Dysbiosis of Gut Microbiota and Its Relationship with the Regulatory T Cells in Patients with Aplastic Anemia

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Research Article

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Abstract

Background: The characteristics of gut microbiota (GM) and its relationship with the Regulatory T Cells (Treg) remains unclear in patients with aplastic anemia (AA).

Methods: This study was a cross-sectional survey which included 12 AA patients consisted of 6 with severity aplastic anemia (SAA) and 6 with non-severity aplastic anemia (NSAA) and 6 healthy participants. The GM and its relationship with the Treg cells of AA patients were analyzed.

Results: The results showed that the presence of compositional differences in the GM structure between the AA and Control groups. The bacterial communities were depleted of *Clostridia* class (e.g., *Lachnospiraceae ND3007*, *Lachnospiraceae XPB1014*, *Lachnolostridium*, *Ruminococcaceae UCG 013* and *Butyricicoccus* genus) in AA group, especially in SAA group. Inversely, the relative abundance of *Lactobacillus* and *Streptococcus* genus from *Bacilli* class were increased significantly in patients with SAA. The relative abundance of *Lachnospiraceae* (r=0.663, *p*=0.029), *Clostridiaceae 1* (r=0.619, *p*=0.042) and *Clostridiales vadinBB60 group* family (r=0.674, *p*=0.023) which from *Clostridia* class, were positively correlated with the Treg cell counts.

Conclusion: We speculated that the decrease of some bacteria from *Clostridia* class may participate in the pathophysiological process of AA through reducing the Treg cell counts. Notwithstanding the low sample size, our data provided some clues that the treatment strategy of AA could start by adjusting the imbalance of GM, increasing Treg cell counts to improve the suppression of bone marrow hematopoiesis.

Introduction

Aplastic anemia (AA) is a disease of pancytopenia and bone marrow hypoplasia caused by the decrease of hematopoietic stem cells[1, 2]. The incidence of AA in the China is 7.4/million[3, 4], and the study reported that the mortality rates of severe aplastic anemia (SAA) exceeding 80% in 2 years which indicated the prognosis for SAA is poor[5]. Evidence indicated that AA is an immune mediated or an autoimmune disease[2, 4, 5], immune mediated destruction of hematopoietic stem/progenitor cells (HSPCs) plays a central role in the pathophysiology of AA[6]. T lymphocytes are the main effector cells of the immune system and abnormal T cell subsets play an important role in the occurrence and development of AA[7]. Among T cells, Treg cells are the key lymphocyte subsets for inducing immune tolerance, play an important role in maintaining immune system homeostasis by inhibiting the induction and function of autoreactive T cells[8]. The immunosuppressive deficiency of Treg cells may lead to the damage of hematopoietic function induced by effector T cells, and eventually lead to the apoptosis of HSPCs[9]. However, research shown that, the majority of AA patients have the phenomenon of decreased Treg cells[10], and Treg cells decreased with the increase of disease severity[11].

Gut microbiota (GM) has gained increasing interest in the pathogenesis of immune-related diseases. Dysbiosis of the gut, which is characterized by the outgrowth of potential pathogenic bacteria or a decrease in the number of beneficial bacteria, has been implicated in various autoimmune disorders, including ulcerative colitis (UC), rheumatoid arthritis (RA), andsystemic lupus erythematosus (SLE)[12]. In animal models, the mechanism of the effects of the microbiota on immune homeostasis has been widely studied[13–15]. Round *et al.*[16] found that the expression of Treg cells was low in germ-free (GF) mice, and high after the recovery of colonization, suggesting that the imbalance of GM was closely related to Treg cells, and the maintenance of GM homeostasis was of great significance for the production and amplification of Treg cells. Some studies further explored the

mechanism of GM regulating Treg. Arpaia *et al*.[17] found that several commensal microbes and their metabolites, such as butyrate, propionate, and acetate, induced the differentiation and proliferation of Treg cells. The Treg cell counts reduced in the lamina propriaof GF mice and particularspeciesof *Clostridia* class involved in the Treg cell induction in the gut[18, 19]. The colonization of *Bacteroidesfragilis* in GF mice promote the secretion of the polysaccharide A (PSA) which mediated the development of inducible Tregs with a unique genetic signature[16].

In human study, Han *et al*.[20] reported that patients with acute graft-versus-host disease (aGVHD) have GM imbalance and the relative abundance of the *Lachnospiraceae* and *Ruminococcaceae* family which from *Clostridia* class were positively correlated with Treg cell counts. However, the characteristics of GM and the relationships between specific microbiota taxa and Treg cells in patients with AA remains unclear. We hypothesis that patients with AA may have dysbiosis of GM which associated with a reduced Treg cells counts. Here, we performed a study in 12 AA patients, including 6 with SAA and 6 with non-severity aplastic anemia (NSAA) compared to 6 healthy participants, to provide insights into the alterations in the GM of AA patients and the relationship between disease severity and GM, and analyzed the correlation between GM and Treg cell counts, furthermore, to find out the specific microbiota taxa correlation with Treg cells.

