

# Distinguishing Transient From Persistent Brain Structural Changes in Pediatric Patients With Acute Disseminated Encephalomyelitis

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*Neurol Neuroimmunol Neuroinflamm* 2025;12:e200337. doi:10.1212/NXI.0000000000200337

## Abstract

### Background and Objectives

Pediatric patients with acute disseminated encephalomyelitis (ADEM) are at risk of impaired brain growth, with long-term neuropsychiatric consequences. We previously reported transient expansions of cerebral ventricle volume (VV) in experimental autoimmune encephalomyelitis, which subsequently normalized. In this study, we investigated changes in VV in ADEM in relation to other brain structures and clinical outcomes.

### Methods

We investigated brain MRI scans acquired in routine clinical practice from a multicenter cohort of 61 pediatric patients with ADEM, of whom 39 were myelin oligodendrocyte glycoprotein (MOG) antibody-positive. Patients were compared with 1,219 pediatric healthy controls (HCs). Volumes of multiple brain structures were computed using a contrast-agnostic machine learning-based tool and analyzed with mixed-effect models regarding other clinical parameters.

### Results

Patients with ADEM had larger VV than HCs at initial clinical presentation, before immune therapy. Most of the patients showed further VV increases within 2 months after disease onset. Patients had smaller brain volumes than HCs, with specific reductions in deep gray matter structures. These changes were more pronounced in MOG antibody-negative patients. Of the patients with more than 2 MRI scans, 12 of 22 resolved their VV expansion back to within 15% of baseline values while 10 of 22 had persistently increased VV at the last available MRI within 1 year from onset. Patients with persistent VV expansion had greater reductions in volumes of other brain structures at the *last MRI* than patients whose VV resolved and were more likely to have residual neurologic signs. The VV *resolving* and *nonresolving* patients did not differ regarding age, sex, elevated CSF cell counts at baseline, or occurrence of relapses. However, patients with a larger magnitude of VV expansion— $\geq 90\%$  of baseline volume—were more likely to be in the *nonresolving* group.

### Discussion

We could distinguish between 2 outcomes of VV changes in ADEM: one in which the VV expanded but ultimately returned to normal and one in which the expansions continued after disease onset and treatment but failed to resolve. The latter was associated with reduced brain volume, particularly in deep gray matter structures. This highlights the necessity for patients with ADEM to undergo regular MRI scans to assess whether developing VV expansions indicate a greater risk of permanent brain atrophy.

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Go to [Neurology.org/NN](https://www.neurology.org/NN) for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

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## Glossary

**ABCD** = Adolescent Brain Cognitive Development; **ADEM** = acute disseminated encephalomyelitis; **ADEMON** = ADEM with optic neuritis; **BV** = brain volume; **CoV** = coefficient of variation; **EAE** = encephalomyelitis; **EDSS** = Expanded Disability Status Scale; **HCS** = healthy controls; **LME** = linear mixed effect; **MDEM** = multiphasic acute disseminated encephalomyelitis; **MOG** = myelin oligodendrocyte glycoprotein; **MS** = multiple sclerosis; **NEOS** = anti-NMDAR Encephalitis One-Year Functional Status; **RRMS** = relapsing-remitting multiple sclerosis; **VV** = ventricle volume; **WM** = white matter.

## Introduction

Acute disseminated encephalomyelitis (ADEM) is an acquired demyelinating syndrome that is frequently associated with an infectious trigger.<sup>1</sup> ADEM is more common in children than in adults and is associated with polyfocal CNS signs and encephalopathy.<sup>2</sup> While ADEM is usually monophasic with resolution of clinical signs, pediatric patients with ADEM show impaired brain growth and are at risk of long-term neuropsychiatric consequences.<sup>3</sup>

We reported that pediatric ADEM is associated with brain volume (BV) loss and a failure in age-expected brain growth.<sup>4</sup> We associated negative effects of the pathology with reduced BV and increased cerebral ventricular volume (VV), which was already observed on disease onset.<sup>4</sup> Previously, we had reported profound enlargement of VV in the experimental autoimmune encephalomyelitis (EAE) mouse model of neuroinflammation.<sup>5,6</sup> This occurred before clinical onset, normalized on remission of clinical signs, and then expanded again during subsequent relapses.<sup>6</sup> We hypothesized that this transient VV expansion and resolution was related to short-term inflammatory processes superimposed on long-term neurodegeneration. The observation in the animal model was clinically relevant, as we showed that most patients with relapsing-remitting multiple sclerosis (RRMS) who had undergone monthly MRI scans over 1 year showed transient increases in VV and seemed to be at an earlier stage of disease.<sup>6</sup> The VV fluctuations we observed in patients with RRMS, while significant, were less pronounced than the fluctuations observed in EAE mice. The EAE model we used<sup>5,6</sup> has many features that resemble RRMS pathology, although it has been suggested to be an even more accurate model of acute demyelinating neuroinflammatory diseases such as ADEM.<sup>7</sup>

In this study, we examined longitudinal brain MRI scans of pediatric patients with ADEM and assessed how changes in VV relate to changes in other brain structures and clinical outcomes. Given the rarity of ADEM, we used a heterogeneous multicenter cohort of patients, with data acquired in routine clinical practice. We aimed to distinguish transient VV changes from persistent expansions associated with brain atrophy, to identify patients at greater risk of permanent deficits.

## Methods

### Patients

Data from 66 patients with suspected ADEM were obtained from 35 pediatric neurology clinics in Germany, Austria, Italy, Switzerland, and Canada:  $n = 66$ ; 34 of 66 were girls; age =  $6.32 \pm 4.24$  years (mean  $\pm$  sd range 0.66–19.6). 5 patients were subsequently rediagnosed with neuromyelitis optica spectrum disorder, multiple sclerosis (MS), CIS, or longitudinally extensive transverse myelitis. Of the remaining 61 patients, 51 with ADEM, 5 with ADEM with optic neuritis (ADEMON), and 5 with multiphasic acute disseminated encephalomyelitis (MDEM) were included. This is a retrospective cohort obtained from routine clinical practice. CSF cell counts were obtained by routine procedures, and myelin oligodendrocyte glycoprotein (MOG) antibody titers were measured in serum samples obtained at initial clinical presentation, as described.<sup>4,8</sup> The anti-NMDAR Encephalitis One-Year Functional Status (NEOS) score was recorded at initial presentation.<sup>9</sup> The presence of residual clinical signs and Expanded Disability Status Scale (EDSS) scores were recorded at the last available observation for each patient. Additional details are given in Table 1.

### Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the Ethics Committee of the University Witten/Herdecke, Germany. Caregivers of all patients gave informed consent; this included consent for international sharing of the data.

### MRI Acquisition and Analysis

Brain MRI data were acquired on initial clinical presentation (baseline) before administration of steroids or other therapies. MRI scan parameters varied among centers, with variable timing of follow-up scans (eTable 1). Within the ADEM cohort, 15 of 61 patients had 1 MRI scan, 46 of 61 patients had at least 1 follow-up scan, and 22 of 61 patients had  $\geq 2$  scans. In total, there were 180 patient scans. Among the 22 patients with  $>2$  scans, a total of 94 scans within the first 12 months were available. All scans were manually screened for quality control, given that scans acquired for diagnostic purposes might not be suitable for volumetric analysis. Scans that were cut off or corrupted by artifacts were excluded. In 16 of 180 scans (from 6 patients), brain volumes were calculated but

