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Effect of treatments on skin microbiota in patients with atopic dermatitis: a protocol for systematic review

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ABSTRACT

Introduction Atopic dermatitis (AD) is a chronic inflammatory skin disease and skin microbiota dysbiosis shows an important role in the pathogenesis of AD. Effects of treatment on skin microbiota for patients with AD have been evaluated in recent years; however, the results remained controversial across studies. This systematic review will summarise studies evaluating the effect of treatments on skin microbiota among patients with AD.

Methods and analysis We will search PubMed, EMBASE, Web of Science, ClinicalTrials.gov and Chinese Clinical Trial Registry in November 2021; other data sources will also be considered, including searching specific authors and screening references cited in the enrolled articles. Interventional studies, which enrolled patients with AD receiving treatments and reported treatment-related skin microbiota changes, will be included. Our primary outcomes include skin microbiota diversity and treatment-related differential microbes; the secondary outcomes include microbiota functions and microbial interactions. Risk of bias assessment will be performed using Cochrane risk-of-bias tool for randomised trials, risk of bias in non-randomised studies of interventions and methodological index for non-randomised studies. Two researchers will independently perform study selection, data extraction and risk of bias assessment, with disagreements resolved by group discussions. Subgroup analyses will be performed according to different types of treatment for AD.

Ethics and dissemination Ethics approval is not required for this systematic review. Findings will be disseminated via peer-reviewed publication or conference proceedings.

PROSPERO registration number CRD42021246566.

Strengths and limitations of this study

- This systematic review will evaluate the effect of treatments on skin microbiota among patients with atopic dermatitis (AD), including topical therapy, phototherapy, systemic treatment, etc, and may provide insights into future aetiologic research and personalised therapy studies of AD.
- The search strategies for this systematic review are comprehensive, including searching electronic databases: PubMed, EMBASE, Web of Science, ClinicalTrials.gov and Chinese Clinical Trial Registry; other information sources will also be considered, including searching specific authors and screening references cited in the enrolled articles.
- Study selection, data extraction and risk of bias assessment will be conducted by two researchers independently, with disagreements resolved by team discussions, which could diminish potential bias.
- Due to the heterogeneity of methods and indexes used for microbiota evaluation, there may be a limitation to perform a quantitative synthesis.

INTRODUCTION

Rationale

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterised by recurrent eczematous lesions and intense itch with a prevalence of 10%~20% in children and 7%~10% in adults.[1] According to WHO Global Burden of Diseases initiative, at least 230 million people worldwide are suffering from AD.[1] A series of factors have a role in AD pathogenesis, including genetic susceptibility, epidermal barrier dysfunction, immunological dysregulation, skin microbiota dysbiosis, etc.[2] Recently, the role of skin microbiota in the development and treatment of AD has received increased attention.

As the largest organ of the human body, human skin is an epithelial barrier to the external environment and supports diverse microorganisms, including bacteria, fungi, viruses, etc, which compose the skin microbiota.[3] The skin microbiota could provide protective effects against pathogens by directly killing pathogens or altering the virulence of pathogens.[4] Importantly, the skin microbiota could stimulate the host immune response to invading pathogens and skin microbiota–host interactions are critical for host immune response and skin homeostasis.[4–6] As for AD, which is an immune-mediated inflammatory skin disorder, skin microbiota dysbiosis shows an important role in the pathogenesis of AD.[7 8] The skin microbiota dysbiosis in AD includes low diversity, overabundant colonisation of *Staphylococcus aureus (S. aureus)*, low abundance

of other skin commensal bacteria, etc. Studies of skin microbiota profile using high-throughput sequencing showed that the microbial diversity of AD skin decreased compared with controls; further analyses showed that the microbial diversity was inversely correlated to disease severity. Additionally, the diversity also reduced during an AD flare.[7 9] As for *S. aureus*, which is an important pathogenic factor for AD, the prevalence of *S. aureus* colonisation among patients with AD was 70% for lesional skin, 62% for the nose and 39% for non-lesional skin; and the prevalence of *S. aureus* colonisation increased with disease severity for patients with AD.[10] In addition to *S. aureus*, the relative abundance of other species of the genus *Staphylococcus*, such as *S. haemolyticus*, also increased for AD cases.[11] Moreover, AD cases showed decreased relative abundance of multiple genera, including *Streptococcus* spp, *Propionibacterium* spp, *Acinetobacter* spp, etc.[11] In terms of fungal microbiota, which also play a critical role, a reduction in the relative abundance of *Malassezia* spp and an increase of the *M. dermatis* etc were observed for AD.[11]

