Glycan-Based Biomarkers for Lysosomal Storage Disease: The Sensi-Pro® Assay

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Abstract

Historical methods to measure substrate accumulation in lysosomal storage diseases (LSDs) have been hampered by the heterogeneity of the pathologic substrate material as well as the presence of endogenous, non pathologic material. To overcome these challenges, the Sensi-Pro Assay was developed. By selectively amplifying the substrates accumulating due to the deficient enzyme, this method provides an assay with unique capabilities that can be applied across many LSDs. To validate the method, we analyzed substrate in samples of serum, plasma, urine, and cerebrospinal fluid obtained from MPS patients, MPS animals, and normal subjects (data presented here is from MPS I subjects). The results demonstrate that the assay can detect pathologic substrate accumulation with significant differences in substrate levels between untreated MPS subjects, treated MPS subjects, and normal subjects. Matched sample analysis demonstrated enhanced performance relative to assays which do not distinguish between pathologic and non-pathologic substrate. Response to ERT was also demonstrated in serum, CSF, and cortex tissue. Sample sizes of less than 50 microliters were required for robust assay performance.

Background: A Need for Improved Diagnostics

The need for enhanced measurement of substrate accumulation in MPS and other lysosomal storage diseases exists for several critical reasons:

1. **Clinical endpoints can lack sensitivity.** Slowing progressing chronic symptoms with dramatic patient heterogeneity can make assessing clinical response challenging in trials as well as clinical practice.

2. **Accumulating substrates provide an appealing diagnostic target.** Measuring substrate accumulation has direct relevance to the underlying pathology of lysosomal storage diseases.

3. **Existing and novel diagnostic methods are not sufficient.** Available methods for measuring substrate accumulation have limited dynamic range and may not significantly distinguish between untreated, treated, and healthy subjects, making it difficult to ascertain optimal dosing and treatment.

The Diagnostic Challenge: Substrate Heterogeneity

The most significant barriers to using substrate accumulation as a measure of LSD disease are as follows:

1. **Extreme complexity of the accumulating pathologic material.** In MPS, the accumulating material consists of linear polymers with dramatically different lengths and sulfation patterns, thus millions of unique structures can accumulate **(Figure 1)**. Similar challenges exist in LSDs which accumulate lipid-linked glycans. **Figure 2** provides an illustration of the heterogeneity in Fabry Disease.

2. **Presence of endogenous, non-pathological material.** Normal cellular substrate material exists in biological samples from healthy individuals. This creates challenges in distinguishing affected subjects from normal subjects.

