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Mucopolysaccharidosis type I (MPS I): Assessment of disease severity, therapeutic options and early diagnosis

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Plasma and urinary levels of dermatan sulfate and heparan sulfate derived disaccharides after long-term enzyme replacement therapy (ERT) in MPS I: correlation with the timing of ERT and with total urinary excretion of glycosaminoglycans

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ABSTRACT

Introduction Mucopolysaccharidosis type I (MPS I) results in a defective breakdown of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate, which leads to a progressive disease. Enzyme replacement therapy (ERT) results in clearance of these GAGs from a range of tissues and can significantly ameliorate several symptoms. The biochemical efficacy of ERT is generally assessed by the determination of the total urinary excretion of GAGs. However, this has limitations. We studied the concentrations of heparan sulfate and dermatan sulfate derived disaccharides (HS and DS, respectively) in the plasma and urine of seven patients and compared these levels with total urinary GAGs (uGAGs) levels.

Methods Plasma and urine samples were collected at different time points relative to the weekly ERT for three non-consecutive weeks in seven MPS I patients who had been treated with ERT for at least 2.5 years. Heparan and dermatan sulfate in plasma and urine were enzymatically digested into disaccharides, and HS and DS levels were determined by HPLC-MS/MS analysis. uGAGs were measured by the DMB test.

Results The levels of HS and DS were markedly decreased compared with the levels before the initiation of ERT. However, the concentrations of DS in plasma and of both HS and DS in urine remained significantly elevated in all studied patients, while in six patients the level of total uGAGs had normalized. The concentrations of plasma and urinary HS during the weekly ERT followed a U-shaped curve. However, the effect size is small. The concentrations of plasma and urinary DS and uGAGs appeared to be in a steady state.

Conclusions HS and DS are sensitive biomarkers for monitoring the biochemical treatment efficacy of ERT and remain elevated despite long-term treatment. This finding may be related to the labeled dose or antibody status of the patient. The timing of the sample collection is not relevant, at least at the current dose of 100 IU/kg/weekly.

INTRODUCTION

Mucopolysaccharidosis type I (MPS I; OMIM 252800) is an autosomal recessive lysosomal storage disorder caused by a deficiency of the lysosomal hydrolase α -L-iduronidase (IDUA, EC 3.2.1.76). IDUA catalyzes an essential step in the degradation of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate. Clinical signs and symptoms result from continued and widespread accumulation these GAGs throughout the body, leading to organ dysfunction and progressive multisystem disease. The phenotypic spectrum of MPS I ranges from the more attenuated phenotypes, Hurler-Scheie and Scheie syndrome (MPS I-H/S and MPS I-S), which are predominantly characterized by progressive musculoskeletal, pulmonary and cardiac disease, to the severe Hurler syndrome (MPS I-H), with progressive central nervous system (CNS) disease in addition to the somatic manifestations [1,2].

The inability to catabolize heparan sulfate and dermatan sulfate leads to the excretion of these molecules in the urine, and a total urinary GAGs (uGAGs) analysis is usually the first step in the diagnostic process. Determination of uGAGs is generally performed by a quantitative assay with dimethylene blue (DMB) [3]. A definite diagnosis requires the determination of a deficiency of IDUA activity and subsequent mutation analysis [1].

