A Distinct Plasma Microbiome But Not Gut Microbiome in Patients With Systemic Lupus Erythematosus Compared to Healthy Individuals

Warren A. James[1], Elizabeth Ogunrinde[2], Zhuang Wan[2], Diane L. Kamen[3], Jim Oates[3], Gary S. Gilkeson[4], and Wei Jiang[5]

ABSTRACT. **Objective.** Blood microbiome has been analyzed in cancer patients using machine learning. We aimed to study whether the plasma microbiome represents the microbial community in the gut among patients with systemic lupus erythematosus (SLE) and healthy controls (HCs).
Methods. Paired plasma and stool samples from female patients with SLE and female HCs were assessed for microbiome composition by microbial 16S ribosomal RNA sequencing.
Results. Decreased microbial alpha diversity in stool compared to plasma and distinct plasma and gut beta diversity were found in both HCs and patients with SLE. No difference in gut microbial diversity was found; however, plasma alpha diversity was decreased in patients with SLE compared to HCs. The predominant bacteria differed between plasma and stool in both groups. Although the predominant plasma and stool genus bacteria were similar in patients with SLE and HCs, some were clearly different.
Conclusion. Compared to the gut, the plasma microbiome contained distinct community and greater heterogeneity, indicating that the predominant circulating microbiome may originate from sites (eg, oral or skin) other than the gastrointestinal tract. The decreased plasma but not gut alpha diversity in patients with SLE compared to HCs implies an altered plasma microbiome in SLE, which may be important for systemic immune perturbations and SLE disease pathogenesis.

Key Indexing Terms: autoantibodies, autoimmune diseases, autoimmunity, bacteria, systemic lupus erythematosus

[1]W.A. James, MD, College of Medicine, Medical University of South Carolina; [2]E. Ogunrinde, PhD, Z. Wan, MS, Department of Microbiology and Immunology, Medical University of South Carolina; [3]D.L. Kamen, MD, J. Oates, Director and Endowed Chair, MD, Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina, and Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina; [4]G.S. Gilkeson, MD, Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina, and Staff Physician, Ralph H. Johnson VA Medical Center; [5]W. Jiang, MD, Department of Microbiology and Immunology, and Division of Infectious Diseases, Department of Medicine, Medical University of South Carolina, Charleston, South Carolina, USA.

W.A. James and E. Ogunrinde contributed equally to this work.

The authors declare no conflicts of interest relevant to this article.

Address correspondence to Dr. G.S. Gilkeson, 114 Doughty Street, Strom Thurmond Research Building Room 416, Charleston, SC 29403, USA. Email: gilkeson@musc.edu. Or Dr. W. Jiang, 173 Ashley Avenue, BSB208D, Charleston, SC 29425, USA. Email: jianw@musc.edu.

Accepted for publication January 25, 2022.

Most studies have analyzed microbiome in stools or in samples from other mucosal sites that may not represent the systemic microbiome and may not play a major role in systemic immunity. Recently, blood microbiome has been analyzed in patients with cancer using a method of machine learning[1]; notably, the method of machine learning requires a huge sample size (a total of 18,116 samples in this study). Few studies have been conducted on blood microbiome, with relatively small sample sizes.[2,3] We recently published a methodological paper on human plasma microbiome analysis using a substantially expanded set of controls, including technical and biological replicates, blank extractions and amplifications, and a thorough and combined bioinformatics analysis of the resulting control and plasma sequence data.[4] The amplicon sequence variant (ASV) from the water control was subtracted from the plasma microbiome analysis.[4]

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by systemic inflammation and the production of autoantibodies against self-antigens, leading to progressive organ damage. A previous study suggested that patients with SLE had a distinct plasma microbiome composition with increased plasma levels of total microbial translocation that associated with disease pathogenesis.[5] One hypothesis is that the translocation of bacterial products into the systemic circulation

leads to systemic inflammation.[6] The exact etiology of plasma microbial translocation remains unclear, but there is evidence to suggest that the source of blood bacterial translocation stems from increased intestinal permeability.[6] Under normal, healthy conditions, the intestinal epithelium forms a protective barrier with assistance from protective factors such as mucus layers, and secretory IgA and defensins.[6] In some disease states with a compromised barrier, increased levels of plasma microbial translocation may contribute to disease pathogenesis.[5,6]