In terms of treatment for AD, effects of treatment on skin microbiota have been evaluated in recent years. Studies have demonstrated that the skin microbial diversity increased and the abundance of *S. aureus* reduced after treatment of systemic immunomodulating biologics, topical corticosteroids, etc[12–14]; the microbiota structure after treatment was more similar to those of healthy individuals.[12] However, the results remained controversial across studies. For patients with AD with specific characters, the abovementioned changes were not observed in patients after treatment; such phenomena may be associated with low abundance of *S. aureus* in these patients.[15] Additionally, to the best of our knowledge, there is no systematic review to evaluate the effect of treatments on skin microbiota in patients with AD. It is, therefore, warranted to summarise the available studies for understanding the role of skin microbiota in the treatment and prognosis of AD. Our systematic review may provide insights into aetiology studies and personalised therapy studies of AD.

Objectives

The aim of this research protocol is to outline a systematic review, which will evaluate the effect of treatments on skin microbiota among patients with AD, including topical therapy, phototherapy, systemic treatment, etc.

METHODS

This protocol was reported following the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) statement.[16] Reporting items are shown in the PRISMA-P checklist (online supplemental file 1).

Registration

Our protocol for the systematic review has been registered in the International Prospective Register of Systematic Reviews (PROSPERO).

Search strategy

The following electronic databases will be searched from November 2000 to November 2021: PubMed, EMBASE, Web of Science, ClinicalTrials.gov and Chinese Clinical Trial Registry. Other data sources will also be considered, including searching specific authors and screening references cited in the enrolled papers.

The search strategy is a combination of parameters 'atopic dermatitis', 'atopic eczema', 'eczematous dermatitis', 'microbiome', 'microbiota', 'microflora', 'bacterial flora' and 'bacterial community'. The full search strategy is provided in online supplemental file 2.

Eligibility criteria

We will include interventional studies, which enrolled patients with AD receiving treatments and reported treatment-related skin microbiota changes. The inclusion criteria were summarised by using the Population, Intervention, Comparison and Outcome (PICO) strategy.[16]

Population

Our targeted study population is patients diagnosed with AD; skin microbiota samples of patients were collected and skin microbiota characteristics obtained using high-throughput sequencing, including 16S ribosomal RNA (rRNA) gene sequencing, metagenomic sequencing and viral sequencing, were reported. Studies that only reported several specific bacteria will be excluded.

Intervention

The intervention (treatment for AD) includes the following:

1. Topical therapy, such as topical corticosteroids, topical calcineurin inhibitors, antibiotics, emollients, etc.
2. Phototherapy, such as narrow-band ultraviolet B, medium-dose ultraviolet A1, etc.
3. Systemic treatment, includes systemic immunosuppressants and systemic immunomodulating biologics.

Comparator

Our targeted studies evaluate the effects of treatment on skin microbiota. Thus, studies which conducted treatment-related comparisons of skin microbiota will be considered. The eligible comparisons include the following:

1. Before versus after treatment: this comparison could provide changes of skin microbiota after treatment.
2. Treatment versus placebo: such studies could offer the comparison of skin microbiota changes between treatment and placebo.
3. Comparison between different types of treatment: such studies could offer the comparison of skin microbiota changes among different types of treatment.

Outcomes

The primary outcomes of our study include:

1. Skin microbiota diversity (alpha diversity and beta diversity): the alpha diversity indexes include Shannon Index, Chao 1 Index, Simpson Index, Observed Species Index, etc.[17] The beta diversity represents difference between microbial communities.[17]
2. Treatment-related differential microbes: namely, microbes whose abundance increased or decreased after

treatment. Treatment-related microbes in levels of phylum, class, order, family, genus and species will be summarised.

