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Children and young adults with spinal muscular atrophy treated with nusinersen

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ABSTRACT

Introduction: Treatment of children with spinal muscular atrophy (SMA) now includes disease modifying drugs such as nusinersen. Real-world data can provide new insight on the efficacy and safety of nusinersen for treatment of children with SMA.
Aim: The aim of our study is to evaluate the effect of treatment of children and young adults with SMA type I, II and III at various stages of the disease after 14 months of treatment with nusinersen.
Methods: In this prospective, two-center (in Slovenia and Czech Republic) study, data from all patients with a genetically confirmed diagnosis of SMA before 19 years of age who were treated with nusinersen were collected before initiation of treatment, and after 6 and 14 months of treatment. Various standardized motor scales and a questionnaire that focused on daily-life activities were used.
Results: Form both centers, 61 patients from 2 months to 19 years of age were enrolled in the study. Sixteen had SMA type I (median age 5.2 years); 32 had SMA type II (median age 8.9 years); and 13 had SMA type III (median age 8.6 years). Patients had 2-4 copies of the SMN2 gene. One patient died in the study period and one discontinued treatment. After 14 months of treatment, SMA type I ($p = 0.002$) and type II ($p = 0.002$) patients had significantly better outcomes, while type III patients showed a trend towards improvement ($p = 0.051$) on motor scales. Younger age at the initiation of treatment and a higher number of SMN2 copies is related to a better outcome. Younger children also seem to improve faster compared to older children. No serious side effects were reported.
Conclusion: The results of our study which included patients of various SMA types and stages of the disease suggest that treatment with nusinersen benefits patients, regardless of SMA type. Earlier age at the initiation of treatment and a higher number of SMN2 copies were related to a better outcome, however even some patients of higher age and/or later stage of the disease benefited from the treatment. Our study also suggests that nusinersen is safe to use, as no major side effects, requiring discontinuation of treatment, were reported. There is an unmet need for novel standardized tests and biomarkers, which could help guide clinician's decisions on the selection of best treatment options and monitor treatment success.

1. Introduction

Spinal muscular atrophy (SMA) is a rare neuromuscular disease, characterized by degeneration of the motor neurons in the anterior horn of the spinal cord, resulting in atrophy and weakness of the voluntary muscles of the limbs and trunk. Despite being a rare disorder, SMA is the most common genetic cause of infant mortality and a major cause of childhood morbidity, with a pan-ethnic incidence of 1/11.000 [1]. SMA has been categorized into types 0, I, II, III, and IV based on age of symptom onset and maximal achieved motor abilities, ranging in severity from a disease that affects newborns who are born with severe impairment and die within

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weeks of birth (Type 0) to a disease which manifests itself in adult life (Type IV).

SMA is transmitted in autosomal recessive manner and caused by mutations in the survival motor neuron 1 (SMN1) gene, encoding the SMN protein which is essential for motor neuron survival [2]. While SMN1 is the SMA-determining gene, SMN2 gene, a homologous pseudogene of SMN1, is the major modifier of SMA disease severity [3]. Both genes are located in the chromosomal region 5q13[1] and encode proteins with identical amino acid sequences. However SMN2 differs from SMN1 by 5 nucleotides [4]. One of these nucleotide differences occurs in exon 7 of the SMN2 gene, resulting in an alternative splicing pattern that favours skipping of exon 7 [5]. Eighty to 90% of the transcripts produced from the SMN2 gene lack exon 7, resulting in a defective protein, which rapidly degrades [6].

Humans have a variable number of copies of the SMN2 gene.[7]Although SMN2 copy number strongly inversely correlates with SMA disease severity [3], this correlation is not absolute, as many other factors related to splicing, transcription, mRNA stabilization, posttranslational modification and even exogenous factors can influence disease severity [8]. Increasing the levels of SMN proteins produced by SMN2 can have significant disease modifying effects [7].

Nusinersen is an antisense oligonucleotide which can increasing the amount of full-length SMN protein produced from the SMN2 gene by modulating its mRNA splicing pattern [9]. Treatment with Nusinersen can significantly alter the natural history of SMA and improves the motor function across SMA of all types [10]. It has been approved in Europe since June 2017. Prior to approval in Europe, Nusinersen was provided to patients with SMA type 1 within an Expanded Access Program (EAP), which was also available in Slovenia. Nowadays, nusinersen is already wildly available for most type of SMA patients, depending on the country of residence. As there are only limited clinical trial data in older SMA type I, II and III patients there is an enormous need for exact evidence of nusinersen efficacy, its variability and side effects in broader cohort of SMA patients [11].

The aim of our study is to evaluate the effect of treatment of children and young adults with SMA type I, II and III after 14 months of treatment with nusinersen.