Once considered to be a sterile environment, it is now well established that plasma contains bacterial products, under both normal conditions and disease states.[1,5] While the gut microbiome has been studied extensively,[7] few studies have characterized the human plasma microbiome.[1,2,3,8,9,10] To date, no study has directly compared gut and plasma microbiome composition in healthy individuals and patients with SLE. The role of dysbiosis and the circulating microbiome in the development of autoimmunity and SLE is being increasingly studied[11]; however, the source of this microbial translocation has yet to be revealed, though there is evidence to suggest that the gastrointestinal tract is a major source.[11] In this novel study, we compared the microbiome composition using stool and paired plasma samples among patients with SLE and healthy controls (HCs) to further understand the source of microbial translocation.

METHODS

Study participants. A single cohort was examined in this study and the clinical information of patients is shown in Table 1. It consisted of 9 unrelated HCs and 11 patients with SLE. All patients and HCs in this cohort were females. Samples were obtained from the Division of Rheumatology and

Table 1. Demographic, clinical, and laboratory features of patients and controls.

	Mean (SD)
Demographic features	
Controls, n = 9	
Sex, male/female, n	9/0
Race, African American, n	9
Age, yrs	45 (15)
Patients with SLE (n = 11)	
Sex, male/female, n	11/0
Race, African American, n	11
Age, yrs	51.4 (11.0)
Clinical manifestations of patients with SLE	
SLEDAI	1.72 (1.90)
Laboratory manifestations of patients with SLE	
anti-dsDNA, IU/mL	57 (64)
C3 complement, mg/dL	117.2 (21.1)
C4 complement, mg/dL	23.7 (9.2)
Serum albumin, g/dL	3.6 (0.2)
BUN, mg/dL	14 (4)
Serum creatinine, mg/dL	0.8 (0.1)
Urine protein, mg/dL	26.3 (18.4)
Urine creatinine, mg/dL	143.1 (99.6)
Urine protein/creatinine ratio, mg/mg	0.210 (0.112)

BUN: blood urea nitrogen; SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

Immunology at the Medical University of South Carolina. The criteria for inclusion in this study were age ≥ 18 years and the ability to provide informed consent. The criteria for exclusion in this study were individuals who were pregnant or breastfeeding, recent severe illness, contraindications to blood withdrawals, or antibiotic and probiotic treatment within the previous 90 days. This study was approved by the Institutional Review Board of the Medical University of South Carolina (ethics approval number: Pro00082453). All participants signed a consent form.

DNA extraction. The microbial DNA was extracted from 400 μL of plasma and 400 μL of water control using a QIAamp UCP Pathogen Mini Kit (Qiagen), according to the manufacturer's protocol. Bacterial DNA from 1 mg of stool was extracted using a QIAamp DNA Microbiome Kit (Qiagen), following the manufacturer's protocol. PCR primers 515/806 were used to amplify the V4 variable region of the 16S ribosomal RNA (16S rRNA) using a HotStarTaq Plus Master Mix (Qiagen) under the following conditions: denaturation at 94 °C for 3 minutes, followed by 28 cycles at 94 °C for 30 seconds, 53 °C for 40 seconds, and 72 °C for 1 minute, followed by a final elongation step at 72 °C for 5 minutes. Sequencing was carried out using an Ion Torrent PGM (MR DNA) according to the manufacturer's guidelines. A proprietary analysis pipeline was applied to process the sequencing data (MR DNA).

Sequence processing and taxonomic assignment. The microbiome analysis was described in our previous studies.[4,5] In our present study, we applied a quality-filtering strategy to plasma microbiome to efficiently exclude ASVs of contaminations and artifacts. ASVs distinguish sequence variation by a single nucleotide change and these single DNA sequences are created following the removal of erroneous sequences during high-throughput gene sequencing.[5] Briefly, short sequences < 200 bp, ambiguous base calls, and homopolymer runs > 6 bp were removed. Next, singleton sequences and chimeras were removed, sequences were denoised, and ASVs were clustered at 3% divergence (97% similarity). Final ASVs were taxonomically classified using BLASTn against a database derived from the Ribosomal Database Project (RDPII; http://rdp.cme.msu.edu) and the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov). The ASV table of raw counts was normalized to an ASV table of relative abundances, and taxa of the same type were grouped by phylum, class, order, family, genus, and species.

