OPEN ACCESS

Original research

Haematopoietic stem cell transplantation for treatment of relapsing-remitting multiple sclerosis in Sweden: an observational cohort study

Thomas Silfverberg ,[1,2] Christina Zjukovskaja,[3] Per Ljungman,[4,5] Adjmal Nahimi,[6] Erik Ahlstrand,[7] Arta Dreimane,[8] Sigrun Einarsdottir,[9,10] Jan Fagius,[3] Ellen Iacobaeus,[11,12] Hans Hägglund,[3,5] Niclas Lange,[7] Stig Lenhoff,[13] Jan Lycke ,[14] Johan Mellergård,[15,16] Fredrik Piehl,[11,12] Anders Svenningsson,[17,18] Andreas Tolf ,[3] Honar Cherif,[3] Kristina Carlson,[3] Joachim Burman[3]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jnnp-2023-331864).

For numbered affiliations see end of article.

Correspondence to
Dr Joachim Burman, Department of Medical Sciences, Uppsala University, Uppsala, 752 36, Sweden; joachim.burman@uu.se

Received 22 May 2023
Accepted 26 July 2023
Published Online First 25 September 2023

ABSTRACT

Background A growing evidence base supports the use of autologous haematopoietic stem cell transplantation (aHSCT) for treatment of relapsing-remitting multiple sclerosis (RRMS), but it has not yet been integrated into most national clinical guidelines. The objective of this study was to assess efficacy and safety when aHSCT is implemented in routine healthcare.

Methods We assessed 231 patients and the final analysis included 174 RRMS patients who were treated with aHSCT in Sweden before 1 January 2020. Efficacy was evaluated by performing a retrospective analysis of prospectively collected data from the Swedish MS registry. Procedure-related safety was assessed by analysing data from electronic patient records covering a period of 100 days following aHSCT.

Results With a median follow-up time of 5.5 (IQR: 3.4–7.5) years, the Kaplan-Meier estimate for no evidence of disease activity was 73% (95% CI 66% to 81%) at 5 years and 65% (95% CI 57% to 75%) at 10 years. Out of the 149 patients with baseline disability, 80 (54%) improved, 55 (37%) were stable and 14 (9%) deteriorated. The mean number of adverse events per patient was 1.7 (±SD: 1.5) for grade 3 events and 0.06 (±SD: 0.3) for grade 4 events. Febrile neutropenia was the most common adverse event, affecting 68% of patients. There was no treatment-related mortality.

Conclusions Treatment with aHSCT for RRMS is associated with freedom from disease activity in a majority of patients, with acceptable adverse events. This procedure should be considered a standard of care for patients with highly active RRMS.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Autologous haematopoietic stem cell transplantation (aHSCT) is an emerging treatment option for relapsing-remitting multiple sclerosis (RRMS) patients. To date, only one randomised clinical trial has compared aHSCT with standard disease-modifying treatment (DMT) for RRMS. This trial demonstrated a significant advantage of aHSCT over standard DMT in terms of time to progression and neurological disability after 2 years. Moreover, there were no recorded Common Terminology Criteria for Adverse Events grade 4 adverse events or instances of treatment-related mortality. However, it remains uncertain whether these results can be translated into routine healthcare.

WHAT THIS STUDY ADDS

⇒ This study supports and strengthens the evidence from the sole randomised controlled trial on aHSCT for RRMS conducted thus far. More than half of the participants experienced improved disability, and approximately two-thirds displayed no evidence of disease activity over a 10-year period. The incidence of severe adverse events was low, and there was no record of treatment-related mortality, suggesting that aHSCT can be safely implemented within routine healthcare.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ aHSCT has the potential to benefit a larger number of MS patients and should be considered a standard of care for highly active MS. Further research is needed to identify the specific patient populations that would derive the most benefit from aHSCT.

Check for updates

To cite: Silfverberg T, Zjukovskaja C, Ljungman P, *et al*. *J Neurol Neurosurg Psychiatry* 2024;**95**:125–133.

INTRODUCTION

Multiple sclerosis (MS) is a leading cause of permanent neurological disability in young adults.[1] The prevailing theory posits that MS is an autoimmune inflammatory disease mediated by B and T lymphocytes,[2] resulting in demyelination, gliosis and axonal degeneration in the central nervous system (CNS).[3 4] The most frequently observed disease course at onset is relapsing-remitting MS (RRMS), characterised by distinct inflammatory episodes in the CNS that cause varying degrees of residual disability. Over time, RRMS typically transitions into a secondary progressive (SPMS) disease course marked by neurodegeneration and disability accumulation. Natural history studies estimate that

the median time from RRMS onset to secondary progression is approximately 19 years.[5]

Current disease-modifying treatments (DMTs) primarily focus on reducing inflammation to prevent the formation of MS plaques and clinical relapses. However, the extent to which DMTs slow disability accumulation and delay the transition to SPMS remains unknown. It has been proposed that around half of disability worsening in RRMS occurs without an associated relapse. Such progression independent of relapse activity is common; it occurs frequently in early MS and persists even with highly effective MS therapies.[6] An increasingly used treatment goal is to maintain no evidence of disease activity (NEDA), which encompasses the absence of new relapses, new or enlarged lesions on MRI, and confirmed disability worsening (CDW).[7 8]

High-dose chemotherapy followed by autologous haematopoietic stem cell transplantation (aHSCT) has been used to treat MS since the 1990s. The goal of aHSCT is to reset the immune system by eliminating autoreactive lymphocytes, in order to induce long-term remission.[9] Growing evidence supports the efficacy and safety of aHSCT, with two-thirds of treated patients maintaining NEDA up to 4–5 years post-treatment.[10 11] It has also been reported that more than half of patients improve in disability outcomes after aHSCT.[12 13] The treatment-related mortality rate following aHSCT is estimated at 0.2%–0.3%.[11 14 15] The timing of aHSCT is crucial, as it is significantly more effective in RRMS than in progressive forms of MS.[11 15] Efficacy and safety have improved in recent years due to increasing experience, improved patient selection, and optimised conditioning regimens.[16] The Swedish Board of Health and Welfare approved aHSCT for MS in 2016, but in most countries, it has not yet been integrated into clinical guidelines. The outcome of aHSCT for RRMS in broader use outside clinical trials remains undetermined.

The objective of this multicentre retrospective cohort study was to assess the efficacy and safety of aHSCT for RRMS when implemented in a setting of routine healthcare.

METHODS

Data collection

Patients were identified using the Swedish MS registry (SMSreg) and local European Society for Blood and Marrow Transplantation (EBMT) registries at the seven transplantation centres throughout Sweden. The SMSreg is a nationwide registry that has been amassing prospective data on MS patients since the mid-1990s. It currently has an estimated coverage of >80% overall and nearly 100% for patients with advanced therapy such as aHSCT.[17]

Data were extracted from the SMSreg and electronic patient records. A neurologist at each transplantation centre retrospectively reviewed disease course and all outcome data in the SMSreg to ensure their validity. A haematologist collected safety data by systematically analysing medical records from the time of stem cell mobilisation to 3 months following aHSCT. All severe adverse events (AEs), defined as AE of grade 3 or higher, were documented in accordance with version 5.0 of the Common Terminology Criteria for Adverse Events.[18] Anaemia, leucopenia, neutropenia and thrombocytopaenia as well as transient alopecia and amenorrhoea were expected during the first weeks after aHSCT and were not included. A comprehensive overview of all data is given in online supplemental section 1. The study protocol is available in online supplemental file 2.

Inclusion criteria

Inclusion criteria were diagnosis of MS according to the revised McDonald criteria,[19] with a relapsing-remitting disease course,[20] and aHSCT performed for MS at any of the seven Swedish transplantation centres before 1 January 2020.

Exclusion criteria

Exclusion criteria were progressive MS (primary progressive MS or SPMS) according to Lublin *et al*[20] at the time of aHSCT, that the patient did not consent to reporting of data to the EBMT register or failure to meet the minimal dataset. The minimal dataset covered disease course at the time of transplantation, date of transplantation, data on conditioning regimen and at least one follow-up visit (unless early death before first follow-up visit) with data on clinical assessment using the Kurtzke Expanded Disability Status Scale (EDSS) and neuroradiological assessment with MRI.[21]

Endpoints

The primary endpoints were NEDA at 5 years and treatment-related mortality. Secondary endpoints were NEDA at 3 and 10 years; CDW, relapse-free survival and MRI event-free survival at 3, 5 and 10 years; annualised relapse rate after aHSCT; proportion of patients with confirmed disability improvement; and EDSS change between baseline and follow-up at 1, 2 and 3 years. The frequency and grade of severe AEs were used to estimate the safety of the procedure, limited to within 100 days of aHSCT in order to restrict information bias. Definitions of these endpoints are given in online supplemental section 2.

Procedures

Mobilisation

Stem cells were mobilised using a combination of cyclophosphamide 2 g/m^2 and granulocyte-colony-stimulating factor (G-CSF) 5 µg/kg subcutaneously starting on day 5 or 6 until stem cell harvest.

Harvest

Haematopoietic stem cells were harvested by apheresis of peripheral blood. A minimum of 2.0×10^6 CD34$^+$ cells/kg were harvested and cryopreserved, with no ex vivo manipulation.

Conditioning

Two conditioning regimens were used; BEAM-antithymocyte globulin (ATG) and Cy-ATG. The BEAM-ATG comprised carmustine 300 mg/m^2, etoposide 800 mg/m^2, cytarabine arabinoside 3200 mg/m^2, melphalan 140 mg/m^2 and ATG from rabbit (rATG) 10 mg/kg and was given during 7 days. This protocol was mainly used during the first few years of the study period and was then replaced by Cy-ATG at all centres. The Cy-ATG protocol was given during 5 days and included cyclophosphamide 200 mg/kg, rATG 6 mg/kg and 5000 mg methylprednisolone, including tapering after stem cell infusion, and hyperhydration and uromitexan to prevent haemorrhagic cystitis. There was a minimum wash-out time of 48 and 24 hours from the last chemotherapy administration to the reinfusion of the autologous stem cells for BEAM-ATG and Cy-ATG, respectively.

Antimicrobial prophylaxis

Oral ciprofloxacin was used to prevent bacterial infection during the neutropenic phase, except for eight patients who received prophylactic intravenous antibiotics. Prophylaxis against herpes simplex virus and *Pneumocystis jiroveci* was given for a

minimum of 3 months following aHSCT. Monitoring for reactivation of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) was performed for patients with positive serology.

Supportive care

If needed, all patients were given filtered and irradiated blood products until their lymphocyte counts exceeded 1.0×10^9/L.

