The Association Between the Gut Microbiota and Systemic Lupus Erythematosus, a Meta-Analysis

Shate XIANG
Zhejiang Chinese Medical University https://orcid.org/0000-0002-2223-7557
Yiqian Qu
Zhejiang Chinese Medical University
Suhai Qian
Zhejiang Chinese Medical University
Yao Wang
Zhejiang Chinese Medical University
Yibo Jin
Zhejiang Chinese Medical University
Jie Li
Zhejiang Chinese Medical University
Xinghong Ding (dxh@zcmu.edu.cn)
Zhejiang Chinese Medical University https://orcid.org/0000-0001-7090-9445

Research article

Keywords: Systemic lupus erythematosus, Gut microbiota, Diversity, Abundance level, Meta-analysis

Posted Date: June 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-608010/v1

Abstract

Introduction:

Evaluate the changes of gut Microbiota in patients with Systemic Lupus Erythematosus (SLE) and healthy people by meta-analysis.

Methods

We searched the case-control studies of SLE and healthy controls (HCs) for detecting the diversity of gut Microbiota and the abundance level of some microbiota in the two groups. StataMP16 software was applied for this meta-analysis. The Newcastle-Ottawa quality assessment scale (NOS) was used to assess the quality of the included studies.

Results

Eleven case-control studies were included. There were 373 SLE patients and 1288 healthy people, involving 5 countries and 9 different cities. Compared with the HCs, the Shannon-wiener diversity index (WMD=-0.22; 95% CI=-0.32 to -0.13; P = 0.000) and Chao1 richness estimator (SMD=-0.62; 95% CI=-1.04 to -0.21; P = 0.003) of gut Microbiota in SLE decreased, and the abundance level of *Ruminococcaceae* decreased (SMD=–-0.48; 95% CI = 0.76 to-0.21; P = 0.001). Enterobacteriaceae (SMD = 0.39,95% CI = 0.11 to 0.66;P = 0.006) and Enterococcaceae (SMD = 0.55; 95% CI = 0.19 to 0.9; P = 0.03) showed higher abundance levels in comparison with HCs. The subgroup analysis showed the abundance level of *Ruminococcaceae* (SMD=-0.89; 95% CI =-1.34 to -0.45; P = 0.000) was lower and *Enterococcaceae* was higher (SMD = 0.77; 95% CI = 0.34 to 1.21P = 0.001) in Chinese with SLE compared with HCs. In non-Chinese patients with SLE, there were no significant difference between the abundance level of *Ruminococcaceae* (SMD=-0.22; 95% CI=–-0.58 to 0.13; P = 0.216) and *Enterococcaceae* (SMD=-0.08; 95% CI=-0.49 to 0.32; P = 0.682) with HCs. The subgroup analysis also found the level of *Enterobacteriaceae* was affected by the sample size.

Conclusion

Compared with the diversity of healthy people, richness and evenness of gut microbiota in patients with SLE are impaired. There is a decrease in the abundance level of beneficial bacteria and an increase in the harmful bacteria. Thus, gut microbiota in patients with SLE appear disorder, which may lead to metabolic imbalance, destruction of the integrity of the small intestine, immune system disorders and pro-inflammatory. Regulating the abundance of gut microbiota can be used as one of the key strategies for treating SLE.

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease with complex clinical manifestations, which is characterized by excessive activation of B cells, T cells and the production of autoantibodies, which can cause injury in multiple organs and tissues [1]. Clinical manifestations include butterfly erythema, serous effusion, proteinuria, arthritis, oral ulcer and so on. At present, there is no radical cure, only immunosuppression and immunoregulation. Although the medical community is constantly improving diagnostic methods and treatment strategies, the morbidity and mortality of SLE are still increasing [2–3].

The pathogenesis of SLE is not clear. It has been proposed in recent years that gut microbiota may play an important role in the pathogenesis of SLE as gut microbiota can help the development of immune system and has close relationship with innate and adaptive immunity [4–6]. Recently, studies mainly focus on the mechanism of gut microbiota triggering SLE. Some investigators speculate that the occurrence of SLE may be associated with impaired gut microbes and impaired gut barrier [7]. It may be the pathogenesis of SLE that disrupted gut barrier leads to 'leaky gut', activating immune factors and causing systemic autoimmunity [8]. Another point of view is that molecular simulation is the key to the occurrence of SLE induced by gut microbiota. The orthologs of Ro60 in gut microbiota binds to the B cells and T cells, resulting in cross-reaction, so that it stimulates autoimmune diseases [9]. The incidence of lupus is gender-specific. Woman have a higher incidence of SLE compared to man [10]. Through the experimental study in mice, it was found that sex hormones may cause the difference of gut microbiota [11–12].

From that, the disorder of gut microbiota is closely related to the systemic lupus erythematosus, which can be reflected in the changes of diversity of bacteriome and the related microbial abundance. However, the changes of gut microbiota are affected by genetic genes, diet, BMI, region, immunosuppressants and other causes [13–16], which may affect the experiments results about the changes of gut microbiota in patients with SLE and healthy people. For example, Marc JanBonder [17] proposed that gene-diet interaction can regulate the abundance of Bifidobacterium. The experimental results of Chiara Bellocchi [18] and Jingquan He [19] showed there was no significant difference in Shannon-wiener diversity index and Chao1 richness estimator between SLE and healthy controls (HCs). Weifang Zhu[20] drew a different experimental result that chao1 richness estimator of SLE was higher and the Shannon-wiener diversity index was lower compared with HCs.

Thus, in order to verify the changes of gut microbiota in patients with SLE and the factors that may affect the changes of gut microbiota, we performed a meta-analysis to analyzed the relevant research results around the world, providing an evidence-based medicine basis for follow-up research.

Methods

This meta-analysis referred to the Observational Studies in Epidemiology (MOOSE) group, which provide meta guidance for observational studies [21]. This meta-analysis was registered in the International Prospective Record of Systematic Reviews (PROSPERO) on May 17, 2021, with the number CRD42021249607.

Literature Search

A systematic literature search was conducted using the following English and Chinese databases by two researchers (STX and YQQ) respectively: Pubmed, Embase, Cochrance, Web of science, Wanfang database and Chinese National Knowledge Infrastructure databases (http://www.cnki.net/). We use a combination of Medical Subject Headings [MeSH] and synonymous to search studies (full search strategy available in Supplement 3). The retrieval time is from the establishment of the database to March 1, 2021.

Study Selection

The study inclusion criteria for meta-analysis were as follows: (1) population as the research object; (2) the research contents included the changes of gut microbiota diversity or the relative abundance of microbiota between SLE patients and HCs; (3) fecal samples; (4) the experimental results were described by using median, quartile range, average, standard deviation or P value, ensuring the data can be converted. The exclusion criteria were: (1) animal experiment; (2) the research contents were irrelevant; (3) review articles; (4) failure to get complete experimental data.

