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Original research

Factors associated with outcomes following autologous haematopoietic stem cell transplantation for multiple sclerosis

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ABSTRACT

Background Autologous haematopoietic stem cell transplantation (AHSCT) has emerged as a highly effective treatment for relapsing-remitting multiple sclerosis (RRMS), though patient selection remains challenging. The degree to which disease-modifying therapies (DMTs) and procedure-related complications affect treatment outcomes is unclear. The objective of this study was to investigate the factors that might influence outcomes following AHSCT.

Methods Data from the multicentre, retrospective cohort study Haematopoietic Stem Cell Transplantation for Treatment of Multiple Sclerosis in Sweden (AutoMS-Swe) were analysed, comprising 174 patients with RRMS who received AHSCT before 1 January 2020. Primary outcomes included inflammatory disease activity, confirmed disability worsening (CDW) and overall evidence of disease activity. Confirmed disability improvement was investigated as a secondary outcome. Associations between variables of interest and outcomes were assessed using univariable Cox proportional hazards models.

Results Patients who received rituximab as the last DMT before AHSCT had a reduced hazard of inflammatory disease activity (HR 0.18, 95% CI 0.04 to 0.78). Epstein-Barr virus detection was associated with a higher hazard of inflammatory disease activity (HR 2.3, 95% CI 1.05 to 5.07). CDW was associated with longer disease durations (HR 1.09, 95% CI 1.00 to 1.19) and was negatively associated with gadolinium-enhancing lesions (HR 0.08, 95% CI 0.01 to 0.64). No CDW events occurred in treatment-naive patients.

Conclusions Prior rituximab treatment appears to be protective against inflammatory activity after AHSCT. Disease duration and gadolinium-enhancing lesions are major determinants of disability following AHSCT.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Autologous haematopoietic stem cell transplantation (AHSCT) is a potent treatment option for patients with relapsing-remitting multiple sclerosis. Higher age, longer disease duration and the absence of gadolinium-enhancing lesions have all been associated with worse outcomes, but selecting the patients who are likely to benefit most from AHSCT remains a key challenge.

WHAT THIS STUDY ADDS

⇒ This study confirms and supports previous findings by showing that patients who were treated earlier or who had markers of active, inflammatory disease appeared to develop less disability following treatment and also had a higher likelihood of disability improvement. Patients who had received rituximab before AHSCT had a lower long-term risk of clinical relapses or MRI activity following AHSCT.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ AHSCT appears to be most effective at preventing long-term disability when used earlier in the disease, emphasising the importance of timely referral and treatment. Alongside other findings showing that AHSCT is safe and effective in routine healthcare, this study highlights the potential of AHSCT to benefit a larger number of MS patients. Further research is needed to confirm whether rituximab increases the effectiveness of AHSCT and to explore the mechanisms of how this may be mediated.

INTRODUCTION

Advances in therapeutic interventions targeting the immune system have improved outcomes for patients with multiple sclerosis (MS). High-dose chemotherapy followed by autologous haematopoietic stem cell transplantation (AHSCT) is one such intervention. Initially developed for the treatment of haematological malignancies, encouraging results in animal models of MS[1] and reported improvements in those with concomitant malignancy and MS[2] helped pave the way for its specific use in MS by the late 1990s.[3] Several thousand procedures for MS have since been performed.[4] The goal of treatment is to achieve durable, long-term remission, without relapses or worsening of disability.

Both observational[5–12] and interventional trial data[13–16] show that AHSCT is a potent treatment for relapsing-remitting MS (RRMS), with ongoing randomised trials directly comparing it against other highly effective treatments.[17–19] It is increasingly clear that its safety and therapeutic effectiveness

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depend in large part on patient selection with prognostic factors identified thus far including age, disease duration, disease activity and previous treatments.[5–9 20] Other factors, including the complications of AHSCT such as viral reactivation, have received limited attention to date. This is of particular interest considering the crucial role that Epstein-Barr virus (EBV) infection plays in the development of MS.[21–23] Furthermore, EBV infection could well be affected by other disease-modifying therapies (DMTs) such as B-cell depleting agents, and this is yet to be explored in the context of AHSCT outcomes.

The aim of this study was to identify factors associated with treatment outcomes after AHSCT for MS. We used data from the multicentre, observational study Haematopoietic Stem Cell Transplantation for Treatment of Multiple Sclerosis in Sweden (AutoMS-Swe),[12] consisting of investigator-validated data from the Swedish MS registry as well as electronic health records to study the effects of 15 prespecified potentially prognostic factors.

METHODS

Inclusion criteria

The inclusion and exclusion criteria have previously been described in detail.[12] Briefly, all patients had a diagnosis of MS according to the revised McDonald criteria[24] with a relapsing-remitting disease course and had undergone AHSCT for MS in Sweden before 1 January 2020. Data were extracted from the Swedish MS registry and hospital records on 22 May 2022.