Methods

Study participants

Our pilot study was conducted at the *First Affiliated Hospital of Soochow University*, involving 12 participants with a new diagnosis of AA and 6 healthy individuals from November 2018 to May 2019. According to the severity of the disease, AA patients were divided in the SAA and NSAA. The exclusion criteria for the AA group included patients (1) had received oral/intravenous glucocorticoid, other immunosuppressive drugs and antibiotic treatment within 1 month[21]; (2) combined with other immunodeficiency diseases such as UC, RA, SLE[12]; (3) had diarrhea or gastrointestinal dysfunction; (4) had taken products or supplements containing probiotics (oligosaccharides) and yogurt regular to alter GM within 3 weeks before study[22]. The inclusion criteria of the control group included participants (1) without basic lesion[21]; (2) who had not taken any antibiotics within 3 months[21]; (3) who had not taken products or supplements containing probiotics (oligosaccharides) and yogurt regular to alter GM within 3 weeks before study[21]; The study complied with the Helsinki Declaration and ethical approval for the study was obtained from the Soochow University Ethics Committee (ECUS-2019000106) and all patients provided informed consent.

Study protocol

Prior to taking fecal specimens and peripheral blood, the researcher explained the purpose and significance of the study in detail to the participants and made clear that the participants' privacy will be protected. Participants were asked to sign the informed consent. Researcher instructed participants to complete the general information questionnaires which included the socio-demographic information andclinical characteristics. The clinical data were obtained from the medical records of the AA patients; socio-demographic information were self-reported. It took about 5–10 minutes to complete the questionnaires.

DNA Extraction and 16s rRNA Gene Sequencing for Fecal Specimens

The fecal specimens of all participants were taken. Researcher instructed the AA patients to put the first stool on the thick sterile pad after the admission. The healthy participants took stool at any time. The sterile cotton swab was used to take about 20g fresh stool from participants into the sterile feces collection tubes. After the specimenwas collected, it was immediately put into the portable liquid nitrogen ank and frozen for 15 minutes. The specimens were transferred to the − 80℃ frigerator within 24h for preservation[21]. For each fecal specimen, DNA was extracted and purified, and the V3-V4 region of the 16S rRNA genes was polymerase chain reaction (PCR)-amplified using modified universal bacterial primers[23, 24]. Purified PCR products were sequenced with the Hiseq2500 PE250 platform[25]. Sequence data were compiled and processed using QIIME[26]. Sequence data were screened and filtered for quality and then aligned to the full-length 16S rRNA gene, using the SILVA reference alignment as a template[27]. Sequences were grouped into operational taxonomic units (OTUs) of 97% similarity[28]. Phylogenetic classification was used to describe the intestinal composition of each subject in the AA and the control groups[29].

Measurement of Quantities of Tregs from Peripheral Blood

Peripheral blood of AA patients was collected into EDTA anticoagulant tube on the day after admission. Peripheral blood monocytes (PBMCs) in RPMI 1640 were stimulated with 25 ng/ml phorbol 12-myristate 13-acetate, 1 μg/ml ionomycin, and 10 μg/ml Brefeldin A (Sigma, St Louis, MO, USA) at 37°C for 4h. After incubation, PBMCs were washed with PBS twice. Then, the cells were stained with anti-CD_4 at room temperature (RT) in dark for 15 min. After treatment with permeabilizing solution, cells were stained withanti-CD25 and anti-CD_{127} at RT for 45 min without permeabilizing treatment. For each tube, at least 10,000 events were collected in agatecreated around the viable lymphocyte population. Quadrants were applied to the isotype control dot plots to exclude nonspecificstaining. The percentage of $CD_4^+CD_{25}^+$high CD_{127} intensity/dim (Treg) cell were determined.

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation. Relative OTU abundances were calculated using QIIME[26]. Principal Component Analysis (PCA) was used to observe the degree of difference between specimens and the variation rule of difference[30]. Venn diagram made by the abundance of OTUs was used to explore which species are shared or unique among groups[31]. Community richness and diversity were examined in each group using alpha diversity, including Chao 1 index and Faith's PD (Faith's Phylogenetic Diversity)[32], which were calculated from OTUs. The relative abundance between the AA and the control group were compared at the phylum, class, order, family, genus levels. A non-parametric test (Kruskal-Wallis test) was used to compare the statistical significance in GM among the SAA, NSAA and the Control groups. In order to compare the distribution characteristics of specific GM between the AA patients and healthy participants, box plot used for community composition analysis[33]. Statistical correlations between the Treg cells counts and the abundance of specific GM were investigated using bivariate correlation analysis (Spearman). P value less than 0.05 was considered significant. Statistical data were generated using SPSS 22.0. Statistics and drawing are mainly done by R[34], python, java, etc.

Result

Characteristic of participants

Between November 2018 and April 2019, a total of 18 participants were analyzed in this study. There were 6 participants in each of the SAA (2 male and 4 females), NSAA (2 male and 4 females) and Control group (1 male and 5 females). The average age of three groups were 36.37 ± 10.31, 36.33 ± 8.16 and 39.33 ± 11.91 yrs, respectively. The body mass index (BMI) of the three groups were 22.68 ± 2.31, 22.47 ± 1.25 and 22.87 ± 8.04 kg/m^2, respectively. There were no statistical differences in age and BMI among three groups.

Venn diagram analysis of GM and principal component analysis of GM

A total of 72 fecal specimens were collected (4 specimens per participant). From these specimens, a total of 594966 (33054 per specimen) Tags were obtained. The total number of OTUs was 4454.

Venn diagram made by the abundance of OTUs was used to explore which species are shared or unique among groups. As shown in Fig. 1, 3038 OUTs (9.08%) in the AA group and 664 OUTs (3.70%) in the Control group were unique, and 710 OUTs (87.22%) were shared by two groups.

In order to observe the degree of difference between specimens and the variationrule of difference, PCA (Fig. 2) was used. The more similar the sample community composition, the closer they are to each other in the figure. The result confirmed the presence of compositional differences in the GM structure of AA patients and healthy controls.

