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Changes in the bacterial profile and diversity of the gut microbiota in allogeneic hematopoietic stem cell transplant recipients ☆

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ABSTRACT

Objectives: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is associated with significant alterations in gut microbiota (GM) composition, affecting transplant success. This study aimed to correlate these GM changes with post-transplant (post-HSCT) outcomes.

Methods: A prospective multicentre cohort study was conducted between June 2017 and December 2021 in three Spanish hospitals. Stool samples from allo-HSCT recipients were collected before HSCT, and at 14-, 30-, 60-, and 100-days post-HSCT. Bacterial 16S rRNA gene sequences were characterized and microbial diversity assessed.

Results: Analysis of 409 samples from 95 patients revealed significant longitudinal GM shifts. Alpha diversity significantly decreased at days 14 ($P < 0.001$), 30 ($P < 0.001$), and 60 ($P = 0.002$) compared to baseline. A distinct shift in dominant taxonomic profiles was observed, notably a significant decrease in *Blautia* abundance ($P < 0.001$). Patients with acute gastrointestinal graft-versus-host disease (GI-GVHD) ($P = 0.009$), bacteraemia ($P = 0.014$), or death ($P < 0.001$) exhibited significantly lower *Blautia* levels. LEfSe analysis identified 22 differential taxa between deceased and surviving patients; the former showed higher abundance of potential pathogens such as *Enterococcus_H* ($P = 0.026$), *Enterococcus_A* ($P = 0.019$), and *Staphylococcus* ($P = 0.009$).

☆ Key point: Dysregulation of the gut microbiota composition is associated with a worse prognosis in patients receiving allogeneic hematopoietic stem cell transplantation.

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Conclusions: Significant variations in the GM's taxonomic profiles and relative abundances post-HSCT, particularly the decrease in *Blautia* and the increase in certain pathogens, are associated with poorer clinical outcomes.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a well-established therapy for bone marrow diseases [1]. Infections, graft-versus-host disease (GVHD), and hematological malignancy recurrence remain major causes of morbidity and mortality post-transplant (post-HSCT).

Recent studies have highlighted the critical role of the gut microbiota (GM), a complex ecosystem essential for maintaining human health [2–4]. The GM can be altered by numerous factors, such as diet, physical activity, and exposure to different treatments. Notably, the Mediterranean Diet, prevalent in our region and characterized by high fibre and unsaturated fat intake, has been shown to significantly impact the composition of the gut microbiome [5]. However, the potential influence of local dietary habits, alongside other regional factors, on the GM composition in allo-HSCT recipients remains underexplored. It is uncertain whether these factors could modulate the microbiota differently compared to cohorts from other geographic areas, where dietary and environmental conditions may vary.

On the other hand, during allo-HSCT, the gut microbiota undergoes substantial changes, leading to dysbiosis—a loss of diversity. This dysbiosis has been associated with an increased risk of systemic infections [6–10], higher GVHD frequency [11], and increased mortality [10,12,13]. Persistent dysbiosis during granulocyte recovery predicts poor overall survival and disease relapse [13–15].

Therefore, it is particularly relevant to characterize the intestinal microbiota in the context of allo-HSCT within our country to better understand its impact on clinical outcomes. In this line, the present study aimed to characterise GM composition and dynamics during the first 100 days post-HSCT, analysing its association with infectious episodes, GVHD, and mortality.

Methods

Study design and follow-up

A prospective cohort study was conducted between June 2017 and December 2021, including allo-HSCT recipients over 18 years. It was performed in three Spanish teaching hospitals: University Hospital Marqués de Valdecilla (HUMV) (coordinating centre), University Central Hospital of Asturias (HUCA), and University Hospital of Salamanca (HUSAL). All patients provided informed consent.

Follow-up extended from hospital admission to one-year post-HSCT or death. Stool samples were collected before allo-HSCT, upon the patient's admission, and at days 14, 30, 60, and 100 post-HSCT with a tolerance of ± 3 days. All centres followed standard recommendations [16], and samples were frozen at −80 °C until DNA extraction at the coordinating centre.

Clinical data on patient demographics, transplant details, and post-transplantation clinical course were collected at each visit.

Patient management protocols

Each centre employed its own antibiotic prophylaxis protocol. HUMV used none, while HUCA and HUSAL used quinolones (ciprofloxacin and levofloxacin, respectively) during the neutropenic period. No formal dietary restrictions were applied, though one centre recommended a low bacterial load diet avoiding raw meat/fish, unpasteurised cheese, and sausages.

Samples selection criteria

For data analysis and sample processing, we included patients with at least one pre-transplant sample and two collected during post-HSCT follow-up. Patients who died within 30 days post-HSCT were excluded unless at least one sample post-HSCT was available.

16S rRNA library preparation and sequencing

Stool sample DNA was extracted using the QIAamp PowerFecal DNA Kit (Qiagen, Germany), quantified with a Qubit fluorometer (Invitrogen) and stored at −20 °C until library preparation.

The V3-V4 region of the 16S rRNA gene was PCR-amplified [17]. Sample multiplexing, library purification, and sequencing followed the Illumina '16S Metagenomic Sequencing Library Preparation' guide (San Diego, CA, USA) at the molecular biology and microbiology laboratory of the Agricultural Technology Institute of Castilla and León. Libraries were sequenced on an Illumina MiSeq platform, providing 300-bp paired-end reads.