Procedures

Peripheral haematopoietic stem cells were mobilised with a single dose of cyclophosphamide 2 g/m^2 and granulocyte colony-stimulating factor 5 µg/kg (0.5 IE/kg) for 6–7 days. Haematopoietic stem cells were obtained by apheresis of peripheral blood to a minimum yield of 2.0×10^6 CD34$^+$ cells/kg. Stem cells were cryopreserved with no ex vivo manipulation. Two different conditioning regimens were used: the BEAM-antithymocyte globulin (ATG) conditioning regimen (BCNU 300 mg/m^2; etoposide 800 mg/m^2; cytosine-arabinoside 800 mg/m^2; melphalan 140 mg/m^2; ATG from rabbit (thymoglobulin) 10 mg/kg) and the Cy-ATG conditioning regimen (cyclophosphamide 200 mg/kg; rabbit ATG (thymoglobulin) 6 mg/kg). Prophylaxis against bacterial, viral and fungal infection was administered in the neutropenic phase. Prophylaxis against herpes viruses and *Pneumocystis jirovecii* continued for a minimum of 3 months following AHSCT. Patients with positive serology before the start of mobilisation for cytomegalovirus (CMV) and EBV were monitored for virus reactivation in the weeks following conditioning. Quantitative PCR (qPCR) was used to detect viral DNA in serum, whole blood or plasma as per the standard methodology at each university hospital. While time frames for virological testing were not fixed during the study period or between institutions, patients were typically tested weekly for approximately 5–7 weeks following AHSCT.

Study definitions and outcomes

Primary outcomes included inflammatory disease activity, disability worsening confirmed at 6 months (confirmed disability worsening, CDW), and overall evidence of disease activity (EDA). Secondary outcomes included disability improvement confirmed at 6 months (confirmed disability improvement, CDI). Definitions for all outcomes (inflammatory disease, CDW, EDA and CDI) are provided in online supplemental table S1. Patients were censored at the time that they experienced inflammatory disease activity, CDW, EDA and CDI. In those who did not experience these outcomes, the censoring was instead at the time of the last recorded Expanded Disability Status Scale (EDSS) score.

Variables of interest

Factors that could putatively affect the outcome of AHSCT were selected before analysis. Each had previously been identified as risk factors for the onset or progression of MS, or as potentially relating to outcomes following AHSCT. They include information about the patient and their disease at baseline, as well as events occurring soon after conditioning for AHSCT. In total, 15 variables were selected, as listed in figures 1–3 and defined in online supplemental table S1.

When considering the last DMT before AHSCT, patients were divided into three groups: treatment-naive patients, who had never received DMTs; those who had received rituximab as the last DMT before AHSCT; and those who had received any of the remaining DMTs (with a full list of the DMTs before AHSCT available elsewhere[12] and in online supplemental table S2). CMV and EBV DNA detection were defined as detection of CMV or EBV DNA at any level in whole blood, plasma or serum using qPCR. Virological data were only analysed in patients who received AHSCT after 31 December 2012 to mitigate biases arising from older, less sensitive qPCR techniques. There was no attempt to standardise the virologic methods used, although the same method was used for each patient throughout the procedure.

Statistical analyses

Statistical analyses were performed using R (version 4.4.0) with the 'tidyverse', 'survival', 'ggsurvfit', 'table 1' and 'forestploter' packages. Categorical data were summarised using frequencies, while discrete and continuous variables were summarised with medians and IQR. Fisher's exact test was used to determine differences in proportions and the Mann-Whitney U test, differences between two groups. A two-tailed $p<0.05$ was considered statistically significant.

Associations between the variables of interest and the primary and secondary outcomes were assessed using univariable Cox proportional hazard models. These associations are summarised with an HR, 95% CI and likelihood ratio test p value alongside the number of individuals included in each model and number of observed events. HRs for covariates where 95% CIs did not include the line of no effect were considered statistically significant. Survival was estimated using the Kaplan-Meier method.

RESULTS

In total, 174 patients across the seven Swedish transplantation centres were included with a median observation period of 5.5 years (IQR 3.4–7.5 years). The procedure was generally well tolerated and there was no treatment-related mortality.[12] Table 1, online supplemental tables S2 and S3 summarise the baseline clinical and demographic characteristics of the included patients.

Primary outcomes

Inflammatory disease activity

Of the 36 events representing inflammatory disease activity, 17 patients experienced clinical relapses. Of the remaining 19 patients censored with MRI events, 14 of these were due to new or enlarging T2-weighted lesions, while 5 patients had both gadolinium-enhancing T1-weighted lesions and new or enlarging T2-weighted lesions. The median interval between the baseline MRI scan and infusion of autologous haematopoietic stem