Statistical analysis

Statistical analysis was performed with V.3.5.3 of R, using the packages ggplot2, survival, fBasics, ggpubr, moments, survminer, plotrix, grid, gridExtra, lattice and devtools. Data were summarised using frequencies for categorical variables, medians (IQR) for discrete variables and time data unless inappropriate due to rare events, and means (±SD:) for continuous variables. The Mann-Whitney test was used to determine statistically significant differences between two groups, Fisher's exact test was used to determine statistically significant differences between proportions, and the Wilcoxon signed rank test was used to determine statistically significant differences between two time points. Survival was estimated using Kaplan-Meier plots (95% CI). A two-tailed $p<0.05$ was considered statistically significant.

RESULTS

Patient characteristics

Data were exported from the SMSreg on 22 May 2022. We evaluated 231 patients for participation in the study, of whom 174 were included in the final analysis. Fifteen patients did not meet the inclusion criteria and were not analysed further, 30 patients had progressive MS at aHSCT and 12 did not fulfil the minimal dataset; these 42 patients were excluded from the study (figure 1). Baseline patient characteristics are shown in table 1.

The first patient was treated on 25 May 2004. Median age at aHSCT was 31 years (IQR: 26–36) and 64% of the patients were women. Median disease duration was 3.4 years (IQR: 1.0–6.9). The patients had received a median of 2 (IQR: 1–3) DMTs prior to aHSCT, and 23 patients were previously untreated. The median follow-up time was 5.5 years (IQR: 3.4–7.5). A total of 2435 follow-up visits with EDSS scoring and 1785 MRI scans were analysed, with a cumulative follow-up time amounting to 1034 years. After a median of 2.9 years (IQR: 2.1–4.4), 20 patients (11%) received DMT after aHSCT (figure 2). After a median of 4.1 years (IQR: 1.8–5.9) years, 10 patients transitioned from RRMS to SPMS.

Procedures

All patients were mobilised with cyclophosphamide+G CSF. For the conditioning, BEAM-ATG was used in 33 patients and Cy-ATG in 141 patients. The last patient receiving BEAM-ATG was treated in 2015. The median time to engraftment (defined as absolute neutrophil count ≥0.5 and thrombocyte counts >20 and rising, without transfusion of thrombocytes) was 12 days (IQR: 11–13.5). The median time of hospitalisation for aHSCT was 20 days (IQR: 19–22), calculated from the day of admission to the hospital to the day of discharge. Twenty-eight patients received G-CSF in the first week following aHSCT. There was a mean decrease in body weight of 2.2 (±SD: 2.1) kg and a median loss of plasma albumin of 7.5 (5–11) g/L during hospitalisation for aHSCT.

Primary endpoints

The Kaplan-Meier estimate of NEDA at 5 years was 73% (95% CI 63% to 81%). There was no treatment-related mortality (figure 3).

Secondary endpoints

Clinical relapses, MRI events and CDW

The Kaplan-Meier estimates of clinical relapses, MRI events and CDW at different time points are presented in figure 3

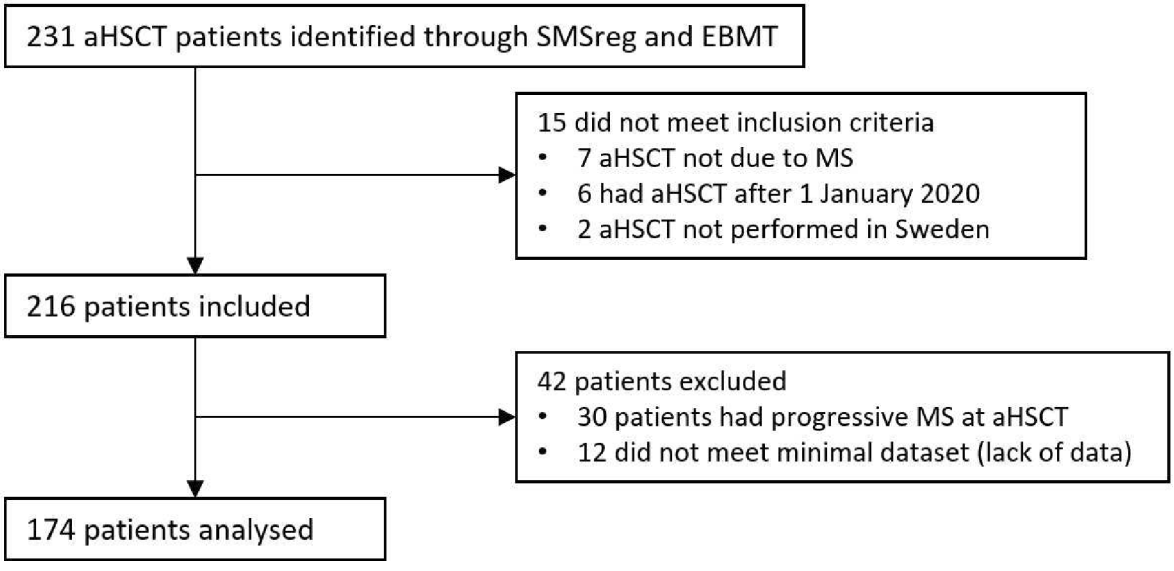

Figure 1 In this study, a total of 231 patients were evaluated for potential inclusion, with 174 ultimately being incorporated into the final analysis. Fifteen patients were excluded as they failed to meet the inclusion criteria, which required a diagnosis of multiple sclerosis (MS), a relapsing-remitting disease course and autologous haematopoietic stem cell transplantation (aHSCT) performed for MS at any of the seven Swedish transplantation centres before 1 January, 2020. Thirty patients had progressive MS at aHSCT and were excluded. An additional 12 did not fulfil the requirements for the minimal dataset, which were data on disease course at the time of transplantation, date of transplantation, data on conditioning regimen and at least one follow-up visit. EBMT, European Society for Blood and Marrow Transplantation; SMSreg, Swedish MS registry.

Table 1 Baseline patient characteristics

n=174	No of patients (%)
Age in years (range: 9–58)	
0–9	1 (0.6)
10–19	6 (3.4)
20–29	70 (40)
30–39	68 (39)
40–49	26 (15)
50–59	3 (1.7)
Sex	
Female	112 (64)
Male	62 (36)
Comorbidities*	
Depression	7 (4.0)
Obesity	5 (2.9)
Asthma	5 (2.9)
Bipolar disorder	4 (2.3)
Anxiety disorder	4 (2.3)
Mb Crohn	3 (1.7)
Hypertension	3 (1.7)
Psoriasis	3 (1.7)
Prior malignancy†	2 (1.1)
Diabetes mellitus	2 (1.1)
Chronic renal disease	2 (1.1)
Rheumatoid arthritis	2 (1.1)
Prior deep vein thrombosis	2 (1.1)
Thyrotoxicosis	2 (1.1)
Ankylosing spondylitis	2 (1.1)
Irritable bowel syndrome	2 (1.1)
No comorbidity	122 (70)
EDSS at aHSCT‡	
0–1.5	23 (13)
2–3.5	88 (51)
4–5.5	38 (22)
6–6.5	16 (9.2)
7–9.5	8 (4.6)
Gadolinium-enhancing lesions at aHSCT§	
0	91 (57)
1–9	44 (28)
10–20	11 (6.9)
>20	13 (8.2)

*Comorbidities with a frequency of more than 1%.
†Two cases of breast cancer.
‡Data missing for one patient.
§Fifteen patients did not have a contrast-enhanced MRI scan at baseline.
aHSCT, autologous haematopoietic stem cell transplantation; EDSS, Expanded Disability Status Scale.

and table 2. Notably, all instances of CDW occurred independent of relapses, and there was no relapse-associated worsening. An ad hoc analysis was made to determine the further clinical course in the 48 patients who displayed evidence of disease activity after AHSCT. A new baseline was set after the first instance of disease activity. After a median follow-up time of 6.2 years (IQR: 5.1–7.4), 5 patients exhibited CDW, 12 suffered from a clinical relapse and 16 patients had an MRI event. The Kaplan-Meier estimate of NEDA was 62% (95% CI 49% to 79%) 5 years after rebaselining.

Annualised relapse rate

The annualised relapse rate was 1.7 (±SD: 1.9) in the year prior to aHSCT and 0.035 (±SD: 0.12) during the follow-up period ($p<0.0001$).

Proportion of patients with confirmed disability improvement

Of the 149 patients with any degree of disability at baseline (EDSS≥2), 80 (54%) improved in disability, 55 (37%) were stable and 14 (9%) deteriorated at the end of follow-up. The proportions of patients with changes in disability at different time points are presented in figure 4.

EDSS change

The median EDSS at baseline was 3.5 (IQR: 2–4). The evolution of EDSS over the first 3 years is shown in figure 5. At the last follow-up, the median EDSS was 2 (IQR: 1–3.5), significantly lower than at baseline ($p<0.0001$).

Safety

To assess the safety of aHSCT, we analysed mortality, need for intensive care and severe AEs. The mean number of severe AEs per patient was 1.7 (±SD: 1.5) for grade 3 events and 0.06 (±SD: 0.3) for grade 4 events. Thirty patients (18%) did not experience any severe AE (those directly related to the procedure excluded). The frequencies of all severe AEs are presented in table 3.

Overall mortality

One patient in the cohort died during the follow-up period. The deceased patient had pre-existing depression and a history of a suicide attempt. The cause of death was attributed to suicide precipitated by substance abuse. The death occurred more than 6 years after aHSCT, and this event was deemed unrelated to the treatment.

Intensive care

Five patients were admitted for intensive care, with a median duration of 2 days (range: 1–2). The reasons for intensive care were correction of hyponatraemia (n=2), sepsis and hypoxia (n=1), febrile neutropenia and hypotonia (n=1), and pulmonary embolism in combination with perimyocarditis with a transient decrease in left ventricle ejection fraction to 30% (n=1).

Febrile neutropenia

Febrile neutropenia was the most frequently observed AE linked to aHSCT, affecting 125 patients (72%) Intravenous antibiotics were administered to 138 patients (79%).

Bacterial infections

Bacterial infection was verified through culture in 61 patients (35%) at any time from stem cell mobilisation to day+100. The most common bacterial species were *Escherichia coli* (n=20 patients), alpha streptococci (n=8) and coagulase negative streptococci (n=6). The most common clinical infections were septicaemia (n=20), urinary tract infections (n=17), and venous catheter-related infections (n=4).

