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Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimisation strategies in haematopoietic stem cell transplant recipients: study protocol for the prospective multicentre OptimBioma study

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ABSTRACT

Introduction Haematopoietic stem cell transplantation (HSCT) is a life-saving treatment for a number of haematological diseases. Graft versus host disease (GVHD) is its main complication and hampers survival. There is strong evidence that intestinal microbiota diversity of the recipient may increase the risk of GVHD worsening survival. Antibiotic regimens used during the early phase of the transplant may influence clinical outcomes by reducing intestinal microbiota diversity. Present guidelines of European Conference on Infections in Leukaemia exhort to optimising antibiotic use in haematological patients including HSCT recipients. The present study aims to investigate if, in HSCT recipients, the optimisation of antibacterial use may preserve intestinal microbiota composition reducing the incidence and severity of acute GVHD and improving relevant clinical outcomes.

Methods and analysis This is a prospective longitudinal observational study of two cohorts of HSCT recipients: (1) the intervention cohort includes patients treated in centres in which a predefined strategy of antibiotherapy optimisation is implemented, with the objective of optimising and reducing antibiotic administration according to clinical criteria and (2) the control cohort includes patients treated in centres in which a classic permissive strategy of antibiotic prophylaxis and treatment is used. Adult patient receiving a first HSCT as a treatment for any haematological condition are included. Clinical variables are prospectively recorded and up to five faecal samples are collected for microbiota characterisation at prestablished peritransplant time points. Patients are followed since the preconditioning phase throughout 1-year post-transplant and four follow-up visits are scheduled. Faecal microbiota composition and diversity will be compared between both cohorts along with acute GVHD incidence and severity, severe infections rate, mortality and overall and disease-free survival.

Ethics and dissemination The study was approved between 2017 and 2018 by the Ethical Committees of participant centres. Study results will be disseminated through peer-reviewed journals and national and international scientific conferences.

Trial registration number NCT03727113

Strengths and limitations of this study

- First-in-class prospective comparative observational multicentre study addressing the effect of two different (centres driven) antimicrobial strategies (optimised vs standard antimicrobial use) on intestinal microbiota diversity, acute graft versus host disease and survival in haematopoietic stem cell transplant recipients.
- Robust design by systematic collection of faecal samples at predetermined peritransplant time points and prospective collection of a wide relevant clinical data set throughout 1-year follow-up with scheduled clinical visits.
- Non-randomised design (for security reasons) with propensity score matching statistical approach to reduce possible bias by confounding variables.
- No causal mechanistic association could be accurately concluded although meaningful cause–effect relationships should be advanced.

INTRODUCTION

Allogeneic haematopoietic stem cell transplantation (HSCT) is a life-saving treatment

for many severe haematological disorders. However, the deep immunosuppression associated to the procedure results in high risk of infectious complications prompting the administration of antimicrobial prophylaxis and therapy. Antibiotics fight off pathogenic bacteria but at the same time they may damage intestinal commensal bacteria leading to changes in intestinal microbiota composition and reduced diversity.[1] There is evidence that loss of intestinal microbiota diversity during HSCT may cause an increased risk of acute graft-versus-host disease (GVHD) worsening of short and long-term clinical outcomes.

Pioneering preclinical studies suggested that selective intestinal decontamination with antibiotics could reduce the incidence of GVHD[2 3] leading to the clinical use of pretransplant antibacterial prophylaxis. These expectations were not subsequently confirmed in the clinical setting and this practice is not currently performed in many transplant centres. However, recent studies suggest that changes in intestinal microbiota composition may play an important role in the development of GVHD and in clinical outcomes of HSCT.[1 4 5]

Therefore, antibacterial therapy strategies currently used in HSCT clinical practice should be re-evaluated in order to avoid as much as possible intestinal microbiota misbalance.[6–8] Our group has recently demonstrated in an academic prospective multicentre randomised clinical trial (How-Long study),[9] that in haematological patients (including HSCT recipients) with febrile neutropenia it is safe to discontinue empirical antibiotic therapy after resolution of fever when patients are clinically stable, irrespective of their neutrophils counts, significantly reducing exposure to antibiotics. On the other hand, the ECIL group (European Conference of Infections in Leukaemia) has proposed[10] specific empirical antibacterial therapy strategies in haematological patients including HSCT recipients, in order to optimised antibiotic use. These recommendations are heterogeneously implemented in the different haematopoietic transplant centres. This study will investigate if a predefined strategy of optimisation of antimicrobial therapy that includes ECIL recommendations[10–12] and the How-Long study,[9] will preserve intestinal microbiota composition and diversity while reducing the incidence and severity of acute GVHD when compared with a conventional permissive antibiotic strategy. In addition, severe infections rate, transplant-related mortality and long-term survival will be compared between both groups.

