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Immunomodulation with Azathioprine therapy in Rasmussen syndrome: a multimodal evaluation

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ABSTRACT

Objective: To verify safety and efficacy of the corticosteroid-sparing drug Azathioprine (AZA) in Rasmussen syndrome (RS), we retrospectively analyzed a cohort of RS patients recruited in a single pediatric neuroscience center.

Methods: We compared outcomes in 30 RS patients who received AZA with 23 patients who were not treated with this drug. We used a multimodal approach to correlate therapy with clinical features (seizures, epilepsia partialis continua [EPC], hemiparesis) and neuroimaging markers of progressive brain atrophy.

Results: AZA was well tolerated; only one patient discontinued treatment due to pancytopenia. In 27/30 AZA patients, all of whom were corticosteroid responders, corticosteroid therapy could be weaned or reduced without worsening of seizures in 89%. AZA patients had a lower prevalence of EPC (42% vs. 67% in controls) and hemiparesis (64% vs. 92%, respectively). Cox regression showed for the AZA group compared to controls a delayed time to: 1) EPC (of about 2 years, *Exp(B)=0.295, 95%CI[0.108, 0.807];p=0.017*), 2) hemiparesis (about one year, *Exp(B)=0.315, 95%CI[0.137, 0.724];p=0.007*), and 3) surgery (about 2 years, *Exp(B)*=2.068, *95%CI[1.012,* 4.227*];p=0.046*). However, there were no group differences in cognitive decline over time (IQ change per year) or in hemispheric grey matter atrophy on serial MRI scans.

Conclusion: AZA treatment appears to slow clinical progression of Rasmussen syndrome in steroid responders; this will give most advantage in patients in the early stages of the disease in whom surgical decision-making may require further time.

Classification of Evidence: This study provides Class III evidence that ~~in~~ for pediatric RS patients AZA is well tolerated and slows hemiparesis and appearance of EPC.

Introduction

Rasmussen Syndrome (RS) or Rasmussen's encephalitis is a rare, chronic inflammatory disease of the brain, of unknown etiology that affects only one cerebral hemisphere, characterized by pharmacoresistant focal seizures, progressive hemiparesis and cognitive decline[1, 2]. The definitive pathogenic mechanism of RS remains unknown; histopathology reveals a chronic encephalitis and suggests that T-lymphocytes have a crucial role in the pathogenesis[3-6].

An effective curative medical treatment has remained elusive. The aim of the current therapeutic approach is to minimize seizure burden and limit neurological decline. For the first goal, antiepileptic drugs with short-term intense immunotherapy, and eventually surgery, play a role. To prevent and limit neurological deficits, in view of the assumed immunological etiology, long-term immunotherapy has been utilized [7, 8]. Azathioprine (AZA) is a cytotoxic agent, which predominantly affects T cell function. It is used for immunosuppression in autoimmune diseases and is widely used for prevention of rejection following organ transplantation. AZA has been utilized in pediatric RS patients as a corticosteroid-sparing agent at Great Ormond Street Hospital for Children, London, since the early 1990s. Here we examined the clinical and neuroimaging outcomes of patients treated with AZA in comparison to a group of patients not treated with AZA (control group).

Material and methods

The aim of this study is to describe safety (class III evidence) and efficacy (class III evidence) of the corticosteroid-sparing drug AZA in RS. We performed a retrospective review of patients followed between 1987 and 2018 at Great Ormond Street Hospital (GOSH) for Children NHS Foundation Trust, a tertiary pediatric hospital in London,

with specific expertise in complex epilepsies and epilepsy surgery. The inclusion criteria were: 1) a consensus diagnosis of definitive RS by a team of pediatric neurologists according to previously accepted diagnostic criteria [8,9] and 2) a minimum follow-up of two years from disease onset. The exclusion criteria were: 1) bilateral disease, 2) metabolic or degenerative progressive neurological diseases, 3) cerebral vasculitis, 4) infectious diseases, 5) other immune mediated encephalitis (for example anti-N-methyl-D-aspartate (NMDA) receptor encephalitis) and 6) ~~2)~~ insufficient clinical data.

Data were collected from medical records, including clinical, neuropsychology, histology and neuroradiology reports. As per institutional protocol, once the diagnosis of RS was established, patients entered a follow-up program that required yearly assessments with MRI brain scan, EEG and neurological evaluation. Follow-up could be adjusted according to individual clinical evolution.

Definition of treatment groups: For this analysis, RS patients were divided into two groups – an *AZA treated group* and a non-treated (*control)* group. For the AZA group a minimum time of treatment of 3 months was chosen based on the drug's mechanism of action, that requires this time to reach efficacy [10]. The decision to start AZA or not was made by the child's lead clinician. There was no selection on the basis of clinical presentation as to who or who was not initiated on AZA. In the majority, those not trialling the medication were historical cases, or patients who after initiation progressed rapidly and moved to surgery. The decision to use AZA was made when there was a certain diagnosis of Rasmussen syndrome. In the initial phase, this was initiated subsequent to a trial of steroids; in the majority of patients, it was initiated when the diagnosis was confirmed and steroids initiated. The patients in the AZA group received open-label AZA (1.5mg/kg/day orally), in most cases with concomitant oral prednisolone (2mg/kg/day to maximum 60mg/day) for six weeks. Steroids were then

weaned to 1mg/kg/day over 6 weeks and weaned to zero over a further 3 months. The control group consisted of a group of RS patients not treated with AZA or who received less than 3 months of AZA treatment. AZA was discontinued at the time of surgery, with no prior discontinuation or wean.

Volumetric MRI analysis

For MRI analysis, patients were included if they had undergone at least two 3D volume T1-weighted MRI scans separated by at least one month, to permit a longitudinal assessment of grey matter (GM) volume changes. Prior to 2007, scans were acquired on a 1.5 T Siemens Vision System (Erlangen, Germany) using a MPRAGE sequence (repetition time 10ms; echo time 4ms; flip angle 12°; voxel size 1.0x1.0x1.25mm). Scans after 2007 were acquired on a 1.5 T Siemens Avanto System, using a 3DFLASH sequence (repetition time 11ms; echo time 5ms; flip angle 15°, voxel size 1.0x1.0x1.0mm). After 2015 all scans were acquired on a 3T Siemens Prisma system. For scans performed elsewhere (about 20% of scans) the exact acquisition parameters were not always available. Thirty-seven (70% of all RS patients) with at least two available MRI scans were included in the MRI analysis (total of 121 scans, median 3 scans per patient, range 2–10). This allowed us to consider the temporal relationship of each MRI scan in relation to the onset and duration of AZA treatment.