Common AEs

Other common AEs included hypokalaemia, which affected 31 patients (18%) and was associated with the use of hyperhydration and furosemide in half of the cases. There were no recorded arrhythmias. Nausea interfering with dietary intake and requiring

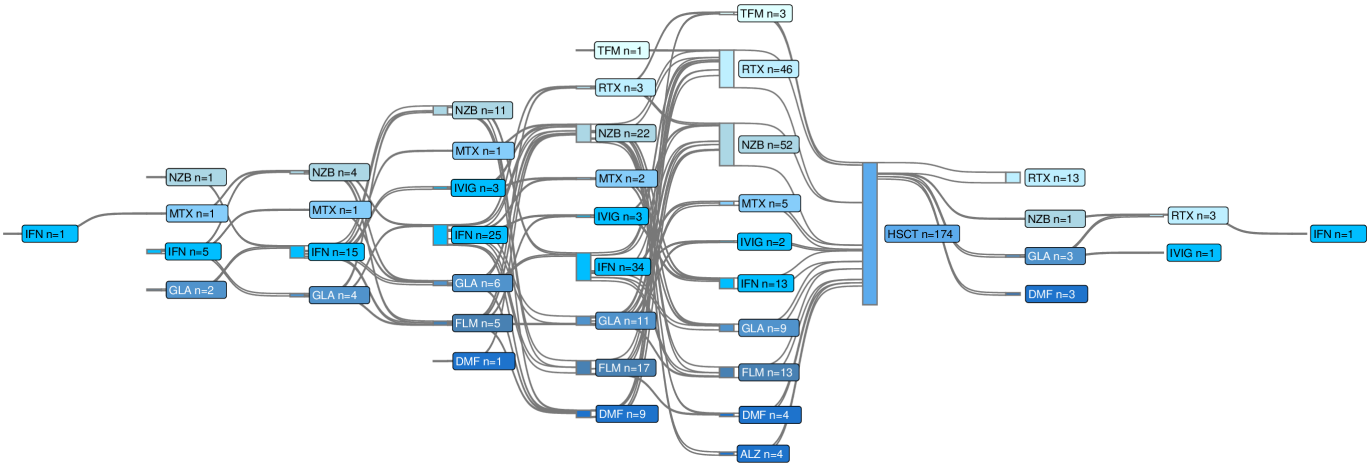

Figure 2 Current and previous treatments. This Sankey diagram shows disease-modifying treatments used before and after autologous haematopoietic stem cell transplantation (aHSCT). Twenty-three patients had not used any disease-modifying treatment prior to aHSCT. ALZ, alemtuzumab; DMF, dimethyl fumarate; FLM, fingolimod; GLA, glatiramer acetate; IFN, interferon; IVIG, intravenous IgG; MTX, mitoxantrone; NZB, natalizumab; RTX, rituximab; TMF, teriflunomide.

parenteral nutrition occurred in 14 patients (8.0%). ATG-associated serum sickness requiring steroids or intravenous fluids affected 11 patients (6.3%). Thromboembolic events occurred in eight patients (4.6%): deep vein thrombosis (n=5), vascular access thrombosis (n=2) and pulmonary embolism (n=1). One case of autoimmune disease (immunological thrombocytopenic purpura) occurred in the first 100 days following aHSCT.

There were few associations between AEs and pre-existing comorbidities. The only grade 4 psychiatric AE happened in a patient with pre-existing bipolar disorder and the only grade 4 thromboembolic event occurred in a patient with heterozygote activated protein C resistance. Gastrointestinal AEs with grade 3 diarrhoea occurred in one patient with pre-existing Crohn's disease and in another who had undergone a gastric by-pass procedure.

EBV and CMV

None of the patients developed EBV-related or CMV-related disease. In 59 patients, at least one PCR test showed detectable EBV levels, while 49 patients had PCR tests positive for CMV. In the majority of cases, low levels of EBV and CMV reactivations (below 900 copies/mL) were detected in a single blood sample from asymptomatic patients. Twenty-one patients had measurable EBV levels in at least two samples, but only one received rituximab treatment as a preventive measure. In the case of CMV, eight patients had persistent, measurable levels in at least two samples. Five of these patients received pre-emptive oral treatment for CMV, while one patient required intravenous therapy. Data were unavailable for five patients.

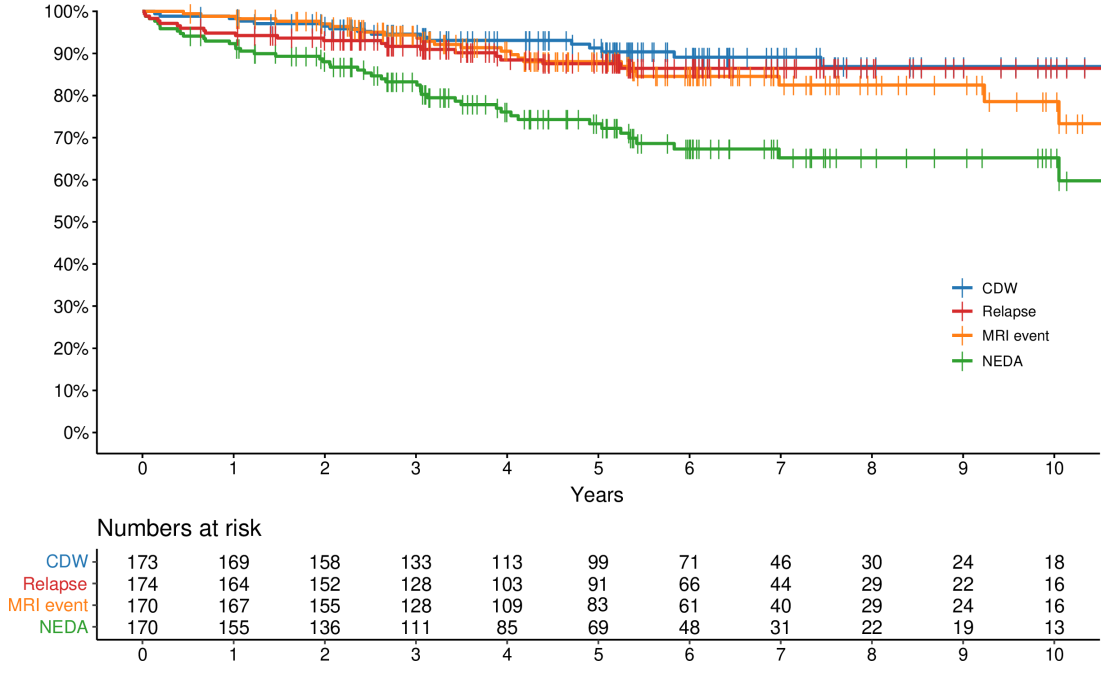

Figure 3 Primary and secondary endpoints. Kaplan-Meier curves for the primary endpoint: no evidence of disease activity (NEDA), and for the secondary endpoints: freedom from MRI events, freedom from clinical relapses and freedom from confirmed disability worsening (CDW).

Table 2 Kaplan-Meier estimates of NEDA and freedom from clinical relapses, MRI events and CDW by year

	Clinical relapse		MRI event		CDW		NEDA	
Year	KM	95% CI	KM	95% CI	KM	95% CI	KM	95% CI
1	95%	92% to 98%	99%	97% to 100%	98%	96% to 100%	92%	88% to 96%
2	92%	89% to 96%	97%	94% to 100%	96%	94% to 99%	87%	82% to 93%
3	91%	87% to 96%	94%	91% to 98%	95%	91% to 98%	83%	77% to 89%
5	87%	82% to 93%	88%	83% to 94%	91%	87% to 96%	73%	66% to 80%
10	86%	80% to 92%	79%	69% to 90%	87%	80% to 94%	65%	56% to 74%

CDW, confirmed disability worsening; KM, Kaplan-Meier estimate; NEDA, no evidence of disease activity.

Other viral and fungal infections

Other viral infections were verified in 23 patients (13%). Respiratory viruses (rhinoviruses, adenoviruses and coronaviruses) were found in eight patients. Four patients tested positive for BK virus, with three cases detected exclusively in urine and one case found in both urine and plasma. All four of these patients had undergone cyclophosphamide conditioning. Notably, no instances of haemorrhagic cystitis were observed. Herpes zoster reactivation was documented in three patients. In the full cohort, only 1.7% (n=3 patients) had a confirmed localised fungal infection. Two of these patients presented with oral candidiasis, while the third had vaginal candidiasis. Importantly, no cases of invasive fungal infection were seen.

DISCUSSION

A key finding of this cohort study of 174 RRMS patients is that treatment with aHSCT was followed by maintenance of NEDA over 5 years in 73% of patients, without compromising safety. There was no treatment-related mortality and AEs were manageable. These findings support what is currently the only randomised controlled trial of aHSCT for RRMS,[12] suggesting that the results are generalisable to routine healthcare settings.

One of the advantages of aHSCT is that it is a one-time treatment, allowing for comparison with immune replacement therapies such as alemtuzumab and cladribine. While direct trial comparisons are generally discouraged, factors such as age, disease duration, annualised relapse rate and the percentage of patients with gadolinium-enhancing lesions at baseline were reasonably comparable between the CARE-MS trials and our study. In the CLARITY trial, however, patients were older, had longer disease durations and a lower percentage of participants had gadolinium-enhancing lesions at baseline, suggesting a slightly less inflammatory disease. Disability was lower in both CARE-MS trials and CLARITY, indicating that patients in our trial had more severe disease. The proportion of patients maintaining NEDA over 2 years (88%) was considerably higher than that reported in the CARE-MS I and II studies of alemtuzumab (32%–39%)[22 23] and the CLARITY study of cladribine (47%).[24] A previous study comparing outcomes of patients treated with aHSCT or alemtuzumab at two Swedish centres reported similar results, with the Kaplan-Meier estimate of NEDA at 3 years being 88% for aHSCT and 37% for alemtuzumab.[13]

A substantial number of patients were followed for more than 5 years. At the 10-year mark, 65% of patients still maintained