Enzyme replacement therapy (ERT) with recombinant IDUA (laronidase, Aldurazyme[®]) is approved for the treatment of the non-neurological manifestations of MPS I. Weekly infusions with laronidase result in the clearance of stored GAGs from a range of tissues in MPS I patients [4-9], and is currently the treatment of choice in patients with MPS I-H/S and MPS I-S [10]. uGAGs was used as an endpoint in the pivotal trials demonstrating the clinical efficacy of laronidase treatment [4-9]. A rapid decline within the first few weeks to months of treatment, with a near normalization in the majority of patients, is generally observed. There are, however, several limitations to the use of uGAGs as a biomarker for therapeutic efficacy. First, the DMB test detects all GAGs in the urine including keratan sulfate and chondroitin sulfate in addition to heparan and dermatan sulfate. Recent studies have shown that the urinary dermatan sulfate:chondroitin sulfate ratio may be a more suitable biomarker for MPS I [11]. Second, uGAGs may reflect renal storage rather than the total body burden of GAG accumulation. Blood-derived biomarkers may therefore be more suitable. The serum heparin cofactor II–thrombin (HCII-T) complex, the formation of which largely depends on the concentration of dermatan sulfate, and to a lesser extent heparan sulfate, is a promising biomarker [12-15] and may reflect short-term treatment outcome [16,17]. Recent reports suggest that heparan sulfate and dermatan sulfate derived disaccharides in plasma may also be useful biomarkers in MPS I [18,19]. Another issue is that the timing of the urine collection or blood sampling for the determination of biomarkers relative to the timing of ERT is generally not specified, and whether the levels of biomarkers are influenced by the time between an ERT infusion and sample collection has not been addressed in detail.

Here, we study the concentrations of heparan sulfate and dermatan sulfate derived disaccharides (HS and DS, respectively) in both urine and plasma and total uGAGs at different time points relative to the infusion of laronidase in patients who have been treated with laronidase for more than 2 years. We show that plasma and urinary HS and DS are significantly more sensitive biomarkers than uGAGs and that the timing of sample collection with respect to timing of the infusion of the recombinant enzyme is not relevant at the current dose of 100 IU/kg once weekly.

MATERIALS AND METHODS

Ethics board approval

This project was approved by the Ethics Committee of the AMC, Amsterdam (#10/253). Written informed consent was obtained from all parents and all patients above the age of 12.

Patients

Patients from all three phenotypic groups were approached. Eight patients and/or families were invited to participate. One patient chose not to participate. The remaining seven patients were included. All patients (1 MPS I-H, 2 MPS I-H/S, 4 MPS I-S) had a confirmed diagnosis of MPS I as documented by enzymatic and genetic analyses and had received laronidase for a minimum of 2 years and 7 months at a dose of 100 IU/kg/week (Table 1). The clinical course after the initiation of ERT was considered stable in all patients by the treating physician (FAW), except in patient 6 (Hurler phenotype) who showed a slowly progressive cognitive decline.

Table 1. Patient characteristics

	Gender	Phenotype	Genotype	Age (y)	Time on ERT	Port-a-Cath	Antibody titer
Patient 1	F	MPS I-S	R388H/W402X	15	6 y 4 m	N	1:128
Patient 2	M	MPS I-S	Q70X/R383H	37	2 y 9 m	N	1:32
Patient 3	F	MPS I-S	Q70X/R383H	34	2 y 9 m	N	Negative
Patient 4	F	MPS I-H/S	P533R/P533R	6	2 y 8 m	Y	1:64
Patient 5	F	MPS I-H/S	P533R/P533R	6	2 y 8 m	Y	1:512
Patient 6	M	MPS I-H	W402X/W402X	13	7 y 0 m	Y	1:1024
Patient 7	F	MPS I-S	474-2A>G/R383H	17	2 y 7 m	Y	Negative

Control plasma and urine samples

Control plasma samples were obtained for measurement of plasma HS and DS (n=35; age range: 0.08-52.6 years), and control urinary samples for measurement of HS and DS (n=63; age range: 0.01-54.7 years), and total uGAGs (n=97; age range: 0.01-77.9 years).

Baseline samples

Baseline EDTA plasma, collected prior to the initiation of ERT, was available from all patients except patient 6. Baseline urine samples were available from all patients. Samples had been stored at -80 °C until analysis.

Sample collection

Samples were collected during three non-consecutive weeks (Figure 1). Blood was obtained immediately prior to infusion and on days three and five of each study week. First morning voids of patients 1, 2, 3, 4, 5 and 7 were collected daily during the three study weeks. The urine samples of patient 6 were not first-morning-void samples. Three patients had a Port-a-Cath (PAC), which was used for all blood collections. In all other patients, blood samples were collected by vein puncture. All samples were collected at the site of the weekly ERT or at home. The urine was immediately stored at -15 to -20°C. Blood samples were transported on the day of collection and stored at 4°C until processing. All blood samples were processed within 48 hours after collection.