Data Extraction

Two researchers (STX and YQQ) extract relevant data from the included study and cross-verify it. If any disagreement was raised, it was resolved through discussion and consultation with a third researcher (SHQ). The contents include: (1) the first author, the year of publication, the country and region in which the study was published; (2) the characteristics of the subjects (sex, average age, population, drugs currently taken and related doses) (3) Experimental methods (diagnostic criteria, sample size, gut microbiota assessment technique); (4) data on alpha diversity and Beta diversity between patients with SLE and HCs. (5) data of the relative abundance of gut microbiota. Since we cannot get in touch with the author of studies, the data we used were all public.

Data conversion

Some studies do not provide relevant experimental data directly, GetDataGraphDigitizer2.25 software (http://getdata-graph-digitizer.com/) was applied to extract the sufficient data. Refer to the operation method proposed by Xiang Wan [22] and carry out data conversion.

Quality Assessment

The two researchers (STX and YQQ) independently rated the quality of the included studies and cross-checked it. If encounter disagreement, we sought third-party adjudication. The nine-star Newcastle-Ottawa Quality Assessment Scale (NOS) for case-control studies was used to assess the quality of the included studies [23]. The NOS scale includes seven items: (1) Adequate Definition of Cases; (2) Representativeness of the Cases; (3) Selection of Controls; (4) Definition of Controls; (5) Ascertainment of Exposure; (6) Same method of ascertainment for cases and controls; (7) Non-Response Rate. With a total score of 9. We selected studies with a score of 6–9 points, as high-quality articles, and include them in our research.

Statistical Analysis

The collected or converted data (including Shannon-wiener diversity index, Chao1 richness estimator and the relative abundance of gut microbiota) were sorted out. The number of studies for data were four or more data would be statistically analyzed by Stata software version 16.0. The results were displayed by forest plot. When the data included are continuous variables and the measurement methods are the same, we choose weighted mean differences (WMD) as the effect scale. When there is a large difference in the mean or standard deviation between the included studies, we choose standardized mean differences (SMD) as the effect scale [24].

Statistical heterogeneity was evaluated using the chi-square-based Q statistic test. I^2 was used as an index to evaluate heterogeneity, of which 25%, 50% and 75% were the boundaries of moderate, large and extreme heterogeneity, respectively. When $I^2 > 50\%$, random effect model was selected for data analysis, while $I^2 < 50\%$, fixed effect model was selected for data analysis [25]. In our meta-analysis, $I^2 > 25\%$ was taken as the level of statistical significance of heterogeneity. The level of significance was set at $p < 0.05$.

Subgroup analysis were performed by year of publication, sample size, population, percentage of women in the sample size, and whether or not taking drugs. By deleting one study at a time and combining the effect values of the remaining studies, sensitivity analysis was performed to evaluate the effect of a particular study on the overall results.

Publication bias was evaluated by Egger test [27], Begg test [28] and funnel chart. Among them, Egger test and Begg test showed that there was no publication bias when $P > 0.05$, and there was publication bias when $P < 0.05$. We take the result of Egger test as the reference value when the result of Egger test is inconsistent with that of Begg test. However, due to the limited number of studies included, the published bias assessment results may be not sufficiently reliable.

Results

Study selection and Characteristics

A total of 720 studies were retrieved. Deleting duplicate studies by Endnote, 459 articles were remained. After manual review of the title and abstract, a total of 425 articles which were not related to the research

content and whose experimental subjects were animals or reviews were deleted. To further read the full text of the literature, we deleted a total of 3 studies whose experimental results were incomplete, the experimental procedure was unclear, or the sample was non-human feces. In the remaining 31 studies, the data of Shannon-wiener diversity index, Chao1 richness estimator and abundance level of some gut microbiota were analyzed. There was no difference in the experiment results of Beta diversity between patients with SLE and HCs in various studies, so it was not included in the meta-analysis. Among them, twenty-four studies left after deleted eight studies in which the data could not be collected or gut microbiota was described as log10 bacteria per gram of feces or LDA SCORE. After quality evaluation, eleven studies were included in meta-analysis. The systematic search process is summarized in Fig. 1.

A total of eleven studies were included in this study (nine in English and two in Chinese). This meta-analysis involved 373 patients with SLE and 1288 HCs. The experimental samples used in the eleven studies were human feces and all available studies described the gut microbiome by 16S rRNA-gene amplicon sequencing. The diagnostic criteria of SLE patients refer to the criteria set by American College of Rheumatology (ACR). Detailed studies characteristics are showed in Table 1. All the eleven studies were observational case-control studies. According to the NOS scale, we evaluated the quality of each study to ensure that the included studies was of high quality. The evaluation results are shown in Table 2.

Table 1
The characteristics of included studies

Study	Location	SLE case/HC case			SLE case
	country / race	Number	Female sex (%)	Mean Age ± SD	Medication/dosage
Chiara Bellocchi et al[18]. (2019)	Italy / Not ChineXse	27/27	88.9%/74.1%	47.7 ± 16.6/52.5 ± 10.0	Prednisone(< 10 mg per day)/ Immunosuppressant / HCQ/ Statin
Taco A. van der Meulen et al[29]. (2019)	Netherlands / White/European ethnic background	35/965	94.3%/58.0%	47.0 ± 14.0/45.0 ± 13.0	Proton pump inhibitors/ NSAIDS/ Corticosteroids(<7.5mg per day)/ Antimalarial
Xin M. Luo et al[30]. (2018)	America / African American(not Caribbean) and Caucasian, non-Hispanic.	14/17	71.4%/NA	43.2 ± 18.1/NA	HCQ / belimumab / MMF / MTX / rituximab / AZA / tacrolimus
Arancha Hevia et al[31]. (2014)	Spain / Caucasian, origin	20/20	100%/100%	49.2 ± 10.7/46.9 ± 8.6	NA
Feng Wei et al[32]. (2019)	China / Chinese	14/16	92.9%/87.5%	40.7 ± 13.9/38.6 ± 14.5	NA
Zhixing He et al[33]. (2016)	China / Chinese	45/48	100%/100%	46.0 ± 1.8 /43.5 ± 2.4	NA
Mengchen Guo et al[34]. (2020)	China / Chinese	17/20	100%/100%	34.4 ± 3.4/30.4 ± 1.9	NA
Yao Li et al[35]. (2019)	China / Chinese	40/22	100%/100%	37.5 ± 14.2/37.2 ± 14.7	NA
Weifang Zhu et al[20]. (2018)	China / Chinese	32/26	93.8%/87.5%	33.8 ± 14.2/NA	Immunosuppressant / hormone drugs (no record)

Study	Location	SLE case/HC case			SLE case
	country / race	Number	Female sex (%)	Mean Age ± SD	Medication/dosage
Bei-di Chen et al[19]. (2020)	China / Beijing	117/115	72.8%/84.3%	30.8 ± 10.9/32.4 ± 11.3	NA
Zhidong Sun. et.al[36]. (2019)	China / Heilongjiang	12/12	NA	NA	NA

Table 2 Score of studies included in this meta-analysis based on NOS

(The table was uploaded as additional file 1 because it was larger than one page.)

