UPLC-MS/MS Analysis of Urinary Free Oligosaccharides for Lysosomal Storage Diseases: Diagnosis and Potential Treatment Monitoring

Rongrong Huang,1 Sara Cathey,2 Laura Pollard,1 and Tim Wood1*

BACKGROUND: The glycoproteinoses are a subgroup of lysosomal storage diseases (LSDs) resulting from impaired degradation of N-linked oligosaccharide side chains of glycoproteins, which are commonly screened by detecting the accumulated free oligosaccharides (FOSs) in urine via thin layer chromatography (TLC). The traditional TLC method suffers from limited analytical sensitivity and specificity and lacks quantification capability. Therefore, we developed an analytically sensitive and relatively specific assay using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) for urinary FOS analysis and validated its use for urine screening of glycoproteinoses and other LSDs.

METHODS: Urine volumes equivalent to 30 μg of creatinine were derivatized with butyl-4-aminobenzoate and then purified through a solid-phase extraction cartridge. A 7-min UPLC-MS/MS analysis was performed on a triple quadrupole mass spectrometer using an amide column for separation of derivatized FOS. Urine samples from >100 unaffected controls and 37 patients with various LSDs were studied.

RESULTS: Relative quantification was conducted on 7 selected FOSs using a single internal standard, which allowed the identification of patients with 1 of 8 different LSDs: aspartylglucosaminuria, α-fucosidosis, α-mannosidosis, β-mannosidosis, β-galactosidase deficiency, Sandhoff disease, sialidosis, and galactosialidosis. Patients treated with hematopoietic stem cell transplant show decreased FOS responses compared with untreated patients.

CONCLUSIONS: This UPLC-MS/MS assay offers a valuable tool for screening of glycoproteinoses and other LSDs, with potential use for future treatment monitoring.

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Patients affected by lysosomal storage diseases (LSDs)3 are unable to properly degrade specific macromolecules such as glycosaminoglycans, glycoproteins, and sphingolipids (1). The accumulation of partially degraded substrate(s) in the lysosome results in cellular toxicity, and often can be detected in the urine owing to excess excretion. Various low-cost screening methods have been developed to measure (either qualitatively or quantitatively) these partially degraded substrates as a first-tier test to identify patients with a defect in the degradation of the macromolecules.

Traditional urine-based screening for the glycoproteinoses, a group of LSDs also known as oligosaccharidases, includes qualitative separation of free oligosaccharides (FOSs) via thin layer chromatography (TLC) (2). Accurate diagnosis of a glycoproteinosis disorder using the TLC method suffers from a lack of analytical sensitivity because of the nature of the method and analytical specificity caused by interfering nonspecific bands from medications or diet. The subjective interpretation of the banding patterns can create bias when comparing results from different laboratories (2, 3).

Several groups have applied mass spectrometry to the analysis of FOS in patients with LSDs (4–6). These methods involve reducing-end labeling of FOS; therefore, glycoamino acids accumulated in patients with diseases such as aspartylglucosaminuria are often missed because of the lack of an appropriate reducing end. Xia et al. (7) were the first to apply MALDI-TOF mass spectrometry to the analysis of a large group of glycoproteinoses. Although MALDI-TOF analysis offers comprehensive detection of the various FOSs accumulated in these pa-
tients, this instrumentation is not widely available in clinical laboratories and this technology is not well suited for quantification, limiting its potential use for monitoring treatment efficacy.

Our laboratory has developed and validated an ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method, operating on a standard triple quadrupole instrument, for the detection and relative quantification of specific oligosaccharide species. Derivatization of FOS by butyl-4-aminobenzoate (BAB) was used because of its previous clinical use for quantification of urinary glucose tetrasaccharide for Pompe disease (glycogen storage disease type II) reported by Young et al. (4, 8). Modifications to solid-phase extraction and UPLC separation were made to allow for the retention and detection of the underivatized glycoamino acids observed in patients with aspartylglucosaminuria. A set of 45 glycoprotein-related FOS compositions chosen from the literature (5, 7, 9, 10) were initially analyzed, from which 7 FOSs were selected for clinical validation. Urine samples from 110 unaffected controls and 37 affected patients with LSDs were studied, and age-specific normal ranges were established for each of the 7 FOSs. In a small number of samples collected from patients with α-mannosidosis or α-fucosidosis after hematopoietic stem cell transplant (HSCT) treatment, substantial decreases were observed in disease-specific oligosaccharide responses, indicating that the method may be used to monitor treatment efficacy as novel therapies are developed for this group of conditions.