Bioinformatics and data analysis

Bioinformatics processing is detailed in the Supplementary Material.

To investigate GM dynamics, distinct sample subsets were generated: (1) Longitudinal subset: samples from baseline and days 14, 30, 60, and 100 post-HSCT; (2) Baseline subset: only baseline samples for initial microbiota composition; (3) Neutropenia subset: samples collected from transplantation to day 17.67 post-HSCT (using the median number of days for 500 neutrophil recovery as the cutoff point).

Across subsets, the inverse Simpson index was used to evaluate the richness and evenness. The Bray–Curtis dissimilarity index was used to evaluate compositional dissimilarity between microbial communities, using genus-level relative abundances (filtered at mean >0.001). Principal Coordinate Analysis (PCoA) was utilized to plot beta diversity patterns in two dimensions, and data separation was assessed using a permutation test (adonis [18] or anosim [19], based on beta dispersions). Linear discriminant analysis effect size (LEfSe) [20] was used to identify microbial biomarkers differentiating study groups.

Statistical analysis

Continuous variables were summarised as median and interquartile range (IQR) or mean ± standard deviation (SD). Categorical variables were summarised as number of observations and frequency. Normality was assessed using the Shapiro-Wilk test. Variables not exhibiting normal distribution were analysed using the Wilcoxon rank-sum test (two categorical levels) and Kruskal–Wallis test (more than two categorical levels), with FDR—corrected pairwise tests. Cumulative survival rates were estimated using

the Kaplan–Meier method and compared with the log-rank test (Mantel–Haenszel). For the multivariable analysis, a binomial generalised linear model, adjusted using AIC test with a step-backward selection, was employed. All *P*-values were considered two-sided with a significance level of 0.05. Statistical analyses were performed using either STATA SE 16 [21] or R environment version 4.3.3 (2024-02-29 ucrt) [22].

Ethical considerations

All procedures were performed in accordance with current legislation, Declaration of Helsinki principles and good clinical practice guidelines.

The study was approved by Clinical Research Ethics Committee of Cantabria (Ref: ENT-MFA-2016-01, ENTHERE-SCT; 2017.112, MICROBIOMA-SCT; and NVAL21/17, MITAPH), as reference committee, and Clinical Ethics Committee of the participating centres. Written informed consent was obtained from all patients for data use and follow-up samples collection.

Results

Characteristics of the patients

A total of 95 patients and 409 stool samples were analysed across the participating centres. The clinical and transplantation characteristics of the patients are summarized in Table 1.

Taxonomic profile of microbiota

A total of 519 faecal samples were collected from 107 allo-HSCT patients. After excluding 12 patients due to insufficient samples or bioinformatics issues, 409 samples from 95 patients were analysed. First, we characterized the GM by calculating the mean relative abundance of each taxon at the phylum and genus level across the entire sample set. The 10 most abundant phyla and genera were represented (Supplemental Figure S1), and we observed significant changes over time (Figure 1). Focusing on *Blautia* abundance, Figure 2 shows lower levels in patients with acute gastrointestinal GVHD (GI-GVHD) grade 2 or higher (mean ± SD = 0.132 ± 0.130 in non-acute GVHD, 0.108 ± 0.112 in grade ≤2 GI-GVHD, and 0.069 ± 0.090 in grade >2 GI-GVHD, $P = 0.009$), bacteraemia (0.131 ± 0.121 in patients without bacteraemia vs 0.108 ± 0.121 in whose with, $P = 0.014$), and death (0.127 ± 0.126 in survived patients vs 0.076 ± 0.090 whose died, $P < 0.001$). A detailed analysis of *Blautia* abundance across visits and according to clinical variables was included in the Supplementary Material.

To corroborate this association, a multivariable analysis adjusted for age, sex, conditioning intensity, progenitor source, hematopoietic cell transplantation-specific comorbidity index (HCT-CI) Score, and prior antibiotic treatment was performed (details on antibiotic exposure are presented in the Supplementary Material). The results remained significant after adjustment for both GI-GVHD (OR: 0.046; 95% CI: 0.003 to 0.508) and patient mortality (OR: 0.010; 95% CI: 0.000 to 0.234). Although statistical significance was not reached for bacteraemia, the observed trend was maintained (OR: 0.448; 95% CI: 0.037 to 5.427) (Figure S2).

Alpha diversity analysis

Alpha diversity was assessed at each visit, revealing significantly higher baseline diversity (mean ± SD= 19.46 ± 12.73) compared to days 14 (8.69 ± 6.78, $P < 0.001$), 30 (10.34 ± 6.97, $P < 0.001$), and 60 (13.64 ± 8.48, $P = 0.002$). A recovery in diversity was observed by day 100 (mean ± SD= 15.87 ± 7.59, $P = 0.175$) (Figure 3).

Table 1
Clinical characteristics of the study population ($N = 95$).

