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# Spinal cord atrophy as a measure of severity of myelopathy in adrenoleukodystrophy

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## Abstract

All men and most women with X-linked adrenoleukodystrophy (ALD) develop myelopathy in adulthood. As clinical trials with new potential disease-modifying therapies are emerging, sensitive outcome measures for quantifying myelopathy are needed. This prospective cohort study evaluated spinal cord size (cross-sectional area - CSA) and shape (eccentricity) as potential new quantitative outcome measures for myelopathy in ALD. Seventy-four baseline magnetic resonance imaging (MRI) scans, acquired in 42 male ALD patients and 32 age-matched healthy controls, and 26 follow-up scans of ALD patients were included in the study. We used routine $T_1$-weighted MRI sequences to measure mean CSA, eccentricity, right-left and anteroposterior diameters in the cervical spinal cord. We compared MRI measurements between groups and correlated CSA with clinical outcome measures of disease severity. Longitudinally, we compared MRI measurements between baseline and 1-year follow-up. CSA was significantly smaller in patients compared to controls on all measured spinal cord levels ($P < .001$). The difference was completely explained by the effect of the symptomatic subgroup. Furthermore, the spinal cord showed flattening (higher eccentricity and smaller anteroposterior diameters) in patients. CSA correlated strongly with all clinical measures of severity of myelopathy. There was no detectable change in CSA after 1-year follow-up. The cervical spinal cord in symptomatic ALD patients is smaller and flattened compared to controls, possibly due to atrophy of the dorsal columns. CSA is a reliable marker of disease severity and can be a valuable outcome measure in long-term follow-up studies in ALD.

**Synopsis:** A prospective cohort study in 42 adrenoleukodystrophy (ALD) patients and 32 controls demonstrated that the spinal cord cross-sectional area

**Abbreviations:** ALD, X-linked adrenoleukodystrophy; AP, anteroposterior; CSA, cross-sectional area; DTI, diffusion tensor imaging; EDSS, expanded disability status scale; MRI, magnetic resonance imaging; RL, right-left; SSPROM, severity scoring system for progressive myelopathy.

Stephanie I. W. van de Stadt and Wouter J. C. van Ballegoij contributed equally to this work.

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of patients is smaller compared to healthy controls and correlates with severity of myelopathy in patients, hence it could be valuable as a much needed surrogate outcome measure.

**KEYWORDS**

cross-sectional area, MRI, myelopathy, neurodegeneration, spinal cord atrophy, X-linked adrenoleukodystrophy

## 1 | INTRODUCTION

X-linked adrenoleukodystrophy (ALD) is a rare inborn error of metabolism, caused by mutations in the *ABCD1*-gene.[1] Pathogenic *ABCD1*-mutations result in defective peroxisomal beta-oxidation causing accumulation of very long-chain fatty acids in plasma and tissues.[2] The clinical spectrum in male ALD patients ranges from isolated adrenocortical insufficiency to devastating cerebral demyelination (cerebral ALD).[3,4] Virtually all male and most female patients develop progressive myelopathy and peripheral neuropathy in adulthood.[5-7] Clinical features of this myelopathy include incontinence and a gait disorder due to spastic paraparesis and sensory ataxia.[8,9] Pathologically, there is axonal degeneration of mainly the corticospinal tracts and dorsal columns.[10,11] In men, symptoms usually become apparent in the third decade of life.[1] However, there is variability in age of onset and rate of progression of myelopathy, even within families.[7,12]

Currently, no disease-modifying treatment is available to halt or slow the progression of myelopathy in ALD, but new potential therapies are under development (eg, NCT03231878, www.clinicaltrials.gov). Clinical trials to determine efficacy are difficult because current measures for the severity of myelopathy are not sensitive to small changes in disease severity (requiring long studies with large numbers of patients),[7,13] are affected by floor and ceiling effects (which means patients at the extreme ends of the disease spectrum cannot be included in trials) and are not disease specific (ie, walking tests can be influenced by other diseases such as joint arthrosis). Therefore, new surrogate outcome measures are needed.

