Audit of Lysosomal Storage Diseases Testing at the National Health Laboratory Service in Johannesburg from 2011-2020.

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Medicine

Johannesburg, 2023

Declaration

I Michael Novellie, declare that this research report is my own, unaided work. It is being submitted in partial fulfilment of the Degree of Master of Medicine in the branch of Medical Genetics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

……

(Signature of candidate)

12th day of October 2023 in Johannesburg, South Africa.

Abstract

Lysosomal Storage Diseases (LSDs) are a group of Inborn Errors of Metabolism (IEM), due to the lack of a lysosomal enzyme. This results in toxic accumulation of metabolic waste products in various organs leading to neurodevelopmental regression, organ failure and premature death in the absence of treatment. Treatments for LSDs are limited. This study audited LSD diagnostic test requests received by the Division of Human Genetics, National Health Laboratory Service (NHLS) in Johannesburg from 2011 to 2020 with the aim of understanding the demand, appropriateness, and patient management of suspected LSD cases. A quantitative survey of all samples (1861 tests) referred to NHLS Johannesburg during the study period was performed. A total of 198 (13.3%) samples were rejected for testing mainly because of faulty sample collection. Of the 1663 that were accepted for testing 1457 (87.6%) tested negative, 73 (4.4%) were inconclusive and 133 (8.0%) tested positive. Fifty-five (3.1%) patients with LSD test requests, all of which were positive, were known to a Clinical Genetics unit. The most frequently requested test was for Fabry disease: 620 (33.3% of all requests), even though this disease is not the most prevalent LSD. Of the 603 accepted test requests for Fabry disease, only 6 (1.0%) tested positive. This suggests that some referring clinicians had unrealistic expectations of encountering this disease. It should be noted, however, that testing for Fabry disease is part of a broad diagnostic workup that may be applied even if the indication for testing is not specific. Access to LSD testing was unequal: private facilities were proportionally over-represented compared to public facilities; certain provinces with large referral centres (in KZN and Gauteng) were over-represented compared to smaller centres. Feedback and education of referring clinicians regarding indications for testing and importance of patient follow up, especially by clinical genetics services, are recommended. Follow up of positive MPS screening tests with specific diagnostic tests is essential. A system should be implemented where a medical geneticist phones the referring clinician and discusses further sample requirements (blood for enzyme analysis) and referral to a genetics clinic for all positive LSD screening tests. Future consideration should be given to designing a more systematic testing process, with the introduction of molecular testing to supplement biochemical testing.

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Abbreviations

ERT	Enzyme Replacement Therapy
GLC	Genetics Logistic Centre
IEM	Inborn Error of Metabolism
JHB	Johannesburg
LSD	Lysosomal Storage Disease
MPS	Mucopolysaccharidosis
MPS 1	Hurler syndrome, Hurler-Scheie syndrome and Scheie syndrome
MPS 2	Hunter syndrome
MPS 3	Sanfilippo syndrome
MPS 4	Morquio syndrome
MPS 6	Maroteaux-Lamy syndrome
MPS 7	Sly syndrome
NHLS	National Health Laboratory Service
NGS	Next Generation Sequencing

CHAPTER 1 INTRODUCTION

1.1 Background

Lysosomal Storage Diseases (LSDs) comprise approximately 70 conditions caused by various defects in the functioning of lysosomes within a cell [1,2]. They constitute a subset of a larger group of conditions termed Inborn Errors of Metabolism (IEM). IEMs are genetic conditions resulting from abnormal enzyme function [1]. Individual LSDs are rare, but as a collective the global incidence has been estimated at between 1 in 4000 and 1 in 9000 [3]. LSDs, like many other IEMs, are mainly inherited as autosomal recessive conditions [1]. Notable exceptions include Fabry disease and Hunter syndrome (MPS 2), which show X-linked inheritance [2].

A key role of the lysosome is to maintain cellular homeostasis by removal of waste products including lipids, proteins, carbohydrates, and nucleic acids [4]. This is achieved by a variety of enzymes, transporters and activators operating within the lysosome (Figure 1.1). Defects can occur at any level of lysosomal function. The result is the accumulation of substances that are toxic to the cell [4-6] which, depending on the cell type affected, can cause the patient to show severe and debilitating features. Any organ system can be affected, with neurodevelopmental decline, skeletal dysplasia, organomegaly and dysmorphic facial features commonly seen [2]. Phenotypes and severity can vary significantly between the different types of LSDs [4,5]. The variability, atypical presentation and often subtle presenting features may lead to a missed or delayed diagnosis [6].

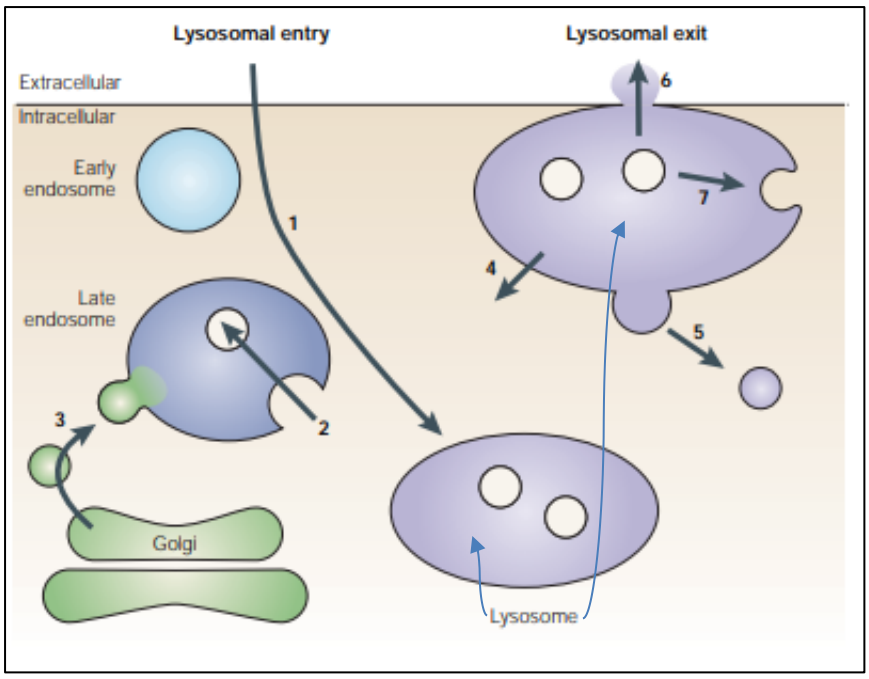

Figure 1.1 Diagrammatic representation of the function of the lysosome in removing waste products, adapted from Futterman and Meer [4] (p.561). Extracellular and intracellular waste enters the cell and fuse with vesicles to form endosomes (labels 1 and 2). Hydrolytic enzymes from the Golgi bodies fuse with endosomes, assisting with enzymatic digestion of waste products (label 3). Waste products are packaged into vesicles, recycled, or removed from the cell by exocytosis (labels 4-7).

1.2 Broad classification of LSDs

LSDs can be classified according to the specific cellular waste product that accumulates within a cell [2]. Some common diseases relating to each group are outlined below and summarized in Table 1.1

Table 1.1 Lysosomal Storage Diseases classified by accumulated waste products, common disease examples and associated genes.

	Accumulated waste product	**Common diseases**	**Associated genes**
Sphingolipidoses	Various types of lipids.	Gaucher disease Niemann-Pick disease Fabry disease Krabbe disease Tay–Sachs disease	*GBA1* *NPC1/NPC2* *GLA* *GALC* *HEXA*
Mucopolysaccharidoses	Glycosaminoglycans	MPS1 (Hurler syndrome, Hurler-Scheie syndrome and Scheie syndrome) MPS 2 (Hunter syndrome)	*IDUA* *IDS*

		MPS 3 (Sanfilippo syndrome) MPS 4 (Morquio disease) MPS 6 (Maroteaux-Lamy syndrome) MPS 7 (Sly syndrome)	*SGSH, NAGLU, HGSNAT, GNS* *GALNS, GLB1* *ARSB* *GUSB, HYALI, SUMF1*
Glycogen storage disease type 2	Glycogen	Pompe disease	*GAA*

Oligosaccharidoses	Glycoproteins/oligosaccharides	Mannosidoses (alpha and beta) Fucosidosis Galactosialidosis Sella disease Sialuria Aspartylglucosaminuria, Schindler disease Pycnodysostosis Mucolipidosis (types 1,2,3,4)	*MAN2B1*, *MANBA*, *FUCA1*, *CTSA*, *SLC17A5*, *GNE*, *AGA*, *NAGA*, *CTSK*, *NEUL*, *GNPTAB*, *GNPTAG*, *MCOLN1*

1.2.1 Sphingolipidoses

Sphingolipidoses result from the accumulation of glucocerebroside (a lipid subtype) in macrophages within cells due to the deficiency of enzymes or activator proteins [2,6]. Sphingolipids are key structural elements of plasma lipoproteins and cell membranes and are found in many organ systems [6]. Sphingolipidoses include Gaucher disease, Niemann-Pick disease, Fabry disease, Krabbe disease and Tay–Sachs disease [6].

Gaucher disease is the most common LSD with three subtypes described. The underlying pathology involves the accumulation of glucocerebroside in the macrophages due to an inability of the lysosomes to breakdown sphingolipids [1,6]. Type 1 is associated with variable age onset of diverse symptoms such as hepatosplenomegaly, haematological abnormalities, skeletal disease, and occasionally neurological symptoms [2,6]. Clinical phenotypes can vary from mild to severe disability [1,6]. Types 2 and 3 are associated with early neonatal or infantile death from neurological dysfunction and follow a relentless clinical course with poor feeding, failure to attain developmental milestones and seizures commonly observed.

Fabry disease is a relatively common LSD with variable severity and age of onset [6]. Affected males with the classic type typically present with severe neuropathic pain precipitated by exercise or variation in temperature [6]. Characteristic skin lesions (angiokeratomas) can occur in up to 80.0% of individuals. Cardiac complications (cardiomyopathy) and renal failure are the main causes of morbidity and mortality. Female carriers may present with symptoms, but milder and of later onset. Males with non-classic Fabry disease also present with mild or atypical symptoms [6].

Krabbe disease usually presents with neuro-regression before the age of 1 year [2]. The underlying pathology is the accumulation of abnormal lipids in the brain which results in demyelination of neurons [2]. The infantile form typically presents initially with non-specific symptoms such as irritability, increased crying, poor feeding, and fevers, making a timely diagnosis challenging.

Tay-Sachs disease is an example of a GM-2 Gangliosidosis. Tay-Sachs disease can also present with neuro-regression before the age of 1 year. However, juvenile, and adult-onset forms are

also described [1,2,6]. The result of the accumulations of GM-2 Gangliosides in brain neurons is severe neurological dysfunction seizures, hearing loss and early death [6].

1.2.2 Mucopolysaccharidoses

Mucopolysaccharidoses (MPS) results from the accumulation of glycosaminoglycans within cells due to a deficiency in the enzymes that break down these molecules [4,6]. Glycosaminoglycans are essential constituents of connective tissue including cartilage and vessel walls [6]. Due to the widespread presence of connective tissue and thus glycosaminoglycans, many different organ systems may be affected in MPS. Signs and symptoms may include bone dysplasia, hepatosplenomegaly, coarse facial features, neurological abnormalities and cardiac disease [1,6]. Skeletal features are most severe in MPS 1, MPS 2, MPS 4 and MPS 6 and can complicate with restrictive lung disease [2]. Coarse facial features are common to most MPS subtypes [2]. Deposition of glycosaminoglycans in cardiac valves may result in cardiomyopathy, most common in MPS 6 [2]. The severity of the phenotype can vary significantly between subtypes of MPS [6]. MPS subtypes may not be distinguishable based on clinical features making molecular and biochemical diagnostics important [6].