**Table 1** Demographic and Clinical Details

| Patients                                 | Total<br>n = 61 | Ventricle expansion resolving <sup>a</sup><br>n = 12 | Ventricle expansion nonresolving<br>n = 10 |
|--|-----------------|--|--|
| <b>Age at first MRI (y)</b>              |                 |  |  |
| Mean (SD)                                | 6.32 (± 4.24)   | 7.49 (± 2.90)  | 5.92 (± 4.58)                              |
| Range                                    | 0.66–19.65      | 2.94–11.71   | 0.89 to 13.51                              |
| <b>Sex (%)</b>                           |                 |  |  |
| Female                                   | 31 (50.8)       | 6 (50)   | 6 (60)                                     |
| Male                                     | 30 (49.2)       | 6 (50)   | 4 (40)                                     |
| <b>Diagnosis (%)</b>                     |                 |  |  |
| ADEM                                     | 51 (83.6)       | 10 (83.3)  | 7 (70)                                     |
| MDEM                                     | 5 (8.2)         | 2 (16.7)   | 2 (20)                                     |
| ADEMON                                   | 5 (8.2)         | 0 (0)  | 1 (10)                                     |
| <b>MRI</b>                               |                 |  |  |
| Onset to first MRI median (IQR) time (d) | 18 (8–30)       | 26 (15.5–30)   | 15 (4–21.5)                                |
| 1 MRI scan (female)                      | 15 (?)          | 0  | 0  |
| ≥2 MRI scans (female)                    | 46 (?)          | 0  | 0  |
| >2 MRI scans (female)                    | 22 (12)         | 12 (6)   | 10 (6)                                     |
| <b>MOG antibody (%)</b>                  |                 |  |  |
| Positive                                 | 39 (63.9)       | 8 (66.7)   | 6 (60)                                     |
| Negative                                 | 22 (36.1)       | 4 (33.3)   | 4 (40)                                     |
| <b>Neurologic residuals (%)</b>          |                 |  |  |
| Yes                                      | 21 (34.4)       | 2 (16.7)   | 9 (90)                                     |
| No                                       | 35 (57.4)       | 10 (83.3)  | 1 (10)                                     |
| Missing                                  | 5 (8.2)         | 0 (0.0)  | 0 (0.0)                                    |
| <b>Last EDSS score</b>                   |                 |  |  |
| Median (IQR)                             | 0 (0–1)         | 0 (0–0)  | 1 (0–3.5)                                  |
| EDSS score >0                            | 16 (26.2)       | 2 (16.6)   | 6 (60)                                     |
| Missing                                  | 5 (8.2)         | 1 (8.3)  | 1 (10)                                     |
| <b>Transverse myelitis (%)</b>           |                 |  |  |
| Yes                                      | 19 (32.2)       | 6 (50)   | 1 (10)                                     |
| No                                       | 39 (63.9)       | 6 (50)   | 8 (80)                                     |
| Missing                                  | 3 (4.9)         | 0 (0.0)  | 1 (10)                                     |
| <b>CSF cell count</b>                    |                 |  |  |
| Median (IQR)                             | 37 (10–73)      | 37.5 (11.5–78.5)                                     | 11 (7–47.5)                                |
| Missing (%)                              | 4 (6.6)         | 0 (0.0)  | 2 (20)                                     |
| <b>Oligoclonal bands (%)</b>             |                 |  |  |
| Yes                                      | 6 (9.8)         | 2 (16.7)   | 2 (20)                                     |
| No                                       | 53 (86.9)       | 9 (75)   | 8 (80)                                     |
| Missing                                  | 2 (3.3)         | 1 (8.3)  | 0 (0)                                      |

Continued

**Table 1** Demographic and Clinical Details (*continued*)

| Patients                            | Total<br>n = 61        | Ventricle expansion resolving <sup>a</sup><br>n = 12 | Ventricle expansion nonresolving<br>n = 10 |  |
|-------------------------------------|------------------------|--|--|--|
| <b>NEOS score</b>                   |                        |  |  |  |
| Median (IQR)                        | 2 (2-3)                | 2 (2-2)  | 2 (2-2)                                    |  |
| <b>Modified Ranking Scale score</b> |                        |  |  |  |
| Median (IQR)                        | 4 (4-5)                | 4 (4-5)  | 5 (4-5)                                    |  |
| <b>Immune therapy (%)</b>           |                        |  |  |  |
| Yes                                 | 50 (82.0)              | 8 (66.7)   | 8 (80)                                     |  |
| No                                  | 6 (9.8)                | 3 (25)   | 2 (20)                                     |  |
| Missing                             | 5 (8.2)                | 1 (8.3)  | 0 (0)                                      |  |
| IVMP                                | 50 (82.0)              | 8 (66.7)   | 8 (80)                                     |  |
| IVIG                                | 8 (13.1)               | 2 (16.7)   | 2 (20)                                     |  |
| <b>Healthy controls</b>             | <b>Total n = 1,219</b> | <b>NIH data repository n = 652</b>                   | <b>NIH data repository n = 445</b>         | <b>Pixar study open source n = 122</b> |
| <b>Age at first MRI (y)</b>         |                        |  |  |  |
| Mean (SD)                           | 11.83 (± 5.12)         | 14.44 (± 4.06)                                       | 9.42 (± 4.82)                              | 6.71 (± 2.33)                          |
| Range                               | 0.17–21.92             | 5.58–21.92   | 0.17–18.58                                 | 3.50–12.33                             |
| <b>Sex (%)</b>                      |                        |  |  |  |
| Female                              | 642 (52.7)             | 351 (53.8)   | 227 (51.0)                                 | 64 (52.5)                              |
| Male                                | 577 (47.3)             | 301 (46.2)   | 218 (49.0)                                 | 58 (47.5)                              |
| <b>No. of scans</b>                 |                        |  |  |  |
| 1 MRI scan                          | 858                    |  |  |  |
| ≥2 MRI scans                        | 427                    |  |  |  |
| >2 MRI scans                        | 241                    |  |  |  |

Abbreviations: ADEM = acute disseminated encephalomyelitis; IQR = interquartile range; IVMP = intravenous methylprednisolone; IVIG = intravenous immunoglobulin; MDEM = multiphasic acute disseminated encephalomyelitis; MOG = myelin oligodendrocyte glycoprotein; NEOS = anti-NMDAR Encephalitis One-Year Functional Status.

<sup>a</sup> Ventricle expansion *resolving* patients was defined as those patients with MRI scans at >2 time points, in whom ventricle volume had returned to within 115% of the baseline volume by the last MRI measurement available. *Nonresolving* patients were those whose ventricle volumes at the last MRI exceeded 115% of baseline.

intracranial and CSF volumes were excluded, because of incomplete coverage of the skull. We defined a 2-month period after baseline as an acute phase.

Healthy control (HC) brain MRI data were derived from 3 pediatric cohorts. This ensured sufficient data for age-matched comparison with patients. Two HC cohorts were from the NIH Pediatric MRI Data Repository, Adolescent Brain Cognitive Development (ABCD) Study, held in the NIMH Data Archive (NDA) (*first cohort* n = 464, scanned at 1.5T; *second cohort* n = 652, scanned at 3.0T).<sup>10</sup> A *third cohort* (n = 122, scanned at 3.0T) was from the open-source Pixar study.<sup>11</sup> The ABCD Study is an initiative to investigate normal brain development and to serve as control data for studies of pediatric disorders. In total, 1,219 HCs were included, with a mean age of 11.83 ± 5.12 years (range 0.17–21.92).

All patient and HC MRI data underwent fully automated brain segmentation using the convolutional neural network–based brain segmentation tool SynthSeg. The synthetic training data for SynthSeg used randomized contrast and resolution, and therefore, this tool is agnostic for MR contrast and resolution.<sup>12,13</sup> SynthSeg 2.0 was used with the “robust” option, given the heterogeneous patient data set. All segmentations were examined for quality control. Scans of HCs were processed using the same version of SynthSeg; segmentations from 19 controls were excluded because of poor image quality.

### Data Analysis and Statistics

Data were analyzed using R v4.2.1.<sup>14</sup> Categorical data (sex, residual neurologic signs yes/no, relapses yes/no, elevated CSF counts yes/no, transverse myelitis yes/no) were analyzed by the  $\chi^2$  test comparing VV resolving and nonresolving

patients. Continuous data (ventricle volumes, all brain volumes, coefficient of variation [CoV]) were analyzed by the nonparametric Mann-Whitney or Friedman test, with Wilcoxon post hoc tests. Correlations between z-scores of VV and z-scores of other brain volumes were assessed using the Spearman correlation. The false discovery rate (Benjamini-Hochberg) was used to control for multiple comparisons.

Patients were matched 1:10 with HCs by sex and age within  $\pm$  12 months to calculate z-scores as follows: patient volume minus mean volume of the matched HC subcohort, divided by SD of the HC subcohort. Mean z-scores of 10 iterations of random sampling were used to ensure robust z-score calculations. For subsequent analysis, patients were matched 1:5 with HCs, to ensure a unique set of controls for each patient. Of the 46 patients who had at least 2 MRI scans, we selected time points with the maximum VV expansion within the 2-month *acute phase* after baseline to compare patients and HCs. For this analysis, 29 of 46 patients had an MRI scan within the *acute phase* and were included while 17 of 46 patients had their second MRI scan later than 2 months from the baseline and were not included in this comparison. We defined the last scan within 12 months of the baseline MRI as the *last MRI* for longitudinal assessment.

Linear mixed-effect (LME) modeling was used to account for the variable number and timing of follow-up scans, in patients and HCs. VV, whole BV (excluding ventricles), and volumes of the other structures available were each used as dependent variables. Group (ADEM or HC), sex, and age were used as fixed effects. To account for the nonlinearity of normal BV growth over time, we included the logarithm (age at scan) as a fixed effect. Additional fixed effects tested included age at baseline, MOG antibody status, CSF cell counts (normal or elevated), and occurrence of relapses. These additional fixed effects were added stepwise to the LME model in various combinations. The factors were retained if they yielded a significant improvement in the model fit, based on the restricted maximum likelihood, Akaike information criterion scores, and analysis of variance. Subject ID was the grouping variable, and age at scan was included as a random effect. The R packages *lme4*<sup>15</sup> and *flexplot*<sup>16</sup> were used for LME modeling and model comparisons. *p* values of the regression coefficients were estimated by the Satterthwaite method using the R package *afex*.<sup>17</sup> *p* Values <0.05 were considered significant.