Figure 1. Glycosaminoglycan Heterogeneity in MPS

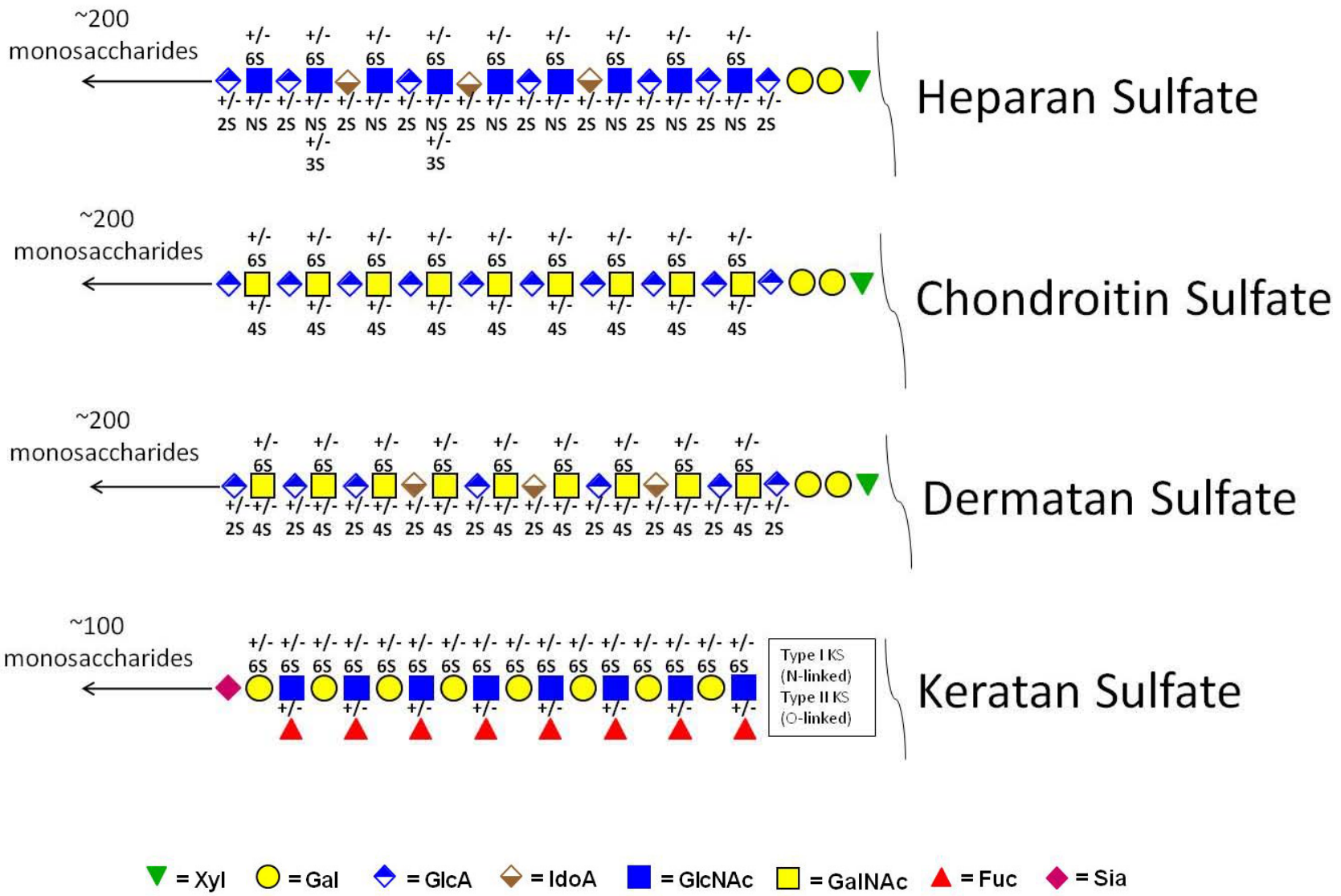
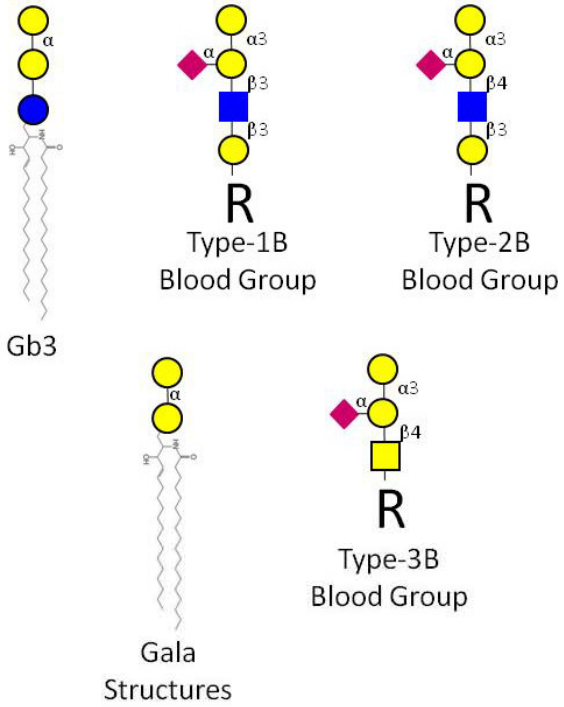


Figure 2. Glycan Heterogeneity in Fabry Disease

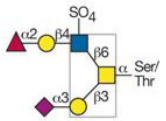


Accumulating Glycan Heterogeneity in Fabry Disease		
Glycan Variability		Unique Forms
	Sialylation	2
	Sialic acid form (Ac/Gc)	~3
	Glass class (lipid linked, O-linked, N-linked)	3
Lipid Variability		
	Sphingosine modifications	~3
	Length of fatty acid (C14 to >30)	16
	Loss of fatty acid (Lyso-)	2
	Saturation	~10
Total Possible Accumulating Structures		>10,000

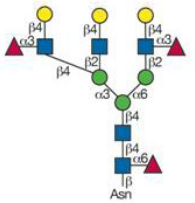
R = Variable glycan class including:

Lipid Linked

O Linked



N Linked



▼ = Xyl ○ = Gal ◆ = GlcA ◆ = IdoA ■ = GlcNAc □ = GalNAc ▲ = Fuc ◆ = Sia

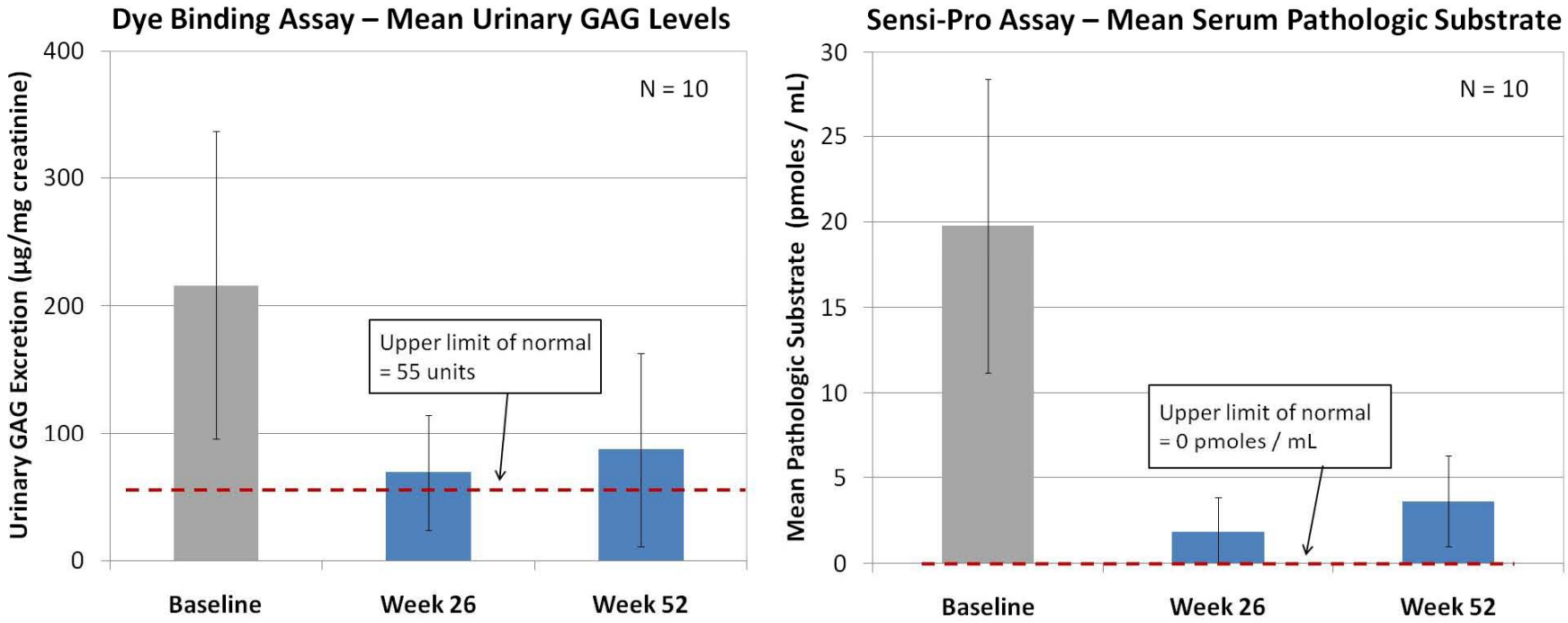
The Sensi-Pro Assay: Introduction

The Sensi-Pro Assay employs novel sample preparation techniques and analytical methods[1] to amplify only the substrates accumulating due to the deficient enzyme (i.e., pathologic substrate). This results in an assay with the following performance characteristics:

- Detects only pathological substrate material
- Applicable to many glycan-related LSDs
  - MPS, Fabry, Gangliosidoses, Gaucher, others
- Capable of discriminating LSD classes
  - Different markers for each class of LSD
- Quantitative analysis
- HPLC or mass spectrometry instrument platforms
- Wide range of sample types (urine, blood, CSF, brain, kidney, etc.)
- Small sample sizes (< 50 microliters)