1.2.3 Glycogen storage disease type 2

Glycogen storage disease type 2 (Pompe disease) results from an accumulation of glycogen in the lysosomes due to a deficiency of the lysosomal enzyme acid alpha glucosidase [4]. Pompe disease occurs as a spectrum, with highly variable age of onset, extent of organ involvement and rate of disease progression. Pompe disease is sub classified into infantile, juvenile and adult forms. Classic infantile form presents with hypotonia (floppy baby), and hypertrophic cardiomyopathy [6]. Juvenile and adult forms mainly present with myopathy. The adult form can involve the diaphragm and often presents with respiratory failure [6].

1.2.4 Oligosaccharidoses

Oligosaccharidoses storage disorders have several clinical features that overlap with MPS. The diagnostic distinction between an MPS and oligosaccharidoses is usually made on urine analysis (oligosaccharidoses will be negative for glycosaminoglycans). These follow a progressive and debilitating course with severe cognitive impairment occurring in most of the oligosaccharidoses [6].

1.3 Epidemiology

The incidence of LSDs varies between different countries, partly due to the varying rates of consanguinity globally and the presence of various founder variants in populations [7,8,9]. In South Africa, three population groups have founder variants for Gaucher disease (Afrikaners, Black African and Ashkenazi Jews) [10]. Due to these founder variants, the incidence of Gaucher disease is reported to be higher in these groups. A local study done by Arndt *et al*.[11] on the mutation profiles of black African patients with Gaucher disease revealed three novel variants as being likely pathogenic. This study also showed that African patients are less likely to have type 2 Gaucher disease compared to patients with European ancestry and that type 1 Gaucher disease tends to be more severe in patients of African ancestry compared to those of European ancestry.

Not much is known about the incidence of LSDs in developing countries, including South Africa, due to limited studies and limited diagnostic capability [9,10]. A 2022 review by Castillon *et al*. reviewed the global literature on Gaucher disease from 2012-2022. This study concluded a data gap in Africa [12]. A Brazilian study done in 2017 attempted to estimate the incidence of LSDs in that country. A minimum frequency of 1 LSD in 19 942 live births was estimated, however, due to few laboratories investigating LSDs in Brazil, an accurate incidence could not be concluded [13]. The same study showed that the number of cases diagnosed in a region was heavily dependent on the diagnostic technology and sample management expertise available [13]. Specifically, this study showed the number of MPS cases diagnosed had more than doubled, over a comparative time period, after the introduction of a professional network that manages and coordinates suspected MPS cases [13].

Some smaller studies have been done in other developing countries, including a hospital-based study done in Pakistan from 2008-2012. This study described 421 patients clinically suspected of having an IEM, but, due to cost, a diagnosis could only be established in 85 patients. Only 20 patients out of this cohort received some form of treatment [14]. An Egyptian study attempted to describe the relative frequency and disease spectrum in a cohort of Egyptian children. From this study a crude estimate was made that over 80.0% of Egyptian children with LSDs do not have access to appropriate diagnostic services [8]. These studies indicate challenges with diagnosis and management of LSDs in the developing world. Similar challenges are likely to occur in South Africa.

1.4 Overview of diagnosing LSDs

The rarity and complexity of LSDs make diagnosis challenging. In resource-limited developing countries, like South Africa, diagnosis may occur late or be missed entirely [13, 14]. Most affected individuals do not show clinical features at birth, and present in childhood with neurodegenerative conditions [1,6]. The impact of a missed diagnosis is not only limited to medical complications, but a family may experience significant psychological distress if they have a child with an apparent but undiagnosed illness and no information for future pregnancy planning. Since LSDs are inherited predominantly in an autosomal recessive manner [1] (a few are X-linked), making an early diagnosis will result in appropriate genetic counselling and enable better family planning by understanding recurrence risks and prognosis. Family planning could also involve prenatal testing for a specific LSD, if available. An early and accurate diagnosis is therefore important, even if treatment is not available. A multi-disciplinary medical team approach including medical geneticists, neurologists, ophthalmologists, and radiologists is important in diagnosing LSDs [15].

The diagnostic approach to an LSD varies depending on the subtype and clinical presentation. Clinical overlap exists between LSDs and other common medical conditions such as congenital or acquired neonatal infections, or complications due to prematurity. For example, Krabbe disease often presents with poor feeding, irritability, and fevers [2]. These non-specific symptoms are commonly seen with neonatal sepsis. An awareness of LSDs as a differential diagnosis for non-specific presentations is therefore important. Accurate clinical assessment and phenotyping helps to direct the most appropriate investigations [6]. For example, an infant with Pompe disease may initially present with hypotonia and poor weight gain. The differential diagnosis for this presentation includes several genetic conditions including Prader-Willi syndrome, congenital myopathies (including Spinal Muscular Atrophy), congenital dystrophies (including Myotonic Dystrophy) as well as structural brain abnormalities [16]. Investigations such as creatinine kinase levels, brain sonar and echocardiogram may assist with structuring the differential diagnosis [16].

The diagnosis of an LSD can be made by measuring the activity of a particular enzyme [4,6]. If an enzyme has reduced activity, it leads to the accumulation of various substrates. Substrates can be measured to screen for a particular LSD or group of LSDs. Substrates that are associated with a particular IEM and are measurable in a laboratory are called biomarkers [6]. For example,

in the case of MPS, the presence of glycosaminoglycans in the urine is a biomarker. If a patient has clinical features that are consistent with an MPS, it would be appropriate to start by screening for the presence of glycosaminoglycans in the urine [6]. If glycosaminoglycans are present, more specific assays would be conducted (urine electrophoresis and enzyme assays) to narrow down the diagnosis. If glycosaminoglycans are not present, then MPS is excluded. Biomarkers are known for various IEM and are used in screening or diagnostic assays [4].

Dried blood spot cards can screen for multiple LSDs quickly and simultaneously by measuring enzyme activity using mass spectrometry [6]. These advantages have led to the widespread use of dried blood spot cards as a means of screening (newborns) and/or diagnosing IEM in many developed countries. For this reason, newborn screening programs have been implemented to varying degrees globally [17]. Knowledge of the LSDs included and not included in a specific newborn screening program is essential to avoid false negative diagnoses.

Mutational analysis by DNA sequencing is another diagnostic option for LSDs if the mutation profile is well described. However, not all DNA mutations for LSDs are detectable on sequencing [17]. Additionally, DNA sequencing results in large datasets that require interpretation and review to differentiate pathogenic variants from variants of uncertain significance. This can lead to diagnostic uncertainty and is time consuming [6]. The cost of DNA sequencing is another limitation, especially when comparing to biochemical testing. For example, measuring 7-ketocholesterol levels is a cheap, rapid, and sensitive biochemical test to diagnose Niemann- Pick Disease Type C, compared to DNA sequencing [6,17].

1.4.1 Current LSD diagnosis at NHLS, JHB

The National Health Laboratory Service, Division of Human Genetics, Johannesburg (hereafter referred to as NHLS, JHB) aims to provide high quality, affordable laboratory diagnostic services in medical genetics. The NHLS, JHB, is the only public laboratory providing routine LSD testing in South Africa. The University of Cape Town in conjunction with the NHLS provides some testing for IEM, however, the University does not test for any LSDs [18]. The website of Potchefstroom Laboratory for Inborn Errors of Metabolism [19] states that screening and testing for a wide variety IEM (including all common LSDs) are provided.

Molecular testing, in the form of Next Generation Sequencing (NGS) has only been intermittently available at NHLS JHB since 2019 owing to an inadequate and sporadic supply of reagents.

Table 1.2 lists the LSDs for which screening and diagnostic testing are conducted at NHLS, JHB.

Table 1.2 List of LSD tests conducted at NHLS reviewed in this study:

Urine glycosaminoglycan assay
Urine MPS electrophoresis assay
Serum fluorometric enzyme assays for: • Hurler Syndrome (Alpha-L-iduronidase enzyme) • Sanfilippo Syndrome (N-Acetyl glucosaminidase enzyme) • Morquio Syndrome (Beta- galactosidase enzyme) • Maroteaux-Lamy Syndrome (N-acetylgalactosamine-6-sulfatase enzyme) • Gaucher Disease (Beta glucosidase enzyme) • Pompe Disease (Alpha glucosidase enzyme) • Fabry Disease (Alpha galactosidase enzyme) • Metachromatic Leukodystrophy (Arylsulfatase A enzyme) • GM1 Gangliosidosis (Beta galactosidase-1 enzyme) • Tay Sachs (Hexosaminidase A enzyme) • Chitotriosidase screening assay • Galactosialidosis (Beta galactosidase enzyme)

Tests for Tay-Sachs disease can be done on blood or amniotic fluid. However, within the study period, no records for Tay-Sachs disease testing on blood samples were recorded. Testing for mucopolysaccharidoses at NHLS, JHB is limited to certain subtypes and can be grouped as

screening or diagnostic tests. Testing for mucopolysaccharidoses does not happen systematically. No formal gatekeeping or communication with requesting clinicians exists.

Mucopolysaccharidoses testing at NHLS, JHB:

Screening tests:

1.) Spot urine glycosaminoglycan (all mucopolysaccharidoses subtypes have raised glycosaminoglycans).
2.) Urine electrophoresis for heparin and dermatan sulfate banding patterns. The same banding pattern is seen in Hunter, Hurler and Maroteaux-Lamy diseases. A different banding pattern is seen in all other forms of mucopolysccaridoses.

Diagnostic tests:

1.) Following from the above screening tests, a blood sample is used for fluorometric enzyme analysis for Hurler, Sanfilippo B, Morquio B and Maroteaux-Lamy syndromes. Diagnostic enzyme analysis for Hunter, Sanfilippo A, Sanfilippo C, Morquio A, and Sly syndromes is not available at the NHLS, JHB.

Figure 1.2 below summarizes some of the testing options for mucopolysaccharidoses at NHLS, JHB. The figure is not used as a testing algorithm that is followed systematically. Rather, the choice of testing done at NHLS, JHB depends mainly on the type of sample received: if a urine sample is received, a screening test is done, if a blood sample is received, a diagnostic test is done.