We performed brain tissue volume extraction using the voxel-based morphometry (VBM) module of Statistical Parametric Mapping version 12 (SPM12) software after segmenting scans into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF), using previously described method [11]. The data were normalized into the International Consortium of Brain Mapping space and resampled to a voxel size of

1.5x1.5x1.5mm. Global brain GM and hemispheric GM volumes were extracted using the Easy volume function.

Volumetric Measures: We focused here on GM changes as they represent the most sensitive parameter of cerebral atrophy in RS [11, 12] and extracted the following measures, according to previous works [12-14]:

- GM interhemispheric ratio (GM-HRvol): absolute GM volume of the affected hemisphere (AH) divided by absolute GM volume of the unaffected hemisphere (UH) for each scan (GM-HRvol = AH / UH). GM-HRvol equal to one means that the two hemispheres have the same volume, whereas GM-HRvol values <1 mean a certain degree of atrophy of the AH. This relative measure of GM atrophy has been shown to be most sensitive and specific to RS compared to other epilepsy syndromes [14].
- GM-HRvol change per year: this value was obtained by dividing the difference between a GM-HRvol for two consecutive scans by the time (in years) between the scans.

Additionally, for every scan, we determined concurrent AZA and corticosteroid therapy, to define a subgroup of patients who had serial volumetric MRI scans performed before and during AZA treatment (considered after at least 3 months of therapy).

We performed two separate analyses of the ratio of interhemispheric atrophy. First, we performed a longitudinal analysis in all cases with at least one scan before and one scan during AZA treatment compared to all control cases with at least two serial scans (total n=28). A second analysis compared the change in the interhemispheric ratio in all serial scans, depending on whether patients were on or off AZA during the

intervening period. This analysis was aimed at increasing the power of the comparison, as more patients could be included (total n=36).

Neuropsychological measures

We included patients with at least two independent assessments during the follow-up period and analyzed the scores of neuropsychological assessments of intellectual functioning using the age-appropriate version of the Wechsler Intelligence Scales (WISC-IV, WISC-III, WISC-R, WPSSI-R, WPSSI-III, WAIS-III, WASI). The general ability index (GAI) was calculated in about half of the sample, because FSIQ could not be determined. In those cases, this index was used instead of FSIQ score. In an analogy to the volumetric MRI analysis, we performed a longitudinal (before and during AZA) comparison and a serial IQ comparison (change scores normalized by time between assessments) taking into account AZA use in between assessments across all data points.

Outcome measures

The primary outcome measures were: 1) efficacy on corticosteroid weaning and sustained seizure control, 2) neurological decline (EPC and hemiparesis), 3) cognitive decline (IQ scores), 4) quantitative analysis of hemispheric brain atrophy on MRI (GM-HRvol), and 5) time to surgical treatment. Secondary outcome measures were: 1) safety of AZA treatment, and 2) long-term outcome at last observation. For further details on the definitions, see supplementary material (appendix e-1).

Statistical analysis

Statistical analysis was performed using SPSS 25 for Windows (SPSS Inc., Chicago, IL, USA). The threshold for statistical significance was set to $p < 0.05$.

For analysis of survival curves, Kaplan-Meier plots were computed and differences were tested using the Mantel-Cox test. Mann-Whitney U tests were used for continuous variables without a normal distribution. Categorical data were compared between treatment groups using Chi^2-tests. Repeated-measures ANOVA was conducted to investigate the effects of treatment group and time (before AZA, during AZA) on GM-HRvol, adjusting for time from disease onset to first scan. Group differences in IQ change scores with AZA treatment were evaluated using the Mann-Whitney test.

To examine the effect of concomitant AZA treatment on longitudinal change in IQ and GM-HRvol, (irrespective of whether pre-treatment assessments were available as in the longitudinal analysis above) a linear mixed model with random intercept and restricted (residual) maximum likelihood estimation procedure was computed using the MIXED module in SPSS. This allowed test results to account for correlated observations nested within individuals. Independent variables included current AZA treatment and duration from onset to assessment.

Fully automated pipelines were used for the analysis of MRI volumetric data, minimizing any bias. Similarly, assessments of neuropsychological functions were blind to AZA medication status. All analysis was performed by SP, who was not involved in clinical management.

Classification of Evidence

This study provides Class III evidence that AZA is well tolerated and slows motor decline and appearance of EPC in pediatric RS patients.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the UCL Institute of Child Health/Great Ormond Street Hospital Research Ethics Committee. STROBE (Strengthening the reporting of

observational studies in epidemiology) checklist for cohort studies was completed for this study.

Data availability

An anonymized database was created to investigate the objective of this observational, retrospective study, which is available from the authors on request.

Results

Demographic and clinical data

Between 1987 and 2018, 60 patients received a diagnosis of probable or definite Rasmussen's syndrome (RS). We identified 53 patients who met inclusion criteria. A total of seven patients were excluded, four because they did not fulfil the diagnostic criteria, three because of lack of clinical data. Thirty patients were treated for at least 3 months with Azathioprine and denoted as the AZA group, while the remaining 23 cases represented the control group. Table 1 summarizes the principal demographic and clinical characteristics of the treatment groups and table 2~~e 1~~ the follow-up data.

The average number of antiepileptic drugs was 2 (range 0-5). One child was commenced on ketogenic diet but discontinued without adequate trial due to poor compliance.

Immunotherapy

A total of 48 of 53 patients (90% of cohort) were treated with different immunomodulatory therapies following diagnosis, mainly with corticosteroids (see table 3~~e 2~~). Corticosteroid treatment was given to 47 patients, mostly as prednisolone (2 mg/kg/day orally for six weeks) followed by tapering. Median time between onset of RS and first corticosteroid administration was 1.7 years (range: 0-7.8 years). Corticosteroids were effective in reducing seizures in about 70% (33/47). Note that as

data on seizure frequency was not consistently available, seizure response was defined qualitatively as “response” (clear improvement in seizure frequency or intensity) or “no response”. AZA was administered at the same time or soon after introduction of corticosteroid therapy, with a mean gap of 0.5 years. In only 3 children AZA was not concomitant to steroids. In the control group patients received corticosteroids with a shorter duration of the treatment (see table 1). Median time from disease onset to commencing AZA was 2.8 years (IQR 1-4). Median time from acute phase to AZA therapy was 0.4 years (IQR 0.1-1.1). Mean duration of AZA therapy was 3.2 years (range 0.5-10.5 years). Nine patients, who have not yet come to surgery, continued on AZA at last follow-up. Most patients received AZA before surgery; however 3 patients, who underwent a lobar resection early in their history without improvement in seizure control, received AZA after the first surgery. AZA was stopped in one patient because of side effects.