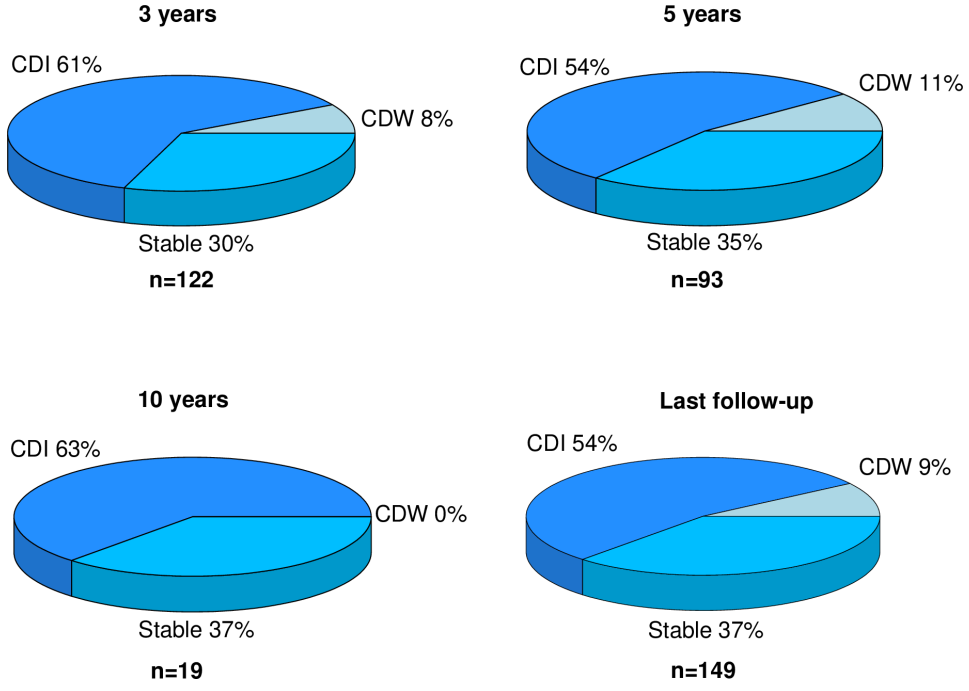

Figure 4 Changes in disability over time. Proportions of patients with confirmed disability improvement (CDI), stable disability and confirmed disability worsening (CDW) at different timepoints. Scores on the Kurtzke Expanded Disability Status Scale (EDSS) at baseline were compared with the EDSS scores at 3, 5 and 10 years as well as with the EDSS scores at last follow-up. Only patients with some degree of disability (EDSS≥2) were taken into account for this analysis.

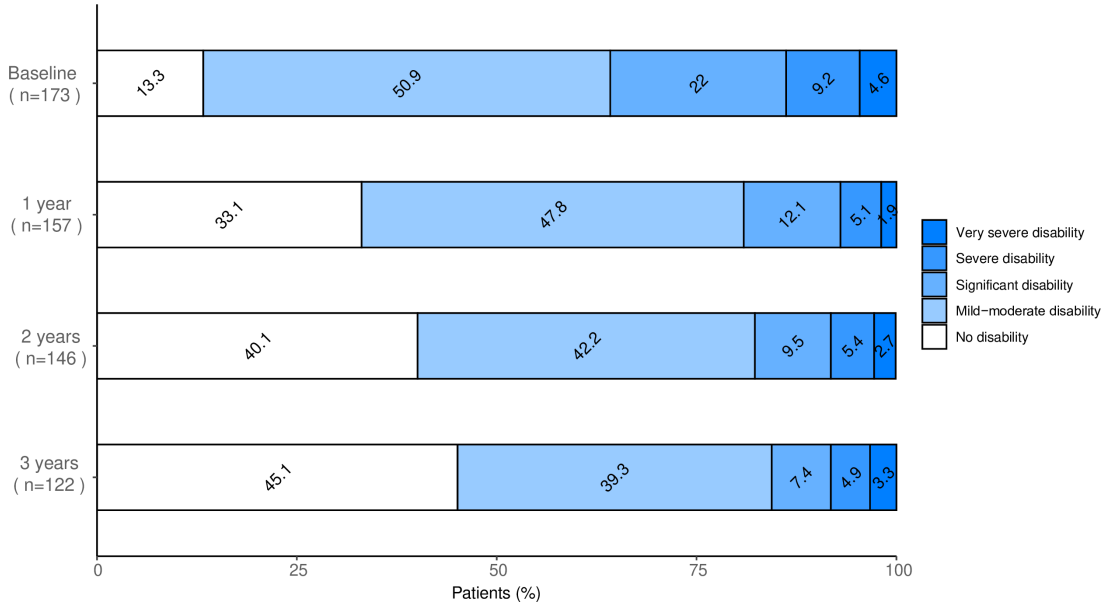

Figure 5 Proportions of patients with different levels of disability over time. Disability strata defined using the Kurtzke Expanded Disability Status Scale (EDSS) from baseline and over the first 3 years after autologous haematopoietic stem cell transplantation. No disability was defined as EDSS 0–1.5, mild-to-moderate disability as EDSS 2–3.5, significant disability as EDSS 4–5.5, severe disability as EDSS 6–6.5 and very severe disability as EDSS 7–9.5.

NEDA and 88% did not experience disability worsening. These results indicate a durable response, further emphasised by the low number of patients requiring additional treatment after aHSCT and the low conversion rate from RRMS to SPMS. These figures are slightly more favourable than those reported in a study of Italian patients treated with the BEAM conditioning regimen,[25] which may reflect differences in disease severity and emphasises the importance of early intervention.

The indication for aHSCT in Sweden evolved during the study period. Initially considered a rescue treatment, reserved for patients with the most aggressive forms of MS where other treatment options were considered futile.[26] The indication was later changed to include patients with active disease despite adequate course of treatment with at least one DMT or with rapidly evolving severe RRMS defined by at least two disabling relapses in 1 year with evidence of MRI disease activity, similar to the European Medicines Agency label for natalizumab. Consequently, this group represents individuals with a poor long-term prognosis; nevertheless, aHSCT was associated with an improvement in EDSS at group level. Nearly half of the patients were free from disability 3 years after aHSCT, a significant increase from the 13% who had no disability at baseline. Over half of those with disability at baseline improved after aHSCT, and only 9% had worsened at the last follow-up. This improvement distinguishes aHSCT from standard DMTs. Although this was an observational study where parts of the improvement can be explained by regression to the mean, the proportion of patients with improvement in EDSS was very similar to that reported in the randomised controlled Multiple Sclerosis International Stem Cell Transplant trial.[12]

The comprehensive analysis of medical records allowed us to obtain high-resolution safety data, presenting the full range of severe AEs directly related to the procedure. All AEs could be managed using standard procedures at a tertiary referral hospital. Several commonly anticipated AEs, such as hypokalaemia resulting from loop diuretic use, serum sickness after rATG administration and elevated hepatic transaminases following cyclophosphamide treatment, were all resolved before hospital discharge. Hypokalaemia episodes did not lead to any observed arrhythmias. Only six patients (3.4%) experienced grade 4 AEs, which occurred evenly throughout the study period.

The most frequent severe AEs were febrile neutropenia and infectious complications, both of which are generally expected during the transient impairment of the immune system after conditioning. We observed a higher incidence of febrile neutropenia compared with most previous studies. One potential explanation could be that oral ciprofloxacin, instead of intravenous antibiotics, is used for bacterial prophylaxis after aHSCT in Sweden. Eight patients in this study received prophylaxis with intravenous antibiotics and had comparable rates of febrile neutropenia, but none of them had any positive bacterial cultures. We defined febrile neutropenia as measurements of fever during neutropenia, and not, as in several previous studies, as positive bacterial cultures. Conversely, earlier research indicated a high prevalence of EBV and CMV reactivations following aHSCT for MS,[27 28] while our study found a very low frequency of clinically relevant EBV and CMV reactivations despite active monitoring. Taking together data from our and previous studies, although varying types of infections are unavoidable in conjunction with aHSCT, we believe that the low risk of severe complications should not deter patients from undergoing this procedure in situations where the clinical benefits are considered to be high.

Acknowledging a low capture rate of AEs by review of medical records, we deliberately chose not to gather data on AEs beyond the initial 3 months. For reference, we recently published a registry linkage study on a largely overlapping patient cohort treated with aHSCT (n=139), and compared safety outcomes with patients treated with alemtuzumab (n=132) and a large reference population (n=2486) treated with regular DMTs.[29] Data from SMSreg were linked to a series of national demographic and health registers with excellent coverage, including the mortality register, cancer register and national patient register. The study revealed a moderately elevated risk of infections requiring hospital care with aHSCT, also beyond the first 6 months. Thyroid disease was also more common compared with the reference population, but significantly lower than with

Table 3 Severe adverse events by frequency

n=174	Grade 3 (%)	Grade 4 (%)
Febrile neutropenia*	119 (68.4)	6 (3.4)
Hypokalaemia†	31 (17.8)	–
Nausea	14 (8.0)	–
Serum sickness	11 (6.3)	–
Oral mucositis	9 (5.1)	–
Diarrhoea	9 (5.1)	–
Elevated transaminases	9 (5.1)	–
Hypoalbuminaemia	7 (4.0)	–
Hypotension	6 (3.4)	–
Fatigue	5 (2.9)	–
Anorexia	5 (2.9)	–
Hyperglycaemia	5 (2.9)	–
Pericarditis	2 (1.1)	1 (0.6)
Depression	2 (1.1)	1 (0.6)
Thromboembolic event	2 (1.1)	1 (0.6)
Skin/soft tissue infection (non-neutropenic)‡	3 (1.7)	–
Vascular access thrombosis	3 (1.7)	–
Pneumonia (non-neutropenic)‡	3 (1.7)	–
Catheter-related infection (non-neutropenic)‡	3 (1.7)	–
Cytokine release syndrome	3 (1.7)	–
Myalgia	3 (1.7)	–
Vomiting	3 (1.7)	–
Elevated gamma-GT	3 (1.7)	–
Heart failure	1 (0.6)	1 (0.6)
Hyponatraemia	1 (0.6)	1 (0.6)
Sepsis (non-neutropenic)‡	2 (1.1)	–
Infectious enterocolitis (non-neutropenic)‡	2 (1.1)	–
Fever	2 (1.1)	–
Seizures	2 (1.1)	–
Urticaria	2 (1.1)	–
Syncope	2 (1.1)	–
Abdominal pain	2 (1.1)	–
Postherpetic pain	2 (1.1)	–
CMV reactivation	1 (0.6)	–
EBV reactivation	1 (0.6)	–
Varicella zoster	1 (0.6)	
Pyelonephritis (non-neutropenic)‡	1 (0.6)	–
Non-infectious enterocolitis	1 (0.6)	–
Atrial fibrillation	1 (0.6)	–
Hypoxia	1 (0.6)	–
Pulmonary infiltrates	1 (0.6)	–
Interstitial oedema in lungs	1 (0.6)	–
Renal insufficiency	1 (0.6)	–
Allergic reaction	1 (0.6)	–
Leucocytosis	1 (0.6)	–
Immunological thrombocytopaenia	1 (0.6)	–
Mania	1 (0.6)	–
Hallucinations	1 (0.6)	–
Hemichorea	1 (0.6)	–
Skeletal pain	1 (0.6)	–
Genital herpes simplex infection	1 (0.6)	–
Vaginal haemorrhage	1 (0.6)	–
Elevated alkaline phosphatase	1 (0.6)	–