Statistical analysis. The microbiome diversity (alpha diversity) was computed using the Simpson diversity index and the Shannon index in R (Version 1.2.5001; R Foundation for Statistical Computing). Significance was tested using the Wilcoxon rank-sum test in R, which was applied to compare relative abundances. *P* values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method. Statistical significance of beta diversity was determined with permutational multivariate analysis of variance (vegan R package with the Adonis function).

RESULTS

Plasma has a distinct microbial community profile and increased species heterogeneity compared to the gut. To understand the potential source of the circulating microbiome, we examined differences in alpha and beta diversities of the gut and plasma microbiomes. The plasma microbiome of our cohort demonstrated significantly increased variance of species diversity compared to the gut microbiome ($P < 0.0001$), as measured by the Simpson diversity and Shannon-Wiener indices (Figure 1A). This difference was observed in either all samples or separated groups based on whether they had SLE or were HCs. Consistently, beta diversity showed distinct plasma and gut microbial communities, both in all samples and when separated into groups as reflected by principal coordinates analysis
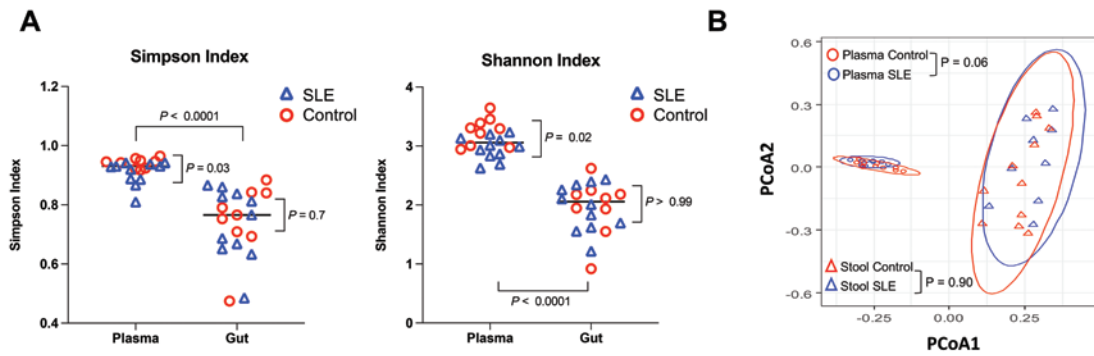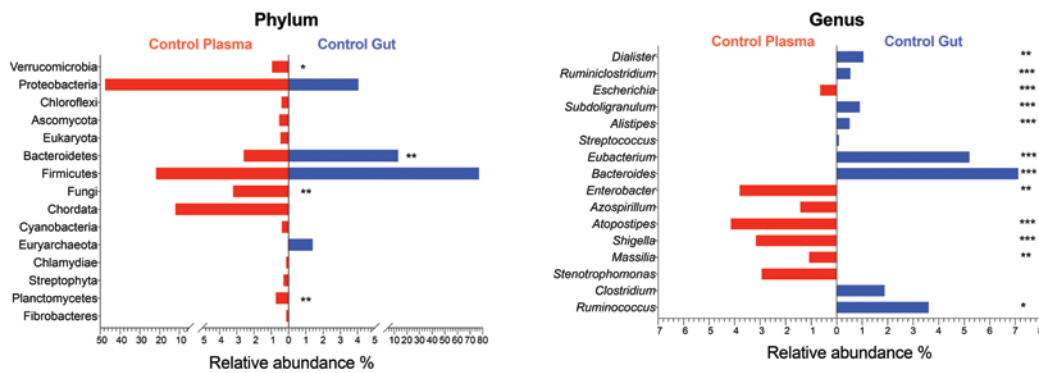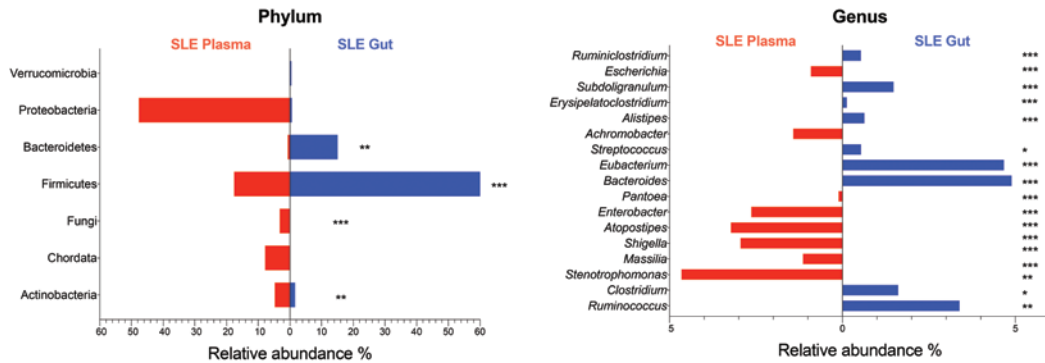
Figure 1. Distinct plasma microbiome from gut microbiome. (A) Alpha diversity measured using Simpson and Shannon indices by site and patient status. (B) PCoA of beta diversity grouped by site and patient status. Median percentages of relative abundance at the phylum and genus levels in gut and plasma from (C) healthy controls and (D) patients with SLE. PCoA: principal coordinate analysis; SLE: systemic lupus erythematosus.