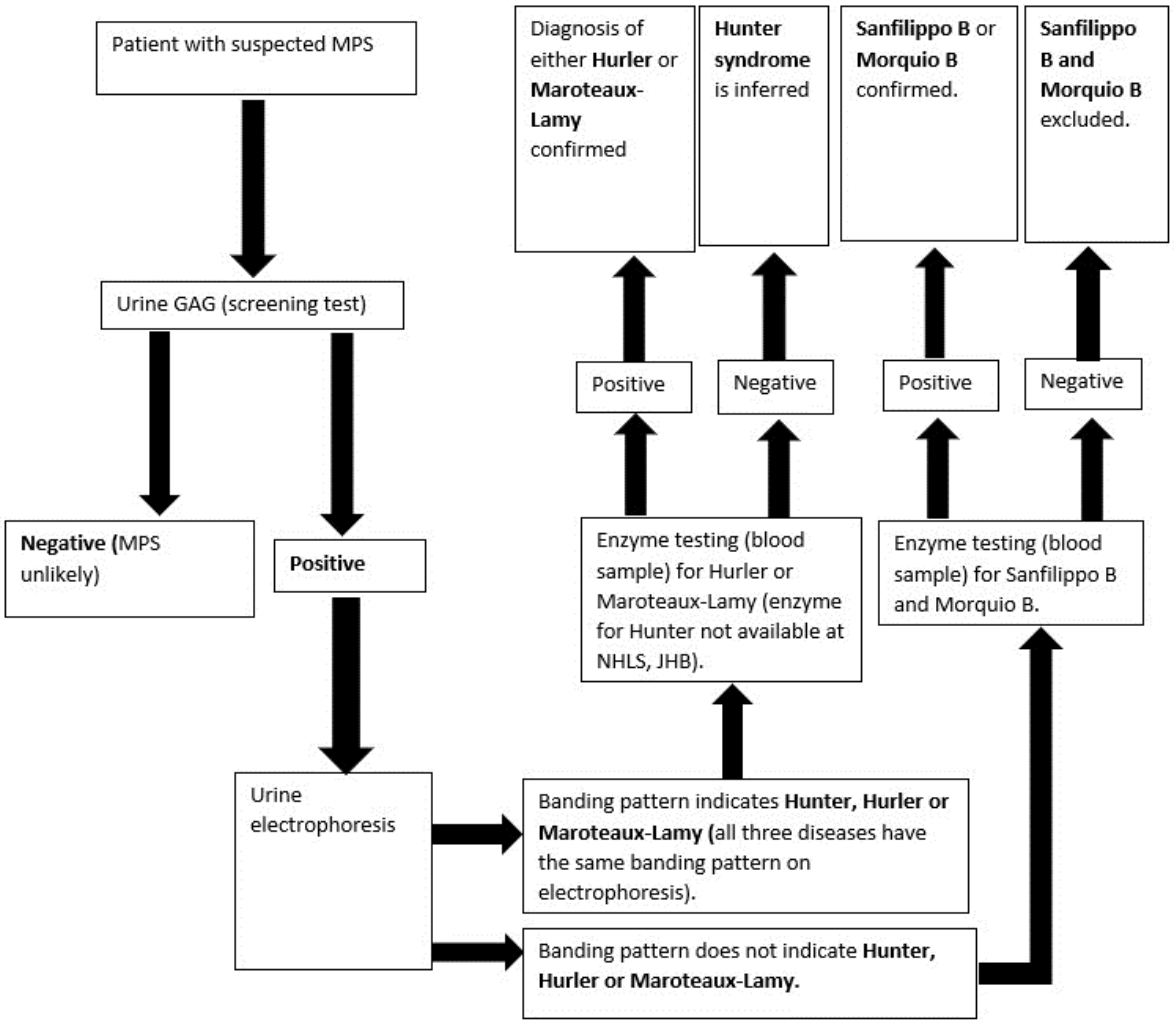

Figure 1.2 Summary of testing options for mucopolysaccharidoses conducted at NHLS, JHB.

1.5 Overview of management of LSDs

Recent medical advances have improved the diagnostic sensitivity and reduced the cost of testing for LSDs and have resulted in some effective treatments for certain LSDs [2,20]. Treatment strategies include replacing a deficient enzyme, substrate reduction therapy and pharmacological chaperones [2,6,20]. These interventions need to be initiated as early as possible before irreversible organ damage or neurological damage has occurred [6]. Treatment can only be initiated if a specific diagnosis has been made. Examples of treatable LSDs include Gaucher disease, Fabry disease, Pompe disease, mucopolysaccharidosis type 1 (Hurler syndrome), type 2 (Hunter syndrome), Type 4 (Morquio syndrome) and type 6 (Maroteaux-Lamy syndrome) [20].

These treatments are lifelong, therefore patient access and availability for clinical follow up at a healthcare facility is required.

Factors such as ease of diagnosis, age of onset, and severity of symptoms as well as treatment availability make LSDs a potential target for new-born screening or for screening population groups at increased risk due to founder effects [21,22]. In fact, newborn screening programs are ideal for early detection of LSDs because a diagnosis can be made before symptoms occur [19].

The costs of treating LSDs are high, especially as treatment often requires frequent administration of an expensive enzyme intravenously [1,6,20]. Other complicated and expensive treatments include bone marrow transplantation, organ transplantation (often liver transplant) or gene therapy [18]. If patients go untreated and develop complications, the cost to the healthcare system may be even higher, especially given the frequent requirement of complex investigations, surgery, and prolonged hospital admissions [9].

1.5.1 Current LSD management at NHLS, JHB:

NHLS, JHB Clinical and Counselling section of the Division of Human Genetics manages patients with LSDs at various hospitals around Johannesburg (Charlotte Maxeke Johannesburg Academic Hospital, Rahima Moosa Mother and Child Hospital, and Chris Hani Baragwanath Academic Hospital). Services include genetic counselling, clinical management, prenatal genetic testing and referral/collaboration with other specialist clinics.

Management guidelines for Fabry disease, Gaucher disease and Pompe disease have been published in South Africa [7,23,24] in 2012 (for Gaucher disease) and 2014 for (Fabry disease and Pompe disease). The data used to establish these guidelines came largely from those developed overseas. Treatment guidelines for Fabry disease and Gaucher disease both recommend the use of Enzyme Replacement Therapy (ERT). However, in South Africa, the only ERT available for use in the public sector is imiglucerase (for Gaucher disease). Imiglucerase is only available at Charlotte Maxeke Johannesburg Academic Hospital in Gauteng and due to diagnostic and referral challenges only a minority of patients with Gaucher disease receive imiglucerase.

1.6 Rationale of research

The ability of the NHLS, JHB to diagnose and screen for LSDs is limited. Diagnostic and screening tests are only offered for certain groups of LSDs. Especially in view of resource

limitations, it is important to assess the past services provided by NHLS, JHB with a view to updating and improving the diagnostic program, as well as the technology upon which it relies. This requires an audit of the demand, scope, value, and limitations of the current service. An audit could provide guidelines for planning and budgeting services for future LSD testing at NHLS, JHB. A further consideration is that recent medical advances in LSD diagnosis (such as next generation sequencing) and treatment (ERT) offer a basis for improving patient management within the constraints of resource availability.

1.7 Study aim

To assess the utilization of laboratory testing, outcome of testing and follow up of patients with suspected LSDs at NHLS, JHB.

1.8 Study objectives

1.) To obtain data on the number and types of tests requests and test outcomes for LSDs at NHLS, JHB.
2.) To assess the appropriateness of LSD test requests.
3.) To obtain data on the geographic location of the referring hospitals that request LSD tests.
4.) To obtain data on the number of patients with a suspected diagnosis of an LSD that were followed up or referred to a medical geneticist or genetic counsellor.

CHAPTER 2 METHODOLOGY

2.1 Research Questions

The following questions were constructed to address the study objectives and achieve the overall aim of the study:

1. How many tests were referred to NHLS, JHB for LSD testing from January 2011 to December 2020.
2. What were the LSD subtypes for which testing was requested?
3. How many tests were rejected and what were the reasons for rejection?
4. Of all the approved test requests, what percentage tested positive, negative, and inconclusive?
5. What are the main referring centres that requested LSD testing?
6. What were the clinical indications for LSD testing?
7. How many patients that had LSD testing requested, were followed up or referred to a medical geneticist or genetic counsellor and what number of patients received ERT, or had prenatal screening or cascade carrier testing done?

2.2 Research Design

All samples referred to NHLS Johannesburg from January 2011 to December 2020 with a request for diagnostic LSD testing were ascertained. All LSD tests conducted at NHLS, JHB over this period were reviewed. The specific LSD subtypes (listed in Appendix A) included enzyme assays for Gaucher disease, Niemann-Pick disease type C, Fabry disease, Tay-Sachs disease, Pompe disease, Hurler syndrome, Sanfilippo B syndrome, Metachromatic Leukodystrophy, GM 1 Gangliosidosis, Morquio B syndrome, and Maroteaux-Lamy syndrome. Screening assays for mucopolysaccharidoses included spot urine glycosaminoglycan screen and urine electrophoresis (as shown in Figure 1.2).

Ethics clearance was obtained from WITS University (Appendix B).

2.3 Materials and Methods

A standardised, electronic data capturing sheet was created using REDCap v12.1.2 [25] (a secure web application for building and managing online surveys and databases) (see Appendix C). For each LSD test request audited, a separate electronic data capturing sheet was used. Each

data capturing sheet was labelled with a participant number rather than a name or surname, therefore ensuring RedCap records were deidentified. The data capturing sheet was pre-tested, and contained text boxes, tick boxes and drop-down lists to capture data from the following three databases, respectively:

2.3.1 Manual and electronic records of the Biochemistry Laboratory, NHLS, JHB

Standard manual NHLS test request forms available to clinicians at referring hospitals, are used to request specific LSD testing. In addition to the LSD test requested, the referring clinician provides patient identifiers and a brief clinical history on the form. Each request form is accompanied with a sample from the patient (usually blood or urine). Each test request is identifiable through a specific laboratory reference number. The laboratory reference numbers, patient identifiers and test results are kept in electronic records (Microsoft Excel 2019 spreadsheets) in the Biochemistry Laboratory.

2.3.2 NHLS TrakCare test results viewer or DISA electronic database

NHLS TrakCare is the current NHLS laboratory information system and has been in use since 2013. Requesting physicians and medical scientists have access to TrakCare to view laboratory testing reports. NHLS TrakCare contains additional clinical information not found in manual and electronic laboratory records (including indication for testing). DISA electronic database was the information system used by NHLS prior to 2013.

The electronic records contained all LSD tests, for which test requests were approved.

2.3.2 Manual and electronic clinical patient records

Handwritten notes are made by the Clinical and Counselling section of the NHLS, JHB, during a patient consultation, which takes place at genetic clinics at various public hospitals in JHB. They are stored in manual form (a patient file) in an archive at the NHLS, JHB. The REDCap v12.1.2 clinical database, created by the Clinical and Counselling Section of NHLS, JHB in September 2018 contains clinical patient details, including summaries of patient management and results of testing from that date. Manual records (handwritten in laboratory books) contained data on MPS screening assays (urine glycosaminoglycan spot urine screen and electrophoresis for dermatan and heparin sulfate, Tay-Sachs disease amniocentesis testing

(Hexosaminidase A enzyme testing) and all tests that were rejected. Manual and electronic patient records were used to address research question 7, above.

2.4 Selection Criteria

2.4.1 Inclusion criteria:

All recorded samples referred to the NHLS, JHB with a request for LSD testing, from January 2011 to December 2020 were reviewed. This includes samples that were later rejected for any reason. Samples with a request for an LSD subtype that NHLS, JHB does not offer (due to lack of capacity) were included.

2.4.2 Exclusion criteria:

Biochemical testing for non-LSD IEM such as Galactosaemia were not examined. Heterozygote carrier screening tests for LSDs were excluded.

2.5 Data Collection

2.5.1 Manual and electronic records in the Biochemistry Laboratory

All data were collected by the principal investigator at NHLS, JHB. From manual and electronic records in the Biochemistry Laboratory, the following data were collected: data on laboratory reference numbers, type of LSD testing, date of testing, sample type and the biochemical result/value of the assay performed.

2.5.2 NHLS TrakCare test results viewer

Laboratory reference numbers (obtained from manual and electronic records in the Biochemistry Laboratory) were used to search NHLS TrakCare test results viewer. Data on referring centre, indications for testing, laboratory test result report and turnaround time were captured for the period between 2013-2020.

The name of each referring centre and the indications for testing were typed into a textbox on the REDCap electronic data capturing sheet. When the referring centre or clinical indication for testing was left blank, “unknown” was typed into the text box.

The laboratory test result report was downloaded from TrakCare and the outcome of the test was recorded as “positive”, “negative” or “inconclusive”.

Owing to complex login requirements, the researcher was unable to access the DISA electronic database. Thus, for samples prior to 2013, the referring centre and indication for testing was recorded as “unknown”. For samples prior to 2013 the outcome was not specifically recorded as being positive, negative or inconclusive. However, for this period the test outcome could be derived by examining whether the biochemical value found in the electronic laboratory records was above or below the reference range for a positive test.

2.5.3 Clinical patient records and REDCap clinical database search

Clinical patient records were searched at the NHLS Johannesburg file archive using patient identifiers such as hospital number, name, surname, and date of testing. Information such as clinical review by a medical geneticist, genetic counselling offered, and other management was obtained for selected patients (patients seen in Gauteng and in the public sector). In addition to an archive search, for samples referred after 2017, a REDCap clinical database search was done. Patient identifiers such as hospital number, name, surname as well as key word searches (such as “LSD” or “MPS”) were typed into the REDCap search box to obtain further clinical data.

2.6 Data Analysis

Data for each sample audited was entered on an electronic data capturing sheet. Data reports were generated in REDCap clinical database from previously captured data. Key word searches and filters were used to extract specific datasets. REDCap data was exported to Microsoft Excel 2019 spreadsheet to create figures and tables. Data was descriptive and categorical and therefore, no statistical analysis was required.