Materials and Methods

MATERIALS AND METHODS AND INSTRUMENTS
Sodium cyanoborohydride, BAB, and 2-acetamido-1-N-(β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (GlcNAc-Asn) were purchased from Sigma-Aldrich, and 3,4-ununsaturated uronic acid-N-acetyl-glucosaminyl (ΔuA-GlcNAc) was purchased from Iduron. Solvents used for UPLC were LC-MS grade purchased from Fisher Scientific. All other solvents and reagents were of analytical grade and commercially available. Sep-Pak Aminopropyl 1-mL Vac cartridge (1.7 μm, 2.1 mm × 50 mm), and VanGuard Glycan BEH Amide 1.7-μm guard column all were purchased from Waters. The UPLC-MS/MS analysis was performed on a Waters Acquity UPLC System (I-Class) equipped with a Waters Xevo TQS electrospay ionization triple quadrupole tandem mass spectrometer.

Urine samples from affected patients were collected either as part of the Longitudinal Studies of the Glycoproteinoses (ClinicalTrials.gov Identifier: NCT01891422) or for routine clinical testing in the Biochemical Genetics Laboratory at the Greenwood Genetic Center. We analyzed 53 urine samples from 33 untreated patients with 1 of 8 LSDs: aspartylglucosaminuria [Online Mendelian Inheritance in Man (OMIM 208400)] (n = 10), α-fucosidosis (OMIM 208400) (n = 4), α-mannosidosis (OMIM 248500) (n = 21), β-mannosidosis (OMIM 248510) (n = 1), β-galactosidase deficiency [GM1 gangliosidosis (OMIM 230500) (n = 5)] and Morquio B (OMIM 253010) (n = 3), GM2 gangliosidosis [Sandhoff disease (OMIM 268800)] (n = 2), sialidosis (OMIM 256550) (n = 5), and galactosialidosis (OMIM 256540) (n = 2). An additional 7 urine samples were collected from 3 patients with LSDs who had undergone HSCT: α-fucosidosis (n = 6) and α-mannosidosis (n = 1). Institutional review board approval was obtained from the Self Regional Healthcare Institutional Review Board, Self Regional Hospital, Greenwood, SC. Urine samples (n = 110) from normal controls with a wide age range (2 days to 63 years of age) were either clinical samples with normal TLC screening results or collected from healthy adult volunteers. All controls were anonymized.

URINE SAMPLE PREPARATION
Urine samples were centrifuged, and a volume of supernatant equivalent to 30 μg of creatinine was dried under nitrogen, to which 60 μL of deionized water containing 2 μg of internal standard (IS), ΔuA-GlcNAc, was added for reconstitution. A blank sample (no urine added) and 2 quality control (QC) samples were assayed in each batch to monitor system performance. QC1 was prepared by combining equal volumes of urine samples from each of 5 known affected patients with α-mannosidosis, α-fucosidosis, β-galactosidase deficiency, aspartylglucosaminuria, and mucolipidosis type III, respectively. QC2 was prepared by combining equal volumes of urine samples from 5 known unaffected patients.