Meta-Analysis of Standardized Mean Difference

We extracted continuous data from studies for meta-analysis, including the relative abundance levels of *Ruminococcaceae, Enterobacteriaceae*, *Lachnospiraceae*, *Enterococcaceae*, *Bacteroides*, and alpha diversity of gut microbes (Shannon-wiener diversity index and Chao1 richness estimator) in SLE patients compared to healthy people. Since there was a difference of more than 10 times in the average values of other indicators except for the Shannon-wiener diversity index, we chose SMD as the effect scale.

This meta-analysis showed lower of Shannon-wiener diversity index (WMD=-0.22; 95% CI=-0.32 to -0.13; P = 0.000; ten studies; Fig. 2A) and Chao1 richness estimator (SMD=-0.62; 95% CI=-1.04 to -0.21; p = 0.003; six studies; Fig. 2B) in patients with SLE than that of HCs. We analyzed the different taxa of gut microbiota in SLE patients, revealing that *Ruminococcaceae* (SMD=–0.48; 95% CI=-0.76 to -0.2; p = 0.001; five studies; Fig. 2C)exhibited decreased abundance and *Enterobacteriaceae* (SMD = 0.39; 95% CI = 0.11 to 0.66; P = 0.006; five studies; Fig. 2D) exhibited increased abundance. *Lachnospiraceae* (SMD = 0.05; 95% CI=-0.46 to 0.57; P = 0.843;four studies□Figure 2E), *Enterococcaceae* (SMD = 0.33; 95% CI = 0.19 to 0.84; P = 0.218; four studies; Fig. 2F) and *Bacteroides* had no significant difference compared with HCs.

Sensitivity analysis

The results of meta-analysis showed that the heterogeneity of Chao1 richness estimator (I^2 = 70.2%), *Enterobacteriaceae* (I^2 = 59%), *Lachnospiraceae* (I^2 = 67.1%), *Enterococcaceae* (I^2 = 66.2%) and *Bacteroides* (I^2 = 74.7%) were all greater than 25%. Further, the source of heterogeneity was evaluated using sensitivity analysis (the results of all sensitivity analyses were presented in additional file 2). Removing one study, the heterogeneity of Chao1 richness estimator (Fig. 3A), *Lachnospiraceae* (Fig. 3B), *Enterococcaceae* (Fig. 3C) and *Bacteroides* (Fig. 3D) all decreased or disappeared. Among them, the

results of Chao1 richness estimator (p = 0.000), *Lachnospiraceae* (p = 0.327) and *Bacteroides* (p = 0.756) were not affected. However, we found that after sensitivity analysis, the P value of *Enterococcaceae* (SMD = 0.55; 95% CI = 0.19 to 0.9; I^2%=43.9%; three studies; Fig. 3C) was 0.03, indicating the difference was statistically significant (p < 0.05), that is, the relative abundance of *Enterococcaceae* in feces of the SLE group was higher compared to the control group.

Subgroup analysis

Removing some of the included studies could not reduce the statistical heterogeneity of *Ruminococcaceae* and *Enterobacteriaceae*. In addition, after sensitivity analysis, the heterogeneity of *Enterococcaceae* still higher than 25%. Subgroup analysis was conducted stratified by year of publication, the size of the sample, the population, the percentage of women in the sample size, and whether or not to take drugs.

When the sample size (n) was greater than 20 or less than 20 for subgroup analysis, the source of heterogeneity of *Enterobacteriaceae* could be excluded (Fig. 4A). In the subgroup with n < 20, the relative level of *Enterobacteriaceae* in patients with SLE was higher than that in the HCs when in the subgroup with n > 20, the relative abundance of *Enterobacteriaceae* was not different.

The population identified subgroups with different results in *Ruminococcaceae* and *Enterococcaceae.* The relative abundance of *Ruminococcaceae* in patients with SLE was lower than that in HCs (SMD=-0.89; 95% CI =-1.34 to -0.45; P = 0.000), but there was no significant difference in non-Chinese subgroup (SMD=-0.22; 95% CI=–0.58 to 0.13; P = 0.216). In the subgroup classified as Chinese, the abundance level of *Enterococcacea* in patients with SLE was higher than that in healthy people (SMD = 0.77; 95% CI = 0.34 to 1.21; P = 0.001), while there was no difference between the subgroups classified as non-Chinese (SMD=-0.08; 95% CI=-0.49 to 0.32; P = 0.682).

Table 3
Results of subgroup analyses of *Ruminococcaceae*

	No. of studies	SMD	P Value(%)	I^2 (%)	P value for heterogeneity	95% CI
Year of publication						
2014	1	-0.422	0.188	NA	NA	-1.049 to 0.206
2018	2	-0.617	0.05	45.7	0.175	-1.047 to -0.186
2019	2	-0.377	0.092	73.1	0.054	-0.815 to 0.061
Sample size						
n<20	2	-0.581	0.029	52.0	0.149	-1.102 to -0.061
n≥20	3	-0.446	0.007	49.0	0.141	-0.771 to -0.121
Population						
Chinese	2	-0.895	0.000	0.0	0.752	-1.337 to -0.452
Non- Chinese	2	-0.223	0.216	0.0	0.714	-0.576 to 0.130
Take drugs or not						
Take drugs	3	-0.404	0.018	52.6	0.121	-0.739 to -0.069
Not take drugs	2	-0.652	0.008	22.4	0.256	-1.138 to -0.167
percentage of women						
100%	1	-0.422	0.188	NA	NA	-1.049 to 0.206
< 100%	4	-0.499	0.001	51.2	0.105	-0.806 to -0.192

Table 4
Results of subgroup analyses of *Enterobacteriaceae*

	No. of studies	SMD	P Value(%)	I^2 (%)	P value for heterogeneity	95% CI
Year of publication						
2014	1	0.281	0.377	NA	NA	-0.343 to 0.904
2018	2	0.618	0.005	4.3	0.307	0.19 to 1.05
2019	2	0.194	0.388	85.1	0.307	-0.246 to 0.633
Sample size						
n<20	2	0.984	0.000	0.0	0.868	0.446 to 1.521
n≥20	3	0.171	0.295	38.5	0.197	-0.150 to 0.493
Population						
Chinese	2	0.642	0.004	30.2	0.231	0.208 to 1.076
Non- Chinese	3	0.385	0.247	66.9	0.049	-0.146 to 0.568
Take drugs or not						
Take drugs	3	0.293	0.087	69.9	0.036	-0.042 to 0.628
Not take drugs	2	0.577	0.019	54.6	0.138	0.093 to 1.062
percentage of women						
100%	1	0.281	0.377	NA	NA	-0.343 to 0.904
< 100%	4	0.411	0.009	68.8	0.022	0.103 to 0.718