2.6.1 Data analysis for indications for test requests

When a referring clinician requests testing, the manual request form has a section labelled “reason” or “indication” for test request. This indication is then typed from the request form into NHLS TrakCare database by laboratory clerks at the receiving offices at NHLS, JHB or at the receiving office at the referring hospital.

Data recorded for indication for testing had to be analysed manually (each indication reviewed individually). Indications were classified either as appropriate, inappropriate, insufficient, or unknown. An appropriate indication for testing was defined as an indication that could reasonably be associated with the condition for which testing was requested. For example, "floppy infant" as an indication for Pompe disease was appropriate. Vague, less specific clinical indications such as "renal failure" or "CVI" were recorded as insufficient. Many tests had a question mark followed by the name of the requested test as the indication for testing (for example, "? Fabry", or "? Gaucher"), these tests were recorded as insufficient. Indications that were obviously inappropriate such as "? Galactosaemia" as indication for Gaucher disease testing or "? heterozygote carrier" as an indication for biochemical testing, with no enzyme specified, were recorded as inappropriate.

2.7 Methodological Limitations

2.7.1 Manual and electronic record limitations

Data on test rejections or test referral to another laboratory were not recorded in electronic records. However, test rejection data were kept in written documents from 2013-2020 (monthly report statistics). These written documents only contained the number of tests rejected per month, name of the referral centre and reasons for test rejection. No patient identifiers were recorded therefore no additional clinical information could be obtained from rejected samples. No rejection data exist for LSD testing prior to 2013 in the Biochemistry Laboratory and thus no rejection data prior to 2013 were captured.

2.7.2 NHLS TrakCare test results viewer and DISA limitations

NHLS TrakCare test results viewer had limited data on indications for testing. Additionally, prior to 2013, data on referring centre, indication for testing, turnaround time and laboratory test result reports were not available. As noted above, DISA records could not be accessed.

The dataset on NHLS TrakCare labelled "date sample received" was the date the sample was registered at the referring centre, rather than the date the sample arrived at NHLS, JHB. No data on date of arrival at NHLS, JHB could be found. Turnaround time was therefore calculated as the period between the date of registration by the referring centre to the date of posting of the tests results on TrakCare by the NHLS, JHB medical scientist.

2.7.3 Limitations in patient management records

Availability of clinical patient records, both electronic and manual, at NHLS, JHB were limited. The REDCap and manual databases included some records of clinical management, but numerous inconsistencies in the records suggested that these do not represent a complete record. Thus, the total number of patients who received clinical follow up, genetic counselling, cascade screening or ERT could not be reliably determined. In some cases, on TrakCare the referring centre was recorded as a Genetic Clinic or the referring clinician's name was known by the principal investigator to be a medical geneticist or genetic counsellor practicing in South Africa. In these cases, it could be inferred that the patients were known to a clinical genetic unit. However, the management of these patients was unrecorded.

CHAPTER 3 RESULTS

3.1 Total Number of LSD test requests and number of subtypes requested

A total of 1861 tests were requested from 2011-2020, of these, 198 (10.6%) were rejected. 1663 (89.3%) of samples were analysed at an average of 3.2 tests performed per week. The LSD test requested most frequently was for Fabry disease (alpha galactosidase enzyme analysis) accounting for 620 (33.3%) of requests. Gaucher disease was the fifth most commonly requested LSD with 226 (12.1%) of requests.

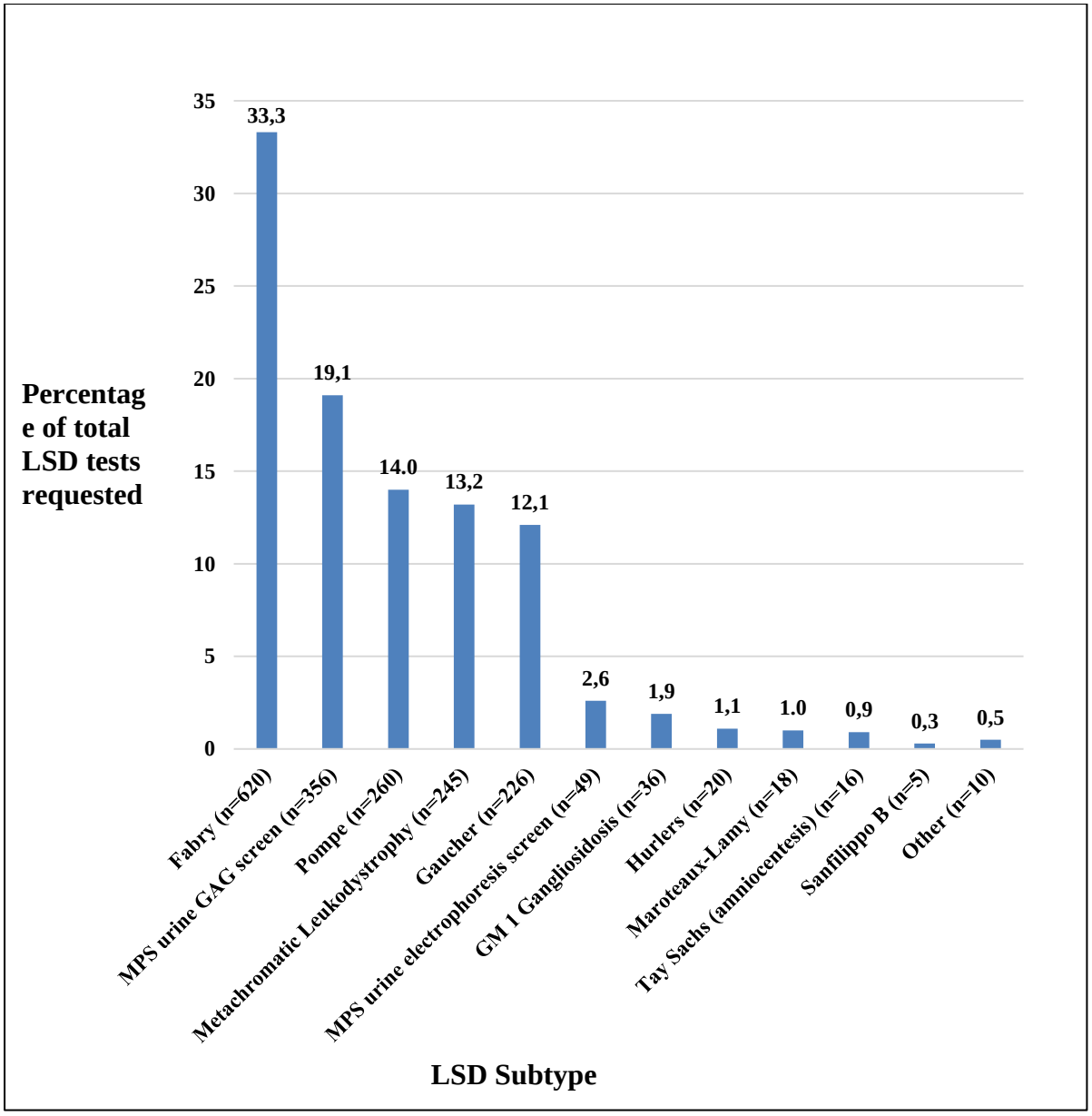

Figure 3.1. Total Number and percentage of LSD test requests submitted to NHLS, JHB, categorized by disease subtype (n=1861). The category 'other' included chitotriosidase assay (a biomarker used to monitor Gaucher disease treatment) and galactosialidosis enzyme testing.

3.2. Total number of rejected tests and reasons for test rejection

Test rejection data were only available from 2013-2020. During this time, a total of 198 samples (13.3%) were rejected (total of 1494 tests requested, and 1296 accepted from 2013 to 2020). The most common reason for test rejection was that the sample was too old based on the laboratory standard for each test. This could occur for various reasons, for example a delay in the transport from the referring hospital to the receiving office at NHLS, JHB, or a delay in

transit from the receiving office to the biochemistry laboratory. In addition, the referring centre was often not recorded. Thus, the data were insufficient to determine the exact cause of the delay. Delays are beyond the control of the laboratory itself.

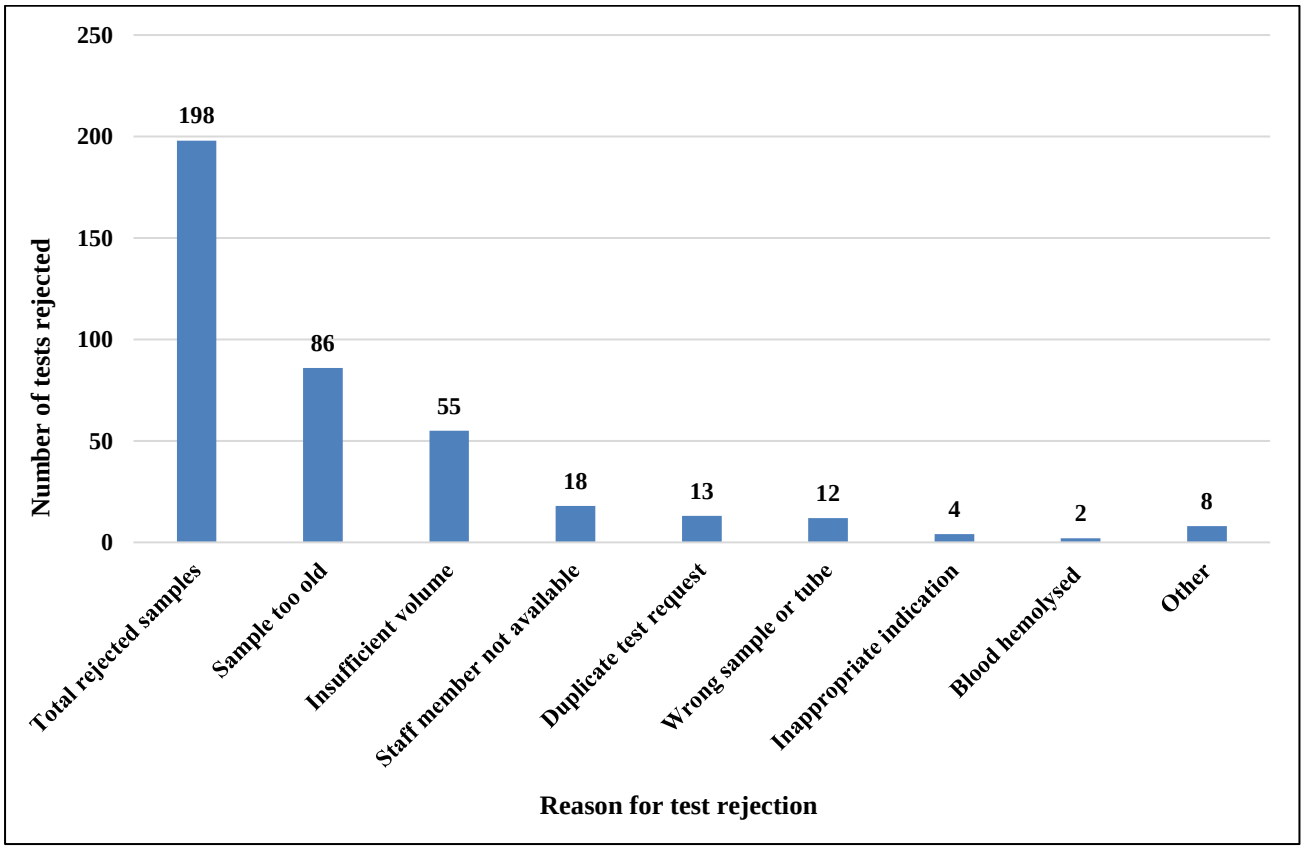

Figure 3.2 Total number of tests rejected by NHLS, JHB and reasons for test rejection. The category 'other' included chitotriosidase assay (a biomarker used as a screen for Gaucher disease) and galactosialidosis enzyme testing.

