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A role for diet and gut microbiota metabolites in autologous hematopoietic cell transplant recipients

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Abstract

Introduction: The gut microbiome has an established role in allogeneic hematopoietic cell transplantation (allo-HCT), but not in an auto-HCT setting. We have hypothesized that fecal short-chain fatty acids (SCFA) and urinary 3-indoxyl sulfate (3-IS), which are metabolites derived from the action of the gut microbiome on dietary fiber, play a role in auto-HCT outcomes.

Methods: This was a single-center prospective study involving auto-HCT recipients. Baseline patient and disease details, diet diaries, and antibiotic exposure were recorded in consenting patients. Serial (pre-HCT, week two, and week four post-HCT) SCFA and urine 3-IS levels were measured using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). HCT outcomes were correlated with these metabolites.

Results: Thirty patients (myeloma, n=13; lymphoma, n=17) were analyzed. The levels of urinary 3-IS, fecal acetate, propionate, and butyrate were found to be decreased at week two and were recovered by week four post-HCT. Those with low median nadir fecal butyrate levels at week two also had significantly lower pre-HCT and week four butyrate levels. Recipients with low butyrate levels had more grade ≥2 mucositis (80% vs. 33%, p=0.01) and low fiber intake (10.4 g vs. 13.6 g, p=0.04). They also had more carbapenem exposure (93% vs. 47%, p=0.005) and prolonged antibiotics (11 days vs. 8 days, p=0.008). There were no differences in the time to neutrophil or platelet engraftment, mortality, or disease response.

Conclusion: Low pre-HCT fecal butyrate levels tend to persist post-HCT and they are associated with mucositis, dietary fiber intake, and antibiotic exposure. The gut microbiome and its modulation may play a role in auto-HCT settings.

Key words SCFA, 3-IS, gut microbiome, auto-HCT

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Introduction

Dysbiosis of the gut microbiome is common in recipients of hematopoietic cell transplantation (HCT). This imbalance in the gut microbiome is associated with morbidity and mortality post-allogeneic HCT[1]. However, data on the importance of the gut microbiome in autologous HCT (auto-HCT) is still evolving. Initial exploratory studies have shown that a similar loss of gut microbial diversity in auto-HCT recipients around the engraftment period was associated with regimen-related toxicities, such as mucositis, vomiting, and diarrhea[2,3]. Subsequent studies have shown an association between lower diversity/particular microbe dominance, minimal residual disease (MRD), and progression-free survival (PFS) in multiple myeloma[4-7]. All these studies examined the gut microbiome diversity using 16S rRNA sequencing. Only one previous study has additionally examined fecal short-chain fatty acid (SCFA) (butyrate) at three months post-HCT and found it to be associated with diet and negative MRD. Indigestible carbohydrates from plant-based diets are metabolized

and fermented to SCFAs (acetate, propionate, and butyrate) by commensals in the colonocytes. These SCFAs play a role in maintaining the protective mucus lining of the colon, influencing regulatory T cells, and mucosal barrier homeostasis[8]. Similarly, dietary tryptophan is converted by the action of the intestinal microbiota to 3-indoxyl sulfate (3-IS) and has been applied in allo-HCT. Fecal SCFAs and urinary 3-IS are good surrogate markers of gut microbial diversity in allo-HCT recipients and are correlated with allo-HCT outcomes[9-11]. In this exploratory study, we have prospectively examined serial fecal SCFA and urinary 3-IS levels in auto-HCT recipients and their association with diet and HCT outcomes.

Material and Methods

This was a single-center prospective study in consecutive auto-HCT recipients aged ≥12 from 2021 to 2022. This study obtained approval from the Postgraduate Institute of Medical Education and Research, Chandigarh,India Institutional Ethics Committee (IEC-04/2020-1622). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Serial urinary 3-IS and fecal SCFA levels were collected prospectively at the following time points: pre-HCT and weeks two and four post-HCT. All of the samples were stored at -80℃ until further processing. Recipient demographics, diseases, and transplant details were recorded. Myeloma patients received melphalan (200 mg/m^2), whereas lymphoma patients received standard (BEAM) carmustine, etoposide, cytarabine, and melphalan (140 mg/m^2) as a conditioning regimen. Disease response was assessed pre-HCT and on day+100 after HCT using PET-CT. A detailed diet diary was maintained using the Indian Council of Medical Research (ICMR) Nutrify India app. Daily averages of calorie, carbohydrate, resistant starch, and dietary fiber intake were calculated. Peri-transplant use of a broad-spectrum antibiotic (carbapenem) and the incidence of drug-resistant infections were also recorded. Group comparisons were performed using 2-sided Mann-Whitney U-tests/chi-squared tests. GraphPad Prism 8.0.2 version was used for all statistical analyses.

