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CLINICAL RESEARCH ARTICLE

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Impaired diaphragmatic motility in treatment-naive adult patients with spinal muscular atrophy improved during nusinersen treatment

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Abstract

Introduction/Aims: The leading clinical feature of 5q-associated spinal muscular atrophy (SMA) is symmetric, proximal muscle weakness. Muscles involved in ventilation exhibit a specific pattern of denervation: intercostal muscles are severely atrophic, whereas the diaphragm muscle is less affected. The aim of this study was to investigate the involvement of diaphragmatic function by ultrasound imaging in adult patients with SMA and to quantify dynamics of diaphragmatic function during nusinersen treatment.

Methods: Diaphragmatic thickness, thickening, and excursion during quiet breathing were assessed in 24 adult patients with SMA type 2 and 3 by diaphragm ultrasound imaging before and during nusinersen treatment and were correlated with spirometric parameters.

Results: Diaphragm thickness was not reduced, but increased in a remarkable proportion of patients, whereas diaphragm thickening and excursion were reduced in about 20% to 30% of nusinersen-naive, adult patients with SMA types 2 and 3. During 26 months of nusinersen treatment, diaphragm thickening fraction and excursion improved.

Discussion: Diaphragm ultrasound imaging can provide disease- and treatment-relevant information that is not identified during routine clinical assessments and may therefore be a valuable complementary outcome measure.

KEYWORDS
diaphragm ultrasound, diaphragmatic dysfunction, nusinersen, respiratory outcome, SMA

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale—Revised; BMI, body mass index; DTF, diaphragm thickening fraction; DUI, diaphragm ultrasound imaging; FEV_1, forced expiratory volume within the first second of forced expiration; FRC, functional residual capacity; FVC, forced vital capacity; HFMSE, Hammersmith Functional Motor Scale Expanded; IQR, interquartile range; L, left-sided; PEF, peak expiratory flow; R, right-sided; RULM, Revised Upper Limb Module; SD, standard deviation; SMA, spinal muscular atrophy; *SMN1*, *survival of motor neuron 1* gene (telomeric); *SMN2*, *survival of motor neuron 2* gene (centromeric); TV, tidal volume; Δabs, absolute difference.

1 | INTRODUCTION

5q-associated spinal muscular atrophy (SMA) is a rare neuromuscular disorder caused by a loss-of-function mutation of the survival motor neuron 1 gene (*SMN1*), leading to death of lower motor neurons.[1] In addition to the characteristic symmetric muscle weakness and

TABLE 1 Study group characteristics

	SMA ($N = 24$)
Age (years), mean (SD), min–max	37.2 (11.9), 17–57
Age of onset (years), median (IQR), min–max	1.5 (1.0–3.0), 0.5–15
Disease duration (year), mean (SD), min–max	34.0 (11.9), 15–54
Sex, *N* (%)	
Female	13 (54)
Male	11 (46)
SMA type, *N* (%)	
2	10 (42)
3	14 (58)
SMN2 copy number, (%)	
2	2 (8.3)
3	14 (58.3)
4	8 (33.3)
Weight (kg), mean (SD), min–max	58.0 (15.5), 27–90
Height (cm), mean (SD), min–max	162.5 (13.6), 140–187
BMI (kg/m^2), mean (SD), min–max	21.8 (5.0), 13.2–34.2
Scoliosis, *N* (%)	
Present	19 (79.2)
Not present	5 (20.8)
Spondylodesis, *N* (%)	
Present	15 (62.5)
Not present	9 (37.5)
Mobility, (%)	
Never able to walk	10 (41.7)
Lost ability to walk	9 (37.5)
Still able to walk	5 (20.8)

Abbreviations: BMI, body mass index; IQR, interquartile range; min–max, minimum-to-maximum values; SD, standard deviation; SMA, spinal muscular atrophy; *SMN2*, survival of motor neuron 2 gene.