Blood	X		X		X		
Urine	X	X	X	X	X	X	X
Weekdays	1	2	3	4	5	6	7




Figure 1. Study protocol (repeated for three non-consecutive weeks)

Sample processing

Blood was collected in 5 ml serum tubes and 4.5 ml EDTA tubes. Blood samples were centrifuged for 10 minutes at 4°C at 1,600 × g. Serum and plasma samples were stored at -20 °C until analysis.

HS and DS assays

HS and DS were determined according to the methods initially reported by Oguma and co-workers [20] with minor modifications.

Expression of chondroitinase B and heparinases I, II and III

Chondroitinase B from Pedobacter heparinus

(Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) was selected as it is the only GAG lyase known to specifically digest dermatan sulfate without activity towards chondroitin sulfate [21]. We cloned chondroitinase B from *Pedobacter heparinus* into pET19b (Novagen-EMD4 Biosciences, Madison, WI, USA). Expression

plasmids (pET15b or pET19b) containing the coding sequence of the mature heparinases were a generous gift from Dr. Ding Xu (University of California, CA, USA).

All enzymes were expressed as His-tagged fusion proteins in *E. coli* (BL21 AI, Invitrogen) in Terrific Broth medium with 8 g/L glycerol at 22°C. The enzymes were purified on HisLink Protein Purification Resin (Promega) according to the manufacturer's protocol. The purified enzymes were dialyzed against a buffer containing 50 mM Tris (pH 7.5), 10 mM CaCl₂, 200 mM NaCl and 2 mM DTT. Thereafter, 126 g/L glycerol and 2 mg/mL BSA were added, and aliquots were snap-frozen in liquid nitrogen and subsequently stored at -80°C.

Before each experiment, the activity of the enzymes was tested. Heparinase I and II activity was measured at 30°C in an incubation medium (1 mL final volume) containing 25 mM HEPES (pH 7.0), 100 mM NaCl, 1 mM CaCl₂, 2 mM DTT and 3-4 mIU enzyme. The reaction was started by the addition of 0.2 mg/mL heparin, and the introduction of the double bond was followed over time by monitoring the absorbance at 232 nm. The activity was calculated using an extinction coefficient of 5,200 L·mol⁻¹·cm⁻¹. The activity of heparinase III and chondroitinase B were measured essentially as described above using de-O-sulfated heparan sulfate or dermatan sulfate as substrates, respectively.

Plasma and urinary HS and DS analysis

The relative abundances of D0A0, D0S0, D0A6+D2A0 and D0S6+D2S0, following the nomenclature of Lawrence et al. [22], in the heparan sulfate standard (Sigma-Aldrich) were 39.5, 26.3, 11.1 and 23.1%, respectively, as determined by HPLC-MS/MS analysis. Using these relative abundances, we calculated the average MW of the heparan sulfate standard as 425.48 Da. The relative abundances of D0a4 and D0a10 [22] in the dermatan sulfate standard (Sigma-Aldrich) were 94 and 6%, respectively, as determined by HPLC-MS/MS analysis. The average MW of the dermatan sulfate standard was calculated as 464.18 Da. These average MWs were used to calculate the exact concentration of digestible heparan sulfate and dermatan sulfate. The maximum enzymatic digestion of the heparan and dermatan sulfate standards (as used to spike a control sample and to calculate analyte recovery) was determined by monitoring the complete digestion at 232 nm (extinction coefficient of 5200 L·mol⁻¹·cm⁻¹) using excesses of heparinase I, II and III and chondroitinase B, respectively. We found that the digestion of these GAGs was incomplete but reproducible; typically, 44% of the heparan and dermatan sulfate standards were digested. This is likely explained by the fact that certain domains within heparan sulfate and dermatan sulfate and/or the shortened products (e.g., tetra- and hexamers) may be poor substrates for the heparinases and chondroitinase B, respectively.