Alpha diversity

To assess differences in GM between the AA patients and the healthy participants, we investigated the diversity and richness of the ecosystem between the AA and the Control groups. Alpha diversity, as measured by the Chao 1and PD index, were all higher in the AA group when compared to the Control group (p<0.01,Fig. 3A, Fig. 3B). In this study, the top 10 abundances of GM were selected to perform the inter-group comparisons in different levels of phylum, class, order, family and genus (Fig. 4), which indicated that there were differences in the composition of GM between the AA patients and healthy participants.

The difference of Treg cell counts between the NSAA and SAA groups

Compared with the normal reference range of Treg cells (7.49–11.51%),[35] the Treg distributions were lower in most AA patients (83%), and even close to zero in two patients (Table 1). As the Table 2 shown, the number of Treg cell counts of SAA significantly lower than that in the NSAA group (2.35 ± 2.06 vs. 7.38 ± 2.69, p = 0.005).

Table 1
The severity of disease and the distributionof Treg cells (%) in patients with AA

Numbers	Severity of disease	Percentage	Numbers	Severity of disease	Percentage
1	NSAA	4.30	7	AA	7.30
2	SAA	0.70	8	AA	9.50
3	SAA	0.00	9	AA	11.40
4	NSAA	6.80	10	SAA	2.20
5	SAA	5.90	11	AA	5.00
6	SAA	2.90	12	SAA	2.40
NSAA: non-severity aplastic anemia; SAA: severity aplastic anemia.					

Table 2
The comparison of the Treg counts between the NSAA and SAA patients

Severity of disease	Treg
NSAA(n = 6)	7.38 ± 2.69
SAA(n = 6)	2.35 ± 2.06
t	3.641
p	0.005

NSAA: non-severity aplastic anemia; SAA: severity aplastic anemia.

The comparison of the relative abundances of GM among the SAA, NSAA and Control groups

Comparisons of the relative abundances of GM among the SAA, NSAA and Control groups were contributed to the understand the relationships between the severity of disease and GM. The results found (Table 3) that the difference of GM between the AA patients and healthy participants was mainly reflected in the *Firmicutes* Phylumn. Although the relative abundance of the *Firmicutes* Phylum ($p = 0.567$) level was no significantly difference between the groups, its class i.e., *Bacilli* ($p = 0.003$) was higher in the AA patients and significantly increased in SAA; *Negativicutes* ($p = 0.03$) and *Erysipelotrichia* ($p = 0.024$) were lower in the AA patients and significantly decreased in SAA. Its orders i.e., *Lactobacillales* ($p = 0.03$) was higher in the AA patients and and significantly increased in SAA; *Selenomonadales* ($p = 0.03$) and *Erysipelotrichales* ($p = 0.024$) were lower in the AA patients and and significantly decreased in SAA. Its family i.e., *Lactobacillaceae* ($p = 0.03$) and *Streptococcaceae* ($p = 0.03$) were higher in the AA patients and significantly decreased in SAA; *Veillonellaceae* ($p = 0.06$) and *Erysipelotrichaceae* ($p = 0.024$) were lower in the AA patients and significantly decreased in SAA. Its Genus i.e., *Ruminococcaceae UCG 013* ($p = 0.025$, Fig. 5A), *Butyricicoccus* ($p = 0.012$, Fig. 5B) which all from *Clostridia* Class were lower in the AA patients and significantly decreased in SAA; the relative abundance of *Lachnospiraceae XPB1014 group* ($p<0.001$, Fig. 5C), *Lachnospiraceae ND3007 group* ($p<0.001$,Fig. 5D), *Lachnolostridium* ($p = 0.001$, Fig. 5E) also from *Clostridia* class in the AA group were significantly lower (almost

none) than the Control group; *Lactobacillus* ($p = 0.03$, Fig. 6.1) and *Streptococcus* ($p = 0.03$, Fig. 6.2) were higher in the AA patients and significantly increased in SAA; in addition, *Dialister* ($p = 0.001$) in the AA group was significantly lower (almost none) than that in the Control group.

Table 3
The comparison of relative abundances of GM [*M*(*P25,P75*)] among the SAA, NSAA and the Control group