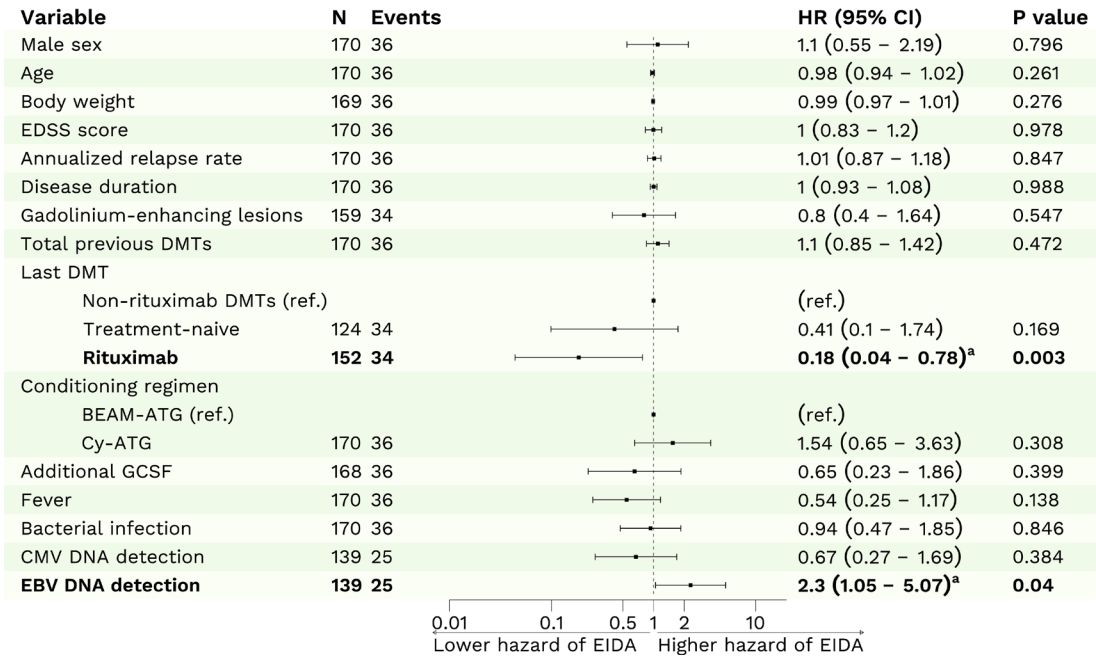

Figure 1 Evidence of inflammatory disease activity (EIDA). Single variable Cox proportional hazards models for the outcome of inflammatory disease activity. HRs for each variable and their 95% CIs are presented as a forest plot. For each variable, the total number of patients in the model and the total number of events observed, including those in any comparison groups, are shown under 'N' and 'events', respectively. P values for the likelihood ratio tests are also presented. The reference groups for categorical variables are denoted by '(ref.)'. [a]Statistically significant covariate (also indicated by bold typeface). CMV, cytomegalovirus; Cy-ATG, cyclophosphamide with antithymocyte globulin; DMT, disease-modifying therapy; EBV, Epstein-Barr virus; EDSS, Expanded Disability Status Scale; GCSF, granulocyte colony-stimulating factor.

cells was 62 days (IQR 40–102 days), while the median interval between infusion of autologous haematopoietic stem cells and the first follow-up MRI scan was 178 days (113–227 days).

The univariable Cox proportional hazards models for inflammatory disease activity are summarised in figure 1. Rituximab as the last DMT prior to AHSCT was associated with a significantly

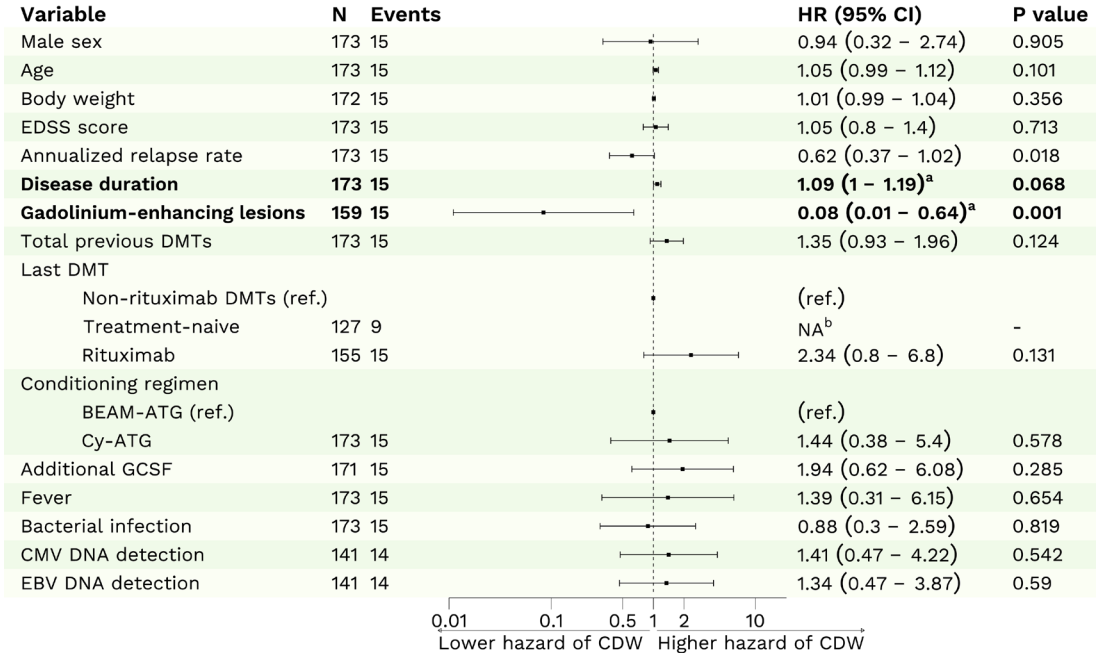

Figure 2 Confirmed disability worsening (CDW). Single variable Cox proportional hazards models for the outcome of CDW. HRs for each variable and their 95% CIs are presented as a forest plot. For each variable, the total number of patients in the model and the total number of events observed, including those in any comparison groups, are shown under 'N' and 'events', respectively. P values for the likelihood ratio tests are also presented. The reference groups for categorical variables are denoted by '(ref.)'. [a]Statistically significant covariate (also indicated by bold typeface). [b]HR unable to be calculated as no observed events in the treatment-naive group. CMV, cytomegalovirus; Cy-ATG, cyclophosphamide with antithymocyte globulin; DMT, disease-modifying therapy; EBV, Epstein-Barr virus; EDSS, Expanded Disability Status Scale; GCSF, granulocyte colony-stimulating factor.