Characteristic	Patients, no. (%)
Sex	
Male	61 (64.21)
Female	34 (35.79)
Age at allo-HSCT (year)	
Median	53.69
Range	18.09-82.50
HCT-CI score, median [IQR]	2 [1–3]
EBMT score, mean ± SD	3.64 ± 1.27
Underlaying Disease	
Acute myeloid leukaemia	24 (25.26)
Acute lymphoid leukaemia	19 (20.00)
Myelodysplastic or myeloproliferative syndrome	15 (15.79)
Non-Hodgkin's lymphoma	10 (10.53)
Primary myelofibrosis	7 (7.37)
Hodgkin's lymphoma	5 (5.26)
Plasma cell dyscrasias	4 (4.21)
Chronic lymphocytic leukaemia	3 (3.16)
Chronic myeloid leukaemia	2 (2.11)
Other	6 (6.32)
Number of pre-treatment lines, median [IQR]	1 [1–3]
Underlaying disease status	
Complete response	45 (47.37)
Complete response with MRD+	16 (16.84)
Partial response	13 (13.68)
Stable disease	15 (15.79)
Progression	6 (6.32)
Type of conditioning	
Myeloablative	58 (61.05)
Non-myeloablative	37 (38.95)
Conditioning regimen	
Flu/Bu	29 (30.53)
Flu/Mel	13 (13.68)
Flu/Cy/TBI	12 (12.63)
Cy/TBI	10 (10.53)
Flu/TBI	7 (7.37)
Flu/Treo	6 (6.32)
Others	18 (18.95)
GVHD prophylaxis	
CNI+MMF+Cy	32 (33.68)
CNI+MTX	30 (31.58)
CNI+MMF	20 (21.05)
Cy post	11 (11.58)
Others	2 (2.11)
Cell source	
Bone marrow	40 (42.11)
Peripheral blood	55 (57.89)
Donor	
HLA-Identical	60 (63.16)
HLA Non-identical	6 (6.32)
HLA-Haploidentical	29 (30.53)
Antibiotic prophylaxis	11 (11.58)
Hospitalization (days), mean ± SD	30.45 ± 17.62
Acute GVHD	58 (61.05)
Gastrointestinal involvement	19 (32.76)
Chronic GVHD	37 (38.95)
Death (1 year after HSCT)	21 (22.11)
Main causes of death	
GVHD	7 (33.33)
Disease recurrence	6 (28.57)
Infection	6 (28.57)
Follow-up of survivors (months)	
Median	12.22
IQR	7.10-12.25

Data are presented as no. (%) unless otherwise indicated.
Abbreviations: Bu, busulfan; CNI, calcineurin inhibitor; Cy, cyclophosphamide; EBMT, European group for blood and marrow transplantation; Flu, fludarabine; GVHD, graft-versus-host disease; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; IQR, interquartile range; Mel, melphalan; MMF, mycophenolate mofetil; MRD, minimal residual disease; MTX, methotrexate; SD, standard deviation; TBI, total body irradiation; Treo, treosulfan.

