Sanfilippo disease (mucopolysaccharidosis type III): Early diagnosis and treatment

de Ruijter, J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
FIVE
HEPARAN SULFATE DERIVED DISACCHARIDES IN PLASMA AND TOTAL URINARY EXCRETION OF GLYCOSAMINOGlycANS CORRELATE WITH DISEASE SEVERITY IN SANFILIPPO DISEASE

Jessica de Ruijter¹, Lodewijk IJlst², Wim Kulik², Henk van Lenthe², Tom Wagemans¹², Naomi van Vlies¹², Frits A. Wijburg¹

¹Department of Pediatrics and Amsterdam Lysosome Centre ‘Sphinx’ and ²Lab. Genetic Metabolic Diseases, Department of Clinical Chemistry and Pediatrics, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

ABSTRACT

Background: Sanfilippo disease (Mucopolysaccharidosis III) is a neurodegenerative lysosomal disorder characterized by accumulation of the glycosaminoglycan heparan sulfate (HS). MPS III has a large phenotypic variability and early assessment of disease severity is difficult. We investigated the correlation between disease severity and the plasma concentration of HS (pHS, defined by the sum of the heparan sulfate derived disaccharides obtained after enzymatic digestion) and urinary total GAGs level (uGAGs, measured by the dimethylene blue test) in a cross-sectional cohort of 44 MPS III patients.

Methods: Disease severity was established on the basis of the age of complete loss of independent walking and of full loss of speech in all patients. Hazard ratios (HR) were obtained with cox-regression analysis. In order to allow prediction of a severe phenotype based on a cut-off value for pHS, patients were divided in two groups (severely affected and less severely affected) based on predictive mutations or on the age of full loss of speech. Receiver operator characteristics (ROC) were obtained for pHS.

Results: pHS and uGAGs were independently and linearly associated with an increased risk of speech loss with a HR of 1.8 (95% CI 1.3-2.7) per 500 ng/ml increase of HS in plasma ($p=0.002$), and a HR of 2.7 (95% CI 1.6-4.4) per 10 mg/mmol creatinine increase of uGAGs ($p<0.001$). pHS and uGAGs were less strongly associated with loss of walking. The area under the ROC curve for pHS was 0.85, indicating good discrimination.

Conclusion: pHS and uGAGs may be useful biomarkers for prediction of severity in MPS III.
INTRODUCTION
Sanfilippo disease (Mucopolysaccharidosis III, MPS III) is the most prevalent type of the mucopolysaccharidoses (MPSs) \(^1\)\(^5\), a subgroup of the group of inherited lysosomal storage disorders (LSDs). MPS III is caused by deficient catabolism of the glycosaminoglycan (GAG) heparan sulfate (HS), and is clinically characterized by neurodegeneration, initially with progressive cognitive impairment and behavioural problems, later followed by motor impairment, loss of communication end early demise. Somatic signs and symptoms in MPS III are relatively mild. Four enzymes are specifically involved in the degradation of HS and, depending on the deficient enzyme, four subtypes (A to D) of MPS III can be recognized (OMIM numbers: 253000, 252920, 259230, and 259240 respectively). Type A is the most common subtype in north-west Europe, and type B is the most frequent type in south-east Europe \(^2\)\(^3\)\(^6\). Subtypes C and D are much rarer.

While the pattern of signs and symptoms generally follows the same course, MPS III is a clinically heterogeneous disease in terms of rate of disease progression. Indeed, MPS III patients with a remarkably mild phenotype are increasingly recognized \(^7\)\(^-\)\(^10\).

Based on the genetic defect, the expression of functional enzyme appears to be the crucial determinant for the clinical phenotype \(^11\), and mutation analysis allows prediction of clinical severity in some patients \(^8\)\(^,\)\(^9\)\(^,\)\(^12\). However, more than 200 different mutations have been reported in MPS III patients \(^13\)\(^-\)\(^15\) and, especially in MPS IIIB, many families carry their own private mutations obstructing prediction of the phenotype at an early age.