Urinary 3-Indoxylsulfate assessment

Detection and quantification of urinary 3-IS were performed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) as described previously[12]. 3-IS was eluted from the reaction mixture by reverse-phase liquid chromatography and detected by linear ion trap quadrupole LC-MS/MS (Shimadzu) in the negative-ion multiple reaction monitoring modes. Solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) were used as the mobile phases, and isatin was used as the internal standard.

Table 1. Comparison of pre-HCT and week 2 dietary parameters and metabolites

	Pre-HCT	Week 2	*p*-value
Calories (Kcal/day)	1,966 ± 277	1,045 ± 327	<0.0001
Carbohydrate (gm/day)	252 ± 36	146 ± 37	<0.0001
Dietary fiber (gm/day)	25 ± 4	12 ± 4	<0.0001
Resistant starch (gm/day)	11 ± 2	5 ± 2	<0.0001
3-Indoxyl sulfate (μmol/ mmol creat)	26.1 ± 18.7	14.4 ± 24.0	0.004
Acetate (μmol/g dry weight)	19.0 ±10.9	9.7 ± 10.2	0.001
Propionate (μmol/g dry weight)	7.6 ± 5.3	3.5 ± 3.7	0.001
Butyrate (μmol/g dry weight)	6.2 ± 4.6	2.7 ± 3.2	<0.001

HCT, Hematopoietic Cell Transplantation

Fecal short-chain fatty acid assessment

As described previously, fecal SCFAs were quantified using LC-MS/MS in the negative ion mode[13,14]. 3-Nitrophenylhdrazine hydrochloride with N-(3-dimethyl aminopropyl)-N'-ethyl carbodiimide (EDC) as a catalyst was used for the derivatization of SCFAs. D3- acetic acid, D5- propionic acid, and D7- butyric acid (TRC, Canada) were used as internal standards. 100-200 mg of fecal sample was weighed and homogenized for 10 min with 60% acetonitrile. The clear supernatant obtained after centrifugation was used to determine the unknown concentrations in the patient samples. Mobile Phase A-0.1% formic acid in 10 mmol ammonium formate and mobile Phase B-0.1% formic acid in methanol were used in this study.

Results

A total of 30 patients (myeloma n=13 and lymphoma n=17) were included in this study over the study period. As expected, dietary calories, carbohydrates, fiber, and resistant starch intake decreased significantly at week two after HCT when compared to pre-HCT intake (**Table 1**). The mean 3-IS levels were also significantly lower in week two when compared to pre-HCT levels (14.4 ± 24.0 vs. 26.1 ± 18.7 μmol/mmol creat, p=0.004). The 3-IS levels were found to be recovered by week four (22.6 ± 18.6 μmol/mmol creat). A similar trend was observed for fecal SCFA. The mean acetate, propionate,

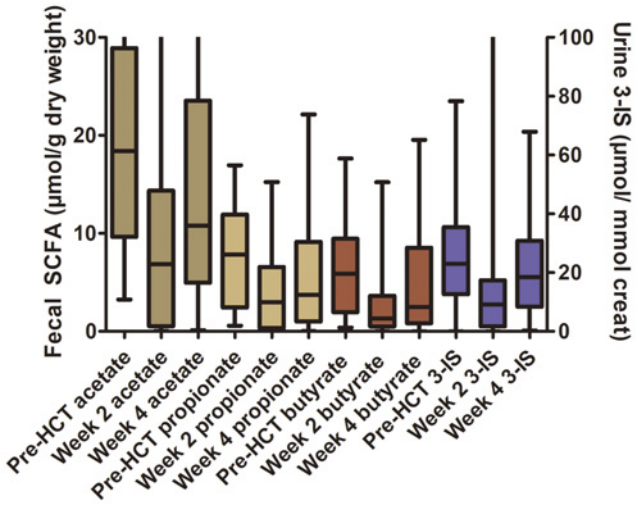

Figure 1. Serial fecal SCFA and urine 3-IS in auto-HCT recipients (nadir at week two post-HCT)