The secondary outcomes of our study include:

1. Microbiota functions: analyses of microbiota functions refer to the prediction of functional profiling of microbial communities using bioinformatics method, such as Phylogenetic Investigation of Communities by Reconstruction of Unobserved States[18] and Tax4Fun.[19]
2. Microbial interactions: we will discuss how microbes interact with each other and the dynamic changes during the treatment of AD.[20]

Additionally, for types of studies, only human studies will be considered. No language restrictions will be applied. Conference abstracts will be excluded as limited information was reported.

Study selection

The identified literature will be imported to EndNote, which is a standard software for managing references. First, duplicate records will be removed. Then the records will be screened through title and abstract; the irrelevant records will be excluded, including reviews, conference abstracts, editorials, letters, irrelevant original articles, etc. Then candidate records will be assessed for eligibility based on full text. Two researchers (YG and K-yZ) will independently evaluate the records in each step. Discrepancies will be solved through group discussions. The preliminary flow chart of study selection process is shown in the PRISMA flow diagram (figure 1).

Data extraction and management

Using a predesigned standardised data abstraction form (online supplemental file 3), two researchers (X-lJ and YG) will independently extract characteristics of include studies, with any disagreements resolved by team discussions. The following information will be collected from the include studies.

Basic information of included studies

1. Authors, publication year, journal, title and region;
2. Aims of the study;

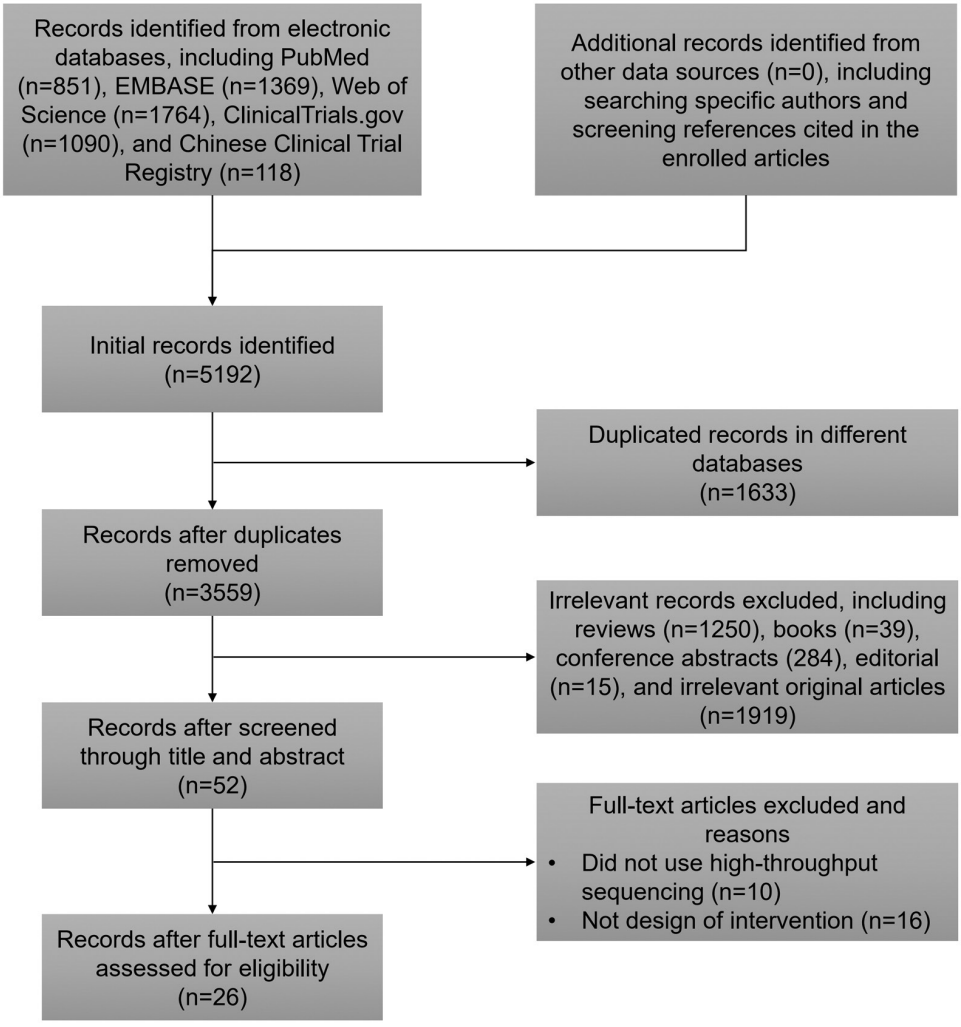

Figure 1 PRISMA flow diagram for study selection. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

3. Study design (randomised interventional study, non-randomised interventional study or single-arm interventional study);
4. Inclusion and exclusion criteria for patients
5. Sample size;

Characteristics of enrolled patients

1. Age, sex and race.
2. Evaluation index of AD: Investigator Global Assessment, SCORing Atopic Dermatitis, Eczema Area and Severity Index and others.
3. Comorbidities.

