Cerebrospinal fluid characteristics of patients treated with intrathecal nusinersen for spinal muscular atrophy

Rotem Orbach MD[1,2] | Liora Sagi MD[1,2] | Efraim Sadot MD[2,3] | Itay Tokatly Latzer MD[1,2] | Anna Shtamler BSN, MHA[1] | Tehila Zisberg BA[1] | Aviva Fattal-Valevski MD, MHA[1,3]

[1]Pediatric Neurology Institute, Dana-Dwek Children's Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

[2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

[3]Pediatric Intensive Care Unit, Dana-Dwek Children's Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Correspondence
Rotem Orbach, MD, Pediatric Neurology Institute, Dana-Dwek Children's Hospital, Tel Aviv Sourasky Medical Center, 6 Weizmann St., Tel Aviv, 6423906, Israel.
Email: rotemorb@gmail.com

Abstract

Introduction/Aims: There is limited information on the potential effects of repeated intrathecal antisense oligonucleotide drug delivery on cerebrospinal fluid (CSF) biochemical and blood cell profiles. This study aimed to examine longitudinal changes in the biochemical components (glucose, protein) and blood cell counts in the CSF of spinal muscular atrophy (SMA) patients treated with intrathecal nusinersen.

Methods: We collected and analyzed clinical and CSF parameters (cell count, protein, glucose, culture) of 50 individuals with SMA during nusinersen treatment (22 type 1, 17 type 2, and 11 type 3).

Results: The median protein concentration at baseline and during treatment was within the normal range but rose during treatment and was significantly above baseline at the time of the ninth intrathecal injection ($p = 0.02$, two-tailed Wilcoxon matched-pairs test, and $p = 0.0015$, Friedman test for repeated measures). Further analysis showed that the increase in CSF protein concentration was evident for SMA types 2 and 3 patients, but not for type 1. This observation was also demonstrated by a significant correlation between the *SMN2* gene copy number and the increase in CSF protein concentration (Spearman rank correlation test).

Discussion: Our results demonstrate that a delayed increase in CSF protein concentration is expected during nusinersen treatment for SMA types 2 and 3. This might reflect the medication's effect and a possible therapeutic biochemical marker.

KEYWORDS
biomarker, cerebrospinal fluid, nusinersen, pediatric, spinal muscular atrophy

1 | INTRODUCTION

The development of nusinersen, an antisense oligonucleotide that modifies pre-mRNA *SMN2* splicing, has changed the course and prognosis of spinal muscular atrophy (SMA).[1–5] Pre-mRNA *SMN2* modification induces better expression of the functional SMN protein, which is necessary for the survival of anterior horn cells, playing an essential role in RNA processing and metabolism.[6,7]

The intrathecal administration of nusinersen, requiring the removal of cerebrospinal fluid (CSF) before injecting the medication, has led to the emerging field of CSF biomarker studies in SMA.[8,9] Ever since the US Food and Drug Administration (FDA) approved nusinersen in 2016, numerous studies attempting to identify biomarkers of clinical significance have been published.[10–16] The CSF protein composition is unique: while most plasma proteins are excluded by the blood-CSF barrier, the choroid plexus and the brain parenchyma comprise an exclusive source for

List of Abbreviations: CHOP INTEND, Children's Hospital of Philadelphia INfant TEst of Neuromuscular Disorders; CSF, cerebrospinal fluid; HFMSE, Hammersmith Functional Rating Scale Expanded; HINE, Hammersmith Infant Neurological Examination; RULM, Revised Upper Limb Module; SMA, spinal muscular atrophy; SMN, survival motor neuron.

certain proteins, such as transthyretin, tau, NSE, S-100B, cystatin, and more.[17]

The influence and effects of repeated intrathecal injections of a synthetic oligonucleotide, such as nusinersen, on blood–brain-barrier function and CSF content, are not well established. Studies by Wurster et al. showed an increase in the total CSF protein in the spinal fluid of adults diagnosed with SMA types 2 and 3.[15,18]

This study aimed to evaluate routine biochemical components (glucose, protein) and cell counts in the CSF of SMA patients treated with nusinersen. Our hypothesis is that repeated intrathecal administration of nusinersen might cause detectable changes within basic CSF parameters in relation to protein synthesis pathways induced by the medication or, alternatively, by inflammation/blood–brain-barrier breakdown caused by the repeated punctures.