and butyrate levels were significantly lower in week two compared to pre-HCT levels (19.0 ± 10.9 vs. 9.7 ± 10.2, p=0.001, 7.6 ± 5.3 vs. 3.6 ± 3.7, p=0.001, and 6.2 ± 4.6 vs. 2.7 ± 3.2 μmol/g dry weight, p=0.0007, respectively) (**Figure 1**). The SCFA levels had recovered by week four post-HCT (15.8 ± 14.9, 6.0 ± 6.1, and 4.9 ± 5.5 μmol/g dry weight, respectively). There was a positive correlation between dietary fiber and fecal butyrate levels at week two post-HCT (r=0.35, p=0.05). Similarly, resistant starch intake positively correlated with fecal propionate level at week two with post-HCT (r=0.534, p=0.0023).

Since butyrate levels have been previously correlated with outcomes in allo-HCT recipients, we decided to compare two groups of patients separated by the median nadir (week two post-HCT) fecal butyrate levels (1.3 μmol/g dry weight). Both cohorts were matched

Table 2. Comparison of patient characteristics and HCT outcomes by nadir fecal butyrate levels

Characteristics	Butyrate ≤ 1.3 μmol/g dry weight n=15, Median (IQR), n (%)	Butyrate >1.3 μmol/gdry weight n=15, Median (IQR), n (%)	*p*-value
Age (years)	46 (27-55)	32 (20-52)	0.3
Males	8 (53%)	10 (67%)	0.4
Females	7 (47%)	5 (33%)	
Multiple myeloma	6 (40%)	7 (47%)	0.7
Lymphoma	9 (60%)	8 (53%)	
Pre-HCT disease status			
Myeloma PET-negative	3/6 (50%)	3/7 (43%)	1.0
Lymphoma CMR	8/9 (89%)	8/8 (100%)	
CD34 Counts (×10^6/kg)	5.6 ± 2.5	6.5 ± 3.1	0.4
Pre-HCT butyrate levels	3.9 ± 3.6	8.4 ± 4.5	0.01
Week 2 butyrate levels	0.5 ± 0.5	4.8 ± 3.3	<0.0001
Week 4 butyrate levels	2.9 ± 3.4	6.9 ± 6.6	0.02
Week 2 3-Indoxylsulfate levels	11.6 ± 20.2	17.2 ± 27.8	0.25
Week 2 Acetate levels	5.6 ± 7.8	13.9 ± 10.9	0.0085
Week 2 Propionate levels	2.0 ± 3.0	5.1 ± 3.8	0.0083
Oral Mucositis			
Grade 1	8 (53%)	14 (93%)	0.03
Grade ≥ 2	7 (47%)	1 (7%)	
Diarrhea			
Grade 1	6 (40%)	12 (80%)	0.06
Grade ≥ 2	9 (60%)	3 (20%)	
Dietary fibers (week 2) (g)	10.4 ± 4.6	13.6 ± 2.6	0.04
Resistant starch (week 2) (g)	4.5 ± 2.4	4.6 ± 1.8	0.9
Post-HCT drug resistant bacteremia	6 (40%)	2 (13%)	0.2
Post-HCT carbapenem exposure	14 (93%)	7 (47%)	0.005
Antibiotic duration (days)	11 (14-9)	8 (10-7)	0.008
Neutrophil engraftment (days)	12 (13-11)	11 (12-11)	0.1
Platelet engraftment (days)	13 (17-10)	12 (14-11)	0.3
Hospitalization duration (days)	16 (20-14)	15 (16-13)	0.04
Mortality	2 (13%)	0 (0)	0.1
Day+100 post-HCT disease status			
Myeloma PET-negative	3/6 (50%)	3/7 (43%)	1.0
Lymphoma CMR	7/9 (78%)	7/8 (87%)	

HCT, Hematopoietic cell transplantation; PET, positron emission tomography; CMR, complete metabolic response

Table 3. Summary of studies exploring the role of gut microbiome analysis in auto-HCT settings