Table 5
Results of subgroup analyses of *Enterococcaceae*

	No. of studies	SMD	P Value(%)	I^2 (%)	P value for heterogeneity	95% CI
Year of publication						
2014	1	0.085	0.789	NA	NA	-0.536 to 0.705
2018	1	0.877	0.002	NA	NA	0.334 to 1.420
2019	2	0.066	0.765	85.1	0.307	-0.366 to 0.499
Sample size						
n<20	1	0.588	0.117	NA	NA	-0.147 to 1.323
n≥20	3	0.259	0.118	75.7	0.016	-0.066 to 0.584
Population						
Chinese	2	0.775	0.001	0.0	0.231	0.208 to 1.076
Non- Chinese	3	-0.085	0.682	0.0	0.049	-0.146 to 0.568
Take drugs or not						
Take drugs	3	0.546	0.440	NA	NA	-0.746 to 0.324
Not take drugs	1	-0.211	0.003	43.9	0.168	0.189 to 0.903
percentage of women						
100%	1	0.085	0.789	NA	NA	-0.536 to 0.705
< 100%	3	0.381	0.027	75.6	0.017	0.042 to 0.719

Analysis of Publication Bias

In order to determine the credibility of this meta-analysis, we assessed the risk of publication bias through Egger's test, Begg's test and observing the symmetry of the funnel chart. The results show that there is no

publication bias in each study, which means that the conclusions of the Meta-analysis are relatively robust. (Funnel charts were shown in additional Fig. 2.)

Discussion

At present, the research on the pathogenesis and treatment of SLE is a difficult point in the medical field. A growing number of studies have demonstrated that human gut microbiota is one of the important factors affecting the development of autoimmune diseases [37]. In patients with SLE, the increase of some gut microbiota can inhibit the production of IL-12p70 and enhance the response of IL-8, IL-6, IL-10 and TNF-α, such as *Streptococcus* and *Veillonella*. They lead to pro-inflammatory response [35–38]. In addition, abnormally enriched, antigen mimicry and metabolic response of gut microbiota can cause the disorder of immune response [19, 39]. Thus, we tried to include as many studies as possible to obtain and extracted experiment results of alpha diversity and some gut microbiota abundance between SLE patients and healthy people, analyzing the changes of gut microbiota in SLE patients by meta-analysis. Our meta-analysis contained eleven case-control studies, comprising three hundred and seventy three SLE patients and one thousand two hundred and eighty eight healthy people, involving five countries and nine different cities.

Meta-analysis results

Our meta-analysis showed that both Shannon wiener diversity index and Chao1 richness estimator, which are used to measure diversity in specific areas or ecosystems of the intestine, decreased in patients with SLE. Chao1 richness estimator is an indicator of species richness [40]. Shannon-wiener diversity index is an index to measure the uniformity of intestinal microorganisms [41]. This means the abundance and uniformity of the gut microbiota of patients with SLE are impaired. As a result, the stability of the micro-community is reduced, the structure and function of the micro-community are weakened, and the ability to resist changes is reduced.

Compared with healthy people, the relative abundance of *Ruminococcaceae* in patients with SLE decreased. It is an beneficial flora. *Ruminococcaceae* is belongs to Firmicutes, one of the cellulose-degrading bacteria (CDB). It can produce short-chain fatty acids (SCFA), which considered as potential orchestrators of the cross talk between gut microbiota and the host metabolism [43]. The lower relative abundance of *Ruminococcaceae* may lead to the imbalance of SCFA production, affecting the metabolism in human body. By studying lupus-prone mice, SCFA can inhibit B cell AID and Blimp1 expression, plasma cell differentiation, autoantibodies' class switching, and prevent IgG1/IgG2 deposition in the kidney and prevent lupus skin lesions [44]. Therefore, the decrease in the relative abundance of *Ruminococcaceae* may cause some complications in patients with lupus. The study also found that SCFA can protect the integrity of the small intestinal epithelial cell membrane. The decrease of the relative abundance of *Ruminococcaceae* may also lead to 'leaky gut'.

The results of Meta-analysis showed that the relative abundance of *Enterobacteriaceae* increased after suffering from SLE. *Enterobacteriaceae* is one of the families of Proteobacteria, in which most of the flora have pathogenicity and can produce inflammatory reaction. For example, *Enterobacteriaceae* is the most common pathogen causing abdominal infection, and the extended-spectrum beta-lactamases (ESBLs) production is the main mechanism of its pathogenesis [47]. Studies have shown that high levels of fS100A8-A9 in the intestine of infants can reduce the abundance level of *Enterobacteriaceae* in the intestine through the expansion of Tregs, and promote the good development of the intestinal microflora [48]. It can be seen that the abundance level of *Enterobacteriaceae* is associated with T cells, which may be the mechanism by which changes in the abundance level of *Enterobacteriaceae* affect the occurrence and development of SLE. *Enterobacteriaceae* is also one of the main pathogens causing pulmonary infection and lung infection [49]. Increasing relative abundance of *Enterobacteriacea* with SLE may be one of the factors leading to multiple infections such as lung and abdominal cavity. *Enterococcaceae*, one of the strains of Firmicutes, is a beneficial microflora and plays a certain role in alleviating gastrointestinal damage. According to the analysis, the relative abundance of this flora in patients with SLE is higher than that in healthy people. The result might indicate that there may be bacteria can reconstruct gut homeostasis by potential compensatory regulation [42, 51]. Furthermore, there was no significant difference in *Lachnospiraceae* and *Bacteroides* between SLE patients and healthy controls in the included study.

Results of sensitivity analysis and subgroup analysis

Of the eleven studies included, six provided data on the Chao1 index. Weifang Zhu (2018) [20] was a source of heterogeneity through sensitivity analysis. Some non-antibiotic drugs can have an impact on gut microbiota taken by SLE patients, such as glucocorticoids. We found that the study includes SLE patients with newly diagnosed patients (who had not used any drugs) and revisited patients (who had used hormone or immunosuppressive therapy). But it had not recorded the amount of immunosuppressants or hormones used in every SLE patients, while the other studies had recorded that the maximum dosage of hormones used should not exceed 10 mg. It has been reported by Mengchen Guo [34] that the gut microbial of glucocorticoid treatment was similar to that of the HC group. Among the included SLE patients, the dose of prednisone was up to 20 mg, which indicated that glucocorticoid could recovered gut microbiota stability in patients with SLE. In addition, some researchers have found that when the dose of glucocorticoids in SLE patients is greater than 15mg per day with longer duration, the possibility of osteonecrosis of the femoral head will increase [52]. Hence, we suspect that the dose of corticosteroids in patients with SLE may have an impact on the results of the experiment.

After remove the article Chiara Bellocchi (2019) [18], the heterogeneity of *Enterococcaceae* meta was eliminated. In this study, 70.37% of the patients were taking hydroxychloroquine (HCQ), while the other three studies did not record that. A short-term treatment with high-dose or long-term use of HCQ can change gut microbiota, resulting in the decrease of relative abundance of Firmicutes [53–54]. Hence, the use of HCQ in patients with SLE may have an effect on the abundance of gut microbiota.

