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RESEARCH REPORT

Measurement of Red Blood Cell Sorbitol Dehydrogenase Activity for Fast Screening of SORD-Related Neuropathies

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ABSTRACT

Background and Aims: To develop an assay to measure red blood cell (RBC) sorbitol dehydrogenase (SORD) activity for fast laboratory screening of SORD-related neuropathies, and to provide additional phenotypic arguments in case of ambiguous genetic results.

Methods: Hemolysates were incubated in the presence of fructose and NADH to produce sorbitol and NAD^+. The decrease in absorbance at $\lambda = 340$ nm was monitored throughout the course of the reaction. Results were expressed as % activity compared to a within-run control. A cohort of 38 individuals was evaluated over 13 runs: 13 individuals served as within-run controls, 3 were diagnosed with SORD deficiency, 2 were highly suspected of having SORD deficiency, 1 exhibited incidental genomic findings in the *SORD* gene, and 19 presented with neuropathy or myopathy of undetermined etiology.

Results: RBC SORD activity was significantly reduced in individuals diagnosed with SORD deficiency when compared to within-run controls, whereas in contrast, individuals without SORD deficiency did not exhibit this reduction. A comparison of these two groups showed that activities were significantly lower in deficient individuals ($p = 0.00006$). A test cutoff of $< 30\%$ yielded 100% sensitivity and specificity to detect deficient individuals.

Interpretation: This test provides a useful alternative to measuring plasma or urine sorbitol with mass spectrometry, allowing clinicians to directly evaluate SORD activity in RBC. As a result, it enables quick and convenient screening for SORD-related neuropathies during standard clinical procedures.

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1 | Introduction

First described in 2020, peripheral neuropathies related to pathogenic variants in the gene encoding for sorbitol dehydrogenase (SORD) are considered the most frequent inherited recessive neuropathies in humans [1]. SORD catalyzes the interconversion of sorbitol into fructose, which is associated with the reduction of NAD^+ to NADH (sorbitol + $NAD^+ \leftrightarrow$ fructose + NADH). Sorbitol accumulation within cells as a result of SORD deficiency is widely recognized as being neurotoxic to peripheral nerves, contributing to the development of Type 2 Charcot–Marie–Tooth disease (CMT2) or distal hereditary motor neuropathy (dHMN) [2]. The genetic diagnosis of SORD related neuropathies can be challenging due to frequent variants of unknown significance (VUS) and the presence of the *SORD2P* pseudogene. The measurement of plasma/urine sorbitol concentration by mass spectrometry (MS), which shows a marked increase in affected individuals, has improved diagnostic yield [1, 3, 4]. Nevertheless, this measurement does not directly assess SORD activity and requires qualified personnel and expensive equipment. In addition, a novel therapeutic agent, govorestat, is currently undergoing clinical evaluation (ClinicalTrials.gov ID: NCT07191912) and calls for a fast and precise diagnosis to treat patients at the earliest. In this letter, we describe and evaluate a straightforward spectrophotometric assay for the determination of SORD enzymatic activity in red blood cells (RBC).

2 | Materials and Methods

The assay is adapted from Morsches *et al.*'s [4], using fructose and NADH as substrates (i.e., in the non-physiological way). In practice, 2.5 – 5 µL of hemolysate was incubated in a mix containing Tris–HCl buffer (pH 7.4), fructose (final concentration 0.51 mol/L) and NADH (final concentration 0.216 mmol/L) (final volume: 1 mL). The variation of absorbance at $\lambda = 340$ nm (A_{340}, NADH maximum absorbance) and at $\lambda = 700$ nm (A_{700}, background absorbance) was recorded with a spectrophotometer (UV MC^2, SAFAS, Monaco) at +37°C over 20 min. For determination of enzymatic activity, the variation of A_{340}—A_{700} per minute (ddA/min) was calculated. Before analyzing patients, the production of sorbitol throughout the reaction was evaluated by MS [3] in three test samples, which confirmed that the assay resulted in sorbitol production from fructose (see Supporting Information).

RBC SORD activity (index test) was measured prospectively in 38 individuals over 13 runs. In total, three samples from individuals with known genetically-confirmed SORD deficiency were analyzed (Table 1, Individuals 1, 2, 5), as well as two samples from individuals with strong clinical suspicion of deficiency (Table 1, Individuals 3 and 4), one sample from an individual with intellectual disability and an incidental finding of homozygous exon 8 deletion in *SORD* (NC_000015.10:g.45072266_45072488del) on whole-genome sequencing (WGS), and 19 samples from individuals with neuropathy/myopathy of unknown origin. Plasma sorbitol concentration was evaluated for each individual using mass spectrometry (MS), which served as the reference standard [3], with a result < 5 µmol/L (lower limit of quantification) being not in favor of deficiency. Each run also included one within-run control. These controls corresponded to leftover samples which were previously analyzed for other routine tests in our laboratory, without any selection criteria regarding their clinical status. While their SORD status was unknown, it is highly unlikely that they would have been deficient considering the rarity of the disease.

