

Perspective

Summary of Scientific and Statistical Methods, Study Endpoints and Definitions for Observational and Registry-Based Studies in Hematopoietic Cell Transplantation

Abraham S. Kanate^{1,*}, Arnon Nagler², Bipin Savani³

¹Hematopoietic Malignancy and Cellular Therapy, West Virginia University Cancer Institute, Morgantown, WV, USA

²Chaim Sheba Medical Center, Tel Aviv University, Tel-Hashomer, Ramat Gan, Israel

³Department of Hematology-Oncology, Vanderbilt University Medical Center, Nashville, Tennessee, USA

ARTICLE INFO

Article History

Received 05 Nov 2019

Accepted 03 Dec 2019

Keywords

Hematopoietic cell transplantation

Scientific methods

Study endpoints

Study definitions

ABSTRACT

Due to the increasingly strict word/character restrictions enforced by most scientific publications, we identified a need for a master document illustrating the “Scientific Methods” that may be applicable to the majority of observational and registry-based studies in hematopoietic cell transplantation (HCT). The purpose of this study is to serve as a reference document that describes the data source, study endpoints and statistical analyses utilized most commonly in retrospective studies in HCT. To this end we compile, define and reference the methodology commonly used in HCT research. While it is recognized that the scientific methodology may periodically require updates due to the evolving landscape of transplant research, such as newer study endpoints and statistical methods, this manuscript describes frequently used methodologies.

© 2019 International Academy for Clinical Hematology. Publishing services by Atlantis Press International B.V.
This is an open access article distributed under the CC BY-NC 4.0 license (<http://creativecommons.org/licenses/by-nc/4.0/>).

1. INTRODUCTION

The field of hematopoietic cell transplantation (HCT) is a very dynamic one, and has been instrumental in significant scientific contributions in hematology/oncology over the last 4 decades. Prospective and retrospective studies rely on well-established and sophisticated scientific and statistical methods, which are vital to the accurate interpretation of data and implementation into clinical practice. Considering the increasingly strict space constraints and word/character restrictions enforced by most scientific publications, we identified a need for a master document illustrating the “Scientific Methods” of the most common type of HCT study, namely observational studies. The purpose of this project is to provide a synopsis of the data source, study endpoints (including definitions) and statistical analyses utilized uniformly across the board, in registry-based retrospective studies.

2. GENERAL PRINCIPLES

1. The current iteration of this manuscript is applicable to retrospective/observational studies evaluating outcomes in patients undergoing HCT. Other forms of cellular therapy such as

chimeric antigen receptor (CAR)-T cell and T-cell receptors (TCRs) are not considered in this paper.

2. HCT is defined as the intravenous infusion of hematopoietic cells derived from bone marrow, peripheral blood or cord blood. It is preceded by a conditioning or preparative regimen aiming to allow engraftment and to reduce tumor burden [1,2].
3. Criteria for inclusion and exclusion of patient-, disease- and transplant-characteristics are recognized as being protocol-dependent and, hence, will differ between studies and are beyond the purview of this manuscript.
4. All study protocols are supposed to be approved by the relevant institutional review board (or its equivalent) at each participating site and comply with country-specific regulatory requirements. All studies should be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

3. STUDY ENDPOINTS AND DEFINITIONS

It is recognized that the primary and secondary endpoints are likely to vary depending on the specific study protocol. The day of hematopoietic cell infusion (day 0) is considered the commencing time point in HCT outcomes research. For the purposes of this manuscript, the commonly reported post-HCT study endpoint outcome measures are described. It may be noted that most

*Corresponding author. Email: askanate@hsc.wvu.edu

Peer review under responsibility of the International Academy for Clinical Hematology

post-transplant outcomes (apart from survival outcomes using the Kaplan-Meier method) are reported now as cumulative incidence with a competing risk model, as opposed to reporting crude proportions [3,4]. In a competing risk model, the first event is considered the observed failure, precluding the occurrence of the other event (e.g., a post-HCT patient dying from infection precludes any further risk of disease relapse) [5].

1. Engraftment: Neutrophil and platelet engraftment or recovery are one of the earliest measurements of post-HCT recovery. The traditional definition for neutrophil engraftment is the first of 3 consecutive days with an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9/L$, after the post-transplantation nadir. Platelet engraftment is defined as the first of 7 consecutive days with a platelet count $\geq 20 \times 10^9/L$ without platelet transfusion. They may be described as median time to engraftment per the above definition, and also as cumulative incidence of engraftment at various time points (e.g., day +30). Early death is a potential competing factor for engraftment when calculating cumulative incidence [3,4]. The failure to achieve a sufficient neutrophil count after HCT results in graft failure.