3.3 Outcome of accepted test requests

A total of 1663 LSD tests were performed from 2011-2020 with 133 testing positive (8.0%). Test requests for Fabry disease had the lowest percentage of positive results: 6/603 (1.0%). Pompe disease testing showed the highest percentage of inconclusive test results 30/230 (13.0%).

Table 3.2 Number of LSD subtype testing performed at NHLS, JHB, with percentage that tested positive.

Number of LSD subtype testing performed	Percentage positive

Fabry disease (n=603)	1.0% (n=6)
MPS urine glycosaminoglycans (n=308)	12.3% (n=38)
MPS electrophoresis (n=55)	63.6% (n=35)
Pompe disease (n=230)	5.2% (n=12)
Gaucher disease (n=193)	15.0% (n=29)
MLD (n=179)	2.2% (n=4)
GM Gangliosidase 2 (n=32)	9.4% (n=3)
Hurler syndrome (n=18)	16.6% (n=3)
Maroteaux-Lamy syndrome (n=18)	5.5% (n=1)
Tay Sachs disease (Amniocentesis) (n=16)	0.0% (n=0)
Sanfilippo B syndrome (n=5)	0.0% (n=0)
Other (Galactosialidosis and chitotriosidase) (n=6)	33.3 %(n=2)
Total (n=1663)	**8.0% (n=133)**

Out of 1663 tests performed, 1457 tested negative (87.6%) and 73 tested inconclusive (4.4%). The 73 inconclusive results were mainly due to intermediate enzyme levels. When an enzyme level was lower than that of a healthy control but higher than expected for an affected individual, the result was reported as inconclusive. In these cases, test reports commented that it was unknown whether the patient had a milder form of the disease or one of the variant types of LSD. Alternatively, an intermediate enzyme level could have occurred due to the sample being too old, a lack of refrigeration or other deficiencies in sample handling. The medical

scientist could not differentiate between these different causes of intermediate enzyme levels and thus would report these as inconclusive.

Pompe disease testing showed the highest percentage of inconclusive test results 30/230 (13.0%). MPS electrophoresis screening assay showed the highest percentage of positive results, 35/55 (63.6%) (Figure 3.3). The high percentage positive of MPS electrophoresis is to be expected because it is a reflex test done after a positive MPS spot urine test for glycosaminoglycans (see Fig 1.2). The category ‘other’ included chitotriosidase assay (a biomarker used to monitor Gaucher disease treatment) and galactosialidosis enzyme testing.

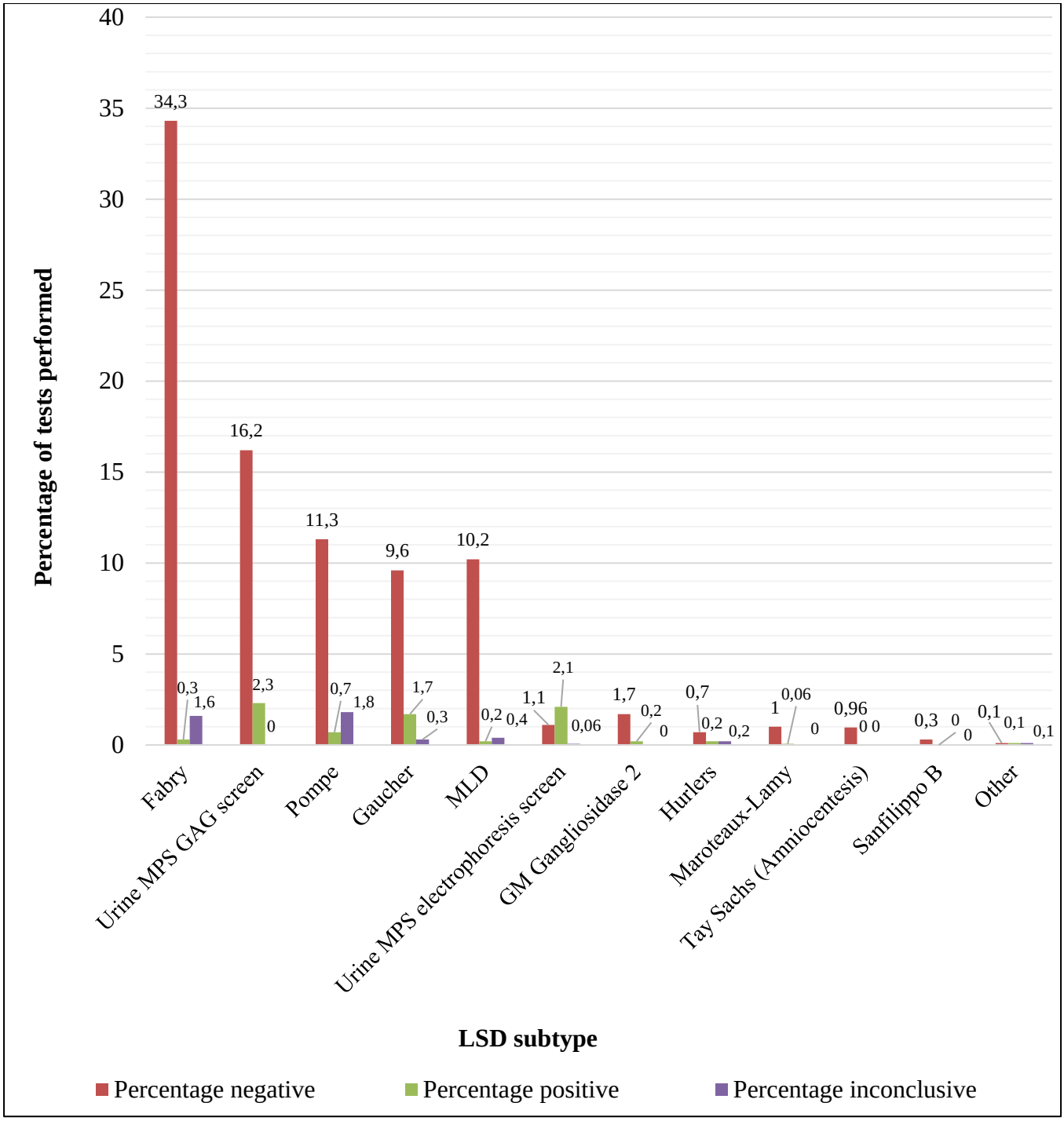

Figure 3.3 Percentage of accepted test requests submitted to NHLS, JHB that tested positive, negative, and inconclusive, categorized by disease/syndrome**.** The category 'other' included chitotriosidase assay (a biomarker used to monitor Gaucher disease treatment) and galactosialidosis enzyme testing.

3.3.1 Follow up of positive urine MPS glycosaminoglycan screens:

MPS urine glycosaminoglycan screening showed 38/308 (12.3%) positive results. 34/38 (89.5%) of these positive urine glycosaminoglycan screening samples went on to test positive on MPS electrophoresis screening. One positive MPS glycosaminoglycan screening test showed a negative MPS electrophoresis and 3/35 had no MPS electrophoresis testing done. Only 4/35 (11.4%) of positive MPS glycosaminoglycan screening tests had follow up enzyme testing or overseas molecular testing with one testing positive for Maroteaux-Lamy syndrome and one testing inconclusive on molecular testing with a variant of unknown significance detected (the MPS subtype was not recorded).

3.3.2 Follow up of positive urine MPS electrophoresis screens:

For MPS electrophoresis screening assays, 35/55 (63.6%) yielded positive results. The reason for this high percentage is that 34/35 positive MPS urine electrophoresis screening tests had already tested positive on an MPS glycosaminoglycan spot urine screen. See Figure 1.2 and Section 3.3. 6/35 (17.1%) of positive MPS urine electrophoresis tests had follow up enzyme analysis performed. Some MPS electrophoresis tests were done without a preceding glycosaminoglycan spot test. This happened when the referring clinician specifically requested electrophoresis only, without a preceding glycosaminoglycan spot test. It is known that in no case was a spot test done elsewhere. Conducting electrophoresis without a preceding screening spot test is not ideal practice.

3.4 The main referral centres that requested LSD testing

The private sector accounted for 783 (42.0%) of test requests, with the public sector accounting for 264 (14.2%). In the remaining 814 (43.8%) of audited samples, the referring centre was either unrecorded or it was done prior to 2013 (no accessible records) (Figure 3.4). An unrecorded referring centre is likely due to poor record keeping at NHLS, JHB. For samples with unrecorded referring centre, no other identification could be found to indicate if the sample was from private or public facilities. Testing from the public sector was mainly from Kwa-Zulu Natal and Gauteng provinces (Figure 3.5). The two public hospitals with the most test requests were Kalafong Hospital and Inkosi Albert Luthuli Central Hospital (IALCH). Kalafong Hospital requested 55/1663 (3.3%), with 2/55 (3.6%) testing positive, however both samples were from the same patient (one positive urine MPS glycosaminoglycan and one

positive urine electrophoresis). IALCH requested 90/1663 (5.4%), with 1/90 (1.1%) testing positive. Both hospitals requested LSD testing more frequently than any other public hospital.

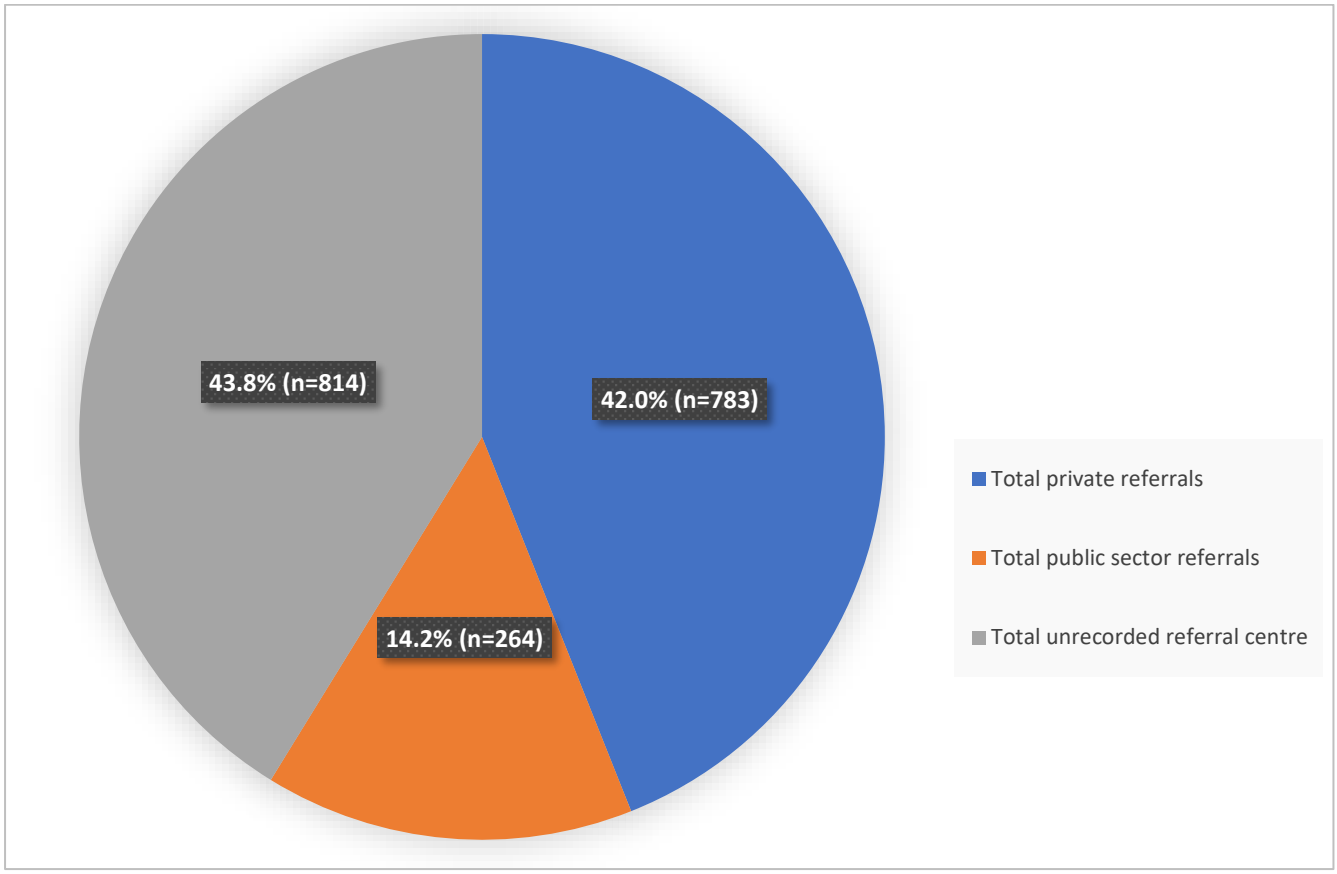

Figure 3.4 Pie chart showing origins of LSD referrals to NHLS, JHB: private sector, public sector, and unknown (unrecorded) (n=1861).