DERIVATIZATION OF OLIGOSACCHARIDES
The oligosaccharides, including the IS, were derivatized with BAB using a modified version of a previously reported method (8). The derivatizing reagent, containing BAB (54 mg), sodium cyanoborohydride (47 mg), acetic acid (0.11 mL), and methanol (1.76 mL), was prepared fresh daily as required. The reconstituted urine samples were mixed with 140 μL of reagent and incubated at 80 °C in darkness for 45 min. After cooling, the samples were diluted with 0.8 mL of acetonitrile. Excess reagent was removed using solid-phase extraction. Samples were loaded onto an aminopropyl cartridge, which was preconditioned with 1 mL of deionized water followed by 1 mL of acetonitrile/water (85/15, v/v). After sample application, the cartridge was washed with 1 mL of acetonitrile/water (85/15, v/v), and then BAB-labeled oligosaccharides and nonlabeled glycoamyloids were eluted with 1 mL of acetonitrile/water (5/95, v/v) containing 50-mmol/L ammonium acetate. The eluate was
then dried under nitrogen and reconstituted in 1 mL of acetonitrile/water (75/25, v/v) before UPLC-MS/MS analysis.

UPLC-MS/MS ANALYSIS
The UPLC separation was performed on an amide column using 50-mmol/L ammonium formate in water as mobile phase buffer A and pure acetonitrile as mobile phase buffer B. The flow rate was set at 0.5 mL/min, and a linear gradient was applied as follows: 0 to 0.5 min, 22% A; 0.5 to 4.0 min, 22% to 52% A; 4.0 to 4.3 min, 52% to 100% A; 4.3 to 5.1 min, 100% A; 5.1 to 5.6 min, 100% to 22% A; 5.6 to 6.8 min, 22% A. The total analysis time was approximately 7 min/sample. The mass spectrometry analysis was performed in electrospray ionization positive ion mode, with a source temperature of 120 °C, capillary voltage of 3.0 kV, and cone voltage of 40 V. The single reaction monitoring (SRM) transitions were created to detect 7 disease-related FOSs, as well as the IS (Table 1), with a dwell time of 50 ms set for each transition.

TOTAL ION CHROMATOGRAM INTERPRETATION AND RELATIVE QUANTIFICATION
Data processing was conducted using MassLynx (version 4.1), for which a total ion chromatogram (TIC) was extracted for each sample and interpreted by comparison with previously collected TICs of positive LSDs and normal controls. TargetLynx was used for peak integration with a signal-to-noise ratio of 5 set as the cutoff for peak detection limit. Relative quantification was assessed through the software reported response, which was calculated as FOS peak area/IS peak area × 1000. Results

METHOD DEVELOPMENT
Reducing-end labeling methods were widely applied to overcome the difficulty involved in the electrospray ionization–SRM analysis of native oligosaccharides, such as poor ionization and sparse fragmentation. Because reducing-end labeling methods require oligosaccharides to have a free reducing end, aspartyl-oligosaccharides, a group of glycoamino acids known to accumulate in urine from patients with aspartylglucosaminuria, are not derivatized and retained on traditional reversed-phase columns (i.e., C18) because of their high hydrophilicity but are easily retained on normal phase columns. However, BAB-labeled FOSs are well retained on both reversed-phase and normal phase columns. Therefore, the Glycan BEH Amide column (Waters), a normal phase column specifically designed for glycan separations, was chosen for this study because of its previously demonstrated superior retention and high selectivity of reducing-end labeled glycans (11–13).

Targeted SRM experiments were conducted to screen 43 selected FOS compositions of interest (see the Materials file in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue12). Because of the structural complexity of these FOSs and the limited availability of authentic standard, a disaccharide not naturally present in human urine, ΔUA-GlcNAc, was chosen as the IS. Initial screening of all 43 FOSs was conducted on 8 known patients with LSDs (aspartylglucosaminuria, α-fucosidosis, α-mannosidosis, β-mannosidosis, β-galactosidase deficiency, Sandhoff disease, sialidosis, and galactosialidosis) and 4 normal controls.