The age at full loss of speech and at full loss of independent walking have been used as hallmarks to classify patients into different severity categories \(^8\)\(^,\)\(^9\).

In addition, the age of diagnosis was identified as a reliable indicator of disease severity \(^16\)\(^,\)\(^17\).

While no proven disease modifying therapy is yet available for MPS III, a number of potential therapies are currently studied \(^18\)\(^-\)\(^21\). Assessment of treatment efficacy in this slowly progressive disorder with variable phenotypic severity will at least partially depend on the response of biomarkers. In addition, biomarkers in MPS III may help to predict the natural course of the disease, which is again important for assessment of treatment efficacy, but also for counselling of families with MPS III.

In several other MPSs, including MPS I (OMIM numbers: 607014, 607015, 607016), MPS II (OMIM number: 309900) and MPS VI (OMIM number: 2523200), total urinary excretion of GAGs, generally determined by the dimethylene blue assay \(^22\), has been used as biomarker to evaluate the response to enzyme replacement therapy (ERT) \(^23\)\(^-\)\(^25\). However, measuring uGAGs may be inadequate to study therapeutic efficacy in these complex multi-system disorders, because uGAGs may primarily reflect renal involvement rather than neurological, musculoskeletal, cardiac disease or lung function, all significantly related to the quality of life of patients. Promising other biomarkers for MPS I, II and VI are the plasma heparin cofactor II-thrombin complex (HCII-T) and the urine dermatan sulfate:chondroitin sulfate (DS:CS) ratio, as they are significantly increased at diagnosis and show a decrease in response to treatment with ERT \(^26\)\(^,\)\(^27\). However, the DS:CS ratio fully, and the HCII-T concentration largely, depend on elevation of DS, a GAG that is not significantly elevated in patients with MPS III.
Here we investigate the concentrations of the sum of the HS derived disaccharides and the total concentration of uGAGs in a large cohort of MPS III patients, and show that they correlate with disease severity.

MATERIALS AND METHODS

Patients
All patients were diagnosed by appropriate enzymatic studies. Blood samples were obtained from 44 patients (28 male, 16 female) at the time of this study. The median age of patients was 15 years (range 3-67 years). Twenty-four patients had MPS IIIA, 11 MPS IIIB, and 9 MPS IIIC. Thirty (13 MPS IIIA, 9 MPS IIIB and 8 MPS III C) of these 44 patients had participated in a trial on efficacy of genistein in MPS III, and blood and urine samples obtained at baseline were included in this study. The ages at diagnosis, at full loss of the ability to walk several steps independently and at full loss of speech were retrieved for all patients from the parents or medical records when applicable. Results of mutation analysis were available from 42 patients.

Blood sample collection
Blood samples were collected by venipuncture. Collection was in EDTA containing standard tubes and the samples were processed following standard protocols. Plasma was stored at -20 °C until further studies.

Measurement of pHS
Heparan sulfate concentration in plasma was measured as the sum of the seven heparan sulfate derived disaccharides obtained after enzymatic digestion of heparan sulfate by heparinase I, II and III followed by quantitation by HPLC-MS/MS analysis as described previously.

uGAGs measurement
Total GAGs in urine were measured by the DMB test which involves binding of GAGs to the dye dimethylene blue (DMB) and subsequent spectrophotometric analysis of the GAG-DMB complex.

Data analysis
The values of pHS were subtracted by the upper value of the 95% confidence interval (CI) for age of reference values. The transformed data were subsequently categorized into quartiles. Concentrations of uGAGs were transformed by subtracting the highest reference value according their age-group, (≤10 years: 15 mg/mmol creatinine, >10 years: 8 mg/mmol creatinine) and subsequently categorized in quartiles. Patient characteristics (age, gender and MPS subtype) were summarized across these quartiles: median and ranges for continuous non-normal variables, and frequencies and percentages for categorical variables. Differences among patient characteristics across pHS and uGAGs quartiles were evaluated using the Kruskal-Wallis test for continuous non normal variables (age), and χ² test for gender and MPS subtype (Tables 1 and 2).
Survival analysis of full loss of speech and full loss of walking were assessed across the quartiles of pHS and uGAGs by Kaplan–Meier curves and log-rank test.