2 | METHODS

2.1 | Patients and samples

The medical records of genetically confirmed 5q-associated SMA patients treated with nusinersen in the Dana-Dwek Children's Hospital and routinely followed-up at the SMA multidisciplinary clinic were reviewed. Clinical and routine laboratory spinal fluid data, as well as specific functional motor test scores, were collected at baseline and after approximately 1 y of treatment. The functional motor tests were performed by the multidisciplinary SMA clinic physical therapists (done routinely every 4 mo) and included the Children's Hospital of Philadelphia Infant Test of Neuromuscular disorders (CHOP INTEND) and The Hammersmith Infant Neurological Examination (HINE) for the SMA type 1 patients, and the Hammersmith Functional Rating Scale Expanded (HFMSE) and the Revised Upper Limb Module (RULM) for the SMA types 2 and 3 patients.

The intervals between CSF sampling followed a globally recognized treatment protocol for loading and maintenance intrathecal administration of nusinersen.[1,19]

CSF samples were collected by routine inter laminar lumbar puncture, or, if necessary, by a computerized tomographic (CT)-guided transforaminal approach.

2.2 | Sample exclusion

To assure the quality of the results, we excluded samples with more than 500 red blood cells ([RBCs]/μL) or samples with missing RBC information.

2.3 | Statistical analyses

Statistical analyses were performed by GraphPad prism version 8.1.2 (San Diego, CA) to recognize exceptions compared to known norms and individual changes in CSF protein, glucose, and cell counts concentrations during treatment. Considering the non-normal distribution of the measured variables (Kolmogorov–Smirnov test), non-parametric tests (paired Wilcoxon matched-paired signed Rank test, Friedman test, and Spearman rank test) were applied for analytic statistics with a two-sided p value ≤0.05 as the threshold for statistical significance.

The study was approved by the Ethics Committee of the Tel Aviv Sourasky Medical Center, which waived informed consent for this retrospective analysis.

3 | RESULTS

The cohort included 50 SMA patients, of whom 22 (44%) were diagnosed with SMA type 1, 17 (34%) with SMA type 2, and 11 (22%) with SMA type 3. Their demographic and clinical characteristics are given in Table 1. The median number of intrathecal injections per patient was nine (Table 1). Considering all standing quality criteria samples, a total of 402 CSF samples comprised the basis for the descriptive/analytical statistics. Table 2 provides the number of patients included in each subgroup analysis.

The median WBC count at baseline (i.e., before treatment initiation) and during treatment was 0 cells/mm^2. Significant pleocytosis of more than 5 cells/mm^2, was present in only three samples of different patients, all of whom had glucose and protein levels within the normal range and sterile cultures. The median protein concentration at baseline and during treatment was within the normal range, but increased during treatment, rising significantly compared to baseline by the ninth injection (Table 2, Figure 1, and Supplementary Figure 1). The Friedman test for repeated measures also demonstrated a significant increase in the CSF protein content ($p = 0.0015$).

Kruskal-Wallis test at baseline (S1) excluded any significant differences of protein concentrations between the SMA types before treatment initiation ($p = 0.17$). With time, statistical differences between the subtypes appeared starting from S7 (Table 2). The number of *SMN2* copies correlated significantly with the increase in protein (Spearman rank: $r = 0.51$, $p = 0.03$, CI 0.06–0.79, n = 19). There were no significant correlations between the degree of protein increment and the improvement in the functional motor test scores, that is, CHOP/HINE for SMA type 1 and HMFCS and RULM for SMA types 2 and 3. Finally, age as a potential modifier was not correlated with protein level at baseline in any of the SMA groups.

4 | DISCUSSION

This study shows that over the first 22-mo treatment period with nusinersen, a gradual increase in CSF protein concentration can be expected for SMA types 2 and 3 patients, but not for SMA type 1.

Analyses of repeated measures revealed a significant increase in the CSF protein concentration. This finding confirms and expands the results of Wurster et al.,[15] who indicated an increase of total protein in the CSF after the fourth administration of nusinersen in patients with