Interventions and comparisons

1. Treatment for AD (topical therapy, phototherapy, systemic treatment or others);
2. Period of treatment;
3. Follow-up time;
4. Comparison of skin microbiota (before vs after treatment, treatment vs placebo or comparison between different types of treatment).

Outcomes

1. Skin microbiota sample collection method and evaluation method of skin microbiota (16S rRNA gene sequencing, metagenomic sequencing or viral sequencing);
2. Major findings of primary outcomes: alpha diversity, beta diversity and differential microbes;
3. Major findings of secondary outcomes: microbiota functions and microbial interactions.

Risk of bias assessment

Two researchers (X-lJ and YG) will independently perform risk of bias assessment, with disagreements resolved by team discussions. The following tools will be used in our study (online supplemental file 4).

Cochrane risk-of-bias tool for randomised trials (RoB 2)

The RoB 2 tool[21] will be used to assess risk of bias for randomised trials. The RoB 2 tool is structured into five domains, including (1) bias arising from the randomisation process, (2) bias due to deviations from intended interventions, (3) bias due to missing outcome data, (4) bias in measurement of the outcome and (5) bias in the selection of reported result; a series of signalling questions were asked in the five domains. Based on the answers to the signalling questions, an overall evaluation of bias will be given, including 'low risk of bias ', 'some concerns' or 'high risk of bias'.

Risk Of Bias In Non-Randomised Studies - of Interventions (ROBINS-I)

The ROBINS-I tool[22] will be used to assess risk of bias for non-randomised studies. The tool covers seven domains through which bias might be introduced, including (1) bias due to confounding, (2) bias in selection of participants into the study, (3) bias in classification of interventions, (4) bias due to deviations from intended interventions, (5) bias due to missing data, (6) bias in measurement of outcomes and (7) bias in selection of the reported result; several signalling questions will be asked for each domain. Accordingly, a final judgement will be provided and the categories for risk of bias judgements are 'low risk', 'moderate risk', 'serious risk' and 'critical risk' of bias.

Methodological Index for Non-randomised Studies (MINORS)

In our systematic review, single-arm studies focusing on skin microbiota change by comparing pretreatment and posttreatment might be enrolled; the Methodological Index for Non-randomised Studies (MINORS)[23] will be used to assess risk of bias for the single-arm studies. The MINORS consists of 12 indexes: (1) a clearly stated aim, (2) inclusion of consecutive patients, (3) prospective collection of data, (4) endpoints appropriate to the aim of the study, (5) unbiased assessment of the study endpoint(s), (6) a follow-up period appropriate to the aim of the study, (7) loss to follow-up less than 5%, (8) prospective calculation of the study size, (9) an adequate control group, (10) contemporary groups (control and studied group should be managed during the same time period, no historical comparison), (11) baseline equivalence of groups and (12) an adequate statistical analyses. The items were scored 0 if not reported, 1 when reported but inadequate and 2 when reported and adequate. For single-arm non-comparative studies, the indexes (1)~(8) will be applicable and the global score will be 0~16 for such studies. A higher score represents a lower risk of bias. Scores of '13~16', '7~12' and '0~6' are classified as 'low risk', 'moderate risk' and 'high risk' of bias.