These variables were utilized as a dichotomized categorical variable (loss versus maintenance of function). Cox proportional hazards modelling were performed for pHS and uGAGs as continuous variable and as quartiles.

In order to allow prediction of the ‘severe phenotype’ based on a cut-off value for pHS and uGAGs, patients were divided in two groups (severely affected and less severely affected) based on a predictive mutation, and when this was not applicable based on the age of full loss of speech (age <12 years). Correlation of pHS and uGAGs was tested with a Pearson test. Receiver operator characteristics (ROC) were obtained for pHS.

The correlation between disease severity (severe, intermediate and attenuated) as predicted by the genotype according to the previously reported genotype-phenotype correlations, and the level of pHS or uGAGs were tested with the Kruskal-Wallis test for all three severity groups and the Mann-Whitney U-test was used as a post-hoc test on each pair of groups. Finally, Mann-Whitney test was performed to detect differences between age of diagnosis (< 6 years and ≥ 6 years) and the level of pHS and uGAGs.

All analyses used two-sided tests with an overall significance level of α=0.05. All data analyses were conducted using SPSS (version 16.0, SPSS Inc., Chicago, IL).

RESULTS

pHS and uGAGS and loss of function

Median HS concentration in plasma of age-matched control subjects (4-52 years) was 118 ng/mL (range 79-480 ng/mL, n=35, Figure 1). The median plasma heparan sulfate concentration in patients was 1103 ng/ml (range 529-2674, Figure 1). The median concentration of total GAGs in urine of patients was 34.6 mg/mmol creatinine (range 11.9-83.9); reference values in our laboratory: 2-10 years 5-15 mg/mmol creatinine, and 10-100 years 1-8 mg/mmol creatinine.

pHS concentration and age at loss of function

We subsequently determined if pHS levels were associated with the decline of speech and motor functions in the 44 patients of whom plasma was available. To this end, pHS levels were divided into quartiles (Q): Q1: <562; Q2: 562-838; Q3: 838-1207; Q4: >1207. Age and MPS III subtype were not evenly distributed among the quartiles. In the lowest two quartiles patients had a higher median age compared to the higher quartiles. MPS III B subtype was also more present in the two lower quartiles (Table 1).

Kaplan-Meier analysis of the data indicated that patients with a lower pHS level had maintained speech significantly longer (Figure 2A and Table 3). pHS levels were significantly associated with the age of full loss of speech (log-rank test p=0.003). The risk for speech loss was assessed by multivariable Cox proportional hazards analysis. With increasing quartiles of pHS, the hazard ratios (HR) for loss of speech were 3.9 (95% CI: 0.8-19.5, p=0.097),...
### Table 1. Baseline characteristics by quartile pHS.

<table>
<thead>
<tr>
<th>Participants (n)</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>Test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>KW=8.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$X^2$= 5.5</td>
<td>0.139</td>
</tr>
<tr>
<td>Male</td>
<td>9 (82)</td>
<td>4 (36)</td>
<td>8 (73)</td>
<td>7 (36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2 (18)</td>
<td>7 (64)</td>
<td>3 (27)</td>
<td>4 (64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS III$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$X^2$= 12.7</td>
<td>0.048</td>
</tr>
<tr>
<td>A</td>
<td>7 (64)</td>
<td>7 (64)</td>
<td>4 (36)</td>
<td>6 (55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4 (36)</td>
<td>4 (36)</td>
<td>2 (18)</td>
<td>1 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>5 (46)</td>
<td>4 (36)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Number of cases (%)

KW= Kruskal-Wallis test, $X^2$=Chi-square statistic

Quartiles (Q): Q1: <562 ng/ml; Q2: 562-838 ng/ml; Q3: 838-1207 ng/ml; Q4: >1207 ng/ml.