Plasma and urine heparan sulfate and dermatan sulfate were enzymatically digested into disaccharides in a mixture containing 100 mM NH₄Ac (pH 7.0), 10 mM Ca(Ac)₂, 2 mM DTT, 5 mIU each of heparinase I, II, III, 50 mIU chondroitinase B and 50 µL EDTA plasma or 50 µL urine diluted to 0.2 mM creatinine in a final volume of 150 µL. After 2 hours of incubation at 30°C, 15 µL of 150 mM EDTA (pH 7.0) was added along with

125 ng of the internal standard, 4UA-2S-GlcNCOEt-6S (HD009, Iduron, Manchester, UK), and the reaction was stopped by boiling for 5 min to denature the proteins. The reaction mixture was centrifuged at $20,000 \times g$ for 5 min at room temperature. The supernatant was subsequently applied to an Amicon Ultra 30K centrifugal filter (Millipore) and centrifuged at $14,000 \times g$ for 15 min at 25°C. The filtrate was stored at -20°C until analysis. The disaccharides were quantified on a Waters Quattro Premier XE (tandem) mass spectrometer (Waters Corporation, Milford, MA, USA) coupled to an Acquity UPLC system (UPLC-MS/MS). The disaccharides were separated on a Thermo Hypercarb HPLC column (100 × 2.1 mm, 5 μm). The mobile phase consisted of 10 mM NH_4HCO_3 (pH 10), and the disaccharides were eluted with an acetonitrile gradient of 0% to 20% for 2.5 min, held at 20% for the next 2.5 min, with 2 min of equilibration at 0% before the next injection; the flow rate was 0.2 mL/min, and the total run time was 7.1 min. All disaccharides were detected and quantified in the MRM acquisition mode, using the transitions m/z 378.1>175.1 for D0A0, 416.1>138.0 for D0S0, 458.1>97.0 for D0A6 and D2A0, 496.0>416.0 for D2S0 and D0S6, 458.0>299.9 for D0a4, 538.0>458.0 for D0a10 and 472.0>97.0 for the 4UA-2S-GlcNCOEt-6S internal standard. All samples were digested and analyzed in triplicate. Furthermore, in each experiment, a control sample was spiked with the heparan and dermatan sulfate standards (2 μg/mL each) to calculate the recovery after enzymatic digestion, which was typically $43 \pm 2\%$, in good agreement with the maximal enzymatic digestion determined spectrophotometrically (44%). The concentrations of D0A0, D0S0, D0A6+D2A0, D0S6+D2S0 and D0a4 and D0a10 were calculated using a calibration curve for each of the disaccharides with 4UA-2S-GlcNCOEt-6S as an internal standard.

The total DS in the plasma and urine samples was calculated as the sum of D0a4 and D0a10. The total HS in the urine was calculated as the sum of D0A0, D0S0, D0A6+D2A0 and D0S6+D2S0. For the assessment of HS in the plasma, D0A0 was used as a marker because samples that were collected via PAC were contaminated with exogenous heparin. Heparin consists of highly sulfated disaccharides, mostly D2S6, and contains very little D0A0. In contrast, D2S6 is undetectable in endogenous heparan sulfate present in plasma or urine. Therefore, we determined the ratio of D2S6 to D0A0 in heparin (100:6.96) and used this ratio and the quantity of D2S6 in each sample obtained via PAC to correct the D0A0 value for exogenous heparin. In our samples, this correction was less than 5% of the total D0A0.

Measurement of total urinary GAGs

Total uGAGs were measured by the dimethylene blue (DMB) test, which involves binding of GAGs to the dye DMB followed by a spectrophotometric analysis of the GAG-DMB complex [3].

Antibodies to laronidase

IDUA-reactive antibodies were measured in the serum collected prior to infusion by IgG ELISA and confirmed by western blot analysis as described previously [23].