plots, which were calculated using the weighted UniFrac metric (Figure 1B).

Decreased plasma but not gut alpha diversity in patients with SLE compared to HCs. We found that the gut microbiome was similar in HCs and patients with SLE. However, plasma from patients with SLE contained significantly decreased species diversity compared to HCs (Simpson diversity index, $P = 0.03$; Shannon-Wiener index, $P = 0.02$; Figure 1A). Moreover, the beta diversity of plasma microbiome was marginally different in HCs and patients with SLE ($P = 0.06$), but the beta diversity of gut microbiome was similar in both groups ($P = 0.90$; Figure 1B).

Distinct microbiome in the plasma from the gut. The predominant microbiome in the plasma differed from that in the stool. There was a significant difference in the relative abundance of bacteria at each level of taxa between the gut and plasma microbiome. The largest difference was observed at the genus level. At the phylum level, we found that the plasma microbiome was more enriched in Proteobacteria than the gut microbiome, and the gut microbiome was more enriched in Firmicutes and Bacteriodetes than that of the plasma (Figure 1C,D). The ratio of gut enrichment of Firmicutes to Bacteriodetes is 4.58 vs 6.39 in patients with SLE and HCs, respectively. The same was true when comparing

the pooled samples from both groups as well as when comparing by group (Figure 1C,D). The ratio of plasma enrichment of Firmicutes to Bacteriodetes is 25.87 vs 11.0 in patients with SLE and HCs, respectively.

Microbiome composition between patients with SLE and HCs. Next, the relative microbial abundance of both gut and plasma tended to be similar between patients with SLE and HCs but with exceptions (Figure 2A–D). After adjusting for FDR, the gut microbiome of patients with SLE was significantly less enriched in *Dialister* and *Azospirillum*, and significantly more enriched in Verrucomicrobia phylum and *Streptococcus* genus than those in HCs (Figure 2A,B). The plasma microbiome of patients with SLE was significantly more enriched in Gemmatimonadetes phylum only (Figure 2C).