5.7 (95% CI: 1.2-27.0, $p=0.03$) and 13.1 (95% CI: 2.6-66.8, $p=0.002$) compared to the lowest quartile of pHS. This indicates a gradual, significant, and independent association between pHS and speech loss. PHS was also independently and linearly associated with an increased risk of speech loss when used as a continuous variable. Adjusted HR was 1.8 (95% CI: 1.3-2.7) per 500 ng/ml increase of HS in plasma ($p=0.002$, Figure 3A).

**pHS concentration and loss of walking**

Kaplan-Meier analysis of the data showed a relation between the age at full loss of walking independently and concentration of pHS, but this was less strong than for speech loss.

**Figure 1.** Heparan sulfate in plasma for controls and MPS III patients. The dashed lines represent the 95% predicted CI of the fitted curve.
(Figure 2B and Table 3). Log-rank test nearly reached statistically significance ($p=0.05$) for loss of walking. The risk for loss of walking was assessed by multivariable Cox proportional hazards analysis. With increasing quartiles of pHs, the hazard ratios for loss of walking were 2.7 (95% CI: 0.5-13.2), 2.0 (95% CI: 0.3-12.0) and 8.8 (95% CI: 1.5-52.8) compared to the lowest quartile of pHs ($p$-values 0.23, 0.46 and 0.02 respectively).

Figure 2. Kaplan-Meier analysis in MPS III patients of (A) age of loss of speech according to quartiles of heparan sulfate in plasma (pHS). (B) age of loss of walking according to quartiles of pHS. (C) age of loss of speech according to quartiles of urinary GAGs (uGAGs). pHS quartiles (Q): Q1: <562; Q2: 563-838; Q3: 838-1207; Q4: >1207 uGAGs quartiles (Q): Q1: <8.3; Q2: 8.3-21.3; Q3: 21.3-34.3; Q4: >34.3.
In contrast to its association with loss of speech, pHS was not linearly associated with an increased risk of loss of walking when used as a continuous variable HR 2.7 \( p=0.14 \) per 500 ng/ml increase of HS in plasma.

**uGAGs concentration and age at loss of function**

We determined if uGAGs levels were associated with the decline of speech and motor functions in the 30 patients of whom urine was available. uGAGs levels were first divided in quartiles: Q1: <8.3; Q2: 8.3-21.3; Q3: 21.3-34.3; Q4: >34.3. Age and MPS III subtype were not evenly distributed among the quartiles. In the lowest two quartiles patients had a higher median age compared to the higher quartiles. MPS III B subtype was more present in the two lower quartiles, and subtypes A and C were more present in the higher quartiles (Table 2).

**uGAGs concentration and loss of speech**

The uGAGs quartiles showed a significant difference when related to the age at which patients lost their ability to talk: univariate analysis, log-rank test \( p<0.001 \), Figure 2C, and Table 3). Cox regression analysis revealed the following HRs: second quartile HR 3.7 (95% CI: 0.3-42.6), third quartile HR 44.3 (95% CI: 2.1-943.3) and fourth quartile HR 224.7 (95% CI: 8.1-6247.2), all compared to the lowest quartile \( p \)-values 0.29, 0.02 and 0.001 respectively). uGAGs were also independently and linearly associated with an increased risk of speech loss when used as a continuous variable. HR was 2.7 (95% CI 1.6-4.4) per 10 mg/mmol creatinine increase of uGAGs \( p=0.001 \), Figure 3B).

**uGAGs concentration and loss of walking**

Log-rank test showed significant differences when comparing the age of loss of walking for the four quartiles \( p=0.04 \). However, no analysis could be done to assess HRs for the quartiles as none of the patients in the lowest quartile had lost their ability to walk at the time of this study.

