spleen, or bone marrow. Muscle biopsies may also show secondary mitochondrial changes. Bone marrow biopsies are mainly performed when malignancy is suspected and are not a primary focus here.

Conclusions

Hepatomegaly and splenomegaly (HSM) are common manifestations of many LSDs and can sometimes be the only clinical finding in attenuated cases. Overlooking LSDs in patients with HSM can delay diagnosis and timely treatment. HSM is often readily apparent in young children, whereas adults may present incidentally through routine labs or imaging. When all common hematologic and metabolic conditions are excluded, LSDs should be considered in the differential diagnosis.

Abbreviations

ASMD	Acid sphingomyelinase deficiency
CESD	Cholesterol esterase storage disease
ERT	Enzyme replacement therapy
GAGs	Glycosaminoglycans
HSCT	Haematopoietic stem cell transplantation
HSM	Hepatosplenomegaly
IMDs	Inherited metabolic disorders
LSDs	Lysosomal storage diseases
NPC	Niemann–Pick disease type C
MPS	Mucopolysaccharidosis
TLC	Thin layer chromatography

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