GM	SAA(n = 6)	NSAA(n = 6)	Control(n = 6)	H	*p*
P-*Firmicutes*	0.478(0.103,0.748)	0.622(0.505,0.726)	0.675(0.547,0.773)	1.135	0.567
C- *Bacilli*	0.164(0.031,0.263)	0.049(0.025,0.128)	0.003(0.002,0.003)	11.942	0.003
O-*Lactobacillales*	0.163(0.030,0.261)	0.048(0.024,0.126)	0.002(0.001,0.003)	11.942	0.003
F-*Lactobacillaceae*	0.011(0.010,0.015)	0.009(0.007,0.013)	0.000(0.000,0.000)	11.802	0.003
G-*Lactobacillus*	0.011(0.010,0.015)	0.009(0.007,0.013)	0.000(0.000,0.000)	11.789	0.003
F-*Streptococcaceae*	0.095(0.003,0.235)	0.027(0.007,0.103)	0.001(0.000,0.002)	11.486	0.003
G-*Streptococcus*	0.090(0.005,0.235)	0.027(0.007,0.102)	0.001(0.000,0.002)	11.486	0.003
C-*Clostridia*	0.301(0.063,0.464)	0.559(0.356,0.684)	0.598(0.372,0.678)	4.643	0.098
O-*Clostridiales*	0.301(0.063,0.465)	0.559(0.356,0.684)	0.598(0.372,0.678)	4.643	0.098
F- *Ruminococcaceae*	0.211(0.027,0.334)	0.253(0.194,0.442)	0.412(0.125,0.566)	2.351	0.309
G-*Ruminococcaceae UCG 013*	0.001(0.000,0.001)	0.003(0.002,0.004)	0.005(0.001,0.012)	7.404	0.025
G-*Butyricicoccus*	0.001(0.000,0.001)	0.001(0.001,0.002)	0.007(0.003,0.015)	8.842	0.012
F- *Lachnospiraceae*	0.078(0.033,0.111)	0.091(0.071,0.234)	0.146(0.080,0.305)	2.117	0.347
G-*Lachnospiraceae XPB1014 group*	0.000(0.000,0.000)	0.000(0.000,0.000)	0.006(0.003.0.013)	16.126	0.001
G-*Lachnospiraceae ND3007 group*	0.000(0.000,0.000)	0.000(0.000,0.000)	0.009(0.004.0.018)	16.152	0.001
G-*Lachnolostridium*	0.000(0.000,0.000)	0.000(0.000,0.000)	0.002(0.001,0.004)	13.472	0.001
C-*Negativicutes*	0.008(0.004,0.011)	0.009(0.004,0.015)	0.070(0.027,0.184)	11.474	0.003
O-*Selenomonadales*	0.008(0.004,0.011)	0.009(0.004,0.015)	0.070(0.027,0.184)	11.474	0.003
F-*Veillonellaceae*	0.008(0.003,0.010)	0.009(0.003,0.014)	0.026(0.015,0.053)	10.327	0.006
G-*Dialister*	0.000(0.000,0.000)	0.000(0.000,0.000)	0.003(0.001,0.035)	14.237	0.001
C-*Erysipelotrichia*	0.005(0.004,0.008)	0.008(0.005,0.013)	0.014(0.007,0.029)	7.450	0.024
O-*Erysipelotrichales*	0.005(0.004,0.008)	0.008(0.005,0.013)	0.014(0.007,0.029)	7.450	0.024
F-*Erysipelotrichaceae*	0.005(0.004,0.008)	0.008(0.005,0.013)	0.014(0.007,0.029)	7.450	0.024
P-*Fusobacteria*	0.000(0.000,0.000)	0.000(0.000,0.000)	0.002(0.001,0.013)	10.949	0.004

GM: gut microbiota; P: phylum; C: class; O: order; F: family; G: genus. NSAA: non-severity aplastic anem ia; SAA: severity aplastic anemia. Kruskal-Wallis test was used to compare the statistical significance in GM among the three groups. NSAA: non-severity aplastic anemia; SAA: severity aplastic anemia.

The specific GM correlate with the Treg cell counts

The Treg cell counts decreased in the majority of patients with AA. In this study, Treg cell counts were positively correlated with the relatively abundance of *Lachnospiraceae (*r = 0.663, *p* = 0.029*)*, *Clostridiaceae 1* (r = 0.619, *p* = 0.042) and *Clostridiales vadinBB60 group* family (r = 0.674, *p* = 0.023), all of them belong to *Clostridia* class. In addition, Treg cell counts were positively correlated with the relatively abundance of *Burkholderiaceae* (r = 0.633, *p* = 0.036), *Pseudomonadaceae* (r = 0.647, *p* = 0.031) and *Aeromonadaceae* family (r = 0.651, *p* = 0.030), three of them from *Gammaproteobacteria* class. No correlation was found between Treg cell and *Bacteroidetes fragilis* (r=-0.250, *p* = 0.459), *Ruminococcaceae* (r = 0.017, *p* = 0.961), *Lachuospiraceae* (r = 0.355, *p* = 0.309) and *Enterobacyeriaceae* (r = 0.101, *p* = 0.768) in this study (Table 4).

Table 4
Correlation between the relative abundances of GM and the Treg cell counts

GM (family)	**Treg**	
	r	*p*
Lachnospiraceae	0.663	0.029
Clostridiaceae 1	0.619	0.042
Clostridiales vadinBB60 group	0.674	0.023
Burkholderiaceae	0.633	0.036
Pseudomonadaceae	0.647	0.031
Aeromonadaceae	0.651	0.030
Bacteroidetes fragilis	-0.250	0.459
Ruminococcaceae	0.017	0.961
Enterobacyeriaceae	0.101	0.768
GM: gut microbiota.		

Discussion

Human disease is attributable not only to single pathogens but also to global changes in our microbiome[36], and dysbiosis of the GM can cause various autoimmune diseases[12]. In animal experiments, the mechanism of the effects of commensal microbiota on immune homeostasis has been studied extensively[13, 17, 37]. AA is immune mediated disease or an autoimmune disease, which may be associated with the dysbiosis of the GM. We observed that the GM is associated with the development of AA. In the present study, we found that the presence of compositional differences in the GM structure of AA patients and healthy controls, the diversity of GM in the AA group was richer than that in the Control group, which is consistent with Chen's[21] findings, this results indicated that the taxonomic composition of the GM may be more complex in the AA patients with more distinct members. In terms of the relative abundance of GM, we found that *Lachnospiraceae XPB1014 group*, *Lachnospiraceae ND3007 group* and *Lachnolostridium*s genus in the SAA and NSAA groups (almost zero) were significantly lower than that in the Control group, all of the microbiota above mentioned were from *Lachnospiraceae* family. The study conducted by Chen *et al*.[21] showed that the relative abundance of

Lachnospiraceae family in AA patients was lower than that of healthy people, which is similar with the result of this study, and *Lachnospiraceae* family increased after allogeneic hematopoietic stem cell transplanation (allo-hsct). The change of other microbiota in the AA patients in this study has not been confirmed by other studies.

