STATISTICAL ANALYSIS PLAN

Evaluating associations between antibiotic exposures and graft-versus-host disease in hematopoietic stem cell transplant recipients

Version History

Version	Date	Reason for Update	Time Stamp
V1.0	MAR-22-2019	Finalized initial SAP (adult analysis)	MAR-22-2019
V2.0	SEP-04-2019	Addition of pediatric HSCT recipients	SEP-04-2019
V3.0	OCT-21-2019	Primary outcome has been modified to be liver and/or gut GVHD. This allows the date of onset to be specific to the outcome (i.e. different date of onset in skin GVHD vs gut/liver GVHD).	OCT-21-2019

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BACKGROUND

Hematopoietic stem cell transplantation (HSCT) is used to treat a variety of malignant and non-malignant conditions in children and adults.[1] In autologous HSCT, the recipient's own stem cells are transplanted, while the stem cells are taken from a genetically-similar donor in allogeneic HSCT. Despite optimization of histocompatibility matching using high-resolution DNA typing and prophylaxis with immunosuppressive medications, graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality among allogeneic HSCT recipients.[2,3] GVHD is characterized by damage of epithelial surfaces by T cells recognizing host tissue antigens.

In the past, broad-spectrum antibiotic usage in allo-HSCT recipients was believed to be protective against GVHD, prompting clinicians to administer such combinations in order to reduce the gut microbial burden. However, several recent studies suggest that exposure to anaerobic antibiotics may increase GVHD risk, presumably through alteration of the gut microbiome. Most notably, Shono et al. reported that antibiotics with an anaerobic spectrum of activity (e.g. imipenem-cilastatin, piperacillin-tazobactam) were associated with higher 5-year GVHD-related mortality.[4] These findings were supported by murine experiments demonstrating pathological evidence of GVHD in the colon of mice who underwent allogeneic HSCT and were treated with piperacillin-tazobactam.[4]

RESEARCH DESIGN & METHODS

A. Data Source and Procedures

This research will make use of databases maintained by the Duke University Pediatric Blood and Marrow Transplant Program and the Department of Hematologic Malignancies and Cellular Therapy at Duke University, as well as the Duke Enterprise Data Unified Content Explorer (DEDUCE) - a web-based environment that allows Duke-based investigators access to administrative, financial, and clinical information generated during patient care within the Duke University Health Care System.[5] The data elements that will be reviewed include demographics (e.g. sex, date of birth), diagnoses (e.g. GVHD), procedures (e.g. infusion of stem cells), and medication orders (e.g. antibiotics). Data will be exported from the transplant program databases or DEDUCE as excel and csv files and stored on the study's secure, web-based database (REDCap) on servers hosted by Duke University. Data within these files will be analyzed using SAS (Statistical Analysis System; SAS Institute, Cary, NC). Only study investigators will have access to these files through the REDCap database. Moreover, all analyses of data contained within these files will be performed on an encrypted computer, and no files with protected health information will be saved and stored anywhere other than within the REDCap database.

B. Study Participants and Methods

B.1. Study Subjects

Eligible subjects will have undergone their first allogeneic HSCT between January 1, 2005 and December 31, 2016. Patients who died prior to or on day +28 after HSCT and patients with any diagnosis of GVHD prior to onset of febrile neutropenia will be excluded. Patients will be classified into the following mutually-exclusive groups: 1) received an anaerobic antibiotic regimen (piperacillin-tazobactam OR a carbapenem) for a febrile neutropenia episode; 2) received a non-anaerobic antibiotic regimen (ceftazidime AND/OR cefepime AND/OR aztreonam) for febrile neutropenia and did not receive an anaerobic antibiotic regimen; 3) received only another antibiotic regimen for febrile neutropenia; or 4) did not receive antibiotics for a febrile neutropenia episode. Patients in groups 3) and 4) will not be included in the analyses presented herein.

B.2. Exposures

We will record antibacterial agents received by patients between days -7 and +28 in relation to allogeneic HSCT. Medication data will be recorded based on review of medication orders and progress notes in the electronic medical record and the transplant program databases. Antibacterial agents that were administered for febrile neutropenia and received by at least 5% of the study population will be recorded.

B.3. Primary Outcome

Diagnoses of and deaths from acute and chronic GVHD will be determined based upon review of transplant program databases and electronic medical records using a standardized data collection form. Acute GVHD diagnoses will be determined using criteria developed by Glucksberg et al. and later refined at the 1994 Consensus Conference on Acute GVHD Grading.[6,7] The primary outcome will be acute GVHD of the gut or liver (regardless of stage).

