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Long-term outcomes of allogeneic haematopoietic stem cell transplantation for adult cerebral X-linked adrenoleukodystrophy

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The adult cerebral inflammatory form of X-linked adrenoleukodystrophy is a rapidly progressive neurodegenerative disease, as devastating as childhood cerebral adrenoleukodystrophy. Allogeneic haematopoietic stem cell transplantation has been demonstrated to provide long-term neurological benefits for boys with the childhood cerebral form, but results in adults are sparse and inconclusive. We analysed data from 14 adult males with adult cerebral adrenoleukodystrophy treated with allogeneic haematopoietic stem cell transplantation on a compassionate basis in four European centres. All presented with cerebral demyelinating lesions and gadolinium enhancement. Median age at diagnosis of adult cerebral adrenoleukodystrophy was 33 years (range 21–48 years). In addition to cerebral inflammation, five patients had established severe motor disability from adrenomyeloneuropathy affecting only the spinal cord and peripheral nerves (Expanded Disability Status Scale score $\geqslant 6$). Eight patients survived (estimated survival $57 \pm 13\%$) with a median follow-up of 65 months (minimum 38 months). Death was directly transplant-/infection-related ($n = 3$), due to primary disease progression in advanced adult cerebral adrenoleukodystrophy ($n = 1$), or secondary disease progression ($n = 2$) after transient multi-organ failure or non-engraftment. Specific complications during stem cell transplantation included deterioration of motor and bladder functions ($n = 12$) as well as behavioural changes ($n = 8$). Arrest of progressive cerebral demyelination and prevention of severe loss of neurocognition was achieved in all eight survivors, but deterioration of motor function occurred in the majority ($n = 5$). Limited motor dysfunction (Expanded Disability Status Scale score < 6) prior to transplantation was associated with significantly improved survival [$78 \pm 14\%$ ($n = 9$) versus $20 \pm 18\%$($n = 5$); $P < 0.05$] and maintenance of ambulation (Expanded Disability Status Scale score < 7) post-transplant (78% versus 0%; $P = 0.021$). In contrast, bilateral involvement of the internal capsule on brain MRI was associated with poorer survival [$20 \pm 18\%$ ($n = 5$) versus $78 \pm 14\%$ ($n = 9$); $P < 0.05$]. This study is the first to support the feasibility, complications and potential long-term neurological benefit of allogeneic haematopoietic stem cell transplantation in adult cerebral adrenoleukodystrophy. Further studies are warranted to attempt to improve outcomes through patient selection and optimization of transplantation protocols.

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Abbreviations: AACS = adult X-ALD clinical symptom score; ACALD = adult cerebral inflammatory form of X-linked adrenoleukodystrophy; AMN = adrenomyeloneuropathy; CCALD = childhood cerebral inflammatory form of X-linked adrenoleukodystrophy; EDSS = Kurtzke Expanded Disability Status Scale; GVHD = graft-versus-host disease; HSCT = haematopoietic stem cell transplantation; X-ALD = X-linked adrenoleukodystrophy

Introduction

X-linked adrenoleukodystrophy (X-ALD) is an inherited peroxisomal disorder caused by a defective *ABCD1* gene leading to a characteristic accumulation of saturated very long chain fatty acids in blood and tissues. The white matter in the brain, spinal cord, adrenal cortex, Leydig cells and hair follicles is typically affected (Moser, 1997; Kemp *et al.*, 2012; Wiesinger *et al.*, 2015). With an estimated combined incidence of 1:17 000 in males and females, X-ALD is one of the most common peroxisomal diseases.

Although pronounced phenotypic variation may occur within kindreds, two major forms of X-ALD can be differentiated (Moser, 1997; Kemp *et al.*, 2012; Wiesinger *et al.*, 2015). First, a chronic neuronopathic form, called adrenomyeloneuropathy (AMN), involving spinal cord and to a lesser extent peripheral nerves in adult males as well as heterozygous females, and characterized by a slowly progressive spastic paraplegia with sensory ataxia. Second, a cerebral demyelinating variant with neuro-inflammation that causes white matter destruction. Cerebral demyelination results in rapid loss of neurocognitive, motor and sensory functions with vegetative state and death within a few years of onset. To date, no triggers or other factors have been identified to predict the onset of this acute inflammatory cerebral form of X-ALD, which affects about a third of all males in childhood (childhood onset cerebral ALD, CCALD). Primary isolated cerebral disease in adolescence or adulthood is uncommon (5–10%), although up to 63% of males with AMN develop secondary cerebral demyelination within 10–15 years (de Beer *et al.*, 2014). For the purposes of this study, primary and secondary cerebral demyelination with gadolinium enhancement of lesions in adulthood is termed adult cerebral ALD (ACALD). ACALD is as devastating as CCALD: patients rapidly lose cognitive and motor function leading to death.

Allogeneic haematopoietic stem cell transplantation (HSCT) is an established long-term treatment in male children with CCALD (Aubourg *et al.*, 1990; Shapiro *et al.*, 2000; Baumann *et al.*, 2003; Peters *et al.*, 2004; Beam *et al.*, 2007; Miller *et al.*, 2011), although the mechanism of action is not fully understood (Moser and Mahmood, 2007; Schonberger *et al.*, 2007; Cartier *et al.*, 2014). Survival and neurological outcome of CCALD patients after HSCT is clearly superior compared to untreated patients (Mahmood *et al.*, 2007; Miller *et al.*, 2011). Despite the significant risks of HSCT, no other effective therapeutic option exists for CCALD. Only a few anecdotal reports of allogeneic HSCT in ACALD are available (Hitomi *et al.*, 2005; Fitzpatrick *et al.*, 2008). In contrast to male children, the cerebral form in adult males is usually associated with motor and sensory deficits in the lower limbs as well as bladder dysfunction as a consequence of AMN. The pattern of CNS demyelination in adults may also be different (Loes *et al.*, 2003; Eichler *et al.*, 2007). Moreover, bone marrow-derived cells infiltrating the brain may react differently in older patients (Barrett *et al.*, 2015). The extremely poor prognosis with a median overall survival of 2 years once ACALD enters the phase of active neuroinflammation (van Geel *et al.*, 2001; de Beer *et al.*, 2014), combined with lack of any other effective treatment, has justified the extension of allogeneic HSCT into the older age group.

We report a retrospective analysis of the feasibility, toxicity and long-term neurological outcomes of 14 adult males treated with allogeneic HSCT for ACALD in four European centres. Based on our combined experience, we propose preliminary guidelines for patient selection and treatment protocols for allogeneic HSCT in this setting.

Patients and methods

Participants

Fourteen adult males underwent HSCT for ACALD in four haematopoietic stem cell transplant centres in Germany,