Statistical analyses

The major data for data synthesis were alpha diversity indexes and relative abundance of differential microbes. We anticipate that different methods for high-throughput sequencing were used to evaluate skin microbiota, such as 16S rRNA gene sequencing, metagenomic sequencing, etc. In addition, different indexes representing microbial diversity were used, including Shannon Index, Phylogenetic Diversity Index, Chao 1 Index, Abundance-based Coverage Estimators Index (ACE Index), etc. Therefore, changes of alpha diversity indexes and relative abundance of differential microbes between before treatment and after treatment will be reported; only studies using the same index and method of high-throughput sequencing will be included for further meta-analysis. The mean differences with 95% CI will be calculated as effect measurements. Between study statistical heterogeneity will be assessed using the I^2 statistic. If a meta-analysis is not possible due to limited number of studies using the same index and method of high-throughput sequencing, a narrative synthesis will be provided and we will summarise major findings according to the included articles. In terms of subgroup analyses, findings will be summarised and reported according to different types

of treatment for AD, including topical therapy, phototherapy, systemic treatment, etc.

Ethics and dissemination

As a systematic review, this study is based on published information and will not collect individual patient data. Therefore, the ethical approval is not required. Findings of our study are expected to be published in peer-reviewed journals or will be presented at a professional conference. Major findings will be summarised as shown in online supplemental file 5.

Patient and public involvement

No patients or public will be involved in the design, conduct or dissemination of this systematic review.

DISCUSSION

In recent years, multiple studies have demonstrated that skin microbiota dysbiosis plays a critical role in the development of AD, such as low microbial diversity, overabundant colonisation of *S. aureus*, low abundance of other commensal bacteria, etc.[9] However, the impact of treatment on skin microbiota among patients with AD remained unclear and the results remained controversial across studies.[12–15] Therefore, it is warranted to summarise the available studies to understand the role of skin microbiota in the treatment and prognosis of AD. We will systematically review studies focusing on the effect of treatments on skin microbiota among patients with AD, including topical therapy, phototherapy, systemic treatment, etc.

Findings of this study have several potential clinical implications. First, our study will report alterations of skin microbiota after treatment and potential new biomarkers of microbiota will be found; thus, our study may provide new insights for pathogenesis of AD and therapeutic strategies in terms of microbes. Second, we anticipate that several microbes were associated with the prognosis of AD according to enrolled studies, and prognostic biomarkers of AD may be reported. Moreover, we will include studies assessing different types of treatment and the comparisons of them will offer variant alterations of skin microbiota due to different types of treatment. Accordingly, these findings may provide evidences for personalised therapy for patients with AD.

We acknowledge several limitations. We anticipate that different methods for high-throughput sequencing and different indexes representing microbial diversity were used for assessment of skin microbiota. Due to the heterogeneity of methods and indexes used for microbiota evaluation, there may be a limitation to perform a quantitative synthesis.

In conclusion, this research protocol outlines a systematic review focusing on the effect of treatments on skin microbiota among patients with AD. The systematic review will provide a collective summary of impact of different types of treatment on skin microbiota for patients with AD.

Contributors YG and BY conceived and designed the systematic review protocol. YG and K-yZ wrote the search strategy, did the pilot literature search and will participate in study selection. X-lJ and YG will conduct the data extraction and risk of bias assessment. YG wrote the initial draft of the manuscript. XD, YZ and BY advised on protocol design and revised the manuscript. BY is the guarantor of this systematic review. All authors read and approved this final manuscript.

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Disclaimer The sponsor has not been involved in the design of this systematic review and the writing of the protocol.

Competing interests None declared.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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Online Supplemental File 1

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	NA
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2 and 6
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	13
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	NA
Support:			
Sources	5a	Indicate sources of financial or other support for the review	13
Sponsor	5b	Provide name for the review funder and/or sponsor	13
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	13
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	4-5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	5

METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6-7
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	5 and online supplemental file 2
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8-9
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7 and figure 1
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8-9 and online supplemental file 3
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8-9 and online supplemental file 3
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	9
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	9-11
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	11
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	11
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	11
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	11
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	NA
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	NA

