Effect of Anti-Iduronate 2-Sulfatase Antibodies in Patients with Mucopolysaccharidosis Type II Treated with Enzyme Replacement Therapy

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Objective To assess the relationship between anti-Iduronate 2-sulfatase (IDS) antibodies, *IDS* genotypes, phenotypes and their impact in patients with enzyme replacement therapy (ERT)-treated Mucopolysaccharidosis type II.

Study design Dutch patients treated with ERT were analyzed in this observational cohort study. Antibody titers were determined by enzyme-linked immunosorbent assay. Neutralizing effects were measured in fibroblasts. Pharmacokinetic analysis of ERT was combined with immunoprecipitation. Urinary glycosaminoglycans were measured using mass spectrometry and dimethylmethylene blue.

Results Eight of 17 patients (47%) developed anti-IDS antibodies. Three patients with the severe, neuronopathic phenotype, two of whom did not express IDS protein, showed sustained antibodies for up to 10 years of ERT. Titers of 1:5120 or greater inhibited cellular IDS uptake and/or intracellular activity in vitro. In 1 patient who was neuronopathic with a titer of 1:20 480, pharmacokinetic analysis showed that all plasma recombinant IDS was antibody bound. This finding was not the case in 2 patients who were not neuronopathic with a titer of 1:1280 or less. Patients with sustained antibody titers showed increased urinary glycosaminoglycan levels compared with patients with nonsustained or no-low titers.

Conclusions Patients with the neuronopathic form and lack of IDS protein expression were most at risk to develop sustained anti-IDS antibody titers, which inhibited IDS uptake and/or activity in vitro, and the efficacy of ERT in patients by lowering urinary glycosaminoglycan levels. *(J Pediatr 2022;248:100-7)*.

Mucopolysaccharidosis type II (Hunter disease; MPS II, OMIM #309900), a progressive X-linked lysosomal storage disorder characterized by impaired lysosomal degradation of the glycosaminoglycans (GAG) heparan and dermatan sulfate, is caused by variants in the *Iduronate 2-sulfatase* (*IDS*) gene that encodes IDS enzyme. Two phenotypes are distinguished: (1) Patients with the neuronopathic form of MPS II develop severe mental disability during childhood, show a gradual decline in motor performance, and generally die before the age of 20 years if left untreated,[1] (2) Patients with the non-neuronopathic form show a slower progression of the disease, a close to normal mental development, and generally survive into adulthood.[1,2] Enzyme replacement therapy (ERT) with recombinant idursulfase (Elaprase, Shire Human Genetic Therapies), registered for patients with MPS II since 2007, has shown positive effects on overall survival, growth, physical and respiratory function, and reduction of GAG levels in the spleen, liver, heart, and urine.[3-8] The clinical effects may vary considerably among patients because ERT cannot pass the blood-brain barrier and brain disease will progress over time.[9] Blood-brain barrier-penetrating ERT, intrathecal and intracerebroventricular ERT are approved and under development.[10-14] Another factor in treatment variability is the formation of antibodies against the recombinant enzyme. Muenzer et al highlighted a possible association between the presence of antibodies and a hampered reduction of urinary GAG (uGAG) levels.[15] Multiple subsequent studies have reported on the presence of antibodies in approximately 50% of patients undergoing ERT treatment for MPS II, but no significant effect of antibodies on outcome measures could be found at a group level owing to large variations between patients.[15-18] Open-label trials revealed the importance of monitoring antibodies in patients with MPS II treated with ERT.[15-17] The level of the antibody peak titers and the neutralizing effect and sustained presence of antibodies are important to take into account to judge the effect of antibodies on outcome variables of ERT as

CRIM	Cross-reactive immunologic material	IAR	Infusion-associated reaction
DMB	Dimethylmethylene blue	IDS	*Iduronate 2-sulfatase*
DS	Dermatan sulphate	MPS II	Mucopolysaccharidosis type II
ERT	Enzyme replacement therapy	PBS	Phosphate-buffered saline
GAG	Glycosaminoglycan	uGAG	Urinary glycosaminoglycan
HS	Heparan sulphate	uDS	Urinary dermatan sulfate
		uHS	Urinary heparan sulfate

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A.v.d.P. had no conflict of interests concerning any aspect of the current study. However, she gives advice to several pharmaceutical companies about the implementation and development of innovative therapies, mainly for Pompe disease, but also for other LSDs and neuromuscular disorders. Furthermore, she received funds for research via agreements between Erasmus MC and pharmaceutical companies. She also advices public or private charities, who aim to improve the care for patients with metabolic diseases. The other authors declare no conflicts of interest.

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neutralizing antibodies significantly hampered the reduction in liver size and uGAG levels.[5,19-21]

IDS disease-associated variants are generally good predictors of the neuronopathic phenotype.[22] A distinction can be made between variants that disrupt IDS protein expression and variants that allow IDS protein to be expressed although with impaired enzymatic activity. The first class of variants classify as cross-reactive immunologic material (CRIM) negative, the latter as CRIM positive. CRIM negative *IDS* variants are predicted to elicit higher anti-recombinant IDS antibody titers because endogenous IDS protein is not produced and recombinant IDS applied during ERT is foreign to the patient.[23,24] In the present study, the relationships between predicted CRIM status; the level, duration, and neutralizing effects of anti-IDS antibodies; and the effects on uGAG levels have been analyzed in the Dutch MPS II patient cohort with an ERT treatment duration of up to 10 years.