<table>
<thead>
<tr>
<th>FOS ID</th>
<th>FOS composition</th>
<th>SRM transition</th>
<th>RT, min</th>
<th>Disorder</th>
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<tbody>
<tr>
<td>FOS 1</td>
<td>Hex1HexNAc2Asn</td>
<td>498.2 &gt; 133.03</td>
<td>2.5</td>
<td>Aspartylglucosaminuria</td>
</tr>
<tr>
<td>FOS 2</td>
<td>Hex3HexNAc2Fuc</td>
<td>1234.45 &gt; 399.25</td>
<td>1.7</td>
<td>α-Fucosidosis</td>
</tr>
<tr>
<td>FOS 3</td>
<td>Hex3HexNAc1</td>
<td>885.4 &gt; 399.25</td>
<td>1.0</td>
<td>α-Mannosidosis</td>
</tr>
<tr>
<td>FOS 4</td>
<td>Hex1HexNAc1</td>
<td>561.25 &gt; 399.25</td>
<td>0.4</td>
<td>β-Mannosidosis</td>
</tr>
<tr>
<td>FOS 5</td>
<td>Hex3HexNAc2</td>
<td>1088.45 &gt; 399.25</td>
<td>1.5</td>
<td>β-Galactosidase deficiency</td>
</tr>
<tr>
<td>FOS 6</td>
<td>Hex2HexNAc3</td>
<td>1129.45 &gt; 399.25</td>
<td>1.3</td>
<td>GM2 (Sandhoff disease)</td>
</tr>
<tr>
<td>FOS 7</td>
<td>Neu5Ac1Hex3HexNAc2</td>
<td>1379.55 &gt; 399.25</td>
<td>2.0 and 2.1</td>
<td>Sialidosis/galactosialidosis</td>
</tr>
<tr>
<td>IS</td>
<td>ΔHexA-GlcNAc</td>
<td>557.28 &gt; 399.25</td>
<td>0.5</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Hexose.
* N-acetyl hexose.
* Asparagines.
* Fucose.
* Sialic acid.
* Unsaturated hexuronic acid.
* Not applicable.
(5 months to 21 years of age). Subgroups of FOS with similar structures were observed for each LSD (see Table 2 in the online Data Supplement), consistent with previous findings \((5, 7, 9, 10)\). Age-dependent FOS responses were observed in normal controls.

**SCREENING BY TIC PATTERNS**

Each of the 7 FOSs (Table 1) was carefully selected based on its relative abundance in affected individuals, as well as the retention time to ensure adequate peak separation between each FOS to use a single TIC for pattern screening. Shown in Fig. 1 are representative TICs, consisting of 8 SRM transitions, including the IS, for 1 normal control (Fig. 1A) and 8 patients with LSDs (Fig. 1, B–I). A unique TIC pattern was observed for each disorder except for sialidosis and galactosialidosis, which produced similar TIC patterns. The increase in the appropriate FOS in affected patients created a dominating peak with a unique retention time in the corresponding TIC compared with the normal control (note the scale differences of signal intensity in the y axis for each TIC), which was easily distinguished from the normal control and the other LSDs. Although the FOS specific for Sandhoff disease (FOS 6) at a retention time of 1.3 min was not the most abundant peak in the TIC from the affected patient (Fig. 1F), the pattern could still be differentiated from that of the normal control and other LSDs. Additionally, a baseline-separated peak was obtained in the corresponding SRM transition as presented in the inserted extracted ion chromatogram (Fig. 1F), allowing interference-free peak integration for relative quantification.

Two baseline-separated peaks were detected for the corresponding Neu5Ac1Hex3HexNAc2 (FOS 7) at retention times 2.0 min and 2.1 min with an approximate...
peak height ratio of 1:10. Our hypothesis was that these peaks were \( \alpha(2-3) \) and \( \alpha(2-6) \) sialic acid linkage isomers, with the \( \alpha(2-3) \) species eluting before the \( \alpha(2-6) \). This was observed in several previously reported studies using amide columns for separation of sialic acid linkage isomers labeled with various reducing-end derivatizing reagents\((14, 15)\). Although no such data are available for BAB-labeled oligosaccharides, the trend could be inferred based on the chemistry of amide columns and sialylated oligosaccharide derivatives. Additionally, the relative peak height ratio observed here was also consistent with the previously reported ratio for patients with sialidosis\((16)\).