Table 1 Patients demographics and disease characteristics

Patient ID[a]	Age at ACALD (years)	EDSS-Score[b] (max. 10)	Addison's disease	MRI pattern[c] Occipital/frontal/ pyramidal tracts/others	Loes-Score (max. 34)	Clinical symptoms (at age in years)
11P	23	1	Yes	+ / – / – / –	2.5	Addison's disease (10), mild spastic paraplegia (24)
01B	45	6.5	No	** / – / ** / –	7	Sensory symptoms (44.4), spastic paraplegia (45.5), CNS symptoms: attention deficit, disinhibition (46.1). Gadolinium enhancement on first MRI
12P	27	4	Yes	** / – / – / –	5.5	Addison's disease (16), mild spastic paraplegia (25)
02H	48	2.5	Yes	– / + / – / –	2	Addison's disease (18.6), mild spastic paraplegia (45.6)
03B	35	3.5	No	** / – / + / –	9.5	Intermittent bladder dysfunction (33.0), spastic paraplegia (35.2). Gadolinium enhancement on first MRI
04S	32	7	Yes	** / + / ** / + (temporal, thalamus)	14	Family screening (24.8), lost to follow-up; CNS symptoms: impaired hearing/vision (prosopagnosia, field defect), dysphasia, ataxia (31.9), Addison's disease (33.4). Weight loss
13P	42	6	No	– / – / ** / + (centrum semiovale)	5	Family screening (35), spastic paraplegia (35)
05B	33	4	No	+ / (+) / – / ** (olivo–ponto–cerebellar atrophy)	9	Ataxia (25.5), spastic paraplegia (30), severe bladder dysfunction. Weight loss
06B	31	3	Yes	(+) / + / – / –	6	Addison's disease (17.0), sensory symptoms (25.6), CNS symptoms: mild attention deficit, mild impaired vision (field defect) (31.8)
14P	21	1	No	** / – / + / –	12	CNS symptoms: learning difficulties (14.5), family screening (18.8). Cerebral demyelination without Gadolinum enhancement on first MRI
07B	32	4	No	** / – / + / –	11	Spastic paraplegia (31.3), CNS symptoms: seizure (32.4), attention deficit, dysarthria (32.8), impaired vision. Weight loss. Gadolinum enhancement on first MRI
08B	42	6.5	Yes	– / ** / ** / –	10.5	Spastic paraplegia (23.8), Addison's disease (25.8), CNS symptoms: disinhibition, attention deficit (42.4). Weight loss
09B	46	6.5	No	– / + / ** / + (cerebellum)	4.5	Spastic paraplegia (24.0), ataxia (44.5). Weight loss
10B	25	4	No	+ / – / + / –	5	Family screening (14.4), spastic paraplegia (21.4), drug abuse (23)

[a]Patients in order of transplantation date. Capital letters indicate transplant centre: P = Paris, B = Berlin, H = Hannover, S = Sheffield.
[b]EDSS: Kurtzke Expanded Disability Symptom Score (EDSS) just prior to HSCT.
[c]MRI pattern: MRI lesions differentiated for involvement of parieto-occipital, frontal, pyramidal long tract fibres in internal capsule or pons, and other white matter as indicated. Degree of involvement: –, no; +, moderate; **, extensive.

France and UK. Diagnosis of X-ALD was based on elevated concentrations of fasting plasma very long chain fatty acids, and additionally on mutations of the *ABCD1* gene (HGNC: 61) in nine patients. Diagnosis of ACALD required the detection of gadolinium enhancement in cerebral demyelinating lesions by brain MRI.

Patient characteristics are summarized in Table 1. Patients had detailed neurological, neurophysiological, and neuropsychological evaluation as well as MRI exams before and sequentially after HSCT. All patients were offered HSCT on an individually selected compassionate basis in accordance with the practice guidelines of the Working Party on Inborn Errors of the European Group for Blood and Marrow Transplantation (Peters and Steward, 2003) with written informed consent for HSCT. The use of stem cell collections from unrelated donors and cord blood, where appropriate, were approved by the medical advisory boards of the respective national donor search programmes for this indication.

Allograft selection, preparative regimen, and graft-versus-host disease prophylaxis

Twelve of 14 patients received allogeneic HSCT from a matched donor after myeloablative conditioning. A matched donor was either a genotypical HLA-identical sibling ($n = 3$) or a $\geqslant$9/10 HLA-matched unrelated donor ($n = 9$) confirmed by high-resolution DNA typing of HLA class I (HLA A, B, Cw) and class II (HLA DRB1, DQB1) alleles. Ten of these 12 patients were transplanted with bone marrow, and two patients received granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells based on donor

Table 2 Patients hematopoietic stem cell transplantation characteristics

Patient ID	Δ ACALD to HSCT, months	Conditioning	Donor	Source	Donor chimerism (Day +100: >90% donor)	Acute GVHD	Significant infection	Other significant non-neurological toxicity	Outcome
11P	6	MAC + S	MSD	BM	Yes	Grade I	Life-threatening	Haemorrhagic cystitis, thrombotic microangiopathy	Alive
01B	8	MAC + S	MSD	BM	Yes	No	–	–	Alive
12P	12	RIC + S	UD	1 × CB	Not tested (aplastic)	N/A	Fatal (fungal)	–	Dead (TRM)
02H	3	MAC + S	MUD	BM	Yes	Grade I	Life–threatening	Transient multi–organ failure	Dead (Progress)
03B	14	MAC + S	MUD	BM	Yes	No	Severe	–	Alive
04S	15	MAC + S	MSD	BM	Yes	No	–	–	Dead (Progress)
13P[a]	9	RIC	UD	2 × CB	Yes (Day +60) No (<1% donor later)	No	Life–threatening	Immune nephrotic syndrome, end–stage renal failure	Dead (Progress)
05B	9	MAC + S	MUD	BM	Yes	No	Severe	–	Alive
06B	11	MAC + S	MUD	BM	Yes	Grade I	–	–	Alive
14P	6	MAC	MUD	BM	Yes	No	–	–	Alive
07B	12	MAC + S	MUD	BM	Yes	Grade I	Severe	Pneumonia	Alive
08B	9	MAC + S	MUD	PBSC	Yes	No	Life-threatening	Pneumonia, haemorrhagic cystitis, polyserositis, multi-organ failure	Dead (TRM)
09B	5	MAC + S	MUD	PBSC	Not tested	Grade IV	Fatal	Sepsis/pneumonia, haemorrhagic cystitis, multi-organ failure	Dead (TRM)
10B	11	MAC + S	MUD	BM	No (80% donor)	Grade I	–	–	Alive

Δ ACALD to HSCT: Time interval between first detection of gadolinium enhancement in MRI (onset of ACALD) and HSCT in months.
Conditioning: MAC (+S) = myeloablative conditioning (plus serotherapy), i.e. busulfan 16 mg/kg orally (or busulfan i.v. targeted dose in Patient 14P) and cyclophosphamide (120 mg/kg over 2 days or 200 mg/kg over 4 days) (plus rabbit anti-thymoglobulin Genzyme® or Fresenius®). RIC (+S) = reduced intensity conditioning (+serotherapy), i.e. clofarabine 200 mg/m^2, busulfan 4 mg/kg orally, melphalan 140 mg/m^2, and alemtuzumab in Patient 12P; cyclophosphamide 50 mg/kg, fludarabine 200 mg/m^2, total body irradiation with 2 Gy in Patient 13P.
Donor: MSD = matched sibling donor; (M)UD = (matched) unrelated donor.
Source: BM = bone marrow; PBSC = peripheral blood stem cells; CB = cord blood with one (1 ×) or two (2 ×) units.
Acute GVHD: acute graft-versus-host disease with maximum grade. Significant infection: refers to infection ⩾ 3 according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v.3.0) criteria.
Significant non-neurological toxicity: refers to non-neurological toxicity ⩾ 3 according to CTCAE v.3.0 criteria.
Outcome: TRM = transplant-related mortality, progress = ACALD progression.
[a]Patient 13P: initially autologous hematopoietic regeneration, since Day +30 detection of mixed donor chimerism (>90% donor chimerism at Day +60, 50% donor at Day +120, thereafter <1% donor). Toxicity due to allogeneic cord blood transplantation/non-engraftment suspected.

preference. Myeloablative conditioning consisted of busulfan and cyclophosphamide. In two patients it was not possible to identify a suitably HLA-matched donor, and they underwent unrelated cord blood transplantation after reduced-intensity conditioning. All but two patients received additional serotherapy for graft-versus-host disease (GVHD) prophylaxis: polyclonal anti-thymocyte globulin from Genzyme® ($n = 1$), Fresenius® (now Neovii®; $n = 10$), or the monoclonal antibody alemtuzumab (Genzyme®; $n = 1$). Basic transplant characteristics of all patients are summarized in Table 2. GVHD prophylaxis and supportive care measures were delivered according to standard of care protocols at the individual centres.