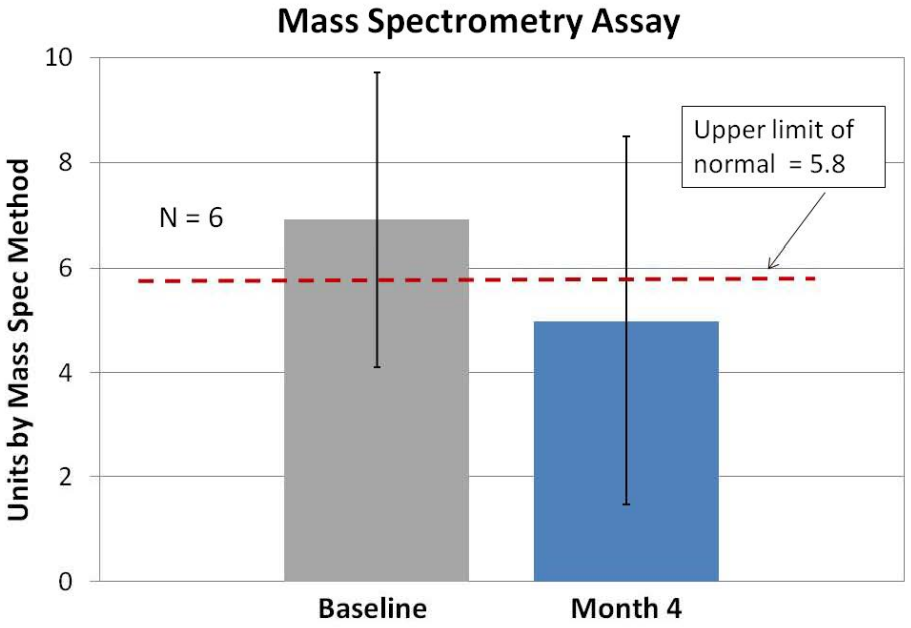
[1] Manuscript submitted

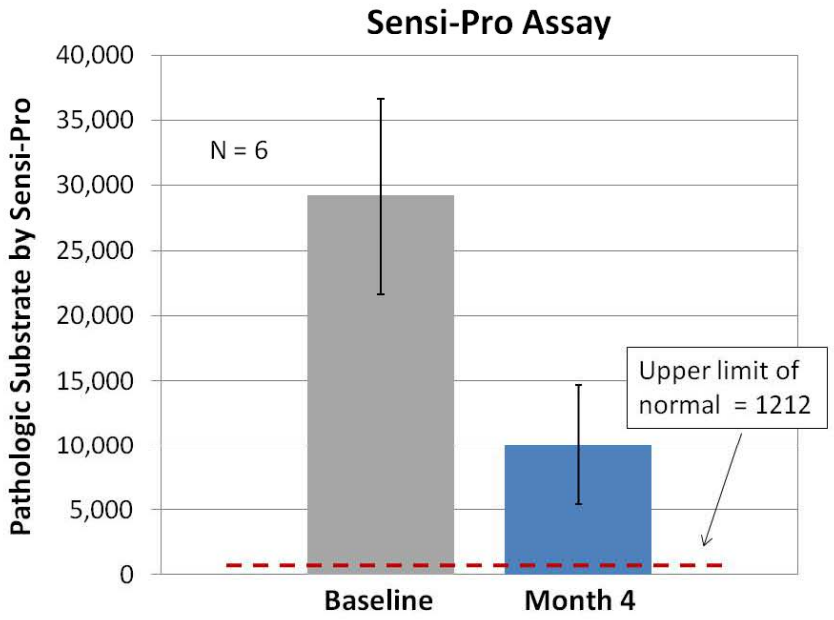
Comparison of Dye Binding Assay and Sensi-Pro Assay
Blinded Analysis of Samples From the Laronidase Phase I / II Clinical Trial*



* Ten MPS I patients treated with IV laronidase weekly for 52 weeks. Urinary GAG levels are the values recorded in the clinical trial database. Serum pathologic substrate levels were generated by analyzing stored serum samples using the Sensi-Pro Assay. Analysis remained blinded until results reported to the investigator. Error bars are standard deviation.