## Data Availability

Anonymized segmented data will be made available to qualified investigators on request.

## Results

### Patients With ADEM Have Significantly Larger Ventricles at Disease Onset

Of the 61 patients with ADEM, 5 presented with ADEMOM and 5 with MDEM; 39 of 61 (63.9%) were MOG antibody-

positive (Table 1). The median time between initial clinical presentation and the first MRI scan was 18 days. In all patients, the first MRI scan was acquired before anti-inflammatory treatment. Patients with ADEM showed larger absolute VV compared with HCs (Figure 1A). For each patient, z-scores of the baseline VV were calculated relative to 10 randomly sampled age-matched and sex-matched HCs. Median VV z-scores were significantly higher in patients with ADEM than in HCs ( $p < 0.0001$ , Figure 1B). Patients with ADEM showed greater variability in VV over time, with a significantly greater CoV (median = 18.6%,  $n = 22$ ) compared with HCs (7.1%,  $n = 241$ ), calculated for patients and HCs with  $\geq 2$  scans ( $p < 0.001$ , Figure 1C).

### Changes in Ventricles and Brain Volumes Continue After Disease Onset

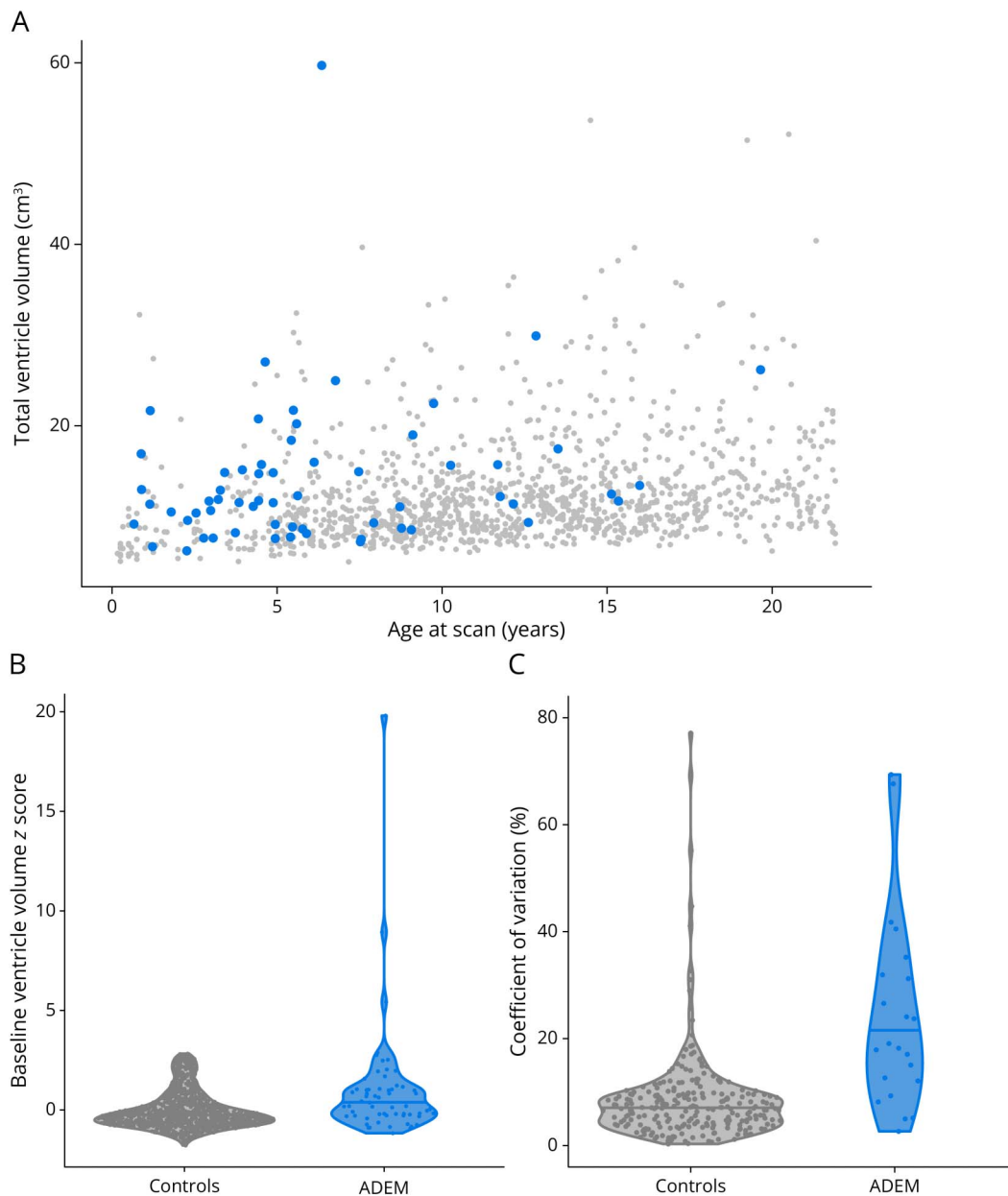
Most patients with ADEM (46/61) and 35% of HCs (427/1,219) had at least 2 MRI scans. Plots of VV vs age at scan are shown in Figure 2 (top left panel); lines connect serial measurements of patients (blue) or HCs (gray). Most patients with at least 2 MRI scans (33/46, 71.7%) showed further increases in VV after initial presentation, indicating expansions in VV beyond the enlargement of VV relative to HCs observed at baseline. The rest of the patients (13/46, 28.3%) showed a decrease in VV from baseline, with no subsequent VV expansion, indicating a resolution of the VV, which was elevated at baseline. Most of the patients with continued VV expansion (25/33, 75.8%) did not have relapses at any time point for which we have data while 6 (13.0%) had at least 1 relapse and 2 (6.1%) had no available relapse information. Information on the specific timing of the relapses was not available, so no association with VV expansions could be made. Of the patients who showed continued increases in VV beyond baseline, 28 of 33 patients (84.8%) received steroid treatment and 5 of 33 patients (15.2%) did not get steroids after their first MRI. Of the patients who showed a decrease in VV after baseline, 6 of 13 (46.2%) received steroid treatment after the first MRI scan while 4 of 13 (30.6%) did not (3/13 with missing information on steroid treatment).

Beyond VV, we investigated absolute volumes of the whole brain and substructures (Figure 2) for a more granular comparison of differences between ADEM and HC and how these structures relate to VV changes. We used LME modeling to investigate differences between ADEM and HC, to account for the variable number and frequency of measurements of patients and HC cohorts (Table 2). The LME analysis accounted for the impact of sex, age, and the logarithm of age (which models the nonlinear relationship between age and normal brain growth). As expected, on average, male patients had larger volumes than female patients for all structures (Table 2).

The LME analysis confirmed that patients with ADEM had significantly larger VV than HCs (regression coefficient  $\beta = 2.434$ , effect size  $t = 4.421$ ,  $p < 0.0001$ ); i.e., VVs of ADEM were on average 2.434 cm<sup>3</sup> larger than that of HC, independent of sex or age (Table 2). Total CSF volume



**Figure 1** Patients With ADEM Have Enlarged Brain Ventricles at Disease Onset



(A) Total ventricle volume at the first clinical presentation of patients with ADEM ( $n = 61$ , blue) compared with healthy controls at first MRI ( $n = 1,219$ , gray). (B) Ventricle volume z-scores of patients with ADEM are significantly greater than those of age-matched and sex-matched controls ( $p < 0.0001$ ). (C) The coefficient of variation of ventricle volumes in patients with ADEM (median = 18.6%,  $n = 22$ ) is significantly greater than in controls (7.1%,  $n = 241$ ), calculated for patients and controls with more than 2 MRI scans ( $p < 0.001$ ). ADEM = acute disseminated encephalomyelitis.