Assessment of engraftment, graft-versus-host disease, and toxicity

Engraftment with full donor chimerism was defined as >90% donor cells in total nucleated cells at Day +100 post-HSCT as detected by DNA-based techniques (i.e. short tandem repeats analysis). Diagnosis of acute GVHD was primarily based on clinical criteria; overall staging of acute and chronic GVHD was done according to published criteria. Transplant toxicity was described according to the National Cancer Institute common terminology criteria for adverse events version 3.0 (CTCAE v.3.0) with severe toxicity recorded for adverse events grade ⩾ 3.

Data acquisition and assessment of neurological outcome

Patient-related clinical information was obtained from retrospective review of medical records in each transplant centre and from their neurologists.

Assessment tools were used to analyse changes in disease status: the Adult X-ALD Clinical Symptom score (AACS) (Köhler and Sokolowski, 1999) to quantify the overall X-ALD neurological impairment, the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) to assess motor dysfunction, the modified Rankin scale (Rankin, 1957) to describe disability status in daily activities, and the Loes MRI severity score (Loes *et al.*, 1994) to measure the extent of cerebral demyelination. Neurological examination covered

the following areas: motor function, sensation, bladder function, as well as overall CNS function including vision, hearing, and speech. AACS, EDSS, and modified Rankin scale were determined for the time points pre-HSCT, worst status during first 6 months post-HSCT, and status ~24 months post-HSCT (minimum ⩾12 months) for evaluable patients. In four patients (Patients 1B, 3B, 4S and 7B), no MRI scans were available before the onset of ACALD, while in three patients (Patients 12P, 8B and 9B), post-HSCT MRI could not be obtained due to poor clinical status and early death. In addition to Loes score, MRI scans were reviewed for gadolinium enhancement and patterns of demyelination (parieto-occipital ± long tract pyramidal involvement versus all other patterns). Lesion progression was calculated from sequential MRI scans. Neuropsychometric assessment was performed pre-HSCT and, whenever possible, at various time points post-HSCT. Normalized measures of IQ were generated by appropriate tools according to the centres' preference.

Patients unable to walk without aid or rest for ~100 m (EDSS ⩾6), were classified as advanced AMN. Stable motor function post-HSCT was defined as increment in EDSS <1 point with preserved/maintained ambulation (EDSS <7). Severe deterioration in motor function was classified as increment in EDSS ⩾2 points or to EDSS ⩾7. Stable neurocognition post-HSCT was defined as deterioration in IQ <15 [<1 standard deviation (SD)] or no cognitive deterioration as detected by care-givers; severe deterioration in intellectual function was classified as obvious cognitive decline or inability to test for IQ anymore. Moderate deterioration was defined as any deterioration less than severe. For the purpose of this study, events were defined in relation to ACALD progression, i.e. event-free survival was used in this study for surviving patients who retained a stable cognitive function after HSCT.

Statistical analysis

Survival was compared by Kaplan-Meier estimates, and comparisons done by the log-rank method. Categorical variables were compared using the z-test or Fisher's exact test. Comparison of continuous variables was performed by non-parametric tests (Mann-Whitney signed rank test, the Kruskal-Wallis ANOVA with following pair-wise comparisons according to Dunn's method). Before and after analyses were performed for single time points with the Wilcoxon signed rank test or for multiple time points with the Friedman repeated measures ANOVA followed by multiple pair-wise comparisons according to the Tukey's test. Calculations were done using Sigmaplot 11.0 (Systat Inc., San José, CA, USA).

Results

Vital status, engraftment, disease progression-related mortality and transplant-related mortality

Fourteen patients with ACALD transplanted between 2003 and 2012 at a median age of 34 years (range 21–48 years) were included in this study. Characteristics of HSCT are summarized in Table 2.

Eight of the 14 patients were alive with a median follow-up of 65 months (range 38–116 months). The estimated overall survival probability was 57.1 ± 13.2% (mean ± SD) (Fig. 1A). There were no survivors after cord blood transplantation with reduced intensity conditioning ($n = 2$) nor after myeloablative conditioning and peripheral blood stem cell transplantation ($n = 2$), while 8 of 10 males after myeloablative conditioning and bone marrow transplantation survived (estimated survival probability 80.0 ± 12.6% (log-rank test 11.48, $P < 0.001$) (Fig. 1B). The small numbers did not permit detection of relevant transplant differences between patients with matched donors.

Full donor chimerism (>90% donor cells) was detected in 10 of 11 evaluable patients receiving myeloablative conditioning; one patient showed 80% donor cells (Table 2). Two males who received cord blood after reduced intensity conditioning did not permanently engraft. In the 12 patients receiving myeloablative conditioning, neutrophil recovery (peripheral neutrophil count ⩾ 500/μl for three consecutive days) occurred at a median of 19.5 days (range: 10–34 days). The incidence of severe GVHD was low: only one patient (8% of all males engrafting with donor cells) developed acute GVHD grade ⩾ II (grade 4 of the skin), while chronic GVHD was observed in limited form only in four patients.

There was one death from primary disease progression without relevant HSCT complications (Patient 4S). Two further patients died from secondary disease progression after life-threatening infections with transient multi-organ failure (Patient 2H) or graft-rejection (Patient 13P). Three early deaths (21%) were directly transplant- or infection-related, either associated with non-engraftment (Patient 12P) or immobility due to advanced AMN (Patients 8B and 9B).

Survival was related to baseline motor dysfunction prior to HSCT: limited AMN (EDSS < 6) was significantly associated with superior survival compared with advanced AMN [estimated survival probability 77.8 ± 13.9% ($n = 9$) versus 20.0 ± 17.9% ($n = 5$); log-rank test 3.91, $P = 0.048$; Fig. 1C]. After excluding from the analysis the two patients who failed to engraft, the EDSS gained further prognostic significance with the estimated survival probability increasing to 87.5 ± 11.7% ($n = 8$) for EDSS <6 versus 25.0 ± 21.7% ($n = 4$) for EDSS ⩾6 (log-rank test 5.16, $P = 0.003$).

Neurological status at diagnosis of ACALD and before stem cell transplantation

Disease characteristics of the 14 ACALD males before HSCT are summarized in Table 1.

Cerebral disease was first detected at a median age of 33 years (range: 21–48 years). Ten X-ALD males had been monitored in the years before the onset of ACALD. Four