Spinal cord atrophy measured by conventional magnetic resonance imaging (MRI) has been studied in various neurodegenerative disorders. In diseases such as hereditary spastic paraplegias, multiple sclerosis and amyotrophic lateral sclerosis a significant smaller spinal cord cross-sectional area (CSA) was found in patients compared to healthy controls.[14-16] Furthermore, CSA was associated with disease severity and progression in multiple sclerosis.[17] In addition to CSA, morphometric spinal cord parameters, such as eccentricity, right-left (RL) and anteroposterior (AP) diameters, have been used for a more detailed description of structural changes in the spinal cord in neurological diseases like Friedreich's ataxia and amyotrophic lateral sclerosis.[18-21] Although spinal cord degeneration is the pathological hallmark of ALD and atrophy has been previously described,[22,23] only one dedicated study on quantifying spinal cord atrophy has been performed to date. Cervical and thoracic CSA was reduced 26%-40% in 13 ALD males compared to 12 healthy controls, but the degree of reduction did not correlate to a clinical disability or disease duration.[24] Confirmation of these data in larger cohorts and longitudinal data are lacking.

The main objective of this prospective cohort study was to quantify the degree of spinal cord atrophy in ALD. We measured different spinal cord MRI metrics (ie, CSA, eccentricity and RL and AP diameters). We correlated spinal cord CSA with conventional clinical outcome measures and evaluated this parameter as a potential surrogate outcome measure for the severity of myelopathy in ALD.

## 2 | METHODS

### 2.1 | Study design and patient selection

This study was part of a prospective cohort study ("the Dutch ALD cohort") performed at the Amsterdam University Medical Centres (location AMC, Amsterdam, The Netherlands), the national referral centre for ALD. Patients were recruited at the outpatient neurology clinic between June 2015 and February 2018. For this particular study, all men over 16 years of age were eligible to participate. We excluded patients with active cerebral ALD (defined as gadolinium-enhancing white matter lesions on cerebral MRI) or other neurological diseases interfering with the assessment of myelopathy. History, neurological examination and outcome measures were assessed at baseline and 1-year follow-up, as described previously.[7] The follow-up protocol of the natural history study was modified to include spinal cord imaging, therefore, for 16 of 42 patients only baseline MRI scans were available at the time of analysis. Healthy volunteers were 32 age-matched male individuals without any clinical evidence of neurologic disease. Written informed consent was obtained from

all participants. The study protocol was approved by the local Institutional Review Board (METC 2014_347). All procedures followed in this study were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

## 2.2 | Clinical assessment

All patients underwent a structured history, focused on symptoms of myelopathy, and extensive neurological examination as described previously.[7] Based on neurological history and examination, patients were classified as symptomatic or asymptomatic. Symptomatic patients were defined as having signs and symptoms of myelopathy.[5,7] Four outcome measures were used to assess the severity of myelopathy: Expanded Disability Status Scale (EDSS), Severity Scoring system for Progressive Myelopathy (SSPROM), timed up-and-go and 6-minute walk test. The EDSS measures neurological disability and ranges from 0 (no disability) to 10 (death).[25] The SSPROM scores symptoms of myelopathy and ranges from 0 to 100 with lower scores indicating a higher degree of impairment.[26] The timed up-and-go and 6-minute walk test are timed walking activities. Timed up-and-go measures the time to get up from an armchair, walk 3 m, turn around, walk back and sit down again.[27] The 6-minute walk test measures the maximum walking distance in 6 min.[28] Moreover, semi-quantitative measurements of vibration sense were performed with a Rydel-Seiffer tuning fork at the hallux and internal malleolus. All examinations, including MRI assessment, were performed on the same day.