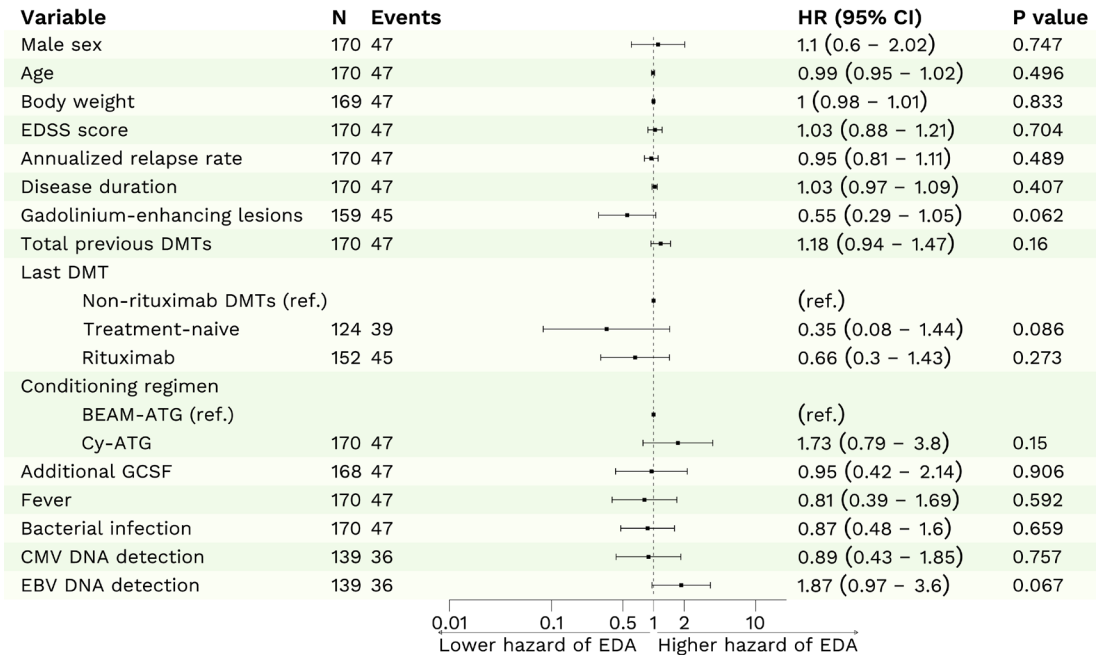

Figure 3 Evidence of disease activity (EDA). Single variable Cox proportional hazards models for the outcome of EDA. HRs for each variable and their 95% CIs are presented as a forest plot. For each variable, the total number of patients in the model and the total number of events observed, including those in any comparison groups, are shown under 'N' and 'events', respectively. P values for the likelihood ratio tests are also presented. The reference groups for categorical variables are denoted by '(ref.)'. CMV, cytomegalovirus; Cy-ATG, cyclophosphamide with antithymocyte globulin; DMT, disease-modifying therapy; EBV, Epstein-Barr virus; EDSS, Expanded Disability Status Scale; GCSF, granulocyte colony-stimulating factor.

lower risk of inflammatory disease activity compared with other DMTs (HR 0.18, 95% CI 0.04 to 0.78) while EBV DNA detection was associated with an increased hazard for inflammatory disease activity (HR 2.3, 95% CI 1.05 to 5.07). Figure 4A,B shows the Kaplan-Meier survival estimates for inflammatory disease activity stratified by the last DMT prior to AHSCT and EBV DNA detection respectively, while online supplemental figure S6 shows Kaplan-Meier estimates for inflammatory disease activity for each of the DMT groups.

Confirmed disability worsening

There were 15 recorded events of CDW, all occurred independently of clinical relapses. The univariable Cox proportional hazards models are summarised in figure 2. The factors associated with a lower hazard of CDW included shorter disease duration (HR 1.09, 95% CI 1.00 to 1.19) and the presence of gadolinium-enhancing T1-weighted lesions at baseline (HR 0.08, 95% CI 0.01 to 0.64). Figure 4C shows the Kaplan-Meier estimates for CDW with respect to gadolinium-enhancing lesions. No CDW events were observed in treatment-naive patients throughout follow-up (figure 2, online supplemental figure S7).

Evidence of disease activity

In total, there were 47 recorded events of disease activity. The Cox proportional hazards models for EDA are summarised in figure 3. There were no statistically significant associations with EDA.

Secondary outcomes

Confirmed disability improvement

There were 122 patients with an EDSS score of 2.0 or above at the time of AHSCT and 64 recorded events of CDI after the intervention. The Cox proportional hazards models for CDI are summarised in figure 5. Factors significantly associated with CDI include age (HR 0.95, 95% CI 0.91 to 0.98), annualised relapse rate (HR 1.23, 95% CI 1.11 to 1.36), disease duration (HR 0.91, 95% CI 0.84 to 0.98), the presence of gadolinium-enhancing lesions (HR 1.89, 95% CI 1.13 to 3.15, online supplemental figure S8), the total number of previous DMTs (HR 0.78, 95% CI 0.62 to 0.97). Patients who were treatment-naive had a substantially higher hazard of improvement following AHSCT (HR 3.55, 95% CI 1.86 to 6.75) than the comparison group, shown as a Kaplan-Meier plot in figure 4D.

Post hoc analyses

Rituximab before AHSCT was associated with a low proportion of EBV DNA detection after AHSCT

The strength of the association between rituximab and the reduced risk of inflammatory disease activity was striking and not evident when other DMTs were considered independently (online supplemental figure S6). A series of post hoc analyses were, therefore, performed in order to better characterise this correlation.