All grades 3 and 4 adverse events according to CTCAE V.5.0 for all patients from start of mobilisation to day +100 after aHSCT. Anaemia, neutropenia, leucopenia and thrombocytopaenia as well as transient alopecia and amenorrhoea were expected during the first weeks after aHSCT, and were excluded. Neurological adverse events assessed as manifestations of MS were not included. There were no grade 5 adverse events.
*Febrile neutropenia comprises all episodes of fever (according to CTCAE V.5.0) regardless of clinical infection occurring during the neutropenic phase following stem cell mobilisation and conditioning.
†Hypokalaemia was associated with furosemide treatment after hyperhydration in 15 patients.
‡Occurring outside the neutropenic phase.
aHSCT, autologous haematopoietic stem cell transplantation; CMV, cytomegalovirus; CTCAE, Common Terminology Criteria for Adverse Events; EBV, Epstein-Barr virus.

alemtuzumab. Importantly, no malignancies were noted in the aHSCT group. Apart from the same fatal case reported here, there were no additional mortalities with aHSCT, compared with four with alemtuzumab.[29]

This study was an observational cohort study, inherently limited by the absence of a control group. Some data may have been missing, potentially leading to underreporting, primarily of AEs. To ensure data accuracy in SMSreg, an on-site neurologist cross-verified each patient's register data with their medical health records. When any patient's medical record is accessed in Sweden, the patient's vital status is automatically synchronised with the Swedish Tax Agency's records, which are updated daily. Consequently, we have complete and up-to-date mortality data for all patients, regardless of their last follow-up visit, and hence can confirm that no patient in the study cohort died from COVID-19 during the recent pandemic. The absence of a control group precludes definitive conclusions about the effect size in comparison to other RRMS treatments and makes it impossible to estimate the magnitude of the regression to the mean. Conversely, notable strengths of this study are the near-complete coverage, high data density and granularity of the SMSreg.

In summary, our findings demonstrate that aHSCT for RRMS is feasible within regular healthcare and can be performed without compromising safety. Our study corroborates the results observed in the only randomised controlled trial conducted to date.[12] We believe that aHSCT could benefit a greater number of MS patients and should be included as a standard of care for highly active MS.

Author affiliations
[1]Medical Sciences, Uppsala universitet Medicinska och farmaceutiska vetenskapsomradet, Uppsala, Sweden
[2]Center for Clinical Research Dalarna, Falun, Sweden
[3]Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[4]Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden
[5]Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska Comprehensive Cancer Center, Karolinska University Hospital Huddinge, Stockholm, Sweden
[6]Department of Neurology, Rehabilitation Medicine, Memory Disorders, and Geriatrics, Skåne University Hospital Lund, Lund, Sweden
[7]Department of Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
[8]Department of Hematology, Linköping University Hospital, Linkoping, Sweden
[9]Institute of Medicine, University of Gothenburg, Gothenburg, Sweden
[10]Department of Hematology and Coagulation, Sahlgrenska Sjukhuset, Gothenburg, Sweden
[11]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[12]Department of Neurology, Karolinska University Hospital, Stockholm, Sweden
[13]Department of Hematology, Oncology & Radiophysics, Skåne University Hospital Lund, Lund, Sweden
[14]Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy, Gothenburg, Sweden
[15]Department of Neurology, Linköping University, Linköping, Sweden
[16]Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden
[17]Department of Clinical Sciences, Karolinska Institutet Institutionen för kliniska vetenskaper Danderyds sjukhus, Stockholm, Sweden
[18]Department of Neurology, Karolinska Institutet Institutionen för kliniska vetenskaper Danderyds sjukhus, Stockholm, Sweden

Correction notice This article has been corrected since it first published online. The authors state in their "Methods" section that "The Cy-ATG protocol was given during 5 days and included cyclophosphamide 200 mg/kg, rATG 10 mg/kg and 5000 mg methylprednisolone, including tapering after stem cell infusion, and hyperhydration and uromitexan to prevent haemorrhagic cystitis." The stated dosage of rATG 10 mg/kg is incorrect and should instead be 6 mg/kg, so that the corrected sentence reads "The Cy-ATG protocol was given during 5 days and included cyclophosphamide 200 mg/kg, rATG 6 mg/kg and 5000 mg methylprednisolone, including tapering after stem cell infusion, and hyperhydration and uromitexan to prevent haemorrhagic cystitis." The 10 mg/kg dosage stated for BEAM-ATG regimen in the same section remains correct.

Acknowledgements This study was presented as an abstract and oral presentation at the European Society for Blood and Marrow Transplantation 49th Annual Meeting, Paris, 23-26 April 2023.

Contributors JB is the guarantor of the study and accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. The study was conceptualised by JB. The methodology was planned by JB, KC, PL, HC and TS. Funding was acquired by JB and TS. Data were collected by JB, TS, PL, SE, EA, SL, EI, JF, JL, NA, JM, NL and AD. Data were validated by JB, TS, EI, JF, JL, NA, JM, NL and FP. Data curation and data analysis were performed by JB, CZ and TS. Supervision and coordination of the study were performed by JB and TS. Visualisations were made by JB, CZ and TS. The original draft of the manuscript was written by JB and TS. Reviewing and editing were performed by all coauthors.

Funding The study was funded by the Center for Clinical Research Dalarna with grant numbers CKFUU-936620 and CKFUU-976388, the Marianne and Marcus Wallenberg Foundation, Region Stockholm, the Swedish Research Council, the Swedish Society for Medical Research (2020-02700), the Swedish Society of Medicine and Uppsala-Örebro Regional Research Council (RFR-967904).

Disclaimer None of the funders had any role in the study design, the collection, analysis, or interpretation of data, the writing of the manuscript, or the decision on where to publish the article.

Competing interests EI has received speakers fee from Merck and honoraria from advisory boards for Sanofi-Aventis, Biogen and Merck. FP previously received research grants from Merck KGaA, Janssen and UCB outside this study. FP has received payment for expert testimony from Novartis. FP has participated in Data Monitoring Committee for clinical trials from Chugai, Lundbeck and Roche. JM has received lecture honorarium from Merck. NL has received honoraria from Sanofi. All other individual authors declare that there is no conflict of interest.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and the Ethical Review Authority in Sweden granted approval for the study on 14 April 2021 (ref: 2021-01530). As this is a retrospective observational study, a specific consent for this study was not obtained, but every patient involved in the study gave their written consent, permitting their data to be reported to the European Society for Blood and Marrow Transplantation (EBMT) registry. Although participation in national quality registries like SMSreg is obligatory for Swedish citizens receiving publicly funded healthcare, patients retain the option to opt out of research conducted using data from these registers.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Deidentified individual participant data supporting the findings presented in this article, including text, tables, figures and appendices, will be accessible alongside the study protocol for a period of 5 years, starting 9 months after the article's publication. Researchers with a sound proposal can request access to this data by contacting joachim.burman@uu.se. To obtain access, those requesting data will need to sign a data access agreement.

ORCID iDs
Thomas Silfverberg http://orcid.org/0000-0002-3829-285X
Jan Lycke http://orcid.org/0000-0002-7891-8466
Andreas Tolf http://orcid.org/0000-0002-0580-8821

REFERENCES

1 Noseworthy JH, Lucchinetti C, Rodriguez M, *et al*. Multiple sclerosis. *N Engl J Med* 2000;343:938–52.
2 Weiner HL. Multiple sclerosis is an inflammatory T-cell-mediated autoimmune disease. *Arch Neurol* 2004;61:1613–5.
3 Compston A, Coles A. Multiple sclerosis. *Lancet* 2008;372:1502–17.
4 Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* 2015;15:545–58.
5 Rovaris M, Confavreux C, Furlan R, *et al*. Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurol* 2006;5:343–54.
6 Lublin FD, Häring DA, Ganjgahi H, *et al*. How patients with multiple sclerosis acquire disability. *Brain* 2022;145:3147–61.
7 Smith AL, Cohen JA, Hua LH. Therapeutic targets for multiple sclerosis: current treatment goals and future directions. *Neurotherapeutics* 2017;14:952–60.
8 Nixon R, Bergvall N, Tomic D, *et al*. No evidence of disease activity: indirect comparisons of oral therapies for the treatment of relapsing-remitting multiple sclerosis. *Adv Ther* 2014;31:1134–54.
9 Muraro PA, Martin R, Mancardi GL, *et al*. Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis. *Nat Rev Neurol* 2017;13:391–405.
10 Sormani MP, Muraro PA, Saccardi R, *et al*. NEDA status in highly active MS can be more easily obtained with autologous hematopoietic stem cell transplantation than other drugs. *Mult Scler* 2017;23:201–4.
11 Muraro PA, Pasquini M, Atkins HL, *et al*. Long-term outcomes after autologous hematopoietic stem cell transplantation for multiple sclerosis. *JAMA Neurol* 2017;74:459–69.
12 Burt RK, Balabanov R, Burman J, *et al*. Effect of nonmyeloablative hematopoietic stem cell transplantation vs continued disease-modifying therapy on disease progression in patients with relapsing-remitting multiple sclerosis: a randomized clinical trial. *JAMA* 2019;321:165–74.
13 Zhukovsky C, Sandgren S, Silfverberg T, *et al*. Autologous haematopoietic stem cell transplantation compared with alemtuzumab for relapsing-remitting multiple sclerosis: an observational study. *J Neurol Neurosurg Psychiatry* 2021;92:189–94.
14 Sharrack B, Saccardi R, Alexander T, *et al*. Autologous haematopoietic stem cell transplantation and other cellular therapy in multiple sclerosis and immune-mediated neurological diseases: updated guidelines and recommendations from the EBMT autoimmune diseases working party (ADWP) and the joint accreditation committee of EBMT and ISCT (JACIE). *Bone Marrow Transplant* 2020;55:283–306.
15 Burt RK, Han X, Quigley K, *et al*. Real-world application of autologous hematopoietic stem cell transplantation in 507 patients with multiple sclerosis. *J Neurol* 2022;269:2513–26.
16 Das J, Sharrack B, Snowden JA. Correction to: autologous haematopoietic stem cell transplantation in multiple sclerosis: a review of current literature and future directions for transplant haematologists and oncologists. *Curr Hematol Malig Rep* 2019;14:136.
17 Hillert J, Stawiarz L. The Swedish MS registry – clinical support tool and scientific resource. *Acta Neurol Scand* 2015;132:11–9.
18 National Institutes of Health, National Cancer Institute. Common terminology criteria for adverse events (CTCAE) V5.0: US Department of health and human services. 2017. Available: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf
19 Thompson AJ, Banwell BL, Barkhof F, *et al*. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* 2018;17:162–73.
20 Lublin FD, Reingold SC, Cohen JA, *et al*. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology* 2014;83:278–86.
21 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444–52.
22 Coles AJ, Twyman CL, Arnold DL, *et al*. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* 2012;380:1829–39.
23 Cohen JA, Coles AJ, Arnold DL, *et al*. Alemtuzumab versus interferon beta 1A as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* 2012;380:1819–28.
24 Giovannoni G, Cook S, Rammohan K, *et al*. Sustained disease-activity-free status in patients with relapsing-remitting multiple sclerosis treated with cladribine tablets in the CLARITY study: a post-hoc and subgroup analysis. *Lancet Neurol* 2011;10:329–37.
25 Boffa G, Massacesi L, Inglese M, *et al*. Long-term clinical outcomes of hematopoietic stem cell transplantation in multiple sclerosis. *Neurology* 2021.
26 Burman J, Iacobaeus E, Svenningsson A, *et al*. Autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: the Swedish experience. *J Neurol Neurosurg Psychiatry* 2014;85:1116–21.
27 Nash RA, Dansey R, Storek J, *et al*. Epstein-Barr virus-associated posttransplantation lymphoproliferative disorder after high-dose immunosuppressive therapy and Autologous Cd34-selected hematopoietic stem cell transplantation for severe autoimmune diseases. *Biol Blood Marrow Transplant* 2003;9:583–91.
28 Mehra V, Rhone E, Widya S, *et al*. Epstein-Barr virus and monoclonal gammopathy of clinical significance in autologous stem cell transplantation for multiple sclerosis. *Clin Infect Dis* 2019;69:1757–63.
29 Alping P, Burman J, Lycke J, *et al*. Safety of alemtuzumab and autologous hematopoietic stem cell transplantation compared to noninduction therapies for multiple sclerosis. *Neurology* 2021;96:e1574–84.