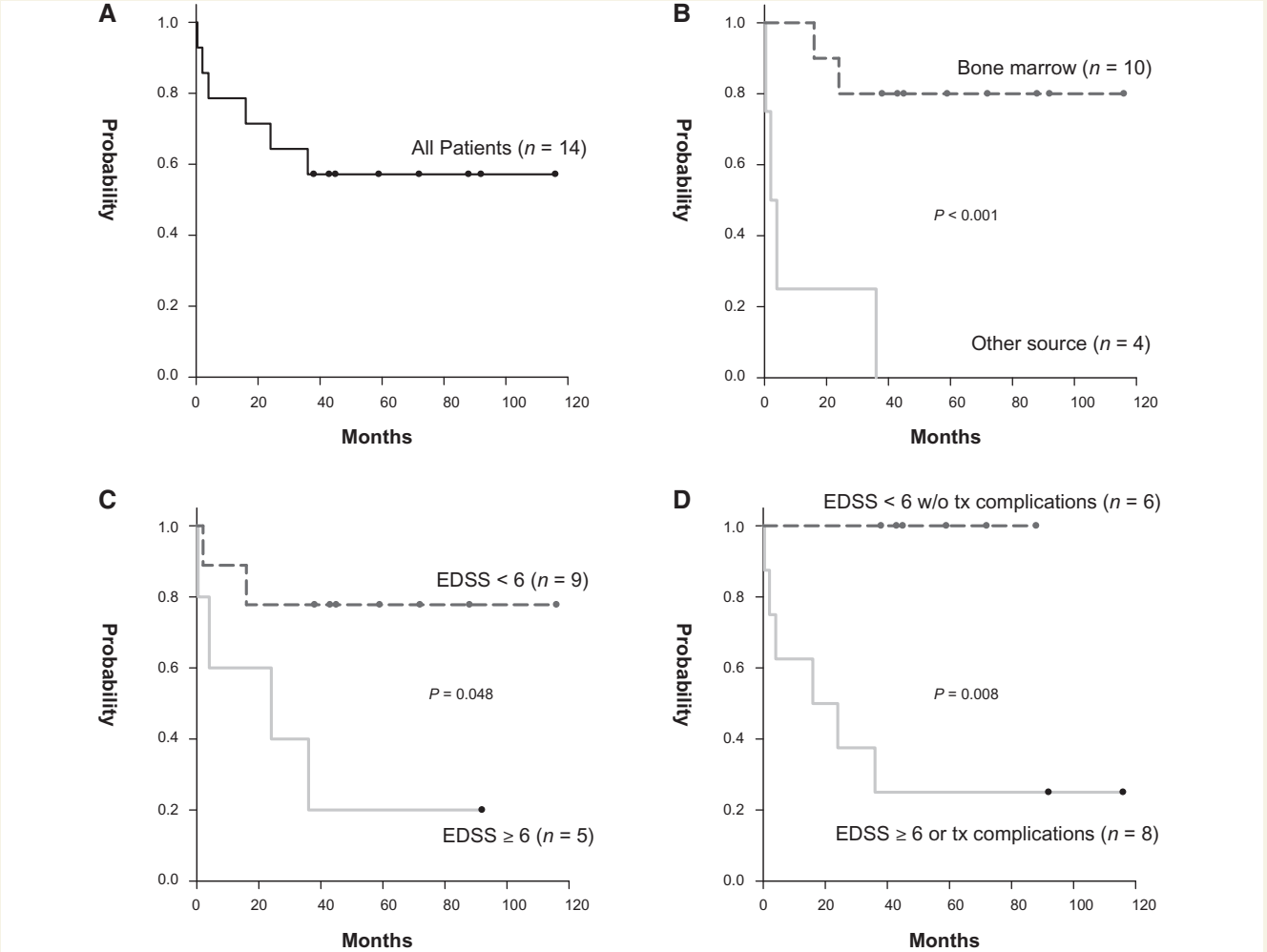

Figure 1 Kaplan-Meier estimates for survival of ACALD patients after HSCT based on various transplant and patient characteristics. (**A**) Survival of all 14 patients. (**B**) Patients stratified by stem cell source, i.e. bone marrow versus peripheral blood stem cells or cord blood. (**C**) Patients stratified by extent of motor dysfunction (EDSS) at admission for HSCT. (**D**) Patients stratified for EDSS at admission for HSCT and occurrence of life-threatening or fatal infections during first 6 months post-HSCT. Dots on probability lines indicate censored patients.

patients were first diagnosed with Addison's disease during adolescence [median age at diagnosis 16.5 years (range: 10–18 years)] in the absence of AMN at that time. Two patients had been diagnosed with AMN at the age of 24 and 31 years because of spastic paraparesis. Three patients were identified by family screening with either no symptoms (Patient 10B; age 15 years), increasing learning difficulties (Patient 14P; age 18 years), or gait disturbances in combination with undiagnosed Addison's disease (Patient 8B; age 23 years). At diagnosis of X-ALD, Patient 14P presented with extensive parieto-occipital leukodystrophy and marked ventricular dilatation but without gadolinium enhancement as indication for arrested CCALD at that time. The median period between diagnosis of X-ALD and onset of ACALD was 132 months (range: 19–266 months) among these 10 patients. In contrast, four patients (Patients 1B, 3B, 4S and 7B) already had evidence of ACALD at their first MRI examination. Although Patient 4S had been originally diagnosed at the age of 24 years by family screening, he was not seen again until 8 years later at which stage he had developed visual and auditory deficits and ataxia. Patient 7B presented with seizures as first symptom. The history of AMN symptoms among these four tended to be shorter than in the other 10 patients (median duration 15.5 months versus 37 months; $P = 0.088$).

All but one patient (Patient 14P) presented with AMN symptoms, which varied considerably in duration (median 24 months, range 0–266 months) and severity: while three patients had severe motor dysfunction (EDSS $\geqslant 6$), two patients displayed no motor dysfunction, and two others showed only a mild limitation in running. There was no correlation between EDSS and age, but some linear correlation between duration of AMN symptoms and EDSS was observed ($r = 0.735$; $P = 0.003$). Bladder dysfunction was common and present in 12 patients, with marked

Table 3 Disease status within 6 months after allogeneic hematopoietic stem cell transplantation and MRI outcome

Patient ID	EDSS before	Early changes at months post HSCT	EDSS at 0–6	MRI outcome at months post-HSCT
11P	1	Motor dysfunction ↑↑ immediately after treatment, walks only few metres with 2 crutches at 6; bladder dysfunction ↑↑ (parallel to BK virus cystitis); behavioural disinhibition until at 12; vision↓	7	New lesions and progression, LS 12 at 13; thereafter stable
1B	6.5	Conditioning: walks only few steps without help until at 3; fever after first serotherapy: seizure, bladder/stool incontinence (until at 1); severe depression until at 6	8	No change, minimal Gd+, LS 7(stable) at 6; new lesions (frontal), LS 10 at 18
12P	4	Motor/bladder dysfunction stable until shortly before death in aplasia at 2 (TRM); severe behavioral changes	7	Not tested
2H	2.5	Sepsis after first serotherapy: seizure, disorientation, coma, renal failure, bedridden at 1; major improvement until at 3; still walks only with two crutches; thereafter further deterioration	9.5	Many new lesions (PO, frontal, CC), LS 14 at 6; progression and atrophy, LS 20 at 12
3B	3.5	Sepsis at 0.5: bladder/stool incontinence, agitated; walks only few steps without aid at 1; thereafter motor/bladder function↑; moderate disinhibiton	6.5	Moderate lesion progression (PO), LS 11 at 16; stable, CC atrophy, LS 12 at 59
4S	7	Motor dysfunction↑↑, hemiplegic at 3; vision↓; complete hearing loss at 2; major confusion/psychosis at 1; severe depression at 6; no further deterioration at 3–6	8.5	Lesion progression, +atrophy, LS 15.5 at 8
13P	6	Spastic paraparesis ↑; moves from wheel-chair to bed with difficulties at 6; bladder dysfunction stable; severe behavioral changes	8.0	New lesions and progression, LS 8 at 12; minimal progression, LS 9 at 24
5B	4	Conditioning: Gait ataxia↑, needs urine catheter; walks only few steps without aid at 1, thereafter motor function↑; moderate depression until at 6	6.5	New lesions (PO, frontal), LS 11.5 at 7; fewer lesions detected (PO, CC, visual), LS 8.5 at 34
6B	3	Normal motor function; minimal bladder dysfunction unchanged; moderate disinhibition at 1	3	New lesions (PO), LS 8 at 6; no change at 37
14P	1	Normal neurological function preserved; severe behavioural disinhibition at 6	1	No progression, LS 12 (stable) at 14
7B	4	Sepsis at 0.5: walks only few steps with aid, bladder incontinence, attention deficit; able to walk 300 m, bladder function↑ at 6; major depression until at 6; anticonvulsive prophylaxis	7.5	Lesion progression, LS 12 at 9; fewer lesions detected, LS 10 at 24
8B	6.5	Conditioning: motor dysfunction ↑, bladder/stool incontinence; sepsis at 0.5: bedridden, disoriented, 'frontal brain syndrome', cognition↓; continued decline until death at 4 (TRM)	9.5	Not tested
09B	6.5	Conditioning: severe dizziness, bladder/stool incontinence, dysarthria; sepsis at Day 2: cannot stand; bedridden; GVHD at day 7: severe disorientation, hallucination; death at 0.5 (TRM)	9.5	Not tested
10B	4	No change at 1; ataxia↑ at 3 (needs bilateral aid); rarely stool incontinence at 6	6.5	Internal capsule↑, LS 5 (stable) at 38

EDSS at 0–6 = maximum EDSS score during first 6 months post-HSCT.
MRI: LS = Loes score; PO = parito-occipital; CC = corpus callosum.
TRM = transplant-related mortality.

impairment in two of them. Sensory dysfunction was overall mild in 13 patients and in Patient 6B was the only AMN symptom.