atrophy, which preferentially affect proximal muscle groups and trunk,[2] distal muscle groups, bulbar function, and ventilatory muscles are also affected.[3] Depending on disease severity and progress, respiratory impairment ranges from mild to severe, eventually requiring mechanical ventilation in some cases.[4] In the natural history of the disease, respiratory function progressively declines in childhood after disease onset and respiratory failure is the major cause of morbidity and mortality in SMA type 1 and 2.[5] For those who survive into adulthood, respiratory function tends to stabilize during early adulthood, with different trajectories depending on SMA type.[6,7]

The diaphragm is innervated by the phrenic nerve. Its motor neuron somas are located in the anteromedial cluster at the C3 to C5 level and seem to be relatively resistant to *SMN1* mutation.[8] In contrast to the less affected diaphragm muscle, intercostal muscles have been found to be more denervated and atrophic in autopsy studies.[9–11] Particularly in SMA with earlier onset, this imbalance of ventilatory muscles results in a bell-shaped chest and characteristic "diaphragmatic breathing."[12,13] The diaphragm contributes most to inspiratory volume,[14] which may explain the relatively late respiratory failure in SMA type 1 and the remarkably preserved respiratory function in milder SMA types compared with the overall markedly reduced motor function. Due to the fact that ventilatory impairment is more distinct and more frequently fatal in patients with early onset, information on pathoanatomy has been generated by autopsy studies exclusively examining human postmortem tissue from patients with early-onset SMA. Nevertheless, ventilatory impairment also affects adult patients and patients with an initially less severe SMA type and may also lead to respiratory failure later in the course of disease. Therefore, additional outcome measures of respiratory function may be valuable in monitoring adult patients with SMA during disease-modifying drug treatment. Diaphragm ultrasound imaging (DUI) allows an assessment of diaphragm anatomy and function,[15] including assessment in adult patients with SMA.[16]

The aim of this study was to investigate the involvement of diaphragmatic function by ultrasound imaging in adult patients with SMA and to quantify the dynamics of diaphragmatic function during nusinersen treatment.

2 | METHODS

2.1 | Study design

This prospective, single-center, observational study was performed between September 2017 and September 2021 at the Department of Neurology of the University Hospital Carl Gustav Carus Dresden. Inclusion criteria were the presence of 5q-associated SMA with molecular genetic proof of homozygous deletion or other mutation in the *SMN1* gene, at least 18 years of age, treatment with nusinersen, and at least one DUI measurement after the DUI measurement at baseline before initiation of nusinersen. Exclusion criteria were missing baseline DUI measurement, spinal surgery, initiation of ventilatory support by invasive or noninvasive ventilation, or treatment discontinuation/change within the observation period. All participants gave informed consent. The study was approved by the institutional review board (EK 393122012).

2.2 | Diaphragm ultrasound imaging

The DUI measurement was done by a pulmonology specialist (S.L.) experienced in thoracic sonography at baseline before nusinersen treatment and was repeated once a year when possible. Missing measurements were due to shorter treatment period, treatment discontinuation, or switch to risdiplam. DUI was performed in the supine position during quiet breathing, using an ultrasound scanner (Xario 200 Model TUS-X200, Toshiba) and diaphragm thickness and excursion were measured and recorded. Patients were asked to breathe quietly and relaxed with normal effort, and diaphragm thickness was measured separately for the left and right sides in end-expiratory (functional residual capacity, FRC) as well as in end-inspiratory (tidal volume, TV) position at the zone of apposition (eighth/ninth intercostal space between the anterior axillary and midaxillary lines) with B-mode using a linear higher frequency transducer (7.5 to 10 MHz). Reference ranges were from Boussuges et al.,[17,18]