Data analysis

To examine the longitudinal changes in the HS and DS levels in plasma and urine and in the total uGAGs within the supposed steady state, we first constructed individual longitudinal plots of these measurements. Then, for each outcome (i.e., HS and DS in plasma and urine and total uGAGs), we fitted mixed effect models allowing for both a U-shaped trend and a constant trend and tested which of the two models better fitted the data at hand for each outcome in the following manner. We fitted mixed effects models for each outcome, including a random intercept for each patient and a fixed effect of phenotype based only on a constant trend. After examining the residuals for each of these models, we then chose for each outcome measure a) no transformation, b) a square root transformation, or c) a natural log transformation of the outcome, allowing for possible heteroscedasticity. After choosing a suitable transformation, these constant trend models were updated. Using the outcome transformation chosen in the first step, we then fitted the mixed effects models allowing for a U-shaped trend over time, including a fixed effect of a second-degree polynomial fitted on the day of the week during the steady state (allowing for a U-shape) in addition to the terms already in the constant trend models. The fits of the constant trend and U-shaped trend were then compared using a standard likelihood-ratio test, choosing the best fitting model for each outcome. Due to the limited amount of data, we did not use a more complex polynomial and did not include an interaction between the phenotype and day of the week during the steady state.

From the selected best-fitting models for each outcome, we calculated the individual predictions, which were added to the longitudinal plots. For the U-shaped trend models, we also calculated Cohen's *d* as a measure for effect size based on the maximum difference in the curves with the day of the week compared with the predicted level at day 1 and relative to the standard deviation of the measurements on that day.

We compared the concentrations of HS and DS in plasma and urine and total uGAGs in the MPS I patients with the controls in the following manner. We regressed each marker on age in the controls, using asymptotic regression models including an intercept, asymptotic level and rate of decline with age. From these models we calculated 95% prediction intervals, and defined the "upper limit" of normal for each marker as the upper limit of the 95% prediction interval. Subsequently, we compared measurements in each MPS I patient with the upper limit of normal, for each marker, relative to the age of the patient.

The analyses were performed using the statistical package R, version 2.14.2.

RESULTS

Plasma and urinary HS and DS levels and total uGAGs in the controls

The concentrations of HS and DS in plasma and urine and total uGAGs in controls decrease significantly during the first years of life and stabilize before 20 years of age (Figures 2a-e). The upper limit of the 95% prediction interval was considered to be the upper limit of normal.

Plasma and urinary HS and DS levels and total uGAGs in the patients at baseline (prior to the initiation of ERT)

Data at baseline are listed in Table 2.

Table 2. Plasma and urinary HS and DS levels and total uGAGs at baseline (prior to the initiation of ERT)

Patient	HS _p (ng/ml)	DS _p (ng/ml)	uHS (µg/mmol creatinine)	uDS (µg/mmol creatinine)	Total uGAGs (mg/mmol creatinine)
1	475	1029	9977	13925	69
2	352	631	5197	7094	25
3	383	974	10257	13560	51
4	546	1321	16401	19329	87
5	648	1342	16808	18975	89
6	N.A.	N.A.	27887	27754	98
7	422	997	6947	8533	50

HS_p = HS plasma*, DS_p = DS plasma**, uHS = urinary HS***, uDS = urinary DS**, Total uGAGs = total urinary GAGs as measured by the DMB test, * = represented by the disaccharide D0A0, ** = represented by the sum of the disaccharides D0a4 and D0a10, *** = represented by the sum of the disaccharides D0A0, D0A6 + D2A0, D2S0 and D0S6 + D2S0. N.A. = not available

Plasma and urinary HS and DS levels and total uGAGs in the patients at t=0 (prior to the infusion of ERT)

To allow comparison with controls, we plotted the results in relation to age (Figures 2a-e). The mean plasma HS levels (represented by D0A0) at t=0 were elevated in two of the seven patients (1.2- to 1.5-fold higher than the upper limit of normal) compared with controls (Figure 2a). The mean plasma DS levels (represented by the sum of D0a4 and D0a10) at t=0 were elevated in six of the seven patients (1.1- to 3.3-fold higher than the upper limit of normal) compared with controls (Figure 2b).