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

Online Supplemental File 2

Literature search strategy

Database	Search strategy
PubMed < November 2000 - November 2021 >	((microbiota [Mesh]) OR "microbiome"[Title/Abstract] OR "microbiota"[Title/Abstract] OR "microflora"[Title/Abstract] OR "bacterial flora"[Title/Abstract] OR "bacterial community"[Title/Abstract])) AND ("dermatitis, atopic"[Mesh] OR "atopic dermatitis"[Title/Abstract] OR "atopic eczema"[Title/Abstract] OR "eczematous dermatitis"[Title/Abstract])
EMBASE < November 2000 - November 2021 >	('microbiome':ti,ab,kw OR 'microbiota':ti,ab,kw OR 'microflora':ti,ab,kw OR 'bacterial flora':ti,ab,kw OR 'bacterial community':ti,ab,kw) AND ('atopic dermatitis'/exp OR 'atopic eczema':ti,ab,kw)
Web of Science (SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC) < November 2000 - November 2021 >	(TS=(microbiome) OR TS=(microbiota) OR TS=(microflora) OR TS=(“bacterial flora”) OR TS=(“bacterial community”)) AND (TS=(“atopic dermatitis”) OR TS=(“atopic eczema”))
ClinicalTrials.gov < November 2000 - November 2021 >	Condition or disease: atopic dermatitis OR eczema OR eczematous dermatitis Study type: Interventional Studies
Chinese Clinical Trial Registry < November 2000 - November 2021 >	Public title: atopic dermatitis OR eczema OR eczematous dermatitis

Online Supplemental File 3

Data Extraction Form

I. Basic information of included studies

Authors: _____

Publication year: _____

Journal: _____

Title: _____

Region: _____

Aims: _____

Study design: ☐Randomized interventional study

☐Non-randomized interventional study

☐Single-arm interventional study

Inclusion criteria for patients: _____

Exclusion criteria for patients: _____

Sample size: _____

II. Characteristics of enrolled patients

Age: ☐mean ± SD _____

☐median (IQR)_____

Sex: _____(male : female)

Race: _____

Evaluation score: ☐IGA _____

☐SCORAD _____

☐EASI _____

☐Other score _____

Comorbidities:_____

III. Interventions and comparisons

Treatment for AD: ☐Topical therapy _____

☐Phototherapy _____

☐Systemic treatment _____

Period of treatment: _____

Follow-up time: _____

Comparison of skin microbiota:

☐Before *v.s.* after treatment _____

☐Treatment *v.s.* placebo _____

☐Comparison between different types of treatment _____

IV. Outcomes

Skin microbiota sample collection method: _____

Evaluation method of skin microbiota:

☐16S rRNA gene sequencing _____

☐Metagenomic sequencing _____

☐Viral sequencing _____

Indexes used for alpha diversity:

☐Shannon ☐Chao 1 ☐Observed species

☐Simpson ☐Pielou evenness ☐Sobs index

☐Other _____

Major findings about alpha diversity:

_____

Method used for beta diversity: _____

Major findings about beta diversity:

_____

Major findings about differential microbes:

☐Phylum_____

☐Class _____

☐Order _____

☐Family _____

☐Genus _____

☐Species _____

Method used for microbiota function:

☐PICRUSt ☐PICRUSt2 ☐Tax4Fun

☐Other _____

Major findings about microbiota functions:

_____

Major findings about microbial interactions:

_____

V. Information of data extraction

Reviewer name: _____

Date of data extraction: _____

Online Supplemental File 4

Risk of bias tools

Cochrane risk-of-bias tool for randomized trials (RoB 2)

Bias domain and signaling question	Response options		
	Lower risk of bias	**Higher risk of bias**	**Other**
1. Bias arising from the randomization process			
1.1 Was the allocation sequence random?	Yes/ Probably Yes	No/ Probably No	No Information
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Yes/ Probably Yes	No/ Probably No	No Information
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	No/ Probably No	Yes/ Probably Yes	No Information
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias arising from the randomization process?			
2. Bias due to deviations from intended interventions			
2.1 Were participants aware of their assigned intervention during the trial?	No/ Probably No	Yes/ Probably Yes	No Information
2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	No/ Probably No	Yes/ Probably Yes	No Information