Recent studies suggested that the onset of AA is associated with immune damage of Tregs[11, 38–42]. Previous data have shown inadequate numbers of peripheral blood Tregs in patients with AA[38]. Qi *et al*.[39] reported that the percentage of $CD_4^+CD_{25}^+CD_{127}$ dim Tregs in peripheral blood lymphocytes of AA patients were lower than in normal controls (0.83 ± 0.44% vs. 2.18 ± 0.55%, $P<0.05$). Kordasti *et al*.[40] showed the absolute Treg number was significantly lower in AA patients than in healthy donors (5.5×10^6 vs. 3×10^7; $P = 0.01$). In this study, 83% AA patients had decreased Treg cells, even 20% of AA patients' Treg cells were close to zero, meanwhile, the Treg cell counts of SAA group significantly lower than that in the NSAA group (2.35 ± 2.06 vs. 7.38 ± 2.69, $p = 0.005$), which is consistent with the study of Tong *et al*[10], they found that the Treg cells obviously decreased in patients with SAA when compared to the patients with mild AA. The above mentioned suggested that the Treg cell counts in peripheral blood of AA patients is closely related to severity of AA. Commensal microbial community have been found to potentiate the generation of Treg cells[43, 44]. The results of the animal studies have demonstrated that anti-inflammatory *Clostridia* class could coordinate the Treg/Th17 balance and induce immune tolerance by histone deacetylase inhibition at animal experiment[17–18, 43–45]. Han *et al*.[20] reported that the relatively abundance of the *Lachnospiraceae* and *Ruminococcaceae* family which from *Clostridia* class were positively correlate with Treg cell counts (r = 0.578, r = 0.492, respectively) and the ratio of Treg and Th17 cells (r = 0.469, r = 0.419, respectively) in aGVHD patients. That study also found that the relative abundance of the *Lachnospiraceae* and *Ruminococcaceae* family positively correlated with H3 acetylation (r = 0.484, r = 0.037; $p<0.001$, $p = 0.001$, respectively). Therefore, they speculated that the GM might influence the development the Treg/Th17 balance by coordinating H3 acetylation in CD_4^+ T cells. Atarashi *et al*.[18] showed that colonization of mice with healthy human *Clostridia* class exhibited a robust accumulation of Treg cells in the colon. They also found that clonic Treg cells can be induced by indigenous *Clostridium* species, particularly clusters IV and XIVa of the genus *Clostridium*, promoted Treg cell accumulation[46]. *Clostridia*-derived metabolites, short chain fatty acid (SCFAs), play an important role in inducing the differentiation of Treg cells and modulating the Treg/Th17 balance byhistone acetylation, particularly acetylated H3[17–18,43–46,47,48]. SCFAs increased the expression of anti-inflammatory IL-10 producing Foxp3-expressing Tregs through histone deacetylase inhibition in a GPR43 dependent manner[43]. Simth *et al*.[43] provided SCFAs in the drinking water to GF mice for 3 weeks and found that SCFAs increased colonic Treg frequency and number, did not significantly alter colonic TH1 or TH17 cell numbers. But, the research of the correlation between Treg cell counts and the GM in AA patients have not been reported.

In our study, we observed that there were relationships between the specific microbiota taxa and the Treg cell counts. The relative abundance of *Lachnospiraceae*, *Clostridiaceae 1* and *Clostridiales vadinBB60 group* family which all from *Clostridia* class positively correlated with the Treg cell counts, which is consistent with Han' found[20]. We also found that the relative abundances of *Ruminococcaceae UCG 013*, *Butyricicoccus*, *Lachnospiraceae ND3007 group*, *Lachnospiraceae XPB1014 group* and *Lachnolostridium*s genus which all from *Clostridia* class were lower and decreased significantly with the severity of disease in the AA patients. We speculated that the decrease of *Clostridia* class may change Treg cell counts by reducing SCFAs and may participate in the pathophysiological process of AA. In addition, we found that the relatively abundances of *Burkholderiaceae*, *Pseudomonadaceae* and *Aeromonadaceaes* family were positively correlated with the Treg cell

counts, three of them all from *Gammaproteobacteria* class, which has not been confirmed by other studies. Other studies have confirmed that *Bacteroidetes fragilis*[43], *Ruminococcaceae*[20], *Lachuospiraceae*[20] and *Enterobacyeriaceae*[20] family have a significant correlation with Treg cell counts, but there were no such relationships been found in this study, which may be related to the different study objects and the small sample size.

Our study has several limitations. One limitation is the small sample size which would limit the interpretation of results. Secondly, it is a deficit that we only analyzed the GM of the new diagnosis AA patients. In future studies, the sample size should be enlarged to confirm our results. In addition, animal experiments and prospective observational studies are needed to explore the association between specific GM and the Treg cells, deeply interpret the mechanism of how GM, Treg cells affect the development of AA.

Conclusion

In this study, the diversity of GM in the AA patients was greater high than healthy participants. The relative abundances of some bactaria from *Clostridia* class were lower in the AA patients, and decreased significantly in SAA; their relative abundances of *Lachnospiraceae*, *Clostridiaceae 1* and *Clostridiales vadinBB60 group* family were positively correlated with the Treg cell counts. Therefore, we speculated that the decreased of *Clostridia* class may participate in the pathophysiological process of AA by reducing the Treg cell counts. Although the analysis was conducted at a single time point and on a small sample size, our data highlight a significant dysbiosis of the GM in AA patients, the relationship of GM and the severity of disease and find out the specific microbiota taxa correlation with Treg cell counts. Our data provided some clues that the treatment strategy of AA could start by adjusting the imbalance of intestinal flora, increasing Treg cell counts to improve the suppression of bone marrow hematopoiesis.