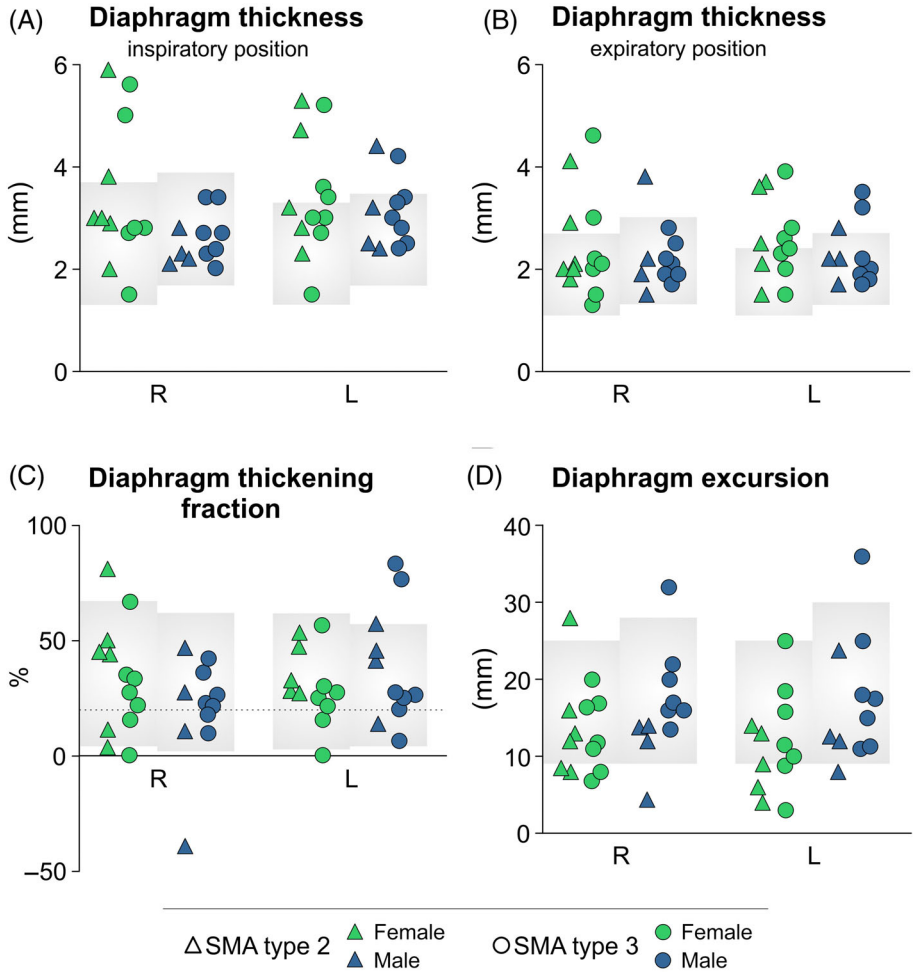

FIGURE 1 Diaphragm thickness (A, B), thickening (C) and excursion (D) of patients with SMA at baseline. Measurements were made for both sides of each patient (R = right side; L = left side). Icon color indicates sex of patients (green = female; blue = male). SMA type is illustrated by the shape of the icons. Gray areas represent the reference range of healthy controls as specified by Boussuges et al.[17,18] taking into account the sex of the patient and the side of measurement. Dashed line (in panel C) marks the cut-off for impaired diaphragm thickening.

TABLE 2 Baseline DUI measurements comparing SMA subtypes

Parameter	SMA type 2 ($N = 10$)	SMA type 3 ($N = 14$)	P value[a]
Thickness R (mm), mean (SD) Expiratory position	2.43 (0.88)	2.27 (0.81)	.841
Thickness L (mm), mean (SD) Expiratory position	2.48 (0.77)[b]	2.41 (0.71)	.829
Thickness R (mm), mean (SD) Inspiratory position	3.00 (1.16)	2.91 (1.17)	.709
Thickness L (mm), mean (SD) Inspiratory position	3.42 (1.10)[b]	3.14 (0.87)	.877
Thickening fraction R (%), mean (SD)	27.94 (33.21)	26.80 (15.98)	.585
Thickening fraction L (%), mean (SD)	38.48 (14.08)[b]	32.24 (24.17)	.179
Excursion R (mm), mean (SD)	12.97 (6.30)	16.24 (6.34)	.138
Excursion L (mm), mean (SD)	11.38 (5.77)[b]	16.17 (8.32)	.159

Note: DUI parameters assessed during quiet breathing.
Abbreviations: DUI, diaphragmatic ultrasound imaging; L, left-sided; R, right-sided; SD, standard deviation; SMA, spinal muscular atrophy.
[a]Calculated by Mann–Whitney *U* test (exact sign).
[b]Left-sided parameters were not measurable in one patient due to severe scoliosis and therefore special anatomical conditions.