METHODS AND ANALYSIS

Study design

A prospective longitudinal observational study of two cohorts of HSCT recipients was established: (1) the intervention cohort includes patients treated at centres using an optimised strategy of antibacterial therapy (see Intervention section), (2) the control cohort includes patients treated at centres using a classical permissive antibacterial therapy strategy (see Intervention section). Each participating centre is allocated in one of the two cohorts according to its clinical practice.

Study settings

Multicentre study conducted at five academic hospitals in Spain, two allocated to the intervention cohort (Virgen del Rocío University Hospital (Seville) and Marqués de Valdecilla University Hospital (Santander) and three to the control cohort (Valencia Clinic Hospital (Valencia), Salamanca University Hospital (Salamanca), Gregorio Marañón University Hospital (Madrid)). A 4-year study period is estimated (2017–2020).

Participants

Eligibility criteria

Inclusion Criteria

- Adult patients admitted to receive their first allogeneic haematopoietic transplant as a treatment for any haematological disease.
- Patients who have signed the study informed consent to participate.
- Patients who have received a previous autologous transplant are not excluded.

Exclusion criteria

- Non-compliance of the patient to sign the informed consent.
- Patients who have initiated the conditioning regimen previously to entering the study will not be included.

Recruitment process

Patients who meet the eligibility criteria and sign the informed consent will be recruited by investigators of the haematology services on the participating sites.

Intervention

Centres allocated to the intervention cohort use an antibacterial systematic approach that includes the following strategies:

1. No routine antibacterial prophylaxis is used.
2. In case of febrile neutropenia:
   - Use of scalation/de-scalation strategy for empiric antimicrobial therapy.[10]
   - Directed simplification in patients with etiological diagnosis according to in vitro susceptibility tests.
   - Switch to a narrower-spectrum agent in patients without an etiological diagnosis and clinical stabilisation on treatment.
   - No broadening the antibacterial spectrum but maintenance of initial antimicrobials therapy in patients with persistent fever if they are clinically stable in the criteria of the physicians in charge of theam. Switch to a narrower-spectrum agent in patients without an aetiological diagnosis and clinical stabilisation on treatment
3. Early (in 72 hours) withdrawal of combined treatments, when clinically indicated.

4. Antibacterial therapy withdrawal regardless neutrophils count and expected duration of neutropenia when patient meets all the following criteria (How-Long strategy).
   i. Afebrile for ≥72 hours.
   ii. Complete resolution of signs, symptoms and alterations in complementary tests secondary to the infection (cough, abdominal pain, diarrhoea, pulmonary infiltrate, etc) for ≥72 hours.
   iii. Normal vital constants (blood pressure, heart rate, respiratory rate and diuresis and, in patients with respiratory involvement, oxygen saturation by pulse oximetry) for ≥72 hours.
5. Short (7 days) aetiological therapy for primary or related to central venous (with catheter removal) no complicated bacteraemia, and 14 days for *Staphylococcus aureus* non complicated bacteremia if good clinical response and good clinical evolution.

In centres allocated to the control cohort the antimicrobial therapy approach does not include any of the strategies used in the optimisation cohort but the following management:

1. Use of antibacterial prophylaxis: levofloxacine 500 mg/24 hours (PO) or ciprofloxacine 500 mg/24 hours (PO) since the start of conditioning or day 0, until neutrophils count in peripheral blood is ≥0.5×10E9/L or empiric antimicrobial therapy is started.
2. In case of febrile neutropenia:
   - Use of early broad-spectrum antimicrobial therapy without systematic use of escalation/de-escalation strategy.
   - Optional antibiotic simplification in patients with etiological diagnosis according to in vitro susceptibility tests.
   - No switching to a narrower-spectrum agent in patients without etiological diagnosis and clinical response.
   - Broadening the spectrum of initial antimicrobials in patients with persistent fever even without clinical worsening.
3. No early discontinuation of the combined empirical antimicrobial therapy even in case of clinical response.
4. No discontinuation of empirical antimicrobial therapy until neutropenia recovery.
5. Prolonged aetiological treatment for primary or related to central venous catheter no complicated bacteraemia, even in case of early clinical response.

