Nutrition 83 (2021) 111093

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Possible clinical outcomes using early enteral nutrition in individuals with allogeneic hematopoietic stem cell transplantation: A single-center retrospective study

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ARTICLE INFO

Article History:
Received 15 August 2020
Received in revised form 20 October 2020
Accepted 11 November 2020

Keywords:
Enteral nutritional support
Allogeneic hematopoietic stem cell transplantation
Graft-versus-host-disease
Intestinal microbiota

ABSTRACT

Objectives: Intensive nutritional support during allogeneic hematopoietic stem cell transplantation (allo-HSCT) yields improved clinical outcomes. However, the clinical implications of early enteral nutrition (EN) in allo-HSCT remain unclear. This retrospective study was conducted to determine the significance of early EN in individuals who underwent allo-HSCT, and the association between early nutritional intervention and clinical outcomes, including the status of the intestinal microbiome.
Methods: Thirty-one participants received EN before conditioning. The intestinal microbiota was examined by meta 16S rRNA gene sequencing of fecal samples.
Results: The median body mass variation was only −0.35 kg on day 60. The probability of 2-y overall survival was 61.1%. The cumulative incidence of treatment-related mortality was 17.4%, and those of acute graft-versus-host disease were 32.3% (grades II–IV) and 3.2% (grades III–IV). Chronic graft-versus-host disease was observed in four participants. Dysbiosis of the intestines and acute graft-versus-host disease occurred simultaneously, and *Enterococcus* species were abundant.
Conclusions: Our results suggest that early nutritional support can improve the outcomes for individuals who have undergone allo-HSCT and can maintain homeostasis of their intestinal microbiome. Future prospective clinical trials are required to elucidate the role of EN in allo-HSCT and the association between the intestinal microbiome and EN.

S. Iyama, H.T., and T. Shiraishi designed the study, collected and analyzed the data, and wrote the manuscript. M.Y. and A.T. collected the data and reviewed the manuscript. A.E., T.I., and Y.S. performed microbiome analysis and reviewed the manuscript. H.O. performed multivariate analysis and reviewed the manuscript. S. Ibata, A.G., S.K., K.N., H.H., C.F., H.I., K.T., T.N., T. Sato, Y.K., S.Y., and M.K. reviewed the manuscript and provided critical input.

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https://doi.org/10.1016/j.nut.2020.111093
0899-9007/

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a promising treatment strategy for intractable hematologic malignancies. Conditioning regimens comprise high-dose chemotherapy and total-body irradiation (TBI), resulting in gastrointestinal mucosal injury, which requires clinical vigilance. Disruption of the gastrointestinal mucosal barrier facilitates bacterial translocation of microorganisms into the bloodstream and increases the risk of sepsis and treatment-related mortality (TRM) [1]. We have previously reported that dietary supplements including glutamine, fiber, and oligosaccharides remarkably decrease the severity of intestinal mucosal damage [2]. However, conditioning encompasses severe malnutrition due to gastrointestinal symptoms such as nausea, vomiting, anorexia, and diarrhea [3]. Malnutrition in individuals who have undergone allo-HSCT adversely affects their prognosis [4,5]. Intensive nutritional support, which has been widely accepted, is indispensable for improving clinical outcomes [6]. If gastrointestinal functions are preserved, enteral nutrition (EN) may be as efficient as parenteral nutrition (PN) [7]. Several studies have supported EN over PN in allo-HSCT [8,9]. Fewer complications, particularly infection, are observed with EN compared to PN [9]. Advantages of EN versus PN include maintenance of the gut barrier and fewer infectious complications such as bacterial translocation [6]. In a comparison of different options for performing EN, recipients appear to prefer percutaneous endoscopic gastrostomy to nasogastric tubes (NGT) [7]. However, in people with cancer, complication rates have been shown to be lower with NGT than with percutaneous endoscopic gastrostomy [10]. Moreover, the clinical implication of EN via a feeding tube in allo-HSCT outcomes is unclear [11].

A recent study reports differences in the intestinal microbiome between individuals receiving EN and PN after HSCT [12]. Those who had minimal oral intake for a longer period had lower microbial diversity and different overall microbial profiles. These data suggest that enteral microbial diversity improves with continuous EN using an NGT. Therefore, this retrospective study was conducted to investigate the significance of early EN in individuals with hematologic diseases undergoing allo-HSCT and to determine the association between early nutritional intervention and clinical outcomes in allo-HSCT recipients.

Materials and methods

Study cohort and ethics

Thirty-one patients who underwent allo-HSCT at Sapporo Medical University Hospital between March 2015 and December 2017 were retrospectively assessed. The study was approved by the institutional review board and conducted in accordance with the Declaration of Helsinki. All participants provided informed consent. Historical control data without EN were extracted from all participants who underwent allo-HSCT between June 2011 and December 2014. Informed consent for historical control data was obtained in the form of an opt-out option on the website. The additional study was approved by the institutional review board. The test cohort was compared with the historical control group. Individuals were eligible for transplantation if they had any hematologic diseases and were at a high risk of relapse or had suitable related or unrelated donors of bone marrow/peripheral blood/umbilical cord blood available within a reasonable period relative to their disease condition. All patients who underwent allo-HSCT during the study were included in our analysis.

Transplantation procedures

The allo-HSCT procedure was performed in accordance with the standard protocol of our hospital. Cyclophosphamide (CY)/intravenous busulfan (ivBU) or CY/TBI was administered as myeloablative conditioning (MAC). Fludarabine (FLU)/melphalan or FLU/ivBU was administered as the reduced-intensity conditioning (RIC) regimen. Antiemetics were provided when each conditioning regimen—such as high-dose CY, TBI, ivBU, or melphalan—was administered. Posttransplant graft-versus-host disease (GVHD) prophylaxis included tacrolimus plus methotrexate or cyclosporine and methotrexate or tacrolimus plus mycophenolate mofetil. Antiinfective prophylaxis administered from the beginning of the conditioning regimen comprised ciprofloxacin, valacyclovir, and a triazole antifungal or micafungin. *Pneumocystis jirovecii* and toxoplasmosis prophylaxis comprised sulfamide-based treatment for neutrophil recovery.