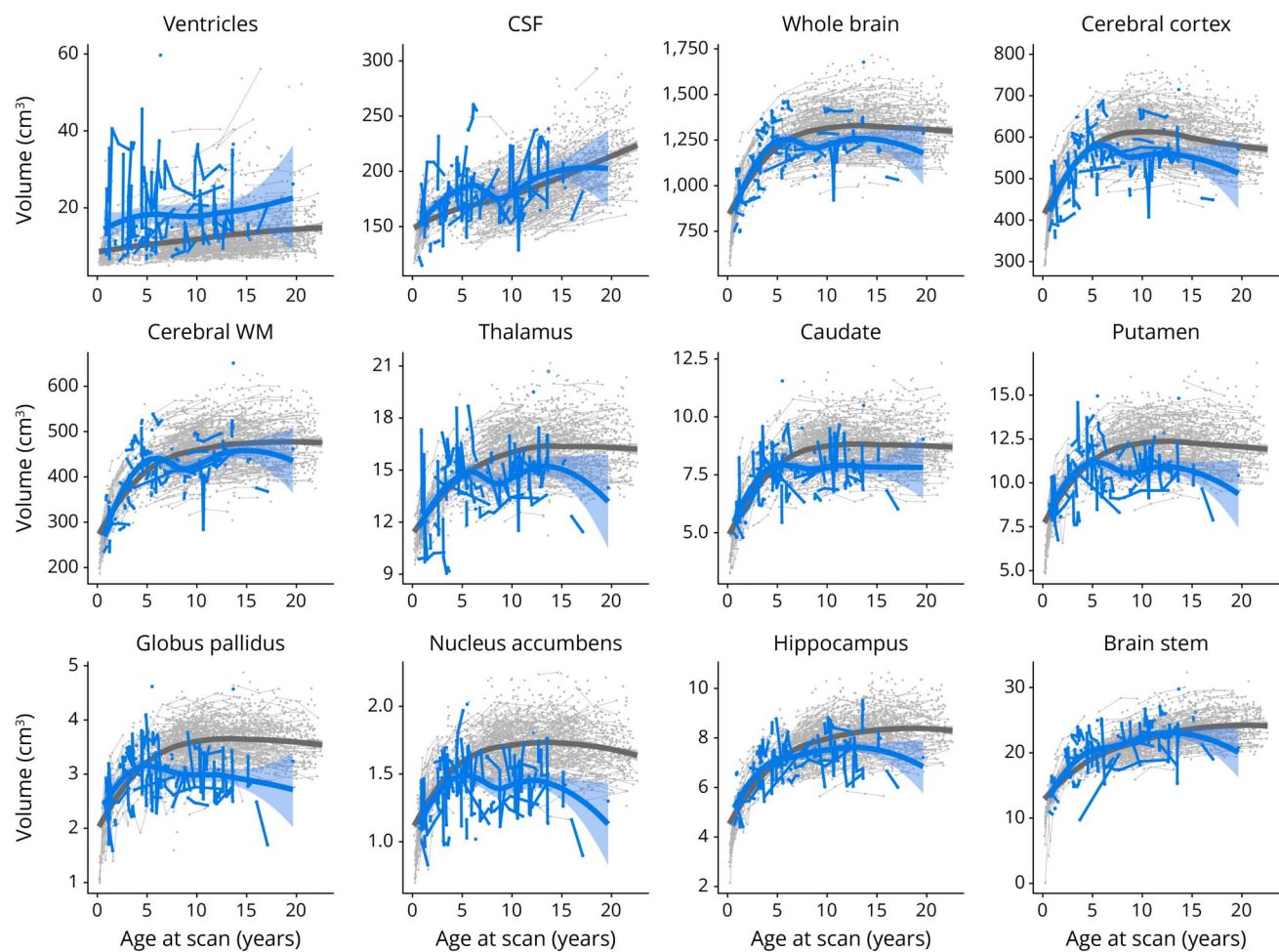
(ventricles plus extraventricular CSF) was greater in patients than in HCs (Table 2). Patients with ADEM had significantly smaller whole brain volume (BV) and cerebral cortex volume, but no difference in cerebral white matter (WM) compared with HCs (Table 2). Differences between patients and HCs were observed for deep gray matter structures, including the thalamus, caudate, putamen, globus pallidus, and nucleus (nuc.) accumbens (Figure 2, Table 2).

We tested whether other available clinical parameters had any significant effects on the dependent variable volumes. MOG

antibody status of patients with ADEM did not have any significant effect on VV. However, MOG antibody-negative patients had significantly smaller whole BV and smaller basal ganglia volumes (caudate, putamen, globus pallidus, nuc. accumbens) compared with MOG antibody-positive patients (Table 2).

Other available clinical parameters did not significantly improve the LME model. The presence or absence of relapses had no significant effect on the LME model. While age at scan was significant (considering the associated growth), baseline age did not improve the fit of the LME model. Baseline CSF

**Figure 2** Longitudinal Changes in Volumes of Brain Structures



Plots show absolute volumes ( $\text{cm}^3$ ) of ventricles, CSF, whole brain, and other brain structures. Light lines connect serial measurements of individual patients with ADEM (blue,  $n = 61$ ) and healthy controls (gray,  $n = 1,219$ ); heavy lines show mean  $\pm$  95% CI. The X-axis shows age of patients and controls (years).

cell counts yielded inconsistent results, and any significant nominal  $p$  values did not survive correction for multiple comparisons. EDSS scores could not be considered as a potential predictive factor because this was only available for the final time point. We did not include steroid treatment as a fixed effect because  $>80\%$  of patients received steroids.

Given that many patients with ADEM with  $>1$  MRI scan showed continued VV expansion beyond baseline, we identified the point of maximum VV for each patient during the 2-month *acute phase* (in Methods) to compare differences between patients and HCs (Table 3). At baseline, the mean absolute VV of patients with ADEM ( $n = 55$ ) was  $14.45 \pm 8.75 \text{ cm}^3$ , compared with  $11.21 \pm 5.52 \text{ cm}^3$  in a 1:5 set of age-matched and sex-matched HCs ( $p < 0.0001$ , Table 3), reflecting a difference of  $+28.9\%$ . At the *acute phase*, the difference between ADEM ( $n = 29$ ) and HC increased to  $+92.9\%$  (Table 3). The cerebral cortex volume was  $-4.6\%$  smaller in patients with ADEM compared with HCs at baseline and  $-7.3\%$  smaller during the *acute phase* (Table 3). Similarly, volumes of the caudate, putamen, globus pallidus,

and nuc. accumbens showed a greater difference between ADEM and HC at the *acute phase* than at baseline.

Within the ADEM cohort, 22 of 61 patients (36.1%) had  $>2$  MRI scans. To compare later disease outcomes with those at the baseline and *acute phase* in patients, we considered the *last MRI* that was available after the *acute phase*, but within 12 months after baseline (termed *last MRI*). At this point, the VV difference between patients and matched HCs was  $+75.2\%$ , reverting from the difference at the *acute phase* (Table 3). Similar observations were made for the cerebral cortex, putamen, and nuc. accumbens between patients with ADEM and matched HCs at the *last MRI* compared with the *acute phase*, but in the opposite direction (Table 3). For the caudate and globus pallidus, a unidirectional decrease in volume was observed from baseline, through the *acute phase*, to the *last MRI* (Table 3).

### Ventricle Expansion Resolves in Some Patients With ADEM but Persists in Others

The observation that the differences in VV between patients with ADEM and HCs at the *last MRI* were less severe than at