## 2.3 | Imaging acquisition and measurements

Imaging of the cervical spinal cord was performed on a 3T MR scanner (Philips Ingenia; Philips Medical Systems, Best, Netherlands) with a 20-channel head-neck-spine coil. 3D $T_1$-weighted fast field-echo sequences were used for analysis. Detailed acquisition parameters included: 189 slices; field of view $256 \times 256 \times 170$ mm$^3$; voxel size $0.9 \times 0.9 \times 0.9$ mm$^3$; TE (echo time) 4.1 ms; TR (repetition time) 8.9 ms; acquisition time 04:17.3 min. Sagittal image reconstructions with voxel size $0.5 \times 0.5 \times 0.9$ mm$^3$ were used for spinal cord metric extraction. The outside body image background was out-thresholded from the region of interest. Then, the scan was bias-field corrected,[29] normalised to intensity value range from 0 to 1000 and resampled to isotropic voxel size $0.5 \times 0.5 \times 0.5$ mm$^3$ with a cubic spline interpolation method. Automatic axial 2D slice-by-slice spinal cord segmentation was performed with a "deepseg" method[30] followed by semi-automatic vertebral level labelling[31] where superior-inferior positions of all present inter-vertebral discs were manually marked. The segmentation, labelling and following quantitative spinal cord anatomy metric extraction were utilised with Spinal Cord Toolbox (SCT, version: 4.0.0).[32] Mean spinal cord CSA (mm$^2$), mean eccentricity, mean AP diameter (mm) and mean RL diameter (mm) were measured for each separate C1-Th2 level for all participants. Eccentricity is a mathematical measure characterising the shape of a conic section, such as an ellipse approximating the spinal cord contour. It is defined as the square root of $1 - (d/D)^2$, where $D$ is the largest (RL) diameter and $d$ is the smallest (AP) diameter of the ellipse. Values closer to 1 indicate a flatter ellipse, as the eccentricity of a circle is 0.

## 2.4 | Statistical analysis

Data were summarised as means with SDs or medians with interquartile ranges (IQR), depending on the distribution. The normality of data was assessed by visual inspection and Shapiro-Wilk and Kolmogorov-Smirnov tests for normality. Differences between patients and controls were assessed using Student's $t$ test (normally distributed data) or Mann-Whitney $U$ test (non-normally distributed data). Differences between controls, asymptomatic and symptomatic patients were assessed with a one-way analysis of variance with post hoc testing with Tukey correction for multiple comparisons. Spearman's rank-order correlation coefficient was used to calculate the correlation between CSA and clinical measures (non-normally distributed data). For longitudinal analysis, we used paired $t$ test or Wilcoxon signed-rank test to assess the difference in clinical measures and mean CSA between baseline and follow-up. $P$-values lower than .05 were considered statistically significant. IBM SPSS Statistics Version 25 was used for data analysis.

# 3 | RESULTS

## 3.1 | Baseline characteristics

The Dutch ALD cohort consists of 61 male ALD patients. Nineteen were excluded for this study: 15 because of age <16 years, three did not give consent and one due to poor quality of the spinal cord MRI. Baseline imaging was available for 74 subjects: 42 patients and 32 controls. The mean age of patients 45.9 (±16.1) and healthy controls 43.3 (±16.7) was not statistically significantly different ($P = .551$).

Details on the clinical characteristics of this cohort are described in detail elsewhere.[7] In summary, 30 (71%) patients had signs and symptoms of myelopathy and were, therefore, classified as symptomatic. Median disease duration was 15.0 years (IQR 8-21). Patients had a median EDSS of 3.5 (IQR 2-6) and SSPROM of 84.5 (IQR 77-99), indicating a moderate degree of disability. Median time on the timed up-and-go was 6.9 s (IQR 3.5-10.2) and mean distance on the 6-minute walk test was 536.3 m (±188.6).