Abbreviations

GM: gut microbiota; Treg: Regulatory T Cells; AA: aplastic anemia; SAA: severity aplastic anemia; NSAA: non-severity aplastic anemia; HSPCs: hematopoietic stem/progenitor cells ; UC: ulcerative colitis; RA: rheumatoid arthritis; SLE: andsystemic lupus erythematosus; GF: germ-free; aGVHD: cute graft-versus-host disease; PBMCs: Peripheral blood monocytes; PCA: Principal Component Analysis.

Declarations

Ethics approval and consent to participate

The study complied with the Helsinki Declaration and ethical approval for the study was obtained from the Soochow University Ethics Committee (ECUS-2019000106) and all patients provided informed consent.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Xiao-hua Wang contributed to the study conception and design. Meng-xiao Ren and Yong-qin Ge contributed to the data acquisition, analysis, and interpretation, and drafting of the manuscript. Jin-dan Qi contributed to the data acquisition. Sheng-li Xue, Miao Miao, De-pei Wu and Yu-hui Huang contributed to revising the manuscript and enhanced the intellectual content. All authors agreed to publish.

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Figures

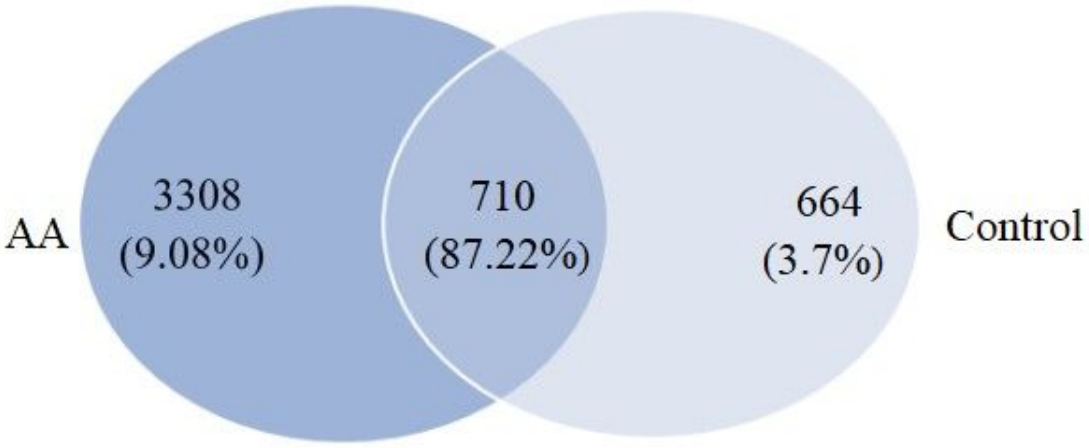

Figure 1

Venn diagram analysis. There were 3038 OUTs (9.08%) in the AA group and 664 OUTs (3.70%) in the Control group which were unique, and 710 OUTs (87.22%) shared by two groups. (AA: aplastic anemia)

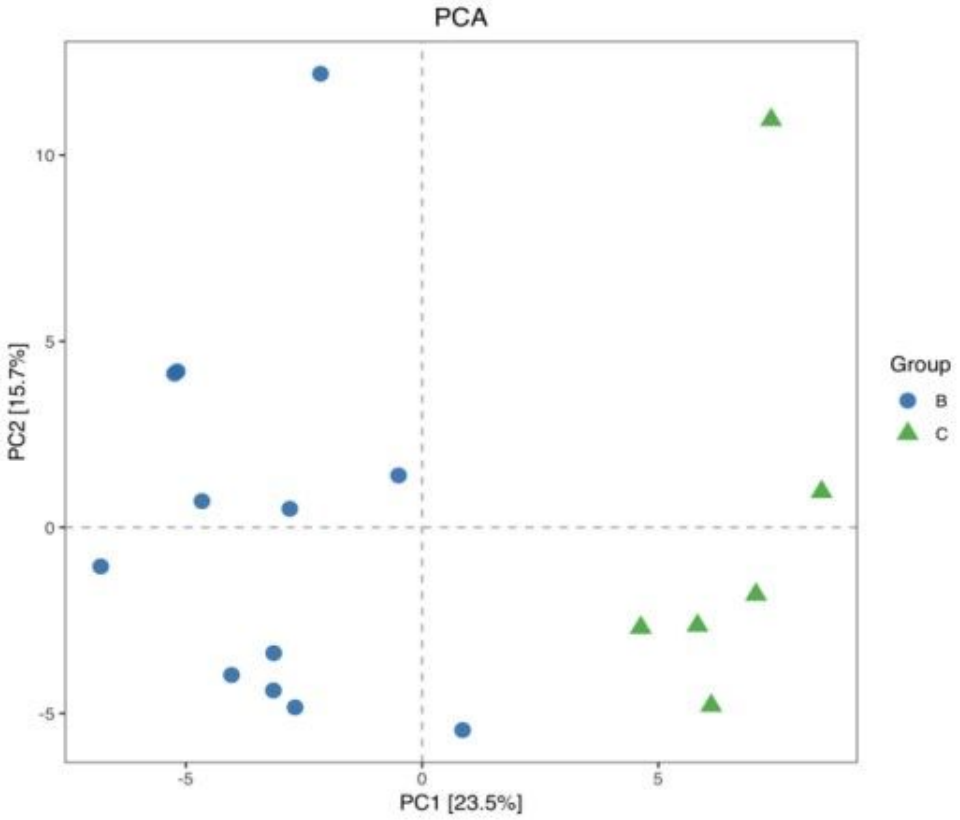

Figure 2

See the Supplemental Files section for the complete figure caption.