Nutritional support

During the study period, all participants underwent implantation with a central venous catheter (CVC) and an NGT (CORFLO 8Fr feeding tube, Nipro, Osaka, Japan) before conditioning chemotherapy. EN or PN was not regulated in this study and was coordinated on an individual basis. Nutritional support was given to all patients who underwent allo-HSCT starting in March 2015. The dietary supplements, including glutamine, fiber, and oligosaccharides, were provided to patients three times per day as appropriate [2]. EN through an NGT was delivered before conditioning chemotherapy and gradually scaled up. EN was introduced when patients were unable to consume an adequate oral diet. If oral dietary intake and EN were poorly consumed or tolerated, total parenteral nutrition was administered via the CVC. A nutritional proposal was provided by the nutritional support team to attain adequate daily energy. Adequate energy requirements were estimated between 1.0 and 1.4 times basal energy expenditure, as calculated by the Harris–Benedict method based on the physical activity and therapeutic regimen.

Data collection and definition of criteria

We described the following detailed definition in the supplemental methods. The data collected included participant characteristics such as sex, age at the time of SCT, diagnosis, disease status at the time of allo-HSCT, complete remission (CR)/non-CR at SCT, conditioning regimen, human leukocyte antigen (HLA) disparity, and donor source. Episodes of oral mucositis, diarrhea, documented bacteremia, severity of acute GVHD (aGVHD) or chronic GVHD (cGVHD), and days to neutrophil recovery were recorded. Oral mucositis and diarrhea were assessed in accordance with the Common Terminology Criteria for Adverse Events, version 4.0, as previously described [2]. Documented bacteremia was defined by positive blood cultures of specimens simultaneously drawn from the CVC and the peripheral vein [13], except for only single coagulase-negative staphylococci. We defined aGVHD and graded it in accordance with the standard criteria, with grades 0–IV [14]; cGVHD was defined by the standard criteria of absent, limited, or extensive [15]. The day of neutrophil recovery was defined as the first of three consecutive days on which the neutrophil count exceeded 500/μL. Nutritional evaluation was based on daily measurements of body mass, body mass index (BMI), daily calorie intake, and rapid turnover proteins (RTPs), including transferrin, transthyretin, and retinol binding protein. Nutrient adequacy was calculated using the following formula [16]: total calories/1.4 × basal energy expenditure. Overall survival (OS) was calculated from the day of SCT until death from any causes. Relapse was defined as the time to onset of hematologic recurrence. TRM was defined as any death other than death from disease exacerbation.

Fecal microbiota analysis

We obtained stool samples and performed microbiome analysis of four randomly selected participant samples. We collected stool samples before conditioning chemotherapy, and on days 0, 14, 30, and 60 after SCT. Stool samples were stored at −80°C. The detailed methods are described in the supplemental methods. Briefly, DNA was extracted from the fecal samples, and the V1-V2 variable regions (approximately 300 base pairs) of the 16S rRNA gene sequences of the extracted DNA were used as a target for meta 16S rRNA sequencing. Sequence data were quality-filtered and then analyzed using the open-source software package QIIME 2. The 16S rRNA gene sequences were assigned to operational taxonomic units using SILVA high-quality ribosomal RNA databases (version 123). The α-diversity analysis, including Shannon species diversity identification, was performed using QIIME 2.

Statistical analysis

The end point of this study was the clinical outcomes, including nutritional status, OS, TRM, and incidence of GVHD. Data for surviving participants were censored at the last follow-up visit. The OS, cumulative survival rates, and incidences of aGVHD were determined by the Kaplan–Meier method. Medians and ranges were determined for continuous variables and percentages with categorical variables. Mann–Whitney *U* tests were performed to evaluate differences. The data analyzed between days 30 and 60 were as follows: median body mass variation, median BMI variation, median RTPs, median caloric-intake variation, median actual caloric intake, and median nutrient-adequacy ratio. The correlation between microbial diversity and nutritional status using RTPs was analyzed using the Pearson product-moment correlation coefficient. A P value <0.05 was considered statistically significant. All statistical analyses were performed using

GraphPad Prism software version 5.0b (GraphPad, Inc., San Diego, CA, USA and IBM Corp., Armonk, NY, USA) and SPSS (SPSS, Inc., Chicago, IL, USA).

Results

Participant characteristics

The characteristics of the participants and historical controls are shown in Table 1 [17]. Thirty-one individuals with acute leukemia, myelodysplastic syndromes, lymphoma, chronic leukemia with blast phase, and adult T-cell leukemia/lymphoma were analyzed. Their median age was 53 y (range, 18–66 y). Of the 31 participants in the EN group, 15 were at high risk and 10 were in the non-CR group. In the MAC regimen, CY plus TBI 12 Gy ($n = 10$) and BU plus CY ($n = 3$) were included. In the RIC regimen, all participants except for one ($n = 17$) were administered FLU plus BU. Complete HLA-matched donors were available for 19 participants (61.3%). Neither antithymocyte globulin nor posttransplant CY was used for mismatched transplantation. In this cohort, participants underwent HSCT with unrelated bone marrow ($n = 23$), unrelated peripheral blood ($n = 3$), related peripheral blood ($n = 3$), and umbilical cord blood ($n = 2$). Of the 38 participants in the control group, 16 were at high risk and 15 were in the non-CR group. In the MAC regimen, CY plus TBI 12 Gy ($n = 9$), BU plus CY ($n = 2$), and FLU plus BU4 ($n = 12$) were included. FLU plus BU ($n = 7$), FLU plus melphalan ($n = 6$), and FLU plus CY ($n = 2$) were included in the RIC regimen. Complete HLA-matched donors were available for 10 participants.