### 3.2 | Between-group differences

On visual examination, the spinal cord of ALD subjects looked smaller and flattened compared to healthy controls (Figure 1). Indeed, spinal cord CSA was significantly smaller in patients compared to controls on all measured levels (Figure 2). The absolute reduction was most pronounced at the C3 level (mean difference 12.92 $mm^2$), while the relative reduction was most pronounced at

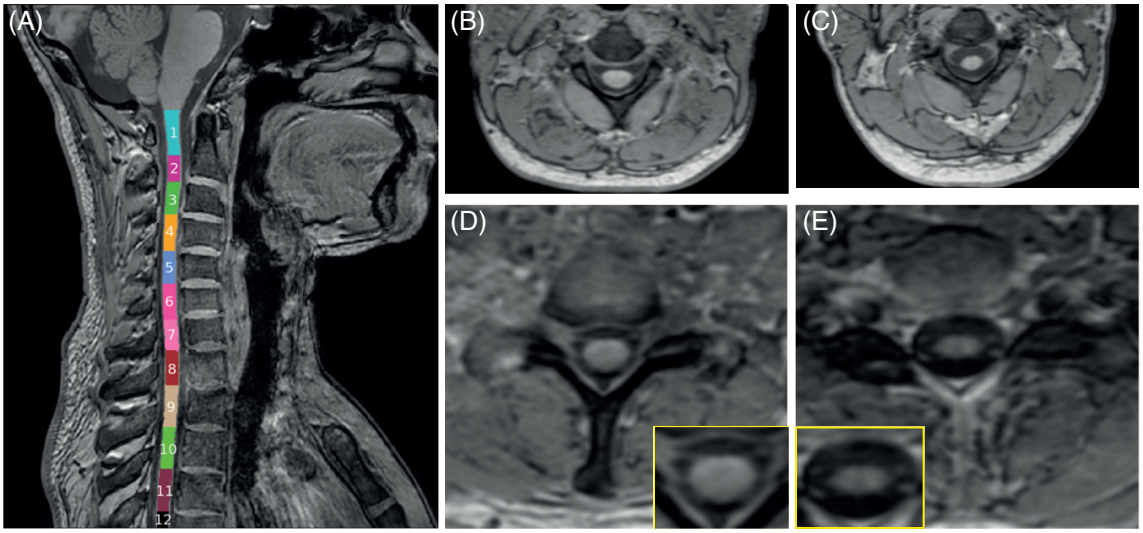
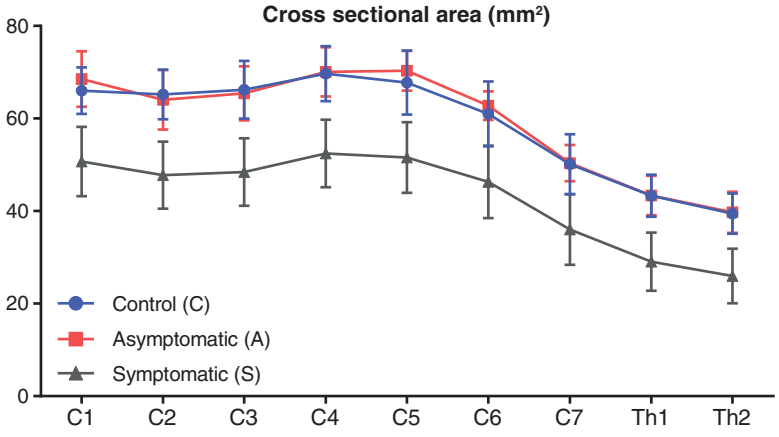



**FIGURE 1** Example of MR images. A, Semi-automatic vertebral labelling performed with the Spinal Cord Toolbox and spinal cord anatomy expert. Upper right: Spinal cord atrophy at C2-C3 in subjects with similar age. B, Healthy control and (C) patient with EDSS 7.0. Lower right: Difference in the eccentricity of the spinal cord at the cervicothoracic junction in subjects with similar age. D, Healthy control, mean eccentricity of 0.82 and (E) patient with EDSS 7.0, mean eccentricity of 0.90