AZA group-specific outcomes

Efficacy on corticosteroid weaning and sustained seizure control

Twenty-seven patients (90% of the AZA group) were already on corticosteroid treatment when AZA was initiated, or the two therapies were initiated at the same time. In all patients, clinicians attempted to wean steroid therapy after a period of around 6 weeks. In 17 patients (57% of the AZA group) the dose of steroids was greatly reduced (in most cases to twice weekly pulsed dose), and in 10 patients (34%) steroids were completely weaned without apparent worsening in seizure control. Of those patients in whom corticosteroids were weaned/reduced, 24/27 (89%) showed a sustained seizure response, predominantly regarding focal to bilateral tonic-clonic seizures (18/21 patients) and convulsive and non-convulsive status epilepticus (6/8 patients). One

patient maintained seizure freedom and in 4/18 patients a sustained reduction in EPC severity was observed.

The median number of AEDs used per patient (n=2) did not change from before to one year after AZA initiation.

Safety

Three patients on AZA therapy (10%) had side effects, in all cases hematological, and in only one (3%) this was clinically significant to require discontinuation. In two patients (7%) a low lymphocyte count was not considered pathologic and AZA was continued without any complications. In the third patient, AZA was started in close relation to carbamazepine, an antiepileptic drug that may also cause hematological side effects. This patient developed pancytopenia, with consequent febrile neutropenia and multiple macular hemorrhages that required a hospital admission. Measurement of Thiopurine methyltransferase (TPMT) activity revealed a low enzyme activity (carrier range). Both AZA and carbamazepine were discontinued, and the hematological problem subsequently resolved. TPMT activity was not routinely assessed in all patients prior to commencing AZA.

Outcomes across treatment groups

Time from onset of RS to development of EPC

Development of EPC was common in this cohort (31/53, 58%), observed in 52% of controls and 63% of the AZA group. In two patients, EPC was the presenting symptom, in 29% within 6 months and in 48% within 18 months from disease onset. AZA was commenced prior to EPC onset in eight patients. Therefore, we divided patients into two groups:

- Group with prior AZA: patients in whom AZA was started without clinical signs of EPC (n=19). Eight children later developed EPC in this group (42%).
- Group without prior AZA: controls (n=23) and those with AZA after onset of symptoms (n=11, total n=34). Twenty-three children developed EPC in this group (67%).

Figure 1A provides a flow chart to show numbers of cases for this analysis.

While there was no difference in mean age at EPC onset between groups (without prior AZA:.1 y ± 3.2, with prior AZA: 8.4 y ±3.9; 95%CI of difference [-1.65, 5.05];T-test p=0.307), time from onset of RS symptoms to EPC was shorter in the prior AZA group compared to controls (1.6 y ± 1.5 VS 3.3 y ± 2.4; 95%CI of difference [0.20, 3.31]; T-test p=0.029).

In a Cox-regression model, we found a significant delay in time to EPC onset in the group who received AZA prior to EPC development versus the (no prior AZA) control group (B= -1.22, Exp(B)=0.295, 95%CI[0.108, 0.807]; p=0.017) when adjusted for steroid response (yes or no) and duration of corticosteroid therapy (Figure 1~~1~~2A).

Time from onset of RS to development of hemiparesis

Of the 45/53 patients (85% of the total cohort) who developed a fixed hemiparesis prior to hemispherotomy, four patients developed hemiparesis within the first month from onset and 24 within 1.5 years.

To analyze the effect of AZA on the development of hemiparesis we divided the RS population in two groups (see Figure 1B):

- Group with prior AZA: patients in which AZA was started before onset of hemiparesis (n=14). Nine subsequently developed hemiparesis on medical treatment in this group (64%, between 6 and 2.3 years from AZA initiation).

- Group without prior AZA: controls (n=23) and those with AZA after onset of symptoms (n=16, total n=39). Thirty-six developed hemiparesis prior to surgery (92%).

While there was no difference in mean age at hemiparesis onset between these adjusted groups (with prior AZA: 10.1 y ± 2.8, without prior AZA: 8.3 y ± 3.6; 95%CI of difference [-0.65, 4.38]; T-test p=0.132), we found a difference in time from onset of RS symptoms to hemiparesis (with prior AZA: 3.1 y ± 1.4, without prior AZA: 1.8 y ± 1.6; 95%CI of difference [0.04, 2.57]; T-test p=0.043).

In a Cox-regression model, we found a significant delay in time to development of fixed hemiparesis in the group who received prior AZA compared to controls (B= -1.16, Exp(B)=0.315, 95%CI[0.137, 0.724]; p=0.007), when adjusted for corticosteroid response and duration of steroid therapy (Figure 2B~~2~~).

Time to hemispherotomy

Patients in the AZA group underwent hemispherotomy at a later stage in the disease course than those in the control group (Table 1). Because the decision to proceed to surgery was determined by multiple factors, we included additional variables into the Cox-regression model for time-to-hemispherotomy: rate of clinical progression (slow vs rapid), presence of EPC, presence of hemiparesis, presence of cognitive decline, affected side (dominant and non-dominant) and group (AZA vs controls). Figure ~~3~~2C shows a delayed time to surgery among those receiving AZA treatment compared to controls (B=0.73, Exp(B)=2.068, 95%CI [1.012, 4.227]; p=0.046), after adjustment for these variables.

Volumetric analysis of brain atrophy

Longitudinal volumetric analysis of hemispheric grey matter atrophy: In the AZA group 16 patients had consecutive T1 volume scans before and during the therapy and 12 control patients had serial scans with a similar time between scans (mean group difference=0.6y, 95%CI=[-0.3, 1.4], t(df=34)=0.18, p=0.402). Importantly, AZA patients had their first volume scan at a later stage in the disease than controls (mean difference=2.2y, 95%CI= [0.8, 3.6], t(df=34)=3.2, p=0.003). Nevertheless, the change in interhemispheric ratio over time was not different between treatment groups, after adjusting for time from onset to first scan (Figure 43A). This finding was unchanged when excluding patients with prior steroid exposure.