Table 1
Participant characteristics

Characteristic	EN group	Historical control group	*P*
Sex ratio (male/female)	17/14	21/17	>0.9999
Age at SCT (y)			
Median (range)	53 (18–66)	53 (22–65)	0.7392
Diagnosis			0.8701
AML	12	16	
ALL	9	12	
MDS	3	4	
Lymphoma	3	4	
CML (BP)	2	1	
ATLL	2	1	
Disease risk			0.6341
Standard risk	16	22	
High risk	15	16	
CR/non-CR			0.6186
CR	21	23	
Non-CR	10	15	
Conditioning regimen			0.1506
MAC	13	23	
CY/TBI	10	9	
BU/CY	3	2	
FLU/BU4	0	12	
RIC	18	15	
FLU/BU	17	7	
FLU/MEL	1	6	
FLU/CY	0	2	
HLA disparity			0.2273
8/8 matched	19	17	
mismatched	12	21	
1 locus mismatched	10	17	
2 or more loci mismatched	2	4	
Donor source			0.5556
Unrelated BM	23	26	
Related BM	0	3	
Unrelated PBSC	3	1	
Related PBSC	3	3	
CB	2	5	

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATLL, adult T-cell leukemia/lymphoma; BM, bone marrow; BU, busulfan; CB, cord blood; CLL, chronic lymphocytic leukemia; CML (BP), chronic myeloid leukemia, blastic phase; CR, complete remission; CY, cyclophosphamide; EN, enteral nutrition; FLU, fludarabine; HLA, human leukocyte antigen; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MEL, melphalan; MPN, myeloproliferative neoplasms; PBSC, peripheral blood stem cell; RIC, reduced-intensity conditioning; SCT, stem cell transplantation; TBI, total-body irradiation.
Standard risk was indicated by acute leukemia in first or second complete remission, chronic myeloid leukemia in first chronic phase, Hodgkin or non-Hodgkin lymphoma in complete or partial chemotherapy-sensitive remission, and myelodysplastic syndrome without excess blasts. All other diseases were defined as high risk according to the schema proposed by the American Society for Blood and Marrow Transplantation in 2006 [17].

Clinical outcomes

Five participants (16.1%) died within 100 d after the allo-HSCT, and the median survival was not reached in this study cohort. Four participants showed evidence of treatment-related adverse events: three had septic shock and one had acute respiratory distress syndrome. One participant presented with disease progression. The probability of 2-y OS was 61.1% in EN group and 39.1% in the historical control group (Fig. 1A). The cumulative incidence of TRM at day 100 in the EN group and the historical control group, respectively, was 13.3% and 18.8%. The 1-y TRM in the EN group and the historical control group, respectively, was 17.4% and 38.2% (Fig. 1B). Other clinical outcomes, such as hospital time, duration of EN/PN, intestinal mucosal injuries (oral mucositis and diarrhea), days to neutrophil recovery, documented bacteremia, severity of aGVHD or cGVHD, and relapse rate, are shown in Table 2. Further, we performed subgroup analysis (MAC versus RIC, HLA matched versus mismatched, and young versus aged). However, the results were not significant (Supplementary Fig. 1). All microbiologically documented infections were bloodstream infections; no invasive fungal or systemic viral infections were observed. The cumulative incidence of aGVHD (grades II–IV) in the EN group and the historical control group, respectively, was 32.3% and 36.8%, and for aGVHD (grades III–IV), 3.2% and 21.1% (Table 2, Fig. 2). We calculated the hazard ratio (HR) for OS using univariate and multivariate Cox regression models. The presence of aGVHD significantly increased the risk of OS with EN (grades II–IV: HR, 2.71; 95% confidence interval [CI], 1.39–5.34; $P = 0.003$; grades III–IV: HR, 2.32; 95% CI, 0.96–5.01; $P = 0.043$). We observed cGVHD in four participants in the EN group. Of the participants with cGVHD, three had mild oral cGVHD and one had mild skin cGVHD. No participants with cGVHD required systemic steroid intervention.

Nutritional evaluation

There were no significant differences in body mass loss or BMI variation between the EN and historical control groups on day 30 (respectively, $P = 0.230$ and 0.371) or day 60 ($P = 0.212$ and 0.598). The median caloric intake was significantly increased in the EN group compared to the historical control group on both days 30 and 60 ($P < 0.0001$ and $P = 0.005$); total caloric intake, EN, and oral intake were significantly increased on day 30 (respectively, $P < 0.0001$, $P < 0.001$, and $P = 0.003$) and day 60 ($P = 0.001$, 0.001, and 0.002). The median adequate nutrient intake was significantly increased in the EN group on days 30 and 60 (respectively, $P < 0.0001$ and $P = 0.002$; Table 3). We determine the trend in caloric intake throughout the study period (Fig. 3A). Recovery of oral diet intake was observed from day 30. All caloric intake decreased on day 60 because of the decrease in PN. The historical control data are shown in Figure 3B.