B.4. Secondary Outcomes

Secondary outcomes will include: skin GVHD diagnosis, acute GVHD mortality, chronic GVHD diagnosis, and chronic GVHD mortality.

B.5. Hypothesis

Primary hypothesis: receipt of an antibiotic regimen with an anaerobic spectrum of activity (piperacillin-tazobactam OR carbapenem) will be associated with a higher hazard of acute gut/liver GVHD than receipt of only a non-anaerobic antibiotic regimen (ceftazidime AND/OR cefepime AND/OR aztreonam).

Secondary hypotheses: receipt of an antibiotic regimen with an anaerobic spectrum of activity (piperacillin-tazobactam OR carbapenem) will be associated with:

- acute skin GVHD
- 1-year acute GVHD mortality
- chronic GVHD diagnosis
- 5-year chronic GVHD mortality

B.5. Statistical Analysis

We will describe characteristics of the study population using frequencies and percentages for categorical variables and medians and interquartile ranges for continuous variables. Our primary analysis will use Cox proportional hazards regression to compare the hazard of acute gut/liver GVHD in patients who received an anaerobic antibiotic regimen (group 1) to patients who received a non-anaerobic antibiotic regimen only (group 2). This will be an adjusted model including the following covariates: year of HSCT, age, sex, race, HSCT indication, HSCT donor/source, HLA matching, preparative regimen intensity, and GVHD prophylaxis regimen. All covariates will be assessed at the time of the HSCT, and therefore will not be time-varying. Unadjusted Cox proportional hazard models will be run as a sensitivity analysis.

To ensure confidence in our models, we will assess the proportional hazards assumption by graphing the log(-log(survival)) curve of the Kaplan-Meier function. If the lines are parallel, the proportional hazard assumption will be met. This will be performed for each covariate. Additionally, the Schoenfeld residuals will be produced for each covariate. A scatterplot of each of the Schoenfeld residuals and time will be assessed for a linear relationship. If the slope of the Schoenfeld residuals over time is statistically different from 0, there will be evidence of non-proportionality for that covariate. If either the Schoenfeld residuals or

the log-minus-log curve suggest evidence that the proportional hazards assumption is violated for a continuous covariate (e.g. age at time of HSCT), an extended Cox regression model will be run. The covariate will be stratified into piecewise functions, and there will be multiple hazard ratios calculated for each piecewise function. If the proportional hazards assumption is violated in a categorical covariate, a stratified model will be used. For acute GVHD outcomes, patients will be censored at any of the following time points: 1) on the date of death; 2) on the day prior to the date of a second allogeneic HSCT; 3) 1 year after HSCT; 4) on the date of the last encounter within the Duke University Health System. For chronic GVHD outcomes, patients will be censored at any of the following time points: 1) on the date of death; 2) on the day prior to the date of a second allogeneic HSCT; 3) 5 years after HSCT; 4) on the date of the last encounter within the Duke University Health System; 5) at the end of the study period (December 31, 2018).

We will then evaluate out primary outcome with anaerobic antibiotic exposure for febrile neutropenia considered as a continuous variable (i.e. calendar days of exposure to antibiotics with anaerobic activity). As such, if patients received two anaerobic antibiotics from the same group on a specific day (e.g. piperacillin-tazobactam AND imipenem), this would count as one unique day of anaerobic antibiotic therapy. Also, if an anaerobic antibiotic is started and stopped on the same calendar day, the patient will be recorded as having one unique day of anaerobic antibiotic exposure.

Finally, we will evaluate for associations between patient group and each of our secondary outcomes using the same approach. There will be a total of 4 secondary outcomes, and each will be tested with the categorical predictor (patient antibiotic exposure group) and the continuous predictor. Therefore, there will be 8 secondary hypotheses. To account for multiple testing, the Bonferroni correction will adjust the significance level of the secondary hypotheses. The survival interval will be defined as the time between HSCT and the date of the GVHD outcome or censoring event. For outcomes related to GVHD mortality, we will additionally run Cox models that consider competing risks from other common causes of mortality. Common mortality causes will be grouped into categories; there will be an ‘other’ category that contains rare causes of death in this dataset. The Lunn-McNeil approach will be used, and it will be assumed that competing risks are independent from each other. These analyses will be performed as a sensitivity analysis, and the competing risk hazard ratios will not be presented if the interpretation is the same as the traditional Cox proportional hazards models.

References

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