**Table 2** Longitudinal Volume Differences From LME Models

|                        | ADEM vs HC | MOG Ab neg vs pos | Age at scan | log10 (age at scan) | Male vs female |
|------------------------|------------|-------------------|-------------|---------------------|----------------|
| <b>Ventricles</b>      |            |                   |             |                     |                |
| $\beta$                | 2.434      | -0.650            | 0.210       | 0.624               | 2.182          |
| $t$                    | 4.421      | -0.734            | 5.564       | 2.236               | 10.789         |
| $p$ Value <sup>a</sup> | <0.0001    | 0.463             | <0.0001     | 0.0298              | <0.0001        |
| <b>CSF</b>             |            |                   |             |                     |                |
| $\beta$                | 7.102      | 0.225             | 2.598       | 5.451               | 15.014         |
| $t$                    | 2.710      | 0.051             | 15.874      | 5.312               | 14.286         |
| $p$ Value              | 0.0083     | 0.959             | <0.0001     | <0.0001             | <0.0001        |
| <b>Whole brain</b>     |            |                   |             |                     |                |
| $\beta$                | -33.118    | -47.649           | -13.53      | 217.993             | 124.816        |
| $t$                    | -2.345     | -2.026            | -21.303     | 44.628              | 22.923         |
| $p$ Value              | 0.0225     | 0.0431            | <0.0001     | <0.0001             | <0.0001        |
| <b>Cerebral cortex</b> |            |                   |             |                     |                |
| $\beta$                | -30.206    | -17.352           | -9.418      | 101.369             | 53.971         |
| $t$                    | -4.626     | -1.591            | -27.665     | 40.397              | 20.905         |
| $p$ Value              | <0.0001    | 0.112             | <0.0001     | <0.0001             | <0.0001        |
| <b>Cerebral WM</b>     |            |                   |             |                     |                |
| $\beta$                | -6.707     | -18.557           | -2.807      | 76.320              | 49.494         |
| $t$                    | -1.135     | -1.896            | -10.211     | 36.764              | 22.065         |
| $p$ Value              | 0.257      | 0.0681            | <0.0001     | <0.0001             | <0.0001        |
| <b>Thalamus</b>        |            |                   |             |                     |                |
| $\beta$                | -0.660     | -0.256            | -0.051      | 1.715               | 1.421          |
| $t$                    | -3.090     | -0.747            | -4.342      | 20.098              | 19.262         |
| $p$ Value              | 0.00239    | 0.455             | <0.00001    | <0.0001             | <0.0001        |
| <b>Caudate</b>         |            |                   |             |                     |                |
| $\beta$                | -0.360     | -0.578            | -0.102      | 1.644               | 0.590          |
| $t$                    | -2.534     | -2.516            | -13.887     | 30.910              | 11.804         |
| $p$ Value              | 0.012      | 0.012             | <0.0001     | <0.0001             | <0.0001        |
| <b>Putamen</b>         |            |                   |             |                     |                |
| $\beta$                | -0.619     | -0.572            | -0.157      | 2.131               | 1.066          |
| $t$                    | -3.763     | -2.143            | -16.863     | 31.496              | 17.994         |
| $p$ Value              | 0.00026    | 0.0324            | <0.0001     | <0.0001             | <0.0001        |
| <b>Pallidum</b>        |            |                   |             |                     |                |
| $\beta$                | -0.256     | -0.282            | -0.047      | 0.707               | 0.290          |
| $t$                    | -4.894     | -3.366            | -13.892     | 27.711              | 15.528         |
| $p$ Value              | <0.0001    | 0.00079           | <0.0001     | <0.0001             | <0.0001        |
| <b>Nuc. accumbens</b>  |            |                   |             |                     |                |
| $\beta$                | -0.157     | -0.094            | -0.021      | 0.280               | 0.130          |

Continued



**Table 2** Longitudinal Volume Differences From LME Models (continued)

|                    | ADEM vs HC | MOG Ab neg vs pos | Age at scan | log10 (age at scan) | Male vs female |
|--------------------|------------|-------------------|-------------|---------------------|----------------|
| <i>t</i>           | -6.216     | -2.325            | -14.397     | 25.487              | 14.827         |
| <i>p</i> Value     | <0.0001    | 0.0236            | <0.0001     | <0.0001             | <0.0001        |
| <b>Hippocampus</b> |            |                   |             |                     |                |
| $\beta$            | -0.213     | -0.199            | -0.051      | 1.463               | 0.528          |
| <i>t</i>           | -1.843     | -1.072            | -8.453      | 33.427              | 13.288         |
| <i>p</i> Value     | 0.0919     | 0.331             | <0.0001     | <0.0001             | <0.0001        |
| <b>Brainstem</b>   |            |                   |             |                     |                |
| $\beta$            | 0.284      | -0.084            | -0.014      | 3.757               | 1.969          |
| <i>t</i>           | 0.957      | -0.176            | -0.803      | 28.684              | 18.500         |
| <i>p</i> Value     | 0.474      | 0.86              | 0.492       | <0.0001             | <0.0001        |

Abbreviations: ADEM = acute disseminated encephalomyelitis; HC = healthy control; MOG = myelin oligodendrocyte glycoprotein; WM = white matter.  
<sup>a</sup> *p* Values corrected for multiple comparisons.

the *acute phase* suggested that in some patients the VV expansion had resolved. We stratified the patients with >2 MRI scans into 2 subcohorts based on the change in VV between baseline and *last MRI*, for each patient. We considered 15% as a normal range of variation in VV because 90% of HCs with >2 MRI scans had a CoV less than 15% (Figure 1C). Therefore, patients whose VV had recovered within this range, i.e., to 115% of baseline values, were considered to have resolved.

Of the 22 patients with more than 2 MRI scans, 12 (55%) had VV that returned to within 115% of their baseline values (termed *resolving*) while 10 had persistent VV expansion >115% at the last available observation (termed *nonresolving*) (Figure 3). Seven of these 22 patients had follow-up MRI measurements beyond 1 year, although only measurements from the last scan within the 12-month period were used for the *last MRI* analysis, to avoid age bias from longer term follow-up scans. However, the VV resolution status remained consistent in all cases; i.e., patients identified as *resolving* at 12 months did not show any subsequent VV expansion after 1 year, and patients identified as *nonresolving* at 12 months had persistently elevated VV that did not resolve back to baseline levels within the follow-up data available.

There was a clear distinction between the *resolving* and *nonresolving* patients in the absolute volume changes of other brain structures from baseline to *last MRI* (Figure 3A). *Nonresolving patients* showed a significantly greater delta (i.e., volume at baseline minus volume at *last MRI*), compared with *resolving* patients, for the whole brain, cerebral cortex, thalamus, caudate, putamen, globus pallidus, and nuc. accumbens (Figure 3A). There was no difference in the delta volume between baseline and *last MRI* for the cerebral WM, hippocampus, or brainstem (Figure 3A).

We examined whether there was any relationship between the magnitude of VV and the volumes of other brain structures at the *last MRI*. Given the wide ranges of absolute values, we assessed the correlation between VV z-scores and the z-scores of the other brain structures, separately for *resolving* and *nonresolving* patients. For the *nonresolving* patients, there were significant correlations between the z-score of VV at the *acute phase* and the z-scores of the thalamus, putamen, globus pallidus, and brainstem at the *last MRI* (Figure 3B). This was not the case for *resolving* patients for any structures. The correlation of the VV z-score with the z-score of the nuc. accumbens was marginal ( $p = 0.0666$ ) in *nonresolving* patients. No correlations between VV z-scores at the *acute phase* and z-scores of other volumes at the *last MRI* were significant for the *resolving* patients (Figure 3B). There were no significant correlations between z-scores of baseline VV and z-scores of other brain structures at the *last MRI*, for either *nonresolving* or *resolving* patients (data not shown).

### Clinical Implications and Predictive Value of Ventricle Expansion in Patients With ADEM

A representative example of images from a *resolving* patient with ADEM shows a transient increase to 171% of baseline VV by day 28 after initial clinical presentation, which subsequently resolved to within 115% of baseline by day 219 (Figure 4A). This patient did not show any residual neurologic signs. By contrast, images from a *nonresolving* patient show a greater magnitude of VV expansion: an increase to 483% of baseline, which had failed to resolve by day 224 after initial presentation (Figure 4B). This *nonresolving* patient had residual neurologic signs (with EDSS = 1), with persistent cognitive impairment at the time of the *last MRI*.

Most of the patients with ADEM (40/61, 65.6%) showed clinical recovery, with no residual neurologic signs at the *last MRI*. However, around a third of the ADEM cohort (21/61, 34.4%) did have residual neurologic signs at the time of last