After omitting the study of Taco A. van der Meulen (2019) [29], the heterogeneity between *Lachnospiraceae* and *Bacteroides* was completely eliminated, which shows that this study was the source of heterogeneity of the two analysis results. We found among the thirty SLE patients included in the study, twenty-eight were White/European ethnic background, but none of the healthy controls had White/European ethnic background. In addition, none of the SLE patients were born in the Netherlands, but nine hundred twenty-nine of the nine hundred and sixty five healthy people in the control group were born in the Netherlands. Except for the influence of disease on gut microbiota, diet, environment and race also have certain effects on it [55–56]. Therefore, the environmental and ethnic differences between the experimental group and the control group will affect the results. This suggests that the conditional differences between the included controlled studies should be controlled in follow-up clinical studies.

Our subgroup analysis showed that the relative abundance of *Ruminococcaceae* and *Enterococcaceae* in patients with SLE may be affected by population. In the Chinese subgroup, *Ruminococcaceae* showed a significant decrease, but in the non-Chinese subgroup, this phenomenon was not prominent [33]. Several investigators analyzed gut microbiota and human genome data, and showed the influence of lineage on microbial composition [57–58]. Quantitative trait loci (QTLs) analysis was performed on Six hundred and forty-five cross-line mice, and thirteen genetic loci that were significantly related to microbial abundance were detected, further confirmed the close relationship between heredity and intestinal microorganisms. However, some research suggested selective influence of genes on gut microbiota. For example, JuliaK.Goodrich (2016) [60]. analyzed one thousand one hundred and twenty-six pairs of gut microbiota from British twins, found that the *Christensenellaceae* was the most highly heritable taxon. It is speculated that *Ruminococcaceae* and *Enterococcaceae* families may also be one of the strains with heredity. Therefore, the different abundance level of gut microbiota varies with different populations when suffering from SLE. Meta-analysis showed the prevalence rate of a disease increases with the increase of sample size, which indicates that the sample size has an effect on the experimental results [61]. Subgroup analysis showed there is a certain difference in the meta-analysis results of *Enterobacteriaceae* when the sample size was less than twenty or it was greater than twenty. This means that the impact of clinical sample size on the results of the study should be taken into account in follow-up clinical studies. When the sample size is greater than twenty, there was still some heterogeneity. Increasing the grouping may get satisfactory results. However, due to the lack of studies included in this meta-analysis, it is impossible to carry out further research.

Limitation

Whether the dose range of glucocorticoid therapy can significantly change the diversity or abundance of intestinal microorganisms has not been studied. Weifang Zhu [20] studies have shown that there is no significant statistical difference in intestinal dominant flora between SLE patients who have not been treated with drugs and SLE patients who have been treated with drugs. However, Mengchen Guo [34] pointed out that glucocorticoid therapy can regulated the balance of gut microbiota. In clinical treatment of systemic lupus erythematosus, there is a large difference in hormone dose between active stage and

remission stage, so our meta-analysis lacks the changes of intestinal microorganisms in different stages of the disease, as well as the changes of gut microbiota in different periods.

Conclusion

This study systematically summarized the changes of gut microbiota between patients with SLE and HCs, including the changes of diversity and the relative abundance of some gut microbiota. Through the study, we found that there was a disorder of gut microbiota in patients with SLE, including the decrease of some probiotics and the increase of harmful bacteria, but there may be compensatory microflora regulating intestinal stability at the same time. This suggests that it can be used as one of the effective methods for the treatment of SLE by modulating the abundance of gut microbiota. In addition, we also studied the sources of heterogeneity through sensitivity or subgroup analysis, so as to provide reference for future clinical research.

Abbreviations

SLE Systemic Lupus Erythematosus

HCs Healthy controls

NOS Newcastle-Ottawa quality assessment scale

MOOSE Observational Studies in Epidemiology

PROSPERO International Prospective Record of Systematic Reviews

MeSH Medical Subject Headings

WMD Weighted mean differences

SMD Standardized mean differences

ACR American College of Rheumatology

CDB Cellulose-degrading bacteria

SCFA Short-chain fatty acids

ESBLs Extended-spectrum beta-lactamases

HCQ Hydroxychloroquine

QTLs Quantitative trait loci

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Pubmed, Embase, Cochrance, Web of science, Wanfang database and Chinese National Knowledge Infrastructure databases (http://www.cnki.net/).

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Competing interests

The authors declare that they have no competing interests

Funding

This research was funded by the National Natural Science Foundation of China (No. 81774179 and 81973778)

Authors' contributions

ST consulted and screened out the literature needed for this research, then evaluated the quality of the literature. ST also extracted and meta-analyzed the data in the literature, and finally completed the writing of the paper.

YQ consulted and screened out the literature needed for this research, then evaluated the quality of the literature. YQ also participated in the writing of the paper.

SH acts as the third party to mediate disputes in this study, and was responsible for completing the processing of pictures and tables in the paper.

WY, YB, LJ were responsible for the translation of the paper language.

XH put forward the idea of the paper and relieved the difficulties encountered in the process of writing the paper.

Acknowledgements

Not applicable

References

1. Kiriakidou M, Ching CL. Systemic lupus erythematosus. Ann Intern Med. 2020;172(11):81–96.
2. Durcan L, O'Dwyer T, Petri M. Management strategies and future directions for systemic lupus erythematosus in adults. Lancet. 2019;393(10188):2332–43.
3. Jorge AM, Lu N, Zhang Y, Rai SK, Choi HK. Unchanging premature mortality trends in systemic lupus erythematosus: A general population-based study (1999–2014). Rheumatology (Oxf), 2018, 57(2): 337–344.
4. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107(26):11971–5.
5. Umesaki Y, Setoyama H, Matsumoto S, Okada Y. Expansion of alpha beta t-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. Immunology. 1993;79(1):32–7.
6. De Luca F, Shoenfeld Y. The microbiome in autoimmune diseases. Clin Exp Immunol. 2019;195(1):74–85.
7. Silverman GJ. The microbiome in sle pathogenesis. Nat Rev Rheumatol. 2019;15(2):72–4.
8. Mu Q, Kirby J, Reilly CM, Luo XM. Leaky gut as a danger signal for autoimmune diseases. Front Immunol. 2017;8:598.
9. Greiling TM, Dehner C, Chen X, Hughes K, Iñiguez AJ, Boccitto M, et al. Commensal orthologs of the human autoantigen ro60 as triggers of autoimmunity in lupus. Sci Transl Med. 2018;10(434):eaan2306.
10. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110–21.
11. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science. 2013;339(6123):1084–8.
12. Yurkovetskiy L, Burrows M, Khan AA, Graham L, Volchkov P, Becker L, et al. Gender bias in autoimmunity is influenced by microbiota. Immunity. 2013;39(2):400–12.
13. Cuervo A, Hevia A, López P, Suárez A, Sánchez B, Margolles A, et al. Association of polyphenols from oranges and apples with specific intestinal microorganisms in systemic lupus erythematosus patients. Nutrients. 2015;7(2):1301–17.
14. Ma Y, Shi N, Li M, Chen M, Niu H. Applications of next-generation sequencing in systemic autoimmune diseases. Genom Proteom Bioinf. 2015;13(4):242–9.
15. Rojo D, Hevia A, Bargiela R, López P, Cuervo A, González S, et al. Ranking the impact of human health disorders on gut metabolism: Systemic lupus erythematosus and obesity as study cases. Sci Rep. 2015;5:8310.
16. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222–7.