Methods

The Center for Lysosomal and Metabolic Diseases at the Erasmus University Medical Center in Rotterdam is the single treatment center for patients with MPS II in the Netherlands. All Dutch patients with MPS II treated with ERT since market approval of idursulfase and until study closure were included. Diagnosis was based on a combination of clinical findings (IDS deficiency in leukocytes or fibroblasts) and a confirmed *IDS* disease-associated gene variant as previously reported.[22] Patients with a total *IDS* deletion, a nonsense and/or a frame shift variant were predicted to be CRIM negative, and patients with a missense or a small in-frame deletion were predicted to be CRIM positive. Patients received 0.5 mg/kg recombinant human IDS (idursulfase) by intravenous infusion once a week. Infusion was, as recommended by the manufacturer, started at 4 mL/h, and gradually increased to an infusion rate of 40 mL/h (**Table I**; available at www.jpeds.com). During the first 6 months of ERT treatment, antihistamine drugs (clemastine 0.025 mg/kg) were administered before every infusion. This study is part of an ongoing, single-center, prospective, open-label study approved by the ethical committee of the Erasmus Medical Center. All patients signed informed consent. Inclusion criteria for this study were administration of idursulfase in the Netherlands for at least 1 year at October 1, 2016, and the availability of at least 2 blood samples at the following timepoints: before the start of ERT; at weeks 4, 8, 12, 24, and 52 in the first year of ERT treatment; and yearly thereafter.

Enzyme-linked Immunosorbent Assay

Serum was stored at −20°C or −80°C until use. A 96-well plate (Nunc, F96 Maxisorp) was coated with 100 μL/well idursulfase at a concentration of 5 μg/mL of phosphate-buffered saline (PBS) (pH 7.4). Samples were incubated for 2 hours at room temperature. After incubation, the idursulfase solution was removed and the coated wells were blocked overnight at 4°C with 375 μL/well of 1% bovine serum albumin (Sigma A7030) in PBS. Subsequent steps were performed at room temperature. Plates were washed 6 times at room temperature with 200 μL washing buffer per well (0.05% Tween in PBS). Next, plates were incubated with 50 μL of patients' serum for 1 hour using a 4-fold dilution series. The sera of individuals without MPS II were used as negatives controls, and a polyclonal anti-idursulfase rabbit antiserum (R250/R251, Eurogentec) was used as a positive control. After incubation for 1 hour, plates were washed again as described before and a 100 μL conjugate was added to each well: for the human sera, the conjugate was polyclonal anti-human-(immunoglobulin [Ig]G, IgA and IgM)-horseradish peroxidase (Acris, Origene) in a 20 000-fold dilution; for the rabbit antiserum, the conjugate was anti-rabbit-IgG-horseradish peroxidase (Sigma) at a 10 000-fold dilution. After incubation for 1 hour, 100 μL Tetramethylbenzidine Microwell Peroxidase substrate (Kirkegaard and Perry Laboratories) was added, and after 10 minutes the incubation was stopped by addition of 100 μL 1 mol/L H_3PO_4. Absorbance was measured at 450 nm using a spectrophotometer (Thermo Electron corporation). Background values of the enzyme-linked immunosorbent assay were determined by omitting the coating of the plates with elaprase and was found to be 1:640. The titer was determined as the maximal dilution at which absorbance was at least double the absorbance of the mean of the negative control sample plus 10%.

In Vitro Neutralizing Effects

The assay was adapted from a neutralizing assay for anti-acid alpha glucosidase (anti-GAA) antibodies in Pompe disease.[25] To fibroblasts of a patient who was IDS deficient (patient 6), 20 μL of patient serum was added plus 20 nmol elaprase in a total volume of 200 μL Ham's-F10 (Lonza Group) medium containing 3 mmol/L PIPES and antibiotics. After 24 hours of incubation at 37°C at 5% CO_2 for 24 hours, cell lysates were prepared as described.[22] The IDS activities in the medium and cell lysates were measured as described and were expressed as the percentage relative to the activity in fetal calf serum.[22]

Pharmacokinetic Analyses

Blood was drawn at the following timepoints; before the start of idursulfase infusion, 15 minutes before the end of idursulfase infusion, at the end of the infusion, and at 15, 30, 60, 120, 180, and 330 minutes thereafter. Plasma was prepared and directly stored at −20°C until further analysis. To measure the percentage of antibody-bound idursulfase, 100 μL of serum samples was diluted 1:1000 in PBS/bovine serum albumin (1 mg/mL), and 20 μL of Sepharose CL-4B beads with protein A or beads without protein A were added (GE Healthcare). The mixture was incubated under continuous rotation for 1 hour at room temperature. Subsequently, the beads were removed by centrifugation for 2 minutes at 12 000 RPM. Enzyme activity was measured in the supernatant as described.[22]

Urinary Glycosaminglycans

Total uGAG was determined by the dimethylmethylene blue (DMB) assay according to De Jong et al.[26] To quantify the specific storage products, dermatan sulphate (DS) and heparan sulphate (HS) were determined by mass spectrometry as described previously.[22] All uGAG values were corrected for the urinary creatinine concentration, which was determined enzymatically by a creatinine assay using a commercially available kit (Roche Modular P) on an autoanalyzer.

Results

Seventeen of 18 patients treated with ERT were eligible and completed the study. One patient was excluded because of a lack of samples to measure antibody titers (Table II). Of the 17 male patients, 9 were classified as neuronopathic. The median age at start of ERT treatment was 5.5 years (range, 1.0-47.3 years). The treatment duration ranged from 1.1 to 9.4 years (median, 4.1 years). Age at latest visit ranged from 5.0 to 48.9 years. Patients 4 and 5 were siblings; no other patients were related. Two of the patients who were neuronopathic died during follow-up at ages of 15.7 (patient 10) and 16.7 years (patient 12) owing to cardiorespiratory failure. ERT was stopped in 2 patients who were non-neuronopathic after 2 years and 18 months, either because of infusion-associated reactions (IARs) (patient 15) or because the patient decided that the benefit of treatment did not outweigh the burden (patient 17).