Figure 3.5 Figure showing breakdown of LSD test requests to NHLS, JHB per province from 2011-2020 (n=264). Map adapted from [The South Africa Homepage (nouahsark.com)]

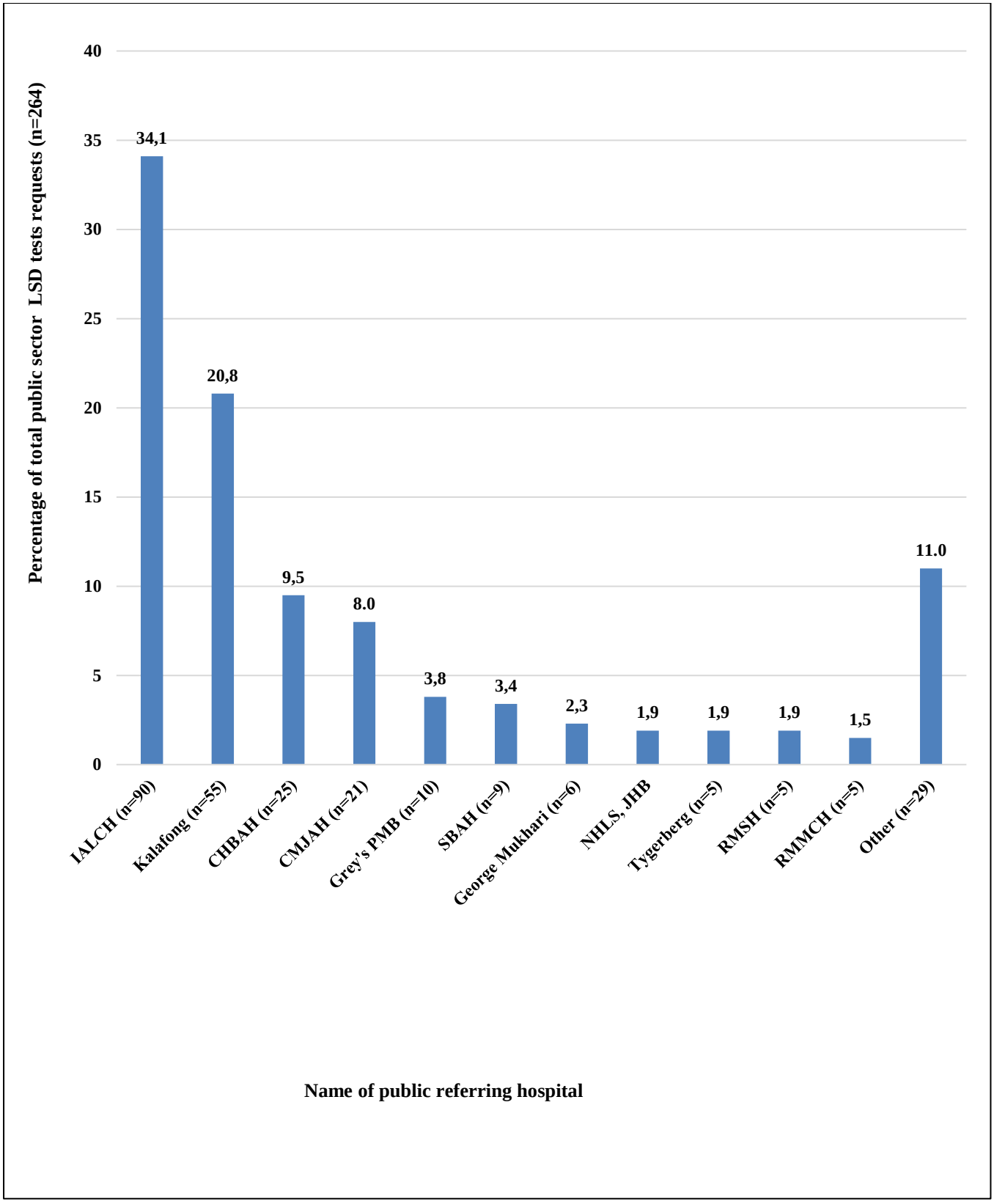

Figure 3.6 Distribution of LSD test requests submitted to NHLS, JHB from public referring hospitals. IALCH = Inkosi Albert Luthuli Central Hospital (KwaZulu-Natal); Kalafong = Kalafong Provincial Hospital (Gauteng); CHBAH = Chris Hani Baragwanath Academic Hospital (Gauteng); CMJAH = Charlotte Maxeke Johannesburg Academic Hospital (Gauteng);

Grey's PMB = Grey's Tertiary Hospital (KwaZulu-Natal); Steve Biko Academic Hospital (Gauteng); George Mukhari = George Mukhari Academic Hospital (Gauteng); NHLS, JHB = National Health Laboratory Service (Gauteng), Tygerberg = Tygerberg Tertiary Hospital (Western Cape), RMSH = Robert Mangaliso Sobukwe Hospital (Northern Cape); RMMCH = Rahima Moosa Mother and Child Hospital (Gauteng).

3.5 Clinical indication for testing

Of the 1861 tests requested during the study period, 1225 (65.8%) had either an unknown reason for referral or an unrecorded reason for referral. Only 40 (2.1%) of referred samples had appropriate indication for testing (see section 2.6.1). A total of 596 (32.1%) of requests had an inappropriate indication for testing (Figure 3.7).

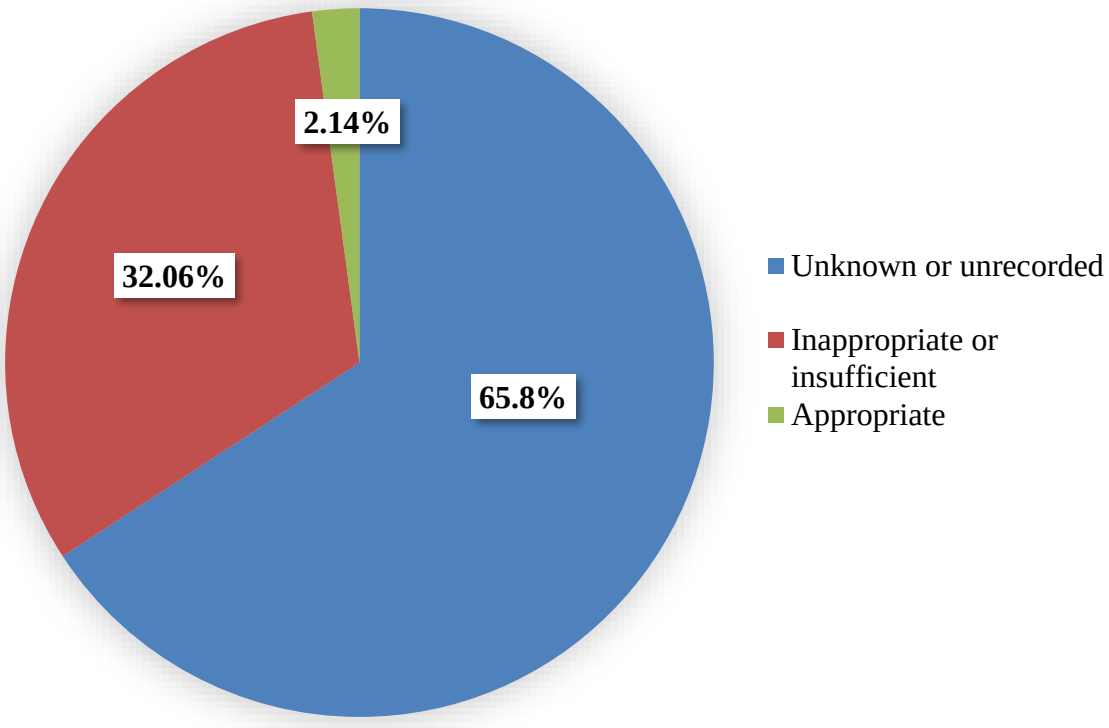

Figure 3.7 Pie chart showing breakdown of clinical indication for testing

3.6 Management and follow up of patients at genetic clinics

A total of 55 patients (3.1%) out of all patients audited were known to a clinical genetic unit in South Africa (from centres in Johannesburg, Cape Town, Bloemfontein and Durban). From the REDCap database it was determined that, out of these 55 patients, 11 were confirmed to have received formal genetic counselling and two patients had cascade testing done on family members (all from southern Gauteng Hospitals). Only one confirmed record could be found of a patient currently receiving ERT. Out of the patients known to Genetic Clinics 19 out of 55 (34.5%) had a positive diagnosis (15 with an MPS, two with Metachromatic Leukodystrophy, one with Fabry disease and one with Gaucher disease).

3.7 Turnaround time

As noted, turnaround time was calculated as the period between the date of registration by the referring centre and the date of posting of the tests results on TrakCare by the NHLS, JHB medical scientist (Figure 3.8). This was because there were no records of the date of arrival at NHLS, JHB. Because of this data limitation turnaround time is not comparable with standard laboratory turnaround times. The mean turnaround time was 6. days, standard deviation= 4.9 (N=720, range 1-30 days). Turnaround time was variable—mostly short, but in rare cases more than three weeks.

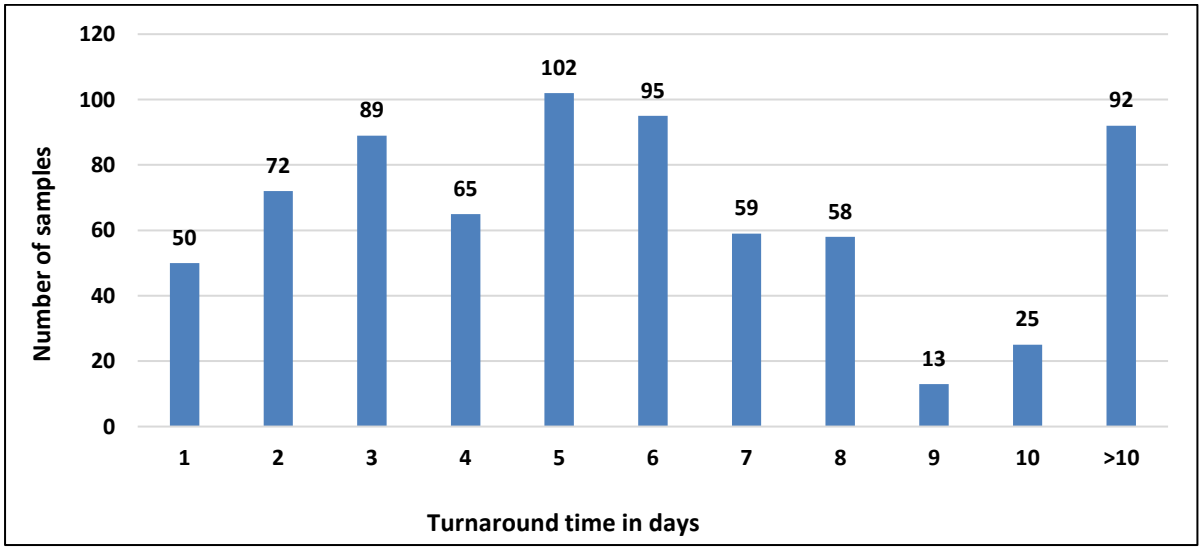

Figure 3.8 Histogram showing turnaround time (period between the date of registration by the referring centre and the date of posting of the tests results on TrakCare) against the number of samples accepted for LSD testing at NHLS, JHB (N=720).