Supplement 1 – Data points

Baseline data

- birth date
- gender
- transplantation clinic
- clinical course (RRMS, SPMS or PPMS)
- disease onset
- diagnosis date
- previous DMT(s)
- number and dates of relapses prior to aHSCT
- results and dates of MRI investigations prior to aHSCT
- results and dates of EDSS scoring prior to aHSCT
- wash-out period prior to aHSCT
- comorbidities

aHSCT data

- date of mobilisation
- date of stem cell harvest
- date of admission and discharge for aHSCT
- date of aHSCT
- drugs used in mobilisation and conditioning including dosage
- G-CSF given post aHSCT
- days to engraftment
- intensive care (yes/no) including reason
- body weight at admission and discharge
- plasma-albumin at admission and discharge
- specific days with fever
- clinical diagnosis of infection
- reactivation of CMV, EBV or other herpes viruses
- bacteremia (species)
- culture negative fever
- hemorrhagic cystitis (yes/no)
- days with intravenous antibiotics including substance
- fungal infection (culture or clinical diagnosis)
- serious adverse events grade three or higher according to the CTCAE.

Follow-up data

- number and dates of relapses after aHSCT,
- results and dates of MRI investigations after aHSCT
- results and dates of EDSS and MSIS-29 scoring after aHSCT
- serious adverse events grade three or higher according to CTCAE until day +100
- subsequent DMT
- date of last follow-up.

Supplement 2 - Definitions

Annualised relapse rate
The number of relapses occurring during a time period divided by the number of years in that time period. E.g. 5 relapses occurring in a time period of 2·5 years equals an ARR of 2 (5/2·5=2).

Confirmed disability improvement
If baseline EDSS ≤ 5·5: A decrease in EDSS score with at least 1 point from baseline that is sustained between two follow-up visits separated in time by no less than six months.
If baseline EDSS ≥ 6: A decrease in EDSS score with at least 0·5 points from baseline that is sustained between two follow-up visits separated in time by no less than six months.

Clinically isolated syndrome
According to the Lublin *et al* criteria from 2014.[25]

Confirmed disability worsening
If baseline EDSS ≤ 5: An increase in EDSS score with at least 1 point from baseline that is sustained between two follow-up visits separated in time by no less than six months.
If baseline EDSS ≥ 5·5: An increase in EDSS score with at least 0·5 points from baseline that is sustained between two follow-up visits separated in time by no less than six months.

Clinical relapse
A period of acute worsening of neurological function lasting ≥ 24 hours not attributable to an external cause such as increased body temperature or acute infection.

EDSS
The Kurtske Expanded Disability Status Scale (EDSS) is a method of quantifying disability in multiple sclerosis. The EDSS is a composite of disability in eight functional systems.[26]

Engraftment
ANC 0.5 or higher and TPK >20 and rising, without transfusion of thrombocytes.

MRI event
The appearance of any T2 lesion > 3 mm or gadolinium enhancing lesion in the brain or spinal cord not present on the baseline scan.

Multiple sclerosis, diagnosis
Diagnosis according to the revised McDonald Criteria from 2017.[24]

No evidence of disease activity (NEDA)
The absence of clinical relapses in addition to absence of confirmed disability worsening and MRI events.

Primary Progressive multiple sclerosis (PPMS)
According to the Lublin *et al* criteria from 2014.[25]

Progressive disease
According to the Lublin *et al* criteria from 2014.[25]

Relapsing/remitting multiple sclerosis
According to the Lublin *et al* criteria from 2014.[25]

Treatment related mortality (TRM)
Death due to any transplantation-related cause other than disease progression.

Supplement 3 – Study Protocol

UPPSALA UNIVERSITET

CKF CENTRUM för KLINISK FORSKNING DALARNA

RESEARCH PROTOCOL

Hematopoietic stem cell transplantation for treatment of multiple sclerosis in Sweden

– a register-based retrospective observational study

Project Manager
Thomas Silfverberg, MD. Center for Clinical Research Dalarna/Department of Medical Sciences, Uppsala University

Principal investigator and scientific supervisor
Joachim Burman, MD, PhD. Ass Prof. of Neurology at the Department of Medical Sciences, Uppsala University

Entity responsible for the research
Department of Medical Sciences, Uppsala University, Region Uppsala

Date 2021-03-08

Title

Hematopoietic stem cell transplantation for treatment of multiple sclerosis in Sweden - a register-based retrospective observational study

Introduction

Multiple sclerosis (MS) is a debilitating disease affecting mainly young individuals, with a peak incidence around 30 years of age. In Sweden 20 000 persons suffer from MS and worldwide an estimated 2.5 million. MS is considered the most common cause of neurological disability in young adults.[1] Untreated, it often leads to severe disability and premature death.[2-5] MS is considered an inflammatory and autoimmune disease of the central nervous system (CNS).

The cause of MS is unknown, but epidemiologic and genetic studies indicate that MS is triggered in genetically susceptible individuals following exposure to environmental factors. This eventually leads to loss of tolerance and activation of myelin-specific T cells. These auto-reactive immune cells will attack oligodendrocytes, resulting in myelin destruction, secondary axonal damage and neuronal loss. The course of MS is heterogeneous and usually involves an early, predominantly inflammatory disease phase of relapsing-remitting MS (RRMS). After a variable period, RRMS evolves into a progressively degenerative stage (secondary progressive MS, SPMS) with neurodegeneration, CNS atrophy and accumulation of disability.

Current treatments for MS aim to reduce inflammation in the CNS, but have several drawbacks. They have to be administered repeatedly, have potentially severe side effects (including death) and cannot suppress disease activity entirely. Moreover, they mainly benefit patients with RRMS and have very little effect once the neurodegenerative process has started. Approved treatments are expensive and constitute a heavy burden for the health care system.

Hematopoietic stem cell transplantation (HSCT) has been in use for treatment of malignancies since the 1950's.[6,7] In 1990, Edward Donnall Thomas was awarded the Nobel Prize in Physiology or Medicine for the development of HSCT as a treatment for leukemia. The first transplantations were allogenic transplantations with graft from a donor. Autologous hematopoietic stem cell transplantation (AHSCT) was developed to restore remission or chronic phase in patients with advanced leukemia without a sibling donor. The breakthrough in its use came after finding circulating stem cells in peripheral blood in patients with chronic myeloid leukemia,[8,9] which enabled a simplified method of harvesting hematopoietic stem cells. To harvest hematopoietic stem cells and then reintroducing them to the patients allowed for higher doses of chemotherapy to be administered, thus creating a deeper disease response. Since the 1980s AHSCT has become standard treatment in many lymphoid malignancies and some childhood solid tumors. One side effect of high dose chemotherapy and AHSCT is that the procedure resets the immune system leading to loss of acquired immunity including (most) memory cells.

In recent years, AHSCT has been utilized for treatment of autoimmune diseases such as MS. AHSCT aims to treat the disease to such a depth that remission is obtained and to remain in remission for as long as possible. For autoimmune diseases, the idea is to diminish the patients' immune system by depleting or eliminating mature lymphoid and myeloid cells with specific phenotypes in the adaptive immune system as well as changing the immunological environment. The procedure starts with harvesting the patients' hematopoietic stem cells by apheresis after mobilizing them from the bone marrow to the peripheral blood using a combination of the cytotoxic drug cyclophosphamide and granulocyte-colony-stimulating factor (G-CSF). The hematopoietic stem cells are identified by

expression of the surface molecule CD34 using immunophenotyping. The cells are then cryopreserved. 3-4 weeks later the patient is treated with high-dose chemotherapy referred to as *conditioning* that result in bone marrow aplasia. When the cytotoxic drugs has been eliminated from the body, the autologous $CD34^+$ cells are reinfused to inhabit the empty bone marrow. In MS, the two most commonly used conditioning regimens are BEAM-ATG and Cy-ATG (see intervention below). The purpose of anti-thymocyte globulin (ATG) is to eliminate T-cells present in the graft. The patient becomes vulnerable for infections during the neutropenic phase following conditioning until engraftment, which normally occurs after 10-14 days. Consequently, the immune system is reconstituted with permanent and beneficial changes in the immune repertoire.

Current data suggest that the procedure is superior at maintaining a disease-free state in comparison to standard disease modifying drugs.[10] At the same time, the safety profile seems to be acceptable, with a low rate of serious adverse events.[10] As a first, the Swedish Board of Health and Welfare approved AHSCT for treatment of RRMS on the national level in 2016[11] and HSCT is now available as a therapeutic option in routine health care in Sweden.