C*hange in the interhemispheric grey matter ratio (GM-HRvol) and concomitant AZA use:* This analysis was restricted to all scans taken after 2 years from disease onset, because only very few early scans were available for the AZA group. The change between serial scans tended to decline with increasing disease duration for both groups (Figure 43B), approaching zero between 8 and 10 years from onset. Linear mixed modeling confirmed this effect for time from onset on GM-HRvol change (estimate =0.0032 SE = 0.00138, t=2.31, p=0.024), but the change in hemispheric atrophy was not different between groups (estimate = -0.0003 SE = 0.0074, t=-0.46, p=0.964). Adding concurrent steroid therapy to the model (which itself was not significant at p=0.754), did not alter this finding.

We examined subgroups based on the hemisphere affected by RS. There was no difference in volumetric change in patients with left or right-hemispheric RS. While both groups lost volume to the same degree, no effect of AZA was seen in either group.

Comparison of serial IQ scores

Longitudinal IQ comparison: In the AZA group 18 patients had at least one IQ assessment before and during treatment, while only 5 controls had serial assessments (Figure ~~5~~3C and D). Groups did not differ in the time from onset to first assessment (mean difference = 0.77 y 95%CI=[-1.47, 3.01], Mann-Whitney test: p=0.538) and time between serial assessments (mean difference = 0.3 y 95%CI=[-.69, 1.30], Mann-Whitney test: p=0.491). The change scores in FSIQ over the follow-up period of 1.5 years between controls (mean change = -4.30 (SD 6.79)) and the AZA group (mean change = -3.36 (SD 7.28), however, was not different (Mann-Whitney test, p=0.857).

We also examined the effect of affected hemisphere on verbal and non-verbal IQ scores showing that longitudinal verbal IQ was reduced by left-hemisphere involvement (p=0.002), but the effect of right hemisphere disease on performance IQ failed to reach significance (p=0.206). Neither AZA, nor steroid use had any measurable impact on scores when side of injury was factored in.

Change in IQ scores and concomitant AZA use: The change in IQ scores between consecutive assessments also tended to diminish over follow-up time (estimate = -1.8146, SE = 0.4404, t=4.12, p<0.0001), crossing the zero line about 8 to 10 years after onset (Figure 3D), however, with no effect of AZA use (estimate = -2.4001, SE = 2.4005, t=0.571, p=0.570).

Discussion

RS is a rare disease with a variable and prolonged clinical course, which makes it difficult to conduct randomized controlled trials. Retrospective studies can provide useful clinical guidance and reference data for future intervention studies. Here we report the largest pediatric cohort of RS patients treated with AZA.

This retrospective study showed that AZA is a safe, long-term corticosteroid-sparing therapy in RS resulting in better seizure control and delay in time to onset of EPC, fixed hemiparesis and time to hemispherotomy. AZA, however, did not appear to halt progression of hemi-atrophy and cognitive decline.

The most commonly used and probably most efficacious immunotherapy in RS are *corticosteroids.* Courses of high-dose intravenous methylprednisolone, often followed with chronic oral prednisone, have been reported to be effective in reducing seizure frequency and improving neurological status [7, 15, 16]. While short-term use is usually effective and tolerated, the long-term usage is often problematic because of side effects. Many patients in our cohort started AZA in combination with steroid therapy and it is therefore difficult to attribute with certainty the reduction of seizures to one or to the other treatment. However, we observed a clear improvement in seizure frequency after initiating steroid therapy in the majority of cases [15, 17] that persisted after reduction of steroids and during AZA maintenance therapy. Further, although there is limited data, in comparison to other immunotherapies studied in RS, AZA seems to be one of the better-tolerated [15, 18-20], specifically in comparison to corticosteroids. In a retrospective study on long-term corticosteroid therapy in RS [21], all eleven patients treated with bolus of methylprednisolone and then oral prednisolone had mild to moderate steroid-related side effects: Cushing syndrome, osteoporosis, hypertension, and infections. Takahashi et al. reported discontinuation of regular pulsed steroid therapy in 62% of patients due to frequent hospitalization and reduction of school attendance[17]. Other authors observed that, when steroid therapy was commenced and was effective on seizures, the attempts to wean the treatment in the long-term resulted in seizure recurrence [15]. Thus, long-term steroid therapy in RS causes a therapeutic dilemma due to high incidence of side effects. In our cohort, it was possible to reduce the dose of corticosteroid therapy in all patients,

and in more than one-third, steroids were completely discontinued without worsening of seizure control. This suggests that AZA may have a role as a steroid-sparing agent in the medical management of RS.

In addition, we observed that AZA use resulted in a clear delay in the onset of neurological decline: patients treated with AZA had later onset of EPC and fixed hemiparesis, as well as time to hemispherotomy. In this context, AZA demonstrated a dual effect, both on epilepsy and neurological function.

Other immunomodulatory therapies have been utilized in RS but only with limited success. *Intravenous immunoglobulin (IVIG)* efficacy has been reported in a few patients [15, 16], however, with no consistent and durable effect and a poor neurological outcome. *Plasmapheresis or protein A immune-absorption* have been used on the premise of removal of potentially pathogenic circulating antibodies, with limited benefit in the long term [15]. *Tacrolimus* [17-19], with a similar target of action to AZA, has been shown to reduce seizures and to lead to a good functional outcome. Single case reports, small case series and rare trials in RS have demonstrated inconsistent efficacy of other immunotherapies: newer monoclonal antibodies such as *Rituximab* [22-24], *Natalizumab* [25], *Adalimumab* [20], mycophenolate mofetil [26], methotrexate alone or the combination of *Alemtuzumab* and intrathecal methotrexate [27] have also been used with mixed results.

Ketogenic diet has been described only in a few patients with pediatric Rasmussen syndrome: in two cases it led to a 50% seizure reduction [28]; in another two the diet was effective in refractory status epilepticus [29, 30]; however in one case only temporarily [30].