The mean urinary HS levels (represented by the sum of D0A0, D0S0, D0A6+D2A0 and D0S6+D2S0) at t=0 were elevated in all patients (2.0- to 7.3-fold higher than the upper limit of normal) compared with controls (Figure 2c). The mean urinary DS levels (represented by the sum of D0a4 and D0a10) at t=0 were elevated in all patients (2.7- to

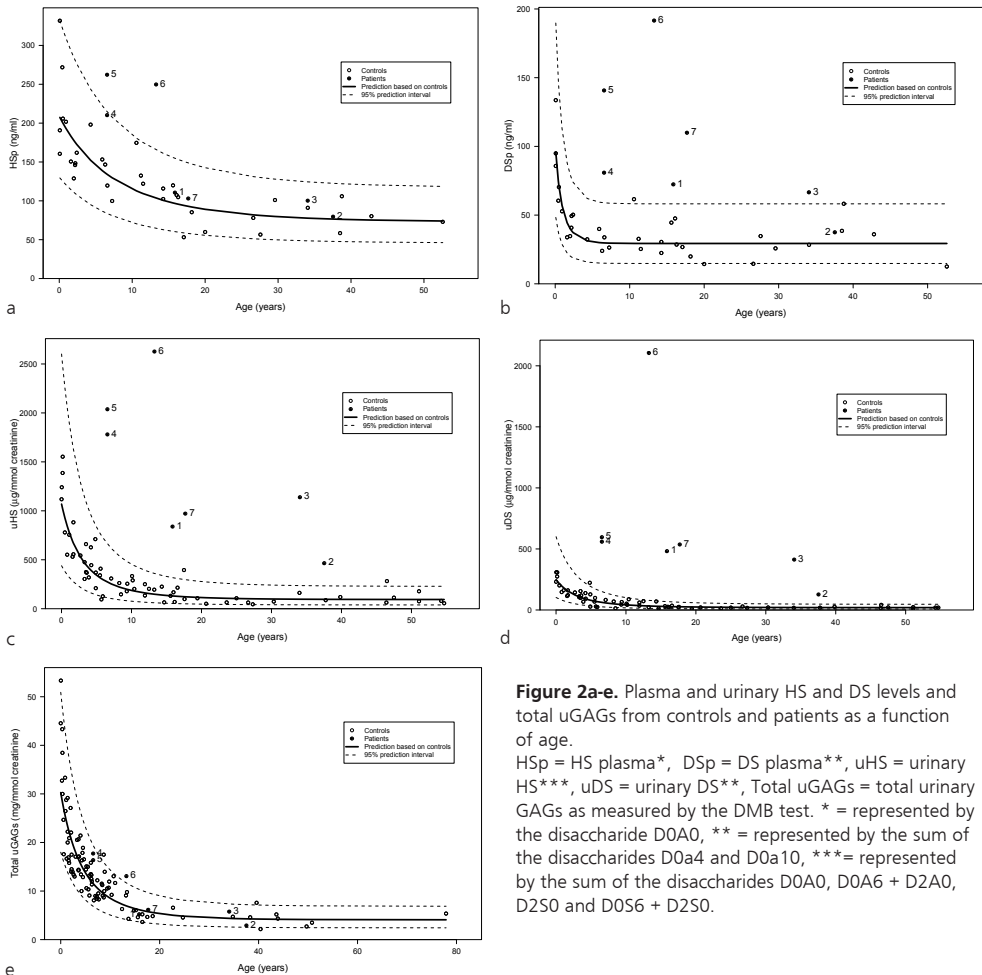


Figure 2a-e. Plasma and urinary HS and DS levels and total uGAGs from controls and patients as a function of age. HS_p = HS plasma*, DS_p = DS plasma**, uHS = urinary HS***, uDS = urinary DS**, Total uGAGs = total urinary GAGs as measured by the DMB test. * = represented by the disaccharide D0a0, ** = represented by the sum of the disaccharides D0a4 and D0a10, *** = represented by the sum of the disaccharides D0A0, D0A6 + D2A0, D250 and D056 + D250.

26.7-fold higher than the upper limit of normal) compared with controls (Figure 2d). Six of the seven patients had total uGAG levels at t=0 within the normal range. The uGAG concentration in the remaining patient was mildly increased (1.1-fold higher than the upper limit of normal; Figure 2e).