2.3 If Yes/ Probably Yes/ No Information to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	No/ Probably No	Yes/ Probably Yes	No Information/ Not Applicable
2.4 If Yes/ Probably Yes/ No Information to 2.3: Were these deviations likely to have affected the outcome?	No/ Probably No	Yes/ Probably Yes	No Information/ Not Applicable
2.5 If Yes/ Probably Yes to 2.4: Were these deviations from intended intervention balanced between groups?	Yes/ Probably Yes	No/ Probably No	No Information/ Not Applicable
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Yes/ Probably Yes	No/ Probably No	No Information
2.7 If No/ Probably No/ No Information to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyses participants in the group to which they were randomized?	No/ Probably No	Yes/ Probably Yes	No Information/ Not Applicable
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias due to deviations from intended interventions?			
3. Bias due to missing outcome data			
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Yes/ Probably Yes	No/ Probably No	No Information
3.2 If No/ Probably No/ No Information to 3.1: Is there evidence that the result was not biased by missing outcome data?	Yes/ Probably Yes	No/ Probably No	Not Applicable
3.3 If No/ Probably No to 3.2: Could missingness in the outcome depend on its true value?	No/ Probably No	Yes/ Probably Yes	No Information/ Not Applicable
3.4 If Yes/ Probably Yes/ No Information to 3.3: Is it likely that missingness in the outcome depended on its true value?	No/ Probably No	Yes/ Probably Yes	No Information/ Not Applicable
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias due to missing outcome data?			
4. Bias in measurement of the outcome			

4.1 Was the method of measuring the outcome inappropriate?	No/ Probably No	Yes/ Probably Yes	No Information
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	No/ Probably No	Yes/ Probably Yes	No Information
4.3 If No/ Probably No/ No Information to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	No/ Probably No	Yes/ Probably Yes	No Information
4.4 If Yes/ Probably Yes/ No Information to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	No/ Probably No	Yes/ Probably Yes	No Information/ Not Applicable
4.5 If Yes/ Probably Yes/ No Information to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	No/ Probably No	Yes/ Probably Yes	No Information
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias in measurement of the outcome?			
5. Bias in selection of the reported result			
5.1 Were the data that produced this result analyzed in accordance with a prespecified analysis plan that was finalized before unblinded outcome data were available for analysis?	Yes/ Probably Yes	No/ Probably No	No Information
Is the numerical result being assessed likely to have been selected, on the basis of the results, from:			
5.2 ... multiple eligible outcome measurements (eg, scales, definitions, time points) within the outcome domain?	No/ Probably No	Yes/ Probably Yes	No Information
5.3 ... multiple eligible analyses of the data?	No/ Probably No	Yes/ Probably Yes	No Information
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction bias due to selection of the reported results?			
6. Overall bias			
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the overall predicted direction of bias for this outcome?			

Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I)

Major Components	Response options			
Part 1: Bias due to confounding				
1.1 Is there potential for confounding of the effect of intervention in this study? If No/ Probably No to 1.1: the study can be considered to be at low risk of bias due to confounding and no further signaling questions need be considered If Yes/ Probably Yes to 1.1: determine whether there is a need to assess time-varying confounding:	Yes/ Probably Yes	No/ Probably No		
1.2. Was the analysis based on splitting participants' follow up time according to intervention received? If No/ Probably No, answer questions relating to baseline confounding (1.4 to 1.6) If Yes/ Probably Yes, go to question 1.3.	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? If No/ Probably No, answer questions relating to baseline confounding (1.4 to 1.6) If Yes/ Probably Yes, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
Questions relating to baseline confounding only (1.4 to 1.6)				

1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
1.5. If Yes/ Probably Yes to 1.4: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
Questions relating to baseline and time-varying confounding (1.7to 1.8)				
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
1.8. If Yes/ Probably Yes to 1.7: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information			
Optional: What is the predicted direction of bias due to confounding?	Favors experimental/ Favors comparator/ Unpredictable			
Part 2: Bias in selection of participants into the study				
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? If No/ Probably No to 2.1: go to 2.4	Yes/ Probably Yes	No/ Probably No	No Information	
2.2. If Yes/ Probably Yes to 2.1: Were the post-intervention variables that influenced selection likely to be associated with intervention?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
2.3 If Yes/ Probably Yes to 2.2: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable

2.4. Do start of follow-up and start of intervention coincide for most participants?	Yes/ Probably Yes	No/ Probably No	No Information	
2.5. If Yes/ Probably Yes to 2.2 and 2.3, or No/ Probably No to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information			
Optional: What is the predicted direction of bias due to selection of participants into the study?	Favors experimental/ Favors comparator/ Towards null/ Away from null/ Unpredictable			
Part 3: Bias in classification of interventions				
3.1 Were intervention groups clearly defined?	Yes/ Probably Yes	No/ Probably No	No Information	
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Yes/ Probably Yes	No/ Probably No	No Information	
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Yes/ Probably Yes	No/ Probably No	No Information	
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information			
Optional: What is the predicted direction of bias due to measurement of outcomes or interventions?	Favors experimental/ Favors comparator/ Towards null/ Away from null/ Unpredictable			
Part 4: Bias due to deviations from intended interventions				
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2				
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Yes/ Probably Yes	No/ Probably No	No Information	

