Determination of Cut-off Values Using the MOM Approach in Lysosomal Storage Diseases in Turkey: Findings from a Newborn Frequency Study

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Objective

- Lysosomal storage diseases (LSDs) are a group of rare disorders characterized by the accumulation of substrates within lysosomes due to various enzyme deficiencies. Early diagnosis is critical for timely initiation of treatment. In newborn and other large-scale screening programs, determining accurate cut-off values is essential to reduce false-positive and false-negative results.
- In this study, enzyme activity levels were evaluated using dried blood spot (DBS) samples from 4,000 patients. The aim was to compare cut-off values based on the test kit with values derived using the Multiple of Median (MOM) approach, and to determine the most appropriate thresholds.

Results

- According to the kit-based [NeoLSD®] cut-off values, 203 patients were found to be positive among the 4,000 tested (distributed as follows: ABG: 43, ASM: 74, GALC: 13, IDUA: 20, GLA: 42, GAA: 55).
- However, using the 0.2 MOM threshold, only 38 patients were positive (distributed as: 14, 1, 21, 4, 6, 10 respectively). All enzyme levels in known positive patients were found to be below the 0.2 MOM values (Table 1).
- Figure 1 shows the reference intervals calculated using the patient-based method.

Conclusion

- It is recommended in the literature that each ethnic population and associated laboratory define its own specific cut-off values. Accordingly, confirmation and validation analyses are planned to definitively establish these thresholds.
- When comparing the manufacturer's cut-off values with the 0.2 MOM approach, the latter yields fewer positive results while still identifying all known positive cases. This suggests that the MOM-based approach increases specificity without compromising sensitivity for confirmed cases. Implementing MOM-based, population-specific cut-offs may improve screening accuracy and reduce healthcare costs. Therefore, more comprehensive validation studies are needed to support the adoption of MOM-derived threshold values in clinical and screening settings.

Method

Study Design and Setting

This ***preliminary study*** was designed as a prospective analysis conducted at the Ankara University Rare Diseases Application and Research Center. The study was approved by the Ankara University Human Research Ethics Committee.

Patient Selection

A total of 4,000 **DBS samples were analyzed.** Samples were obtained from newborns from 19 different cities in seven different regions of Türkiye. Samples were kept at -20°C until analysis. In addition to the 4,000 patients, 15 more patients whose diagnoses we knew were included in the study.

Exclusion criteria: Samples that are not homogeneously distributed on the DBS card,

Samples without consent form

Sample Processing and Analyzer Workflow

All samples were analyzed using tandem mass spectrometry (MS/MS). Flow injection-MS/MS was performed on a SCIEX Triple Quad 3500 instrument. A commercial kit from Revitty was used to measure six different lysosomal enzymes [ABG (β-glucocerebrosidase), ASM (Acid sphingomyelinase), GALC (Galactocerebrosidase), IDUA (α-L-iduronidase), GLA (α-galactosidase A), GAA (Acid α-glucosidase)].

Cut-off Values and Retesting

The study focused on analyzing:

- The kit's uncertainty values were used to determine initial cut-off values and identify patients requiring retesting.
- All samples with any test below the cut-off value were repeated.
- In addition to results that showed improvement after repetition, values that did not show improvement after repetition were also used.

Statistical Analysis

Statistical analyses were performed using the Analyze-it software. Reference intervals with **patient-based method** were calculated using median ± IQR, Box-Cox power transformation, and biweight analysis. Patients with previously known diagnoses and positive results based on kit cut-off values were excluded from the main analysis. The 0.2 MOM value of each enzyme was calculated after excluding patients with low enzyme levels, i.e. those to be repeated (n=206), according to the kit cut-off.

Limitations

Clinical validation has not yet been performed in our study. In further studies, positive cases will be supported by genetic and biomarker studies. Furthermore, a reference range calculation based on gender was not possible in Fabry patients. If gender discrimination could be made, the threshold value could be calculated more accurately. This is the preliminary study further study with larger cohorts is required.

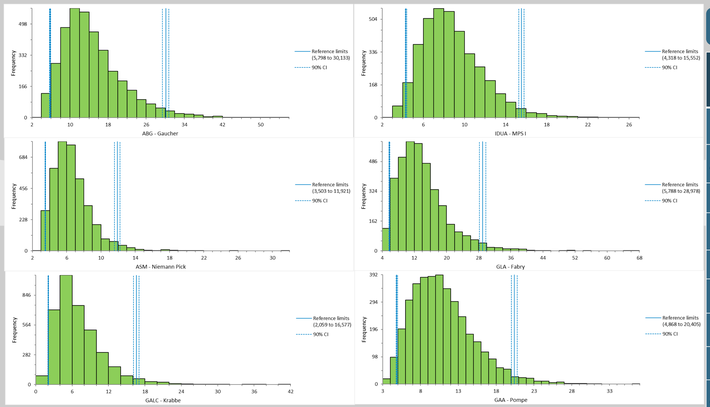

Figure 1. Reference intervals calculated using the patient-based method

Table 1. Enzyme Levels, Cut-off vs. MOM Comparison

Enzyme	Associated Disease	Median (IQR)	Lower Reference Limit	Kit Cut-off	0.2 MOM	Positive by Kit Cut-off (n)	Positive by 0.2 MOM (n)
ABG (β-glucocerebrosidase)	Gaucher Disease	13.42 (7.74)	5.80	3.89	2.68	43	11
ASM (Acid sphingomyelinase)	Niemann-Pick Type A/B	6.25 (2.58)	3.50	2.95	1.25	74	1
GALC (Galactocerebrosidase)	Krabbe Disease	6.15 (4.63)	2.06	0.97	1.23	13	18
IDUA (α-L-iduronidase)	MPS Type I (e.g. Hurler)	8.36 (3.81)	4.32	2.86	1.67	20	4
GLA (α-galactosidase A)	Fabry Disease	12.90 (7.16)	5.79	4.19	2.58	42	5
GAA (Acid α-glucosidase)	Pompe Disease	10.40 (5.32)	4.87	3.77	2.08	55	7
Enzyme	**Associated Disease**	**Enzyme Levels (median-IQR)**		**Known Positive Patients (n)**			
ABG (β-glucocerebrosidase)	Gaucher Disease	0.51 (0.49)		9			
ASM (Acid sphingomyelinase)	Niemann-Pick Type A/B	0.16 (0.09)		2			
GALC (Galactocerebrosidase)	Krabbe Disease	0.04		1			
GAA (Acid α-glucosidase)	Pompe Disease	0.81 (0.74)		3			

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