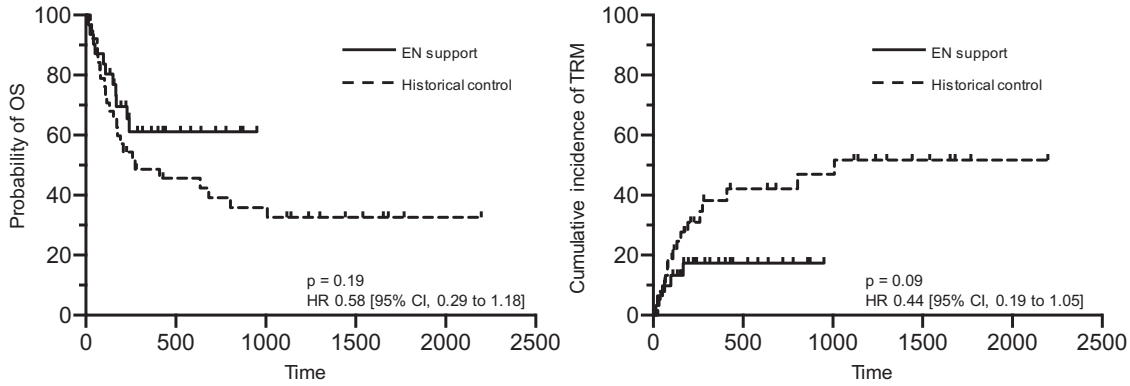

Fig. 1. Kaplan–Meier curves of overall survival (OS) and transplant-related mortality (TRM) for all participants ($n = 31$). (A) Median OS was not reached in the EN group and was 274 d in the historical control group. (B) 1-y TRM was 17.4% in the EN group and 38.2% in the historical control group. CI, confidence interval; EN, enteral nutrition; HR, hazard ratio.

Table 2
Clinical outcomes

Outcome	EN group ($n = 31$)	Historical control group ($n = 38$)	*P*
Time from day 0 to discharge (d)	68 (15–182)	69 (24–274)	0.7124
Duration of EN through an NGT (d)	34 (9–103)	NA	NA
Duration of parenteral nutrition (d)	49 (0–124)	50 (0–166)	0.6946
Mucositis oral grades 2–4	9	30	<0.0001
Duration of mucositis grades ≥2 (d)	0 (0–12)	10 (0–44)	<0.0001
Mucositis oral grades 3–4	3	22	<0.0001
Duration of mucositis grades ≥3 (d)	0 (0–7)	2.5 (0–19)	<0.0001
Diarrhea grades 2–4	19	32	0.0523
Duration of diarrhea grades ≥2 (d)	2 (0–22)	5 (0–40)	0.0288
Diarrhea grades 3–4	10	19	0.1514
Duration of diarrhea grade >3 (d)	0 (0–9)	1 (0–22)	0.0686
Time to neutrophil recovery*, (d)	18 (12–22)	20.5 (13–41)	0.0386
Documented infection	12	17	0.5074
CNS (*Staphylococcus epidermidis*)	8	7	
Enterococcus spp.	3	5	
Corynebacterium jeikeium	1	1	
Klebsiella pneumoniae	1	1	
Escherichia coli	0	2	
Pseudomonas aeruginosa	0	1	
Acinetobacter lwoffii	0	1	
Stenotrophomonas maltophilia	0	1	
Malassezia furfur	0	2	
Candida parapsilosis	0	1	
Invasive pulmonary aspergillosis	0	4	
Acute GVHD grades II–IV	10 (32.3%)	14 (36.8%)	0.8011
Skin	10	11	
Gut	1	9	
Liver	0	2	
Acute GVHD grades III–IV	1 (3.2%)	8 (21.1%)	0.0353
Skin	0	0	
Gut	1	8	
Liver	0	2	
Chronic GVHD	4 (12.9%)	6 (15.8%)	>0.9999
Relapse	13 (41.9%)	14 (36.8%)	0.8048

ANC, all nucleated cells; CNS, coagulase-negative staphylococci; EN, enteral nutrition; GVHD, graft-versus-host disease; NGT, nasogastric tube.
Values are given as median (range), *n*, or *n* (%).
*ANC > 0.5×10^9/L.

Microbial changes analyzed at the genus level

To further evaluate the association between EN and microbiota changes, we studied gut microbial diversity and succession in four participants by analyzing the meta 16S rRNA gene sequence (Fig. 4). Microbial diversity was preserved from the preconditioning phase to 60 d after transplantation in participant #1610, who continued consuming oral and feeding intake. In contrast, microbial diversity was lost in the other three patients from day 30 (participant #1606), day 17 (participant #1611), and preconditioning (participant #1510; Fig. 4). The characteristics of participants whose gut microbiomes were analyzed were as follows: participant #1610 underwent allogeneic peripheral blood stem cell transplantation from an HLA-identical related donor after RIC with FLU plus ivBU. Participant #1606 underwent umbilical cord blood transplantation after RIC with FLU plus ivBU. This participant developed viral encephalitis with consciousness disturbance on day 26 after transplantation, following which all oral intake and

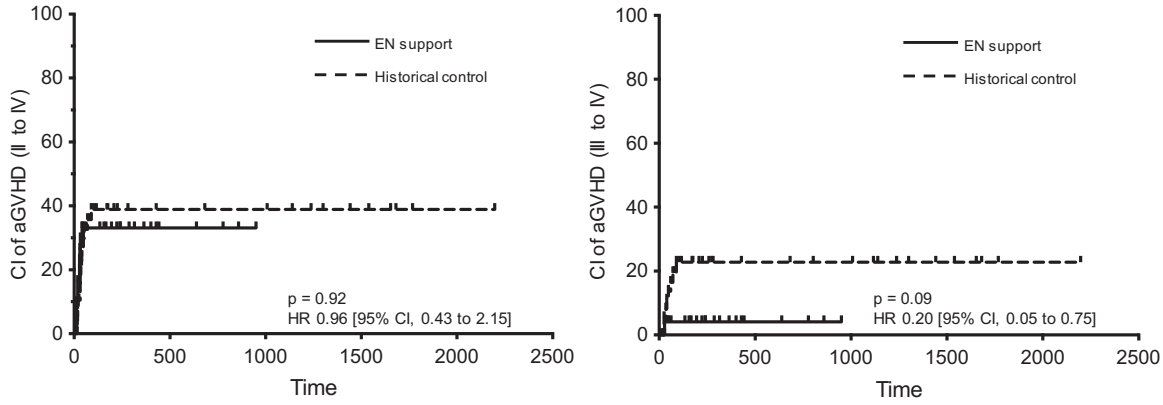

Fig. 2. Cumulative incidences of grades II–IV and III–IV acute graft-vs-host disease (aGVHD). Of the participants with grades III–IV aGVHD, one had gut grade III; no participant had grade IV aGVHD. CI, confidence interval; EN, enteral nutrition; HR, hazard ratio.