We identified 16 different IDS variants: 10 missense variants, 1 silent variant that affects splicing, 3 small deletions that induced a frameshift, 1 nonsense variant, and 1 total deletion of the IDS gene. Thirteen patients were predicted to be CRIM positive, and 4 were predicted to be CRIM negative (Table II).[22]

Antibodies

Eight patients (47%) developed antibody titers above the background titer of 1:640. Median peak titers were 1:5120 (range, 1:1280-1:81 920). In the selection of patients who developed antibody titers above the background titer, the titers started to develop at a median of 18 weeks (range, 8-156 weeks) and first reached maximum values at a median of 36 weeks (range, 8-156 weeks). In 3 patients (2, 6, and 10) who developed antibodies, titers remained 1:2560 or higher until study end (4-10 years after start of ERT) (Figure 1, A). These patients were classified to have sustained antibody titers. Five patients (patient 5, 12, 13, 15, and 16) showed antibody titers at single timepoints followed by normalization to background levels (Figure 1, B). These patients were classified as having nonsustained antibody titers. Nine patients had no-low antibody titers that did not exceed background values (Figure 1, C).

Safety

Idursulfase treatment was generally well tolerated. IARs were reported in 2 patients. Patient 6 experienced mild hyperthermia during ERT infusions from week 8 to week 24. IARs did not occur thereafter. Patient 15 experienced multiple mild episodes of urticaria and/or nausea. In this patient, adaptations of infusion rates and administration of corticosteroids could not prevent IARs. This result in combination with the limited benefit that the patient experienced from ERT was reason to discontinue treatment 2 years after start.

Table II. MPS II patient characteristics

Patients	IDS variant DNA	IDS variant protein	Predicted CRIM*	Neuronopathic	Age at start of ERT (y)	Age at latest visit (y)	ERT duration (y)
1	c.349_351del	p.(Ser117del)	+	Yes	1.0	10.4	9.4
2	c.544del	p.(Leu182Cysfs*31)	-	Yes	2.3	8.8	6.5
3	c.998C>T	p.(Ser333Leu)	+	Yes	2.8	5.5	2.7
4	c.410T>C	p.(Phe137Ser)	+	No	3.6	6.8	3.2
5	c.410T>C	p.(Phe137Ser)	+	No	3.6	6.8	3.2
6	GRCh37/hg19 ChrX:g.(?_148392640)_(149535015_?)del	p.0	-	Yes	4.1	8.2	4.1
7	c.673T>G	p.(Tyr225Asp)	+	No	4.4	5.5	1.1
8	c.1511del	p.(Gly504Alafs*8)	-	Yes	5.2	8.9	3.7
9	c.998C>T	p.(Ser333Leu)	+	Yes	5.5	14.6	9.1
10	c.1375G>T	p.(Glu459*)	-	Yes	8.6	15.7†‡	7.1
11	c.257C>T	p.(Pro86Leu)	+	Yes	9.7	19.0	9.3
12	c.1561G>A	p.(Glu521Lys)	+	Yes	10.9	16.3†‡	5.4
13	c.1265G>A	p.(Cys422Tyr)	+	No	24.5	33.5	9.0
14	c.1122C>T	p.(=)	+	No	25.4	30.7	5.3
15	c.182C>A	p.(Ser61Tyr)	+	No	36.9	39.0†	2.1
16	c.1024C>T	p.(His342Tyr)	+	No	44.6	46.3	1.7
17	c.806A>T	p.(Asp269Val)	+	No	47.3	48.9†	1.6

-, negative; +, positive.
Patients are ranked by age at start of ERT.
*Predicted CRIM.
†Age at time ERT was stopped.
‡Age at death. Patient 10 died at the age of 15.7 years. Patient 12 died at the age of 16.7 years.

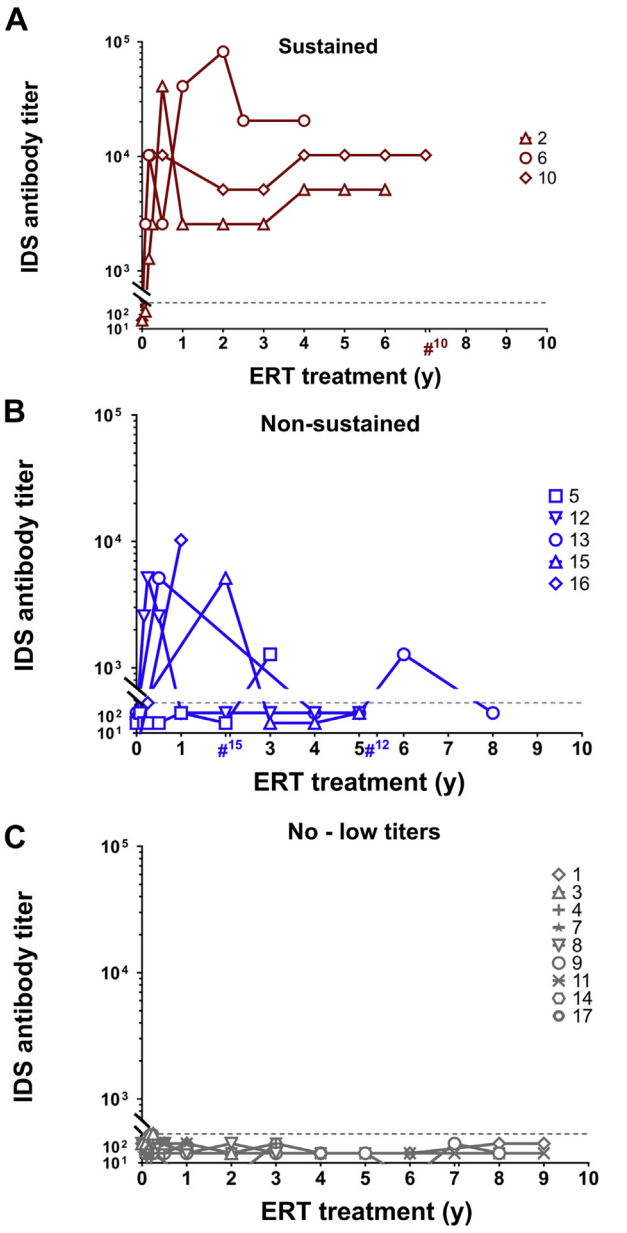

Figure 1. Antibody titers. Classification of patients based on antibody titers. **A,** Sustained titer patients, indicated by *red lines*. **B,** Nonsustained titer patients, indicated by *blue lines*. **C,** No-low titer patients, indicated by grey lines. Technical background of the enzyme-linked immunosorbent assay is indicated with a *dashed grey line*.