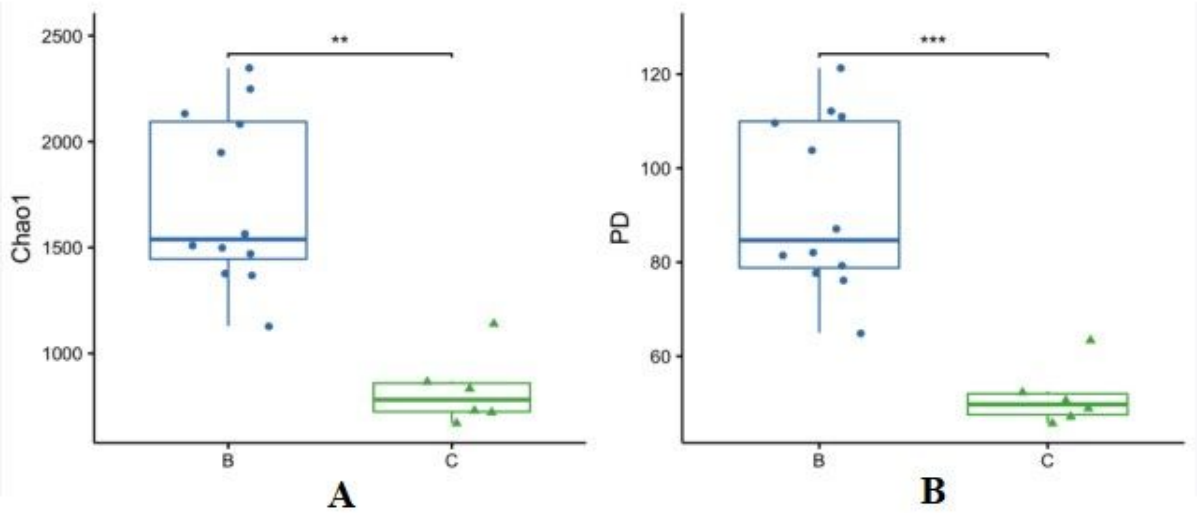

Figure 3

See the Supplemental Files section for the complete figure caption.

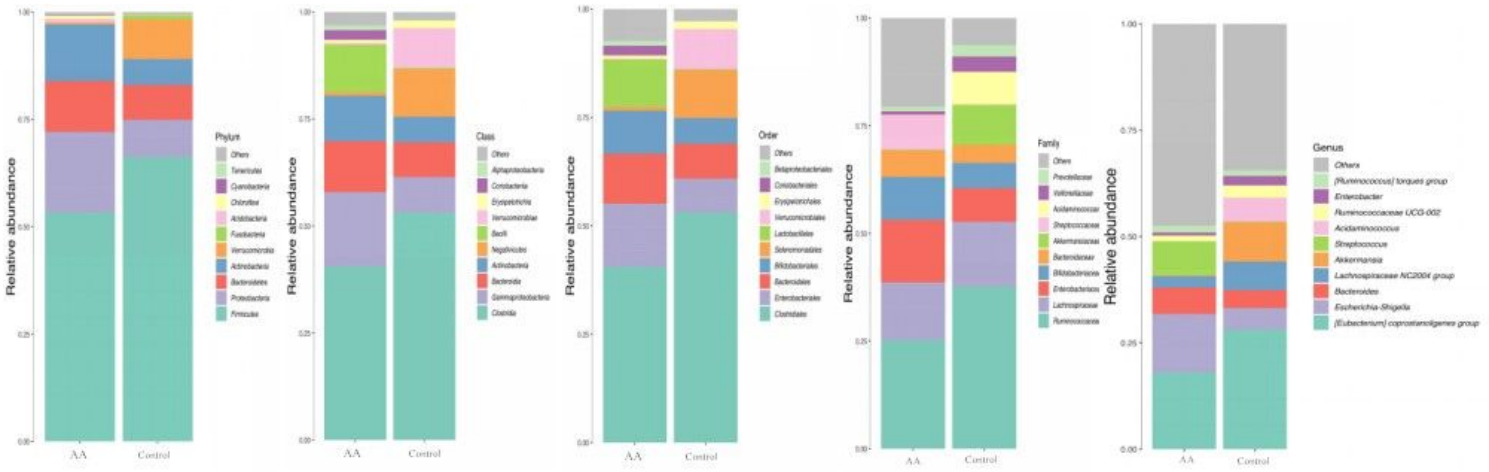

Figure 4

The taxonomics composition distribution profiling histograms between the AA and the Control groups were shown at Phylum, Order, Class, Family, Genus levels separately. There were differences in the composition of the GM between the AA and the Control groups. (GM: gut microbiota)