All experiments were performed at the Clinical biochemistry laboratory of Hôpital Bichat—Claude Bernard, Paris, France. Results were reported as % of control. The results from the index test and reference standard were compared with clinical and genetic data, when available. Statistical analyses included calculation of median, range, and standard deviation, test sensitivity and specificity, detection of outliers by the Tukey Fence method, and comparison of results with the Mann–Whitney test.

TABLE 1 | Laboratory findings in the individuals with confirmed/strongly suspected SORD deficiency reported in this study.

Individual	RBC SORD activity (% of within-run control)	Plasma sorbitol concentration (reference range: < 5 µmol/L)	*SORD* variant(s) transcript NM_003104.6
1 (Figure 1A) 56 y.o. male	13.8%	66.3 µmol/L	c.389G>A,p.Cys130Tyr (class IV variant) Homozygous
2 57 y.o. male	22.0%	295.7 µmol/L	c.757del,p.Ala253Glnfs*27 Homozygous
3 (Figure 1C) 55 y.o. female	9.1%	60.4 µmol/L	c.757del,p.Ala253Glnfs*27 Homozygous
4 25 y.o. male	6.6%	58.6 µmol/L	c.757del,p.Ala253Glnfs*27 Complex rearrangement in *SORD/SORD2P* Compound heterozygous
5 50 y.o. male	6.4%	58.5 µmol/L	c.757del,p.Ala253Glnfs*27 c.786 + 5G>A,p.? (class IV variant) Compound heterozygous

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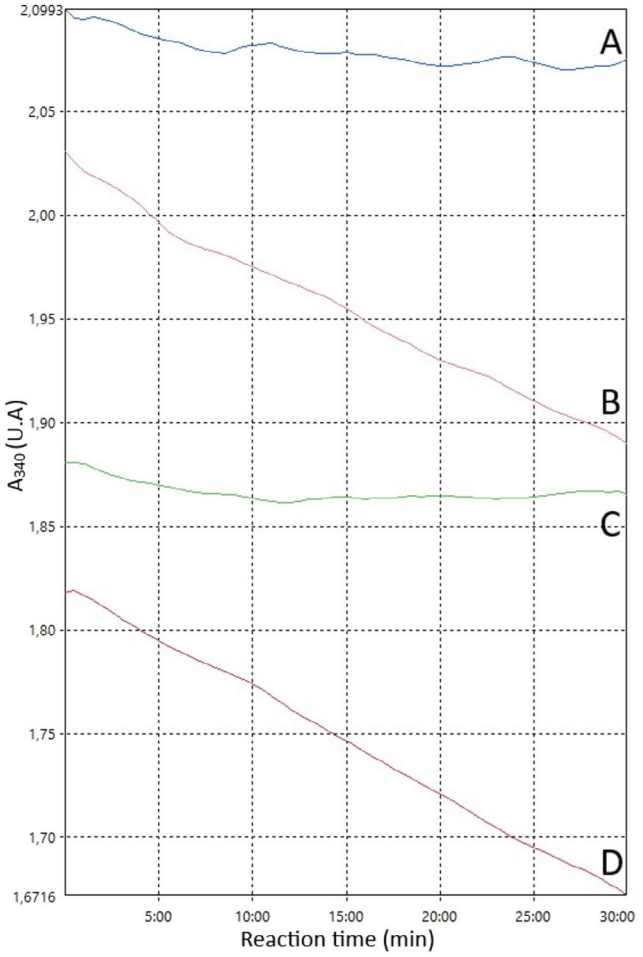

FIGURE 1 | Spectrophotometric determination of red blood cell SORD activity. Variations in absorbance at 340 nm (A_{340}) in samples from 4 individuals (A–D) was measured. Individual A is known to be SORD-deficient (Table 1, Individual 1). Individual B is an individual with neuropathy of unknown etiology and normal plasma sorbitol concentration. Individual C has a strong clinical suspicion of SORD deficiency, and increased plasma sorbitol concentration (Table 1, Individual 3). Individual D is a within-run control obtained from a leftover sample from routine laboratory activity. The enzyme activity of A is markedly decreased compared to the within-run control (13.8%), as is the enzyme activity of C (9.1%). Using the cutoff < 30%, both results are compatible with SORD deficiency. The enzyme activity of B is comparable to the within-run control (90.0%), which is not in favor of SORD deficiency.