DISCUSSION

In the current study, we directly examined the microbiome composition of the gut and plasma in patients with SLE compared to HCs. We found that both microbial alpha and beta diversities of the gut microbiome were not significantly altered between healthy and diseased patients; this finding is in line with some previous studies.[12,13] The fact that diversity was not
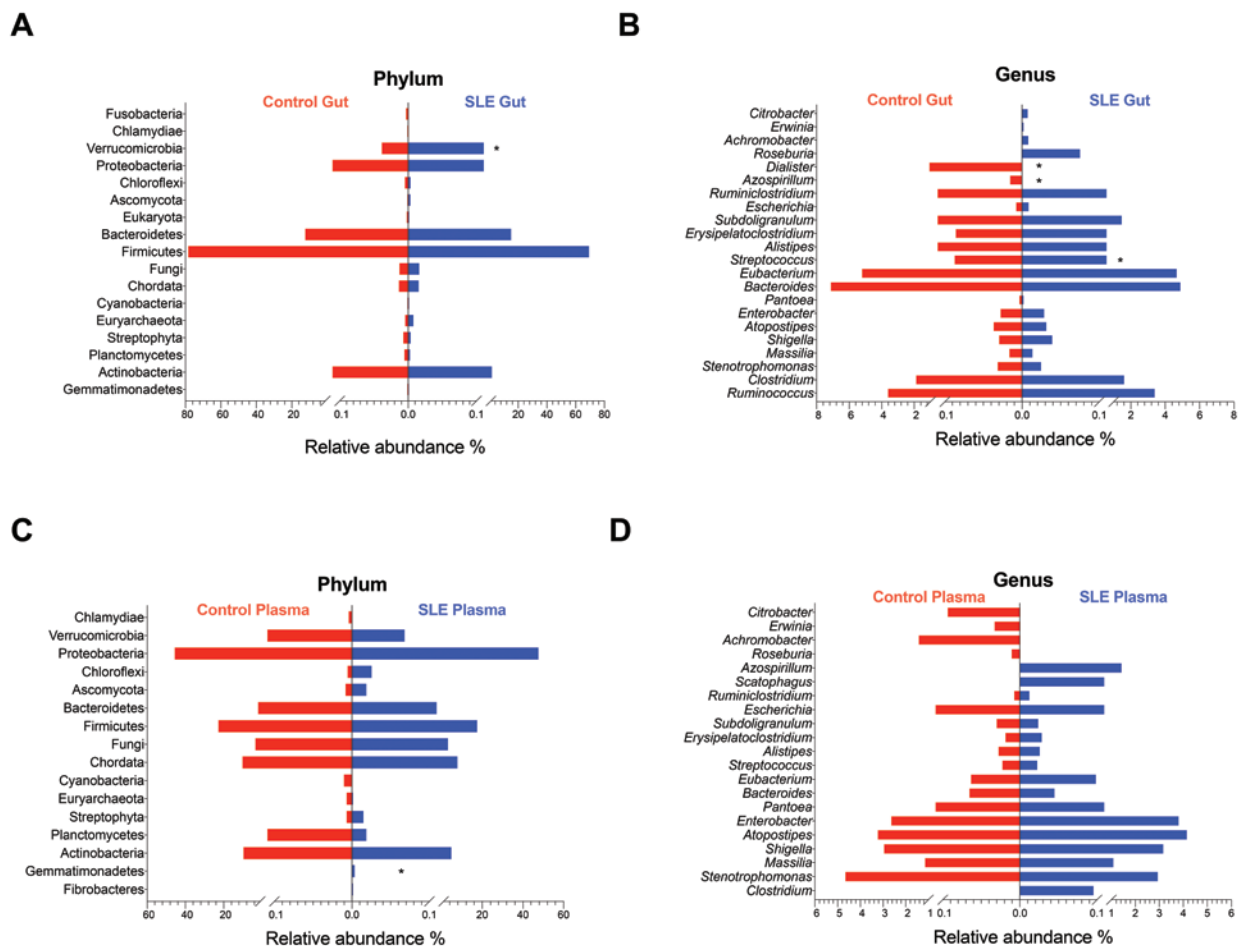

Figure 2. Comparisons of plasma and gut microbiome between healthy controls and patients with SLE. Median percentages of relative abundance of gut microbiome at (A) phylum and (B) genus levels, and plasma microbiome at (C) phylum and (D) genus levels in healthy controls vs patients with SLE. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. SLE: systemic lupus erythematosus.

significantly altered does not mean that the difference in microbiome is insignificant; and indeed, other studies show a difference in gut microbiome between patients with SLE and HCs.[12,13] Nonetheless, we did find that plasma microbial alpha diversity was significantly decreased in patients with SLE, and beta diversity was marginally different in patients with SLE compared to HCs. Notably, the plasma microbial composition of both groups was significantly different from that of their own gut.