Author, Country, Year	Patients and methods Samples timing	Results
Montassier et al., France, 2015	Lymphoma (n=28) 16S rRNA NGS Pre-conditioning and 7 days later (pre-HCT)	Gut microbiome changes associated with mucositis.
El Jurdi et al., USA, 2019	Myeloma (n=15) 16S rRNA NGS day-2, +7, and +30 post auto-HCT	Lower diversity was associated with diarrhea, vomiting, and mucositis.
Pianko et al., USA, 2019	Myeloma (n=34) 16S rRNA NGS after induction therapy	High abundance of *Eubacterium hallii* (butyrate-producing) with MRD-negativity. No association with auto-HCT.
Khan et al., USA, 2021	Myeloma (n=534) 16S rRNA NGS 1 sample between day-30 and +100 auto-HCT	Lower diversity was associated with a high risk of death and poor progression-free survival.
D'Angelo et al., USA, 2022	Myeloma (n=30) 16S rRNA NGS, pre-HCT, engraftment, day+100 9-12 months post-HCT	Lower alpha diversity at engraftment was associated with a partial response. High fiber associated with blautia abundance.
Urvi A. Shah et al., USA, 2022	Myeloma (n=34) 16s rRNA NGS Fecal butyrate GC-MS 3 months post-HCT	High diversity and butyrate concentration was associated with MRD negativity. Plant-based diet correlated with butyrate levels.
Our study	Myeloma (n=13), lymphoma (n=17) Urine 3-IS and fecal SCFA (LC-MS/MS) Pre-HCT, weeks 2 and 4 post-HCT	Low butyrate levels are associated with low intake of dietary fiber, and higher incidence of mucositis, broad-spectrum antibiotic exposures, and hospitalization duration.

rRNA, ribosomal ribonucleic acid; NGS, next generation sequencing; MRD, measurable residual disease; SCFA, short chain fatty acids; 3-IS, indoxyl sulfate

for age, sex, diagnosis, pre-HCT disease status, and CD 34 counts (**Table 2**). Those with low median nadir fecal butyrate levels also had significantly lower pre-HCT and week four butyrate levels. The recipients with low butyrate levels had more grade ≥ 2 GI mucositis (80% vs. 33%, p=0.01) and low dietary fiber intake (10.4 g vs. 13.6 g, p=0.04). They also had more carbapenem exposure (93% vs. 47%, p=0.005), prolonged antibiotics (11 days vs. 8 days, p=0.008), and hospitalization duration (16 days vs. 15 days, p=0.04). However, there was no difference in the incidence of drug-resistant bacteremia, neutrophil count, or platelet engraftment days. The day+100 mortality and disease status were also not different.

Discussion

Gut microbial alterations have been associated with allo-HCT outcomes and are subjected to therapeutic interventions that modify the microbiome. A role of gut microbiome perturbation in auto-HCT outcomes has also been proposed. Although studying the gut microbiome using 16S rRNA next-generation sequencing is cumbersome and expensive, studying metabolites in urine and feces is comparatively easy and also inexpensive. This study has explored the association of these metabolites with dietary content, HCT outcomes, urinary 3-IS, and fecal SCFAs. These metabolites are directly produced by the action of gut commensals on dietary fiber and resistant starch and play a direct role in gut immune homeostasis. This study has confirmed that these metabolites decreased post-HCT by week two (peri-engraftment) and recovered by week four. These metabolites correlated with dietary fiber and resistant starch intake. The low fecal butyrate levels pre-HCT tended to persist after HCT. It is associated with a higher incidence of mucositis and, consequently, lower fiber intake, more broad-spectrum antibiotic exposure, longer antibiotic duration, and hospitalization duration. Its association with GI toxicity has been reported in two previous studies[2,3]. Although the causality of antibiotic exposure, dietary fiber, and butyrate levels is difficult to establish, our study has shown that low pre-HCT butyrate levels beget events (mucositis leading to lower fiber intake and increased use of broad-spectrum antibiotics) that lead to even lower post-HCT butyrate levels[15]. In a small series of patients with heterogeneous diagnoses of myeloma and lymphoma and a short follow-up of 100 days post-HCT, we could not show an association between disease status and mortality, in contrast to other previous studies[4,5]. This might also be due to the rapid recovery of fecal SCFAs around week four post-HCT, which is usually when auto-HCT recipients return to their regular diet and home environment.