### Table 2. Baseline characteristics by quartile of uGAGs.

<table>
<thead>
<tr>
<th></th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>Test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (median, range)</td>
<td>28 (22-67)</td>
<td>19 (11-46)</td>
<td>13 (9-18)</td>
<td>9 (5-16)</td>
<td>KW=18.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( \chi^2 = 0.67 )</td>
<td>0.88</td>
</tr>
<tr>
<td>Male</td>
<td>5 (71)</td>
<td>5 (62.5)</td>
<td>6 (75)</td>
<td>4 (57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2 (29)</td>
<td>3 (37.5)</td>
<td>2 (25)</td>
<td>3 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS III*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( \chi^2 = 22.85 )</td>
<td>0.001</td>
</tr>
<tr>
<td>A</td>
<td>0 (0)</td>
<td>5 (62.5)</td>
<td>4 (50)</td>
<td>4 (57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>7 (100)</td>
<td>1 (12.5)</td>
<td>0 (0)</td>
<td>1 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0 (0)</td>
<td>2 (25)</td>
<td>4 (50)</td>
<td>2 (29)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of cases (%)

KW Kruskal-Wallis test, \( \chi^2 \) Chi-square statistic

Quartiles (Q): Q1: <8.3; Q2: 8.3-21.3; Q3: 21.3-34.3; Q4: >34.3.
Table 3. Speech loss and loss of walking by quartile of pHS and uGAGs.

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Median pHS levels (ng/ml) (range)</th>
<th>Number of patients</th>
<th>Median age of patients until loss (years)</th>
<th>Number of patients</th>
<th>Median uGAGs levels (mg/mmol creat) (range)</th>
<th>Number of patients</th>
<th>Median age of patients until loss (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>531 (219-562)</td>
<td>n=11</td>
<td>2</td>
<td>2</td>
<td>6.2 (3.9-8.3)</td>
<td>n=7</td>
<td>1</td>
</tr>
<tr>
<td>Q2</td>
<td>638 (562-838)</td>
<td>n=11</td>
<td>6</td>
<td>16</td>
<td>17.1 (8.3-21.3)</td>
<td>n=8</td>
<td>2</td>
</tr>
<tr>
<td>Q3</td>
<td>1043 (838-1207)</td>
<td>n=11</td>
<td>8</td>
<td>21</td>
<td>27.4 (21.3-34.3)</td>
<td>n=8</td>
<td>3</td>
</tr>
<tr>
<td>Q4</td>
<td>1452 (1207-2434)</td>
<td>n=11</td>
<td>8</td>
<td>12</td>
<td>48.4 (34.3-68.9)</td>
<td>n=7</td>
<td>5</td>
</tr>
</tbody>
</table>

* No median could be computed of less than 50% of patients had loss of function.

Q= quartile.

Correlation between pHS and uGAGs

There was a strong correlation between pHS and uGAGs levels (both corrected for age) using Pearson correlation: R=0.69 p<0.01.

Receiver operation characteristic for pHS

Of the 44 patients of whom plasma was available, 25 patients could be categorized as severe vs. less severe based on genotype and 15 patients based on full loss of speech (severe: <12 years). Four patients could not be included in this analysis because genotype was not available and they were under 12 years of age and had still retained their ability to speak. Ten patients were classified as severe and 30 patients as less severely affected.

The sensitivity for pHS to determine a severe phenotype was 90% with a specificity of 73.3% at a cut-off value of 990 ng/ml (positive predictive value (PPV) 52.9% and negative
predictive value (NPV) 95.7%). The area under the ROC curve for pHS was 0.85 (Figure 4), indicating good discrimination.

Because of too few data, no reliable ROC-characteristics curve could be calculated for uGAGs.

