1. **Title**

Safety and efficacy of hematopoietic or mesenchymal stem cell therapy in adults with Multiple Sclerosis: A systematic review and meta-analysis

2. **Registration**

This protocol adheres to Preferred Reporting Items for Systematic Reviews and Meta-analyses Protocols (PRISMA-P) guidelines (Shamseer et al., 2015). The review was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 8th September 2015 (CRD42015025951).

3. **Authors and contributions**

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Contributions:
Design and/or conceptualisation of the study: VF, MB, AL, MV. Data collection, analysis and/or interpretation: VF, TD, AR, MB, AL, MV. Drafting and/or revising the manuscript: VF, TD, AR, MB, AL, MV.

4. **Amendments**

Amendments to this protocol and their rationale will be recorded in future versions of this protocol, if needed.

5. **Support**

AL and TD are supported by a National Health and Medical Research Council of Australia Project Grant (ID 1084880). AR is supported by a University of Sydney Early Career Researchers grant (CI Lampit).

6. **Rationale**

Multiple Sclerosis (MS) is a chronic neurological disease affecting the central nervous system accompanied by demyelination, gliosis and axonal loss (Rejdak et al., 2010). MS initiates at the prime of workplace productivity and family commitment with an average age of onset of 29.2 years (Dua et al., 2008). Increase of MS severity from mild to severe is estimated to more than triple an individual’s total healthcare, direct non-medical and indirect costs to €75 000 per annum (adjusted to 2008 rates, Kobelt and Kasteng, 2009). Current immunosuppressive and immunomodulatory treatments decrease relapse and disability by about 68% and 44% respectively in relapsing-remitting MS compared to placebo over 24
months (Filippini et al., 2013). However, for progressive MS no improvement in disability has been found over 12 randomised controlled trials, and tolerance and adherence to these drugs in relapsing-remitting MS remains a challenge (Filippini et al., 2013, Giovannoni et al., 2012). There are hence compelling reasons to develop new treatments to better prevent and reduce disability in MS.

Stem cell therapy has emerged as a novel second-line intervention to assist with refractory MS (Ardeshiry Lajimi et al., 2013). Two therapeutic strategies frequently trialed include autologous (patient-specific) or allogenic (donor derived) hematopoietic or mesenchymal stem cell therapy (HSCT and MSCT, respectively). HSCT aims to diminish autoreactive immune cells using chemotherapy and to replenish the immune system through hematopoietic stem cell infusion back into the individual (Cipriani et al., 2013). Immune system regeneration has been thought to underlie this process with an altered patient t-cell repertoire after HSCT (Muraro et al., 2005). Conversely, MSCT is thought to produce immunomodulatory as well as immunosuppressive actions, with evidence that either autologous or allogenic cells suppress autoreactive immune cells as well as inhibition of inflammatory cytokine release in vitro (Di Nicola et al., 2002). The increasing numbers of clinical trials performing HSCT (Saccardi and Gualandi, 2008) and MSCT (Wei et al., 2013) warrant a systematic evaluation of the efficacy and safety of these interventions in MS.

A recent systematic review and meta-analysis of autologous HSCT for progressive MS found greater rates of progression-free survival (PFS) after lower-intensity compared to high-intensity chemotherapy regimens (Reston et al., 2011). This systematic review was limited to primarily small case-studies while morbidity and mortality rates were restricted to qualitative analysis as authors did not combine safety outcomes. The association between conditioning regimen intensity and efficacy of autologous HSCT is also supported by a previous systematic review (Burt et al., 2008) and a retrospective database survey of the European Blood and Marrow Transplantation Group between 1995-2000 (Saccardi et al., 2006). Another systematic review has included both HSCT and MSCT amongst other stem cell therapies and concludes that HSCT may be effective for progressive MS symptoms; further, the authors discuss the feasibility and safety – but not the efficacy – of MSCT (Ardeshiry Lajimi et al., 2013). However, this systematic review has several limitations, including a combination of animal and human studies, lack of differentiation between study designs, inclusion of reviews as primary evidence and reliance on non-quantitative outcomes. Chances of bias were also high due to limited explanation of excluded studies as well as lack of study quality assessment.