Neutralizing Effect of Antibodies

Neutralizing effects of anti-IDS antibodies were measured by adding elaprase with patient serum to cultured IDS-deficient fibroblasts. Enzyme activity was measured in medium and fibroblast lysates after 24 hours. The intracellular IDS activity reflects the sum of effects of antibodies on cellular uptake and on IDS enzyme activity. The IDS activity in the medium reflects effects of antibodies on IDS activity only. In patients 2, 6, and 10 with sustained antibodies, intracellular IDS activity was strongly inhibited to enzyme levels that were 0%-10% of control values at titers of 1:2560 and higher (**Figure 2**, A; available at www.jpeds.com; red symbols). Patients 5, 12, 13, and 15 with nonsustained antibody titers were tested at timepoints during which the maximum titer was 1:5120; this titer showed variable inhibition of intracellular IDS activity to enzyme levels that ranged from 2% to 66% of control values (**Figure 2**, A; blue symbols).

Inhibition of extracellular activity was in general less prominent (**Figure 2**, B). Only patient 10 with sustained titers showed full inhibition of extracellular activity at a high titer of 1:10 240 and inhibition to enzyme levels that were 20% of control values at 1:51 120. In contrast, patient 6 with sustained titers showed no inhibition of extracellular IDS activity at titers up to 1:10 240. Only at the very high titer of 1:81 920 was inhibition observed to enzyme levels that were 20% of control values. Patient 2 with sustained titers showed maximal inhibition to 55% of control values at a titer of 1:40 960. Patients 5, 12, 13, and 15 with nonsustained titers showed variable inhibition of extracellular IDS activity that ranged from inhibition to 25% of control values at 1:1280 (patient 5) to 70% of control values at 1:5120 (patient 15).

The lack of correlation between inhibition of intracellular and extracellular IDS activity can be seen in **Figure 2**, C. Titers that fully inhibit intracellular activities inhibited extracellular IDS activity to variable extents. This finding suggested that distinct anti-IDS had been formed that inhibited either cellular uptake, IDS enzyme activity, or both.

Pharmacokinetic Analysis

A pharmacokinetic analysis was performed to analyze the effects of anti-IDS antibodies in patients during infusion with elaprase. Three patients were selected as representative examples of the 3 groups with sustained, nonsustained, or no-low titers. Serum samples were collected during ERT, followed by immunoprecipitation with Sepharose-protein A, which precipitates antibody-bound IDS, or control Sepharose. Antibody-free IDS activity was measured in the supernatant. Patient 6 with sustained titers (actual titer at the time of infusion was 1:20 480 at 2.5 years of ERT) showed peak IDS activity at 3 hours of ERT in serum that was immunoprecipitated with control Sepharose (**Figure 3**, A, black line). Immunoprecipitation of serum with protein A-Sepharose completely removed IDS activity from the supernatant, indicating that all infused elaprase was antibody bound (**Figure 3**, A, red line). Patient 5 with a nonsustained titer (actual titer at the time of analysis was 1:1280 at 3 years of ERT) showed similar IDS activities in serum samples that were subjected to immunoprecipitation with Sepharose-protein A (**Figure 3**, B, blue line) or control Sepharose (**Figure 3**, B, black line), indicating that elaprase was not bound by antibodies during ERT. This was also found for patient 4, who had no antibodies and was analyzed at 2.5 years of ERT (**Figure 3**, C). The area under the curve was considerably lower for patient 6 (**Figure 3**, A) compared with patients 5 and 4 (**Figure 3**, B and C). This