| Level | Control (C) (n=32) | Asymptomatic (A) (n=12) | Symptomatic (S) (n=30) | p-values C-A | C-S | A-S | Mean difference C-S |
|---|---|---|---|---|---|---|---|
| C1 | 66.00 (5.04) | 68.52 (5.98) | 50.68 (7.51) | 0.469 | <0.001* | <0.001* | -15.33 |
| C2 | 65.19 (5.37) | 64.05 (6.44) | 47.76 (7.27) | 0.858 | <0.001* | <0.001* | -17.42 |
| C3 | 66.21 (6.22) | 65.42 (5.85) | 48.44 (7.31) | 0.934 | <0.001* | <0.001* | -17.77 |
| C4 | 69.69 (5.94) | 70.09 (5.32) | 52.46 (7.30) | 0.981 | <0.001* | <0.001* | -17.23 |
| C5 | 67.73 (6.94) | 70.33 (4.32) | 51.56 (7.64) | 0.510 | <0.001* | <0.001* | -16.17 |
| C6 | 60.97 (7.03) | 62.79 (3.12) | 46.29 (7.84) | 0.722 | <0.001* | <0.001* | -14.68 |
| C7 | 50.14 (6.48) | 50.36 (3.93) | 36.01 (7.64) | 0.995 | <0.001* | <0.001* | -14.13 |
| Th1 | 43.33 (4.55) | 43.35 (4.27) | 29.07 (6.29) | 1.000 | <0.001* | <0.001* | -14.26 |
| Th2 | 39.46 (4.35) | 39.75 (4.48) | 25.95 (5.90) | 0.983 | <0.001* | <0.001* | -13.51 |

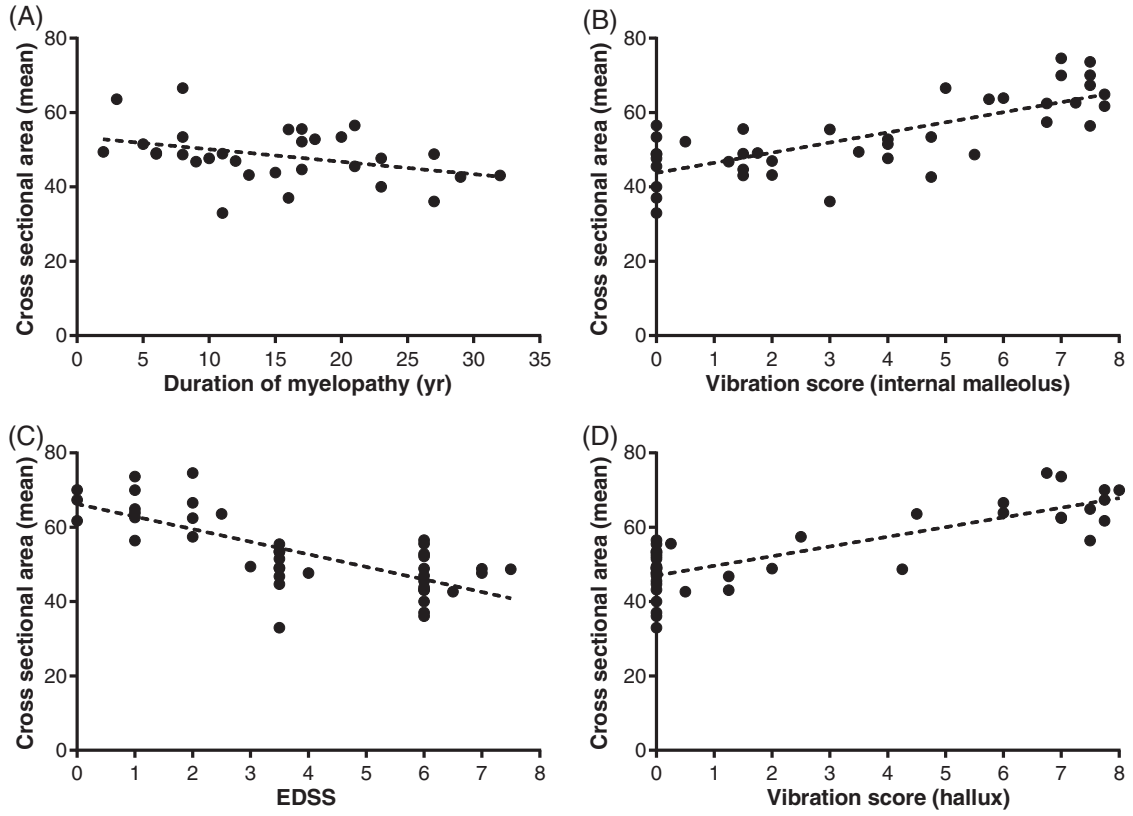
**FIGURE 2** Cross-sectional area in patients and controls. Line chart: Mean CSA in healthy controls (blue circle), asymptomatic patients (red square) and symptomatic patients (grey triangle) with SDs (error bars). Table: Differences in mean CSA between healthy controls, asymptomatic and symptomatic patients. Differences between groups are analysed with one-way analysis of variance. *Statistically significant *P*-value