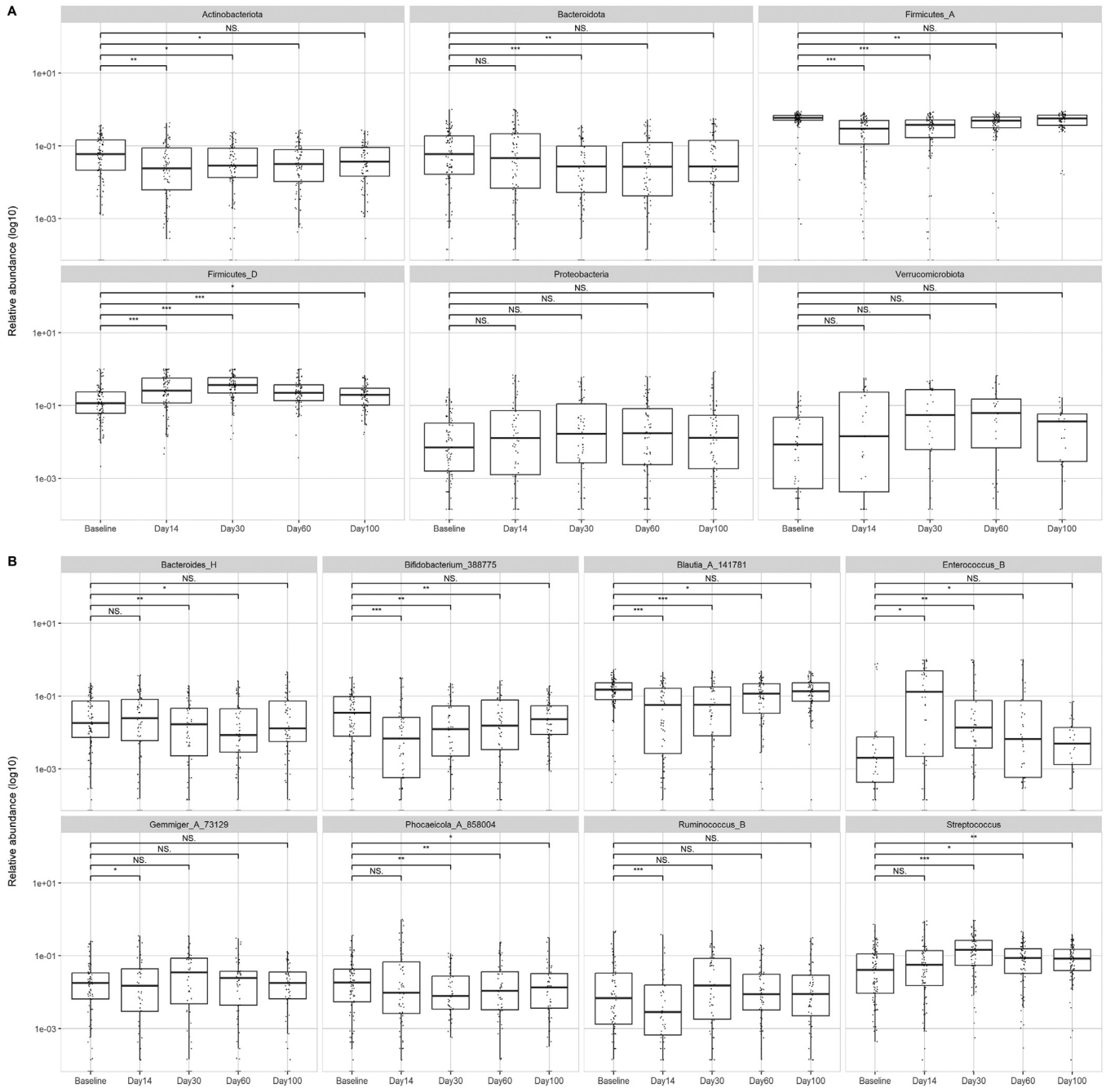

Fig. 1. Changes in the relative abundance of each taxon identified as most prevalent within de sample set, across visits. Relative abundance is plotted on a logarithmic scale (log10). (A) Phylum level. (B) Genus level. Differences in abundance between groups were assessed using Kruskal–Wallis test, followed by post-hoc comparisons using the Wilcoxon rank-sum test with false discovery rate (FDR) correction. *Indicates significant differences between the two groups with a *P*-value <0.05. **Indicates *P*-value <0.01. ***Indicates *P*-value <0.001. NS, denotes non-significant differences in statistical analysis. The mean relative abundance of each taxon at the phylum and genus level was calculated across the sample set. Subsequently, changes in the 10 most abundant phyla and genera were analysed per visit. Phyla *Synergistota, Fusobacteriota, Desulfobacterota* and *Firmicutes_C* have not been included in the representation because they are found in very low proportion. Genera *Escherichia* and *Akkermansia* have not been included because no significant differences in abundance were found during follow-up.

Across the 409 stool samples, no significant differences were found in the overall alpha diversity between the samples from patients who experienced acute GVHD (aGVHD) (mean ± SD= 13.75 ± 9.65 vs 13.71 ± 10.02), acute GI-GVHD (11.89 ± 8.31 vs 13.71 ± 10.02) or chronic GVHD (cGVHD) (13.72 ± 8.59 vs 13.75 ± 10.52) events and those who did not ($P = 0.793$, 0.271 and 0.324, respectively), infection (13.78 ± 9.9 vs 12.46 ± 5.25, $P = 0.997$), bacteraemia (14.02 ± 11.08 vs 13.34 ± 7.62, $P = 0.316$), or transplant-related variables such as cell source type (bone marrow: 13.12 ± 8.60 vs peripheral blood: 14.16 ± 10.51, $P = 0.685$) or conditioning intensity (myeloablative: 14.29 ± 10.49 vs non-myeloablative: 12.81 ± 8.39, $P = 0.352$) during follow-up.

No significant difference was observed between samples from patients with or without relapse (15.36 ± 10.59 vs 13.39 ± 9.58, $P = 0.186$). However, it was significantly lower in samples from patients who died during follow-up compared to those who survived (11.74 ± 9.85 vs 14.19 ± 9.72, $P = 0.008$).