17. Bonder MJ, Kurilshikov A, Tigchelaar EF, Mujagic Z, Imhann F, Vila AV, et al. The effect of host genetics on the gut microbiome. Nat Genet. 2016;48(11):1407–12.
18. Bellocchi C, Fernández-Ochoa Á, Montanelli G, Vigone B, Santaniello A, Quirantes-Piné R, et al. Identification of a shared microbiomic and metabolomic profile in systemic autoimmune diseases. J Clin Med. 2019;8(9):1291.
19. Chen BD, Jia XM, Xu JY, Zhao LD, Ji JY, Wu BX, et al. An autoimmunogenic and proinflammatory profile defined by the gut microbiota of patients with untreated systemic lupus erythematosus. Arthritis Rheumatol. 2021;73(2):232–43.
20. Zhu WF. Study on the changes of intestinal flora in patients with systemic lupus erythematosus. Zhejiang University; 2018.
21. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: A proposal for reporting. Meta-analysis of observational studies in epidemiology (moose) group. Jama. 2000;283(15):2008–12.
22. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol. 2014;14:135.
23. Stang A. Critical evaluation of the newcastle-ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010;25(9):603–5.
24. Bakbergenuly I, Hoaglin DC, Kulinskaya E. Estimation in meta-analyses of mean difference and standardized mean difference. Stat Med. 2020;39(2):171–91.
25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Bmj. 2003;327(7414):557–60.
26. Chavalarias D, Wallach JD, Li AH, Ioannidis JP. Evolution of reporting p values in the biomedical literature, 1990–2015. Jama. 2016;315(11):1141–8.
27. Hayashino Y, Noguchi Y, Fukui T. Systematic evaluation and comparison of statistical tests for publication bias. J Epidemiol. 2005;15(6):235–43.
28. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50(4):1088–101.
29. van der Meulen TA, Harmsen HJM, Vila AV, Kurilshikov A, Liefers SC, Zhernakova A, et al. Shared gut, but distinct oral microbiota composition in primary sjögren's syndrome and systemic lupus erythematosus. J Autoimmun. 2019;97:77–87.
30. Luo XM, Edwards MR, Mu Q, Yu Y, Vieson MD, Reilly CM, et al. Gut microbiota in human systemic lupus erythematosus and a mouse model of lupus. Appl Environ Microbiol. 2018;84(4):e02288-17.
31. Hevia A, Milani C, López P, Cuervo A, Arboleya S, Duranti S, et al. Intestinal dysbiosis associated with systemic lupus erythematosus. mBio. 2014;5(5):e01548-01514.
32. Wei F, Xu H, Yan C, Rong C, Liu B, Zhou H. Changes of intestinal flora in patients with systemic lupus erythematosus in northeast china. PLOS ONE. 2019;14(3):e0213063.

33. 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.
34. Guo M, Wang H, Xu S, Zhuang Y, An J, Su C, et al. Alteration in gut microbiota is associated with dysregulation of cytokines and glucocorticoid therapy in systemic lupus erythematosus. Gut Microbes. 2020;11(6):1758–73.
35. Li Y, Wang HF, Li X, Li HX, Zhang Q, Zhou HW, et al. Disordered intestinal microbes are associated with the activity of systemic lupus erythematosus. Clin Sci (Lond). 2019;133(7):821–38.
36. Sun ZD, Wei F, Xu HF. Study on the changes of intestinal flora in patients with systemic lupus erythematosus. Int J Immunol. 2019;42(6):579–82.
37. Silverman GJ, Azzouz DF, Alekseyenko AV. Systemic lupus erythematosus and dysbiosis in the microbiome: Cause or effect or both? Curr Opin Immunol. 2019;61:80–5.
38. van den Bogert B, Meijerink M, Zoetendal EG, Wells JM, Kleerebezem M. Immunomodulatory properties of streptococcus and veillonella isolates from the human small intestine microbiota. PLOS ONE. 2014;9(12):e114277.
39. Li R, Meng X, Chen B, Zhao L, Zhang X. Gut microbiota in lupus: A butterfly effect? Curr Rheumatol Rep. 2021;23(4):27.
40. Chao A. Nonparametric estimation of the number of classes in a population. Scand J Statist. 1984;11(4):265–70.
41. Chao A, Shen TJ. Nonparametric estimation of shannon's index of diversity when there are unseen species in sample. Environ Ecol Stat. 2003;10(4):429–43.
42. Shen T, Yue Y, He T, Huang C, Qu B, Lv W, et al. The association between the gut microbiota and parkinson's disease, a meta-analysis. Front Aging Neurosci. 2021;13:636545.
43. Rodríguez-Carrio J, López P, Sánchez B, González S, Gueimonde M, Margolles A, et al. Intestinal dysbiosis is associated with altered short-chain fatty acids and serum-free fatty acids in systemic lupus erythematosus. Front Immunol. 2017;8:23.
44. Sanchez HN, Moroney JB, Gan H, Shen T, Im JL, Li T, et al. B cell-intrinsic epigenetic modulation of antibody responses by dietary fiber-derived short-chain fatty acids. Nat Commun. 2020;11(1):60.
45. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of amp-activated protein kinase in caco-2 cell monolayers. J Nutr. 2009;139(9):1619–25.
46. Elamin EE, Masclee AA, Dekker J, Pieters HJ, Jonkers DM. Short-chain fatty acids activate amp-activated protein kinase and ameliorate ethanol-induced intestinal barrier dysfunction in caco-2 cell monolayers. J Nutr. 2013;143(12):1872–81.
47. Mureșan MG, Balmoș IA, Badea I, Santini A. Abdominal sepsis: An update. J Crit Care Med (Targu Mures). 2018;4(4):120–5.
48. Willers M, Ulas T, Völlger L, Vogl T, Heinemann AS, Pirr S, et al. S100a8 and s100a9 are important for postnatal development of gut microbiota and immune system in mice and infants. Gastroenterology.

2020;159(6):2130–45.e2135.