The median interval from detection of ACALD to HSCT was 9 months (range 3–15 months). During this period there was clinical disease progression in six patients: AMN symptoms in one, cognition in two, and both motor and cognitive functions in three patients. Patient 6B developed a visual field defect, while IQ remained stable. Patient 14P still had a strictly normal neurological exam even before HSCT, while five other patients presented with severe motor disability (EDSS $\geqslant 6$). With regard to occupational status, five males had discontinued their established work/profession at onset of ACALD, four of them because of progressive motor dysfunction. In the period prior to HSCT, four more patients were unable to continue working mainly due to neurocognitive decline.

The median IQ before HSCT was 99 (range 72–139). Cognitive deterioration was suspected or reported by relatives in all four patients who showed ACALD in their first brain MRI. Neuropsychological evaluation before HSCT in these patients also suggested deterioration by revealing better verbal IQs than performance IQs (data not shown). In contrast, 7 of 10 patients who were followed up long-term displayed no relevant IQ changes until admission for HSCT ($P = 0.018$).

Table 4 Long-term disease status after allogeneic haematopoietic stem cell transplantation

Patient ID	EDSS before	Long-term outcome at months post-HSCT	EDSS at 24 months	Motor function >36 months	Follow-up in months
11P	1	Disinhibition resolved at 12; cognition stable (PIQ 89), but visual loss (1/10, 2/10 with major cuts in visual fields) at 18; motor function slowly improved after at 24 (walks 500 m with 1 crutch at 108); severe bladder dysfunction unchanged; good QoL despite motor and visual handicap	6	↑	116
1B	6.5	Depression improved at 12; motor dysfunction (↑) at 12: transfers alone to wheelchair; moderate bladder dysfunction unchanged; cognition↓ (IQ 75) at 20; teaches at handicapped institution; no changes after at 24	7	=	92
2H	2.5	Deterioration in all functions since at 3; unable to walk unassisted at 6; diabetes mellitus/chronic inflammation at 12; rapid further deterioration; death from secondary ACALD progression at 16	10	N/A	(16)
3B	3.5	Behavioural changes resolved at 12; cognition stable (IQ 99) at 16; walks without aid >500 m at 53; limited bladder dysfunction unaltered; completely stable at 88; good QoL; retired due to spasticity	4	↑	88
4S	7	Depression improved at 12; further deterioration in motor/bladder/cognitive function after at 6; death from ACALD progression at 24	10	N/A	(24)
13P	6	Repeated septicaemias under dialysis after at 6; paraplegic, unable to transfer from bed to wheelchair alone, can speak and understands, but severe cognitive decline at 24; very poor QoL, aware of his status; death from secondary ACALD progression at 36 (also related to non-engraftment)	9	N/A	(36)
5B	4	Best clinical status at 12: able to walk 200 m unassisted; requires crutch for walking at 41; severe bladder dysfunction unaltered; cognitive function stable (PIQ 108) at 12; depression↓↓, but moody; still unemployed	6	↓	72
6B	3	Stable neurocognition: IQ 111 at 13; no relevant change until at 31; motor function ↑: walks >5 km at 59; no bladder dysfunction at 59; bachelor completed; information processing moderately↓; excellent QoL	3	↑	59
14P	1	Severe behavioural disinhibition resolved at 12; normal neurological status at 14; cognitive function stable (IQ 72 before); back to university (with great difficulties); good QoL	1	=	43
7B	4	Best clinical status at 12: able to walk 100 m unassisted; hemiparesis; requires crutch for walking at 18; bladder incontinence at 18; cognitive function↓ (concentration↓); depression improved at 12; still low QoL	6	↓	45
10B	4	Moderate improvement of ataxia after at 22 (walks >1 km with bilateral aid); intermittent stool incontinence unchanged; cognition: memory↓, attention↓, information processing↓ at 13; formal thinking↑ at 38 (moderate decline in neurocognition also due to drug abuse?); retired; good QoL	6	↑	38

Motor function >36 months (at last follow-up): ↑ improved; = stable; ↓ deteriorated compared to early post-HSCT period. QoL = quality of life.

Neurological outcome early after haematopoietic stem cell transplantation

Significant neurological and/or behavioural changes were observed during the transplant procedure and early follow-up in all but one ACALD patient (Table 3).

Exacerbation of motor disability during the first 6 months post-HSCT was a characteristic feature observed in 12 of 14 patients. The median increment in EDSS was 2.8 points with considerable heterogeneity (range: 0–7 points). Only two patients who did not demonstrate obvious gait disturbances before remained unaffected in their motor function (Patients 6B and 14P). All of the other 12 patients deteriorated further; 10 (71%) of them were at least temporarily unable to walk more than a few meters even with aid (EDSS $\geqslant$7). With the exception of Patient 14P, bladder (and sphincter) dysfunction also became apparent or aggravated during the transplant period, especially with fever caused by serotherapy or infection. Two patients developed seizures during the transplant procedure. Deterioration of neurocognitive function, significant hearing or vision impairment as well as severe disorientation and somnolence occurred in five patients (36%) during early post-transplant phase, four of whom subsequently died. Moderate-to-severe behavioural changes were observed in 11 males (79%), which improved markedly or disappeared in all surviving patients after 6–12 months.

Patients with limited AMN at admission for transplantation (EDSS <6) developed significantly fewer neurological symptoms (other than progressive motor disability) compared to the ones with advanced AMN (1/9 = 11% versus 4/5 = 80%; z = 2.001, P = 0.045).

Long-term neurological outcome after haematopoietic stem cell transplantation

The detailed long-term follow-up for each patient is listed in Table 4, excluding the three patients who died within the

Table 5 Characteristics of patients with or without bilateral involvement of internal capsules on brain MRI

	Group A ($n = 5$) With bilateral involvement of internal capsule	Group B ($n = 9$) Without bilateral involvement of internal capsule	*P*-value
Progressive disease detected	5/5	6/9	ns
Rate of demyelinating lesion progression, Loes score points per year	**2.9** (1–6)	**0.6** (0–6)	0.078
Age at ACALD, years	41.9 ± 5.6*	30.7 ± 8.1	<0.05
Time period AMN to ACALD, months	90 (15–235)	27 (24–49)	ns
Loes score before HSCT	**6.0** (4.4–9.9)	**7.0** (4.9–11.4)	ns
EDSS before HSCT	**6.5** (6.4–6.6)	**3.5** (2.1–4.0)	<0.01
Probability of survival, %	20 ± 18 *	78 ± 14	<0.05

All values in bold represent median values (with 25th and 75th percentile). Significance calculated by rank sum test.
*Age and probability of survival: Mean ± SD. Significance calculated by student *t*-test (age) or log-rank test (survival). ns = not significant.

first 6 months post-transplant. Three patients progressively deteriorated during the first year post-HSCT and died from ACALD progression 16 to 36 months after transplant.

Eight patients stabilized beyond 6 months post-HSCT and became long-term survivors. Two of these did not deteriorate during the transplant procedure and showed a stable motor function thereafter. Exacerbation of motor disability during the first 6 months post-transplant partly reversed in the other six patients. However, only three males (21% of all transplanted patients) could walk without assistance (EDSS < 6) 24 months after HSCT. Patient 1B was still unable to walk more than a few meters even with aid (EDSS = 7). The median gain in EDSS in these males was 1.5 points (range 0.5–5 points) compared to baseline status. After 24 months post-HSCT, motor function further improved in four patients, while two deteriorated and two remained stable. Bladder dysfunction improved partly over time, but five of the eight patients showed deterioration in comparison to the pre-HSCT status.