Conclusion

The low pre-and early HCT SCFA levels associated

with early HCT outcomes may suggest therapeutic intervention using specific diets rich in fiber/resistant starch and limiting broad-spectrum antibiotics. The findings of this study need to be confirmed in a larger homogeneous patient population and a longer follow-up period, with analysis to elucidate the correlation with gut microbial diversity using 16S rRNA sequencing in order to establish the role of these metabolites as biomarkers in an auto-HCT setting.

Author Contributions

SK, AP, PM, and DL conceived the study, and all authors contributed to patient recruitment and sample collection. SK, AP, PM, and DL processed, analyzed the data, and wrote the manuscript. SK and AP contributed equally to this study.

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Conflicts of Interest

The authors have no competing interests to declare relevant to this article's content. Disclosure forms provided by the authors are available on the website.

Informed consent was obtained from all individual participants included in the study.

References

1. 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.
2. El Jurdi N, Filali-Mouhim A, Salem I, Retuerto M, Dambrosio NM, Baer L, et al. Gastrointestinal Microbiome and Mycobiome Changes during Autologous Transplantation for Multiple Myeloma: Results of a Prospective Pilot Study. Biol Blood Marrow Transplant. 2019; **25**: 1511-9.
3. Montassier E, Gastinne T, Vangay P, Al-Ghalith GA, Bruley des Varannes S, Massart S, et al. Chemotherapy-driven dysbiosis in the intestinal microbiome. Aliment Pharmacol Ther. 2015; **42**: 515-28.
4. Khan N, Lindner S, Gomes ALC, Devlin SM, Shah GL, Sung AD, et al. Fecal Microbiota Diversity Disruption and Clinical Outcomes after Auto-HCT: A Multicenter Observational Study. Blood. 2021; **137**: 1527-37.
5. Shah UA, Maclachlan KH, Derkach A, Salcedo M, Barnett K, Caple J, et al. Sustained Minimal Residual Disease Negativity in Multiple Myeloma is Associated with Stool Butyrate and Healthier Plant-Based Diets. Clin Cancer Res. 2022; **28**: 5149-55.
6. Pianko MJ, Devlin SM, Littmann ER, Chansakul A, Mastey D, Salcedo M, et al. Minimal residual disease negativity in multiple myeloma is associated with intestinal microbiota composition. Blood Adv. 2019; **3**: 2040-4.
7. D'Angelo C, Sudakaran S, Asimakopoulos F, Hematti P, El-Gamal D, Safdar N, et al. Perturbation of the gut microbiome and association with outcomes following autologous stem cell transplantation in patients with multiple myeloma. Leuk Lymphoma. 2023; **64**: 87-97.
8. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013; **504**: 451-5.
9. Masetti R, Zama D, Leardini D, Muratore E, Turroni S, Brigidi P, et al. Microbiome-derived metabolites in allogeneic hematopoietic stem cell transplantation. Int J Mol Sci. 2021; **22**: 1197.
10. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med. 2016; **8**: 339ra71.
11. Weber D, Oefner PJ, Hiergeist A, Koestler J, Gessner A, Weber M, et al. Low urinary indoxyl sulfate levels early after transplantation reflect a disrupted microbiome and are associated with poor outcome. Blood. 2015; **126**: 1723-8.
12. Zhu W, Stevens AP, Dettmer K, Gottfried E, Hoves S, Kreutz M, et al. Quantitative profiling of tryptophan metabolites in serum, urine, and cell culture supernatants by liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2011; **401**: 3249-61.
13. Liebisch G, Ecker J, Roth S, Schweizer S, Öttl V, Schött HF, et al. Quantification of fecal short chain fatty acids by liquid chromatography tandem mass spectrometry—investigation of pre-analytic stability. Biomolecules. 2019; **9**: 121.
14. Han J, Lin K, Sequeira C, Borchers CH. An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography-tandem mass spectrometry. Anal Chim Acta. 2015; **854**: 86-94.
15. Pianko MJ. Impact of diet and antibiotics on gut microbiota and outcomes in patients with multiple myeloma treated with autologous hematopoietic stem cell transplantation. Leuk Lymphoma. 2023; **64**: 3-4.

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