**Figure 4.** ROC-curve of pHS for determination of disease severity.

**Correlating disease severity in MPS IIIA (assessed by genotype) with pHS**

Twenty of the 24 patients with MPS IIIA included in this study had a genotype considered to be predictive of the phenotype. Six patients had a genotype predicting a severe phenotype (homozygous or compound heterozygous for the p.R245H, p.Q380R or c.1080delC mutations). Eight patients had a genotype predicting an intermediate phenotype (compound heterozygous for the p.S298P mutation in combination with either the p.R245H, p.Q380R or c.1080delC mutation). Six patients had a genotype predicting an attenuated phenotype (either homozygous for the p.S298P mutation, or compound heterozygous for the p.S298P in combination with the missense changes (p.T421R or p.P180L), or the missense change p.L12Q in combination with the c.1080delC mutation). Four patients could not be categorized according to their mutation as they had previously unreported mutations in combination with one of the three severe mutations (p.R245H, p.Q380R or c.1080delC).

Kruskal-Wallis analysis showed a significant difference between the levels of plasma HS in the three groups of predictive mutations (H(2) =13.4, p=0.001). The median concentration of HS in the severe group was 1434 ng/ml (range 821-1708), in the intermediate group 780 ng/ml (range 531-1133), and in the attenuated group 521 ng/ml (range 514-623). Mann-Whitney tests were used to follow this up. A Bonferroni correction was applied and all effects are reported at a 0.0167 level of significance. Results show that all three genotypes have significantly different HS levels in plasma: severe vs. intermediate genotype (U=4, r=-0.70), severe vs. attenuated genotype (U=0, r=-0.83) intermediate vs. attenuated genotype (U=5, r=-0.66). All p-values were 0.01 or smaller.
The smaller number of patients with MPS IIIB and IIIC, in combination with the general lack of predictive genotypes, precluded studies on correlating severity, based on genotype, with pHS.

**Correlating disease severity (assessed by genotype) in MPS IIIA with uGAGs**

Twelve of the 13 patients with MPS IIIA could be phenotypically categorized on the basis of a predictive genotype. Three patients were classified as severe (homozygous for R245H mutation or compound heterozygous for R245H and Q380R mutation), three as intermediate (compound heterozygous for the S298P with R245H or Q380R mutation) and six as attenuated (either homozygous for the p.S298P mutation, compound heterozygous for the common mutation p.S298P in combination with the missense changes (p.T421R or p.P180L) or the missense change p.L12Q in combination with the c.1080delC mutation). Median uGAGs concentration in the severe group was 50.7 mg/mmol creatinine (range 48.4-55.1), in the intermediate group 28.1 (range 17.5-28.2) and in the attenuated group 17.9 (range 13.6-27.0). Kruskal-Wallis test showed a significant difference in GAGs concentration in the three groups (H(2) = 7.6, \( p = 0.02 \)). However, Mann-Whitney tests showed no significant results after Bonferroni correction.

The smaller number of patients with MPS IIIB and IIIC, in combination with the general lack of predictive genotypes, precluded studies on correlating severity, based on genotype, with uGAGs.

**Age at diagnosis and pHS and uGAGs**

**pHS**

We compared the levels of pHS between patients who were diagnosed before the age of 6 years (group 1) and patients diagnosed \( \geq 6 \) (group 2). Five of the 44 patients were excluded from this analysis, as the diagnosis was made because of sibling studies in four patients. In one patient the diagnosis was made serendipitously, based only on hepatomegaly detected by chance.

Twenty patients were diagnosed before the age of 6 years (group 1), and 19 patients at a later age (group 2). Median age at diagnosis for the whole group was 5.5 years (range 2-66 years); in group 1: median age 4.5 years (range 2.5-5.5), in group 2: median age 9 years (range 5.5-66). A significant difference (Mann-Whitney test) between the levels of HS was detected (group 1: median = 1088 ng/ml; group 2: median = 617 ng/ml, U=105, \( r=-0.39, p =0.017 \)).

**uGAGs**

Twelve of the 30 patients were diagnosed < 6 years and 13 were diagnosed \( \geq 6 \) years. The Mann-Whitney test showed a significant difference in uGAGs levels between patients diagnosed < 6 years (median 28.0 mmol/mg creatinine) and patients diagnosed \( \geq 6 \) years (median 13.7 mg/mmol creatinine), U=36 , \( r=-0.46, p=0.02 \).
DISCUSSION

The study reported here shows that pHS and uGAGs are significantly related to the severity of the disease in MPS III. pHS and uGAGs levels may be particularly useful for predicting disease severity in patients in whom the genotype is not informative, which is generally the case in MPS III B, C and D [7,9,13-15,30] and in a substantial portion of patients with MPS IIIA [8,13].