who assessed a large cohort of healthy controls. They reported mean diaphragm thickness in end-expiratory position (FRC) of 1.9 mm for women and 2.1 mm for men at the zone of apposition on the right side and 1.7 mm for women and 2.0 mm for men on the left side. Lower and upper limits of normal were mean ± 1.95 standard deviations.[17,18] Based on the measurement of diaphragm thickness, diaphragm thickening fraction (DTF) during quiet breathing was calculated as follows:

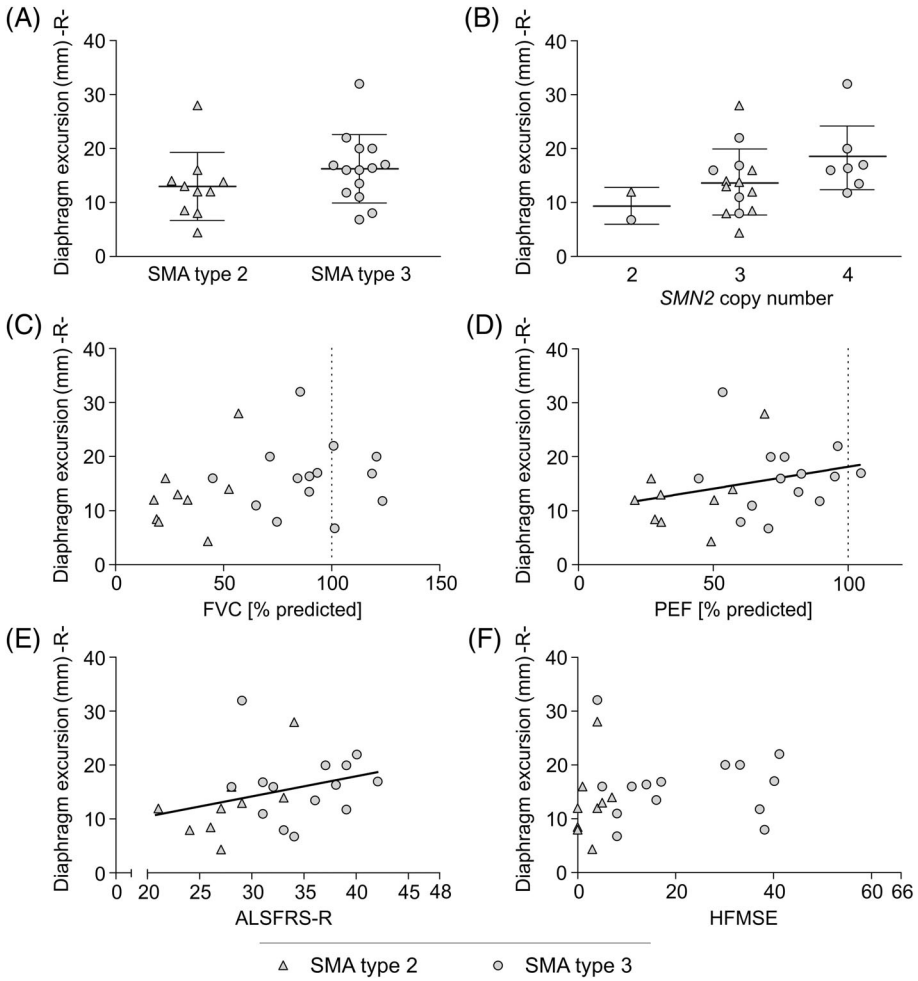

FIGURE 2 Association of right-sided diaphragm excursion (during quiet breathing) with disease severity (A, B, E, F) and spirometric parameters (C, D). Each icon represents a single patient; the shape of the icons distinguishes SMA types 2 and 3. Scatterplots include mean (black horizontal line) and standard deviation (whiskers) (A, B). Spirometric parameters are presented as the percentage of predicted value. The predicted value (100%) is indicated by the vertical, dotted line (C, D), and has been adjusted for patients' age, sex, and height (according to Criee et al.[22]). In the case of significance of the Spearman rank-order correlation coefficient (ρ), a regression line is shown to illustrate the association (D, E).