The above definitions are broadly applicable to ablative conditioning for allogeneic and autologous HCT. However, it should be noted that in non-myeloablative conditioning, the lowest count may remain higher than the above described nadir for neutrophils and, more commonly, platelets, in which case engraftment maybe considered to have occurred on day +1. Post-transplant chimerism may also be utilized in this setting to describe the proportion of hematopoietic elements of donor origin. Chimerism testing involves identifying the genetic profiles of the recipient and of the donor hematopoietic elements by analyzing genetic polymorphisms called short tandem repeat (STR) loci. Traditionally utilized definitions include (i) graft rejection (<5% donor chimerism), (ii) mixed chimerism (5–95% donor chimerism) and (iii) full chimerism (>95% donor chimerism) [6]. It is important to differentiate between unsorted chimerism (all hematopoietic elements) from sorted chimerism, where the chimerism for each hematopoietic element is separately reported (e.g., myeloid *versus* T-lymphocytes).

2. Graft-*versus*-host disease (GVHD): Applicable only to allogeneic HCT, GVHD is one of the most important post-transplant outcome measures. Historically divided into acute and chronic GVHD, based on whether symptoms arise before or after day +100 post-HCT, the current consensus is to classify it based on distinctive clinical features [7]. Grading of acute GVHD in most retrospective studies is based on the modified Glucksberg criteria, which divide acute GVHD into grades I to IV [8]. Grade II-IV acute GVHD is considered clinically significant, and grade III-IV is considered severe acute GVHD. Grading of chronic GVHD into limited or extensive is based on the modified Seattle criteria [9]. The cumulative incidence of acute and chronic GVHD are usually reported with death of the subject or disease relapse considered as competing risk factors.
3. Relapse incidence: Relapse or progression of underlying malignancy is an important measure of failure for any treatment approach. Definitions of relapse/progression vary depending on the underlying malignancy under consideration. For

practical purposes, in acute leukemia, one can define relapse as disease recurrence and appearance of leukemic blasts in the peripheral blood or bone marrow (>5%) after previously achieving a morphological remission. For patients undergoing transplantation without achieving a prior remission, presence of active disease at the time of hematological recovery would be considered as progression of disease. Details of post-relapse therapy are not a requisite for describing relapse incidence. The latter is represented as cumulative incidence, with death from any cause other than relapse being the competing risk factor.

4. Non-relapse mortality: Death from any cause other than disease relapse is defined as non-relapse mortality (NRM). NRM is a composite of complications of the transplantation, that is, treatment related mortality, as well as death from other causes (e.g., suicide). A cumulative incidence calculator with disease relapse being a competing factor is used to depict NRM.
5. Disease-free survival: The most commonly described composite survival outcomes measuring efficacy of the therapeutic strategy are disease-free or progression-free survival (DFS or PFS). Leukemia-free survival (LFS) is used interchangeably with DFS/PFS in studies focusing on leukemia. The Kaplan-Meier method is used for calculation of DFS. DFS is calculated as time from day of hematopoietic cell infusion to disease relapse, death from any cause or last documented follow-up. Other measures of treatment failure that may be considered include time to tumor progression (TTP).
6. Overall survival: A composite end-point measuring survival after the proposed intervention is overall survival (OS). In OS calculation, patient status as alive *versus* dead alone is considered, without accounting for therapeutic failure. OS is calculated as time from day of hematopoietic cell infusion to death from any cause or last documented follow-up.
7. GVHD-/relapse-free survival: A relatively new composite end-point in allogeneic HCT research that is considered to measure the ideal post-transplant recovery without ongoing transplant related morbidity is GVHD-free/relapse-free survival (GRFS) [10]. A refined definition of GRFS thought to better represent registry-based studies was proposed by Ruggeri *et al.* and is now incorporated in many studies. The refined GRFS is defined as survival without the following events: grade III-IV acute GVHD, severe chronic GVHD, disease relapse or death from any cause after allogeneic HCT [11]. Other end-points in this category include off-immunosuppression/relapse free survival (IRFS) and chronic GVHD/relapse free survival (CFRS) [12].

4. STATISTICAL ANALYSIS

As noted above, composite survival outcomes (DFS, OS and GRFS) are estimated by the Kaplan-Meier method. Cumulative incidence functions are used to estimate acute GVHD, chronic GVHD, relapse incidence and NRM. A competing risks model is utilized in the calculation of cumulative incidence as noted under the respective subsections. It may be pointed out that an exhaustive and excellent review of statistical methodology suggested for EBMT studies was published in 2013 by Simona Iacobelli on behalf of the EBMT Statistical Committee [5]. We describe the salient highlights

of statistical methods below and recommend readers to review the above-mentioned manuscript for a more comprehensive and in-depth understanding.