thoracic levels (23.5%). When stratifying patients into groups based on their symptomatic status (asymptomatic vs symptomatic), the analysis showed that difference in spinal cord CSA between patients and healthy control subjects was determined by the effect of the symptomatic subgroup. There was no difference in CSA between asymptomatic patients and healthy control subjects (Figure 2).

In addition, the morphometric analysis confirmed that the spinal cord was significantly flatter (reduced AP compared to RL diameter) in patients compared to controls. On all measured levels mean eccentricity and mean AP diameters differed significantly from controls ($P < .001$), while RL diameters only differed in high cervical and the first two thoracic levels (Table S1).

### 3.3 | Correlation with clinical outcome measures

Figure 3 shows the correlations between clinical outcomes and CSA. Spinal cord CSA correlated strongly with EDSS, SSPROM and vibration sense scores (Spearman's rho >0.7) and moderately with disease




| | r | p-value | N |
|---|---|---|---|
| EDSS[1] | -0.75 | <0.001* | 42 |
| 6MWT[1] | 0.27 | 0.112 | 35 |
| SSPROM[1] | 0.70 | <0.001* | 42 |
| Timed up-and-go[1] | -0.71 | <0.001* | 37 |
| Vibration score[1] (internal mallelous) | 0.71 | <0.001* | 41 |
| Vibration score[1] (hallux) | 0.71 | <0.001* | 41 |
| Disease duration[2] | -0.37 | 0.046* | 30 |
| Age controls[2] | -0.01 | 0.980 | 32 |

**FIGURE 3** Correlations between clinical outcomes and the cross-sectional area. Above: scatterplots of correlation between CSA measured at C3 level and disease duration (A), vibration score measured at the internal malleolus and hallux (B and D) and EDSS (C). Below: correlation coefficients for clinical outcomes and CSA. *Statistically significant *P*-value. [1]Spearman's rank-order correlation test. [2]Pearson's correlation test. Abbreviations: CSA, cross-sectional area; EDSS, expanded disability status scale; SSPROM, severity scoring system for progressive myelopathy; 6MWT, 6 Minute Walk Test

**TABLE 1** Disease progression

| | Baseline (n = 26) | Follow up (n = 26) | Difference (95% CI) | *P*-value |
|---|---|---|---|---|
| *Clinical outcome measures* | | | | |
| EDSS | 3.5 (1.0-6.0) | 3.5 (1.8-6.0) | 0.21 (0.01-0.42) | .042* |
| SSPROM | 91.0 (81.0-100) | 87.0 (75.8-100) | −2.23 (−4.43-0.03) | .052 |
| 6MWT (n = 24) | 562.6 (±198.4) | 555.3 (±197.0) | −8.73 (−13.0-27.5) | .464 |
| TUG (n = 23) | 4.7 (3.5-9.1) | 5.2 (3.7-9.0) | 0.23 (−0.41-0.86) | .045* |
| Quantitative vibration score (internal malleolus) | 4.38 (1.06-6.81) | 3.75 (0.19-6.56) | −0.24 (−0.57-0.09) | .199 |
| Quantitative vibration score (hallux) | 1.63 (0.00-7.00) | 0.75 (0.00-6.50) | −0.30 (−0.50-0.10) | .007* |
| *Spinal cord CSA ($mm^2$)* | | | | |
| C1 | 56.49 (11.03) | 56.16 (10.46) | 0.33 (−0.35-1.00) | .330 |
| C2 | 53.36 (9.95) | 53.29 (10.79) | 0.07 (−0.75-0.88) | .867 |
| C3 | 54.13 (9.81) | 54.04 (10.59) | 0.09 (−0.75-0.93) | .823 |
| C4 | 58.78 (9.96) | 58.43 (10.39) | 0.34 (−0.69-1.38) | .501 |
| C5 | 58.05 (11.08) | 58.18 (11.11) | −0.13 (−0.98-0.73) | .763 |
| C6 | 51.54 (10.49) | 51.98 (10.25) | −0.44 (−1.57-0.68) | .426 |
| C7 | 40.87 (10.10) | 41.66 (10.46) | −0.79 (−1.96-0.37) | .174 |
| Th1 | 33.88 (8.86) | 33.89 (9.38) | −0.01 (−1.03-1.01) | .981 |
| Th2 | 30.88 (8.58) | 30.85 (8.93) | 0.03 (−0.61-0.67 | .917 |