2. Methods

The study and all experimental protocols were approved by University Children's Hospital in Ljubljana, the National Medical Ethics Committee of Slovenia (0120-160/2016-2) and the Medical Ethics Committee of the Motol University Hospital. Informed consent was obtained from all participants and/or their legal guardian/s.

2.1. Patients

In this prospective, two-center study, we collected data from all patients with a clinically and genetically confirmed diagnosis of SMA before 19 years of age who were treated with nusinersen either at the Department of Child, Adolescent and Developmental Neurology at the University Children's Hospital Ljubljana, Slovenia (SI center) or at the Department of Paediatric Neurology, Motol University Hospital, Prague, Czech Republic (CZ center). Both centers are part of the European Reference Network for Rare Neuromuscular Diseases (ERN EURO-NMD).

In Slovenia, the first child (SMA type I) started the treatment in March 2017 through the early access program. Four more children with SMA type I received the medicine through this program in the following months until nusinersen was approved by European Medicines Agency (EMA) and reimbursed by The Health Insurance Institute of Slovenia, which offered the treatment to all Slovene children with SMA, regardless of type and stage of the disease in May 2018. All children and eligible young adults were offered the treatment, and those who decided for treatment between March 2017 and finished 14-months treatment period by June 2020 were enrolled in the study.

In Czech Republic the first child started the therapy in December 2017. Before, seven Czech patients started nusinersen therapy abroad, within the early access program. After the EMA approval, it took 9 months to get the reimbursement for nusinersen therapy from Czech insurance companies, firstly only for children younger than 12 years, and since January 2020 for all types of SMA patients, adults included. In Czech Republic, there are four Centers for nusinersen therapy, our center is the biggest for pediatric patients, treating more than half of all Czech pediatric patients. All patients treated in our center that signed the consent form and started the therapy before November 2018 were enrolled in this study.

2.2. Evaluation

In all patients SMA was genetically confirmed and the number of SMN2 copies was analyzed. All patients were thoroughly evaluated before the initiation of treatment, before the 5th application (after 6 months) and before the 7th application (after 14 months). Before initiation of treatment, all patients were seen by a pediatric neurologist, pulmonologist, gastroenterologist, endocrinologist, and psychologist. The physical capabilities were tested by The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) [12], Hammersmith Functional Motor Scale (HFMS), Expanded Hammersmith Functional Motor Scale (HFMSE) [13] scales or Motor Function Measurement (MFM) [14] test. Testing was performed by a physiotherapist trained to use the tests, depending on the age and capabilities of the patient, before the treatment and at all follow-up examinations. The same test was used for all time-points in each particular patient.

After the 7th application, the parents were given a questionnaire on their observations of changes in their child's condition in several domains, such as movement, sleep, feeding, speech, etc. They were asked whether they noticed changes in any of the domains within the 14 months of treatment. The test we used was not a standardized quality of life test (QoL), as such test designed specifically for the needs of SMA patients does not yet exist, but we included questions in the test we believed have clinical relevance, according to our own experience.

2.3. Treatment with nusinersen

The intrathecal (IT) application of nusinersen was performed in all patients in controlled hospital environments. In patients with severe scoliosis, some of which also had scoliosis surgery and metal rod implantation prior to initiation of treatment, we have performed a 3D CT scan of the lumbosacral region prior to the first application, to better understand the local anatomy for easier IT application of the drug. An atlas of all patients was composed, where the details of each application were stored (position, needle type, location of needle entry relative to local topology and angle), to simplify later applications. In some patients IT application was performed under CT scan.

Most of the IT applications were given either using no sedation or under sedation with midazolam alone or in combination with ketamine. If the children were very anxious or the IT application was very difficult to perform, it could have been performed under general anesthesia. Local skin anesthesia with a lidocaine paste was however used in all patients prior to all IT applications. Children

were advised to be well hydrated for at least one day before the application.

Nusinersen was injected intrathecally on days 0, 14, 30, 60, 180, 300 and 420 in all patients in a standard dose of 5 ml (12 mg/ml), or appropriately reduced dose in smaller children, accordingly to the company's recommendations. After the application, all children were advised to lie prone for 2 h, to reduce the risk of post lumbar puncture (LP) symptoms. All children were monitored for potential side effects throughout the study.

Children were dismissed from the hospital the next day (after the first application) or in the evening of the day of application (all following applications).