Table 3
Nutritional evaluation

	Day 30			Day 60		
Measurement	EN group	Historical control group	*P*	EN group	Historical control group	*P*
Body mass variation (kg)	1.10 (−2.4 to 3.3)	1.80 (−10.2 to 20.0)	0.230	−0.35 (−6.0 to 6.0)	0 (−13.5 to 11.9)	0.371
BMI variation (%)	2.12 (−4.30 to 24.46)	3.42 (−16.2 to 24.2)	0.212	−0.51 (−7.42 to 14.95)	−0.21 (−21.4 to 23.56)	0.598
Caloric-intake variation (%)	22.2 (−19.3 to 180.0)	−6.7 (−91.7 to 214.8)	<0.0001	−1.3 (−69.0 to 63.5)	−29.6 (−98.6 to 31.9)	0.005
Actual caloric intake (kcal)						
Total caloric intake	1936 (1472–2748)	1410 (80–2008)	<0.0001	1580 (472–2012)	1070 (200–2140)	0.001
via EN	400 (0–1600)	0 (0–400)	<0.0001	108 (0–1700)	0 (0–1000)	0.001
via PN	1040 (0–2040)	1120 (0–1940)	0.688	26 (0–1740)	310 (0–1840)	0.374
Oral intake	400 (0–1800)	100 (0–1500)	0.003	1000 (0–2000)	340 (0–1800)	0.002
Nutrient-adequacy (%)	104.3 (71.6–148.5)	77.1 (5.0–107.0)	<0.0001	85.2 (25.2–103.4)	60.5 (9.8–123.7)	0.002

BMI, body mass index; EN, enteral nutrition; PN, parenteral nutrition.
Values are given as median (range).

feeding nutrition were completely discontinued. Participant #1611 underwent allogeneic bone marrow transplantation from an HLA one-locus mismatched unrelated donor after MAC with CY and TBI. This participant had severe nausea and appetite loss at 14 d after transplantation, following which oral food intake and EN were temporarily discontinued. Feeding nutrition with NGT was resumed from day 17, and oral food intake was then reintroduced. Microbial diversity was gradually restored on day 30. Participant #1510 underwent allogeneic bone marrow transplantation from an HLA-identical unrelated donor after RIC with FLU plus ivBU. This participant had severe nausea, appetite loss, and extreme non-cooperation before conditioning chemotherapy. Sinusoidal obstruction syndrome was observed on day 26. EN and oral ingestion could not be continued, and only total parenteral nutrition was continued. No aGVHD was observed in participants #1610 and #1606; in contrast, participants #1611 and #1510 developed aGVHD on day 17 and day 55, respectively.

The following antibiotics were used: participant #1610 was exposed cefepime from days 13 to day 21 and vancomycin from day 17 to day 23. Participant #1606 was exposed to cefepime from day 11 to day 17 and piperacillin/tazobactam and meropenem from day 18 to day 31. Participant #1611 was exposed to cefepime from day 5 to day 14, daptomycin from day 11 to day 14, and doripenem from day 14 to day 22. Participant #1510 was exposed to cefepime from 15 d before transplantation to 2 d before transplantation. Microbial diversity was significantly correlated with nutritional status using RTPs (Fig. 4C)—transferrin: $r^2 = 0.483$, 95% CI, 0.051–0.762, $P = 0.031$; transthyretin: $r^2 = 0.652$, 95% CI, 0.294–0.849, $P = 0.002$; retinol binding protein: $r^2 = 0.494$, 95% CI, 0.066–0.769, $P = 0.027$.

Discussion

Allo-HSCT recipients are often malnourished because of fasting owing to severe gastrointestinal mucosal injury induced by conditioning. We have previously reported that appropriate prevention of mucosal injury using dietary supplements may improve the survival rate of individuals who have received allo-HSCT [2]. Furthermore, a reduction in bacterial translocation with increasing enteral support has been reported using appropriate animal models [18]. Considering this background, we investigated whether aggressive EN using NGT during HSCT has better clinical outcomes and whether intensive EN improves the intestinal microbiome environment.

Several studies have evaluated the effect of body mass loss after allo-HSCT. Body mass loss has been reported in numerous patients, and significant body mass loss is associated with poor clinical outcomes [5]. In the present study, almost one-third of participants lost more than 10% of their body mass from baseline through 3 mo after allo-HSCT; the cumulative incidence of 2-y non-relapse mortality in this malnutrition group was 27.3%. However, the median body mass variation in participants in the EN group was only −0.35 kg, median BMI variation was only −0.51% on day 60, and 2-year TRM was 17.4% (Table 3, Fig. 1). Intestinal mucosal injury was observed; however, the severity of mucositis and diarrhea was mild, and the duration was short. Preventing malnutrition may hasten the recovery of mucosal injury. Indeed, RTPs, which reflect nutritional status, remained nearly unchanged, and variations in total caloric intake remained unaltered on day 60 (data not shown). One limitation in interpreting these results is that RTP levels are significantly affected by various factors, such as