Plasma and urinary HS and DS and total uGAGs during the week following ERT

The results relative to the day of the laronidase infusion are presented in Figure 3. The plasma and urinary HS levels are statistically best described by a U-shaped curve following the administration of ERT but with a small to medium effect size (Cohen's $d < -0.28$ and -0.39 , respectively). The plasma and urinary DS levels and total uGAGs are best described by a flat curve relative to the day of the laronidase administration.

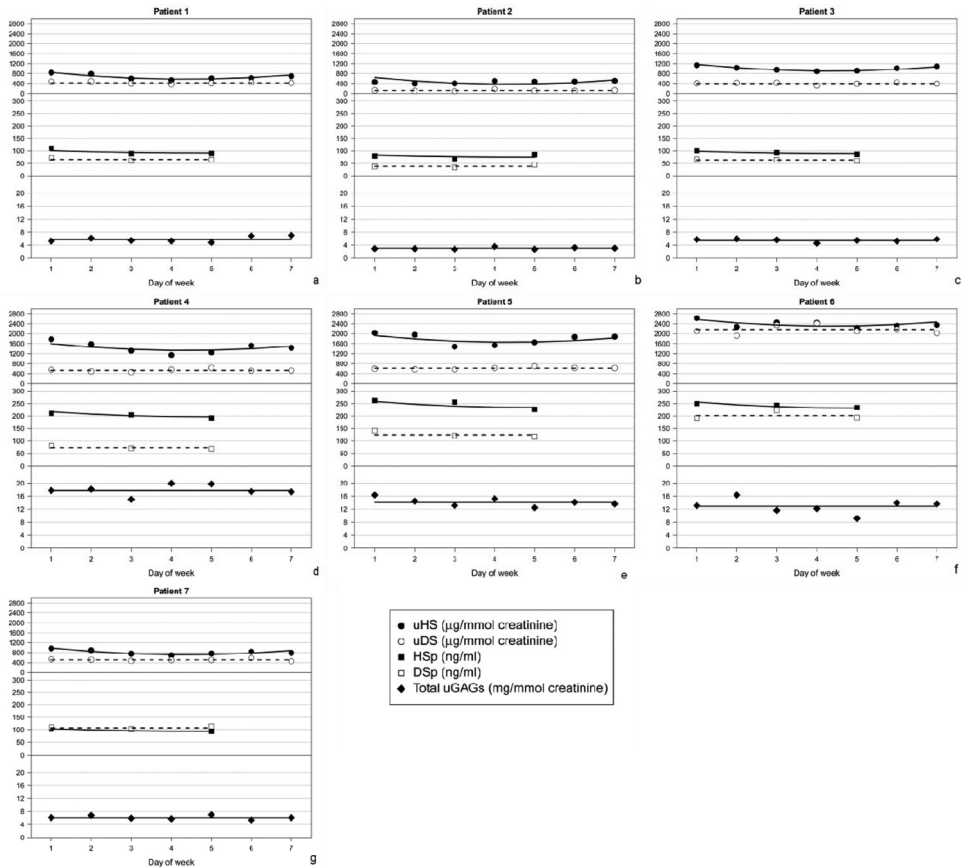


Figure 3a-g. Plasma and urinary HS and DS levels and total uGAGs relative to the day of infusion of laronidase (day 1) in different patients.

HSp = HS plasma*, DSp = DS plasma**, uHS = urinary HS***, uDS = urinary DS**, Total uGAGs = total urinary GAGs as measured by the DMB test, * = represented by the disaccharide D0A0, ** = represented by the sum of the disaccharides D0A4 and D0A10, *** = represented by the sum of the disaccharides D0A0, D0A6 + D2A0, D2S0 and D0S6 + D2S0.

Patient antibody status

Five patients tested positive for IgG antibodies against laronidase (Table 1). The remaining two patients were seronegative.

DISCUSSION

Our results show that despite a very strong decrease after the initiation of ERT, the concentrations of HS and DS in urine and DS in plasma were still significantly elevated in MPS I patients on long-term ERT. In contrast, the total uGAGs excretion normalized in the majority of these patients. This is likely due to the non-specific nature of the DMB assay,

which also detects the relatively abundantly excreted chondroitin sulfate, which may easily obscure increased concentrations of heparan sulfate and dermatan sulfate.