49. de Maio Carrillho CM, Gaudereto JJ, Martins RC, de Castro Lima VA, de Oliveira LM, Urbano MR, et al. Colistin-resistant enterobacteriaceae infections: Clinical and molecular characterization and analysis of in vitro synergy. Diagn Microbiol Infect Dis. 2017;87(3):253–7.
50. Guo H, Chou WC, Lai Y, Liang K, Tam JW, Brickey WJ, et al. Multi-omics analyses of radiation survivors identify radioprotective microbes and metabolites. Science. 2020, 370(6516).
51. Wallen ZD, Appah M, Dean MN, Sesler CL, Factor SA, Molho E, et al. Characterizing dysbiosis of gut microbiome in pd: Evidence for overabundance of opportunistic pathogens. NPJ Parkinsons Dis. 2020;6:11.
52. Chen S, Cai Q, Xu Y, Fu Q, Feng Y, Chen X, et al. Associations between glucocorticoids, antiphospholipid antibodies and femur head necrosis in patients with sle: A directed acyclic graph-based multicentre study. Ther Adv Musculoskelet Dis. 2021;13:1759720x211002677.
53. Pan ZY, Chang YX, Han N, Hou FY, Lee BJY, Zhi FC, et al. Short-term high-dose gavage of hydroxychloroquine changes gut microbiota but not the intestinal integrity and immunological responses in mice. Life Sci. 2021;264:118450.
54. Angelakis E, Million M, Kankoe S, Lagier JC, Armougom F, Giorgi R, et al. Abnormal weight gain and gut microbiota modifications are side effects of long-term doxycycline and hydroxychloroquine treatment. Antimicrob Agents Chemother. 2014;58(6):3342–7.
55. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science. 2016;352(6285):565–9.
56. Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. Nat Med. 2018;24(10):1526–31.
57. Blekhman R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, et al. Host genetic variation impacts microbiome composition across human body sites. Genome Biol. 2015;16(1):191.
58. Ma J, Coarfa C, Qin X, Bonnen PE, Milosavljevic A, Versalovic J, et al. Mtdna haplogroup and single nucleotide polymorphisms structure human microbiome communities. BMC Genom. 2014;15:257.
59. Leamy LJ, Kelly SA, Nietfeldt J, Legge RM, Ma F, Hua K, Sinha R, Peterson DA, Walter J, Benson AK, et al. Host genetics and diet, but not immunoglobulin a expression, converge to shape compositional features of the gut microbiome in an advanced intercross population of mice. Genome Biol. 2014;15(12):552.
60. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE. Genetic determinants of the gut microbiome in uk twins. Cell Host Microbe. 2016;19(5):731–43.
61. Vaisi-Raygani A, Mohammadi M, Jalali R, Salari N, Hosseinian-Far M. Prevalence of cystic echinococcosis in slaughtered livestock in iran: A systematic review and meta-analysis. BMC Infect Dis. 2021;21(1):429.