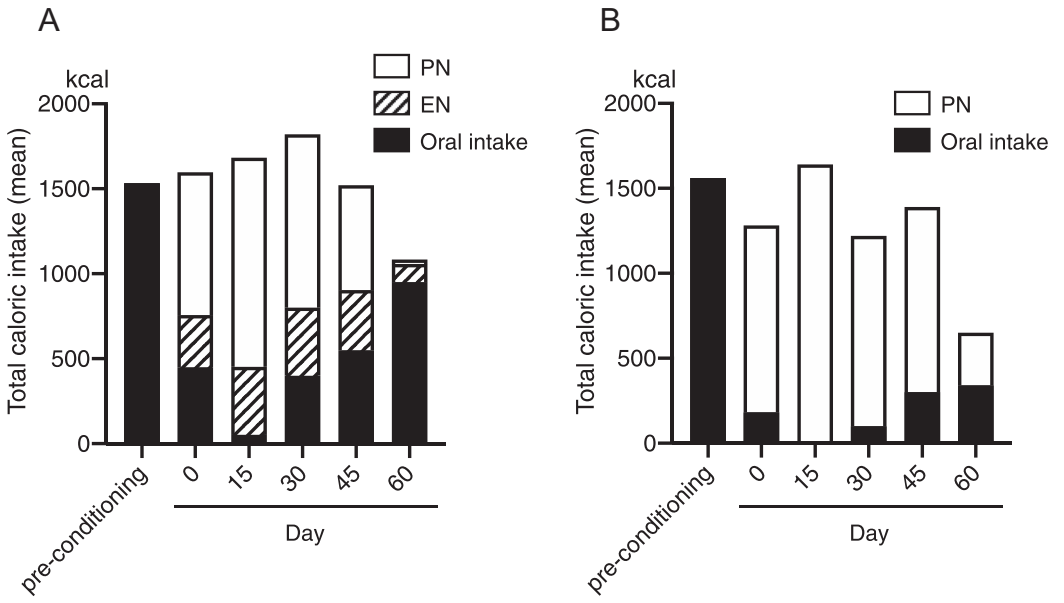

Fig. 3. Total caloric intake throughout the study period in (A) the EN group and (B) the historical control group. EN, enteral nutrition; PN, parenteral nutrition.