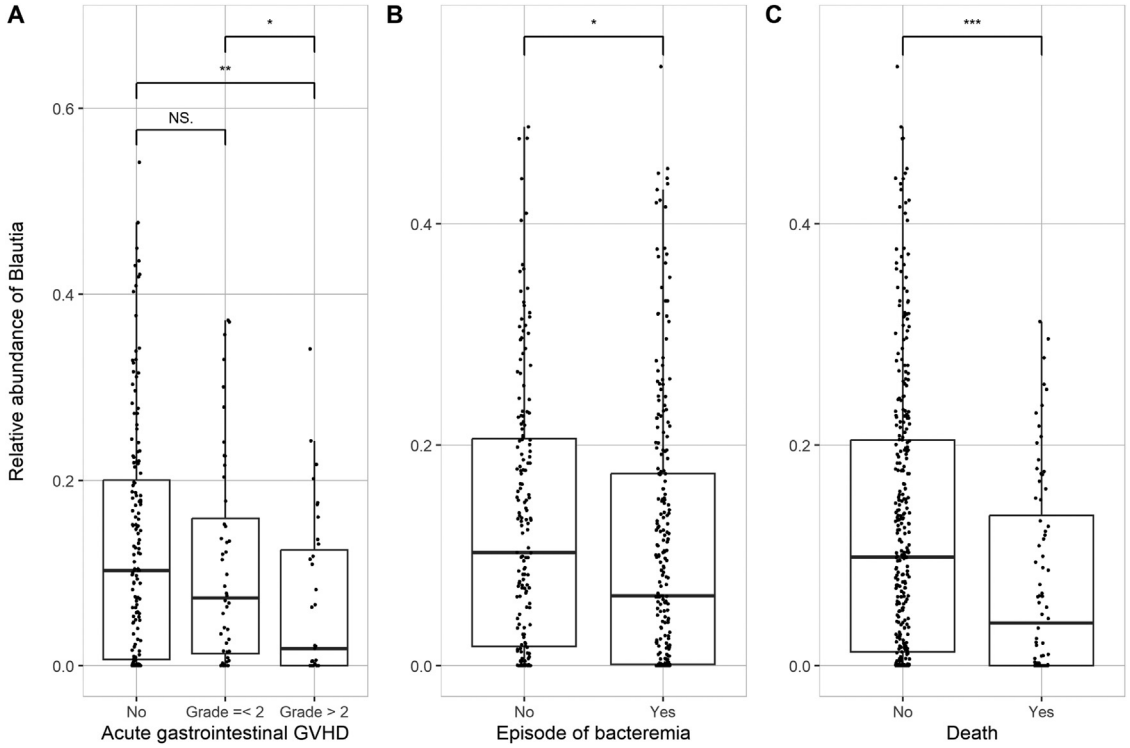

Fig. 2. Relative abundance of the genus *Blautia* in the global set of samples in relation to patient characteristics associated with worse clinical outcomes. Differences in abundance between groups were assessed using the Wilcoxon rank-sum test, and Kruskal–Wallis test, followed by post-hoc comparisons using the Wilcoxon rank-sum test with false discovery rate (FDR) correction. *Indicates significant differences between the two groups with a *P*-value <0.05. **Indicates *P*-value <0.01. ***Indicates *P*-value <0.001. NS, denotes non-significant differences in statistical analysis.

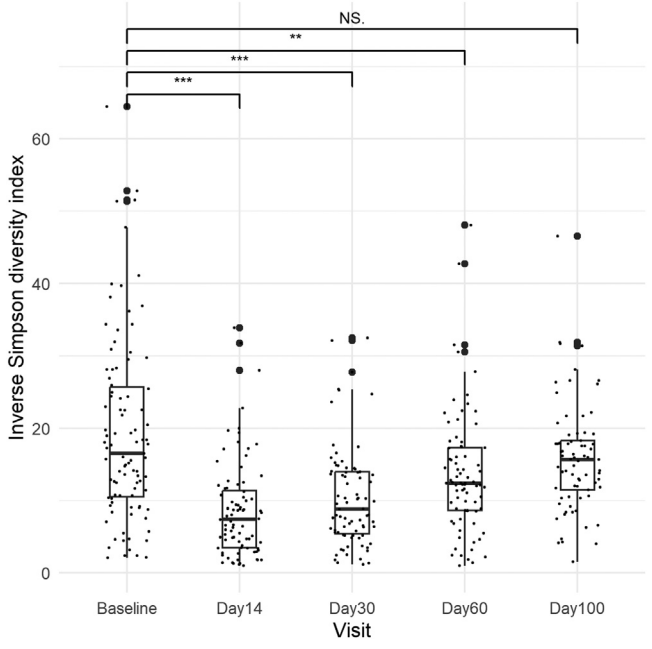

Fig. 3. Alpha diversity analysis was calculated by visit using Inverse Simpson index. Differences in alpha diversity of the GM between samples collected at each follow-up visit and the baseline visit were assessed using the Kruskal–Wallis test, followed by post-hoc comparisons using the Wilcoxon rank-sum test with false discovery rate (FDR) correction. *Indicates significant differences between the two groups with a *P*-value <0.05. **Indicates *P*-value <0.01. ***Indicates *P*-value <0.001. NS, denotes non-significant differences in statistical analysis.

Alpha diversity analysis in the baseline and neutropenia periods

For a more comprehensive analysis, samples collected at baseline and up to 17.67 days post-HSCT (a period characterized by neutropenia) were selected to analyse alpha diversity and its relationship with patient variables at these time points.

Baseline alpha diversity was significantly lower in patients with prior infections (13.75 ± 8.68 vs 22.36 ± 13.51, $P = 0.002$), prior bacteraemia episode (11.87 ± 7.56 vs 20.88 ± 13.03, $P = 0.008$), or had received prior antibiotic treatment (16.08 ± 10.25 vs 22.91 ± 14.13, $P = 0.018$).

Overall survival was also examined in relation to diversity at baseline and during the neutropenia period (see Supplementary Text and Figure S3).

Beta diversity analysis

PCoA based on Bray–Curtis distances at the genus-level at baseline visit showed no clustering by an episode of GI-GVHD, bacteraemia, or death (adonis test, $P = 0.870$, 0.483 and 0.159, respectively). However, significant differences in beta diversity were found between patients with and without prior antibiotic treatment ($P = 0.032$) and between patients with low and high baseline diversity ($P = 0.001$).

During the neutropenic period, significant differences were found in samples from patients who experienced bacteraemia during follow-up ($P = 0.025$) (Figure S4) and between those with low versus high diversity during this period ($P = 0.001$).

Differential abundance analysis

LEfSe analysis was performed to identify taxa that could serve as differential markers between patients who died during follow-

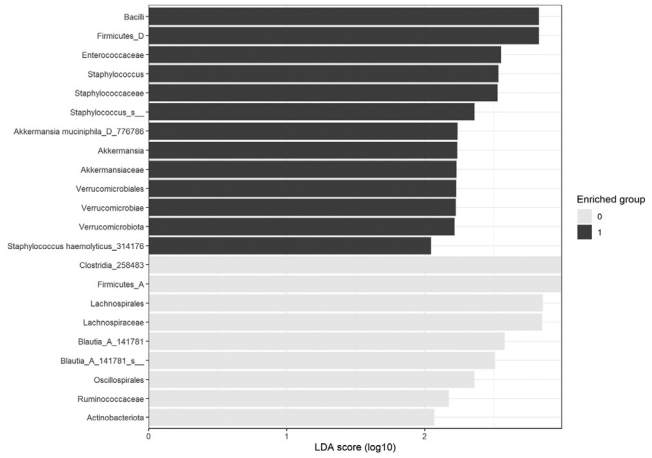

Fig. 4. LEfSe analysis identified 22 differentially abundant taxa between deceased (black bars, 1) and surviving patients (grey bars, 0).