Survey of the field

The first-generation trials investigating AHSCT for MS used heavy myeloablative transplantation regimens, which were associated with high treatment-related mortality. Additionally, the procedure was reserved for patients with treatment resistant progressive forms of MS. It soon became evident that this therapy was not able to stop worsening in patients with progressive disease.[12] In the following years, it became clear that HSCT could be a very effective treatment for RRMS.[13,14] Development of less toxic conditioning regimens and better patient selection has led to a substantial decrease in treatment related mortality, which today has been estimated at 0.3%.[15]

In terms of efficacy, AHSCT compares favorably to conventional treatment. About two-thirds of treated patients reach complete remission with no evidence of disease activity (NEDA) 4-5 years after the procedure.[16] With first line therapy, such as interferon beta, only 7.9 % had NEDA at seven years and even with strong immunosuppression, such as natalizumab or alemtuzumab, only 32-39 % of patients exhibited NEDA after a relatively short follow-up time of two years.[17]

The MIST-trial is the only randomized controlled trial investigating AHSCT vs disease-modifying treatment (DMT), such as natalizumab, dimethyl fumarate, fingolimod, glatiramer acetate, interferon beta-1a, mitoxantrone, teriflunomide in MS-patients to this date. Interim results show a median time to progression of 24 months in the DMT arm compared to 1.92% of the patients in the AHSCT arm showing progression at 24 months.[10] Although the procedure is expensive, it is a one-time treatment. When compared with conventional therapy in a cost-effectiveness analysis, the outcome is highly in favor of AHSCT.[18]

AHSCT has been shown to be more effective for treating RRMS than progressive MS in multiple trials . The current European Society for Blood and Marrow Transplantation (EBMT), the Autoimmune Diseases Working Party (ADWP) and the Joint Accreditation Committee of the International Society for Cellular Therapy (ISCT) points out that the use of AHSCT for primary progressive MS (PPMS) and secondary progressive MS (SPMS) is less effective compared to RRMS. According to the guidelines, AHSCT can be considered for progressive MS only if inflammatory activity is still evident but the benefit is considered very limited, especially for primary progressive MS (PPMS).[19,20]

There has not been any randomized trials comparing conditioning regimens in MS. High intensity regimens such as busulfan + cyclophosphamide + ATG are associated with high risk for considerable toxicity including sinusoid obstruction syndrome.[21] The two most commonly used regimens are the

intermediate intensity BEAM-ATG or Cy-ATG described previously. The current EBMT guidelines recommends both protocols.[20] A Brazilian retrospective study from 2009 compared the conditioning regimens BEAM + horse ATG (hATG) with cyclophosphamide + rabbit ATG (rATG) in 41 patients of which 80% had progressive MS. The study showed a mortality rate of 3 out of 21 treated patients in the BEAM-hATG cohort for patients treated between 2001 and 2004. The high mortality justified a change in the protocol to cyclophosphamide-rATG from 2004 to 2006 and there was no further treatment related mortality until the end of the follow up in late 2007. The overall adverse events where more common in the BEAM-hATG group.[22]

Research question

What is the effectiveness and safety of autologous hematopoietic stem cell transplantation as treatment for relapsing-remitting multiple sclerosis?

Project Outline

This is an observational cohort study with retrospective analysis of prospectively collected data. The study cohort is constituted of all patients with relapsing-remitting multiple sclerosis treated with AHSCT in Sweden from 2004 when the first AHSCT was performed until 31 December 2019. The study aims to describe the effectiveness, safety and patient reported outcomes of AHSCT for MS through real world data. Treatment-related mortality will be analyzed from the start of mobilization until the end of the study. Other adverse events will be described until 3 months post-transplantation. A statistical subgroup comparison of efficacy and safety between the conditioning regimens BEAM-ATG and Cy-ATG will be included within the study.

Methodology

Study population

All individuals with a diagnosis of MS, who was treated with AHSCT in Sweden until 31 December 2019 can be included in this study. Patients will be identified through the local EBMT-registers and the Swedish MS register (SMSreg).

Inclusion criteria

- Diagnosis of multiple sclerosis according to the revised McDonald criteria 2017.[23]
- Autologous hematopoietic stem cell transplantation performed for treating multiple sclerosis at a Swedish transplantation center until 31 December 2019.

Exclusion criteria

- Diagnosis of primary progressive MS or secondary progressive MS according to Lublin *et al*[24] at the time of transplantation.
- Patient not accepted reporting of data to the EBMT register.
- Not fulfilling requirements of the minimal dataset, se below.

Definition of minimal dataset

- Data on disease course of multiple sclerosis at the time of transplantation.
- Transplantation and the following in-patient care performed in Sweden.

- Date of transplantation.
- Data on drugs used in conditioning.
- At least one follow-up visit performed in Sweden* including data on:
    - Clinical assessment
    - The Kurtzke Expanded Disability Status Scores (EDSS)

*Unless early death before first follow-up visit.

Additional note: For a patient to be included in the analysis of treatment effectiveness data on MRI evaluation is needed at least once during follow-up.

Intervention

The therapeutic intervention of AHSCT consists of four parts: the mobilization of hematopoietic stem cells (HSC), the harvest of HSCs, the ablation (conditioning) of the immune system and the reinfusion of autologous HSCs.

In Sweden a combination of cyclophosphamide (2 g/m^2) and G-CSF is used to mobilize the HSCs. The HSCs are identified by immunophenotyping, and cells that express CD34 on their surface are considered to be HSCs. A minimum of 2×10^6 $CD34^+$ cells/kg is harvested and then cryopreserved. No in vitro manipulation is done to the stem cells.

After a few weeks, conditioning is performed with high-dose chemotherapy. The two dominating protocols for conditioning in Sweden are BEAM-ATG and Cy-ATG. The BEAM-ATG protocol consists of carmustine (BCNU) 300 mg/m^2, etoposide 800 mg/m^2, cytarabine arabinoside (ARA-C) 800 mg/m^2 and melphalan 140 mg/m^2 + rATG or hATG. The Cy-ATG protocol include cyclophosphamide 200 mg/kg + rATG/hATG with 1000 mg Metylprednisolone given day -5 to -1 and Mesna given repeatedly to avoid hemorrhagic cystitis. High-dose steroids and hyperhydration is used in most Swedish centers when giving high-dose cyclophosphamide conditioning.

After a minimum of 24 hours after the last administration of chemotherapy have passed, the reinfusion of autologous $CD34^+$ cells is made. Prophylaxis for bacterial infection with the quinolone ciprofloxacin is given during the neutropenic phase. Additional antibiotics may be administrated as needed. Filtered and radiated blood products are used until their lymphocytes exceeds $1{,}0 \times 10^9$/L. Prophylaxis for herpes and pneumocystis is given for a minimum of 3 months. Prophylaxis for hepatitis B-reactivation is given to patients who has tested positive for HBs-ag (hepatitis B surface antigen) and/or anti-HBc (antibodies against hepatitis B core proteins).

Source data verification

To verify the accuracy and completeness of data in EBMT and SMSreg, a verification of source data *vis-à-vis* the medical records will be made by local co-principal investigators.

Data collection

Baseline data on birth date, sex, date of onset, date of diagnosis, disease course, previous treatments, etc. will be collected from the SMSreg (see data points below).

Data on the circumstances of the intervention will be collected from local repositories of the EBMT and supplemented by reviews of the medical records. This includes data points such as doses and

names of drugs used for mobilization and conditioning, dates for administration of these drugs, date of hematopoietic stem cell transplantation, date of hematological milestones, occurrence and grading of adverse events during the first three months after the intervention.

Data on clinical outcome after the first three months of the intervention will be collected from SMSreg. Apart from mortality, long-term complications including autoimmune disease will not be analyzed in this study, since that has already been published in a different study.[25] Mortality at any point after AHSCT will be analyzed through the medical records to determine if it was treatment-related. Data on treatment related mortality will be analyzed until the time of data collection.

Data management

All data collected will be stored in a deidentified data set where name and social security number have been erased and given a coded study number (pseudonym). The data set will be stored at a secure server belonging to the entity responsible for the research. Each patient will only be identified using a separately and securely stored code key only available to the main researchers. All storage, correspondence and analysis with the pseudonymized data set will adhere to current European General Data Protection Regulation (GDPR) guidelines. The code key will be stored until the study is published, not exceeding two years after the end of the data collection, to allow for any relevant data additions and then be destroyed. The data set will be stored for 15 years and then be destroyed.

Ethical Review Authority approval

Approval from the Swedish Ethical Review Authority is pending.

Definitions

Annualized relapse rate (ARR)
The number of relapses occurring during a time period divided by the number of years in that time period. E.g. 5 relapses occurring in a time period of 2.5 years equals an ARR of 2 (5/2.5=2).

Clinical improvement
Baseline EDSS ≤ 5.5
A decrease in EDSS score with at least 1 point from baseline that is sustained between two follow-up visits separated in time by no less than six months.

Baseline EDSS ≥ 6
A decrease in EDSS score with at least 0.5 points from baseline that is sustained between two follow-up visits separated in time by no less than six months.

Clinically isolated syndrome (CIS)
According to the Lublin *et al* criteria from 2014.[24]

Clinical progression
Baseline EDSS ≤ 5
An increase in EDSS score with at least 1 point from baseline that is sustained between two follow-up visits separated in time by no less than six months.

Baseline EDSS ≥ 5.5
An increase in EDSS score with at least 0.5 points from baseline that is sustained between two follow-up visits separated in time by no less than six months.

EDSS
The Kurtzke Expanded Disability Status Scale (EDSS) is a method of quantifying disability in multiple sclerosis. The EDSS is a composite of disability in eight functional systems.

FSMC
Fatigue Scale for Motor and Cognitive Functions (FSMC) is a 20-item scale for evaluating MS-related cognitive and motor fatigue.[26]

MRI progression
The appearance of any T2 lesion > 3 mm or gadolinium enhancing lesion in the brain or spinal cord not present on the baseline scan.

MSIS-29
The Multiple Sclerosis Impact Scale (MSIS-29) is a measure of the physical and psychological impact of MS from the patient's perspective.[27]

Multiple sclerosis, diagnosis
Diagnosis according to the revised McDonald Criteria from 2017.[23]

No evidence of disease activity (NEDA)
'No evidence of disease activity' is defined as absence of relapses in addition to absence of clinical progression and MRI progression.

Primary Progressive multiple sclerosis (PPMS)
According to the Lublin *et al* criteria from 2014.[24]

Progressive disease
According to the Lublin *et al* criteria from 2014.[24]

Relapse
A period of acute worsening of neurological function lasting ≥ 24 hours not attributable to an external cause such as increased body temperature or acute infection.