In contrast to motor and bladder function, basic cognitive functions were preserved in all surviving patients. Pure intellectual function remained stable in five patients (36% of all transplanted patients): IQ testing was unchanged in three patients (Patients 3B, 5B and 6B). Patient 11P had unaltered cognitive function, but was classified as 'moderate deterioration' due to significant visual loss. Three other surviving patients (Patients 1B, 7B and 10B) showed a moderate cognitive decline. The deterioration in information processing and learning aptitude in Patient 10B may also have been influenced by prior excessive drug abuse. Among the five patients, who had maintained their vocational status prior to HSCT, two (Patients 6B and 14P) continued as students, one (Patient 11P) was unable to resume work due to severe motor dysfunction and visual loss and two died following HSCT (Patients 2H and 9B).

IQ before HSCT was not predictive for outcome: the five patients with proven or suspected cognitive impairment before transplantation (Patients 1B, 3B, 4S, 7B and 14P) had a variable transplant course and outcome after HSCT.

MRI results

The Loes MRI severity score varied considerably both at onset of ACALD and before HSCT. When ACALD was detected, median Loes score was 4.5 points (range 2–12 points) of maximum 34 points. The four patients with ACALD at first MRI examination (Patients 1B, 3B, 4S and 7B) tended to have a somewhat higher overall Loes score (median 7.8 points, range 4–9 points). Loes score increased during the time period between first detection of ACALD and HSCT to a median 6.5 points (range 2–14 points; $P = 0.004$). Five patients remained stable, while in six patients (Patients 1B, 4S, 5B, 7B, 8B and 12P) cerebral demyelination progressed by at least 2 points. There was no correlation with progression in Loes score and that of EDSS, AACS, or in Rankin score before HSCT. For the 10 patients with MRI scans performed 6–36 months before the onset of ACALD, the median Loes score progression rate was 1.2 points per year (range 0–5.3 points). For 12 evaluable patients, the median Loes score progression rate between onset of ACALD and HSCT was 2 points per year (0–6 points).

Because of early death or poor condition, three patients did not have MRI follow-up (Table 3). Only Patient 1B showed minimal contrast enhancement 6 months post-HSCT. None of the 11 patients examined beyond 6 months after transplant showed further gadolinium enhancement of cerebral demyelinating lesions. There was no significant increase in Loes score beyond 12 months post-HSCT in comparison to Loes score before HSCT among the eight survivors [median 8 points (range 2.5–12 points) before HSCT; median 10 points (range 5–12 points) > 12 months post-HSCT].

The three patients with late death after HSCT tended to show a greater increase in Loes score (median gain 4 points; range 1.5–18 points) compared with the four experiencing moderate deterioration in cognitive function (median gain 1.5 points; range 0–9.5 points) and the four with stable cognitive function (median gain 0.8 points; range 0.5–2 points). New demyelinating lesions after

Table 6 Changes in neurological performance scales and Loes MRI severity score before and after HSCT according to baseline status and transplant complications

	Group I ($n = 6$): Baseline EDSS < 6 without transplant complications			Group II ($n = 8$): Baseline EDSS ⩾ 6 or early transplant complications[a]		
	Before HSCT	Early post-HSCT, ⩽6 months	Late post-HSCT, ~24 months	Before HSCT	Early post-HSCT, ⩽6 months	Late post-HSCT, ~24 months
EDSS, max. 10 points	**3.8** (3.0–4.0)	**6.5** (3.0–6.5)	**5.0** (3.0–6.0)	**6.3** (3.3–6.5)	**8.3** (7.5–9.5)*,**	**9.0** (6.8–1 0.0)*,** ($n = 5$)
AACS, max. 24 points	**7.5** (7.0–10)	**13.0** (10.0–15.0)*	**9.0** (7.0–12.0)	**11.5** (5.5–12.5)	**18.0** (16.5–22.5)*,**	**21.0** (15.8–24.0)*,** ($n = 5$)
Modified Rankin Score, max. 6 points	**2.0** (1.0–3.0)	**4.0** (3.0–5.0)*	**3.5** (2.0–4.0)	**3.5** (1.5–4.0)	**5.0** (5.0–6.0)*,**	**6.0** (4.0–6.0)* ($n = 5$)
Loes Score, max. 34 points	**9.3** (6.0–11.0)		**9.3** (8.0–11.0)	**5.3** (3.5–8.8)		**11.0** (9.5–16.0) ($n = 4$)

All numbers in bold represent median values (with 25th and 75th percentile). Differences in baseline scores between group I and group II were not significant.

[a]Early transplant complications, i.e. at least life-threatening infections during early transplant phase or graft rejection.

*Friedman repeated measures ANOVA on ranks with following pair wise multiple comparisons (Tukey test): $P < 0.05$ in comparison to respective baseline status before HSCT. Missing values of the three early deceased patients for the late time point were supplemented with the respective values from the earlier time point (last value before death) except for Loes score.

**Kruskal-Wallis ANOVA on ranks with following multiple comparisons versus control according to Dunn's method: $P < 0.05$ in comparison to late post HSCT status of group I patients.

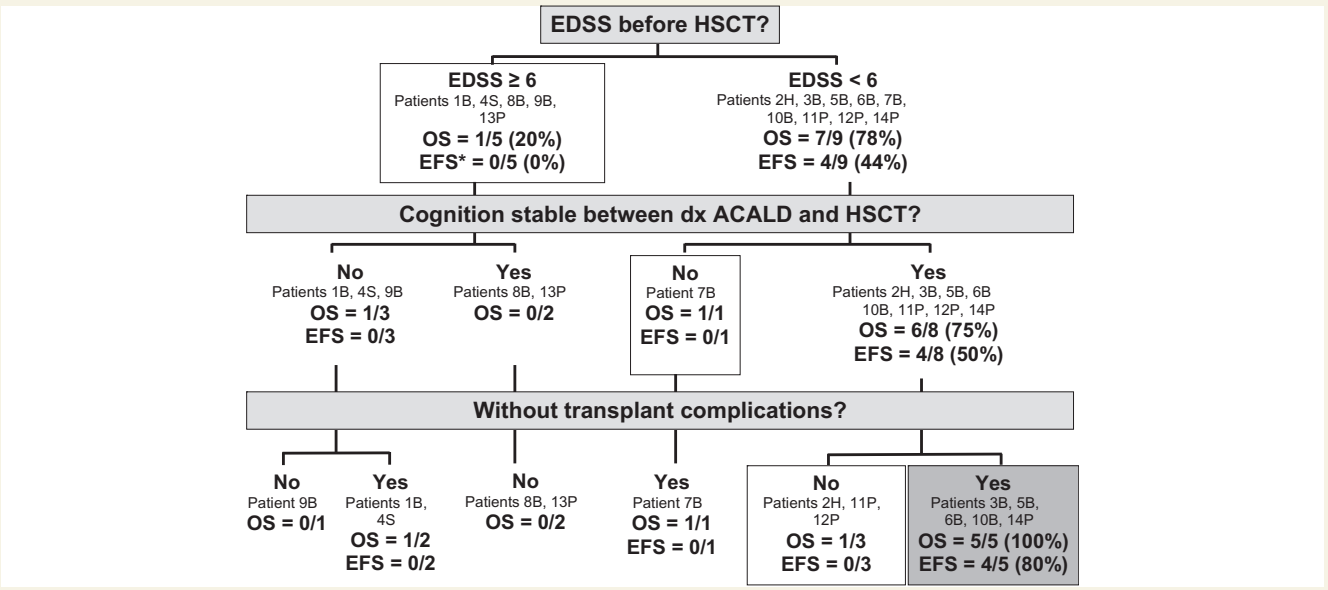

Figure 2 Outcome listed according to EDSS prior to HSCT, stable cognition before HSCT as well as absence of transplant complications. OS = overall survival; EFS = event-free survival, i.e. no cognitive decline post-HSCT.

HSCT were detected in only one of the four cognitively stable patients, whereas lesion progression and new lesions were seen in all three patients experiencing late mortality. However, none of these differences were significant.