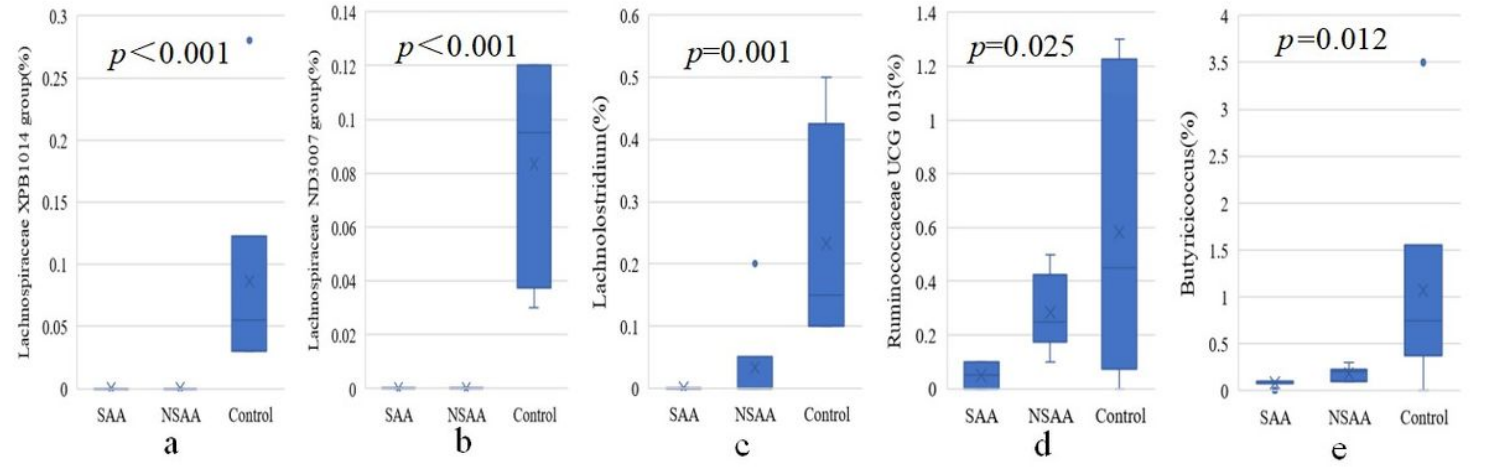

Figure 5

Comparison of the relative abundance of some bactaria from Clostridia class among the three groups.

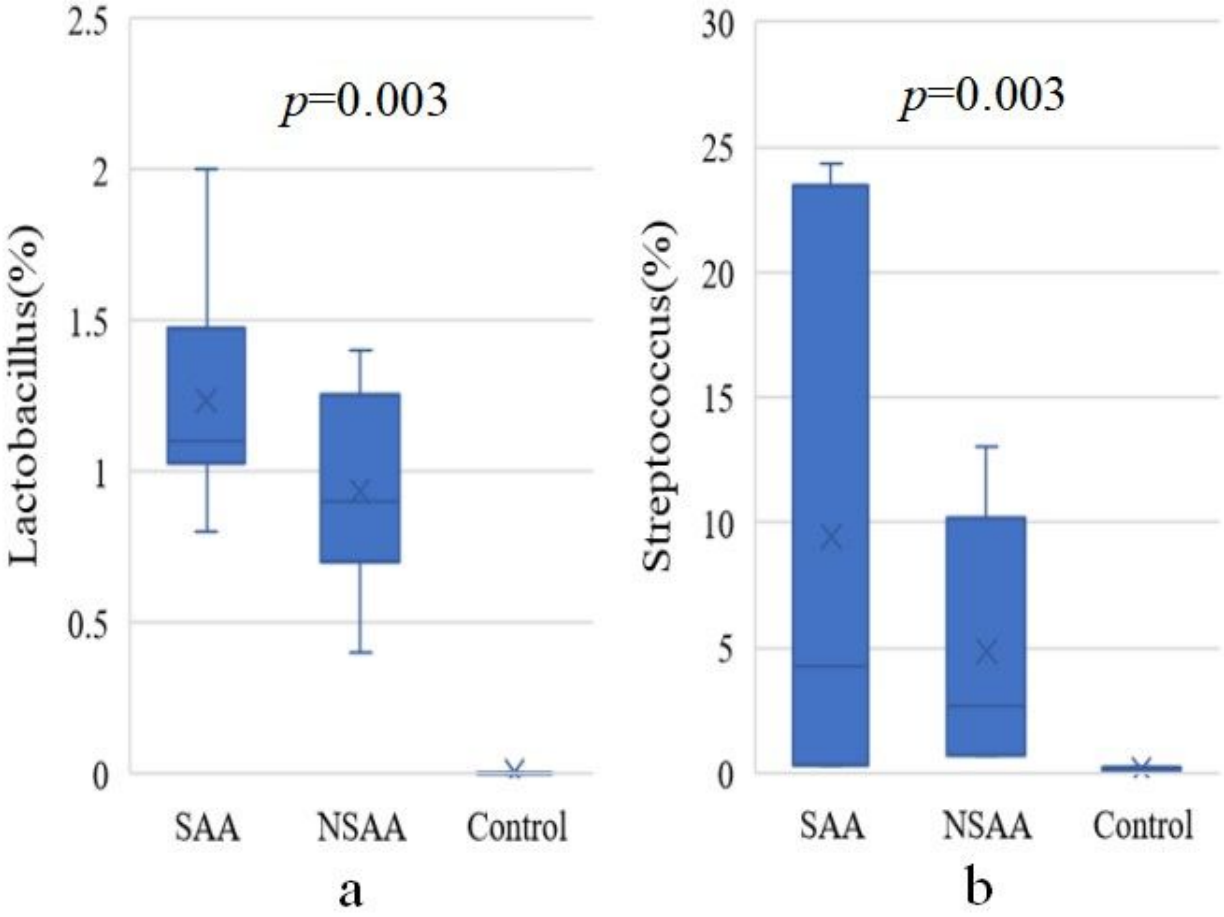

Figure 6

Comparison of the relative abundance of some bactaria from Bacilli class among the three groups.

Supplementary Files

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