$$\text{DTF}\,(\%) = \frac{(\text{end-inspiratory thickness} - \text{end-expiratory thickness})}{\text{end-expiratory thickness}} \times 100\%$$

The DTF was shown to have a wide range in healthy subjects.[17,19] We considered a DTF of less than 20% to be reduced, as suggested previously.[20,21] Diaphragm excursion was measured separately for the right and left sides during quiet breathing, using a convex probe (3.5 MHz). In B mode, the hemidiaphragm was identified through the liver window on the right side or the spleen window on the left side, and then the amplitude of diaphragmatic excursion was recorded using M mode. Mean excursion was 17 to 20 mm in healthy subjects, with a lower limit of normal of 9 mm.[18]

2.3 | Nusinersen administration, spirometry, and clinical routine

Nusinersen was administered intrathecally according to the prescribing information every 4 months after four loading doses within 2 months (day 0, day 14, day 28, and day 63).

Spirometry was performed with each nusinersen administration sitting position using a spirometer (ML3500, MicroLab). Forced vital capacity (FVC), forced expiratory volume within the first second of expiration (FEV_1), and peak expiratory flow (PEF) were recorded. Because the spirometric values are influenced by age, sex, and height, we decided to adjust spirometric measures according to Criee et al.,[22] and to use the percentage of predictive value within the analysis instead of absolute values.

We aimed to capture disease severity by recording the number of *SMN2* gene copies and assessing motor function by using established motor scores (Hammersmith Functional Motor Scale Expanded [HFMSE][23] and Revised Upper Limb Module [RULM][24]). In addition, the ALS Functional Rating Scale—revised (ALSFRS-R)[25] was assessed at each visit. Motor scores comprise several items rating different motor skills with higher scores indicating better function.

2.4 | Statistical analysis

Statistical analysis and data visualization were performed using SPSS version 27 (IBM Corporation) and GraphPad Prism 5 (GraphPad Software, Inc.). Unless otherwise stated, data are presented as