2.4. Statistics

Due to different tests used differing in the number of total score points, percentage scores (number of points achieved from total score) were calculated per time point. Percentage points were logarithmized prior the analysis as antilogarithm of mean value yields geometric mean, the most appropriate measure of central tendency when percentages are calculated from different total score values. The difference between geometric mean is interpreted as % change in percentage scores between time intervals. Logarithmized values were used in further statistical analysis. Children form two study centers were compared in logarithmized percentage scores by Mann-Whitney U test. The difference in logarithmized percentage scores between baseline and 14 months of treatment per SMA group was tested by Wilcoxon test. Multilevel linear regression model with random intercept, time in months, age at first application and their interaction as independent variables and logaritmized percentage score as dependent variable was built to test effect of treatment duration, age of starting the treatment and their interaction on test score. Treatment duration (time in months) was included as continuous variable in the model to take into account unequal time intervals between the three visits. Equal variances at time points were presupposed (Levene test showed no statistically significant differences in variances between time points). To illustrate the interaction effect the children were divided in two groups according to their age at first application: the group bellow the mean age and equal or above mean age when starting the treatment. To investigate the differences within each age group (below average and average or higher) between time points further, multilevel linear regression modelling was repeated. Unequal spacing between time points was neglected and Sidak post hoc test was used to compare differences in logarithmized percentage scores between time points within each age group of children.

Linear regression analysis with difference in logarithmized percentage scores after 14 months of treatment in comparison to baseline as dependent variable and age of onset of treatment and logarithmized percentage score at baseline as independent variables was used to relate age of onset of treatment to the outcome after 14-months of treatment in each SMA type group.

Comparisons with $p < 0.05$ were considered statistically significant. SPSS, version 26 was used for statistical analysis (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Evaluation of patients before the first application of nusinersen

Altogether, 61 patients were enrolled in the study. Of 33 children and young adults eligible for treatment in SI center, 32 have decided to be treated. In CZ center, all 29 eligible children were enrolled in the study. All enrolled patients but one (CZ center) survived the study period; this female patient died suddenly at home at the age of 14 months. She was on therapy for 6m (5 applications), her death was sudden and unexpected, as she had no respiratory infection or other obvious complication. The family described the situation at home as sudden cardiac arrest which followed aspiration of patient's airways. One patient (SI center) discontinued treatment after 4 applications, as she did not consent with further IT applications of nusinersen.

General patient data before the first application of nusinersen, pooled from the two study centers, are summarized in Table 1.

Of the 61 patients, 10 had additional comorbidities: 3 had allergies to inhalatory allergens (of which one was on immunomodulatory therapy), one had Angelman syndrome, one had factor VII deficiency (and needed factor VII application 30 min prior to every IT application of nusinersen), one had gastro-esophageal reflux disease, and one had long QT syndrome. One of the patients had a traumatic right femur fracture for which he had osteosynthetic material implanted after the 5^{th} application of nusinersen.

Although we did not anticipate that populations of patients in the SI and CZ center would be identical, we analyzed the distribution of logarithmized motor scores of children from the two study centers. The logarithmized scores at baseline (before treatment) showed no statistically significant differences between the 2 centers ($p = 0.263$). Other parameters were not compared for this purpose.

3.2. Intrathecal application of nusinersen and complications

Intrathecal application of nusinersen in the lumbar region was performed in all patients at all 7 timepoints, except in the one patient who died (after 5 applications) and the one that discontinued the treatment (after 4 applications). Altogether, 422 IT applications of nusinersen were given. In most patients, nusinersen was given while patients were lying on their side or while sitting and supported by a nurse or a parent; in one child with severe skeletal abnormalities the application was performed in her mother's lap. In 5/61 patients the IT application was performed under CT surveillance (all from the CZ center). In one patient laminectomy L4/5 was performed prior to the first application due to an anatomical obstacle related to the previous spinal surgery.

Our cohort of patients was very diverse in regards of the time lag between the onset of SMA symptoms and the first dose of nusinersen received. The mean time lag in SMA type I patients was 77 ± 63 months (range, 1–173 months), in SMA type II patients 87 ± 56 months (range, 4–216 months), and in SMA type III patients 101 ± 64 months (range, 8–211 months). This data reflect the fact, that nusinersen became available at a time when many patients have already been diagnosed with SMA for months or even years.

Initial application was performed under general anesthesia in 5/61 children who were very anxious. In 3 of these children, latter applications were given under sedation only, while in 2 patients with a very advanced SMA type I all IT applications were performed under general anesthesia (both from SI center). In most patients, sedation with midazolam alone or in combination with ketamine was utilized prior to the IT application; however, some patients did not require neither anesthesia nor sedation.

No serious side effects were reported after any IT application of nusinersen, such as central nervous system (CNS) infection, bleed, paresis, hydrocephalus, thrombocytopenia, renal toxicity, etc. However, of all patients, 24/61 (39.3 %) reported minor side effects at some time-point(s) in the study period. Most of the side effects occurred in the loading phase of nusinersen treatment (first 4 applications / 2 months), with most side effects being observed after the first application (in 13 patients). Eight patients reported side

Table 1
Patient data at the first application of nusinersen. IV – invasive ventilation; NIV – non-invasive ventilation; N/A – not applicable; SMA – spinal muscular atrophy; SMN2 – survival motor neuron 2 gene.