Schedule of visits and collection of faecal samples

The scheduled visits and assessments are described in table 1.

Follow-up is organised in four planned visits: visit 1 (7 days pretransplant), visit 2 (end of antimicrobial therapy or hospital discharge, whichever occurs first), visit 3 (100 days post-transplant) and visit 4 or final visit (1-year post-transplant or mortality, whichever occurs first). A minimum of four faecal samples will be collected: specimen 1, day of starting the conditioning treatment ±48 hours; specimen 2, day of transplantation ±48 hours; specimen 3, day +7 post-transplant ±24 hours; specimen 4, when the first episode of fever at any time from the beginning of the conditioning until the end of antimicrobial therapy or hospital discharge, whichever occurs first (this sample is collected at fever onset or within 48 hours, unless the fever starts on the same day that a scheduled faecal sample is already collected); and specimen 5, day when the antimicrobial therapy is stopped (or within the following 48 hours) or, alternatively, if the patient did not receive antibiotics or continues receiving antibiotics at discharge, the day before discharge (or 24 hours in advance).

The stool samples will be collected at different time points (table 1) in Stool Nucleic Acid Collection and Preservation Tubes (Ref. 45660, Norgen Biotek).

DNA extraction, prokaryotic 16S ribosomal RNA gene (16S rRNA) sequencing and bioinformatics analysis

The microbiome studies will be performed at the laboratory of Infectious Diseases of the Institute of Biomedicine of Seville.

DNA will be extracted from faecal samples using the Stool DNA Isolation kit (Cat. 27600, Norgen Biotek, Ontario, Canadá) according to the manufacturer's

Table 1 Schedule of enrolment and assessments

	VISIT 1 (7 days pretransplant ±48 hours)	Day of transplant (day 0) (±48 hours)	7 days post-transplant (day +7) (±24 hours)	Day of fever onset (if fever occurs) (+48 hours)	VISIT 2 (End antibiotherapy or discharge)*	VISIT 3 (100 days post-transplant) Day +100	VISIT 4 (1-year post-transplant or exitus letalis ±1 week)
Inclusion/exclusion criterion	X						
Signature of informed consent	X						
Clinical data collection	X				X	X	X
Faecal sample collection	Specimen 1	Specimen 2	Specimen 3	Specimen 4	Specimen 5*		

OptimBioma study.
*End of antibiotic therapy or discharge, whichever occurs first.

protocol. All DNA samples will be stored at −20°C until further processing. DNA will be quantified by Qubit fluorometry (Invitrogen Qubit 4 Fluorometer, ThermoFisher Scientific, Spain) and normalised to 5 ng/µL with 10 mM Tris pH 8.5. Bacterial 16S rRNA amplification and library construction will be performed according to the 16S Metagenomic Sequencing Library Preparation guide from Illumina. Briefly, 2.5 µl of total DNA per sample will be amplified using primers targeting the 16S rRNA V3 and V4 regions.[13 14] These regions provide ample information for taxonomic classification of microbial communities. Pooled V3–V4 amplicon libraries will be sequenced using the Illumina MiSeq platform with a V3 reagent kit (Illumina, San Diego, California) and paired-end 300 bp reads. Up to 96 libraries could be pooled together for sequencing. Regarding the bioinformatics analysis of the sequencing data, machine learning libraries from Scikit-learn[15] will be used to filter out and discard poor-quality reads. Processed sequences will be subjected to operational taxonomic unit (OTU) picking against Greengenes (V.13.8),[16] with reads clustered by 97% identity into OTUs using QIIME 2.[17] In-house R scripts (v3.2.2) will be used to visualise the results.

Evaluation of results

In order to assess the impact of both antimicrobial strategies on intestinal microbiota diversity it will be characterised as described in the previous section and biological alpha and beta diversity indexes of samples from both cohorts will be compared.[18] Alpha-diversity and beta-diversity refer to diversity within and between samples, respectively. These secondary bioinformatics analyses will be performed with QIIME 2 and included the calculation of the parameters of alpha-diversity: Shannon's diversity index, frequency of observed OTUs, Faith's Phylogenetic Diversity, and Evenness; and the parameters of beta-diversity: Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance and Unweighted UniFrac distance.