Cognitive decline, the third aspect of disease progression in RS, appeared not to be influenced by AZA therapy. Takahashi et al. described IQ stabilization in a majority of patients receiving different immunotherapies [17]. However, it must be underlined, that cognitive stabilization was defined as a variation of IQ within ± 10 points; which is a

wide range, well exceeding the expected average change over one year of disease progression [11].

An interesting finding from our study was that, while there was a clear delay in onset of EPC and hemiparesis, the rate of hemispheric brain atrophy, did not differ between AZA and control groups. The interhemispheric GM volume ratio is a sensitive marker of disease progression [14] but we did not observe any significant differences over time in patients receiving AZA and those who did not. Subsidiary analyses confirmed this finding also for absolute hemispheric GM volumes (data not shown). One possible explanation is that in the first two years from disease onset MRI data were available from only a few AZA patients. It has already been described that the rate of annual GM atrophy is higher in the early stages of the disease and then declines [13], resulting in a stabilization of the disease that tends not to progress to the same degree over the following years. We confirm this with our analysis here, showing quantitative evidence for stagnation of hemispheric atrophy from around 8-10 years from disease onset.

A correlation between hemispheric MRI volume loss and cognitive (IQ) decline over time has been reported in a sub-sample of this cohort[11]. This correlation is however complicated by the fact that assessments were often performed at different time points.

To date, surgery has been the only effective treatment halting seizures in RS [8] with the most effective procedure being the complete disconnection of the affected hemisphere [7, 17, ~~28~~31]. Limited or focal resections are not effective and often result in relapse with eventual progression to hemispherotomy. Hemi-disconnection results in hemiplegia and hemianopia, hence it can be difficult to offer this effective treatment in functionally preserved patients. Whether surgery should be delayed until a significant functional deficit becomes apparent (with or without debilitating seizures) [7, 15] or should be offered earlier [32~~29~~] is a major clinical dilemma, particularly in dominant hemisphere disease [~~30~~33]. When the language-dominant hemisphere is affected, language decline is

almost inevitable [11]. Nevertheless, language reorganization to the contralateral hemisphere is possible [31, 32]34, 35 and long-term functional language recovery has been documented in some dominant-hemisphere cases [33]36.

In our cohort, patients treated with AZA underwent hemispherotomy later in the disease course in comparison to control patients, most likely related to a better seizure control and delayed neurological decline. In other words, AZA and other immunomodulatory therapies allow an extension of the functionally preserved period before the surgery. However, no benefit was observed for the pre-surgical cognitive trajectory or hemispheric brain atrophy. In addition, the efficacy of surgery for seizure outcome appeared unaffected by prior AZA therapy. Thus, AZA therapy potentially offers an improvement in quality of life of patients with RS and buys time for families to decide about surgical hemi-disconnection, which is the only definitive treatment at present.

AZA was relatively well tolerated in our study. Hematologic side effects are a major concern of AZA [34]37 but affected only 10% of our population. This lower rate in comparison to dermatological cohorts, in which rates up to 48% have been found [35, 36]38, 39, is likely due to the lower dosage used in our patients (1.5 mg/kg vs. 2.5 mg/kg). Only one child required discontinuation for adverse hematological indices, a rate very similar to other studies [35-37]38-40.

There are several limitations with this study. The main limitation is the retrospective study design, with clinical and imaging data collected only for clinical management. The assignment to treatment groups was partially determined by patients' clinical response to corticosteroid therapy and its duration. This is a clear limitation, which we aimed to mitigate by using these variables as covariates in the regression analyses. Furthermore, consistent data on seizure frequency where not available and a sustained seizure response has been defined only in a qualitative way, without a specifying a

timeframe from start of AZA treatment. Another aspect is that clinical practice has changed over the period of observation of 30 years. This inevitably resulted in variability in clinical observations, diagnostic tests, neuropsychological testing and timing of neuroradiological investigations. A major strength of our study is the multimodal approach, which included quantitative measures of brain atrophy and cognition.

Conclusion

Our data suggest that AZA is a well-tolerated therapy, which has a role as steroid-sparing agent in pediatric RS. AZA use not only helped to maintain seizure control but was also associated with delayed emergence of EPC and hemiplegia and extended time to hemispherotomy. However, AZA did not halt cognitive decline or hemispheric GM volume loss. The benefits or not of extending the period of functional preservation and time to surgery requires further consideration.

We postulate that AZA will give the most clinical benefit in patients in the early stages of the disease, who respond to corticosteroids and who have not yet developed EPC and hemiparesis, where decision-making with regard to possible surgery may require further time.

Appendix 1: Authors

Name	Location	Role	Contribution
Serena Pellegrin, MD	University of Verona, Italy; UCL Great Ormond Street Institute of Child Health, London, U.K.	Author	Design and conceptualized study; major role in the acquisition of data; performed statistical and MRI analysis; drafted the manuscript for intellectual content

Torsten Baldeweg, MD, Prof	UCL Great Ormond Street Institute of Child Health, London, U.K.	Author	Design and conceptualized study; interpreted the data; performed statistical and MRI analysis; critically reviewed and revised the manuscript
Suresh Pujar, MD	Great Ormond Street Hospital for Children NHS Foundation Trust, London, U.K.	Author	Collected the data; revised the manuscript for intellectual content
Felice D'Arco, MD	Great Ormond Street Hospital for Children NHS Foundation Trust, London, U.K.	Author	Revised the manuscript for intellectual content
Gaetano Cantalupo, MD, Prof	University of Verona, Italy	Author	Interpreted and analyzed the data; critically reviewed and revised the manuscript
Sophia Varadkar, MD, PhD	Great Ormond Street Hospital for Children NHS Foundation Trust, London, U.K.	Author	Designed and conceptualized study; major role in the acquisition of data; critically reviewed and revised the manuscript
J. Helen Cross, MB ChB, PhD, Prof	UCL Great Ormond Street Institute of Child Health, London, U.K.	Author	Designed and conceptualized study; interpreted the data; critically reviewed and revised the manuscript

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Table 1. Demographic and clinical data. Abbreviations: EPC=epilepsia partialis continua; FSIQ/GAI= Full Scale Intelligence Quotient/General Abilities Index; L=left; R=right; RS=Rasmussen Syndrome. Statistic: t-test with independent sample for the continuous variables and Chi^2-test for categorical variables.