Figures

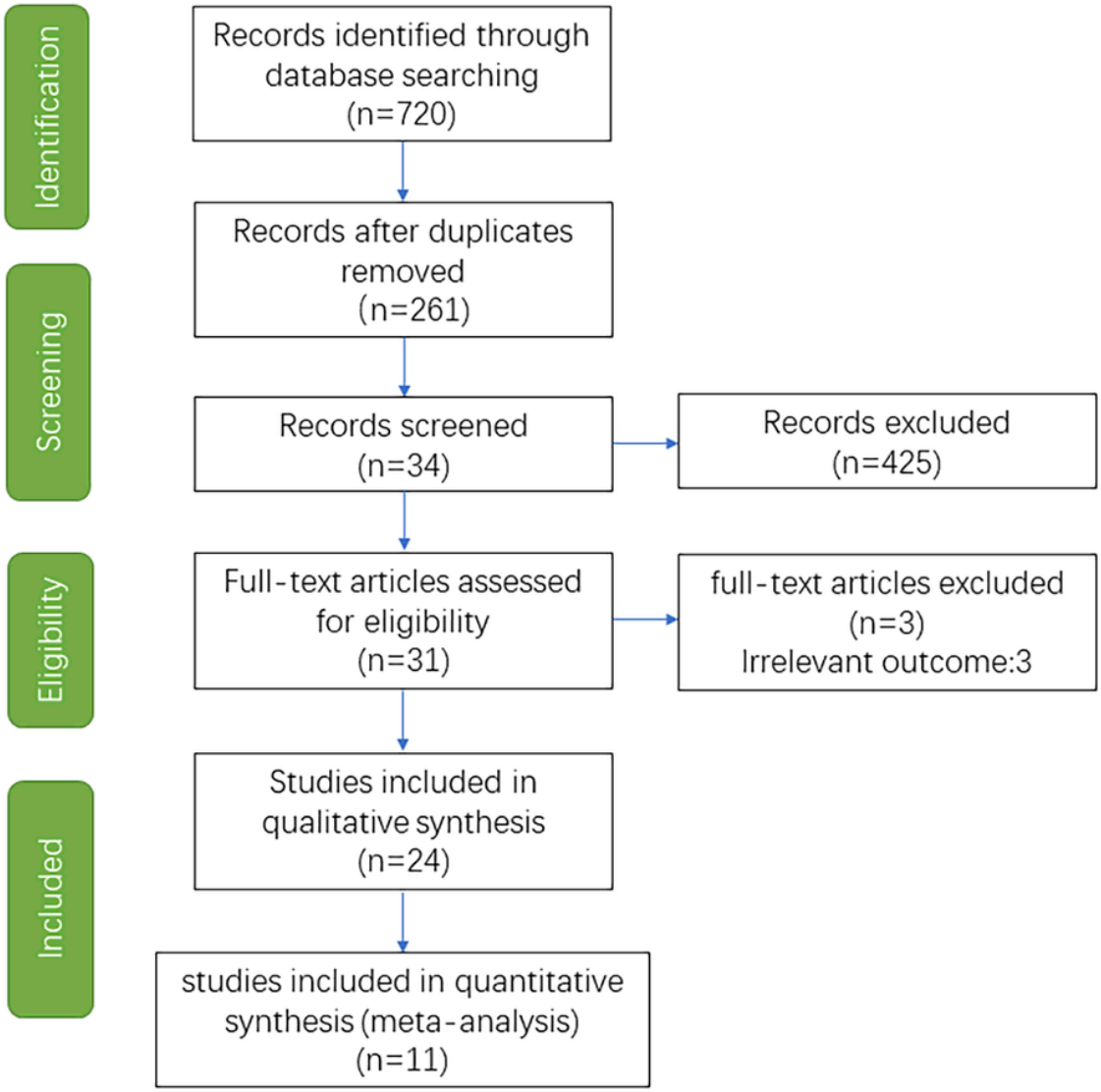

Figure 1

Flow chart of selection and inclusion of studies in this meta-analysis

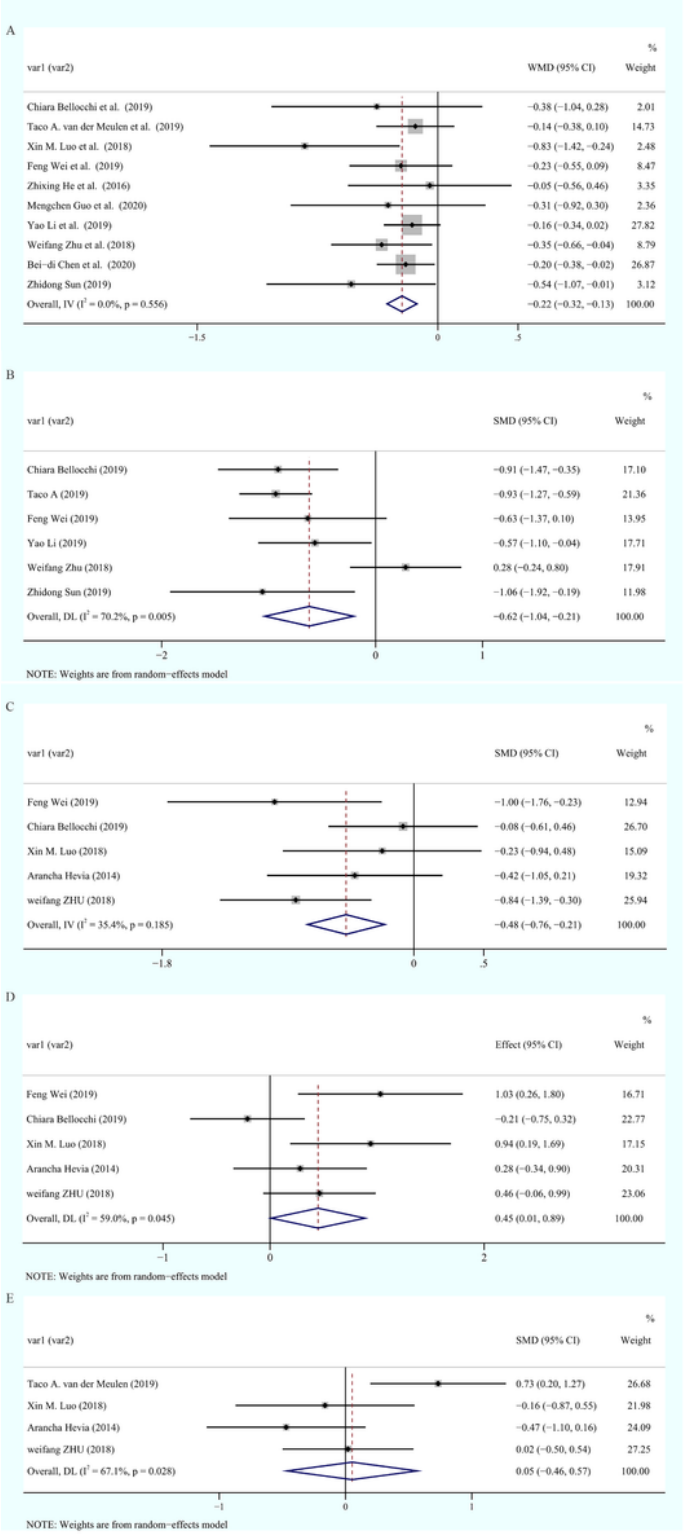

Figure 2

Forest plots of alterations of gut microbiota in patients with systemic lupus erythematosus (SLE) vs. healthy controls (HCs): A. Shannon wiener diversity index B. Chao1 richness estimator C. Ruminococcaceae D. Enterobacteriaceae E. Lachnospiraceae F. Enterococcaceae G. Bacteroides

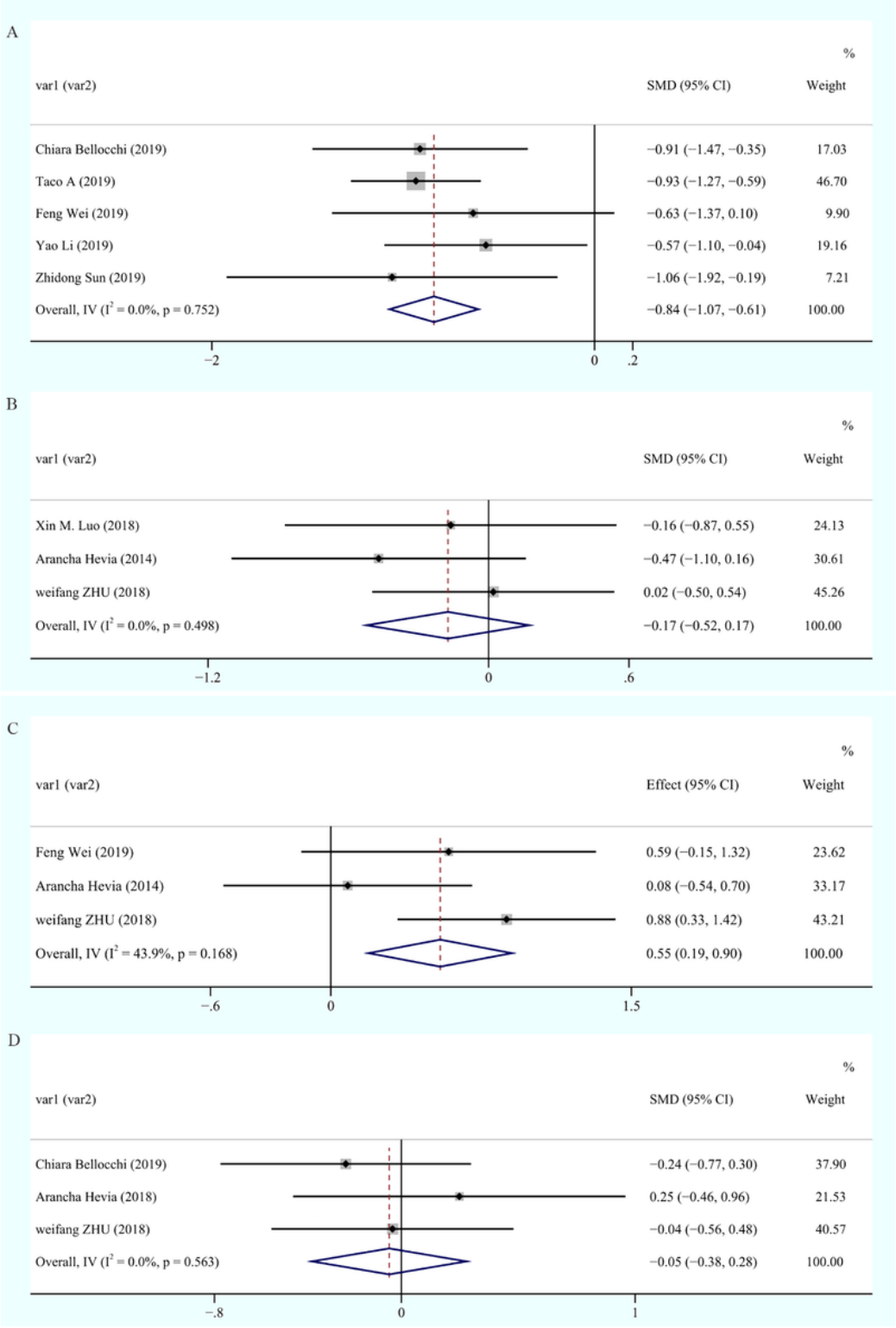

Figure 3

Eliminate sources of heterogeneity by sensitivity analysis. A. Chao1 richness estimator B. Lachnospiraceae C. Enterobacteriaceae D. Bacteroides

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Additionalfile2.docx
- Additionalfile3.docx