	SMA type I	SMA type II	SMA type III	Total N
Number	16 (26.3)	32 (52.4)	13 (21.3)	61
Female	9 (56.3)	13 (40.1)	7 (53.8)	29 (47.5)
Median age (range in years)	5.2 (0.2–14.7)	8.9 (0.8–18.8)	12.6 (1.9–18.6)	8.6 (0.2–18.8)
Median weight (range in kg)	13.7 (6.0–45.0)	19.0 (6.4–62.0)	37.0 (10.0–58.0)	19.0 (6.0–62.0)
SMN2 copies				
2	10 (62.5)	1 (3.1)	0	11 (18.0)
3	5 (31.3)	25 (78.1)	8 (61.5)	38 (62.3)
4	1 (6.2)	6 (18.8)	5 (38.5)	12 (19.7)
Ambulation	0	0	6 (46.2)	6 (9.8)
Ventilation				
Not needed	6 (37.5)	26 (81.3)	13 (100.0)	45 (73.7)
NIV at night	4 (25.0)	4 (12.4)	0	8 (13.1)
NIV night & day	2 (12.5)	2 (6.3)	0	4 (6.6)
IV	4 (25.0)	0	0	4 (6.6)
Gastrostomy	5 (31.3)	3 (9.4)	0	8 (13.1)
Scoliosis	8 (50.0)	22 (68.8)	6 (46.2)	36 (59.0)
Operated for scoliosis	2 (12.5)	8 (25.0)	0	10 (16.4)

effects after more than a single application. Most of the side effects were related to LP, and were reported as follows: lumbar pain (10 patients), headache (8 patients), cerebral spinal fluid (CSF) leakage (4 patients), vomiting (4 patients), irritability (2 patients), rash at the site of LP (1 patient), leg paraesthesia (1 patient). All children with CSF leakage could not lie prone after the IT applications due to their spine deformities. A pillow was placed under their spines, which mostly stopped the leakage. In one patient the CSF leakage after the first application was marked and resolved within next ten days. All other symptoms related to the IT application subsided within the following days.

3.3. Motor capabilities

Patients were examined using one of the standardized motor scales before the initiation of treatment, and after 6 months and 14 months of treatment. Various scales were used, and some children were evaluated using more than one scale at all three time points. However, only one scale was selected for analysis for each particular patient. Of 61 patients, 33 were evaluated using CHOP-INTEND scale, 17 were evaluated with HFMS, 10 were evaluated with HFMSE, and 1 was evaluated with MFM (this patient started the therapy through the early access program in France and continued the treatment at the CZ center). Motor scores at 14m were not available in 2 patients: one died and one discontinued the treatment.

Geometric means of scores on motor scales and standard deviations at each time point, stratified by SMA type are shown in Fig. 1. There was a significant improvement in motor scales after 14 months of treatment in SMA patients type I and II, while type III patients showed a trend towards improvement, which was close to statistical significance. Children with SMA type II seem to benefit from the treatment less than children with SMA type I.

For type I patients, the geometric means of total score on motor scales were 17.0 % (± 5.1 %; range 0–68.8 %) at 0 months, 23.2 % (± 5.5 %; range 0–100 %) at 6 months and 27.5 % (± 4.7 %; range 0–96 %) at 14 months. Fort type II patients, the geometric means were 30.0 % (± 2.0 %; range 6.3–84.4 %) at 0 months, 33.4 % (± 2.1 %; range 6.3–96.9 %) at 6 months and 33.8 % (± 2.1 %; range 6.3–95.3 %) at 14 months. Fort type III patients, the mean percentages were 65.8 % (± 1.6 %; range 22.7–100 %) at 0 months, 75.0 % (± 1.4%; range 42.4–100 %) at 6 months and 78.0 % (± 1.3 %; range 45.5–100 %) at 14 months. There were significant differences in geometric means of total scores on motor scales between 0 months and 6 months for SMA types I, II and III ($p = 0.003$, $p = 0.001$; $p = 0.018$, respectively). Also, there were significant differences between 0 months and 14 months for SMA types I, II and III ($p < 0.002$, $p < 0.002$, and $p = 0.051$, respectively).

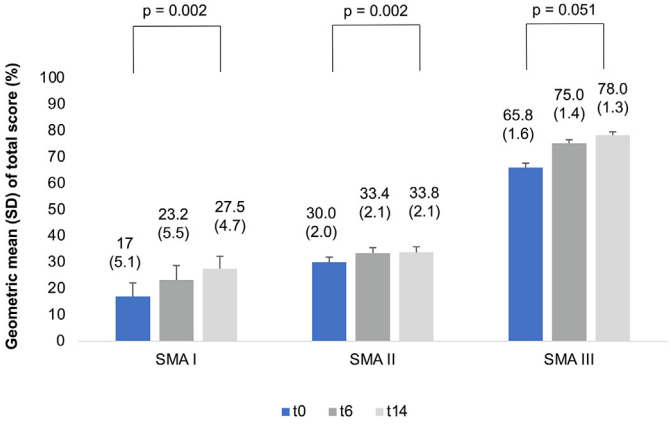

Fig. 1. Geometric mean (SD) of total score on motor scales at various time points, nested by SMA type. The bars represent geometric mean and the whiskers the SD. Lines above the bars represent significant difference in geometric means and an exact p value is given. t0 – before initiation of treatment; t6 – after 6 months of treatment; t14 – after 14 months of treatment.