TABLE 1 Summary of the clinical characteristics of the study group

	Total cohort (n = 50)	SMA type 1 (n = 22)	SMA type 2 (n = 17)	SMA type 3 (n = 11)
No. of intrathecal injections, median (range)	9 (2–13)	9 (5–13)	9 (2–11)	9 (7–10)
Gender (male, %)	21 (42)	8 (36)	9 (53)	4 (36)
Age of symptoms onset, y median (range)	0.5 (0–2.2)	0.08 (0–0.5)	0.7 (0.3–1.3)	1.5 (0.8–2.2)
Age at treatment initiation, y median (range)	7 (0.08–21)	1.7 (0.08–20.8)	11.5 (1–19.3)	10.8 (3.8–17.3)
SMN 2 copies, n (range)	3 (2–4), n = 33	2 (2–3), n = 13	3 (2–4), n = 13	4 (2–4), n = 7
Gastrostomy tube feeding, n (%)	10 (20)	9 (41)	1 (6)	0 (0)
Tracheostomy, n (%)	9 (18)	9 (41)	0 (0)	0 (0)
CT guided intrathecal drug administration, n (%)	7 (14)	0 (0)	7 (41)	0 (0)
CHOP S1, median (range)	–	17 (1–45), n = 21	–	–
CHOP S5-S7, median (range)	–	31 (1–54), n = 20	–	–
HINE S1, median (range)	–	1 (0–4), n = 19	–	–
HINE S5-S7, median (range)	–	2 (0–12), n = 20	–	–
HFMSE S1, median (range)	–	–	5 (0–21), n = 15	49.5 (12–55), n = 10
HFMSE S5-S7, median (range)	–	–	7 (1–21), n = 17	55 (19–61), n = 11
RULM S1, median (range)	–	–	18 (3–22), n = 7	35 (25–37), n = 5
RULM S5–S7, median (range)	–	–	17 (2–24), n = 17	35 (29–37), n = 11

Abbreviations: S1–S7, from baseline to seventh injection.

TABLE 2 CSF protein concentration (mg/dL) in SMA patients over time

CSF sample	Total SMA (n = 50)	SMA type 1 (n = 22)	SMA type 2 (n = 17)	SMA type 3 (n = 11)	*p* value[a]
S1	15 (14–21.5)	18 (14–28.5)	16 (12–17.5)	16.5 (14–20.25)	0.17 (n = 45)
S2	18 (15–25)	18 (15–31)	18 (13.75–22)	17 (15.25–24.75)	0.83 (n = 43)
S3	17 (14–22.5)	18 (13.5–24)	15 (12.5–19)	17 (15–22)	0.52 (n = 45)
S4	17 (14–21)	17 (12.5–23.5)	16 (13.5–20)	18.5 (15.75–23.25)	0.33 (n = 44)
S5	17 (15–23.5)	17 (13.5–22.75)	17 (14.75–21.5)	18 (15–27.75)	0.71 (n = 46)
S6	20 (16.25–23.75)	17 (14–21)	20.5 (18.75–33.5)	22 (19–28)	0.06 (n = 44)
S7	19.5 (16–24.75)	17 (13.5–20)	21 (16.25–25)	24 (21–28)	0.009 (n = 40)
S8	20 (18–25)	18 (16–21.5)	23 (18.5–23)	22.5 (19.25–28.75)	0.04 (n = 35)
S9	23 (18–26)	19 (17–22)	25 (23.5–31.5)	23.5 (18.75–32)	0.02 (n = 26)
p-value[b]	0.02 (n = 25)	0.7 (n = 11)	0.008 (n = 8)	0.03 (n = 6)	

Note: S1–S9 = from baseline to ninth injection.
Note: Median (interquartile range), mg/dL.
[a]SMA subgroups (types) comparison by Kruskal-Wallis test.
[b]Two-tailed Wilcoxon test (S1 vs. S9).

SMA types 2 and 3. Paired Wilcoxon test of protein concentration at each time point versus baseline for the three SMA types revealed a rise in CSF protein concentration for SMA types 2 and 3, starting from the fourth injection, which was enhanced with each repeated intrathecal injection. Comparable result for the SMA type 1 group was not demonstrated. Given an identical treatment protocol for the three SMA subtypes, this finding makes it highly unlikely that the repeated intrathecal injections were the cause of the protein increase.

Comparison of protein concentration among type 1, type 2, and type 3 groups at baseline and at subsequent time points demonstrated that the protein concentration was not significantly different at baseline (S1) or at S2 to S6. This observation excludes that disease subtypes are associated with distinctive protein concentration. However, at the time of the seventh injection (S7) and after that, the protein concentration was significantly different among SMA groups which strengthens the hypothesis that nusinersen injections induce the increase in protein concentration and that it has a cumulative effect. In that context, it was not surprising to find a significant correlation between *SMN2* copy number and the observed changes in the CSF protein concentration.