up and those who survived (Figure 4). Using an LDA score of 2 and a *P*-value cutoff of 0.05, twenty-two markers were identified. Taxa exhibiting the strongest signals, including *Blautia*, were selected, and the relationship between their abundance and mortality was assessed. Patients who died during the study follow-up showed increased abundances of potential pathogens *Enterococcus_H* (mean=0.015 vs 0.009, $P = 0.026$), *Enterococcus_A* (0.008 vs 0.001, $P = 0.019$), and *Staphylococcus* (0.027 vs 0.005, $P = 0.009$). Similarly, *Akkermansia* abundance was also higher in non-survivors (0.054 vs 0.032, $P = 0.017$). Conversely, survivors showed a significantly higher abundance of *Gemmiger* (0.028 vs 0.017, $P < 0.001$) (Figure 5).

Further analyses explored the association of these taxa with clinical outcomes, specifically GI-GVHD and bacteraemia episodes (Figure 2 and Supplemental text and Figure S5).

Discussion

We report the results of a multicentre study that longitudinally profiled the GM of patients undergoing allo-HSCT for various hematological diseases. To our knowledge, this is the first Spanish study to comprehensively profile GM dynamics in this patient population.

Mediterranean diet, rich in vegetables, fruits, legumes, cereals, fish, and healthy fats like olive oil, has been shown to have numerous health benefits. Its impact on the GM is an active area of research. A recent study by Latorre-Pérez et al. [5] characterized the GM of 530 individuals in Spain, aiming to define a 'normal' GM for our country. At the phylum level, *Firmicutes* and *Bacteroidota* were predominant, followed by *Proteobacteria, Verrucomicrobiota*, and *Actinobacteriota*, which is consistent with the taxonomic profile of the GM described by Rajilić-Stojanović and M de Vos [23]. At the genus level, *Bacteroides* was the dominant genus.

Analysis of baseline characteristics in our study population revealed a common taxonomic composition among study patients, regardless of their centre of origin, which differed from that described by Latorre-Pérez et al. [5], even before transplantation. Our patients exhibited low diversity, as measured by the number of identified taxa, which decreased further during the first 30 days post-HSCT. These changes included shifts in the relative abundance of dominant taxa and alterations in alpha diversity as measured by the inverse Simpson index. At the phylum level, the baseline composition was dominated by *Firmicutes*, followed by *Bacteroidota, Actinobacteriota, Proteobacteria and Verrucomicrobiota*; while at the genus level, *Blautia* was the dominant taxon, followed by *Streptococcus*, with *Bifidobacterium* and *Bacteroides* being less abundant. Throughout the study, the overall GM composition was dominated by the genus *Streptococcus*, indicating dynamic alterations in GM taxonomic profiles and alpha diversity. A statistically significant increase in the relative abundance of the *Bacilli* class, dominated by the *Streptococcus* and *Enterococcus* genera, was observed. This shift was particularly evident during the neutropenic period, up to 30 days post-HSCT, and led to a significant reduction in alpha diversity compared to pre-transplant levels refers in previous studies such as Peled et al. [13] and Masetti et al. [24], and a decrease in the abundance of certain taxa associated with better clinical outcomes, such as the genus *Blautia*.

Our results were similar to those of Peled et al. [13], who described a common GM composition in these patients, regardless of the centre of origin, and noted that it differed significantly from the composition found in healthy individuals. Additionally, this alteration was associated with a significant decrease in the abundance of the *Blautia* genus, which has been linked to the development of aGVHD, also described in other studies [11,24]. We observed that patients with characteristics indicative of a poorer outcomes, such as bacteraemia, GI-GVHD, or eventual death, had a