In order to asses clinical outcomes (secondary objectives of the study) the following data will be prospectively recorded: incidence and severity of GVHD (evaluated for any degree, degree-II and degree-III/IV up to day 100 post-transplant), transplant-related mortality and mortality caused by infection (time frame: day 0 to day+30, day+100 and day+365 post-transplant), incidence of severe infections (day 0 to day+30 post-transplant), and overall and disease-free survival (time frame: day 0 to day+30, day+100 and day+365 post-transplant).

Sample size

Assuming a percentage of patients developing grade two or higher acute GVHD[19] in the control cohort of 42%[5] and 20% in the intervention cohort, a power of 80% and an alpha error of 5%, 90 patients in each study cohort should be enough to detect differences between them. However, 100 patients per cohort will be included in order to compensate possible loss of statistical power due to associations between centres and the effect of the optimisation strategy versus standard antimicrobial use (unknown at the time of the study design), to increase the statistical power for the secondary objectives (transplant-related mortality, infections rate, mortality and survival), along with potential withdrawals.[20–22]

Statistical analysis

To determine the impact of both antimicrobial therapy strategies on the intestinal microbiota diversity, in a first step, a multidimensional scaling and a permutational multivariate analysis of variance analyses of both antibiotherapy strategies will be performed using the R statistical package (V.3.2.2).[23] To find out which taxa were most likely to explain the differences between both groups, taxa summaries generated in QIIME 2 will be reformatted for input into LEfSe via the Huttenhower Lab Galaxy Server (https://huttenhower.sph.harvard.edu/galaxy/root). This algorithm performs non-parametric statistical testing of whether individual taxa differed between both groups and then differentially ranked the abundant taxa by their linear discriminate analysis (LDA) log-scores. Differentially abundant taxa that are statistically significant using an alpha error of 5% and LDA log-scores exceeding ±2.0 will be visually represented as bar plots. The median values of taxa abundance and the median percentages of taxa presence in both groups will be calculated, and the Manhattan distances will be used for the clustering analysis. The Kruskal-Wallis rank-sum test will be used to identify significant taxa abundance and Fisher's exact test will be used to identify significant taxa presence in the both groups.

The propensity score will be used to adjust potential confounding effects. To calculate the propensity scores in the logistic model the centre factors and key predictive characteristics identified in the baseline comparability analysis will be taken into account. Propensity scores will be used for all adjusted inferential analyses.

Standard descriptive statistical indices will be used according to the nature of each variable. Continuous variables will be analysed with linear models, binary variables without time factor with logistic models and the time-to-event variables with survival models, all of them incorporating the propensity score as an adjustment factor.

The survival function of both groups will be described using the Kaplan-Meyer method. For the inferential analysis, the stratified log-rank test will be used (with the propensity score value categorised as stratum). HRs and its 95% CIs will be estimated using the Cox proportional hazards regression (including the propensity score value).

The following strategy will be used for time-dependent variables:

1. Continuous variables that follow a Gaussian distribution by means of mixed models for repeated measures (mixed longitudinal model for repeated measurements.

2. Variables that do not comply with the parametric assumptions will be transformed into ranges and analysed analogously to those in section a).
3. Longitudinal binary data will be analogously analysed with the marginal models (generalised estimation equation).

In addition, the following statistical tests will be used when necessary: Fisher's exact test to compare categorical variables between groups, McNemar test or Cochran Q test for the analysis within the groups, dependent or independent t-test for continuous variables when comparing two groups and analysis of variance if comparing more than two groups.

Non-parametric methods will be used in case of deviations from the applicability assumptions: according to the data distribution, Mann-Whitney and/or Kruskall-Wallis tests (independent variables) or Wilcoxon or Friedman tests (dependent variables). Correlations will be done with Pearson or Spearman coefficients according to the data distribution. SAS System (V.9.2) or validated equivalent software will be used. All recruited patients will be included in the main analysis. In addition, a sensitivity analysis will be carried out with those subjects who have complied with the protocol.

Patient and public involvement

Neither patients nor public were involved in the development of the study.