Relapsing/remitting multiple sclerosis
According to the Lublin *et al* criteria from 2014.[24]

Treatment related mortality (TRM)
TRM is defined as death due to any transplantation-related cause other than disease progression.

SDMT
Symbol Digit Modalities Test (SDMT) is a test of cognitive function in MS-patients.[28]

Data points

Baseline data
Birth date, clinical course (RRMS, SPMS or PPMS), disease onset, diagnosis date, previous disease modifying drug (DMD) treatments, number and dates of relapses prior to HSCT, results and dates of MRI investigations prior to HSCT, results and dates of EDSS scoring prior to HSCT. Wash-out period prior to AHSCT.

HSCT data
Transplant date, type of mobilization, conditioning, dosage, days to engraftment, days to discharge, intensive care (yes/no), reactivation of CMV, EBV or other herpes viruses, bacteremia (species), culture negative fever, hemorrhagic cystitis (yes/no) and other serious adverse events grade three or higher according to the NIH common terminology criteria for adverse events (CTCAE).

Follow-up data
Number and dates of relapses after HSCT, results and dates of MRI investigations after HSCT, results and dates of EDSS, FSMC, MSIS-29 and SDMT scoring after HSCT, serious adverse events grade three or higher according to CTCAE until day +100, subsequent DMD treatment.

Endpoints

Recently a survey of Swedish MS patients was made to investigate which aspects of DMD treatment were most important to them. Two of the outcomes that were ranked highest were long-term disability and risk of serious adverse events. The endpoints of this study were deliberately chosen to assess these outcomes.

Primary endpoints
- The Kaplan-Meier estimate of NEDA at five years.
- Treatment related mortality

Secondary endpoints
- The Kaplan-Meier estimate of NEDA at three and ten years.
- The Kaplan-Meier estimate of MRI event free survival at three, five and ten years.
- The Kaplan-Meier estimate of Relapse free survival at three, five and ten years.
- The Kaplan-Meier estimate of Progression free survival at three, five and ten years.
- The annualized relapse rate (ARR) after AHSCT.
- The proportion of patients with clinical improvement.
- The EDSS change between baseline and follow-up at one, two and three years respectively.
- The frequency and grade of serious adverse events within 100 days

Explorative endpoints
- Changes in cognitive function, measured by SDMT at one, two and three years
- Changes in quality of life, measured by MSIS-29 at one, two and three years.
- Changes in MS-related fatigue, measured by FSMC at one, two and three years.

Data analysis

The endpoints will be analysed and described for the whole study cohort. Subgroup analysis comparing BEAM-ATG and Cy-ATG will be conducted to statistically analyse if there are any significant differences in between the two conditioning regimens in terms of efficacy and safety. The explorative endpoints will be analysed if the quality of the data is good enough, as the coverage of these data points in the SMSreg are not known.

Aggregated data will be reported as frequencies for categorical variables and medians with interquartile intervals for continuous variables. The Wilcoxon signed rank test will be used to establish statistical significance between two time points and the Friedman test will be used to establish statistical significance between three or more time points. Survival at different time points will be estimated with Kaplan–Meier survival curves and statistical significance will be established with the log-rank test. A two-tailed p value of < 0.05 will be considered to be statistically significant.

Reporting of data

Reporting of the data will adhere to the STROBE guidelines. Open access will be granted to the reported data.

Work plan

Q3 2021 Start of collection of data

Q1 2021 Completion of collection of data.

Q2 2022 Start of data analysis

Q3 2022 Completion of data analysis.

Q4 2022 Final report

Importance

HSCT is a potentially curative treatment of RRMS and leads to long-term remission in a majority of patients. It has so far not been possible to estimate effectiveness and safety on a large scale, due to its limited use and the heterogeneity of previous reports. In the last years, AHSCT has seen increasing use in Sweden and the Swedish Board of Health and Welfare recently endorsed it. This study of real world data that aims to provide a general picture of the effectiveness and safety of AHSCT for RRMS. The data analysis will include some outcome measures that were identified as of particular importance by stakeholders, such as fatigue. As a result of the excellent coverage of the national SMSreg a minimum of patients will be lost to identification and follow-up, thus providing strength to the report. As this study describes a treatment option, the research question concerns the patients directly and the hypothesis is that the treatment is relatively safe, economically advantageous and effective for treating RRMS. The proposed study will be the largest study of HSCT for RRMS so far, with a size that is equal to all of the previous reports combined. We will be able to estimate the level of serious adverse events on a level of detail that has not been possible before. Thus, the results of this study will be able to provide new and important information, which will enable health care providers and patients to make a better-informed treatment choice. The study will also provide deeper insight in the comparison between the currently two most widely used conditioning regimens used for MS.

Main participants

Project Manager
Thomas Silfverberg, MD (Center for Clinical Research Dalarna/Department of Medical Sciences, Uppsala University)

Principal investigator and scientific supervisor
Joachim Burman, MD, PhD. Ass Prof. of Neurology at the Department of Neuroscience, Uppsala University

References

1. Compston A, Coles A. Multiple sclerosis. *The Lancet.* 2002;359(9313):1221-1231.
2. Brønnum-Hansen H K-HN, Stenager E. Trends in survival and cause of death in Danish patients with multiple sclerosis. *Brain.* 2004;127(Pt 4):844-850.
3. Daumer M, Griffith LM, Meister W, Nash RA, Wolinsky JS. Survival, and time to an advanced disease state or progression, of untreated patients with moderately severe multiple sclerosis in a multicenter observational database: relevance for design of a clinical trial for high dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation. *Multiple Sclerosis.* 2006;12(2):174-179.
4. Hader W. Disability and survival of multiple sclerosis in Saskatoon, Saskatchewan. *Can J Neurol Sci.* 2010;37(1):28-35.

5. Skoog B, Runmarker B, Winblad S, Ekholm S, Andersen O. A representative cohort of patients with non-progressive multiple sclerosis at the age of normal life expectancy. *Brain : a journal of neurology.* 2012;135(Pt 3):900-911.
6. W. MF, Granville NB, Dameshek W. Autologous bone marrow infusion as an adjunct in therapy of malignant disease. *Blood.* 1959;14(5):503-521.
7. Thomas ED, Lochte HL, Jr., Cannon JH, Sahler OD, Ferrebee JW. Supralethal whole body irradiation and isologous marrow transplantation in man. *The Journal of clinical investigation.* 1959;38:1709-1716.
8. Philip T, Armitage JO, Spitzer G, et al. High-dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate-grade or high-grade non-Hodgkin's lymphoma. *N Engl J Med.* 1987;316(24):1493-1498.
9. Reiffers J, Trouette R, Marit G, et al. Autologous blood stem cell transplantation for chronic granulocytic leukaemia in transformation: a report of 47 cases. *Br J Haematol.* 1991;77(3):339-345.
10. Burt RK, Balabanov R, Burman J, et al. Effect of Nonmyeloablative Hematopoietic Stem Cell Transplantation vs Continued Disease-Modifying Therapy on Disease Progression in Patients With Relapsing-Remitting Multiple Sclerosis: A Randomized Clinical Trial. *JAMA.* 2019;321(2):165-174.
11. Vård vid multipel skleros och Parkinsons sjukdom. In: Welfare SBoHa, ed2016:26-30.
12. Burt RK, Cohen BA, Russell E, et al. Hematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based conditioning regimen to prevent disease progression in patients with high disability scores. *Blood.* 2003;102(7):2373-2378.
13. Fagius J, Lundgren J, Oberg G. Early highly aggressive MS successfully treated by hematopoietic stem cell transplantation. *Mult Scler.* 2009;15(2):229-237.
14. Burt R, Loh Y, Cohen B, et al. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. *Lancet Neurol.* 2009;8(3):244-253.
15. Sormani MP, Muraro PA, Schiavetti I, et al. Autologous hematopoietic stem cell transplantation in multiple sclerosis: A meta-analysis. *Neurology.* 2017;88(22):2115-2122.
16. Sormani MP, Muraro PA, Saccardi R, Mancardi G. NEDA status in highly active MS can be more easily obtained with autologous hematopoietic stem cell transplantation than other drugs. *Mult Scler.* 2017;23(2):201-204.
17. Rotstein DL, Healy BC, Malik MT, Chitnis T, Weiner HL. Evaluation of No Evidence of Disease Activity in a 7-Year Longitudinal Multiple Sclerosis Cohort. *JAMA neurology.* 2014.
18. Tappenden P, Saccardi R, Confavreux C, et al. Autologous haematopoietic stem cell transplantation for secondary progressive multiple sclerosis: an exploratory cost-effectiveness analysis. *Bone Marrow Transplant.* 2010;45(6):1014-1021.
19. Muraro PA, Pasquini M, Atkins HL, et al. Long-term Outcomes After Autologous Hematopoietic Stem Cell Transplantation for Multiple Sclerosis. *JAMA neurology.* 2017;74(4):459-469.
20. Sharrack B, Saccardi R, Alexander T, et al. Autologous haematopoietic stem cell transplantation and other cellular therapy in multiple sclerosis and immune-mediated neurological diseases: updated guidelines and recommendations from the EBMT Autoimmune Diseases Working Party (ADWP) and the Joint Accreditation Committee of EBMT and ISCT (JACIE). *Bone Marrow Transplant.* 2020;55(2):283-306.
21. Atkins HL, Bowman M, Allan D, et al. Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. *Lancet.* 2016;388(10044):576-585.
22. Hamerschlak N, Rodrigues M, Moraes DA, et al. Brazilian experience with two conditioning regimens in patients with multiple sclerosis: BEAM/horse ATG and CY/rabbit ATG. *Bone Marrow Transplant.* 2010;45(2):239-248.
23. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* 2018;17(2):162-173.
24. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: The 2013 revisions. *Neurology.* 2014.
25. Alping P, Burman J, Lycke J, Frisell T, Piehl F. Safety Outcomes after Alemtuzumab and AHSCT in Patients with Multiple Sclerosis: A Cohort Study. *Neurology.* 2021.
26. Penner IK, Raselli C, Stocklin M, Opwis K, Kappos L, Calabrese P. The Fatigue Scale for Motor and Cognitive Functions (FSMC): validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler.* 2009;15(12):1509-1517.
27. Hobart J, Lamping D, Fitzpatrick R, Riazi A, Thompson A. The Multiple Sclerosis Impact Scale (MSIS-29): a new patient-based outcome measure. *Brain.* 2001;124(Pt 5):962-973.
28. Benedict RH, DeLuca J, Phillips G, et al. Validity of the Symbol Digit Modalities Test as a cognition performance outcome measure for multiple sclerosis. *Mult Scler.* 2017;23(5):721-733.