To our knowledge, no meta-analysis has critically examined MSCT in MS, nor compared MSCT to HSCT.

As HSCT and MSCT approaches continue to be refined and administered to more MS patient groups, treatment efficacy, safety and potential moderators of effect need to be systematically analysed.
7. Objectives

The aim of this systematic review and meta-analysis is to evaluate the efficacy and safety of autologous or allogeneic HSCT or MSCT for adults with MS and to examine key methodological factors that may underpin differences in effect sizes across studies. The systematic review will specifically:

1) Evaluate and compare efficacy and safety in studies of HSCT and MSCT
2) Identify potential study-level moderators of effect sizes across studies
3) Assess the nature and quality of included studies
4) Provide evidence-based recommendations for future research in the field

METHODS

8. Eligibility criteria

We will include studies or part of studies that meet the following criteria:

Study designs

We will include randomised and non-randomised controlled trials as well as observational studies. Case reports will be excluded.

Participants

We will include studies recruiting adult participants (aged ≥18) with MS (of any type). Studies that examine adults with MS as well as other disease groups or under 18 year olds will be included but analysis will be restricted to adult MS data.

Interventions

Interventions of interest are autologous and allogeneic HSCT and MSCT. There will be no restriction to cell source (e.g., bone marrow, umbilical cord), mobilisation, graft or conditioning procedures used for HSCT. There will be no restriction to route of administration, dosage or number of injected cells for MSCT. Studies examining additional interventions such as other stem cell therapies will be included, but only data from HSCT or MSCT will be included.

Comparators

Any type of control condition will be eligible, including sham/placebo procedures, treatment-as-usual, wait-list or no-contact control groups. If these comparators are not available, baseline data will serve as comparison (i.e. case-control designs). If a study directly compares HSCT and MSCT, data for each intervention will be split and treated as two separate studies (using within-group analysis). If a study includes HSCT and MSCT as well as a control group, the control group will be split into two and treated as separate comparisons towards each intervention (Caldwell et al., 2005). If a study includes both autologous and allogeneic procedures, data for each will be split and treated as two separate studies unless the data for
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each procedure is not available; in this case we will use the intervention provided to the majority of individuals as the type of intervention. If a study compares adult MS to another disease group undergoing either HSCT or MSCT, only the adult MS data will be included.

Outcome

We will include results from studies reporting one or more of the following outcomes post-intervention:

Efficacy

1. **Disability:**
   a. Effect on disability as measured using the Expanded Disability Status Scale (EDSS), reported as continuous outcomes (e.g. median and IQR)
   b. Progression free survival as measured by no increase in disability
   c. Effect on other disability and functionality measures such as Multiple Sclerosis Severity Score (MSSS), Multiple Sclerosis Impact Scale (MSIS) or Multiple Sclerosis Functional Composite (MSFC)

2. **Relapse occurrence:** as defined by post-intervention relapse incidence (e.g. annualized relapse rate) or proportion relapse-free

3. **MRI lesions:** measured as number of new T1-weighted gadolinium-enhancing (Gd+) and/or new or active T2-weighted MRI lesions compared to baseline scan.

4. **Depression, health-related quality of life and psychological wellbeing:** as measured by questionnaires and/or structured interviews

5. **Cognition:** cognitive outcomes in any domain as assessed through tests or batteries.

Safety:

8. **Treatment related and non-treatment related adverse events:** defined as number and percentage of individuals experiencing treatment related or non-treatment related adverse events post-intervention.

9. **Treatment related and non-treatment related mortality:** defined as number and percentage of individuals experiencing treatment related or non-treatment related mortality post-intervention. If treatment related and non-treatment related mortality are not available, overall mortality will be included.

When studies report more than one follow-up, all follow-ups will be included in the review.

Timing

There will be no restrictions for date of publication or language.

9. **Information sources**

Potentially eligible published articles and data will be located by:

1. Electronic database search on Medline (Ovid interface 1946 onwards), Pre-Medline (Ovid interface), Embase (Elsevier interface 1966 onwards), Cochrane Central Register of Controlled Trials (CENTRAL; Ovid interface 1991 onwards).