*Notes*: Values are medians with IQRs or means with SDs. Differences between groups are analysed with Wilcoxon signed-rank test or Paired *t* test. **P*-value statistically significant.

Abbreviations: EDSS, Expanded Disability Status Scale; SSPROM, Severity Scoring system for Progressive Myelopathy; 6MWT, 6 Minute Walk Test; TUG, Timed up-and-go; CSA, cross-sectional area.

duration (Pearson's $r = -0.366$). There was no correlation between CSA and the age of healthy controls (Pearson's $r = -0.005$).

### 3.4 | Disease progression

Follow-up imaging was available for 26/42 patients (62%). The median time between baseline and follow-up scans was 11 months (IQR 9-14). Three of the clinical outcome measures were able to detect disease progression: EDSS (mean change 0.21, $P = .042$), the timed up-and-go (mean change 0.23, $P = .045$) and quantitative vibration score measured at the hallux (mean change −0.30, $P = .007$). However, there was no change in spinal cord CSA between baseline and follow-up on any of the measured levels (Table 1). When looking at the symptomatic subgroup (n = 17), a trend in reduction of CSA measured at C2 was found (−0.39 $mm^2$, 95% CI: −0.04 to 0.83, $P = .073$). For the morphometric measures, only a significant decrease in the AP diameter at C2 level was detected (−0.08 mm, *Z*-value: −2.095, $P = .036$). There was no correlation between baseline CSA and disease progression, measured as the change in EDSS and vibration sense score.

## 4 | DISCUSSION

In this prospective cohort study, we quantitatively assessed spinal cord atrophy as a potential biomarker for the severity of myelopathy in ALD. Our findings showed that the spinal cord is smaller and flatter in ALD patients with symptomatic myelopathy compared to controls. The degree of thinning correlated with clinical outcome measures for myelopathy. We did not detect any change after 1-year follow-up.

CSA was reduced at all levels in ALD patients compared to controls, and this reduction was explained by the symptomatic subgroup. The relative reduction was most pronounced at thoracic levels (23.5%) whereas absolute reduction was more prominent at C2-C3 levels (12.92 $mm^2$). These results are in agreement with previously published data.[24] Moreover, the spinal cord of ALD patients shows AP flattening, as can be seen by visual assessment of MR images. In the patients' spinal cord, relative reduction of AP diameters was greater than the reduction of RL diameters and mean eccentricity was closer to 1, indicating a flatter spinal cord as compared to healthy controls. Comparable results were found in other neurodegenerative disorders, such as spinocerebellar ataxia and Friedreich's ataxia.[19,21] These diseases have

similar pathological mechanisms as ALD, with predominant degeneration of dorsolateral tracts of the spinal cord. Conversely, in amyotrophic lateral sclerosis where corticospinal tracts are mostly affected, this flattening was not seen and eccentricity values for patients and controls were virtually the same.[18] Spinal cord flattening in ALD is thus likely due to dorsal column degeneration.