Univariate analysis is performed using the log-rank test for GRFS, OS and DFS, and Gray's test for cumulative incidence. For subgroups characteristics, comparisons are most often made by using non-parametric tests: the χ^2 test for categorical variables and the Mann-Whitney or Kruskal Wallis test for continuous variables. Multivariate analyses are performed using either the Cox proportional-hazards model or the Fine-Gray model for competing events [5]. Patient-, disease- and transplant related variables to be included in the final model are dependent on the specific proposal (e.g., disease status, age at transplant, graft source, conditioning regimen, etc.). The significance level is fixed at 0.05, and *P*-values are two-sided.

5. CONCLUSION

Here we have compiled most of the “Scientific Methods” including endpoints with definitions and statistical methods routinely used in observation and registry-based studies in HCT. The intent of this manuscript is to be considered as a source document for reference for future studies. Standardized definitions and methodology as presented here will allow uniformity across studies. This white paper may be considered the document of reference for citation purposes, potentially replacing (if needed) or complementing the scientific methods section of future observational reports in HCT. This ought to avoid redundancy, thus opening availability of space for elaborating on results and discussions pertaining to the study. It is recognized that new scientific methodologies may be introduced in the future and, so, periodic updates to this manuscript may be in order, as deemed appropriate. Additionally, authors are always encouraged to provide additional references and descriptions of methodologies which are not covered in this manuscript.

CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

ASK and BS designed and wrote the first draft. All authors reviewed, edited and approved the final draft.

ACKNOWLEDGMENT

The authors would like to acknowledge with gratitude the statistical expertise and advice provided by Prof. Myriam Labopin.

REFERENCES

- [1] LeMaistre, CF, Farnia, S, Crawford, S, McGuirk, J, Maziarz, RT, Coates, J, *et al.* Standardization of terminology for episodes of hematopoietic stem cell patient transplant care. *Biol Blood Marrow Transplant* 2013;19:851–7.
- [2] Majhail, NS, Giral, S, Bonagura, A, Crawford, S, Farnia, S, Omel, JL, *et al.* Guidelines for defining and implementing standard episode of care for hematopoietic stem cell transplantation within the context of clinical trials. *Biol Blood Marrow Transplant* 2015;21:583–8.
- [3] Logan, BR, Zhang, M-J, Klein, JP. Regression models for hazard rates *versus* cumulative incidence probabilities in hematopoietic cell transplantation data. *Biol Blood Marrow Transplant* 2006;12:107–12.
- [4] Kim, HT, Armand, P. Clinical endpoints in allogeneic hematopoietic stem cell transplantation studies: the cost of freedom. *Biol Blood Marrow Transplant* 2013;19:860–6.
- [5] Iacobelli, S, On behalf of the EBMT Statistical Committee. Suggestions on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2013;48:S1–S37.
- [6] Baron, F, Sandmaier, BM. Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Leukemia* 2006;20:1690–700.
- [7] Filipovich, AH, Weisdorf, D, Pavletic, S, Socie, G, Wingard, JR, Lee, SJ, *et al.* National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-*versus*-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005;11:945–56.
- [8] Przepiorka, D, Weisdorf, D, Martin, P, Klingemann, H, Beatty, P, Hovs, J, *et al.* 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995;15:825–8.
- [9] Lee, SJ, Vogelsang, G, Flowers, M. Chronic graft-*versus*-host disease. *Biol Blood Marrow Transplant* 2003;9:215–33.
- [10] Holtan, SG, DeFor, TE, Lazaryan, A, Bejanyan, N, Arora, M, Brunstein, CG, *et al.* Composite end point of graft-*versus*-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood* 2015;125:1333–8.
- [11] Ruggeri, A, Labopin, M, Ciceri, F, Mohty, M, Nagler, A. Definition of GvHD-free, relapse-free survival for registry-based studies: an ALWP-EBMT analysis on patients with AML in remission. *Bone Marrow Transplant* 2015;51:610.
- [12] Pasquini, MC, Logan, B, Jones, RJ, Alousi, AM, Appelbaum, FR, Bolaños-Meade, J, *et al.* Blood and marrow transplant clinical trials network report on the development of novel endpoints and selection of promising approaches for graft-*versus*-host disease prevention trials. *Biol Blood Marrow Transplant* 2018;24:1274–80.