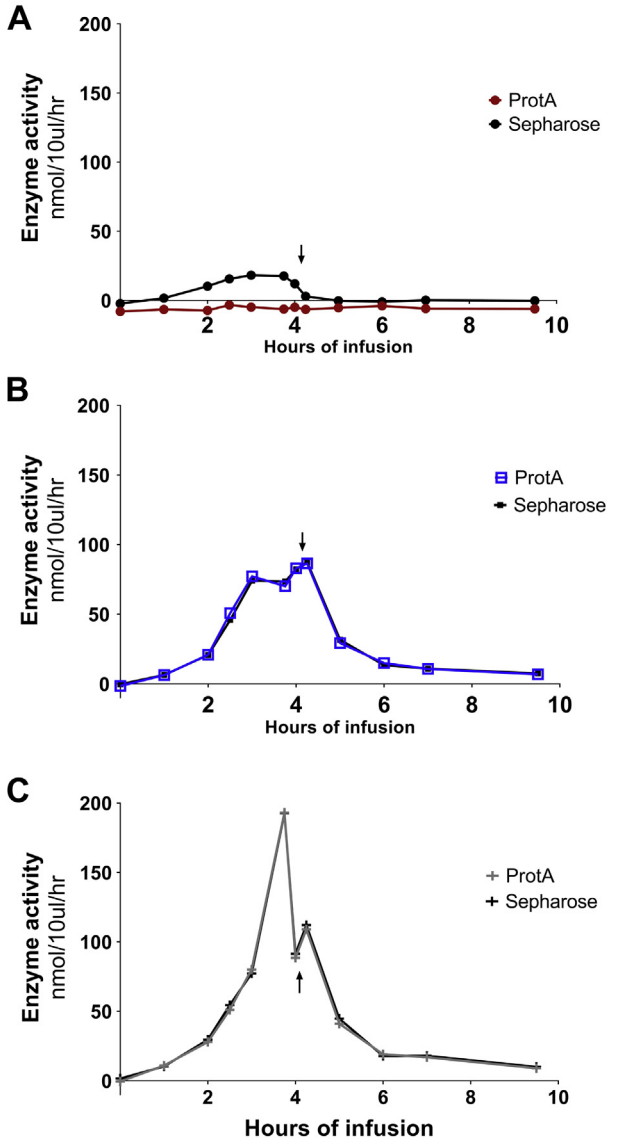

Figure 3. Pharmacokinetic analysis. Antibody binding to IDS during infusion. IDS in plasma was immunoprecipitated with control beads (Sepharose; *black symbols*) or with protein A Sepharose beads (ProtA; *red, blue, and grey symbols*) at several timepoints during ERT to remove antibody-bound IDS. The IDS enzyme activity was measured in the supernatant of the immunoprecipitates. **A,** Patient 6 with sustained antibody titers. The titer at this timepoint of 2.5 years of ERT was 1:20 480. **B,** Patient 5 with nonsustained titers. The timepoint of analysis was 3 years of ERT at an antibody titer of 1:1280 at this timepoint. **C,** Patient 4 with a no-low antibody titer after 2.5 years of ERT with an antibody titer of 1:160 at this timepoint. *Arrows* indicate the end of ERT infusion.

finding suggested that, at the titer of 1:20480 in patient 6, anti-IDS antibodies that bound elaprase partially inhibited IDS activity. In conclusion, circulating antibodies in patient 6 bound and inhibited elaprase during ERT.

GAGs

To assess the impact of antibodies on uGAG levels, DS and HS were measured at baseline and during ERT using mass spectrometry. For comparison, total uGAGs were also measured using the DMB assay. At baseline, the median DS, HS, and total uGAG levels were elevated in all 3 groups (sustained, nonsustained, and no-low titers). The highest DS, HS, and total uGAG levels were measured in the sustained titer group with a median of 22.8-, 35.4-, and 4.9-fold the upper reference limit (normal levels corrected for age, see **Table III** (available at www.jpeds.com)) (**Figure 4**, A-C). In patients 2, 6, and 10 with sustained titers, the median uGAG levels failed to normalize during ERT, and remained elevated 4.8- to 10.5-fold, 3.3- to 13.3-fold, and 1.6- to 4.0-fold for DH, HS, and total uGAGs respectively (**Figure 4**), for individual patients see **Figure 5**, A-C (available at www.jpeds.com). In the remaining patients with nonsustained or no-low titers, median levels of DS, HS, and total uGAGs were only slightly elevated to normal (**Figure 4**, B, C, E-G, I). This was the case for all individual patients (shown in **Figure 5**, B, C, E-G, I), except patient 12. This patient showed initial decrease of DS, HS, and total GAG levels without normalization at 2 years of ERT. His DS, HS, and total GAG levels eventually increased up to 4.2-, 3.0-, and 2.5-fold, respectively at 4 years of ERT (**Figure 5**, B-H) despite the absence of antibodies around these timepoints (**Figure 1**, B).

Discussion

We found that 47% of patients developed anti-elaprase antibodies at any timepoint during follow-up. Five patients (29%) developed nonsustained titers, and 3 patients (17%) developed sustained titers that remained present during a period between 3 and 9 years. Nine patients (52%) did not develop antibodies. These numbers are in line with previous findings in other patient cohorts. In an open-label extension study involving 94 patients with a follow-up of 2-3 years, approximately 50% of patients developed antibodies at any timepoint and 22% developed sustained neutralizing antibodies.[27] In the Hunter Outcome Survey, 50% of patients developed an antibody titer at any timepoint in a one year follow-up study.[20] Similar levels have been found in previous studies.[17,21,28]

There is little information in the literature on individual antibody titers and their timing, because patients are usually scored as being antibody positive or negative. Pano et al found that patients developed antibodies starting at 16 weeks after ERT.[21] Most patients in our cohort who developed antibodies did so before the timepoint of 2 years after start of ERT and remained negative during follow-up, with 2 exceptions. Patient 5, who was initially antibody negative, developed antibodies at a titer of 1:1280 at 3 years after the start of ERT. No further measurements were available for this patient. Patient 13 developed antibodies at the earliest timepoint at 6 months of ERT treatment, was antibody negative at subsequent timepoints (4-5 years of ERT), and showed a