EBV DNA was detected less often in those who had received rituximab before AHSCT than in those who received other DMTs or who were treatment-naive (detected in 15% of patients, 9/46, vs 49%, 47/95; p<0.001, table 1). The median time point at which EBV DNA was first detected was day 25 post-AHSCT (IQR 21–33 days).

Detection of EBV DNA in rituximab-treated patients appears to be related to the interval between the last rituximab infusion and AHSCT

Within the rituximab-treated patients, the interval between the final rituximab dose and date of AHSCT differed substantially between those who did and did not have detectable EBV DNA, with a median interval of 352 days in the detected EBV DNA group, compared with 221 days in those where EBV DNA was

Table 1 Demographic and clinical characteristics of study population

	Total (N=174)	Treatment-naive (N=18)	Rituximab (N=46)	Other DMT (N=110)
Follow-up duration, years				
Median (IQR)	5.5 (3.4–7.5)	4.5 (2.7–7.1)	3.6 (2.6–5.1)	6.3 (5.1–8.4)
Sex				
Female	113 (65%)	14 (78%)	28 (61%)	71 (65%)
Male	61 (35%)	4 (22%)	18 (39%)	39 (35%)
Age, years				
Median (IQR)	31 (26–36)	27 (23–30)	32 (28–39)	32 (26–36)
Body weight, kg				
Median (IQR)	72 (63–84)	68 (64–81)	71 (65–83)	72 (61–84)
Missing	1 (0.6%)	0 (0%)	0 (0%)	1 (0.9%)
Disease duration, years				
Median (IQR)	3.4 (1.0–6.9)	0.28 (0.21–0.32)	3.1 (1.6–9.0)	4.3 (1.7–7.2)
Baseline EDSS score				
Median (IQR)	3.5 (2.0–4.0)	3.3 (2.5–4.8)	3.0 (1.6–3.5)	3.5 (2.5–4.5)
Data missing	1 (<1%)	0 (0%)	0 (0%)	1 (1%)
Gadolinium-enhancing lesions on MRI brain prior to AHSCT				
Present	69 (40%)	14 (78%)	11 (24%)	44 (40%)
Absent	90 (52%)	3 (17%)	31 (67%)	56 (51%)
Data missing	15 (8%)	1 (5%)	4 (9%)	10 (9%)
Annualised relapse rate prior to AHSCT				
Median (IQR)	1.0 (0–2.0)	2.0 (1.0–4.0)	1.0 (0–2.0)	1.0 (0–2.0)
Total number of DMTs prior to AHSCT				
Median (IQR)	2.0 (1.0–3.0)	0 (0–0)	2.0 (1.0–3.0)	2.0 (1.0–3.0)
Conditioning regimen				
BEAM-ATG	33 (19%)	4 (22%)	2 (4%)	27 (25%)
Cy-ATG	141 (81%)	14 (78%)	44 (96%)	83 (75%)
Received additional G-CSF following AHSCT				
Yes	29 (17%)	1 (6%)	7 (15%)	21 (19%)
No	143 (82%)	17 (94%)	39 (85%)	87 (79%)
Data missing	2 (1%)	0 (0%)	0 (0%)	2 (2%)
Fever following AHSCT				
Yes	143 (82%)	16 (89%)	38 (83%)	89 (81%)
No	31 (18%)	2 (11%)	8 (17%)	21 (19%)
Bacterial infection following AHSCT				
Yes	60 (34%)	5 (28%)	15 (33%)	40 (36%)
No	114 (66%)	13 (72%)	31 (67%)	70 (64%)
Serum CMV DNA following AHSCT				
Detected	42 (24%)	2 (11%)	18 (39%)	22 (20%)
Not detected	99 (57%)	13 (72%)	28 (61%)	58 (53%)
Data missing	33 (19.0%)	3 (16.7%)	0 (0%)	30 (27.3%)
Serum EBV DNA following AHSCT				
Detected	54 (31%)	8 (44%)	7 (15%)	39 (35%)
Not detected	87 (50%)	7 (39%)	39 (85%)	41 (37%)
Data missing	33 (19.0%)	3 (16.7%)	0 (0%)	30 (27.3%)

Demographic and clinical characteristics for total study population, and subgroups as stratified by the last DMT prior to AHSCT.
AHSCT, autologous haematopoietic stem cell transplantation; CMV, cytomegalovirus; DMT, disease-modifying therapy; EBV, Epstein-Barr virus; EDSS, Expanded Disability Status Scale; GCSF, granulocyte colony-stimulating factor.

not detected (online supplemental table S4, p=0.032). No individual who received rituximab in the 7 months before AHSCT had detectable EBV DNA. There were no meaningful differences in the cumulative rituximab dosage or number of doses between these groups.