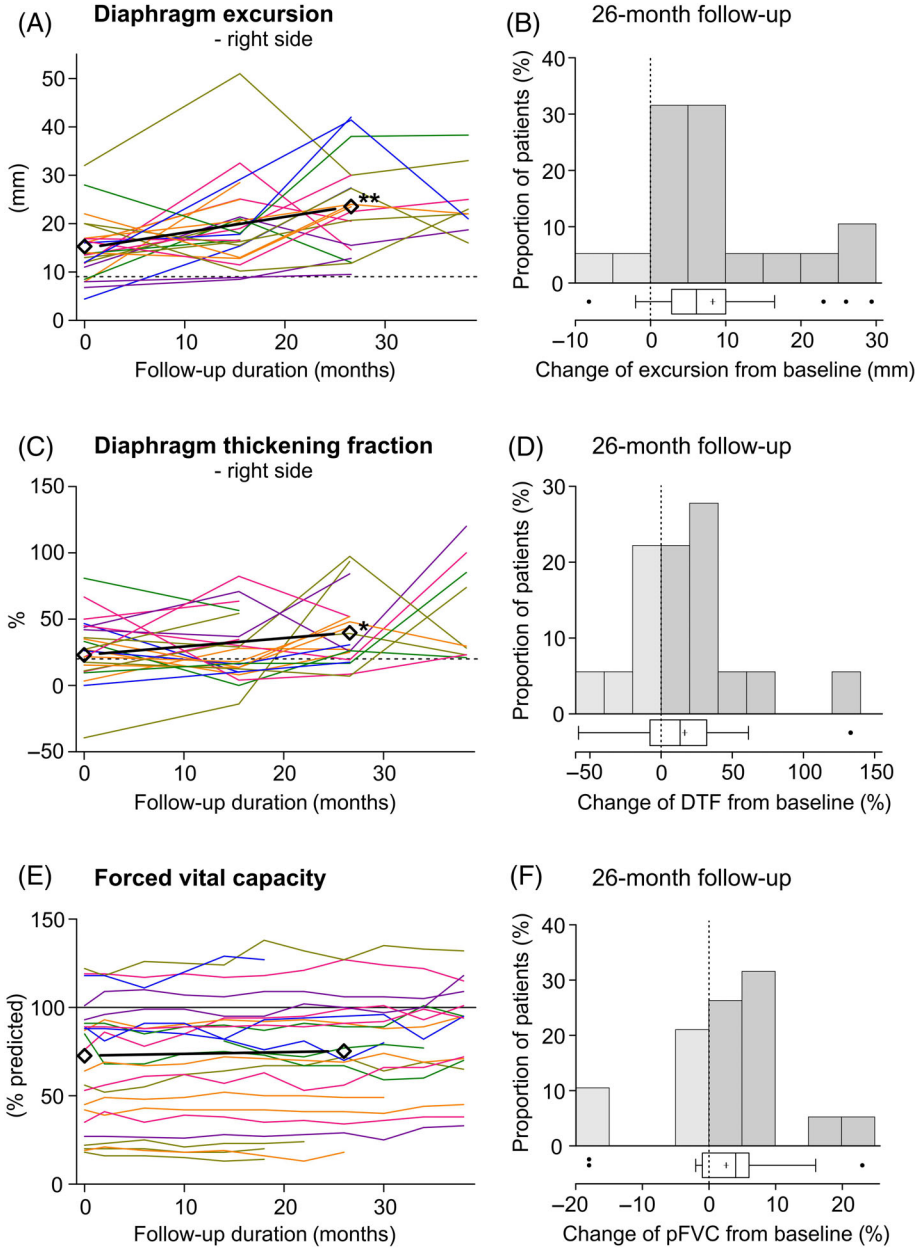

FIGURE 3 Change of diaphragm ultrasound imaging and spirometric parameters during nusinersen treatment. (A) Individual change of right-sided diaphragm excursion during up to 38 months. (B) Change of right-sided diaphragm excursion within 26 months, each bar represents the proportion of patients related to the extent of excursion change. (C) Individual change of right-sided diaphragm thickening fraction (DTF) during up to 38 months. (D) Change of right-sided diaphragm thickening fraction within 26 months, each bar represents the proportion of patients related to the extent of DTF change. (E) Individual change of forced vital capacity (percentage of predicted value) during up to 38 months. The predicted value (100%) is indicated by the solid, thin line and has been adjusted for patients' age, sex, and height (according to Criee et al.). (F) Change of forced vital capacity (FVC, percentage of predicted value) within 26 months, each bar represents the proportion of patients related to the extent of FVC change. (A, C, E) Each colored line illustrates an individual patient; bold black line indicates mean (diamond symbol) change within 26 months. Dashed horizontal line marks lower limit of the reference range. $^{*}P < .05$ and $^{**}P < .01$ calculated by Wilcoxon signed-rank test. (B, D, F) Box-and-whisker plots show median (central line), interquartile range (IQR; boxes), and 1.5 × IQR (whiskers), with individual points representing outliers outside of 1.5 × IQR from the median. + indicates the mean value. Dashed vertical line separates positive from negative changes.

mean ± standard deviation (SD). Some data were not normally distributed according to the Shapiro–Wilk test, and therefore we applied rank-based, nonparametric tests. The Spearman rank-order correlation was used to test for associations between variables. A correlation coefficient (ρ) of $\rho < 0.3$ was considered as a weak, $\rho = 0.3$ to 0.59 as a moderate, and $\rho \geq 0.6$ as a strong correlation (modified from Mukaka[26]). We used the Mann–Whitney U test to investigate group differences. Wilcoxon signed-rank test was employed for longitudinal analysis under nusinersen treatment. We set the observation period at 26 months to be able to include as much data as possible over a sufficient period of time. Data sets with missing values were excluded pairwise for cross-sectional and longitudinal analyses. Statistical significance was set at $P < .05$ (two-sided).