2. Scanning reference lists of previous reviews and included studies to ensure saturation of the literature. As relevant studies are identified, reviewers will check for additional relevant citing articles; and

3. Contacting authors of primary studies for unpublished outcomes.
Potentially eligible unpublished data will be located by:

1. Searching the World Health Organization International Clinical Trials Registry Platform (ICTRP) for ongoing and newly completed trials.
2. Electronically searching and requesting data from the following groups and registries: Center for International Blood and Marrow Transplant Research (CIBMTR), European Group for Blood and Marrow Transplantation (EBMT), International Bone Marrow Transplant Registry (IBMTR) and Autologous Blood and Marrow Transplant Registry (ABMTR).
4. Searching Web of Science Core Collections under Conference Proceedings Citation Index- Science (1990 onwards).

10. Search strategy

Literature search strategies have been created through the use of medical subject headings (MeSH) and text words relating to multiple sclerosis or stem cell transplantation. Syntax and subject headings have been adapted according to database. Quantitative studies will be sought and no search limits will be imposed on date or language. The study design will be limited to humans only while no other study design limit will be applied. Studies in languages other than English will be translated. A Medical Librarian who has expertise in systematic review searching was consulted for search strategy formation. The search term strategy for Medline has been included below and Appendix 1 contains all database search strategies.

The search terms will be:

1. multiple sclerosis, chronic progressive/
2. multiple sclerosis/
3. multiple sclerosis, relapsing-remitting/
4. multiple sclerosis.mp.
5. or/1-4
6. stem cell transplantation/
7. cord blood stem cell transplantation/
8. hematopoietic stem cell transplantation/
9. mesenchymal stem cell transplantation/
10. peripheral blood stem cell transplantation/
11. ((blood or hematopoietic or mesenchymal) adj3 stem cell transplantation).mp.
12. ((cord or peripheral) adj3 blood stem cell transplantation).mp.
13. exp Stem Cells/
14. Bone Marrow Cells/
15. Bone Marrow Transplantation/
16. Bone Marrow Transplant*.mp.
17. or/6-16
18. and/5,17
19. exp Animals/ not humans.sh.
11. Study records

Electronic records obtained from each database will be downloaded into a single EndNote library. VF will conduct the database search, combine records, and remove duplicates. After removal of duplicates, VF will screen search results for initial eligibility against the inclusion criteria based on title and abstract. The full text of potentially eligible studies and studies whose eligibility is unclear based on title and abstract will be assessed independently by VF and an additional reviewer (TD or AR). VF will contact authors when eligibility is unclear based on the full-text article. Disagreements regarding study eligibility will be resolved by a senior reviewer (MB or AL). Reasons for exclusion will be recorded at all stages until a final list of included studies will be composed.

12. Data items

The following information will be extracted for each outcome and recorded in an Excel spread sheet:

1. Study information (first author, year of publication, country, language);
2. Outcomes (name, sample size in each group, events, events rate or mean + SD)
3. Study characteristics (design, risk of bias, trial registration, type of control condition, intervention type for active control, assigned treatment compliance, mean time between follow-ups, type of analysis [ITT/PPC]);
4. Participants’ characteristics (mean age, % female, diagnosis, mean disease duration, % previously refractive to treatment);
5. Intervention HSCT (type and intensity of conditioning regimen, cell source, mobilisation used, graft manipulation inclusion, number of cells given and duration of intervention, supportive care);
   Intervention MSCT (cell source, route of administration, dosage or number of injected cells and duration of intervention, supportive care)

Intention-to-treat data will be preferred if reported. Data collection will be performed by two reviewers independently. Discrepancies will be resolved by consensus.

13. Outcomes

Primary outcomes

   Efficacy:
   1. Effect on disability or functionality as measured by EDSS, MSSS, MSIC or MSFC
   2. Progression free survival
   3. Relapse incidence
   4. Proportion relapse free

Safety
   1. Treatment and non-treatment related adverse events
   2. Treatment and non-treatment related mortality
3. Overall adverse events or mortality

Adverse events will be combined into broad categories unless descriptions of adverse events are not available.