Rituximab-treated individuals with inflammatory disease activity also had detectable EBV DNA

Only a minority of patients receiving rituximab prior to AHSCT experienced inflammatory disease activity, and, incidentally, only a small proportion (7/46, 15%) of rituximab-treated patients later had detectable EBV DNA (online supplemental table S4). Both cases of inflammatory disease activity in the rituximab-treated patients also exhibited EBV DNA in serum following AHSCT. Of all the patients treated with rituximab who did not demonstrate detectable EBV DNA, 0/39 (0%) experienced inflammatory disease activity. The proportion of patients with inflammatory disease activity in the EBV DNA positive, rituximab-treated group (2/7, 29%) was comparable to the proportion

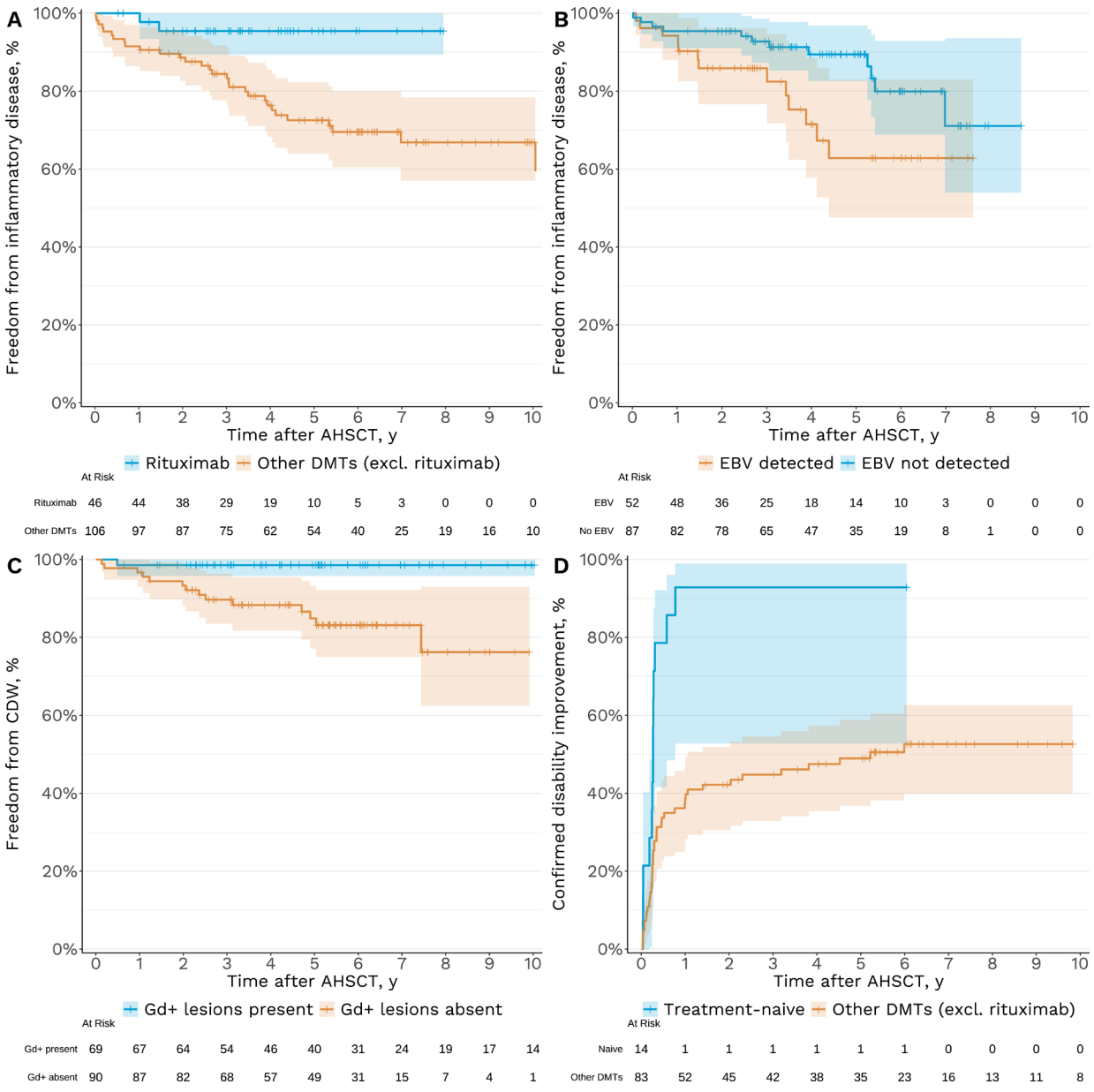

Figure 4 Kaplan-Meier estimates of freedom from inflammatory disease, CDW and confirmed disability improvement, stratified by last disease-modifying therapy, detection of EBV and presence of gadolinium-enhancing lesions before AHSCT. (A) Kaplan-Meier survival estimate of percentage of patients with freedom from inflammatory disease following AHSCT stratified by the last DMT prior to AHSCT; rituximab compared with all other DMTs. (B) Kaplan-Meier survival estimate of percentage of patients with freedom from inflammatory disease following AHSCT stratified by the detection of EBV following AHSCT. (C) Kaplan-Meier survival estimate of percentage of patients with freedom from CDW following AHSCT stratified by the presence of gadolinium-enhancing lesions on T1-weighted MRI before AHSCT. (D) Kaplan-Meier survival estimate of percentage of patients with confirmed disability improvement following AHSCT stratified by the last DMT prior to AHSCT; treatment-naïve patients against non-rituximab DMTs. AHSCT, autologous haematopoietic stem cell transplantation; CDW, confirmed disability worsening; DMT, disease-modifying therapy; EBV, Epstein-Barr virus; Gd +ve, gadolinium-enhancing.

of patients with inflammatory disease activity in EBV DNA positive patients not treated with rituximab (11/34, 32%).

Conditioning regimen did not affect the association between rituximab and inflammatory disease activity

The potential effect of the conditioning regimen on the association between rituximab and outcome was investigated by introducing the type of conditioning regimen as an interaction term (online supplemental table S5). In this model, only rituximab use was shown to be associated with inflammatory disease activity (HR 0.19, 95% CI 0.04 to 0.8). Kaplan-Meier curves for inflammatory disease activity stratified by both rituximab use and the conditioning regimen are shown in online supplemental figure S9.