Of all patients, 43/59 patients (72.9 %) showed improvement on the motor scales after 14 months of treatment with nusinersen; 7/59 (11.9 %) patients showed no overall improvement; and 8/59 (13.6%) showed a decline (Fig. 4). Of those, who showed improvement on motor scales at 14 months follow-up, this improvement was in the range from 1.3 % – to 68.7 % of the maximum score on a particular motor scale. Of the 7 patients with no change, 2 patients had 0% on the motor scales at all time points, 2 patients had 100% on the motor scales at all time points (one additional adult patient had 100% at first 2 timepoints and later discontinued treatment) and the other 3 patients had the same values (6.3 %, 7.5 % and 26.6 %) at all time points. Of those patients, who had worse scores at 14 months follow-up, 1 patient had SMA type I, 5 had SMA type II and 2 patients had SMA III. Two patients had transiently higher 6 months score, compared to the initial or 14 months follow-up evaluation. In some patients the decline could be partly attributed to progressive scoliosis (2 patients), progressive contractures (1 patient), increased weight gain (1), or poor collaboration on tests (1).

Multilevel linear regression model with random intercept, SMN copies, time in months, age at first application and interaction between time and age at first application as independent variables and logarithmized percentage score as dependent variable was built and results are shown in Table 2.

The scaled identity matrix with equal variances at each time points was presupposed. The decision seemed reasonable since there was no statistically significant difference in variances as tested by Levene test ($p = 0.951$). Results show that SMN copies, age at first application, treatment duration (time) and interaction between time and age at first application are statistically significantly associated with logarithmized percentage test score. Children with more SMN copies score higher on the test. Younger children score lower, with time they progress (the score on test increases) and this progress is moderated by the age of a child at first application. Children progress differently according to time when they receive first application of medication – younger children progress faster (gain more points on motor scales in the same amount of time) than older. The interaction effect is illustrated in Fig. 2.

To investigate the differences within each age group (below average and average or higher) between time points further, multilevel modelling is repeated. Unequal spacing between time points is neglected and Sidak post hoc test is used to compare differences in logarithmized percentage scores between time points within each age group of children. Children bellow 8.2 months of age progress statistically significantly after six months ($p = 0.001$) and after 14 months ($p < 0.001$) in comparison to baseline. The difference in progress between 6 months and 14 months is not statistically significant ($p = 0.820$). The difference in progress within age group of 8.2 or higher is not statistically significant after 6 months from baseline ($p = 0.692$), nor after 14 months from baseline ($p = 0.066$). Younger children seem to progress and benefit from the treatment much more and quicker in comparison to older children.

The longitudinal representations of percentages of maximum points on motor scales are presented on Fig. 3 for all SMA subtypes.

Multilevel linear regression model showed that treatment at an earlier age is related to a better motor outcome. When investigating this relationship using multiple linear regression in subgroups of various SMA types, we found that children with SMA type I that were treated earlier had a significantly better motor outcome ($B = -0.11$; $p = 0.008$). This relationship was not significant for SMA type II ($B = -0.002$; $p = 0.866$), or SMA type III patients ($B = -0.01$; $p = 0.480$). The youngest patient in our cohort (SMA type I, aged 2 months at the initiation of treatment) showed the highest improvement on the CHOP-INTEND scales going from 1 point before initiation of treatment to 45 points at 14 months follow-up (from 1.6 %–70.3 % of maximum points). Fig. 4 represents changes of percentage of maximum points on motor scales after 14 months of treatment related to age and nested by SMA type. All patients

Table 2
Effect of age at first application, treatment duration (time) and their interaction on physical capacities measured as logarithmized percentage score (results of multilevel linear regression model).