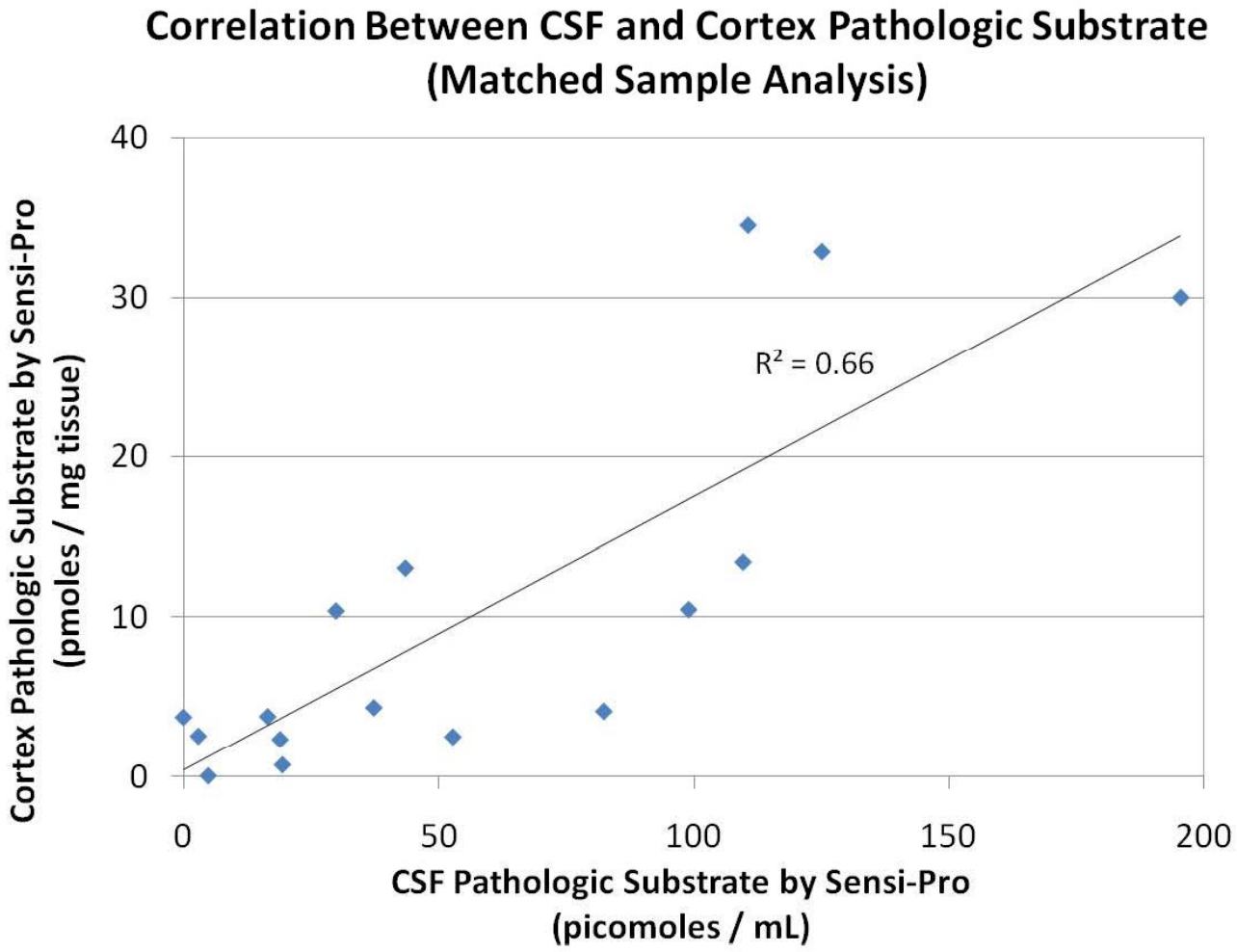
Comparison of Mass Spectrometry Assay and Sensi-Pro Assay
Blinded Analysis of CSF Samples From MPS I Dogs Treated with Intrathecal Laronidase[T]