CHAPTER 4 DISCUSSION AND CONCLUSION

4.1 Total Number of LSD test requests and number of subtypes requested

Regarding the objective of assessing the appropriateness of LSD test requests, the results suggest that some test requests may have been inappropriate. Generally, it would be expected that testing for more common LSDs would be requested more frequently. However, this was not the case, for example Gaucher disease, globally the most common LSD, [3,13,26] was only the fifth most frequently requested. Testing for Fabry disease was the most frequently requested, even though it is less globally prevalent than Gaucher disease [3].

It should be noted that the incidence of LSDs in South Africa, and the exact population size of the area that refers to NHLS, JHB is unknown. Therefore, it is not possible to accurately estimate an expected number of test referrals for the study period. Global birth incidence of Gaucher disease is estimated at 1.5 cases (95% CI: 1.0-2.0) per 100,000 live births [26]. Nevertheless, one might expect that the incidence of LSDs in South Africa may at minimum broadly reflect global incidence. Gaucher disease may be even more common in South Africa due to the presence of founder mutations in Black African, Jewish and Afrikaner populations [11]. NHLS, JHB diagnosed 29 cases of Gaucher disease between 2011 and 2020. Assuming a South African population of approximately 52 million in 2011 [27], an average birth rate of 21 per 1000 population between 2011 and 2020, [27] and using global estimates of Gaucher disease birth incidence, a crude estimation of expected Gaucher disease incidence in South Africa can be made. Using this data, roughly 11 million births occurred in South Africa from 2011 to 2020. Therefore, roughly 165 new cases of Gaucher disease would be expected during that period as a minimum estimate. It should, however, be noted that not all cases would present at birth. The population prevalence (number of existing cases of Gaucher disease in the population) would further add to the number of expected Gaucher disease cases. Considering the amount of Gaucher disease diagnoses made (29 between 2011 and 2020) in relation to the size of the referring population, it is likely that many cases are being missed. Similarly, this principle applies to other LSDs tested at NHLS, JHB.

Explanations for these unexpected results include a possible lack of LSD understanding or experience among referring clinicians. For example, if referring physicians are unfamiliar with the clinical features of Fabry disease, it might make them more likely to request the test. Physicians therefore rely on the laboratory to exclude a condition rather than their clinical findings. A lack of knowledge regarding the rarity of Fabry disease may also contribute to more frequent test requests.

It is uncertain if a single referring physician or a group of referring physicians are responsible for requesting LSD testing. This is because the referring physician's name is rarely recorded on the TrakCare database, usually, only the name of the laboratory referring the sample is recorded. One private laboratory in JHB requested 114 Fabry disease tests, a second private laboratory from an unknown province requested 85 Fabry disease tests, and a private laboratory in Pretoria requested 28 Fabry disease tests. Therefore, three referring centres accounted for a total of 227/601 (37.7%) of Fabry disease test requests. The high volume of Fabry disease test requests could be a result of a small group of physicians continually referring samples for a specific test, despite a very low positive yield, of 1.0%. In recent years some testing has been diverted overseas by commercial companies who provide ERT. It is unknown how this has impacted test requests.

It should be noted that there are various possible reasons for the high number of requests for tests for Fabry disease. This disease has an atypical, late onset variant that can present with complications such as cardiac and renal failure, pain, and fatigue. These complications are commonly seen in general practice and are associated with a variety of underlying clinical causes such as hypertension, diabetes, and autoimmune diseases. This may be an additional reason why physicians who treat adult patients might request Fabry disease testing.

4.2 Total number of rejected tests and reasons for test rejection

Regarding the objective of examining test outcomes, the data showed that a high number of samples had to be rejected. Data from 2013-2020 shows a rejection rate of 13.3% (rejection data prior to 2013 was not available). This high rejection rate could be attributable to the sensitivity of biochemical samples. If a sample does not arrive timeously to the laboratory, enzymes will degenerate, resulting in inconclusive results. The most common reason for test rejection is a sample that is too old (Figure 3.2 in chapter 3) (86 rejections between 2013 and 2020). Biochemical samples are sensitive to temperature variation and biomarkers or enzymes

may start degrading with time. This test rejection data indicates prolonged sample transit time from referring centres to NHLS, JHB. The geographical distance between referring centres and the NHLS JHB would result in increased sample transit time. Poor transport to NHLS, JHB may account for delayed transit. These issues could limit wider access to testing with under resourced and rural areas potentially most affected.

Only four tests were rejected due to inappropriate indication of testing. All four were due to a request for carrier screen on a biochemical sample. This is inappropriate because biochemical values do not accurately reflect carrier status, only molecular genetic testing can confirm carrier status. In addition, a lack of knowledge regarding appropriate sample storage (refrigeration rather than freezing or room temperature storage) may contribute to increased test rejection or inconclusive results.

Eighteen samples were rejected due to a staff member not being available to run the test. Only one qualified medical biochemical scientist currently works at NHLS JHB in the biochemistry laboratory. If that staff member is on leave, samples must be rejected. Limited availability of the staff member at times accounted for long turnaround times for some samples (Figure 3.8).

Other reasons for test rejections such as, incorrect specimen type or tube and insufficient sample volumes, may reflect lack of LSD knowledge or experience among referring physicians. This further limits the diagnostic service available to patients.

4.3 Number of accepted test requests that tested positive, negative, and inconclusive

Test requests for Fabry disease had a lower percentage of positive test results (1.0%) compared to other LSDs (for example Gaucher disease, that had a 15.0% positive test). This suggests that Fabry disease is possibly over-requested based on inappropriate indication for testing. It should be noted, however, that testing for Fabry disease is part of a broad diagnostic workup that may be applied even if the indication for testing is not specific. The expected percentage positive tests will depend on the clinical indication for testing. If a test is only requested when the phenotype clearly matches the suspected disease, a higher percentage of positive tests will be expected. However, if a clinician has a low threshold for testing a lower percentage of positive tests will be expected. Not enough data on clinical indication for testing or patient phenotype could be found to accurately comment if certain conditions were over, or under requested, with the possible exception of Fabry disease.

The MPS urine electrophoresis screening assay recorded the highest percentage of positive test results. The two screening assays used by NHLS JHB (spot urine glycosaminoglycan test and urine electrophoresis)) do not make a diagnosis of a specific MPS, rather they tests for elevated levels of biomarkers associated with the broader group of MPS. A positive screening test therefore must be followed by a confirmatory diagnostic test. As seen in chapter 1, figure 1.2, NHLS JHB only has diagnostic testing available for three MPS subtypes (out of nine known subtypes with some subtypes sub-divided). These include Hurler syndrome, Sanfilippo B syndrome and Maroteaux-Lamy syndrome. The reason given for this limitation in MPS diagnostic testing is that the substrates used to diagnose the other MPS subtypes are expensive and have short expiry times. Diagnostic testing for these MPS subtypes are therefore not performed at NHLS JHB. As a result, many patients who screen positive for MPS, do not get a confirmatory and specific diagnosis. This has implications for management. The absence of a diagnosis limits the provision of informed genetic counselling for these patients and precludes ERT which can only be given in the case of a confirmed diagnosis.

As noted in Chapter 3, the 73 inconclusive results were due to intermediate enzyme levels. The results report advised sending a repeat sample and contacting the Clinical and Counselling section of the Division of Human Genetics for more information or referring the patient to a Clinical Genetics unit. A contact number was also provided. 4/73 (5.5%) of patients with inconclusive results were known to a clinical genetics unit and had additional or repeat testing ordered. Poor follow up by referring clinicians to NHLS, JHB is apparent. Although it is possible that follow up occurred at another laboratory, it is possible that many patients are never followed up. Inconclusive results and lack of follow up of positive results lead to inadequate management and patient anxiety.

4.4 The main referral centres that requested LSD testing

Following from the aim of obtaining data on utilization of testing, data revealed a high percentage of test requests from the private sector. Forty-four per cent of samples referred for LSD testing were from private centres. This result is surprising because the private sector serves a much smaller population than the state sector (less than 20.0% of population). Possible explanations for this discrepancy include the financial limitations in the public sector. Public sector physicians are less likely to request testing if they are uncertain of a diagnosis due to the resource constraints. Public sector physicians are encouraged by managers to reduce

unnecessary expenditure to stay within annual budget. In the private sector, physicians have fewer resource constraints and more patient pressure to make a diagnosis. It is also possible that private sector clinicians are unaware that LSD test requests are diverted to an under resourced state laboratory.

The majority of LSD testing done in the public sector was from tertiary hospitals with access to clinical genetics services (Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath Academic Hospital, Inkosi Albert Luthuli Central Hospital, Kalafong Hospital and Steve Biko Academic Hospital). Kalafong Hospital and Inkosi Albert Luthuli Central Hospital requested LSD testing considerably more frequently than other public hospitals (Figure 1.4). Kalafong Hospital mainly requested Fabry disease testing and IALCH mainly requested MLD testing. Like requests from private centres, it may be that an individual clinician or small group of clinicians repeatedly order one specific test. Referring hospitals either over-request one specific LSD test or under-request LSD tests as a group (Figures 3.3 and 3.5). This could suggest a lack of knowledge or awareness of LSDs in smaller public facilities, a lack of specialists to advise on LSD testing or a lack of laboratory support for sample management. There are indications of inequalities between institutions in terms of access and use of LSD tests.

4.5 Clinical indication for testing

Also following from the objective of obtaining data on the appropriateness of testing, many samples were referred without an indication for testing. Of indications for testing, 65.8 % were unrecorded or unknown.

Requesting physicians send handwritten test request forms to NHLS Johannesburg, thereafter the clinical information, including indication for testing, is entered into an electronic format on NHLS TrakCare by laboratory clerks. If the indication for testing is not recorded on TrakCare, it is uncertain whether the requesting physician provided no indication, or whether the indication was not captured by the laboratory clerk. This led to uncertainty regarding the percentage of referring clinicians that completed the indication for testing section of the manual request form. Poor record keeping may lead to poor service delivery and follow up of test requests.

A lack of LSD knowledge or experience among referring clinicians may have contributed to low percentages of appropriate indications. Only four test requests were rejected for inappropriate indication for testing during the study period. It is possible that, due to infrequent test rejections, referring clinicians have assumed that it was not a prerequisite to provide a clinical indication for an LSD test request. Unlike other tests done at NHLS JHB, biochemical tests do not have clinician gate-keeping and ongoing communication with referring clinicians. This is not ideal laboratory practice as it reduces the value of performing the test.

4.6 Management and follow up of patients

A minimum of 55 patients (3.1%) were seen by either a medical geneticist or a genetic counsellor. These patients were all seen prior to testing for an LSD, during diagnostic workup. This number includes patients that were discussed with the NHLS, JHB Clinical Genetics Unit but seen at other genetics units. Despite some genetic specialists working from private laboratories, this number is lower than expected considering NHLS JHB is the main public referral centre for genetic conditions serving a large population area.

A possible explanation for the lower-than-expected number of patients seen by clinical genetics units includes the lack of available data. Despite limited data, it is suspected that most patients with a clinical suspicion of a LSD do not receive adequate clinical review or management from genetic specialists.

Nineteen of 55 (34.5%) patients known to a genetic clinic tested positive for an LSD. All these patients tested positive after an evaluation by a medical geneticist. This high percentage of positive test results may indicate that clinical review and accurate phenotyping is an important step in directing testing. By the time the patient reaches a medical geneticist, the clinical phenotype tends to be obvious, which may imply that diagnostic testing occurred later than desirable. Early referral is the ideal because earlier treatment offers the opportunity for early intervention and improved prognosis.

4.7 Limitations

4.7.1 Limitations related to availability of data

Prior to 2013, data on referring centres, indications for testing, turnaround times and laboratory test result reports, could not be included due to inaccessibility to the DISA electronic database.