DISCUSSION

In this study, we explored the relationship between a selection of baseline and treatment-related factors and their effects on long-term clinical outcomes following AHSCT treatment for RRMS. We found that disease duration and the absence of gadolinium-enhancing lesions before AHSCT were linked to a greater risk of CDW. Additionally, rituximab treatment prior to ASHCT was associated with a reduced risk of inflammatory disease activity,

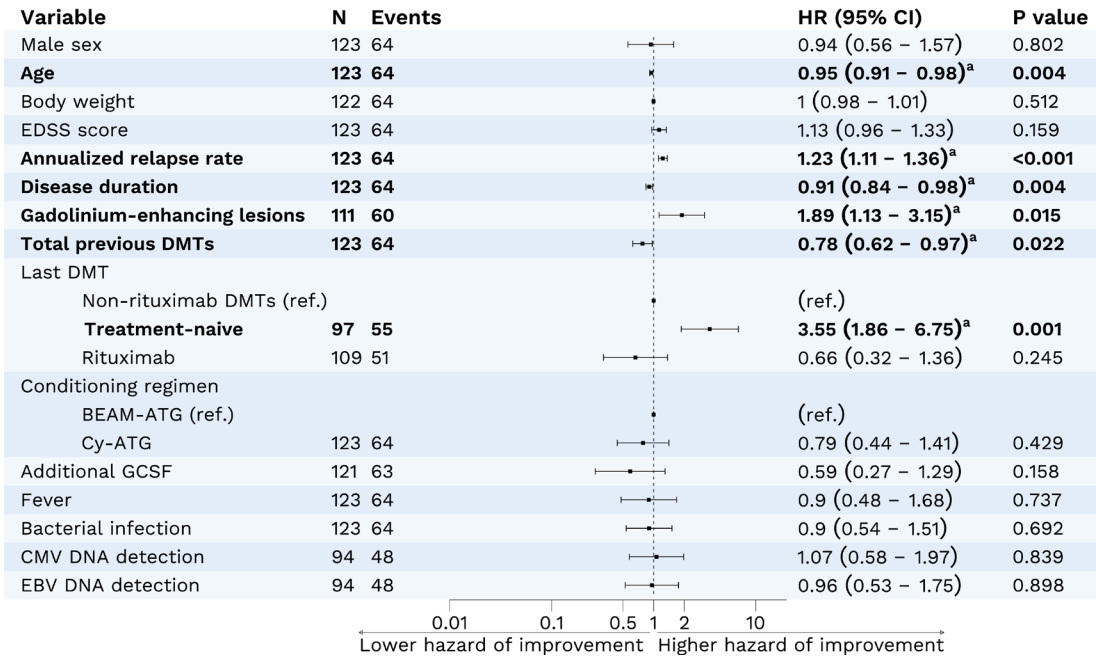

Figure 5 Confirmed disability improvement (CDI). Single variable Cox proportional hazards models for the outcome of CDI. Disability improvement was only assessed in patients with a baseline EDSS score ≥2.0. HRs for each variable and their 95% CIs are presented as a forest plot. The total number of included patients and events for each model, including those in the reference group, are shown in 'N' and 'events' respectively. P values for the likelihood ratio tests are also presented. The reference groups for categorical variables are denoted by '(ref.)'. [a]Statistically significant covariate (also indicated by bold typeface). CMV, cytomegalovirus; Cy-ATG, cyclophosphamide with antithymocyte globulin; DMT, disease-modifying therapy; EBV, Epstein-Barr virus; EDSS, Expanded Disability Status Scale; GCSF, granulocyte colony-stimulating factor.

while the detection of EBV in serum following AHSCT correlated with a higher subsequent risk of inflammatory activity.

Associations between disability worsening following AHSCT with age, disease duration and gadolinium-enhancing lesions have been previously reported.[5 6 9 25 26] Our findings also show this and further add that treatment-naive patients treated with AHSCT appear to have a lower risk of disability worsening. No CDW events were noted over a median follow-up of 4.4 years, though a hazard ratio could not be calculated. In addition to the findings relating to disability worsening, we found that sustained improvements in disability could more frequently be seen in patients with shorter disease durations, higher annualised relapse rates, gadolinium-enhancing lesions and fewer or no previous treatments. A similar finding has also been described in an overlapping case series of treatment-naive patients with 'aggressive', highly-active MS, where substantial improvements in disability following treatment were noted.[27]

It should be noted that the treatment-naive cohort in this study generally had shorter disease durations, higher ARRs and more frequently had gadolinium-enhancing lesions at baseline than the remaining patients. This mirrors the body of evidence that shows that AHSCT is substantially less effective in progressive disease than when compared with RRMS.[5–10 28] Taken together, it is likely that shorter disease durations, higher relapse rates, gadolinium-enhancing lesions and few or no previous treatments act as surrogate markers for active, inflammatory disease, with this phenotype being most amenable to AHSCT treatment. Our findings lend further weight to recommendations for early, first-line use of AHSCT in patients with highly active, rapidly evolving MS[29] in order to avert long-term disability.

A central finding in this study was that rituximab use prior to AHSCT was associated with a reduced risk of inflammatory disease activity compared with all other DMTs. A related finding has been reported by Kvistad *et al*[20] in a cohort that partially overlaps with this present study. They described fewer CDW and EDA events in patients treated with long-acting treatments (predominantly rituximab, but also alemtuzumab or cladribine) than 'short-acting' or no treatment. While these outcomes differ from the associations seen in our study, they lend credence to the possibility that previous treatments might affect treatment outcomes following AHSCT.