	Reg. Coeff. (SE)	P
Intercept	2.50 (0.62)	< 0.001
SMN2 copies	0.48 (0.20)	0.020
Age at first application	-0.06 (0.02)	0.016
Time (months)	0.03 (0.01)	< 0.001
Age x time	-0.002 (0.001)	0.007

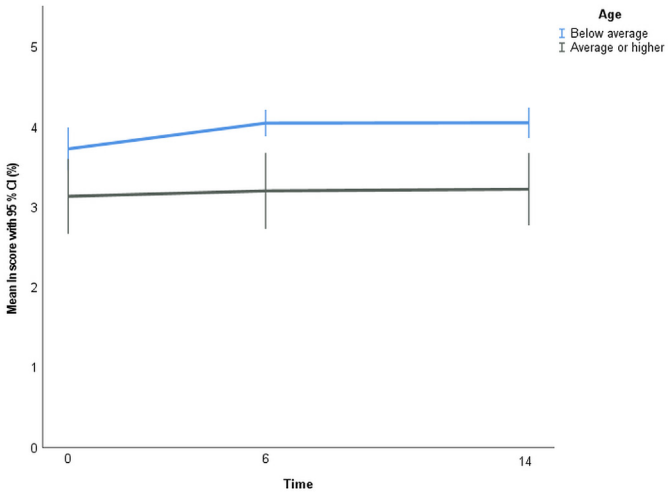

Fig. 2. Logarithmized percentage scores at each time point by age group when receiving first medicine application (mean age = 8.2 months).

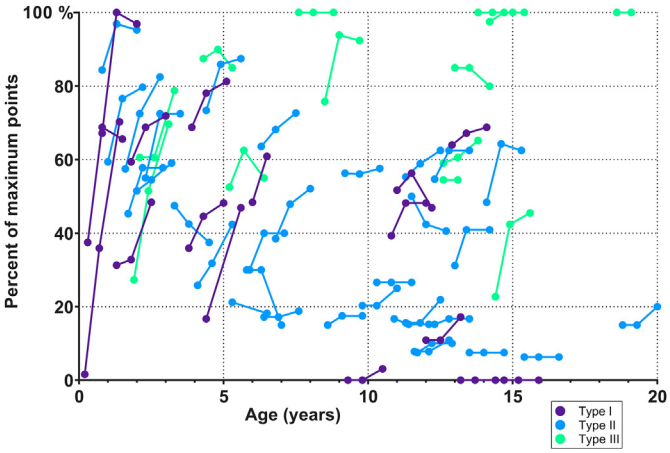

Fig. 3. Longitudinal representations of percentages of maximum points on motor scales at various time points (0, 6 and 14 months) for all SMA subtypes.

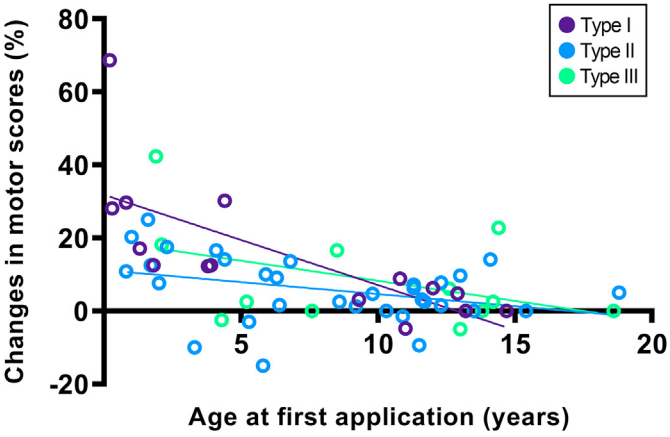

Fig. 4. Change in percentage of maximum points on motor scales between the pre-treatment evaluation and evaluation at 14 months. Lines represent regression lines and suggest that early initiation of treatment corresponds to a better outcome (statistically significant only for SMA type I patients).

that showed an increase in or showed no change in the study time were considered to be treatment responders.

3.4. Ambulation

None of the SMA type I or type II patients were ambulatory at the time of first application of nusinersen, while 6 (46.2 %) patients with SMA type III were. Altogether, only 6/61 patients (9.8 %) were ambulatory at the first application of nusinersen. In the 14-month study period, none of the patients lost ambulation.

In the SI center, 2 patients with SMA type I (2 and 4 copies of SMN2) were able to sit independently after 14 months of treatment; 2 patients with SMA type II were able to stand unsupported for several seconds (both 3 copies of SMN2); 1 patient with SMA type II was able to walk if supported for a short distance (4 copies of SMN2). Of the two patients with SMA type I in a very advaced stage of disease, both needing IV, who showed no movement before treatment except with their eyes, one of the patients could activate the gluteus muscles and could move one finger on both hands, while the other could keep the right leg flexed and head in the middle position if the parents set it in that position, showed some movement with facial and finger muscles (the mother reported she could observe some facial expression for the first time in his life and the patient can communicate by eye blinking now), and both needed less aspirations of the airways during the day.

In the CZ center, 4 patients with SMA type I gained head control (3 patients had 2 copies of SMN2 and one had 3); 3 patients were able to sit unsupported (2 patients had 2 copies of SMN2 and one had 3); 1 patient with SMA type II was able to go into sitting position by herself (3 copies of SMN2) and 2 were able to stand without support (both 3 copies of SMN2), of which one was newly able to move from wheelchair to bed by herself; of the SMA III patients, one re-gained walking after treatment (3 copies of SMN2) and one improved on the 6MWT (4 copies of SMN2).