Based on MRI analysis, 10 patients (71%) showed involvement of pyramidal long tract fibres, reflecting AMN (Table 1). With regard to ACALD, only 7 of 14 patients (50%) were characterized by the typical parieto-occipital pattern without additional involvement of other structures than pyramidal long tract fibres. Among the other seven patients, three patients (21%) demonstrated predominant involvement of the frontal lobe white matter, while four patients (29%) showed demyelinating lesions in other structures (basal ganglia or cerebellum). Although two of the latter (Patients 4S and 5B) displayed additional parieto-occipital involvement, they were classified as an atypical pattern. In contrast to the Loes score itself, the MRI demyelination pattern seemed to have an impact on HSCT outcome: among the 12 males engrafting (cord blood transplantations excluded) the estimated survival of ACALD patients displaying the typical parieto-occipital pattern (with or without projection fibre involvement; $n = 6$) was 100% versus $33.3 \pm 19.2\%$ ($n = 6$; log-rank test 5.57, $P = 0.018$) for all other patterns.

MRI appearances were further analysed according to the study of Eichler *et al.* (2007): Patient 9B had corticospinal tract involvement without corpus callosum involvement, eight patients (Patients 1B, 3B, 4S, 7B, 8B, 10B, 13P and 14P) had corticospinal tract involvement with additional involvement of the splenium or genu, and five patients (Patients 2H, 5B, 6B, 11P and 12P) had no corticospinal tract involvement but other white matter lesions within the brain. Aside from an increased EDSS in the nine patients with corticospinal tract involvement (median EDSS 5.0 versus 2.9), there were no significant differences in age, ratio of progression, rate of lesion progression as well as outcome compared with the other patients. However, the five patients (Patients 1B, 4S, 8B, 9B and 13P) with advanced corticospinal tract axonopathy involving the two internal capsules were older at ACALD onset, had all an EDSS $\geqslant 6$ before HSCT, and had a significantly poorer post-transplant survival compared with the unaffected patients with a trend for a faster rate of Loes score progression before HSCT among these patients (details summarized in Table 5). In this cohort, bilateral involvement of internal capsule identified the same subgroup of patients as the criterion EDSS $\geqslant 6$ just before HSCT (Fig. 1C).

Analysis according to baseline status and transplant complications

To analyse neurological status between patients with excellent versus poor survival further, patients were divided into two groups depending on motor disability before HSCT and early transplant complications, i.e. life-threatening to fatal infections during the first 6 months post-transplant and/or graft rejection:

Group I ($n = 6$) included Patients 3B, 5B, 6B, 7B, 10B and 14P with limited AMN symptoms pre-HSCT (EDSS < 6) and absence of severe early transplant complications.

Group II ($n = 8$) included five patients (Patients 1B, 4S, 8B, 9B and 13P) with advanced AMN before HSCT (EDSS $\geqslant 6$) and three patients (Patients 2H, 11P and 12P) with severe transplant complications.

There was a significant survival advantage for Group I patients with 100% in comparison to Group II patients with $25.0 \pm 15.3\%$ (log-rank test 7.06; $P = 0.008$; Fig. 1D). Neurological and disability status of both patient groups was quantitatively assessed before as well as early (<6 months) and late ($\sim$24 months) after HSCT using EDSS, AACS, modified Rankin, and Loes score (Table 6). The extent of motor dysfunction was a major criterion to define both groups, consequently the median EDSS at baseline tended to be different between both groups (though not significantly). Patients in both groups deteriorated during the early post-HSCT period in both motor and bladder function as well as cognitive function, reflected by an increase in EDSS (not significant for Group I), AACS, and modified Rankin score. Differences between both groups became apparent during the long-term follow-up: all six patients in Group I survived and partly recovered, i.e. these patients had a 67% and 50% chance fully to preserve cognitive and motor function, respectively (Table 4), and did not demonstrate significant differences in EDSS, AACS, and modified Rankin score late post-transplant in comparison to baseline status. In contrast, the majority of Group II patients steadily deteriorated and died while the two long-term survivors remained moderately to severely impaired both in their motor and cognitive function (Patient 11P had only visual impairment with preserved cognitive functions). Accordingly, late post-HSCT status was significantly different between Group I and II patients for EDSS and AACS score. There was no significant difference in Loes score, but data were either missing ($n = 3$) or only available from 8 months post-HSCT ($n = 1$) for half of the Group II patients.

Of the five patients with an EDSS $\geqslant 6$ before HSCT, only Patient 1B survived experiencing further cognitive decline (Fig. 2). A baseline EDSS < 6 identified a group of nine patients with overall good survival. However, cognitive decline between diagnosis of ACALD and transplantation was associated with further cognitive deterioration post-transplant (Patient 7B). For the remaining eight patients, transplant complications had a major impact on outcome. In the absence of transplant complications, all five patients survived with stable cognitive function, whereas of the three who experienced life-threatening infections and/or graft rejection, only Patient 11P survived with loss of vision.

Discussion

This retrospective study of 14 male patients with ACALD provides proof-of-principle that allogeneic HSCT can arrest inflammatory cerebral demyelination allowing survival with preserved neurocognitive function, at least in a subgroup of patients. The estimated mean survival time of the engrafted 12 patients was 81 months, in contrast to 37–41 months reported for untreated patients with cerebral demyelination (van Geel *et al.*, 2001; de Beer *et al.*, 2014). Moreover, four patients in our series (29%) maintained completely stable intellectual function, and another four developed only moderately impaired neurocognition over a median follow-up period of more than 5 years. Patients who did not progress 6 to 12 months post-transplant became long-term survivors with preserved cognitive function despite ongoing AMN symptoms. The results of HSCT therefore appear to be similar to that observed in CCALD patients (Mahmood *et al.*, 2007), if transplant is performed in patients with stable cognitive function as well as limited AMN symptoms. The relatively long interval between first detection of ACALD and HSCT of 9 months in comparison to 5.1 months in male children with CCALD (Miller *et al.*, 2011) may offer future improvement.

The small number of patients allows for only cautious comments regarding factors that may improve outcomes.

Similar to established paediatric protocols, most of our adult cohort were treated with a myeloablative busulfan- and cyclophosphamide-based regimen. Under these conditions, using bone marrow as a stem cell source appeared to be the best single predictor for survival in this series. However, this conclusion may be biased because of confounding factors related to patients treated with peripheral and cord blood stem cells.

Graft failure occurs more frequently with HLA-mismatched grafts compared to matched donors, in non-malignant versus malignant diseases, and after reduced intensity conditioning compared to myeloablation (Olsson *et al.*, 2013). According to the same study, graft failure in non-malignant disorders had no effect on survival, whereas graft rejection in our series was associated with death. In CCALD, immunoablation alone without donor engraftment does not prevent progression of disease (Nowaczyk *et al.*, 1997; Miller *et al.*, 2011). Unrelated cord blood transplantation after myeloablation may be effective in male children with CCALD lacking a matched donor (Beam *et al.*, 2007; Miller *et al.*, 2011). In these studies, however, the overall survival of patients receiving cord blood cells was inferior to other stem cell sources. Therefore, there should be caution regarding unrelated cord blood for ACALD transplant candidates lacking a matched donor, at least with a reduced-intensity conditioning regimen. A promising future alternative option for patients without a matched donor may be gene-modified autologous haematopoietic stem cell transplantation (Cartier *et al.*, 2009).