The origin of the mucopolysaccharides excreted in urine is not well known and blood may be a more appropriate compartment to investigate for potential biomarkers in MPS I. We chose to use an HPLC-MS/MS assay for the detection of HS and DS. This method may provide additional information when compared to the HCII-T complex assay, as our assay also detects HS. In addition, our method allows for reliable quantization of both HS and DS in case of contamination with traces of exogenous heparin after sampling via a PAC. Finally, HS and DS are stable in whole blood for at least 48 hours at 4°C (data not shown), whereas the level of HCII-T declines rapidly unless careful and direct processing is ensured [13].

We also investigated whether the timing of blood and urine sampling relative to the infusion of the recombinant enzyme is relevant. Our data suggest that the concentrations of plasma and urinary DS and uGAGs are in a steady state after long-term ERT at the labeled dose of 100 IU/kg weekly. The concentrations of HS in plasma and urine during the week following ERT are best described by U-shaped curves. However, the effect size is small to medium and, in our opinion, not clinically relevant. The sampling of the studied potential biomarkers can therefore be performed at time points irrespective of the timing of laronidase infusion, at least at the current labeled dose and dosing frequency.

The lack of a full biochemical response to ERT, with clear intra-individual differences, may be related to the generation of antibodies against the infused enzyme. The incidence of an immune response to laronidase in MPS I patients is estimated to be 91% [24], and in a prospective study a suboptimal response of uGAGs was found in patients with high antibody titers (> 1:10,000) [6]. In accordance, in our study patients 5 and 6 with the highest antibody titers had the highest residual HS and DS concentrations. However, patients 3 and 7 were seronegative but still had increased concentrations of DS in plasma. IgG antibody titres alone may not be predictive of functional antibody inhibition, thus it is very difficult to make conclusions about antibody to biomarker correlations in this cohort. Indeed, a recent study showed that antibodies may inhibit enzyme activity even at low titers, and may also inhibit cellular uptake of the infused enzyme [23]. We conclude that factors other than antibody titres, e.g., enzyme dose, are involved in the observed imperfect biochemical response to the treatment. A new dose-optimization study, using HS and DS in plasma and urine in combination with other biomarkers such as the HCII-T complex and the urinary dermatan sulfate:chondroitin sulfate ratio, may determine whether higher doses of laronidase will result in the normalization of biomarkers in seronegative patients.

The discrepancy between the normalization of HS in plasma in the majority of patients, except those with the more severe phenotypes, and the lack of normalization of DS in plasma in all but one patient is remarkable and not understood. In addition, HS is significantly elevated in the urine of all patients, suggesting that urinary HS excretion is not directly related to plasma HS levels.

A limitation of our study is that we did not correlate the decrease in the concentration of biomarkers or the antibody status with the clinical response to the ERT. The small number of patients, the broad phenotypic spectrum and the pleomorphic signs and symptoms precluded any detailed analysis. The young age in patients 4 and 5 and a significant cognitive impairment in patient 6 made reliable assessment of key clinical outcome measures, such as lung function or a six-minute walk test not feasible. In addition, patient 3 was wheelchair bound as a result of compression of the cervical spinal cord before the start of ERT. Nevertheless, the clinical course of the disease after the start of ERT was judged as stable by the treating physician in all patients and therefore no obvious correlation between biomarker response or antibody status with the extent of the clinical response could be detected. Larger-scale studies are needed to determine whether such correlations exist.

In conclusion, we show that HS and DS are sensitive biomarkers for monitoring biochemical treatment efficacy in MPS I and do not normalize in all patients treated with ERT for more than 2.5 yrs. This lack of full efficacy may be related to the labeled dose and, in some patients, to the generation of antibodies against the recombinant enzyme. Finally, the timing of the collection of plasma and urine with respect to the timing of the ERT is not relevant, at least at the current dose of 100 IU/kg once weekly.

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