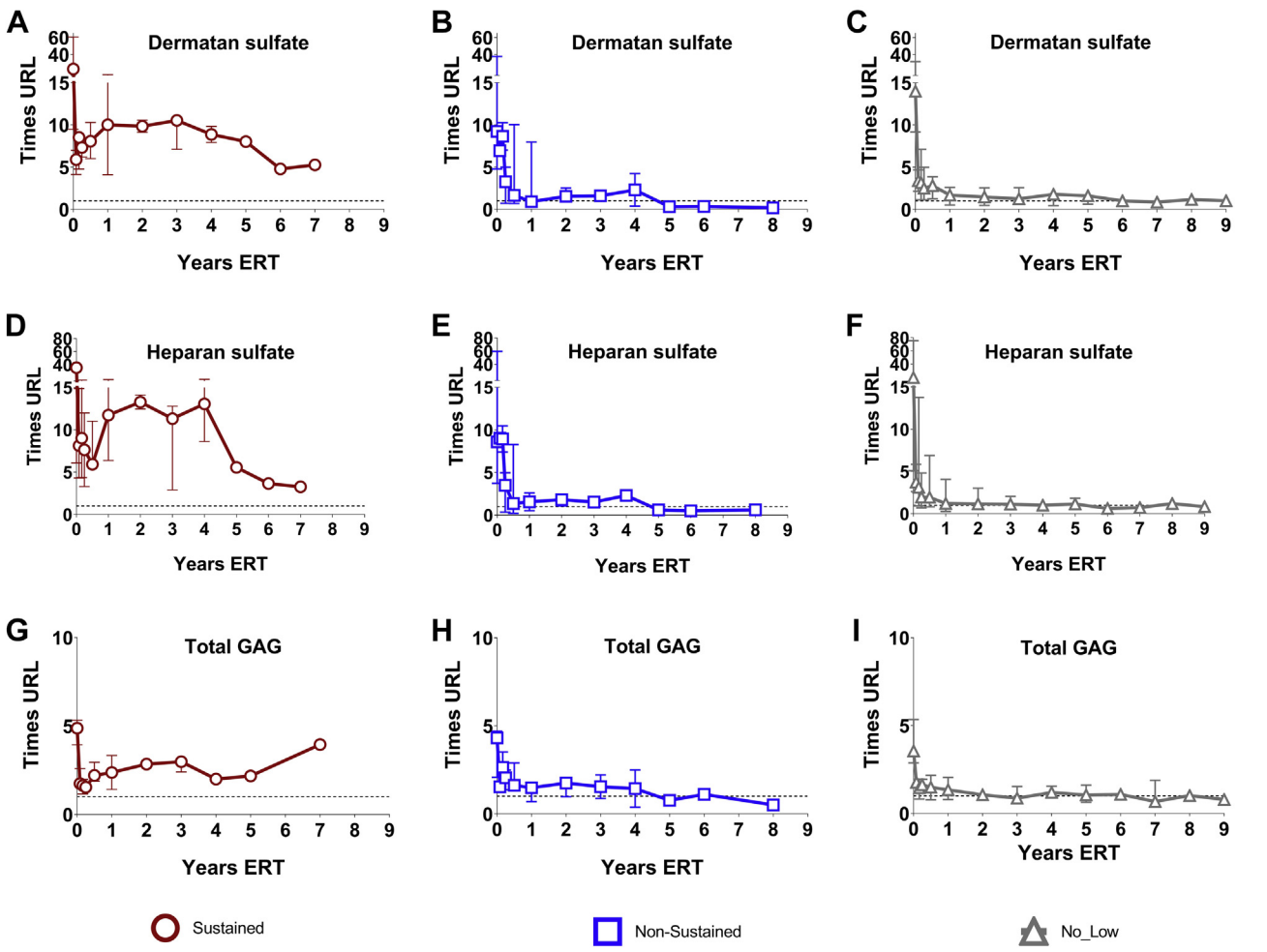

Figure 4. GAG measurements. The median GAG levels expressed as times upper reference limit (URL) for patients classified with sustained (*red*), nonsustained (*blue*), and no-low (*grey*) antibodies. *Dotted lines* represent the URL. Urinary levels of heparan sulfate (**A-C**) and dermatan sulfate (**D-F**) were measured by mass spectrometry. Total uGAG levels (**G-I**) were measured using dimethyl methylene blue (DMB). Ranges are indicated. Timepoints without ranges represent single values. URLs are presented in **Table III**. Noncorrected and individual GAG levels are presented in **Figure 5**.

transient antibody titer at 6 years ERT. Patients with a sustained antibody titer during at least 3 subsequent timepoints remained sustained during the entire follow-up. We found that the height of titers differed substantially between patients who tested positive for antibodies and varied between 1:81 920 and 1:1280. For example, within the group with sustained titers, the maximum variation in titers between different patients was 32-fold. Altogether, this indicates that both the timing and height of antibody titers can vary substantially, arguing for regular monitoring of antibody titers to determine their actual status, in line with previous recommendations.[29,30]

The neutralizing effects of antibodies was assessed in vitro for their combined effects on cellular uptake plus enzyme activity (intracellular IDS activity) and for effects on enzyme activity only (extracellular activity). Titers starting at 1:2560 were capable of complete inhibition of extracellular IDS activity. The data also suggested that different types of anti-IDS had formed. For example, at a titer of 1:5120, patient 13 showed 30% inhibition of intracellular activity, and these values were 75% and 95% for patients 12 and 15, respectively. Previous studies also suggested that different patients can form distinct types of anti-IDS antibodies, ranging from (transient or persistent) non-neutralizing to neutralizing.[20,21,27] In our cohort, antibodies that completely inhibited intracellular activity either showed complete, partial, or no inhibition of extracellular activity (**Figure 2**, C), further highlighting that antibodies with distinct properties had formed in different patients. In this respect, anti-IDS antibodies behaved different from anti-GAA antibodies in Pompe disease, in which inhibition of extracellular and intracellular activity correlated in all but one patient (see Figure S2 in de Vries et al[25]). Evidence that antibodies can interfere with ERT during infusion was obtained by analyzing sera collected at several timepoints during ERT from 3 patients. Patient 6 with sustained

antibody titers and an actual titer of 1:20 480 showed that all infused ERT was bound to circulating anti-IDS antibodies, which inhibited IDS enzymatic activity to a large extent. A titer of 1:1280 in patient 5 did not interfere with ERT during infusion. Increasing the dosing of elaprase would be an option in case of sustained antibodies. It is difficult to determine at which titer elaprase would be in such excess over antibodies that its binding would be irrelevant, because this would require multiple invasive pharmacokinetic analyses. Alternatively immune modulation could be considered for patients with high titers of neutralizing antibodies. This widely debated approach has been proposed for cases of MPS II, and to a larger extent in patients with the classic infantile form of Pompe disease.[24,31,32]