In a state of health, the gut microbiome consists primarily of Firmicutes and Bacteroidetes.[14,15] The ratio of Firmicutes to Bacteroidetes in the gut has been shown to be decreased in patients with SLE compared to HCs[12]; consistently, we found a similar decreased ratio of Firmicutes and Bacteroidetes in the gut but not in the plasma of patients with SLE compared to HCs (Figure 2A,C). Additionally, a *Lactobacillus* strain has been demonstrated to be enriched in the gut from patients with SLE.[16] A previous study on SLE and lupus nephritis demonstrated that the abundance of *Ruminococcus gnavus* in the gut was inversely correlated with disease activity.[17] In the current study, *Lactobacillus* strains and *R. gnavus* were not found to be enriched in either the plasma or gut microbiome in SLE, but *Ruminococcus* strains were found to be increased in the gut microbiome of patients with SLE compared to those in HCs.

Our results are consistent with previously characterized gut microbiome composition. Additionally, we found a large discrepancy in the relative abundance of bacteria contained in the phyla Firmicutes, Proteobacteria, and Bacteroidetes between plasma and stool of both study groups. Although it was once hypothesized that transient or pathological increases in gut permeability were responsible for the circulating microbiome,[18] our results suggest an additional source for bacterial translocation. A possible source is the oral microbiome, which is distinct from the gut microbiome.[19] There is evidence of plasma microbial translocation of oral bacteria following tooth extraction and even flossing in the general population.[20] Further, patients with SLE presented with decreased gut microbiota diversity similar to patients with Sjögren syndrome; in contrast, these 2 patient groups presented with an oral microbial composition that differed substantially.[21] Another possible site is the skin, as its barrier function is disrupted not only in autoimmune diseases such as SLE and psoriasis, but also from cuts, scrapes, and burns.[22] A study by Whittle et al[23] characterizing the blood microbiome of healthy individuals and those with asthma found that blood microbiome was predominated by Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes, consistent with our results. They additionally compared their data with the Human Microbiome Project database and concluded that the blood microbiome is most similar to that of the skin and oropharynx.[23] To our knowledge, ours is the first study directly comparing plasma and gut samples from the same individuals to show that there is little correlation between the human plasma and gut microbiomes.

A previous study showed that bacterial translocation from the small intestine to the liver in mice and in patients with SLE may drive autoimmunity.[11] There is a paucity of data comparing stool and plasma microbiome composition in SLE and other autoimmune diseases. A study by Shukla et al revealed distinct plasma and gut microbiome in patients with myalgic encephalomyelitis/chronic fatigue syndrome and HCs between groups before and following exercise.[24] Proteobacteria and Actinobacteria were relatively more abundant in plasma compared to stool in both groups.[24] Consistently, we found increased relative abundance of Proteobacteria and Actinobacteria in plasma compared to stool in both study groups in our present study.

Enterobacteriaceae or *Escherichia* genus bacteria are believed to be prevalent in the gut microbiome in humans.[25] However, our results show they were found in < 1% of the gut microbiome but were in higher abundance in the plasma microbiome in both groups (Figure 2B,D). Notably, we did not find any *Enterobacteriaceae* or *Escherichia* in the water controls. Nonetheless, previous studies of the human gut microbiome often reported missing or similar low abundance (< 1%) of *Enterobacteriaceae* or *Escherichia* or related species bacteria using 16S sequencing 25 or metagenomic analysis.[26] Thus, the low abundance of *Enterobacteriaceae* or *Escherichia* in the human gut microbiome is unlikely due to the limitation of microbial 16S sequencing. Notably, the previous concept of prevalent bacteria was mostly based on bacterial cultivation; however, bacteria differ largely in susceptibility to in vitro cultivation. Another possibility of the higher abundance of plasma *Enterobacteriaceae* or *Escherichia* compared to stool is that these bacterial antigens may have a greater ability to translocate to the systemic circulation. These hypotheses deserve further investigation.

This study is not without limitations. First, we are limited by the small sample size. Thus, this study could not control for potential confounders, including but not limited to age, race, diet, and treatment regimens. Additionally, our cohort was all female, but there may be sex differences in microbiomes due to anatomical, genomic, biologic, and behavioral factors. Another limitation is the relatively low SLE Disease Activity Index score of our cohort. While ideally patients would have high disease activity at the time of sample collection, there are ethical limitations to altering treatment of patients. Nonetheless, this is the first study to show that the microbiome in the plasma does not correspond with that of the gut in patients with SLE and HCs. This study also opens new directions for future studies linking the disease-associated plasma microbiome and systemic immune perturbations in SLE and other diseases, as well as comparisons of the plasma microbiome with those in other sites (eg, oropharynx, skin, vaginal lavage) to determine which is the main source of plasma microbial translocation.