3.5. Ventilatory support

Of all 61 patients, 45 (73.7 %) did not need any kind of ventilatory support at baseline. Eight patients (13.1 %) needed NIV during the night; 4 patients (6.6 %) needed NIV during day and night, and 4 patients (6.6 %) needed permanent IV. See Table 1 for distribution across various SMA types.

In the 14-month study period, 3 patients who did not need any kind of ventilatory support at baseline, started needing NIV during the night (1 patient was SMA type I, 2 SMN2 copies; 2 patients were SMN type II, 2 and 3 SMN2 copies). Additionally, 1 patients that needed NIV during the night before the first application, started needing NIV day and night after 14 months. There were no additional patients needing IV after 14 months of treatment. No patients could be weaned of NIV or IV in the 14-month study period.

3.6. Scoliosis

Before the first application, 36/61 (59.0 %) patients had scoliosis (a degree of spinal column angulation of more than 30%). Of these, 10 patients (16.4 %) had been operated for scoliosis prior to the first application of nusinersen.

In the 14-month study period, 2 more patients developed significant scoliosis (1 SMN type I, 2 SMN2 copies and 1 SMN type III, 3 SMN2 copies). Four patients with scoliosis underwent surgical stabilization during the study. Of these 4, one patient also started using NIV during the night, and one patient, who was using NIV only during the night, started using NIV during day and night.

3.7. Feeding

To support feeding, 8/61 patients (13.1 %) needed gastrostomy. After 14 months, 1 additional patient (SMA type I with 2 SMN2 copies) needed gastrostomy.

3.8. Subjective measures of common daily activities

Patients and their caregivers were asked to evaluate changes in every-day life capabilities within 14 months of treatment, using a questionnaire we have designed for the purpose of the study. The families were asked whether their children showed any improvement (or worsening) in any topic in the designated time period. None of the caregivers reported worsening in the designated fields. The results are summarized in Table 3.

4. Discussion

This prospective, two-center study provides real-world data on the efficacy and safety of treatment with nusinersen of a heterogeneous group children and young adults with SMA. The results of our study, in which patients of various SMA types and stages of the disease were enrolled, suggest that treatment with nusinersen benefits patients, regardless of the SMA type, and that an earlier age at the initiation of treatment and a higher number of SMN2 copies are related to a better outcome. Furthermore, younger children seem to improve faster (gain more points on motor scales in the same amount of time) compared to older children. Also, our study suggests that nusinersen is safe to use, as no major side effects, requiring discontinuation of treatment, were reported.

We considered treatment to be successful in all cases where patients showed an increase or stable motor scores over the 14-month study period. In our study, treatment was successful in 84.8% of patients: we have seen improvement on motor scales in 72.9% and steady scores in 11.9 % of SMA patients all types. This is an important finding as it shows that SMA patients of all types and stages of the disease have significant potential for improvement in their motor capabilities, if treated with a disease modifying drug, such as nusinersen. This was true even if our patients received nusinersen years after they have been diagnosed with SMA, as most of our patients were not newly diagnosed patients with SMA. Furthermore, our study also suggests that younger patients benefited more from treatment, compared to older patients, which was particularly true for SMA type I patients. Early diagnosis and treatment is therefore crucial in SMA patients, which underlines the importance of proactive diagnostic approaches, such as neonatal screening of SMA.

Table 3
Improvement in daily-life capabilities as evaluated by caregivers after 14 months of treatment with nusinersen. The numbers represent patients that reported improvement in the respected field, with percentages in brackets.

Improvement in…	At 14 months follow-up, N (%)
Movement	39 (63.9)
Endurance	36 (59.0)
Head control	27 (44.3)
Wheelchair control	6 (9.8)
Breathing	14 (23.0)
Aspirations	2 (3.3)
Speech intensity	14 (23.0)
Feeding	16 (26.2)
Salivation	0 (0.0)
Stools	1 (1.6)
Sleep	2 (3.3)
Mood	7 (11.5)
Taking care of the patients	19 (31.1)

The SMN2 gene copies are pivotal for treatment with nusinersen, which modulates alternative splicing of the SMN2 gene, functionally converting it into SMN1 gene, thus increasing the level of SMN protein in the CNS. The results of our study confirm that higher SMN2 copy number is significantly related to better motor outcome. Based on this result we could speculate, that a higher nusinersen dose could have been more beneficial. However, SMN2 copy number cannot be considered a reliable and the only predictor of patient's outcome. Patients with particular SMA type can have various number of SMN2 copies and the course of their disease can be uncertain [15]. One of our patients was a girl who started showing signs of hypotonia within 4 months of life and was diagnosed with SMA by 6 months. She has 4 copies of SMN2 gene, which is unusual for a child diagnosed so early, and was the first child treated with nusinersen in Slovenia at the age of 13 months. Her motor outcome was extremely favorable in spite of the fact that she was treated later than she would be nowadays.