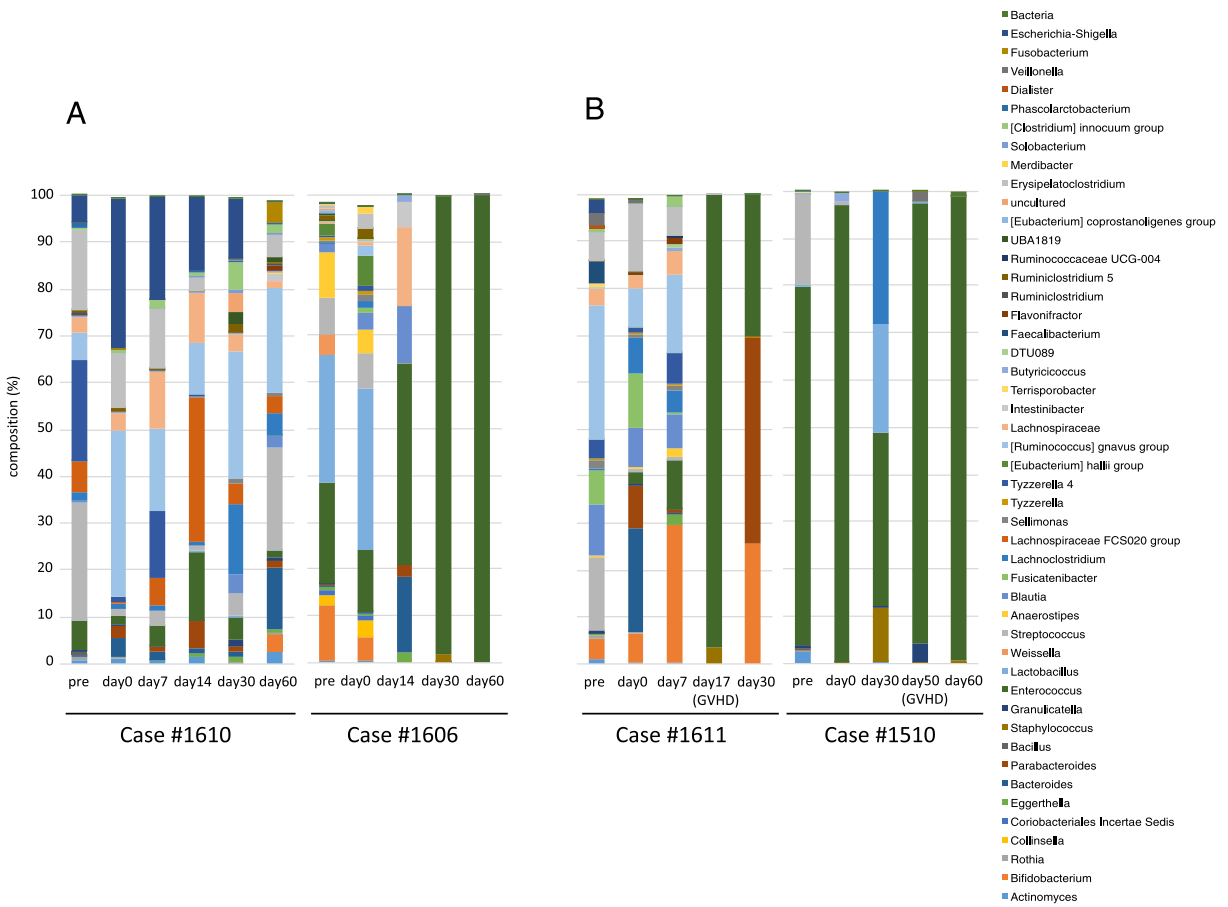

Fig. 4. Changes in the microbiota may have affected the development of acute graft-versus-host disease (aGVHD) in individuals undergoing allogeneic hematopoietic stem cell transplantation. Genus-level relative abundance of microbiota shown from the day before conditioning to day 60 for (A) participants who did not develop acute graft-versus-host disease and (B) those who did. (C) Flora diversity of fecal samples by Shannon index and nutritional status as indicated by transferrin, transthyretin, and retinol binding protein before and after allogeneic hematopoietic stem cell transplantation. Solid lines represent Shannon species diversity, dashed lines represent the concentration of rapid turnover proteins. RBP, retinol binding protein; Tf, transferrin; TTR, transthyretin.

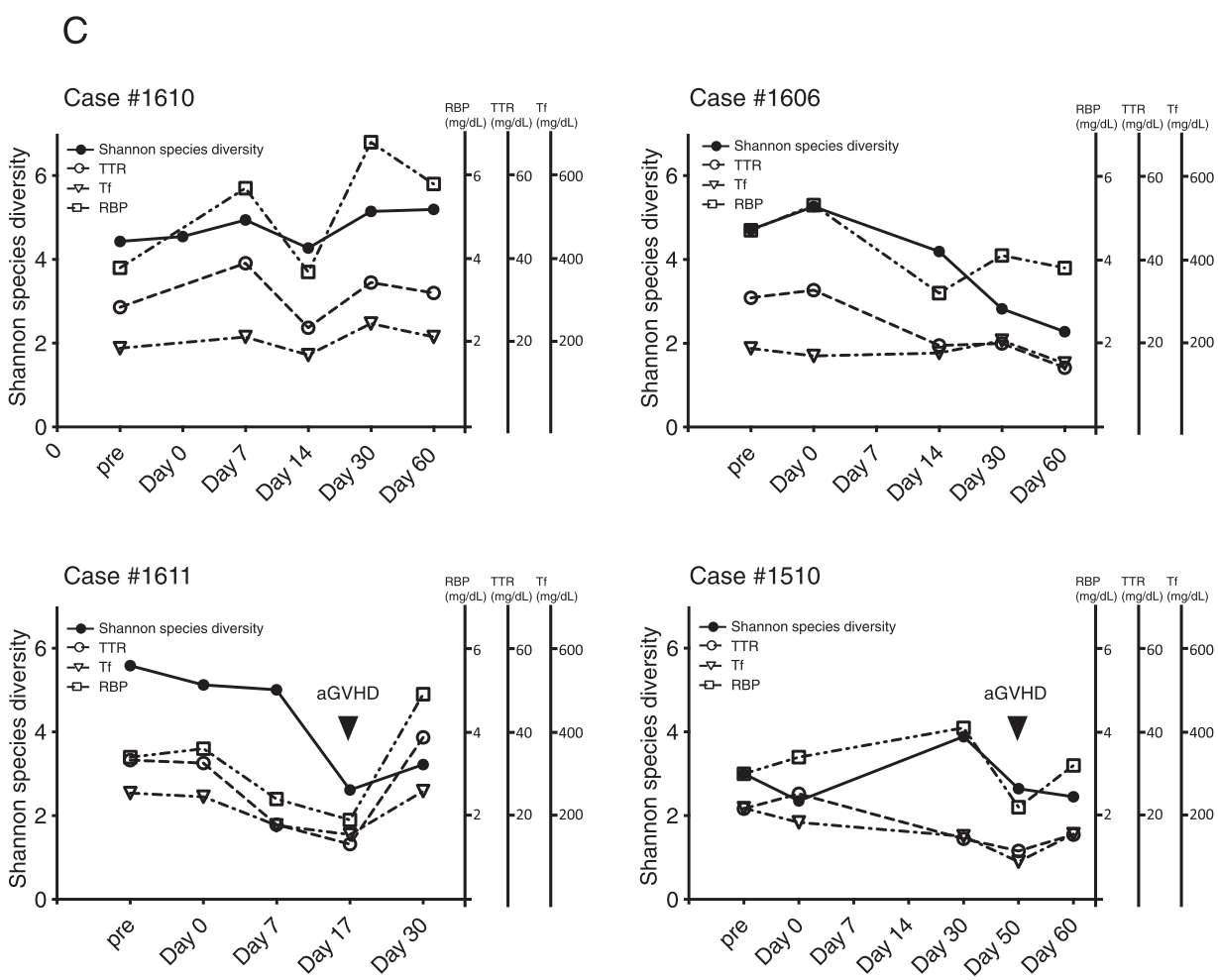

Fig. 4. Continued

inflammation. Furthermore, EN in the actual caloric intake accounted for ~20% of the total nutrition on day 30. We removed NGTs and CVCs to prepare for discharge at around day 60. Starvation of EN or PN led to decreased total caloric intake on day 60 (Fig. 3A). Compared with historical control data (no-EN group; Fig. 3B), EN via NGT contributed to maintaining total caloric intake. Interestingly, recovery of oral diet intake was observed earlier than in the historical control data. Our purpose in using EN with NGT was not to provide all calories required for daily nutrition, but rather to ensure that the gut maintained its function even during the minimal oral intake period. Consequently, the calorie count of oral dietary intake accounted for ~20% on day 30 and increased to over 60% on day 60. Furthermore, early EN may reduce the incidence of complications of hyperglycemia. Glucose intolerance requiring high-dose insulin administration (>50 U/d) was observed in 5 (16.1%) of 31 participants in this group. In contrast, 11 (28.9%) of 38 participants in the historical control group (without early EN) developed glucose intolerance.