**Table 3** Segmented Brain Volumes: Patients With ADEM vs Healthy Controls

|                          | Baseline volume<br>cm <sup>3</sup> | ANOVA<br>F<br>p Value <sup>a</sup> | ADEM vs HC           | Baseline <sup>c</sup><br>n = 55 | Acute phase <sup>d</sup><br>n = 29 | Last MRI<br>n = 22 |
|--------------------------|------------------------------------|------------------------------------|----------------------|---------------------------------|------------------------------------|--------------------|
| <b>Ventricles</b>        |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 14.45 ± 8.75                       | 86.19                              | % Difference         | 28.94                           | 92.98                              | 75.17              |
| HC                       | 11.21 ± 5.52                       | <0.0001                            | p Value <sup>b</sup> | 0.000995                        | <0.0001                            | 0.0000121          |
| <b>Whole brain</b>       |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 1120.29 ± 140.68                   | 5.02                               | % Difference         | -3.06                           | -3.69                              | -3.83              |
| HC                       | 1155.62 ± 171.54                   | 0.0541                             | p Value              | 7,26E-02                        | 1,29E-01                           | 1,90E-01           |
| <b>Cerebral cortex</b>   |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 545.82 ± 64.49                     | 15.4                               | % Difference         | -4.61                           | -7.29                              | -6.51              |
| HC                       | 572.23 ± 76.50                     | 0.000329                           | p Value              | 0.00691                         | 0.00309                            | 0.0156             |
| <b>Cerebral WM</b>       |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 404.05 ± 63.52                     | 0.60                               | % Difference         | -2.64                           | -1.31                              | -0.98              |
| HC                       | 415.01 ± 71.49                     | 0.485                              | p Value              | 0.277                           | 0.589                              | 0.804              |
| <b>Thalamus</b>          |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 14.64 ± 1.89                       | 2                                  | % Difference         | -0.14                           | -4.59                              | -6.60              |
| HC                       | 14.66 ± 2.19                       | 0.224                              | p Value              | 0.648                           | 0.13                               | 0.0219             |
| <b>Caudate</b>           |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 7.72 ± 1.15                        | 7.33                               | % Difference         | -2.76                           | -8.44                              | -9.23              |
| HC                       | 7.94 ± 1.53                        | 0.0169                             | p Value              | 0.0465                          | 0.00374                            | 0.000855           |
| <b>Putamen</b>           |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 10.8 ± 1.23                        | 17.49                              | % Difference         | -4.62                           | -10.48                             | -10.39             |
| HC                       | 11.32 ± 1.82                       | 0.000188                           | p Value              | 0.00308                         | 0.000456                           | 0.000516           |
| <b>Globus pallidus</b>   |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 3.05 ± 0.47                        | 16.31                              | % Difference         | -6.18                           | -11.47                             | -12.81             |
| HC                       | 3.25 ± 0.65                        | 0.000258                           | p Value              | 0.000669                        | 0.000188                           | 0.0000587          |
| <b>Nucleus accumbens</b> |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 1.47 ± 0.21                        | 49.86                              | % Difference         | -8.22                           | -18.08                             | -14.55             |
| HC                       | 1.60 ± 0.25                        | <0.0001                            | p Value              | <0.00001                        | <0.000001                          | <0.00001           |
| <b>Hippocampus</b>       |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 7.06 ± 0.96                        | 0.87                               | % Difference         | -1.56                           | 0.12                               | -4.61              |
| HC                       | 7.17 ± 1.41                        | 0.426                              | p Value              | 0.204                           | 0.724                              | 0.0288             |
| <b>Brainstem</b>         |                                    |                                    |                      |                                 |                                    |                    |
| ADEM                     | 20.20 ± 3.95                       | 2.8                                | % Difference         | 3.95                            | 4.89                               | 1.56               |
| HC                       | 19.43 ± 4.06                       | 0.161                              | p Value              | 0.103                           | 0.231                              | 0.853              |

Abbreviations: ADEM = acute disseminated encephalomyelitis; ANOVA = analysis of variance; HC = healthy control; WM = white matter.

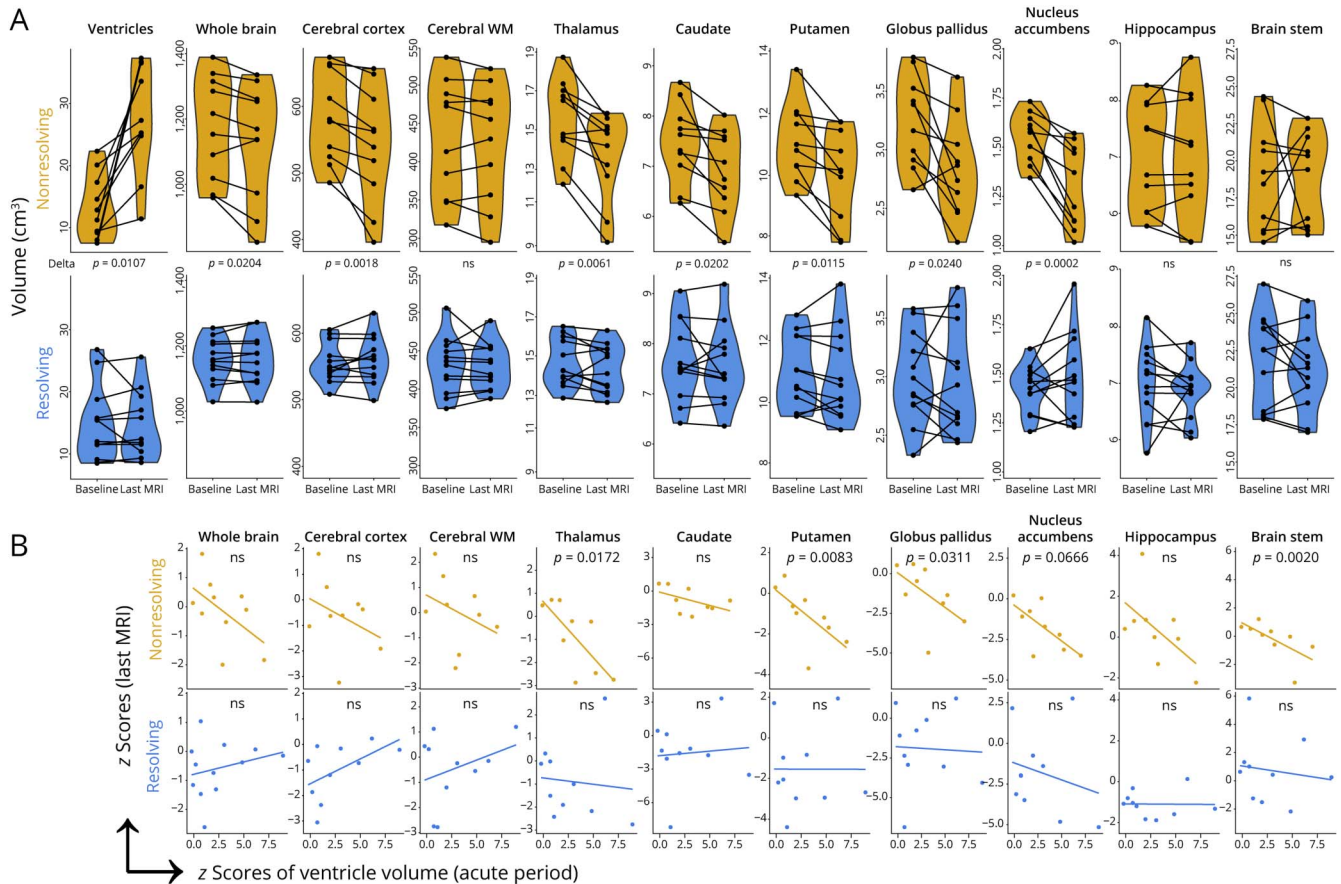
<sup>a</sup> p Values corrected for multiple comparisons by FDR.

<sup>b</sup> Wilcoxon matched pairwise comparison: ADEM vs HC.

<sup>c</sup> Sample size was reduced from 61 to 55 to allow 1:5 matching with healthy controls without repetition of controls.

<sup>d</sup> Patients who had a second MRI within 2 months of baseline.

**Figure 3** Relationship of Ventricle Volumes With Volumes of Other Brain Structures



(A) Ventricle volume nonresolving patients ( $n = 10$ ) show larger reductions in volumes of other brain structures than resolving patients ( $n = 12$ ). The mean difference in volume between the baseline and the *last MRI* (delta) was significantly greater in *nonresolving* patients for nearly all structures examined, with no significant difference in the delta of the cerebral WM, hippocampus, or brainstem. (B) The z-scores of the ventricle volumes at the *acute phase* (within 2 months of baseline) showed a negative correlation with the z-scores of the thalamus, putamen, globus pallidus, and brainstem at the time of last MRI, but only for the *nonresolving* patients. WM = white matter.

observation. A significantly greater proportion of the VV *nonresolving* patients (9/10, 90%) had residual neurologic signs compared with *resolving* patients (2/12, 16.7%,  $p = 0.0006$ ). There was no difference in sex or baseline age between *resolving* and *nonresolving* patients. There was no difference between *resolving* and *nonresolving* patients in MOG antibody status, whether patients had elevated CSF cell counts, and the incidence of relapses or transverse myelitis.