The results of our real-world study are in concordance with the seed studies on treatment of early onset SMA [16] or later onset SMA [17] with nusinersen, as well with single center studies analyzing real-word data [18]. All but one patient survive the study period, in spite of the fact that 14 months represents a long time period in the natural course of SMA type I patients. Furthermore, most of our patients showed improvement on motor scales and some of them showed a significant qualitative change, which could not have been expected relying on the natural course of the disease for a particular SMA type. Five SMA type I patients were able to sit independently after 14 months of treatment, and 5 of our SMA type II patients were able to stand for a short period of time independently of which one could even walk if supported, and one patient with SMA III was again able to walk with support. On the other side, the group of patients from our cohort who lost motor function in the study period, consisted mostly of patients with SMA type 2 who were older than 3 years. All of these patients presented with cofounding factors such as progressive scoliosis, severe contractures, some of them putting on weight, which may influence the scores on motor function tests. It was therefore not possible to claim, that they were non-responders to nusinersen treatment. This group of patients however represents a challenge for the future, as clearly more needs to be done to improve their outcome. Moreover, improvement in motor capabilities does not necessarily corelate with the pulmonary or feeding function. Two of our SMA type 1 patients started the therapy before the age of 1 year needed the NIV after 14-month therapy. One of these patients even need gastrostomy. These patients clearly underline the importance of care provided by a multidisciplinary team.

There is also a clear need to improve the measures with which we measure success of the treatment. The answers to the questionnaire that were given to the caregivers show that standardized tests for motor capabilities do not measure all important aspects of life of these patients and their families. Improvements in daily activities related to nusinersen treatment might be as important as motor capabilities. For example, the improvement of voice intensity is important for the caregivers, as they may leave their child unassisted in a different room and be sure at the same time, that they will be able to help them if needed. We have noticed such improvements already after 2 months of treatment and although these subjective measures can be heavily biased by the parents expectations, the need for novel tests with finer resolution for such changes are needed. This also underlines the need for novel biomarkers, which would provide a better insight into which patients are or are not responding to treatment and have attributes that could potentially decrease their optimal outcome. The current and future therapies of SMA make the landscape of SMA disease more complex, therefore biomarkers will be essential in deciding on optimal treatment or combination of treatments, following progress of treatment and sensing potential difficulties.

No severe side effects of treatment have been reported in any patient during the study period, while 39.3% of all children reported some side effects, most commonly lumbar pain and headache (8 patients). In patients mostly with severely deformed spine, CSF leakage can occur, but this complication can usually be effectively stopped with change in posture of the patient.

Our study has limitations. Real-word data were collected from two centers, however the number of patients was still relatively small and certain analyses were not possible, when patients were stratified in SMA subtypes or age groups. Larger cohorts of patients would be needed to better understand the effect of nusinersen treatment in patients with a slower progress of the disease. Also, patients were evaluated using different motor scales, which was a drawback for direct comparisons, although we minimalized this effect using percentage scores. The questionnaire used to detect finer changes in patients' wellbeing was also not standardized; a standardized quality of life test would be of value for further studies.

5. Conclusion

The results of our study demonstrate the effectiveness of nusinersen therapy in SMA patients of all types, treated in various stages of their disease. Younger age which is related to an earlier initiation of nusinersen treatment in our cohort of patients and a higher number of SMN2 copies were related to a better motor outcome, but older patients starting with the treatment later on can still benefit from the therapy with nusinersen. Our data underline the need for early diagnosis and immediate initiation of treatment, ideally with the help of newborn screening for SMA.

On the other hand, there are limitations to the motor improvement of treated SMA patients. The therapeutic expectations should be realistic before starting the therapy, and there is a clear need for proper education of families which may prevent their future frustrations. Progressive scoliosis or contractures can unfavorably impact physical capabilities even in responders to treatment. Furthermore, even prompt treatment of SMA patients may not prevent the need for ventilatory support and feeding difficulties in the future.

The standardized physical tests of motor capabilities also have limitations, as finer yet important changes that can impact patients' quality of life cannot be picked up by these tests. There is a need for novel standardized tests / questionnaires focusing on patient related outcomes measures as well as biomarkers, that could help guide the selection of patients for various treatment options, monitor success of treatment and detect treatment related problems.

Ethical approval and consent to participate

The study and all experimental protocols were approved by University Children's Hospital in Ljubljana, the National Medical Ethics Committee of Slovenia (0120-160/2016-2) and the Medical Ethics Committee of the Motol University Hospital. Informed consent was obtained from all participants and/or their legal guardian/s.

Consent for publication

All authors have consented to publication of this article.

Availability of data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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