3 | RESULTS

Twenty-four adult patients with SMA were included in this study. Characteristics of the study group are presented in Table 1. DUI measurement was repeated after 15.5 months (SD = 2.2 months; follow-up visit 1; $N = 22$), 26.6 months (SD = 1.7 months; follow-up visit 2; $N = 19$), and 38.3 months (SD = 1.8 months; follow-up visit 3; $N = 9$).

3.1 | Diaphragm thickness and excursion of patients with SMA

At baseline, DUI measurements could be successfully performed in 23 patients. In one patient, left-sided parameters were not measurable

TABLE 3 Change of DUI and spirometric parameters during 26 months of nusinersen treatment

Parameter	*N*	Baseline, mean (SD)	Follow-up, mean (SD)	Mean difference vs. baseline, Δabs (Δ%)	*P* value[a]
Thickness R (mm) Expiratory position	19	2.44 (0.89)	2.59 (0.64)	+0.15 (+6)	.360
Thickness L (mm) Expiratory position	19	2.51 (0.76)	2.46 (0.55)	−0.05 (−2)	.615
Thickness R (mm) Inspiratory position	19	3.00 (1.24)	3.95 (1.86)	+0.95 (+32)	.087
Thickness L (mm) Inspiratory position	19	3.37 (1.00)	4.13 (1.54)	+0.76 (+23)	**.040**
Thickening fraction R (%)	19	23.11 (22.20)	50.48 (54.05)	+27.37 (+118)	**.040**
Thickening fraction L (%)	19	36.03 (22.09)	67.14 (50.47)	+31.11 (+86)	**.031**
Excursion R (mm)	19	15.28 (6.98)	23.55 (9.80)	+8.27 (+54)	**.001**
Excursion L (mm)	19	14.38 (7.77)	19.04 (8.32)	+4.66 (+32)	**.017**
FVC (% predicted)	19	72.73 (30.27)	75.60 (30.92)	+2.87 (+4)	.136
PEF (% predicted)	18	64.95 (20.69)	65.54 (20.81)	+0.59 (+1)	.811
FEV_1 (% predicted)	18	74.53 (28.99)	73.13 (27.70)	−1.40 (−2)	.523

Note: DUI parameters assessed during quiet breathing.
Abbreviations: DUI, diaphragm ultrasound imaging; FEV_1, forced expiratory volume within the first second of forced expiration; FVC, forced vital capacity; L, left-sided; PEF, peak expiratory flow; R, right-sided; Δ%, relative change (%); Δabs, absolute change (mm).
[a]Calculated by Wilcoxon signed-rank test, with significant values indicated in bold.

due to severe scoliosis. The majority of values were within the reference range established by Boussuges et al.[17,18] However, a remarkable proportion of the values showed an increased thickness of the diaphragm, whereas none of the values were below the lower limit of normal diaphragm thickness. The DTF and excursion were reduced in approximately 25% of the measurements and the excursion was increased only in single cases (for details see Figure 1 and Table S1).

3.2 | Right-sided diaphragm excursion correlated with disease severity

Although there was a difference between SMA subtypes in spirometric parameters (Table S2), none of the DUI parameters distinguished patients with SMA types 2 and 3 (Table 2). DUI parameters did not correlate with surrogate markers for disease severity (Table S3) with the exception of the right-sided diaphragm excursion, which correlated moderately with *SMN2* gene copy number, PEF, and ALSFRS-R score (Figure 2 and Table S3).

3.3 | Diaphragmatic motility increased during 26 months of nusinersen treatment

Although diaphragm thickness in end-expiratory position did not change during 26 months of nusinersen treatment, it did change in end-inspiratory position on the left. During 26 months of nusinersen treatment, diaphragm excursion and DTF improved and FVC remained stable (see Figure 3 and Table 3). It is noteworthy that, with the exception of a single patient, all dynamic parameters exceeded the lower limit of the reference range within 38 months (see Table S1 and Figure 3). Interestingly, one patient exhibited a paradoxical end-expiratory diaphragm thickening before start of nusinersen treatment, which normalized within our observation period (Figure 3C).