	AZA group	**Control group**	**p-value**
No.	30	23	
Age at last follow-up assessment, mean ± SD, y	15.7 ± 3.8	13.2 ± 4.2	**0.029**
Follow-up duration from onset, mean ± SD, y	8.9 ± 3.7	7.0 ± 3.8	0.08
Affected side, R : L, n	16 : 14	11 : 12	0.69
Age at first symptom, mean ± SD, y	6.8 ± 2.8	6.1 ± 3.2	0.37
Age at onset of acute phase, mean ± SD, y	9.4 ± 3.4	8.1 ± 4.4	0.27
Age at diagnosis, mean ± SD, y	9.5 ± 2.8	8.8 ± 4.3	0.45
Progression, rapid : slow, n	12 : 18	10 : 13	0.80
EPC, n	19/30	12/23	0.41
Age at onset, mean ± SD, y	9.8 ± 3.5	7.3 ±3.9	0.08
Years from onset of RS mean ± SD, y	2.0 ± 2	1.7 ± 1.7	0.80
Hemiparesis, n	25/30	20/23	0.72
Age at onset, mean ± SD, y	9.0 ± 3.0	8.0 ± 4.0	0.35
Years from onset of RS, mean ± SD, y	2.3 ±1.6	1.7 ± 1.7	0.26
Cognitive decline (reported by parents), n	24/30	21/23	0.12
Age at onset, mean ± SD, y	8.8 ± 2.5	8.2 ± 3.4	0.46
Years from onset of RS, y	2.4 ± 2.4	1.7 ± 1.7	0.31
Change of FSIQ/GAI pre-surgery score per year, mean ± SD	-6.7 ± 14	-12 ± 30	0.59
Last FSIQ/GAI pre-surgery score, mean ± SD	71 ± 13	77 ± 19	0.63
Treated with steroids, n	29/30	18/23	**0.036**

Steroid responders, n	26/29	7/18	**0.001**
Duration of steroid therapy, mean ± SD , y	2.2 ± 2.7	0.2 ± 0.3	**0.001**
Hemispherotomy, n	20/30	18/23	0.35
L side	9/14	8/12	0.17
R side	11/16	10/11	0.90
Hemispherotomy as first surgery, n	18	13	0.12
Age at hemispherotomy, mean ± SD, y	12.5 ±3.7	9.4 ± 4.0	**0.017**
Time from onset to hemispherotomy, mean ± SD, y	6.1 ± 3.5	3.9 ± 2.8	**0.048**
Time from acute phase to hemispherotomy, mean ± SD, y	3.6 ± 2.0	1.9 ± 1.8	**0.014**
Time from diagnosis to hemispherotomy, y	3.0 ± 1.7	1.8 ± 2.2	0.07
Brain biopsy, n	5/20	4/18	0.45

Table 2. Summary of follow-up data: Abbreviations: AED=anti-epileptic drug; EPC=epilepsia partialis continua; FU=follow-up. Statistic: t-test with independent sample or ANOVA for the continuous variables and a Pearson chi-square for categorical variables.

	AZA group n=30		**Control group n=23**		**p-value**
Hemispherotomy	**yes**	**no**	**yes**	**no**	
N	20	10	18	5	
Age at follow-up, y ± SD	15.6 ± 3.7	15.9 ± 4.0	12.8 ± 4.6	14.8 ± 2.6	0.13
Mean follow up duration from onset, y ± SD	9.2 ±3.5	8.3 ± 4.1	7.2 ± 4.0	6.2 s 3.5	0.30
Hemispherotomy, n (%)	20 (67)		18 (78)	-	0.35
Mean time after hemispherotomy, y ± SD	3.1 y ± 2.18		3.3 y ± 2.7		0.92
Seizures at follow-up					
n data available	20	10	17	4	0.09
None, n (%)	15 (75)	-	14 (82)	1 (25)	
Rare, n (%)	1 (5)	-	2 (12)	-	
Monthly, n (%)	2 (10)	5 (50)	-	-	
Daily, n (%)	2 (10)	3 (30)	1 (6)	3 (75)	
EPC, n (%)	-	2 (20)	-	-	
AED use at follow-up					
n data available	20	10	16	4	0.90

None, n (%)	12 (60)	-	9 (56)	-	
1, n (%)	4 (20)	-	2 (12)	1 (25)	
2 or more, n (%)	4 (20)	10 (100)	5 (32)	3 (75)	

Table 3. Seizure response to immunotherapy. Legend: CST = Corticosteroid therapy, IVIG = Intravenous immunoglobulin. CST was given mostly as prednisolone (2 mg/kg/day orally for six weeks) followed by tapering. IVIG were given at 2 g/kg over 2 to 5 days monthly. Some patients received periodic IVIG infusion, while others only a brief course. The effect was predominant after the first cycle of infusion; in most cases the efficacy decreased with time. See appendix e-1 for definition of seizure response. *including Rituximab, Mycophenolate.

	Patients	**Definite response**	**Partial response**	**No response**	**Not known**
CST, n (%)	47	16 (34)	17 (36)	12 (26)	2 (4)
IVIG, n (%)	35	4 (11)	10 (29)	20 (57)	1 (3)
AZA, n (%)	30	9 (30)	16 (53)	5 (17)	-
Plasmapheresis, n (%)	2	-	-	2 (100)	-
Other immunotherapies*, n (%)	6	-	3 (50)	1 (17)	2 (30)

Figure 1. Overview on the patient's selection process. Displayed incidence of EPC (A) and hemiparesis (B) within AZA and control groups. The grey shading indicates the use of AZA in comparison to the appearance of EPC and hemiparesis. Legend: HP=hemiparesis