DISCUSSION

The aim of this study is to prospectively investigate if an antimicrobial therapy in HSCT recipients has lesser impact on the intestinal microbiota composition and diversity than a non-restrictive standard antimicrobial therapy approach and if it correlates with a decrease incidence and severity of acute GVHD leading to improved clinical outcomes as reduced transplant related mortality, severe infections rate and improved survival. If this hypothesis proved to be certain, current antibacterial strategies in HSCT setting may need to be fully reviewed in order to avoid decrease the intestinal microbiota diversity. A prospective longitudinal observational study of two cohorts of patients will be used to address these objectives. The intervention cohort includes patients treated at two centres in which the antimicrobial therapy approach is optimised according to clinical criteria (as specified at intervention paragraph) and the control cohort includes three centres in which the classic management of antimicrobial therapy treatment is used (also specified at intervention paragraph).

One limitation of the study is a non-randomised design. Randomised controlled trials (RCTs) are widely considered the design of choice for the assessment of effectiveness of healthcare intervention as the randomisation process makes the comparison groups equal with respect to both known and unknown prognostic factors at baseline.[24] Nevertheless, RCT design is not applicable in this study. The implementation of a whole antibacterial therapy strategy in this frail setting of patients requires that it is solidly grounded in the daily practice of the clinical team, in order to be safe. The randomisation scenario would implicate the use of unfamiliar antibacterial strategies by the clinical teams. This would be unsafe for patients and then ethically inadmissible. Therefore, an observational study carried out in two groups of centres in which one of the two antibiotherapy approaches is already implemented turns out to be the safest and more feasible design. The propensity score matching statistical technique will reduce the possible bias due to confounding variables.

Another limitation of the study is that no causal association could be accurately described because of the observational design. Nevertheless, as an exhaustive set of prospective clinical data are being recorded for each patient, including start and stopping date of every antimicrobial used, dates of start and resolution of main clinical end points it is likely that meaningful cause–effect relationships might be forwarded.

This is the first prospective multicentre study aiming to address the effect of two antimicrobial therapy strategies on intestinal microbiota and clinical outcomes in HSCT recipients. The systematic collection of faecal samples at predetermined peritransplant time points and the prospective collection of a wide clinical data set with 1-year follow-up based in prescheduled repeated clinical visits will allow to examine changes in the microbiota composition over time and accurate link them to the development of acute GVHD and other clinical outcomes. Another strength of the study is its design based on daily clinical practice. This will provide valuable data on 'real-life patients' in addition to potential recommendations on sampling time points and frequency for further studies. In conclusion, the findings of this study will bring useful insight in the relationship between antibiotherapy use and development of acute GVHD in HSCT recipients helping to design improved strategies expectedly leading to better survival, reduced GVHD and improved quality of life.

Trial status

At submission the study is running and 140 patients are recruited.

Current approved protocol is V.3.0, dated 29/January/2018.

Date recruitment began at 16 January 2018 (first patient in).

Approximate date when recruitment will be completed: November 2019.

Ethics and dissemination

The study was approved between 2017 and 2018 by the five Ethical Committees involved (Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, Comité de Ética de Investigación Clínica de Cantabria, Comité Autonómico de Evaluación de Estudios Posautorización Observacionales, de Seguimiento Prospectivo con medicamentos, Comité de Ética de la investigación con medicamentos del Hospital Univesitario de Salamanca,

Dirección General de Inspección y Ordenación de la Consejería de Sanidad de la Comunidad de Madrid). Each substantial protocol amendment will be notified for approval to the relevant ethics committee(s) prior to implementation. All data collected will be kept strictly confidential and in accordance with all relevant legislation on control and protection of personal information. Study results will be published in peer-reviewed journals as well as national and international scientific conferences.

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Contributors IE conceived, designed and lead the study. IE and CMR-F obtained funding for the research. SJ-J, GL-H, NR-T, FJM-M, MA-G, MEP-I and CMR-F collaborated in methodological aspects of the study. SJ-J, NR-T, CMR-F, MEP-I and CLR coordinated the study. IE, CS, LV-L, MK, JMMB and NR-T were responsible for the inclusion, treatment, clinical monitoring and follow-up of the patients. CLR coordinated sample collection. GL-H, MEP-I and YS were responsible for the sample management. SJ-J wrote the first draft of the manuscript. All authors were involved in critically revising the article and approved the final version.

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