A total of 386/1861 (20.7%) of samples audited were captured on DISA and therefore had limited information. For these samples, only the type of test done, and the results could be obtained.

4.7.2 Limitations relating to test rejection data

Test rejection data prior to 2013 had not been recorded at NHLS, JHB. The quality of data relating to test rejection was limited. Test rejection data post 2013 had been recorded on handwritten documents with dates occasionally omitted. An attempt was made to obtain test rejection data using NHLS TrakCare using filters such as 'biochemical testing' or specific LSD testing such as 'MPS' or 'Gaucher disease'. No additional test rejection data could be found using TrakCare searches. It is not known whether rejection data were not recorded on TrakCare or if it was recorded using reference numbers that were not available. Test rejections may have been underestimated.

4.7.3 Limitations relating to percentage positive, negative, and inconclusive tests

For samples prior to 2013, the type of LSD testing done, and the test result (biochemical enzyme values) were obtained from the electronic laboratory records only. In the absence of a laboratory test report, the result of testing done had to be inferred based on the biochemical value found in the electronic laboratory records. This was done in conjunction with the medical scientist that performed the original testing. Due to the unavailability of laboratory reports prior to 2013, errors in result classification may have occurred.

In some cases, for example, Metachromatic Leukodystrophy [6], a control sample from both parents would assist in clarifying inconclusive results. No evidence was found that controls had been requested in such cases.

Another drawback concerns the sequence of diagnostic testing from urine glycosaminoglycan screen to electrophoresis (to determine MPS subgroup) and then to specific enzyme analysis (as shown in Figure 1.2). Examination of the data suggest that this sequence was not routinely followed. The diagnosis of Hunter syndrome by exclusion (see Figure 1.2) is problematic because it may lead to diagnostic errors.

4.7.4 Limitations related to patient follow up with medical genetics

The quality of data at NHLS, JHB, particularly the clinical archive, was limited. Files are not organized chronologically, with large filing sets missing. The presence of a clinical file in the archive is dependent on clinical staff returning files to the archive after seeing the patient at the Genetic Clinic. There was no clear evidence that cascade family screening had been performed as a follow up. These limitations are likely due to limitations in clerical support at NHLS JHB.

Some patients could have received clinical management at units other than clinical genetics units. For example, Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital Metabolic Clinics have managed patients with LSDs. No clinical files from these units were reviewed. Additionally, Potchefstroom Laboratory for Inborn Errors in Metabolism (PLIEM) has offered free testing and treatment for selected LSDs. It is possible that some patients may have received further testing or management of an LSD at PLIEM with no confirmatory records available at NHLS, JHB.

4.8 Recommendations

Based on the results from this study the following recommendations are made:

- Evidence of cascade screening after positive test results is limited. Follow up of positive MPS screening tests is essential. A system should be implemented where a medical geneticist phones the referring clinician and discusses further sample requirements (blood for enzyme analysis) and referral to a genetics clinic for all positive LSD screening tests.
- The biochemical laboratory does not keep records of family/carrier testing. It is recommended that this should be done in a way that records can be accessed easily.
- NHLS JHB implemented a Genetics Logistic Centre (GLC) in 2021. The function of GLC is to screen samples referred for appropriateness of testing as well as to contact referring clinicians for additional clinical information or provide support and information on appropriate genetic tests. GLC has mainly been used for samples sent for molecular and cytogenetic testing. It is recommended that LSD tests be screened and managed by the GLC as well. This will improve appropriateness of testing, reduce clinician uncertainty, and educate referring clinicians on LSD testing at NHLS JHB. Developed countries have well run referral pathways and support for the diagnosis of LSDs. The challenges that face developing countries regarding LSD management have already been discussed, however, Brazil has had success in managing LSDs using a

national reference centre for IEM [28]. The reference centre toll-free service line is available to any healthcare provider in that country and offers advice on sample collection, sample transport, patient management and referral options [13, 28]. A long-term goal for NHLS, JHB should be to implement a similar national reference centre.

- In view of the high number of rejected samples, the following needs to be communicated to referring centres: information on sample collection procedure, appropriate handling, and transport procedures (especially to avoid the submission of samples that are too old), emphasizing that, when in doubt, the NHLS, JHB Clinical Genetics Unit should be consulted prior to a sample being taken. This underlines the need to involve a medical geneticist before and after testing to reduce test rejection.
- Given that 19 of 55 (34.5%) patients known to a genetic clinic tested positive for an LSD, it is important to emphasize the need to involve a medical geneticist in the diagnostic workup of patients with a suspected LSD.
- The disproportionately high number of requests for testing for Fabry disease, as well as the disproportionately low number of requests for Gaucher disease testing suggests that referring clinicians would benefit from better guidelines on indications for testing.
- Liquid Chromatography-Tandem Mass Spectrometry (LC-MS) using dried blood spot cards has been used in many countries as a means of screening for IEM including LSDs [19]. The low cost and ability to screen for many LSD subtypes simultaneously are advantages of LC-MS. NHLS JHB currently does not make use of LC-MS for LSD work up or screening. Future studies to assess the appropriateness of using LC-MS at NHLS JHB could be considered.
- Molecular testing, in the form of Next Generation Sequencing (NGS) is not routinely provided at NHLS, JHB. Molecular testing could be considered for the diagnosis/confirmation of several LSDs at NHLS JHB. The benefit would be to limit the dependence on an understaffed and technically outdated Biochemistry Laboratory. Additionally, molecular testing would be able to diagnose LSDs that the Biochemistry Laboratory is unable to diagnose currently. Finally, blood samples for molecular testing are less sensitive to degradation compared to biochemical testing. NGS would therefore benefit referring centres in rural or under-resourced areas that are unable to get a biochemical sample to NHLS JHB in time. Research done by Zanetti et al. in 2020 on diagnosing LSDs recommend NGS panels as a first line or one of the first line investigations in the diagnostic approach to LSDs [29]. However, a major limitation of

NGS is variant interpretation. This limitation is applicable to LSDs, if a variant is identified using NGS, complex bioinformatics and careful correlation with clinical features is required to decide on pathogenicity [29,30]. This process can be costly and time consuming. Additionally, NGS has limited ability to diagnose LSDs caused by copy number variations and diagnosing LSDs caused by genes not previously known to be associated with an LSD. It is difficult to state what the full institution of NGS may cost the NHLS, JHB in terms of financial and human resources. A cost comparison between biochemical and NGS diagnostics is therefore not currently feasible. There are risk implications if NGS, once instituted, turns out to be over requested.

- Improvement of technology is recommended. Diagnostic testing at NHLS JHB relies mainly on enzyme analysis using fluorimetry. The fluorimeter currently used has not been upgraded for over 20 years. Neither dried blood spot cards nor mutational analysis (using NGS) has been used routinely for diagnostic purposes for LSDs at NHLS JHB. The availability of NGS at NHLS JHB is dependent on stock of reagents and due to procurement problems, reagents have not consistently been available. This would limit the efficacy of NGS as a first line diagnostic test for LSDs. NGS is essential for the molecular diagnosis of many genetic syndromes in addition to LSDs [29].
- Staff responsible for LSD testing should ideally be expanded to avoid test rejections and long turnaround times resulting from reliance on a single trained staff member. Cross training (the practice of training staff in more than one role or skill) could be appropriate here.
- A future study analysing the appropriateness of providing testing to the private sector should be conducted. Such a study should consider issues such as inequality of access to LSD testing in South Africa as well as the financial benefit of providing LSD testing to private laboratories. The option of co-ordinating testing appropriately between public and private laboratories including communicating results and referring patients for clinical examination should be explored.
- Improvement in record keeping by NHLS JHB, especially for positive, inconclusive and rejected tests. Future record keeping should also include the following: whether test results have been discussed with the referring clinicians, whether clinical follow up at a genetic clinic has been arranged and if confirmatory testing at another facility (such as an overseas laboratory) has been recommended. These details would assist with

overall service delivery and patient care. Clinical information needs to be stored in a database with easy accessibility.

4.9 Conclusion

The result of this research provides evidence of the significant limitations of current biochemical testing for LSDs offered at NHLS, JHB. The strength of this audit is that it identifies important shortcomings and limitations in the diagnosis of LSDs and puts forward recommendations to overcome these. In particular, the audit revealed the need for modernisation of the equipment of a currently outdated laboratory, improvement of record keeping systems (of patient management and test outcomes), as well as increasing the number of genetic professionals (clinical and laboratory-based) to assist with the diagnosis of LSDs.

There is poor gate-keeping and lack of guidance and education of referring clinicians by NHLS JHB. Results show evidence of inappropriate use of biochemical testing by referring clinicians, mainly due to poor patient selection and poor sample collection. Referral for LSD testing in South Africa is very patchy. Inequality of access to LSD testing, between private and public facilities, among different geographical areas (provinces) and between large referral centres and smaller centres is also apparent. These deficiencies will impact on the management of patients affected by or at risk of LSDs.

Results of this study underly the need for involving a medical geneticist in the clinical and laboratory diagnostic processes for LSDs.

Certain LSD subtypes are requested disproportionately frequently with the majority of results negative or inconclusive (especially Fabry disease). Gaucher disease is likely under requested. Within the MPS group, there is a significant lack of appropriate follow up of positive screening results and limited diagnostic and therapeutic options.

National policy guidelines and clinician education may result in broader access and use of LSD testing, reducing inequality issues. Improved sample gatekeeping (including guidance to referring clinicians) and active follow up of positive and inconclusive test results by medical geneticists will improve patient management. More epidemiological studies on the local incidence of LSDs may assist clinicians in patient selection for testing. Use of NGS or liquid

chromatography for improved diagnostic accuracy are possible solutions that could be explored in future studies.

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Appendix A: Ethics certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO:	Dr M Novellie School of Pathology Department of Human Genetics Medical School University E-mail: Michael.Novellie@nhls.ac.za
CC:	Supervisor: Dr L Bhengu and Professor A Krause <Louisa.Bhengu@nhls.ac.za> and <HREC-Medical Research Office@wits.ac.za>
FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	2021/09/23
REF:	R14/49
PROTOCOL NO:	**M210343** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)
PROJECT TITLE:	*Audit of lysosomal storage diseases testing at the National Health Laboratory Service in Johannesburg from 2011-2020*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

Appendix B: REDCap data collection sheet

Confidential

MMed data capturing
Page 1

Form 1

Lab reference number/patient number	_____
Date of testing	_____
Referring centre	_____
Indication for testing	_____
Testing done	☐ Yes ☐ No- test rejected
Reason for test rejection	_____
Type of LSD testing performed:	☐ MPS electrophoresis testing ☐ MPS screening assay (Urine glycosaminoglycans) ☐ Pompe (Alpha-Glucosidase) ☐ Maroteaux-Lamy (LEUCOCYTE ARYLSULPHATASE B) ☐ Gauchers (Beta Glucosidase) ☐ Fabry (alpha Galactosidase A) ☐ Hurler (LEUCOCYTE a-L-IDURONIDASE) ☐ Other LSD testing ({Result of 1st test (positive/negative and value)})
MPS electrophoresis	○ Positive ○ Negative ○ Inconclusive
MPS screening assay (urine GAG)	○ Positive ○ Negative ○ Inconclusive
Pompe	○ Positive ○ Negative ○ Inconclusive
Marateux-Lamy	○ Positive ○ Negative ○ Inconclusive
Gaucher	○ Positive ○ Negative ○ Inconclusive
Fabry	○ Positive ○ Negative ○ Inconclusive

Hurler	◯ Positive ◯ Negative ◯ Inconclusive
Other LSD testing	◯ Positive ◯ Negative ◯ Inconclusive
Turn around time	
Seen by Medical Genetics/ Genetic counselling?	◯ Yes ◯ No
Type of management done	☐ Clinical genetics assessment ☐ Genetic counselling ☐ Cascade screening ☐ Prenatal testing

Appendix C: Turnitin plagiarism report