In addition, pHS and uGAGs might be instrumental for assessment of efficacy of a potentially disease modifying treatment. The recent observations that the plasma HCII-T complex and the urinary DS:CS ratio, both depending on DS levels, reflect treatment effects in MPS I and II, supports our hypothesis that pHS may correlate with treatment efficacy in MPS III. Indeed, our recent study on genistein treatment in MPS III showed a significant negative slope in pHS as a result of treatment with genistein [28]. However, a disease modifying treatment in MPS III will be primarily targeted to the central nervous system (CNS), as clinical disease in MPS III is predominantly caused by CNS involvement, in contrast to the attenuated phenotypes in MPS I and MPS II. Instead of pHS and uGAGs, HS levels in CSF, if proved to be elevated, may be useful as a biomarker to monitor treatment efficacy in MPS III.

Although the concentration of uGAGs has been used as outcome measure in pivotal trials demonstrating efficacy of ERT in MPS I, II and VI [31-33], uGAGs are generally considered to have limited value due to the non-specific nature of the DMB assay. Furthermore, it is not clear if, and to what extent, uGAGs only reflect storage in the urinary tract, an organ system which is clinically not involved in these disorders. Although early studies using ingestion of radio labeled GAGs did reveal partial clearance of these macromolecules via the kidney [34], later studies showed that urinary excretion of GAGs is independent of the serum concentration of GAGs [35]. Our results show that uGAGs do correlate with disease severity in MPS III. In addition, the observation by Langforth-Smith and coworkers [27] that the ratio of DS:CS in the urine is correlated with long-term treatment effects supports the use of urinary excretion of GAGs as a biomarker.

Patient characteristics between the four quartiles of pHS and uGAGs were not evenly distributed for MPS subtype, and age. The higher age of patients in Q1 and Q2 (lowest two quartiles of pHS and uGAGs) is likely to be due to the longer survival of patients with milder phenotypes. The overrepresentation of MPS IIIB patients in these two quartiles can be explained by the higher proportion of MPS IIIB patients with a milder phenotype compared to MPS IIIA [9,10,12,36].

Our study has several limitations. First, all our studies were done cross-sectional. Therefore, we can only speculate that pHS and uGAGs can be used to predict the severity of the phenotype and thus the course of the disease, at diagnosis. Second, in a previous study in MPS III, we showed that the mean variability of pHS was approximately 6% in plasma and for uGAGs 8% [28]. However in some patients fluctuations as large as 21% for pHS and 30% for uGAGS were observed. These remarkable fluctuations may be due to other factors such as intercurrent infections [37]. Undue reliance on a single measurement of pHS and uGAGs should therefore be avoided. Third, the ROC analysis for pHS is based
HS in plasma and GAGs in urine correlate with disease severity in MPS III on relatively few data. Therefore, the proposed cut-off levels for differentiation between severely affected and less severely affected patients should be used with caution.

In conclusion, both pHS and uGAGs show a remarkably good correlation with disease severity in MPS III. Longitudinal data should be collected to identify the true predictive value of these storage products. In addition, it can be of interest to compare pHS and uGAGs with hair morphology, as this was recently reported as a potential biomarker in MPS III 38.

For assessment of treatment efficacy, the use of HS in CSF, in combination with other potentially relevant CSF biomarkers such as the macrophage inflammatory protein 1 alpha (MIP-1α) and phosphorylated tau (P-tau) 39,40 may prove to be essential.
REFERENCES


type II (Hunter syndrome) with idursulfase: the relevance of clinical trial end points. J Inherit Metab Dis. 2011; 34:749-754.