We furthermore evaluated the stability of SORD activity in four lithium heparinate tubes stored at +4°C. The first measurement was realized at 24 h after sampling (this corresponds to the typical delay when the samples are sent from another hospital). Subsequent measurements were performed 6, 10, and 14 days after sampling.

2.1 | Standard Protocol Approvals, Registrations and Patient Consents

This study used data from routine patient care and did not require institutional review board approval. Informed consent was obtained from patients for genetic analyses, as part of routine care.

3 | Results

In all controls, a linear decrease of A_{340}—A_{700} was observed (positive ddA/min; e.g., Figure 1D). In comparison, the three individuals with known SORD deficiency had markedly decreased/null ddA/min, and therefore, markedly decreased RBC SORD activity (Table 1; example run: Figure 1A). In the two individuals with strong suspicion of SORD deficiency, activities were also markedly decreased (Table 1; example run: Figure 1C). Their plasma sorbitol concentrations were found to be increased, compatible with SORD neuropathy (Table 1). When grouping these five individuals, median RBC SORD activity was 9.1% of the within-run control (range: 6.4% to 22.0%; SD: 6.5%).

In contrast, the 19 individuals with neuropathy or myopathy of unknown etiology showed enzyme activities ranging from 55.5% to 236.3% (median: 89.7%, SD: 38.5%) (Figure 1B). The 236.3% sample was identified as a statistical outlier; excluding this point, the range, median, and SD were 55.5 to 119.3%, 86.5%, and 17.7%, respectively for these individuals. These patients' plasma sorbitol concentrations were found to be normal, which was not in favor of SORD deficiency. The person with a homozygous exon 8 deletion in SORD from WGS showed similar activity (76.0%) to these 19 individuals, suggesting the incidental WGS finding was suspicious. Altogether, enzyme activities in individuals with SORD neuropathy were significantly lower ($p = 0.00006$, excluding the outlier) than activities in individuals with neuropathy or myopathy of unknown etiology.

An RBC SORD activity cutoff of < 30% achieved 100% sensitivity and specificity for detecting SORD deficiency (95% CI: 47.8–100% and 82.3–100%, respectively).

In Individual 3, genetic analysis confirmed the presence of a known pathogenic SORD variant at the homozygous state. In Individual 4, a complex rearrangement involving *SORD* and *SORD2P* was found in *trans* of a known pathogenic variant, confirming deficiency.

Regarding the stability of SORD activity in blood samples prior to analysis, median loss (range) of activity compared to Day 1 were −2.5% (−0.1% to −10.3%), −11.7% (−2.8% to −21.1%) and −24.8% (−7.9% to −32.1%) at Days 6, 10, and 14 respectively, indicating that the preanalytical stability is of at least 6 days.

Finally, the cost of analysis was found to be much lower than that of LC–MS/MS in our laboratory (see Supporting Information).

4 | Discussion

SORD activity measurement in RBC is markedly decreased in individuals with known deficiency but not in individuals with neuropathy or myopathy of unknown etiology and normal plasma sorbitol concentration. Normal plasma sorbitol levels combined with only a slight decrease in enzyme activity led to a closer examination of the genetic results for one person who had an incidental finding of a deletion in WGS. It was discovered that the deletion was actually in *SORD2P* and not in *SORD*,

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ruling out any deficiency. This was due to misinterpretation of data due to a misalignment of short reads from the pseudogene.

Analytical validation is still required, and the proposed cutoff of less than 30% for deficiency detection should be reevaluated after more individuals are analyzed. It is also unclear whether intermediate activity levels in heterozygotes might result in false positives, although this risk is lessened since plasma sorbitol would be normal in such cases. Nonetheless, this enzymatic assay appears to be a convenient tool for biochemical screening of SORD deficiency, without pre-analytical difficulties (i.e., at least 6 days of stability at +4°C and no need for sample centrifugation). It is also inexpensive, especially compared to LC–MS/MS (see Supporting Information). The reference laboratory can perform this test alongside plasma or urine sorbitol measurement by MS, offering complementary data and direct enzyme function assessment, which is useful when VUS are detected in genetic analyses (PP4 criterion of ACMG guidelines [5]). Pending confirmation on a larger number of samples, this assay could also serve as a quick first-line test in routine laboratories (turnover time: about 2 h). This could allow for prompt care while awaiting genetic confirmation (which can take up to a year).

For laboratories wishing to implement this assay, the updated version of the protocol is detailed in the Supporting Information.

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Disclosure

The authors have nothing to report.

Ethics Statement

Ethical approval was not sought for the current study because all results are derived from routine patient care.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Sorbitol concentration in the reaction media of three random routine care leftover samples at three timepoints, measured by MS. This was done in three conditions: full reaction mix, reaction mix lacking fructose, and reaction mix lacking NADH.

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