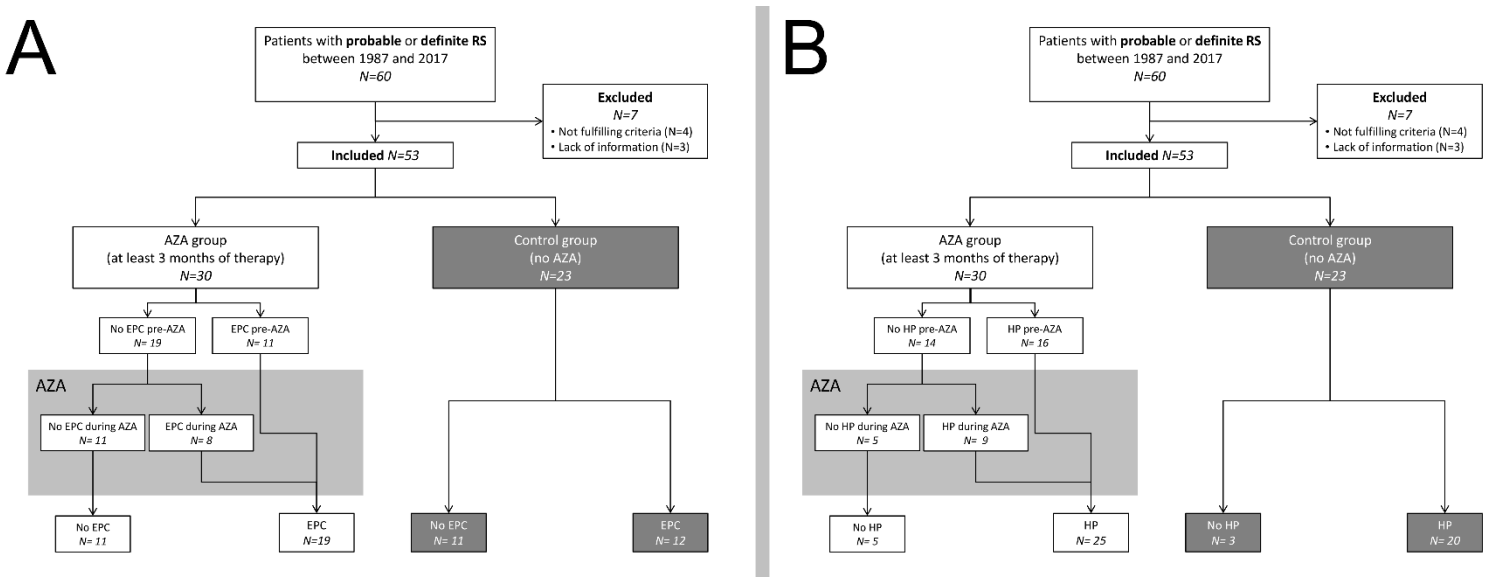


Figure 2.A) Delayed time to EPC in AZA treated patients. This graph represents the Kaplan-Meier plot from the Cox regression model for time from disease onset to EPC. In this plot, drops in the survival curve represents the development of EPC in time. The vertical axis shows the probability of not experiencing EPC. Patients treated with AZA prior to onset (blue line) had a delayed onset of EPC in comparison to patients who did not have AZA at all or only after EPC was already present (red line). B) **Delayed time to hemiparesis and lower prevalence in AZA treated patients.** This graph represents the Kaplan-Meier plot from the Cox regression model for time to hemiparesis. In this plot, drops in the survival curve represents the development of fixed hemiparesis in time. The vertical axis shows the probability of not experiencing hemiparesis. Patients treated with AZA prior to onset (blue line) had a delayed onset of hemiparesis in comparison to patients who did not have AZA at all or only after hemiparesis was already present (red line). C) **Delayed time to hemispherotomy for AZA group.** Patients who receive AZA had a delayed time to hemispherotomy in comparison with controls ($p=0.046$), when adjusting the model for type of progression (rapid/slow), hemisphere affected, presence of EPC, hemiparesis and cognitive decline.