Biochemical assays in patient-derived material are unable to distinguish between non-neuronopathic and the neuronopathic forms of this disease.[22,33-35] However, in many cases, the *IDS* genotype is a good predictor of the phenotype.[22] All patients who are non-neuronopathic are predicted to harbor *IDS* variants that allow some residual expression of enzymatically active IDS protein, and can be classified as CRIM positive. Patients who are neuronopathic have disease-associated *IDS* variants (eg, missense variants or in-frame deletions) that either allow expression of enzymatically inactive IDS protein, leading to a CRIM positive classification, or a variant (eg, frameshifts or large deletions) that abrogates *IDS* expression. The latter can be classified as CRIM negative. In our cohort, all 3 patients with sustained antibodies were predicted to be CRIM negative. These patients indeed had the neuronopathic phenotype. Other patients with the neuronopathic phenotype but with a CRIM positive prediction (patients 1, 3, 9, 11, and 12) had no-low or nonsustained titers, as was the case for all patients who were non-neuronopathic.[4,5,7,13-17] A similar propensity of patients who are CRIM negative for developing anti-IDS antibodies has been reported previously.[21] One exception was patient 8, a patient who was neuronopathic, CRIM-negative who did not develop antibodies. Why this patient did not develop antibodies is not fully understood. We considered the possibility that low amounts of truncated protein may be expressed and, therefore, prevent an immunological reaction on the recombinant IDS applied during ERT treatment. We conclude that the CRIM negative subgroup of patients within the group of patients who are neuronopathic are the most likely to develop sustained anti-IDS antibodies.

We found that median levels of urinary HS (uHS) and urinary DS (uDS) in patients with sustained antibodies were strongly elevated despite ERT, and that these levels were close to normalized in patients with nonsustained or no-low titers. These effects were measured using a mass spectrometry assay, which is more sensitive than the conventional DMB assay. This is the likely reason why in previous studies a relationship between decrease in uGAGs and the level of antibodies was either not found or less prominent.[15,21,27] An analysis of uGAG levels in individual patients showed that patients with the highest antibody titers also had the highest levels of uDS and uHS; patient 6, 10, and 2 (compare **Figure 1** and **Figure 5**). An exception was patient 12. In this patient, the uGAG level initially decreased to close to normal levels. It remains unclear why after this initial decrease the uGAG levels gradually increased between 2 and 4 years of ERT treatment. The results indicate that a titer starting at 1:2560 (as seen in patient 2) can already neutralize elaprase to such extent that uDS and uHS levels remain elevated and ERT has reduced efficacy.

In conclusion, high anti-IDS antibody titers of 1:5120 or more inhibit enzyme uptake and/or IDS activity and the efficacy of ERT. Patients with the neuronopathic form and lack of IDS protein expression were most at risk to develop sustained titers. Therefore we recommend regular (once per 6 months) monitoring of anti-IDS antibodies by enzyme-linked immunosorbent assay and uGAG levels by mass spectrometry. When antibodies are detected, a neutralizing assay to test the effects on cellular uptake and enzyme activity should be performed. Elevation of uDS and uHS levels in combination with the presence of neutralizing antibodies are indicative of decreased efficacy of ERT. It can then be considered to elevate the dosage of ERT, to induce immune suppression, or to stop treatment with ERT. ■

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Data Statement

Data sharing statement available at www.jpeds.com.

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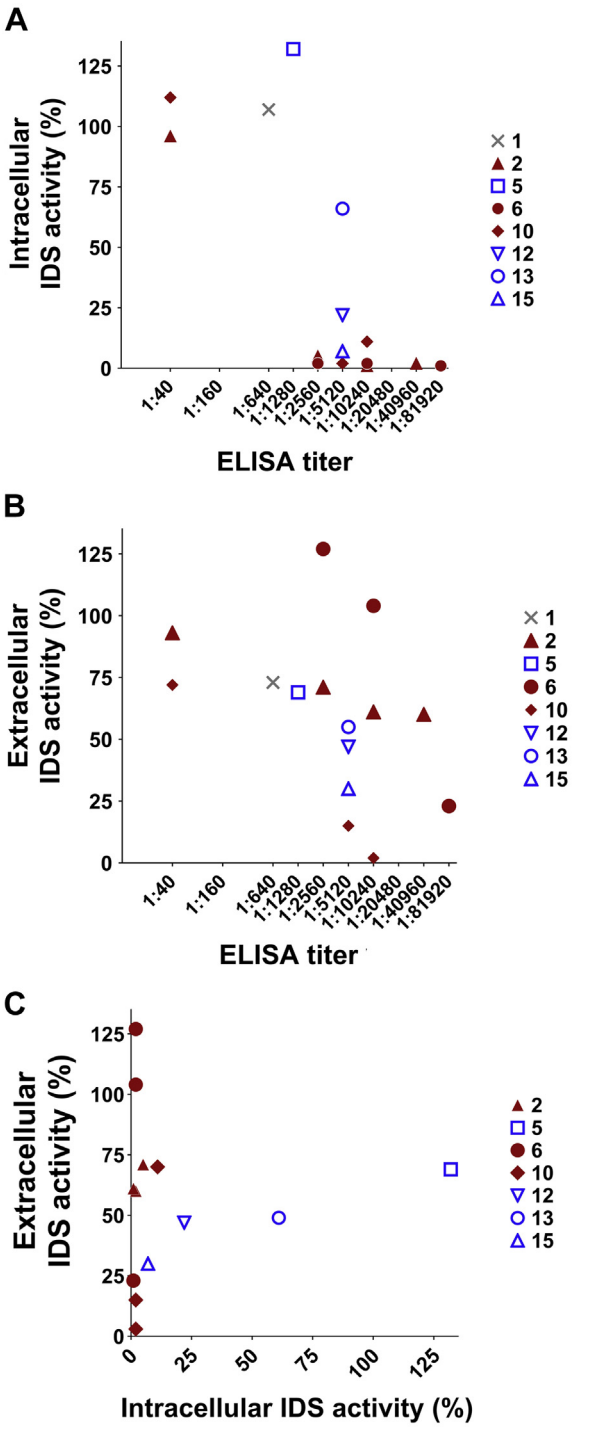

Figure 2. Neutralizing activity of IDS antibodies in vitro. **A,** Intracellular IDS activity was measured after incubation of IDS-deficient fibroblasts with rhIDS and patient serum. **B,** As in **A,** but now for the medium. **C,** Extracellular IDS activity compared with intracellular IDS activity. The samples with an antibody titer of 1:640 or less are not included in **C**. Enzyme activity is expressed as the percentage of enzyme activity using fetal calf serum insetad of patient serum. For the patients with non sustained antibody titers (patients 1, 5, 12, 13, and 15), the peak antibody sample was used. For patients with sustained antibody titers, timepoints 0, 4, 8, 12, 24, and 104 weeks of ERT and the end point were used when available. *ProtA*, protein A Sepharose beads.