**ASSAY VALIDATION**

Like the MALDI-TOF method\((7)\), the patient results were reported only qualitatively (normal or abnormal for each FOS with pattern interpretations). Therefore, validation studies were mainly focused on comparing the clinical sensitivity and specificity between the UPLC-MS/MS method and the TLC method. Because of the lack of reference material and standards for calibrator making, analytical validation studies of the assay were limited.

Superior to the TLC method that typically consumes several milliliters of urine, the urine volumes used for the UPLC-MS/MS method, equivalent to 30 \( \mu \)g of creatinine, generally ranged from 10 to 600 \( \mu \)L, depending on the creatinine concentration. Urine samples were diluted if creatinine values were higher than 3.0 \( \mu \)g/\( \mu \)L or rejected if creatinine values were lower than 0.05 \( \mu \)g/\( \mu \)L.

To assess the analytical specificity, urine samples shown to have interfering bands via TLC analysis were also tested by the UPLC-MS/MS assay. None of the samples had interferences detected for any of the 7 FOSs. In addition, there was no known interference observed for the 7 FOSs based on data collected from all validation urine samples (Fig. 2; see also Table 3 in the online Data Supplement) and from >200 clinical samples tested in our laboratory over a 1-year period (data not shown).

As part of initial validation, the intraassay \((n = 6)\) and interassay \((n = 6\) over a 2-month period) precision was evaluated for each FOS response and the IS peak area in 2 QC urine samples (Table 2). Less than 20% relative SD (%CV) was obtained for the FOS response for most FOSs. Other studies including ion suppression, carryover, and linearity were also assessed as applicable (see Materials file in the online Data Supplement).

Age-specific normal ranges were also developed for each FOS using 110 samples from unaffected controls (Table 3). As expected, FOS response decreased with age. Increases of disease-specific FOS were detected in all 53 urine samples from untreated patients with LSDs compared with age-matched normal controls (Fig. 2). Fold increases were calculated by dividing the FOS responses in urine samples by the corresponding FOS response upper limit of the age-specific normal range. There was 100% clinical sensitivity for the detection of 8 different LSDs: aspartylglucosaminuria (mean, 148-fold increase), \( \alpha \)-fucosidosis (mean, 1733-fold increase), \( \alpha \)-mannosidosis (mean, 87-fold increase), \( \beta \)-mannosidosis (34-fold increase), \( \beta \)-galactosidase deficiency (mean, 82-fold increase), Sandhoff disease (mean, 156-fold increase), sialidosis (mean, 311-fold increase), and galactosialidosis (mean, 348-fold increased). The responses of each FOS in all affected LSD patients are provided in detail in Table 3 of the online Data Supplement.

The clinical specificity of each FOS varied, with FOSs 1 through 3 showing the highest clinical specificity (Fig. 2, A–C), followed by FOS 4 and FOS 6 (Fig. 2, D and F). Slight to marked increases of FOS 5 and FOS 7 were detected in patients with \( \beta \)-galactosidase deficiency, sialidosis, or galactosialidosis (Fig. 2, E and G). However, the ratio of the fold increases between FOS 5 and FOS 7 could be used to differentiate \( \beta \)-galactosidase deficiency from sialidosis or galactosialidosis (see Table 3 in the online Data Supplement), which was also demonstrated by the TIC patterns (Fig. 1, G–I).