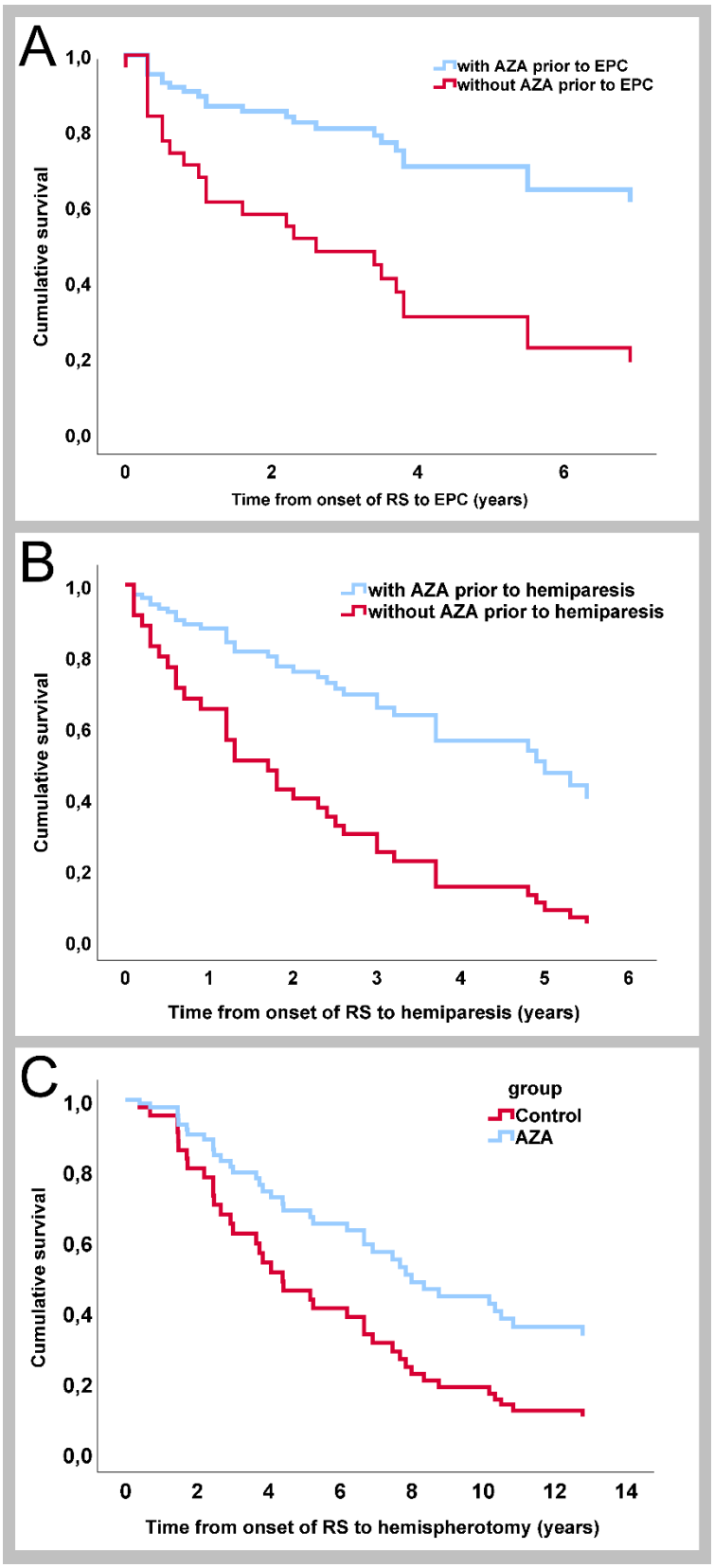
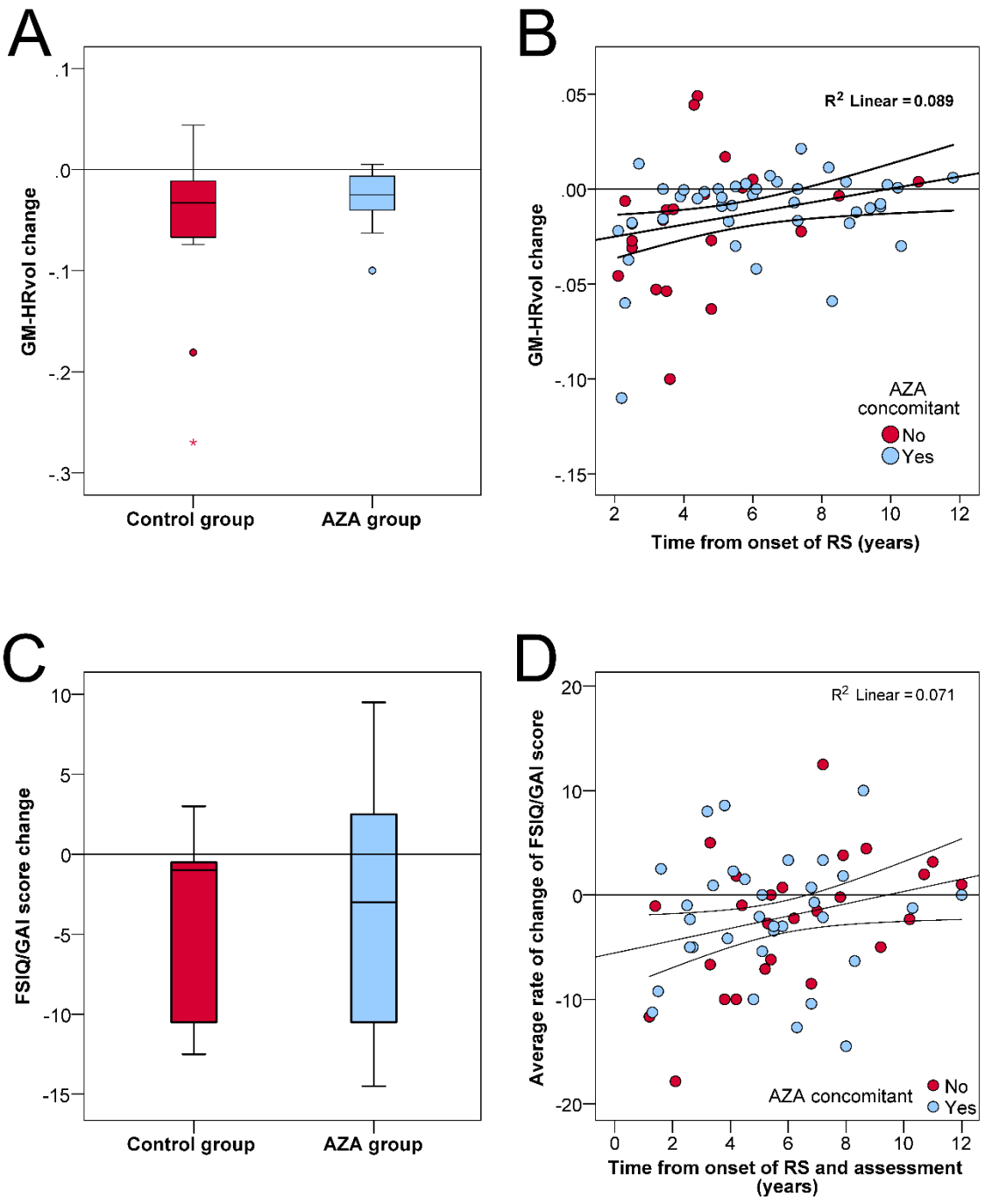

A
1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
with AZA prior to EPC
without AZA prior to EPC
0
2
4
6
Time from onset of RS to EPC (years)
B
1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
with AZA prior to hemiparesis
without AZA prior to hemiparesis
0
1
2
3
4
5
6
Time from onset of RS to hemiparesis (years)
C
1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
group
Control
AZA
0
2
4
6
8
10
12
14
Time from onset of RS to hemispherotomy (years)

Figure 3. Change in GM-HRvol and in FSIQ/GAI scores per year. A) Longitudinal volumetric analysis of hemispheric grey matter atrophy: difference of GM-HRvol (normalized by interscan interval) from before to after AZA treatment (AZA group) compared to a similar interval in the control group (AZA group = -0.030 95%CI=[-0.063, 0.003], control group = -0.053 95%CI=[-0.091, -.014]; group by time interaction effect: F(1,25)=0.748, p=0.395). The two outlier cases in the control group had scans within the first 2 years from onset. B) Change in inter-hemispheric grey matter ratio and concomitant AZA use (blue dots). Data points are displayed after excluding the first two years from onset. C) ~~**Figure 5. Change in FSIQ/GAI scores per year.** A)~~ Mean change of FSIQ/GAI scores over the follow-up period of 1.5 years between control and AZA treatment groups. ~~B~~D) Mean rate of change per year of AZA treated (blue dots) and not treated patients (red dots).


A
GM-HRvol change
.1
.0
-.1
-.2
-.3
Control group
AZA group
B
GM-HRvol change
.05
.00
-.05
-.10
-.15
R² Linear = 0.089
AZA concomitant
No
Yes
2
4
6
8
10
12
Time from onset of RS (years)
C
FSIQ/GAI score change
10
5
0
-5
-10
-15
Control group
AZA group
D
Average rate of change of FSIQ/GAI score
20
10
0
-10
-20
R² Linear = 0.071
AZA concomitant
No
Yes
0
2
4
6
8
10
12
Time from onset of RS and assessment (years)