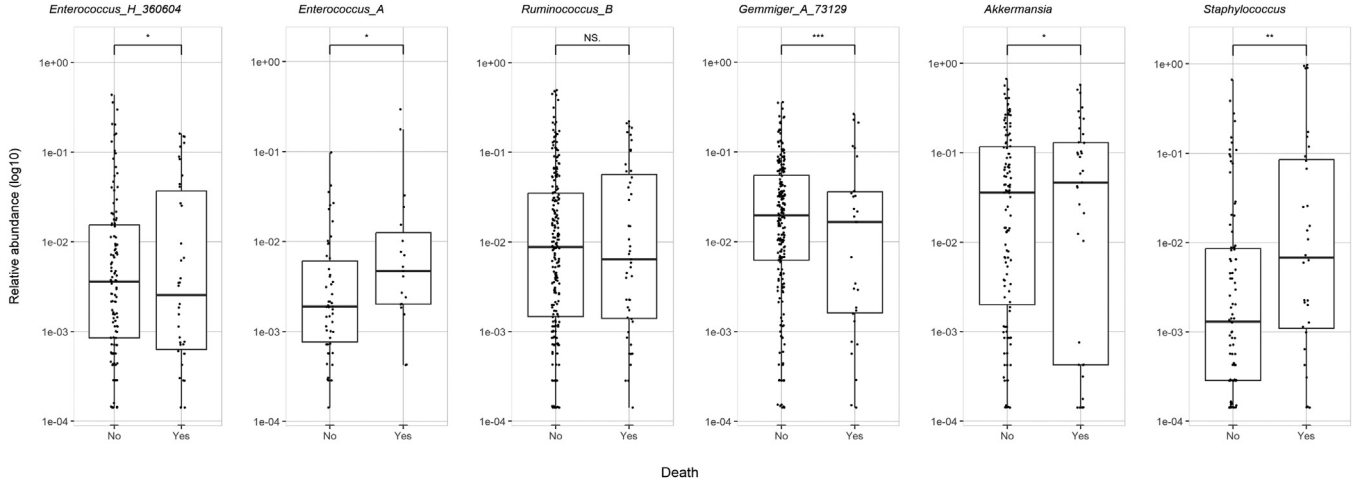

Fig. 5. Differential abundance of each taxa according to mortality. Taxa exhibiting the strongest signals in LEfSe analysis of Fig. 4 were selected. Relative abundance is plotted on a logarithmic scale (log10). Differences in abundance between groups were assessed using the Wilcoxon rank-sum test. *Indicates significant differences between the two groups with a *P*-value <0.05. **Indicates *P*-value <0.01. ***Indicates *P*-value <0.001. NS, denotes non-significant differences in statistical analysis.

lower abundance of the *Blautia* genus compared to those with better outcomes.

These findings suggest that the microbiota composition in these patients may be primarily influenced by their underlying disease and associated treatments, independent of geographic region and dietary variations.

Regarding alpha diversity, no significant associations were observed between overall alpha diversity and patient factors associated with poor prognosis, including aGVHD or cGVHD, infections, or bacteraemia episodes during follow-up, despite these factors being linked to a low alpha diversity in previous studies [14,25]. However, it is important to note that patients who died during follow-up exhibited significantly lower overall alpha diversity compared to those who survived.

A history of pre-transplant infection, bacteraemia, or prior antibiotic treatment were significantly associated with decreased GM diversity. Previous studies consistently linked reduced microbiota diversity to impaired overall survival [10,12,13], a finding replicated in our study. To evaluate the impact of baseline and neutropenia period microbiota diversity on overall survival, patients were stratified based on these metrics. Although not statistically significant, a trend towards lower one-year survival was observed in patients with lower diversity in both periods compared to those with higher diversity, as previously reported by Masetti et al. [24].

PCoA analysis based on genus-level relative abundances did not reveal a distinct clustering of patient samples according to the aforementioned characteristics. Biagi et al. [11] reported similar findings regarding the development or absence of GVHD, which they associated with the presence and abundance of specific taxa. Although we did not identify distinct clusters based on characteristics associated with worse outcomes, our analysis revealed significant clustering between samples from patients with and without prior antibiotic exposure and between those with high and low diversity at both baseline and neutropenia period. These findings are related to the data previously presented and seem to indicate that there are significant changes in the taxonomic profiles of the intestinal microbiota of our patients.

Analysis of the taxa exhibiting the strongest signal in the LEfSe analysis revealed that deceased patients presented a higher abundance of potentially pathogenic genera such as *Enterococcus* and *Staphylococcus*, consistent with findings reported in the literature [9,14,26].

To our knowledge, this is the first study of its kind conducted in Spain. We observed significant variations in the taxonomic profiles and relative abundances of the GM throughout follow-up. The decreased abundance of the genus *Blautia* was notably associated with poorer clinical outcomes. These findings highlight the potential impact of GM dysbiosis on patient outcomes following transplantation. Further research is warranted to elucidate the specific mechanisms underlying these associations and to develop targeted interventions to modulate the microbiota and improve patient prognosis.

Limitations

This multicentre study has inherent limitations due to possible variations in patient management protocols in the different participating hospitals. Nevertheless, allo-HSCT usually follows the recommendations established for treatment and follow-up by national and international scientific societies.

Another limitation of the study is related to sample loss during follow-up, either due to patient death or sample collection failures. This fact may have led to a loss of statistical power, failing to achieve significant values in some of the analyses performed, such as survival curves. Although a trend towards greater survival can be observed in patients with higher GM diversity, this did not reach statistical significance.

Author contributions

Concept and design of the study: MCF, CFA, MFM, MH, and ABR; study conduction, CGR, MCF, MFM, ABR, LVL, and AJGH; clinical data collection and samples collection and processing, all authors; data curation, CGR and CFA; bioinformatic and statistical analysis, CGR, JRG, MH, and CFA; writing—original draft, CGR; writing—review and editing of the manuscript, all authors; funding acquisition, CGR and MCF. All authors critically reviewed the data and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Ethical approval

All procedures were performed in accordance with current legislation, Declaration of Helsinki principles and good clinical practice guidelines. The study was approved by Clinical Research Ethics Committee of Cantabria (Ref: ENT-MFA-2016-01, ENTHERE-SCT; 2017.112, MICROBIOMA-SCT; and NVAL21/17, MITAPH), as reference committee, and Clinical Ethics Committee of the participating centres. Written informed consent was obtained from all patients for data use and follow-up samples collection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in this article are part of a doctoral thesis, raw sequence data will be deposited in the NCBI Short Read Archive upon thesis defense.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.108117.

References

[1] Thomas ED, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, et al. Bone-marrow transplantation (first of two parts). *N Engl J Med* 1975;**292**:832–43. doi:10.1056/NEJM197504172921605.