4 | DISCUSSION

This study has two main results: First, in nusinersen-naive, adult patients with SMA types 2 and 3, the diaphragm thickness did not indicate diaphragm atrophy. However, reduced diaphragmatic thickening and excursion in a proportion of patients suggested diaphragmatic dysfunction. This is in line with observations by Buonsenso et al.,[27] who similarly reported relative sparing of the diaphragm in children with SMA types 1, 2, and 3, but revealed a diaphragmatic dysmotility pattern in nearly three fourths of the patients with SMA type 1 and a gradient of diaphragmatic impairment depending on disease severity. Previous research reported preservation of the diaphragm due to the resistance of phrenic motor neurons to SMN protein deficiency.[8–12] For instance, in SMA type 1, autopsy studies have demonstrated that the size of diaphragmatic myofibers was only minimally altered, whereas myofibers of intercostal muscles were severely atrophic.[9] Notably, we found increased diaphragm thickness in a remarkable proportion of patients in our cohort, and we hypothesize that the diaphragm compensates for the impairment of intercostal muscles during inspiration and therefore changes structurally in terms of hypertrophy. Furthermore, spirometric parameters differed dramatically between SMA types 2 and 3, yet DUI parameters did not, suggesting that the ventilatory impairment is not caused by the diaphragm but due to the other respiratory muscles. This also explains the lack of correlation between DUI and spirometric parameters or motor scores and confirms that the diaphragm degenerates much more slowly compared with the other skeletal muscles. Interestingly,

diaphragm excursion correlated moderately with *SMN2* gene copy number and ALSFRS-R score as surrogate markers of disease severity, reflecting the progression of diaphragmatic dysmotility during the SMA disease course.

Second, diaphragm thickening and excursion representing its motility improved during nusinersen treatment, whereas diaphragm thickness at end-expiration remained stable. All but one patient regained normal diaphragmatic motility during nusinersen treatment and the spirometric parameters remained stable. These findings are consistent with those of others, who reported stable or improved respiratory function in adult patients with SMA types 2 and 3 treated with nusinersen.[28–32] In contrast, natural history data show that respiratory function declines slowly but steadily in adult patients with SMA types 2 and 3a and clarify that some patients become ventilator-dependent in adulthood.[6] Therefore, comprehensive monitoring of respiratory parameters is crucial for early detection of respiratory failure and to provide best medical care.

The main limitation of this study is its relatively small number of participating patients and short duration of the observation period, given that it was designed as a pilot study. In addition, DUI measurement may have been affected by severe scoliosis or chest deformity in some patients. We did not record detailed characteristics of scoliosis, such as the Cobb angle, and therefore did not include them in our analysis. The results of this pilot project must be confirmed in larger, multicenter trials conducted over longer observation periods and in methodological agreement with recent studies on the determination of normal values in healthy individuals.

5 | CONCLUSIONS

Diaphragm ultrasound imaging confirmed relative sparing of the diaphragm, even in long-standing SMA. However, diaphragm motility was reduced in about 20% to 30% of adult patients with SMA types 2 and 3, and was associated with disease severity. During nusinersen treatment, diaphragm motility improved and spirometric parameters remained stable. In conclusion, DUI can provide disease- and treatment-relevant information, which are not identified during routine clinical assessment and may therefore be a valuable complementary outcome measure.

AUTHOR CONTRIBUTIONS

Maren Freigang: Data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Simona Langner:** Data curation; formal analysis; investigation; methodology; validation; writing – review and editing. **Andreas Hermann:** Conceptualization; formal analysis; investigation; methodology; validation; writing – review and editing. **René Günther:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; validation; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

M.F. has received nonfinancial support and a speaker honorarium from Biogen outside the submitted work. A.H. has received personal fees and nonfinancial support from Biogen and Desitin for advisory board meetings. R.G. has received personal fees and nonfinancial support from Biogen, Hoffmann-La Roche, Zambon, and ITF Pharma.

DATA AVAILABILITY STATEMENT

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

ETHICAL PUBLICATION 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.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was approved by the institutional review board at the Technische Universität Dresden (EK 393122012).

INFORMED CONSENT STATEMENT

In accordance with the Declaration of Helsinki all subjects gave informed consent. For patients who were not able to write, consent was confirmed through an independent witness in the presence of the patient.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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