REFERENCES
1. Poore GD, Kopylova E, Zhu Q, et al. Microbiome analyses of blood and tissues suggest cancer diagnostic approach. Nature 2020;579:567-74.

2. Puri P, Liangpunsakul S, Christensen JE, et al; TREAT Consortium. The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. Hepatology 2018;67:1284-302.

3. Schierwagen R, Alvarez-Silva C, Madsen MSA, et al. Circulating microbiome in blood of different circulatory compartments. Gut 2019;68:578-80.

4. Luo Z, Alekseyenko AV, Ogunrinde E, et al. Rigorous plasma microbiome analysis method enables disease association discovery in clinic. Front Microbiol 2020;11:613268.

5. Ogunrinde E, Zhou Z, Luo Z, et al. A link between plasma microbial translocation, microbiome, and autoantibody development in first-degree relatives of systemic lupus erythematosus patients. Arthritis Rheumatol 2019;71:1858-68.

6. Brenchley JM, Douek DC. Microbial translocation across the GI tract. Annu Rev Immunol 2012;30:149-73.

7. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Curr Opin Gastroenterol 2015;31:69-75.

8. Macherey-Nagel. Genomic DNA from blood. User manual. [Internet. Accessed January 25, 2022.] Available from: https://www.bioke.com/blobs/manuals/MN/NS/UM_gDNABlood_NS896.pdf

9. Dinakaran V. Clinical implications of circulating microbiome in cardiovascular disease patients. J Cardiol Curr Res 2017;9:00317.

10. Nejman D, Livyatan I, Fuks G, et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. Science 2020;368:973-80.

11. Manfredo Vieira S, Hiltensperger M, Kumar V, et al. Translocation of a gut pathobiont drives autoimmunity in mice and humans. Science 2018;359:1156-61.

12. Hevia A, Milani C, Lopez P, et al. Intestinal dysbiosis associated with systemic lupus erythematosus. mBio 2014;5:e01548-14.

13. He Z, Shao T, Li H, Xie Z, Wen C. Alterations of the gut microbiome in Chinese patients with systemic lupus erythematosus. Gut Pathog 2016;8:64.

14. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science 2005;308:1635-8.

15. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.

16. Zegarra-Ruiz DF, El Beidaq A, Iñiguez AJ, et al. A diet-sensitive commensal lactobacillus strain mediates TLR7-dependent systemic autoimmunity. Cell Host Microbe 2019;25:113-27.

17. Azzouz D, Omarbekova A, Heguy A, et al. Lupus nephritis is linked to disease-activity associated expansions and immunity to a gut commensal. Ann Rheum Dis 2019;78:947-56.

18. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12:1365-71.

19. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. Protein Cell 2018;9:488-500.

20. Crasta K, Daly CG, Mitchell D, Curtis B, Stewart D, Heitz-Mayfield LJ. Bacteraemia due to dental flossing. J Clin Periodontol 2009;36:323-32.

21. van der Meulen TA, Harmsen HJM, Vila AV, et al. Shared gut, but distinct oral microbiota composition in primary Sjögren's syndrome and systemic lupus erythematosus. J Autoimmun 2019;97:77-87.

22. Sirobhushanam S, Parsa N, Reed TJ, et al. Staphylococcus aureus colonization is increased on lupus skin lesions and is promoted by IFN-mediated barrier disruption. J Invest Dermatol 2020; 140:1066-74.

23. Whittle E, Leonard MO, Harrison R, Gant TW, Tonge DP. Multi-method characterization of the human circulating microbiome. Front Microbiol 2018;9:3266.

24. Shukla SK, Cook D, Meyer J, et al. Changes in gut and plasma microbiome following exercise challenge in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). PLoS One 2015;10:e0145453.

25. Martinson JNV, Pinkham NV, Peters GW, et al. Rethinking gut microbiome residency and the Enterobacteriaceae in healthy human adults. ISME J 2019;13:2306-18.

26. Peña-Gonzalez A, Soto-Giron MJ, Smith S, et al. Metagenomic signatures of gut infections caused by different Escherichia coli pathotypes. Appl Environ Microbiol 2019; 85:e01820-19.