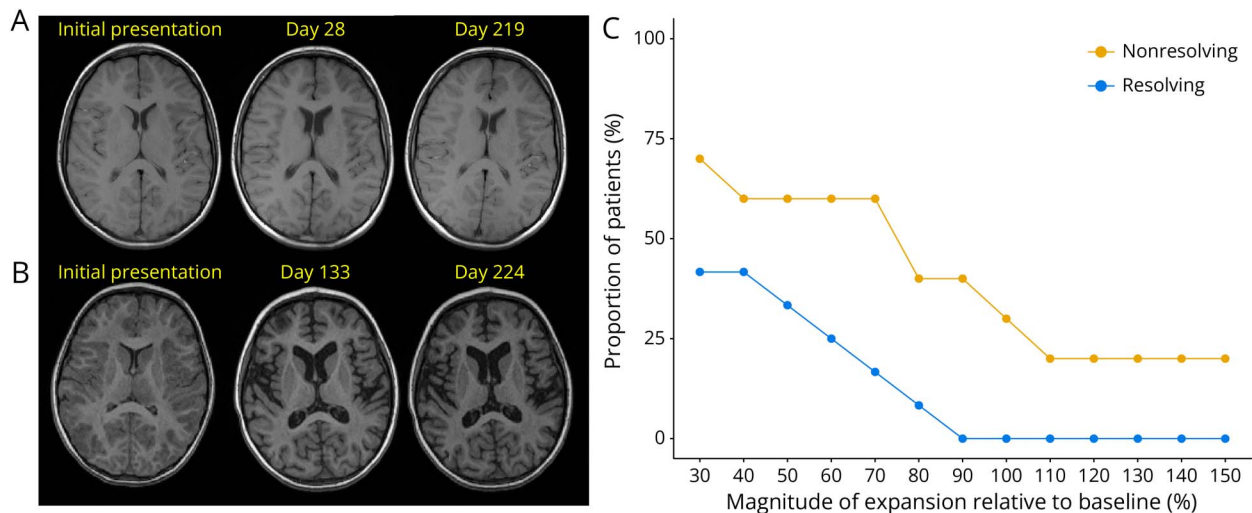
We examined whether the magnitude of the maximum VV expansion experienced by patients could discriminate between the *resolving* and *nonresolving* groups, using a series of thresholds between 30% and 150% of baseline values within 12 months of baseline. There was no difference in the proportion of patients with a maximum VV expansion  $\geq 30\%$  between the *resolving* and *nonresolving* groups. As the threshold was increased, the proportion of resolving patients with VV expansion above the threshold decreased faster than the proportion of nonresolving patients; e.g., at 70% VV expansion, around 60% of patients were in the *nonresolving* group and 13% were in the *resolving* group (Figure 4C). All patients who showed a maximum VV

expansion of  $\geq 90\%$  of baseline volume were in the *nonresolving* group ( $p = 0.0154$ ). This indicates that the magnitude of VV expansion observed could be informative to predict whether patients with ADEM would be in the resolving or nonresolving groups.

## Discussion

In this study, we show that pediatric patients with ADEM have enlarged VV already at baseline, and that most of the patients show further ventricle expansion during the *acute phase* of the disease (2-month period after baseline). In more than half of the 22 patients with at least 3 MRI scans, the VV subsequently normalized between the *acute phase* and *last MRI* (within 12 months of baseline) while the rest showed a persistent expansion—i.e., the VV failed to reduce to within 115% of the baseline volume. While most of the patients with ADEM showed full recovery from clinical signs consistent with expectations, a sizable fraction (34.4%) had residual neurologic signs at the last observation. Crucially, patients whose VV

**Figure 4** Persistent Ventricle Expansion Is Associated With Residual Clinical Signs



(A) Representative axial magnetic resonance images show changes in ventricle volume over time in a resolving patient (m, age = 11.8 years). The ventricles had increased to 171% of baseline at day 28 after initial presentation and had resolved by follow-up at day 219. (B) A *nonresolving* patient (f, age = 3 years) showed an increase in ventricle volume to 483% of baseline at day 133 after initial presentation. This expansion failed to resolve at follow-up (day 224), and the patient showed persistent cognitive deficits and had an EDSS score of 1. (C) Patients with a greater magnitude of ventricle expansion were more likely to be *nonresolving*. The proportion of patients with a maximum ventricle expansion of at least 30% (relative to baseline) was greater in the *nonresolving* group (70%) than in the *resolving* group (41.6%). As the threshold of maximum ventricle expansion increased, the difference in the groups was more exaggerated: 60% of *nonresolving* patients had a  $\geq 70\%$  expansion, compared with only 16.6% of the *resolving* patients; 40% of *nonresolving* patients had a maximum ventricle expansion of  $\geq 90\%$  of baseline volume—none of the *resolving* patients had an expansion this large ( $p = 0.0154$ ). EDSS = Expanded Disability Status Scale.

expansion did not resolve were more likely to have residual neurologic signs, suggesting an association between VV changes and clinical outcomes. Patients with the largest magnitude of VV expansions,  $\geq 90\%$  of baseline volume, were more likely to be in the VV nonresolving group. This underscores the need for patients with ADEM to continue undergoing regular MRI scans after baseline, even when they show improvement from acute neurologic signs. These follow-ups are necessary for early detection of VV expansion and for monitoring further expansions that could indicate a higher risk of persistent damage and to adjust treatment strategies accordingly.

We hypothesized that patients with larger VV expansion already at disease onset may have been at greater risk of worse changes in volumes of other brain structures and worse clinical outcomes. In fact, there was no difference in baseline VV z-scores between the VV resolving and nonresolving groups. There was no difference between the resolving and nonresolving groups in age, sex, occurrence of relapses, or elevated CSF counts at baseline. Because many patients showed further increases, baseline VV values likely did not accurately reflect the severity of pathologic changes. This is consistent with reports that MRI abnormalities in pediatric ADEM may continue to worsen within 3 months of initial presentation, even with recovery from clinical signs.<sup>18</sup>

Patients with ADEM had significantly smaller total BV compared with HCs and significantly smaller volumes of specific brain structures, including the cerebral cortex, thalamus, and basal ganglia. Patients who were MOG antibody-negative had smaller total BV, cerebral cortex volume, and volumes of the

thalamus, caudate, putamen, pallidum, and nuc. accumbens, compared with patients who were MOG antibody-positive. This corroborates the previous report that MOG antibody-negative pediatric patients with ADEM had smaller BV and larger VV than MOG antibody-positive patients<sup>4</sup> and adds specificity attributing these effects to both cortical and deep gray matter structures.<sup>19,20</sup> The LME analysis did not reveal any consistent effects of relapses, baseline age, or baseline CSF cell counts.

Our findings of enlarged VV and reduced total BV are consistent with other reports of reduced BV and impaired brain growth in pediatric ADEM.<sup>4,21</sup> General brain growth impairment has also been shown in pediatric MS,<sup>22,23</sup> as well as region-specific deep gray matter effects, particularly in the thalamus,<sup>24,25</sup> and in pediatric NMDA receptor encephalitis.<sup>26</sup> Several studies report involvement of the basal ganglia in pediatric ADEM,<sup>1,27</sup> with some indicating that this can help discriminate ADEM from pediatric MS.<sup>28,29</sup> These studies describe the presence of MRI lesions in the basal ganglia in ADEM but did not report on volume changes. The relationship between lesions and BV changes remains unclear and warrants further investigation, requiring standardized MRI data protocols to allow for a fair assessment of lesion burden. This is particularly important given that several studies have reported a lack of correlation between ADEM lesions and clinical outcomes.<sup>30–33</sup> Fully automated machine learning–based tools specifically trained to address the challenges of quantifying ADEM lesions—particularly diffuse ones with indistinct borders—would be a great asset, to dissect the link between lesions and volume changes in specific brain structures. Our ADEM cohort showed reduced volume of the thalamus,



caudate, putamen, globus pallidus, and nuc. accumbens. These changes likely reflect specific effects in these structures rather than general brain atrophy because we did not detect differences between ADEM and HC in other brain structures, e.g., cerebral WM and hippocampus. These volume changes were more pronounced in the *nonresolving* group.

Our results suggest that the magnitude of the VV expansion might be predictive for changes in other structures. *Nonresolving* patients with higher VV z-scores at the *acute phase* had lower z-scores for deep gray matter structures and brainstem at the *last MRI*; this correlation was only present in the *nonresolving* group, although the modest sample size warrants cautious interpretation. Notably, there was no correlation between VV z-scores at baseline and volumes at the *last MRI* for either *resolving* or *nonresolving* patients, underscoring the importance of ongoing monitoring after initial clinical presentation to determine whether a patient exhibits large VV expansions that could indicate greater risk of persistent brain atrophy. Decreased volumes of the deep gray matter structures and the cerebral cortex are consistent with reports that patients with ADEM and other acquired demyelinating syndromes can experience neurocognitive deficits, despite showing recovery of acute clinical signs.<sup>3,35-37</sup>

Many patients with ADEM in our cohort showed VV expansions that were substantially larger than what we reported for adult patients with RRMS.<sup>6</sup> The ADEM VV expansions were more similar to what we observed in the first phase of EAE.<sup>6</sup> This may reflect the acute nature of ADEM. The relapsing-remitting EAE model we used involves an acute response to an inflammatory stimulus at the beginning of disease, which is typically followed by milder relapses. Acute inflammation has been associated with ventricle enlargement in an animal model of posthemorrhagic hydrocephalus.<sup>37</sup> In that model, proinflammatory cytokines led to a TLR4-driven upregulation of a Na<sup>+</sup> + K<sup>+</sup> + Cl<sup>-</sup> ion co-transporter on choroid plexus epithelium, resulting in hypersecretion of CSF.<sup>37,38</sup> Hypersecretion of CSF has been proposed to contribute to pediatric hydrocephalus<sup>39</sup> and to posthemorrhagic and postinfectious hydrocephalus in adults<sup>40</sup> but has not yet been associated with pediatric ADEM. This mechanism has not yet been demonstrated in EAE, although we reported that the enlarged ventricles showed altered T<sub>2</sub> relaxation, suggesting an increased free water fraction in the CSF, consistent with inflammation-associated CSF hypersecretion.<sup>5</sup> The choroid plexus is a site of entry of pathologic immune cells from the periphery into the CNS in neuroinflammation.<sup>41</sup> We and others have shown inflammation and disruption of choroid plexus tissue architecture in EAE and MS.<sup>43-45</sup> Recent studies have also shown enlargement of the choroid plexus in MS, including in pediatric cases,<sup>45-48</sup> but this has not yet been reported for pediatric ADEM. In our cohort, only few patients had MRI with sufficient resolution to accurately segment the choroid plexus for volume measurement. Impairment in CSF clearance from the CNS may also contribute to VV enlargement. Perivascular and meningeal inflammation can interfere with the glymphatic system, leading to altered dynamics of

fluid flow through the CNS.<sup>49</sup> While CSF hypersecretion might be an evolved response to acute inflammatory challenges to boost removal of pathogens from the CNS, impaired glymphatic clearance may perpetuate CNS inflammation, because of the failure to remove proinflammatory cytokines and other signals. Sustained enlargement of the ventricles could provoke degeneration in adjacent brain structures due to compression forces.