Secondary outcomes

Efficacy:
1. New MRI lesions or activity
2. Cognition (overall + specific domains)
3. Depression
4. Health-related quality of life
5. Psychological wellbeing

The unit of analysis will be risk-ratios or mean difference (for progression free survival, adverse events and mortality), or standardised mean difference (for all other outcomes). Precision of effect sizes will be estimated using 95% confidence intervals.

14. Risk of bias in individual studies

Risk of bias in individual RCTs will be conducted in accordance with the Cochrane Collaboration risk of bias tool (Higgins and Green, 2011). Low, high or unclear risk of bias will be determined for each of the following categories:

1. Sequence generation
2. Allocation concealment
3. Blinding of outcome assessors, personnel and participants
4. Incomplete outcome data
5. Selective outcome reporting
6. Other sources of bias

Trials with high or unclear risk of bias in items 3 and/or 4 will be considered as having high risk of bias.

Risk of bias in individual non-randomised studies will be assessed using the National Institutes of Health’s study quality tools (National Heart Lung and Blood Institute, 2014).

Assessments will be conducted independently by VF and an additional reviewer (TD or AR). VF will contact authors if study reports do not provide sufficient details to determine risk of bias. Consensus score will be determined by AL.

15. Data Synthesis

Primary and secondary outcomes

Pooling of data into meta-analyses will be done for HSCT and MSCT separately. Analyses will be performed only when at least three studies reported results for the outcome in question. Variance adjustments for intercorrelation among outcomes will be conducted using a tool developed by AL. All other analyses will be conducted using Comprehensive Meta Analysis version 3 (CMA, Biostat Inc., Englewood, NJ) using random-effects model.
**Heterogeneity**

True heterogeneity across studies (i.e., the proportion of variance that reflect variance in true effect sizes rather than sampling error) will be quantified using $I^2$ statistic with 95% confidence intervals (Higgins and Thompson, 2002, Higgins et al., 2003). We will perform subgroup analyses in order to detect possible study design factors that affect outcomes. These analyses will be based on mixed-effects model, and between-subgroup heterogeneity will be tested using Cochrane’s Q (significant at the p<0.05 level). Analyses will be performed both overall and for each outcome in which true heterogeneity is detected. The following between-subgroup moderators will be tested:

1. Regimen intensity (Reston et al., 2011)
2. HSCT procedure (cell source, mobilisation, graft or conditioning procedures, Radaelli et al., 2014)
3. MSCT procedure (cell source, route of administration, dosage or number of injected cells)
4. Early vs delayed treatment (ie. in crossover trials)
5. Nature and intensity of supportive care
6. Date of study/transplantation (before or after 2000, Saccardi et al., 2006)
7. Type of control condition (e.g., sham injection, treatment-as-usual)
8. Study location (region)
9. Gender (% females)
10. Mean time between follow-ups
11. Sample size
12. Year of publication

Subgroup analyses will be performed only when at least two groups with minimal group size of k=5 are available for analyses.

**16. Meta-bias(es)**

For each outcome measure, funnel plots of SMD or risk-ratios vs standard error will be visually inspected for asymmetry that may indicate small study effect (Sterne et al., 2011) and formally tested using Egger’s Test of the Intercepts (Egger et al., 1997). If significant asymmetry will be detected, estimates of unbiased effect sizes will be generated using Duval and Tweedie’s Trim and Fill method (Duval and Tweedie, 2000). In addition, we will conduct mixed-effects subgroup analyses based on risk of bias (high or low risk of bias, see section 14) or quality assessment, as well as trial registration (prospectively, retrospectively or not registered in a clinical trial registry). Analyses of trial registration will be conducted only for trials starting enrolment after July 1 2005 (De Angelis et al., 2004).

**17. Confidence in cumulative estimate**

Overall strength of evidence will be determined by examining quality across studies, precision of effect estimates and evidence for small study effects, including additional sensitivity analyses if warranted. A qualitative summary will be provided based on these findings.
References