[2] Zhang YJ, Li S, Gan RY, Zhou T, Xu DP, Bin Li H. Impacts of gut bacteria on human health and diseases. *Int J Mol Sci* 2015;**16**:7493–519. doi:10.3390/ijms16047493.

[3] Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *N Engl J Med* 2016;**375**:2369–79. doi:10.1056/nejmra1600266.

[4] Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* 2014;**16**:1024–33. doi:10.1111/cmi.12308.

[5] Latorre-Pérez A, Hernández M, Iglesias JR, Morán J, Pascual J, Porcar M, et al. The Spanish gut microbiome reveals links between microorganisms and Mediterranean diet. *Sci Rep* 2021;**11**:21602. doi:10.1038/s41598-021-01002-1.

[6] Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis* 2012;**55**:905–14. doi:10.1093/cid/cis580.

[7] Laterza L, Rizzatti G, Gaetani E, Chiusolo P, Gasbarrini A. The gut microbiota and immune system relationship in human graft-versus-host disease. *Mediterr J Hematol Infect Dis* 2016;**8**:e2016025. doi:10.4084/MJHID.2016.025.

[8] Taur Y, Pamer EG. The intestinal microbiota and susceptibility to infection in immunocompromised patients. *Curr Opin Infect Dis* 2013;**26**:332–7. doi:10.1097/QCO.0b013e3283630dd3.

[9] Holler E, Butzhammer P, Schmid K, Hundsrucker C, Koestler J, Peter K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. *Biol Blood Marrow Transplant* 2014;**20**:640–5. doi:10.1016/j.bbmt.2014.01.030.

[10] Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* 2014;**124**:1174–82. doi:10.1182/blood-2014-02-554725.

[11] Biagi E, Zama D, Rampelli S, Turroni S, Brigidi P, Consolandi C, et al. Early gut microbiota signature of aGvHD in children given allogeneic hematopoietic cell transplantation for hematological disorders. *BMC Med Genomics* 2019;**12**:49. doi:10.1186/s12920-019-0494-7.

[12] Kusakabe S, Fukushima K, Maeda T, Motooka D, Nakamura S, Fujita J, et al. Pre- and post-serial metagenomic analysis of gut microbiota as a prognostic factor in patients undergoing haematopoietic stem cell transplantation. *Br J Haematol* 2020;**188**:438–49. doi:10.1111/bjh.16205.

[13] Peled JU, Gomes ALC, Devlin SM, Littmann ER, Taur Y, Sung AD, et al. Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. *N Engl J Med* 2020;**382**:822–34. doi:10.1056/NEJMoa1900623.

[14] Golob JL, Pergam SA, Srinivasan S, Fiedler TL, Liu C, Garcia K, et al. Stool microbiota at neutrophil recovery is predictive for severe acute graft vs host disease after hematopoietic cell transplantation. *Clin Infect Dis* 2017;**65**:1984–91. doi:10.1093/cid/cix699.

[15] Peled JU, Devlin SM, Staffas A, Lumish M, Khanin R, Littmann ER, et al. Intestinal microbiota and relapse after hematopoietic-cell transplantation. *J Clin Oncol* 2017;**35**:1650–9. doi:10.1200/JCO.2016.70.3348.

[16] Alarcón Cavero T., D'Auria G., Delgado Palacio S., Del Campo Moreno R., Ferrer Martínez M. MICROBIOTA. 59. Del Campo Moreno R (coordinadora). Procedimientos en Microbiología Clínica. Cercenado Mansilla E, Cantón Moreno R, editores. Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). 2016. Available from: https://seimc.org/contenidos/documentoscientificos/procedimientosmicrobiologia/seimc-procedimientomicrobiologia59mod.pdf. Accessed 13 September 2023.

[17] Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;**41**:e1. doi:10.1093/nar/gks808.

[18] Anderson M. A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 2001;**26**:32–46. doi:10.1111/j.1442-9993.2001.01070.pp.x.

[19] Clarke KR. Non-parametric multivariate analyses of changes in community structure. *Austral Ecol* 1993;**18**:117–43. doi:10.1111/j.1442-9993.1993.tb00438.x.

[20] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12**:R60. doi:10.1186/gb-2011-12-6-r60.

[21] StataCorp *Stata statistical software: release 16*, College Station, TX: StataCorp LLC; 2019. Available from: *https://www.stata.com/* Accessed 20 September 2023.

[22] R Core Team. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria; 2023. Available from: https://www.R-project.org/. Accessed 20 September 2023.

[23] Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev* 2014;**38**:996–1047. doi:10.1111/1574-6976.12075.

[24] Masetti R, Leardini D, Muratore E, Fabbrini M, Zama D, Baccelli F, et al. Gut microbiota diversity before allogeneic hematopoietic stem cell transplantation as a predictor of mortality in children. *Blood* 2023;**142**:1387–98. doi:10.1182/blood.2023020026.

[25] Stoma I, Littmann ER, Peled JU, Giralt S, van den Brink MRM, Pamer EG, et al. Compositional flux within the intestinal microbiota and risk for bloodstream infection with gram-negative bacteria. *Clin Infect Dis* 2021;**73**:e4627–35. doi:10.1093/cid/ciaa068.

[26] Stein-Thoeringer CK, Nichols KB, Lazrak A, Docampo MD, Slingerland AE, Slingerland JB, et al. Lactose drives enterococcus expansion to promote graft-versus-host disease. *Science* 2019;**366**:1143–9. doi:10.1126/science.aax3760.