Preliminary evidence for the use of this semiquantitative analysis of FOS for treatment monitoring was obtained by analyzing urine samples from patients treated with HSCT. The Hex3HexNAc2Fuc1 response in a patient with \( \alpha \)-fucosidosis (patient P6) decreased by approximately 45% after transplantation; however, this FOS remained approximately 400-fold increased post-treatment (Fig. 3). Although FOS concentrations cannot be quantitatively measured, we confirmed that the mass spectrometric intensity of Hex3HexNAc2Fuc1 was not at saturation at the concentration presented in the sample, and the peak response remained a good correlation with its concentration between a 1000-fold and 100-fold increase as indicated by the dilution study shown in Table 4 and Fig. 1 in the online Data Supplement. A separate patient with \( \alpha \)-fucosidosis who was also treated with HSCT showed similarly low Hex3HexNAc2Fuc1 response when compared with untreated patients (see Table 3 in the online Data Supplement). A patient with \( \alpha \)-mannosidosis (patient P22) treated with HSCT >5 years before sampling had only a 6-fold increased Hex3HexNAc1 response compared with the increases observed in untreated patients, which range from 44- to 139-fold (see Table 3 in the online Data Supplement). Unfortunately, pretreatment urine samples from these latter 2 patients were not available for analysis.

**Discussion**

In general, the assay demonstrated good clinical specificity for each disorder studied. For 3 FOSs (Hex1HexNAc1Asn, Hex3HexNAc2Fuc1, and Hex3HexNAc1), there were
no gross increases (≥3-fold) noted in urine samples from other nontargeted LSDs. For other FOSs, mild increases (up to 10-fold) were observed in other nontargeted LSD conditions, but the degree of increase was lower compared with that observed in samples from the targeted disorder (range from 30- to 3000-fold; Fig. 2 here and Table 3 in the online Data Supplement). The single exception was Neu5Ac1Hex3HexNAc2 (FOS 7), which was associated with β-galactosidase deficiency, sialidosis, and galactosialidosis. Mild to moderate increases were also observed in patients with the mucopolysaccharidoses (MPS) (up to 60-fold), mucolipidosis types II and III (ML) (4–238-fold), and other lysosomal storage conditions (2–7-fold) (data not shown). Therefore, this FOS may serve as a general marker for lysosomal dysfunction. For patients with a clinical suspicion of MPS, urinary glycosaminoglycans analysis should be used for MPS screening and subtyping.

Sialidosis is a glycoproteinosis disorder caused by the deficiency of the enzyme α-neuraminidase, whereas galactosialidosis is associated with a combined enzyme deficiency of β-galactosidase and neuraminidase. Patients

Fig. 2. For each FOS, the fold increase compared with the upper limit of the age-specific normal range is plotted for both normal controls and patients with LSDs.

FOS 1, Hex1HexNAc1Asn (A); FOS 2, Hex3HexNAc2Fuc1 (B); FOS 3, Hex3HexNAc1 (C); FOS 4, Hex1HexNAc1 (D); FOS 5, Hex3HexNAc2 (E); FOS 6, Hex2HexNAc3 (F); and FOS 7, Neu5Ac1Hex3HexNAc2 (G). A box-and-whisker plot is used to display the minimum, first quartile, median, third quartile, and maximum of each group. The horizontal axis abbreviations represent Sia, sialidosis; GalSia, galactosialidosis; bGal, β-galactosidase deficiency; Sandhoff, Sandhoff disease; aMan, α-mannosidosis; bMan, β-mannosidosis; aFuc, α-fucosidosis.
with these disorders will have similar oligosaccharide patterns when analyzed using TLC. Currently, enzyme and/or molecular testing is used to confirm the diagnosis. Our assay produced similar TIC patterns for patients with sialidosis and galactosialidosis (Fig. 1, H and I). Our initial test of 2 representative patients (patients P33b and P37) showed potential differences between sialidosis and galactosialidosis (see Table 2 in the online Data Supplement). However, given the wide range of Neu5Ac1Hex3HexNAc2 responses presented among the sialidosis patients and the fact that few samples of various patient ages were available, we believe that this FOS should not be used by itself to distinguish sialidosis from galactosialidosis. The analysis of additional FOS species or other bioinformatics processes might allow for the differentiation of these 2 disorders based on FOS response alone in the future.