It is important to note that the rituximab-treated patient group appears similar to the comparator group at baseline in our study, suggesting that these groups are broadly comparable. In addition, the post hoc comparison of freedom from inflammatory activity between rituximab and the disaggregated DMTs supports an association specific to rituximab, and not any other DMT. Intriguingly, the comparison cohort demonstrates inflammatory disease activity for multiple years following AHSCT, whereas the rituximab-treated cohort appears to have persistently lower inflammatory disease, particularly when considering the reported dosing of rituximab and anticipated duration of its therapeutic effect.

One possible explanation for this may relate to EBV biology, the detection of which itself was associated with later inflammatory disease activity in our study. EBV infection is the strongest known risk factor for the development of MS,[21] with memory B cells acting as the predominant site of latent EBV infection. Rituximab acts by binding to CD20 cell surface markers, present on the vast majority of mature B cells (including memory cells) leading to their depletion. Given this overlap, a number of hypotheses linking the effects of this and other DMTs in MS to EBV biology are being explored.[30–32]

In our study, the relationship between EBV, rituximab treatment and outcomes following AHSCT is not entirely clear. On one hand, rituximab was associated with a generally reduced

likelihood of detecting EBV DNA, and those who had detectable EBV DNA despite prior rituximab had a longer interval between their final dose of rituximab and the start of AHSCT treatment. Both of these findings are in keeping with rituximab's B-cell depleting activity. It was noted that the only two patients who demonstrated inflammatory activity in the rituximab-treated group also had detectable EBV DNA. On the other hand, any possible association between EBV detection and treatment outcomes would also be influenced by prior rituximab use. As such, it is not possible to determine whether undetectable EBV DNA is merely an epiphenomenon of rituximab treatment, or whether EBV itself contributes to the development of inflammatory disease following AHSCT. Further complicating this, the detection of EBV DNA is affected by the methodology and type of specimen used.[33] It is plausible that differences between institutions in testing practices, alongside disparities in DMT prescribing, could be a source of confounding between prior DMT use and the detection of EBV DNA following AHSCT.

While EBV reactivation has previously been linked to complications following AHSCT for MS, including EBV-related lymphoproliferative disorders,[9 34] a direct relationship with disease-related outcomes has not been demonstrated. In a cohort of 120 patients treated with AHSCT, Nicholas *et al* showed that disability worsening was associated with monoclonal paraproteinaemia, and this in turn was associated with EBV viraemia.[9] As part of a wider investigation of the EBV-directed T cell repertoire following AHSCT, Massey *et al*[35] showed that 4/9 patients developed low-level EBV plasma viraemia shortly after AHSCT, with two patients also developing detectable EBV DNA many years later. The detection of EBV DNA did not, however, relate to the patients who subsequently experienced relapses.[35]

This is the largest published study of its kind, totalling 174 patients with RRMS across all university hospitals from a single country. The data used in this study were high quality with prolonged follow-up and low rates of missingness. All included data have been independently validated as part of the original AutoMS-Swe study and others,[12 36] strengthening the reliability and homogeneity of these data.

This study also has a number of important limitations to consider. First, as an observational study, the described associations cannot be assumed to be causal, and the degree to which the results are affected by confounding is uncertain. A second limitation is the univariable nature of the statistical analysis. In this study, the small number of outcome events—a result of the particular effectiveness of AHSCT—precluded multivariable analysis with all variables of interest. While this approach can lessen the effect of confounding, it is of limited use when variables are highly collinear. Third, the data in this study span a prolonged period of time, during which the indication for AHSCT has evolved substantially. As elsewhere, in Sweden AHSCT was initially used in those with ongoing disease activity and high levels of disability despite multiple courses of treatment. Its use has since expanded to patients earlier in the disease course, with less advanced disability.[29] Similarly, the conditioning regimen has also switched from BEAM-ATG to cyclophosphamide-ATG, limiting direct comparisons between the two. The testing for viral DNA reflects routine clinical practices during the period studied and thus introduces heterogeneity. Various laboratories employed differing analysis matrices, PCR techniques, monitoring frequencies, and durations, contributing to a potential source of bias. To mitigate this effect, we included only virological data from patients who received AHSCT after 2012, addressing concerns about the lower sensitivity of earlier EBV and CMV detection methods. Finally, the MRI data used in this study did not include measurements that reflect ongoing non-inflammatory disease activity, such as brain or spinal cord atrophy, slowly expanding lesions or paramagnetic rim lesions.[37]

CONCLUSIONS

This real-world cohort study adds to prior investigations of clinical outcomes following AHSCT for RRMS. Disease duration, relapse rate, the presence of gadolinium-enhancing lesions and the first-line use of AHSCT appear to be important determinants of long-term disability. Prior treatment choices also appear to impact AHSCT outcomes, with rituximab treatment before AHSCT linked with a substantially reduced risk of inflammatory disease activity. EBV viraemia following AHSCT may also be associated with inflammatory disease activity and merits further investigation.

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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 and designed by JB and TS. Acquisition, analysis and interpretation of data were performed by YN, CZ, TS, AT, TT, KC and JB. The manuscript was drafted by YN. Statistical analysis was performed by YN and CZ. Funding was obtained by JB. Administrative and technical support were provided by KK. Supervision was performed by JB. All authors critically reviewed the manuscript and contributed intellectual content.

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