In our series, the two patients who had peripheral blood stem cell transplantation did not survive. Although the reason for failure was most likely due to AMN-related immobility with subsequent life-threatening infections, peripheral blood stem cells may also have a negative impact. Transplantation with peripheral blood stem cell in comparison to bone marrow usually results in faster engraftment, more GVHD (Holtick *et al.*, 2015) and higher mortality in non-malignant diseases (Bacigalupo *et al.*, 2015). In ACALD patients, prevention of GVHD may be of particular importance. Rapid clinical deterioration after the onset of severe acute GVHD has been observed in CCALD patients (Peters *et al.*, 2004; Miller *et al.*, 2011) and in a patient with ACALD (Fitzpatrick *et al.*, 2008). In addition, chronic GVHD was associated with temporary deterioration in motor function of an adult patient (Hitomi *et al.*, 2005). Although overall GVHD incidence in this series was low compared to other transplant patients (Socie *et al.*, 2011), the only case of significant acute GVHD was observed after peripheral blood stem cell transplantation.

In summary, we therefore recommend the use of a myeloablative, busulfan-based conditioning regimen in combination with bone marrow for transplanting ACALD patients (Box 1). However, we recognize that future research could focus on potential improvements in the conditioning regimen using other agents to reduce the toxicity of the procedure.

Box 1 Preliminary recommendations for HSCT in ACALD patients

(i) Males at risk of developing ACALD should be routinely monitored by a multidisciplinary team (neurologists, metabolic physicians and transplant haematologists) in specialized centres.
(ii) Potential family donors should be identified in advance to save time once inflammatory cerebral demyelinating lesions (i.e. with contrast enhancement) are identified on brain MRI.
(iii) Stable neurocognitive function prior to transplant and limited AMN symptoms (EDSS < 6) are favourable outcome parameters.
(iv) Patients with bilateral involvement of the internal capsule on brain MRI do not seem to benefit from HSCT.
(v) Stem cell source: bone marrow from a matched related or unrelated donor ($\geqslant$9/10 HLA-match confirmed by high-resolution typing) is preferred over peripheral blood stem cells or cord blood.
(vi) HSCT procedure: busulfan based 'full intensity' or 'myeloablative' conditioning regimens should be used, combined with serotherapy (e.g. antithymocyte globulin) to promote engraftment and limit GVHD. All efforts should be made to prevent fever due to serotherapy or infections, which may be associated with acute neurological deterioration.
(vii) Patients should be treated in experienced transplant centres and registered with the European Group for Blood and Marrow Transplantation (EBMT), the Center for International Blood and Marrow Transplantation Research (CIBMTR) or equivalent transplant registries.

In addition to transplant-related factors, the neurological baseline status of ACALD patients also determines the outcome after allogeneic HSCT. In male children with CCALD undergoing HSCT, more deaths resulted from disease progression in advanced patients than from transplant-related problems (Peters *et al.*, 2004). Although transplant-related mortality in adult patients caused by infection or GVHD may be as high as 30% (La Nasa *et al.*, 2005; Gooley *et al.*, 2010; Peffault de Latour *et al.*, 2012; Bacigalupo *et al.*, 2015), a survival of $<60\%$ in transplanted ACALD patients suggests additional ALD-specific problems. However, an analysis of the impact of neurological status in adults is complicated by the fact that the clinical phenotype of ALD adult males is usually a combination of ACALD itself plus accompanying AMN symptoms.

In ACALD, preserved cognitive function before HSCT was considered to be important for stable long-term outcome. The four patients with progressive cognitive decline between ACALD diagnosis and HSCT, who already displayed cerebral symptoms such as impaired vision and hearing, attention deficits, or seizures before transplant, suggest a disease stage too advanced for satisfactory outcomes from HSCT. This is consistent with a previously-reported Irish adult patient with advanced ACALD (Fitzpatrick *et al.*, 2008) and male children with symptomatic CCALD. The latter display an inferior survival and a greater risk for neurological decline after HSCT than presymptomatic CCALD patients (Peters *et al.*, 2004; Miller *et al.*, 2011). In contrast, the two patients (Patients 3B and 14P) with suspected deterioration in intellectual function before the diagnosis of ACALD but subsequent

stabilization prior to HSCT displayed a stable cognitive function post-HSCT. These courses may indicate cerebral disease with less aggressive dynamics than expected. Uncertainty about the IQ before the onset of ACALD in four patients did not allow definition of a baseline IQ-value as predictor of outcome in this small cohort.

While the Loes score seems to be a valuable predictor of survival and outcome of HSCT in boys with CCALD (Peters *et al.*, 2004; Miller *et al.*, 2011; McKinney *et al.*, 2013), particularly for the typical parieto-occipital demyelination pattern (McKinney *et al.*, 2013), it does not appear to have the same utility in ACALD. In the adults of our series, 'atypical' demyelination patterns were observed in 50% of patients, far more frequent than in childhood with 20–30% (Loes *et al.*, 2003). Whether uncommon MRI patterns and changes in Loes scores immediately prior to transplant predict a poorer survival for ACALD men after HSCT has to be confirmed.

Independent from Loes score, lesion progression in adult patients with X-ALD has been highly associated with corticospinal tract involvement with a mean progression rate of 1 point every 10 months (Eichler *et al.*, 2007). In our cohort, lesion progression before HSCT was observed in an overall higher percentage of patients (71%) independent of corticospinal tract involvement, most likely because gadolinium enhancement was the prerequisite to select patients for transplantation. Contrast enhancement appears to correlate even better with rapidly progressive MRI lesions; median progression rate of Loes score in all 14 adult patients before HSCT was 2.0 points per year (range 0–6 points), as high as in children with cerebral X-ALD (Loes *et al.*, 2003) and at a greater rate than previously reported for adults with corticospinal tract involvement (Eichler *et al.*, 2007). Corticospinal tract involvement alone failed to detect a high-risk group of patients in this series. However, bilateral involvement of the internal capsule identified a subgroup of patients with higher age and EDSS at HSCT as well as a poorer prognosis after transplantation in comparison to all other patients. There was also a trend for more rapid lesion progression in this group.

Bilateral involvement of the internal capsule may therefore mark an end-stage of ascending axonopathy in adult males with AMN. Further studies are needed to determine if AMN patients with isolated involvement of internal capsules develop more severe AMN symptoms and are at high-risk to develop ACALD, and, if so, more rapidly progressive ACALD. Our study suggests, however, that ACALD patients with bilateral involvement of the internal capsule have a significantly poorer prognosis after transplant with little benefit, if any, from HSCT.

Advanced AMN, reflected by bilateral involvement of the internal capsule, appeared to be of at least similar importance for survival than extensive ACALD. Severe motor deficits of the lower limbs prior to transplant were frequently associated with immobility and life-threatening infections during the transplant procedure. Specifically, poor baseline motor function (EDSS $\geqslant$ 6) conferred higher morbidity for survivors, even long after transplantation. Whether some patients became bedridden rapidly after transplant because of myelopathy aggravation or cognitive or psychiatric problems is almost impossible to determine. On the other hand, life-threatening infections and/or non-engraftment may result in severe secondary cerebral disease progression even in patients with limited AMN symptoms prior to transplantation.

Nearly all patients in this cohort developed behavioural changes such as disinhibition or depression at least transiently in the early post-transplant period (Rosebush *et al.*, 1999; Chee *et al.*, 2013), which were associated with an additional burden on the patients, their families, and on the transplant unit. After transplant, these behavioural changes led to fractured relationships. The short- and long-term psychosocial consequences of delivering HSCT to ACALD patients and their families require future consideration.

This study indicates for the first time the feasibility, complications and potential long-term neurological benefit of allogeneic HSCT in ACALD. The poor prognosis of patients with advanced AMN symptoms (EDSS $\geqslant$ 6) may relate to (i) immobility during the transplant procedure with increased risk of infections; (ii) predisposition to rapidly progressive ACALD due to corticospinal tract involvement within the internal capsule; and (iii) cognitive decline and/or psychiatric problems during the transplant procedure that complicates the management of patients during the transplant procedure. Based on our limited experience, we propose preliminary and tentative recommendations for HSCT in ACALD patients (Box 1). Further studies are warranted to improve outcomes through careful patient selection and optimization of HSCT protocols. Given the rarity of this disease, it is essential to have clear referral pathways to specialist centres able to assess and treat patients without delay.

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