A recent randomized study investigated the tolerance of EN with NGT during allo-HSCT and reported that the early NGT cessation rate was ~90% [19], whereas in the present study tube feeding was well tolerated. Our participants started EN with NGT before conditioning chemotherapy; in contrast, in the other recent study NGT was inserted on day 1 after stem cell infusion. To improve EN tolerance, deferment of insertion of the NGT was attempted until the end of conditioning within the first week after transplantation [8]; however, tolerance to NGT may have resulted from the early timing of its insertion. Furthermore, local intolerance such as gastric repletion, nausea, and pharyngeal discomfort was prevented by decelerating the flow of EN and using an NGT made of a soft material.

Interestingly, cases of severe aGVHD were rare, as only one patient had gut aGVHD (grade II). We calculated the HR for OS, TRM, and aGVHD using univariate and multivariate Cox regression models. EN significantly reduced the risk of aGVHD (grades III–IV) considering HLA disparity (HR, 0.12, 95% CI, 0.01–0.93, $P = 0.042$). EN support was found to greatly affect the onset of intestinal GVHD compared to skin or liver GVHD in our study. Adequate EN decreases the incidence of severe and gut aGVHD [8,9,20]; however, the underlying mechanism remains unclear. In recent years, the association between GVHD and the intestinal microbiota of individuals undergoing allo-HSCT has drawn considerable attention. Peled et al. [21] report that higher diversity of the intestinal microbiota is associated with lower GVHD-related mortality in individuals undergoing allo-HSCT. In participants #1611 and #1510, aGVHD and dysbiosis of the intestinal microbiota occurred simultaneously; however, aGVHD was not observed in participant #1610, whose microbial diversity was preserved (Fig. 4A, B). Furthermore, microbial diversity was correlated with nutritional status (Fig. 4C). These data suggest that improving nutrition can induce the restoration of microbial diversity.

Furthermore, we evaluated changes in the microbiota subpopulations upon discontinuing EN and oral ingestion, which revealed a

drastic increase in Enterococcaceae on days 30 and 60 in participant #1606 (Fig. 4A). Furthermore, sinusoidal obstruction syndrome and aGVHD developed on days 26 and 55 in participant #1510. EN and oral ingestion were discontinued, and only total parenteral nutrition was continued without interruption from day 25, resulting in an increase in Enterococcaceae from day 50 (Fig. 4B). The prevalence of Enterococcaceae (order Lactobacillales) markedly increased, whereas that of Lachnospiraceae (order Clostridiales) decreased in participants with aGVHD. Individuals who develop GVHD exhibit microbiota shifts, in which the dominant order deviates from Clostridiales toward Lactobacillales or Enterobacterales [22]. Jenq et al. [23] report a loss in microbiota diversity and expansion of *Enterococcus* species in murine models of GVHD. Dysbiosis of the intestinal microbiota can lead to the development and progression of GVHD through several processes, including the secretion of pathogen-associated molecular patterns, induction of inflammation, activation of macrophages, and coactivation of alloreactive T cells [24]. *Enterococcus* species contribute to the development of intestinal inflammation in mice by impairing the epithelial barrier integrity [25] and induce the production of tumor necrosis factor from macrophages [26].

We additionally administered antiflatulent Biofermin-R tablets (Biofermin Seiyaku, Kobe, Japan) including *Enterococcus faecalis* to each participant as a probiotic. A dozen colonies obtained from three samples (day 60 for participants #1510 and #1606, day 17 for participant #1611) were analyzed by partial 16S rRNA gene sequencing (approximately 500 base pairs, including V2-V3 variable regions) and polymerase chain reaction specific for *E. faecalis* and *E. faecium*. As a result, all colonies were indicated to be *Enterococcus faecium* (Supplementary Fig. 2). These data suggest that *Enterococcus faecium* eliminated *Enterococcus faecalis* from the antiflatulent. Probiotics alone without prebiotics such as EN may not be useful in individuals whose microbial diversity is lost. Furthermore, mouse Paneth cell α-defensins, termed *cryptdins*, exhibit strong antimicrobial activity. Among them, cryptdin-4 exhibits the most potent antimicrobial activity. It eliminates numerous non-commensal bacteria and some commensal bacteria, including *Enterococcus* species [27]. Interestingly, Paneth cells are also targeted by GVHD, thus markedly downregulating α-defensins [28]. Reductions in such antimicrobial peptides may increase the abundance of Enterococcaceae.

Our study has some limitations. First, because of its retrospective nature, some factors may have influenced the outcomes. Second, no comparative data on EN and PN were obtained. Third, microbial diversity was analyzed in only four participants, limiting the generalization of our findings to the entire cohort. Fourth, the historical control cohort involves fundamental confounders. It has more HLA-mismatched transplants and more myeloablative conditioning, suggesting that this group was originally more likely to develop aGVHD than the study cohort treated with early EN. Furthermore, the influence of antibiotics such as piperacillin/tazobactam and carbapenems on the microbiota was not considered.

Conclusions

Our results suggest that early nutritional intervention using NGT can result in promising outcomes for individuals who undergo allo-HSCT. Furthermore, this nutritional support strategy may help maintain homeostasis in the intestinal microbiome. Prospective randomized controlled studies are required to investigate the importance of aggressive EN among allo-HSCT recipients. This clinical trial revealed a potential effect of EN on clinical outcomes in allo-HSCT and a potential association between the intestinal microbiome and EN. Manipulation of the intestinal microbiome via EN may improve clinical outcomes in allo-HSCT.

Acknowledgments

We thank all the medical and nursing staff and nutritional support teams for their contributions to this work and their dedication to the patients.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2020.111093.

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