Besides glycoproteinosis, we also explored other LSDs and genetic conditions for which increases of FOS in urine have been reported. Increases in Hex3HexNAc1 have been described in patients with Gaucher disease (7). To date, our laboratory has analyzed only a single urine sample from a young untreated patient with Gaucher disease. The degree of Hex3HexNAc1 increase in that sample was much less than was noted in all our patients with /H9251-mannosidosis. Analysis of additional samples would be required to determine the degree of increase of Hex3HexNAc1 in untreated patients with Gaucher disease. Increased polyhexoses, including glucose tetrasaccharide, have been detected in urine samples from patients with Pompe disease (see Table 2 in the online Data Supplement), consistent with previous work by Young et al. (4). Additionally, an abnormal tetrasaccharide (17) was detected in patients with 2 forms of congenital disorders of glycosylation [PMM2-CDG (OMIM 212065) and ALG1-CDG (OMIM 608540)] (see Table 2 in the

### Table 2. Intraassay and interassay precision for each free oligosaccharide species and the IS peak area.

<table>
<thead>
<tr>
<th>Intraassay precision</th>
<th>QC1 (n = 6)</th>
<th>QC2 (n = 6)</th>
<th>Interassay precision</th>
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<th>QC2 (n = 6)</th>
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<tr>
<td></td>
<td>Meana</td>
<td>SDa</td>
<td>%CV</td>
<td>Meana</td>
<td>SDa</td>
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<td>15</td>
<td>3</td>
<td>19</td>
<td>22</td>
<td>5</td>
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<tr>
<td>FOS 2</td>
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<td>2</td>
<td>15</td>
<td>6</td>
<td>1</td>
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<td>FOS 3</td>
<td>1411</td>
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<td>5</td>
<td>357</td>
<td>32</td>
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<td>2101</td>
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<td>8</td>
<td>929</td>
<td>340</td>
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<td>71</td>
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<td>16</td>
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<td>13</td>
<td>3</td>
<td>1</td>
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<td>FOS 7</td>
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<td>IS area</td>
<td>21026b</td>
<td>992</td>
<td>5</td>
<td>24151b</td>
<td>1293</td>
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*FOS response = FOS peak area/IS area × 1000.

### Table 3. Age-specific upper limits (mean + 2 SD) of the reference intervals for each free oligosaccharide.a

<table>
<thead>
<tr>
<th>Age</th>
<th>FOS 1</th>
<th>FOS 2</th>
<th>FOS 3</th>
<th>FOS 4</th>
<th>FOS 5</th>
<th>FOS 6</th>
<th>FOS 7</th>
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<tr>
<td>&lt;1 year (n = 27)</td>
<td>201</td>
<td>28</td>
<td>2902</td>
<td>5888</td>
<td>382</td>
<td>15</td>
<td>64</td>
</tr>
<tr>
<td>1-5 years (n = 35)</td>
<td>151</td>
<td>38</td>
<td>1267</td>
<td>3103</td>
<td>231</td>
<td>9</td>
<td>45</td>
</tr>
<tr>
<td>5-15 years (n = 34)</td>
<td>79</td>
<td>8</td>
<td>775</td>
<td>1757</td>
<td>142</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>&gt;15 years (n = 14)</td>
<td>57</td>
<td>15</td>
<td>415</td>
<td>1024</td>
<td>71</td>
<td>2</td>
<td>17</td>
</tr>
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</table>

* Values are FOS response (FOS peak area/IS area × 1000).
eral novel therapeutic approaches have been described for these conditions. Recently, several novel therapeutic approaches have been described for the management of glycoprotein disorders (18, 19), and others are currently in development. Absolute quantification of the FOS species related to a specific disorder could be achieved with the synthesis of standards and labeled ISs. Given the wide clinical spectrum of some of the disorders and our limited cohort size, analysis of additional samples from affected patients is required to further validate the clinical utility of the method.

A substantial reduction in the FOS response was noted in patients with α-fucosidosis and α-mannosidosis after HSCT, even with the current semiquantitative method. This suggests the assay could be used to monitor treatment efficacy for therapeutic interventions as treatments are developed for these conditions. Recently, several novel therapeutic approaches have been described for the management of glycoprotein disorders (18, 19), and others are currently in development.

References