The heterogeneous nature of the ADEM patient cohort, with inconsistent number and timing of follow-up visits and limited sample size, is a limitation of the study. Assembling multi-center patient cohorts is necessary, given the relatively low incidence of ADEM. Ideally, future studies should incorporate a standardized set of MRI acquisition protocols across centers, along with scheduled follow-up scans, even in patients who appear to have recovered from clinical symptoms. This would help mitigate the potential for selection bias, where patients undergoing multiple follow-up scans might represent those with greater disease severity and, therefore, may not accurately represent the general pediatric ADEM population. A larger sample size from future ADEM cohorts will also facilitate stratifying the patient population for a more granular investigation, e.g., separating MOG antibody-positive and antibody-negative patients, as well as patients with a multiphasic disease course (MDEM) that might affect BV changes differently. These groups may represent distinct disease entities, but this level of analysis was not feasible in this study, given the limited sample size. It is also necessary to increase the availability of MRI of HCs, especially for the younger population, to have sufficient sex-matched and age-matched controls. The MRI data lacked consistency among the various clinics, and this increased sample noise may have amplified the differences in VV CoV, compared with the more consistent HC data set. However, the observed volume differences might have also been diminished because of the increased variation in the patient group. With less sample noise, these differences could potentially have been more pronounced. The patient cohort we studied, like many in real-world clinical settings, had some gaps in data records. While such limitations are common, these data remain a valuable resource that is too often overlooked. The use of robust segmentation tools such as SynthSeg that can deal with heterogeneous MRI data of different resolutions and contrasts can mitigate this challenge to unlock the full potential of these data.<sup>12,13</sup> Appropriate statistical methods such as LME modeling can also mitigate the challenges of analyzing real-world clinical data with irregular time intervals between measurements. Improvements in data sharing and availability, including digital health records, are needed to fill in the gaps to fully use routine clinical data. This is also needed to better understand the consequences of steroid and anti-inflammatory treatment on brain growth in children.<sup>50</sup> More detailed information on the timing of steroids and other treatments regarding MRI would allow a more precise evaluation of treatment effects on BV changes, including controlling for pseudoatrophy. Another crucial gap is the need for more neurocognitive evaluation, to assess consequences of both acute and persistent brain changes in patients with ADEM.



Additional insights into the pathogenesis of neuroinflammation can come from correlating MRI findings with soluble factors such as neurofilament light chain or glial fibrillary acidic protein, which have been proposed as surrogate markers of axonal injury, astrocyte damage, and neurodegeneration.<sup>51,52</sup>

In summary, we show that pediatric patients with ADEM have enlarged VV at disease onset, which exacerbates over time in many patients. Some patients have persistent VV enlargement, which failed to resolve and which was associated with residual neurologic signs, possibly indicating permanent brain damage. The magnitude of VV expansion could have predictive power for changes in other brain structures, and patients with the largest VV expansions were more likely to be among the *non-resolving* patients. This argues for regular MRI monitoring of VV of pediatric patients with ADEM, to better observe and understand the dynamic brain changes associated with ADEM and to intercept those patients at the greatest risk of persistent brain changes. We show that pediatric patients with ADEM have reduced volumes of deep gray matter structures, adding to reports of MRI alterations in these structures. In synergy with this, neurocognitive testing for patients with ADEM, regardless of clinical recovery, will be crucial for monitoring long-term effects, providing support, and informing therapeutic decisions.

## Acknowledgment

The following clinicians provided patient data: Mareike Schimmel, MD (Children's Hospital, Klinikum Augsburg, Augsburg, Germany), Verena Kraus, MD (Children's Hospital Schwabing, Technische Universität München, Munich, Germany), Gerhard Kluger, MD (Schoen Klinikum Vogtareuth, Vogtareuth, Germany), Michael Karenfort, MD (University Children's Hospital, Heinrich-Heine-University, Düsseldorf, Germany), Astrid Eisenkölbl, MD (Women's and Children's Hospital, Linz, Austria), Steffen Leiz, MD (Klinikum Dritter Orden, Munich, Germany), and Johannes Koch, MD (Paracelsus Medical University, Salzburg, Austria). Further data used in the preparation of this article were obtained from the Adolescent Brain Cognitive Development<sup>SM</sup> (ABCD) Study ([abcdstudy.org](http://abcdstudy.org)), held in the NIMH Data Archive (NDA). This is a multisite, longitudinal study designed to recruit more than 10,000 children aged 9–10 and follow them over 10 years into early adulthood. The ABCD Study is supported by the National Institutes of Health and additional federal partners under award numbers U01DA041048, U01DA050989, U01DA051016, U01DA041022, U01DA051018, U01DA051037, U01DA050987, U01DA041174, U01DA041106, U01DA041117, U01DA041028, U01DA041134, U01DA050988, U01DA051039, U01DA041156, U01DA041025, U01DA041120, U01DA051038, U01DA041148, U01DA041093, U01DA041089, U24DA041123, and U24DA041147. A full list of supporters is available at [abcdstudy.org/federal-partners.html](http://abcdstudy.org/federal-partners.html). A listing of participating sites and a complete listing of the study investigators can be found at [abcdstudy.org/consortium\\_members/](http://abcdstudy.org/consortium_members/). ABCD consortium investigators designed and implemented the study and/or provided data but did not

necessarily participate in the analysis or writing of this report. This manuscript reflects the views of the authors and may not reflect the opinions or views of the NIH or ABCD consortium investigators.

## Study Funding

This study was partly funded by the German Federal Ministry of Education and Research within the project "Syreal" (Grant No.01/S21069A) (S.W.) and by the European Research Council under the European Union's Horizon 2020 research and innovation program under grant agreement No 743077 (ThermalMR) (J.M.M., S.W., T.N.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Disclosure

J.M. Millward, E. Pilgrim, E. Wendel, I. El Naggari, F. Bartels, C. Finke, T. Niendorf, and S. Waiczies report no disclosures. A. Bertolini has received travel support from Octapharma and advisory board honoraria from Horizon. M. Baumann received compensation for advisory boards and speaker honoraria from Novartis, Biogen, and Roche. K. Rostásy serves as consultant for Roche in Operetta II trial and received speaker honoraria from Merck. F. Paul has received research support from German Ministry for Education and Research (BMBF), Deutsche Forschungsgemeinschaft, Einstein Foundation, Guthy Jackson Charitable Foundation, EU FP7 Framework Program, Biogen, Genzyme, Merck Serono, Novartis, Bayer, Roche. F. Paul has received consulting fees from Alexion, Roche, Horizon, Neuraxpharm. F. Paul has received travel support from Guthy Jackson Foundation, Bayer, Biogen, Merck Serono, Sanofi Genzyme, Novartis, Alexion, Viela Bio, Roche, UCB, Mitsubishi Tanabe, Celgene. F. Paul has participated on Advisory Boards for Celgene, Roch, UCB, Merck. F. Paul has received honoraria for presentations from Almirall, Bayer, Biogen, GlaxoSmithKline, Hexal, Merck, Sanofi Genzyme, Novartis, Viela Bio, UCB, Mitsubishi Tanabe, Celgene, Guthy Jackson Foundation, Serono, Roche. Go to [Neurology.org/NN](http://Neurology.org/NN) for full disclosures.

## Publication History

Received by *Neurology: Neuroimmunology & Neuroinflammation* June 28, 2024. Accepted in final form September 23, 2024. Submitted and externally peer reviewed. The handling editor was Editor Josep O. Dalmau, MD, PhD, FAAN.

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Continued

## Appendix (continued)

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