Furthermore, CSA correlated significantly with all used clinical outcome measures for myelopathy, with more severely affected patients having a smaller CSA. This implies that spinal cord CSA is a reliable biomarker for disease severity and can be used, for example, in multi-centre studies or studies with a longer follow-up period. Since CSA can be derived from routine diagnostic MRI sequences and data processing software libraries are freely accessible, data collection and analysis may be reproducible across sites.

After 1-year follow-up, there was no significant decrease in CSA. Nevertheless, disease duration and CSA were significantly negatively correlated, confirming the initial hypothesis that CSA decreases over time in ALD patients. A sub-analysis in symptomatic patients showed a trend towards smaller CSA after 1 year at C2 level (−0.39 mm, $P = .073$), but the mean change was small and not found at other levels. Furthermore, AP diameter measured at C2 level decreased significantly (−0.08 mm, $P = .036$) but again the mean change was not found at other levels and also, this detected change is below a spatial resolution of the MRI sequence used in our protocol. It is likely that significant changes may be observed after a longer follow-up, also considering the large difference in CSA between symptomatic and asymptomatic patients. A new prospective cohort study is ongoing to confirm this hypothesis.

A few limitations apply to our study. First is the relatively low number of available follow-up MRI scans. Nevertheless, this study is one of the largest prospective cohort studies in ALD. Second, age can be considered as a confounding factor when looking at clinical outcome measures over time. However, in our control group, we did not find a relationship between CSA and age. Therefore, it seems unlikely that the difference we detected is explained by aging. Finally, CSA as the macrostructural quantitative marker is not sensitive enough to detect changes in a presymptomatic stage. With diffusion MRI protocols, namely diffusion tensor imaging (DTI), we were able to detect differences between asymptomatic patients and controls.[33] Correlations between CSA and clinical outcomes were, on the contrary, stronger than correlations between DTI parameters and clinical outcomes. For this reason, CSA is still a reliable marker for disease severity. In the future, advanced diffusion MRI protocols, such as high angular resolution diffusion imaging sampled at multiple q-space shells optimised for spinal cord imaging, can increase the outcome sensitivity and also correlation property for the DTI metrics.[34]

In conclusion, our study shows that the spinal cord in male ALD patients is smaller and flatter compared to controls, likely due to atrophy predominantly affecting the dorsolateral columns. Moreover, spinal cord CSA is strongly associated with disease severity and represents a promising biomarker in the myelopathy of ALD. Due to a slowly progressive disease course, there is no detectable change after 1-year follow-up. In studies with a longer follow-up period or multi-centre studies, CSA can be of use since it requires only routine MRI sequences. Our future research is aimed at identifying more sensitive and dynamic outcome measures able to detect change after a shorter follow-up period, such as optical coherence tomography, body sway measurement and other quantitative MRI techniques like DTI. These studies will hopefully contribute to clinical trial readiness in ALD.

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## CONFLICT OF INTEREST

Stephanie I. W. van de Stadt, Wouter J. C. van Ballegoij, René Labounek, Irene C. Huffnagel, and Igor Nestrasil report no disclosures. Stephan Kemp received unrestricted research grants from Vertex, Blue Bird Bio and SwanBio. Marc Engelen received unrestricted research grants from Minoryx, Vertex, Blue Bird Bio and SwanBio. Received consultancy fees from Minoryx and Blue Bird Bio.

## AUTHOR CONTRIBUTIONS

Stephanie I. W. van de Stadt: data collection; analysis and interpretation of data; drafting and revision of the manuscript. Wouter J. C. van Ballegoij: data collection; analysis and interpretation of data; drafting and revision of the manuscript. René Labounek: analysis and interpretation of data; revision of the manuscript. Irene C. Huffnagel: design and conceptualising study; data collection; revision of the manuscript. Stephan Kemp:

analysis and interpretation of data; revision of the manuscript. Igor Nestrasil: analysis and interpretation of data; revision of the manuscript. Marc Engelen: design and conceptualising study; data collection; interpretation of data; revision of the manuscript.

## DATA AVAILABILITY STATEMENT

The datasets generated or analysed during the current study are available from the corresponding author on reasonable request.

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## SUPPORTING INFORMATION

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

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