The observation that nusinersen affects the protein concentration is consistent with the medication mechanism for altering the

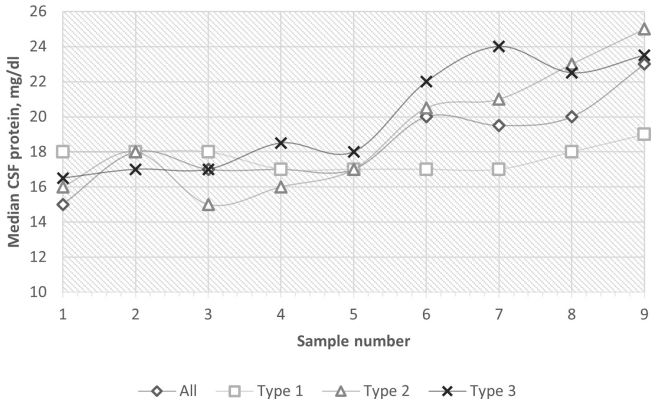

FIGURE 1 Changes in median CSF protein concentration at baseline and during nusinersen treatment. Nine time points are shown. The graph illustrates the trend of protein increase among patients with SMA types 2 and 3. At the same time, the protein concentration among patients with SMA type 1 remained stable. CSF protein concentration normal reference range is15–45 mg/dL

splicing of the *SMN2* gene mRNA, thus increasing the concentration of the functional SMN protein.[2,4] It is also consistent with the fact that the SMN protein plays a vital role in multiple fundamental cellular pathways, including mRNA trafficking and local translation.[6,20] We sought a correlation between clinical improvement, as reflected in specific functional SMA motor tests, with the degree of protein increase; no significant correlations were found, which substantially weakens the clinical importance of the main study observation. The relatively wide time range (S5 to S7) over which the specific motor tests were performed might explain the non-significant results. Individual factors, such as body weight, sex, age, and spinal hardware that might influence the CSF protein concentration[21] were eliminated by applying paired analytic statistics. Advancing age is associated with decreased CSF protein concentration, leading to accepted age-specific related normal references. In the current study, despite "aging" of the patients, an increase in the CSF protein concentration was observed, which strengths the magnitude of this observation.

The study is limited by the relatively small cohort for carrying out subgroup analyses. Unfortunately, concurrent blood samples or additional CSF samples to investigate the CSF protein by a mass spectrometric analysis or a targeted approach for biomarker candidates in SMA such as neurofilaments,[12] gilial fibrillary acidic protein,[22] or chitotriosidase 1[23] were not available.

To conclude, a delayed increase in CSF protein levels can be expected under nusinersen treatment for SMA types 2 and 3, but not for SMA type 1. This study suggests that this is associated with the medication's effect and is a biochemical marker of the therapeutic intervention.

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CONFLICTS OF INTEREST

None of the authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

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

ETHIS STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Rotem Orbach https://orcid.org/0000-0002-1015-1529

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SUPPORTING INFORMATION

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Associations of neuralgic amyotrophy with COVID-19 vaccination: Disproportionality analysis using the World Health Organization pharmacovigilance database

Jee-Eun Kim MD, PhD[1] | **Jin Park MD**[1] | **Young Gi Min MD**[2] | **Yoon-Ho Hong MD, PhD**[3] | **Tae-Jin Song MD, PhD**[1]

[1]Department of Neurology, Seoul Hospital, Ewha Woman's University College of Medicine, Seoul, Republic of Korea

[2]Department of Neurology, Seoul National University Hospital, Seoul, Republic of Korea

[3]Department of Neurology, Seoul National University Metropolitan Government Boramae Hospital, Seoul, Republic of Korea

Correspondence
Tae-Jin Song, Department of Neurology, Seoul Hospital, Ewha Woman's University College of Medicine, 260, Gonghang-daero, Gangseo-gu, 07804 Seoul, Republic of Korea.
Email: knstar@ewha.ac.kr

Abstract

Introduction/Aims: There are limited studies on the association of COVID-19 vaccination with neuralgic amyotrophy (NA). Therefore, we evaluated the association between COVID-19 vaccination and the occurrence of NA.

Methods: We explored unexpected safety signals for NA related to COVID-19 vaccination through disproportionality analysis using VigiBase, the World Health Organization's pharmacovigilance database.

Results: On October 15, 2021, 335 cases of NA were identified in the database. The median time to onset of NA after vaccination was around 2 weeks. A significant signal of disproportionality of NA was observed for the ChAdOx1 nCoV-19 vaccine (AstraZeneca) (information component $[IC]_{025} = 0.33$, reporting odds ratio $[ROR]_{025} = 1.30$) and two

Abbreviations: ADR, adverse drug reaction; CI, confidence interval; IC, information component; MedDRA, Medical Dictionary for Drug Regulatory Activities; NA, neuralgic amyotrophy; PT, preferred term; ROR, reporting odds ratio; WHO, World Health Organization.

Jee-Eun Kim and Jin Park contributed equally to this study.