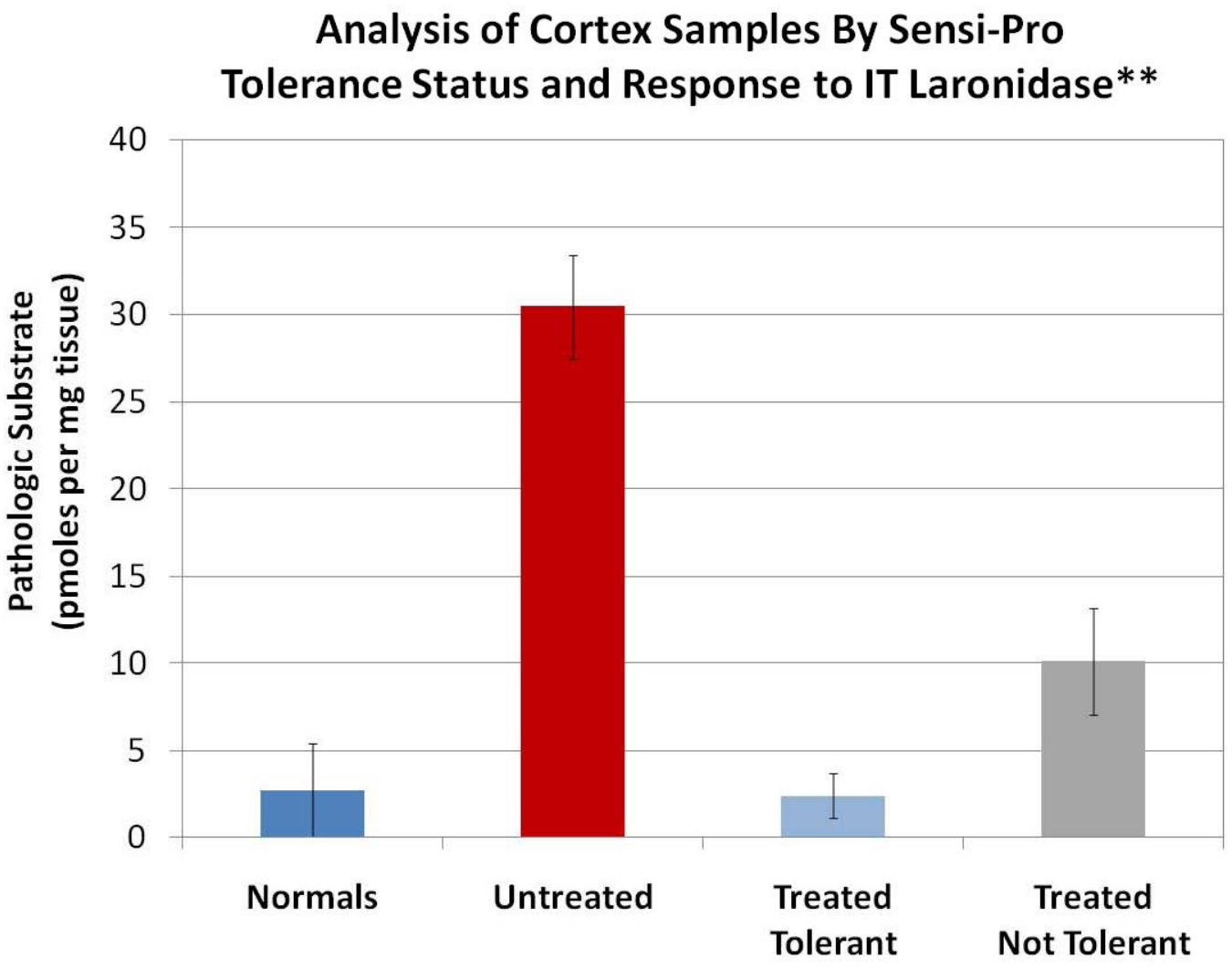


[T] MPS I dogs treated with intrathecal laronidase for four months. CSF substrate levels measured by the mass spectrometry assay employed electro-spray ionization-tandem mass spectrometry to detect oligosaccharides derived from the sample (without distinguishing between pathologic and non-pathologic substrate). Analysis remained blinded until results reported to the investigator. Analysis includes all dogs for which matched samples were available. Error bars are standard deviation.

Analysis of Cortex and CSF Samples From MPS I Dogs Treated with Intrathecal Laronidase*



* MPS I dogs treated with monthly intrathecal infusions of laronidase. Correlation analysis includes all dogs for which matching cortex and CSF samples were available.



** MPS I dogs treated with monthly intrathecal infusions of laronidase. Cut-off value of 20 OD units of anti-iduronidase antibodies per µl of undiluted serum was established to define immune tolerance. Error bars are standard deviation. Normal subjects included carriers.

Conclusions

The performance of the Sensi-Pro Assay was characterized using blinded analysis of matched samples of MPS I subjects:

- Significant differences were detected between samples from untreated, treated, and healthy normal subjects including analysis of CSF and cortex
  - Pathologic substrate in healthy normal subjects was at (or near) zero

- Enhanced performance was demonstrated with the Sensi-Pro Assay relative to alternative methods which do not distinguish between pathologic and non-pathologic substrate
  - Significant differences were not observed using alternative assays

- Pathologic substrate levels detected in cortex were shown to correlate to pathologic substrate levels detected in CSF using the Sensi-Pro Assay
  - This demonstrates the validity of measuring pathologic substrate levels in CSF when evaluating CNS targeted treatment strategies for LSDs

- Also in the MPS I dog model, tolerance status was shown to impact the response to intrathecal laronidase
  - Measuring pathologic substrate may prove useful in optimizing the efficacy of intrathecal ERT for brain disease

- Samples sizes of less than 50 microliters were required for robust performance of the Sensi-Pro Assay