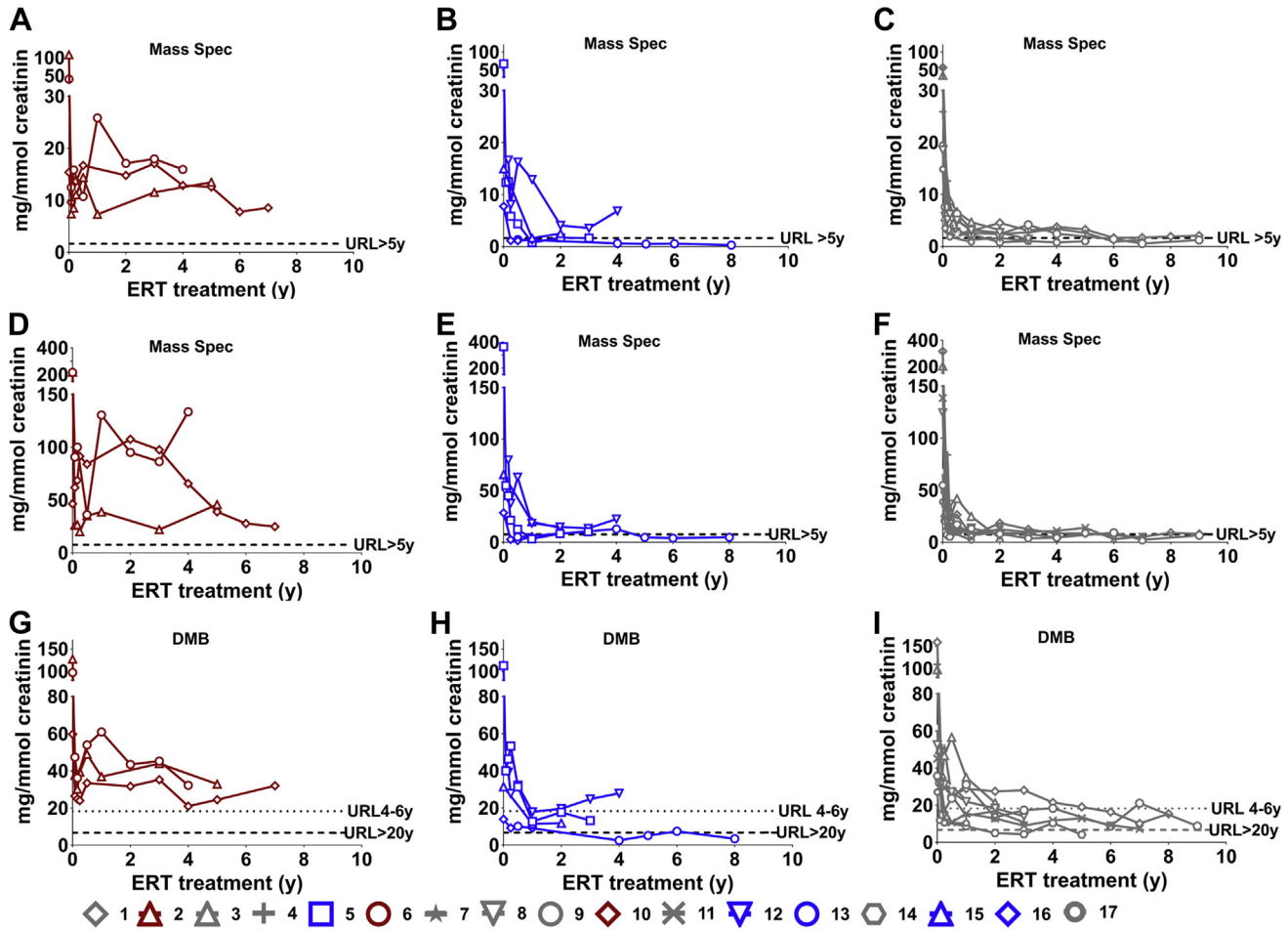

Figure 5. GAG measurements. GAG levels expressed in milligrams per millimole creatinine for patients classified with sustained (*red*), nonsustained (*blue*), and no-low (*grey*) antibodies. *Dotted lines* represent the upper reference limit (URL). Urinary levels of heparan sulfate (**A-C**) and dermatan sulfate (**D-F**) were measured by mass spectrometry (*Mass Spec*). Total uGAG levels (**G-I**) were measured by dimethyl methylene blue (DMB).

Table I. Infusion rate

Infusion rates (mL/h)	Duration (min)
4	30
8	30
16	30
24	15
32	15
40	110

Table III. Upper reference limit of GAGs. Age-dependent upper range limits (URLs) of normal values for DS, HS, and total GAGs, all expressed in mg/mml creatinine

Age	DS	HS	Total GAG
0-1 months	6.7	26.4	71.1
1-2 months	6.7	26.4	54.4
2-6 months	2.4	2.4	54.4
6-12 months	2.4	2.4	36.9
1-2 years	1.8	6.1	32.5
2-4 years	1.8	6.1	26.1
4-5 years	1.8	6.1	18.3
5-6 years	1.6	7.6	18.3
6-10 years	1.6	7.6	15.2
10-15 years	1.6	7.6	11.2
15-20 years	1.6	7.6	8.1
>20 years	1.6	7.6	6.7