4.2. If Yes/ Probably Yes to 4.1: Were these deviations from intended intervention unbalanced between groups and likely to have affected the outcome?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6				
4.3. Were important co-interventions balanced across intervention groups?	Yes/ Probably Yes	No/ Probably No	No Information	
4.4. Was the intervention implemented successfully for most participants?	Yes/ Probably Yes	No/ Probably No	No Information	
4.5. Did study participants adhere to the assigned intervention regimen?	Yes/ Probably Yes	No/ Probably No	No Information	
4.6. If No/ Probably No to 4.3, 4.4 or 4.5: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information			
Optional: What is the predicted direction of bias due to deviations from the intended interventions?	Favors experimental/ Favors comparator/ Towards null/ Away from null/ Unpredictable			
Part 5: Bias due to missing data				
5.1 Were outcome data available for all, or nearly all, participants?	Yes/ Probably Yes	No/ Probably No	No Information	
5.2 Were participants excluded due to missing data on intervention status?	Yes/ Probably Yes	No/ Probably No	No Information	
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Yes/ Probably Yes	No/ Probably No	No Information	
5.4 If No/ Probably No to 5.1, or Yes/ Probably Yes to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	Yes/ Probably Yes	No/ Probably No	No Information	Not Applicable
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information			

Optional: What is the predicted direction of bias due to missing data?	Favors experimental/ Favors comparator/ Towards null/ Away from null/ Unpredictable			
Part 6: Bias in measurement of outcomes				
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Yes/ Probably Yes	No/ Probably No	No Information	
6.2 Were outcome assessors aware of the intervention received by study participants?	Yes/ Probably Yes	No/ Probably No	No Information	
6.3 Were the methods of outcome assessment comparable across intervention groups?	Yes/ Probably Yes	No/ Probably No	No Information	
6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Yes/ Probably Yes	No/ Probably No	No Information	
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information			
Optional: What is the predicted direction of bias due to measurement of outcomes?	Favors experimental/ Favors comparator/ Towards null/ Away from null/ Unpredictable			
Part 7: Bias in selection of the reported result				
Is the reported effect estimate likely to be selected, on the basis of the results, from...				
7.1. ... multiple outcome measurements within the outcome domain?	Yes/ Probably Yes	No/ Probably No	No Information	
7.2 ... multiple analyses of the intervention-outcome relationship?	Yes/ Probably Yes	No/ Probably No	No Information	
7.3 ... different subgroups?	Yes/ Probably Yes	No/ Probably No	No Information	
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information			
Optional: What is the predicted direction of bias due to selection of the reported result?	Favors experimental/ Favors comparator/ Towards null/ Away from null/ Unpredictable			

Overall bias	
Risk of bias judgement:	Low risk of bias/ Moderate risk of bias/ Serious risk of bias/ Critical risk of bias/ No information
Optional: What is the overall predicted direction of bias for this outcome?	Favors experimental/ Favors comparator/ Towards null/ Away from null/ Unpredictable

Methodological index for non-randomized studies (MINORS)*

Major Components	Response options		
1. A clearly stated aim	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
2. Inclusion of consecutive patients	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
3. Prospective collection of data	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
4. Endpoints appropriate to the aim of the study	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
5. Unbiased assessment of the study endpoint	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
6. Follow-up period appropriate to the aim of the study	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
7. Loss to follow up less than 5%	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
8. Prospective calculation of the study size	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
9. An adequate control group	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
10. Contemporary groups	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
11. Baseline equivalence of groups	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
12. Adequate statistical analyses	Not reported (0 point)	Reported but inadequate (1 point)	Reported and adequate (2 point)
Total score			

*The first eight apply to both non-comparative and comparative studies, while the remaining four relate only to studies with two or more groups. The global ideal score being 16 for non-comparative studies and 24 for comparative studies.

Online Supplemental File 5

Summary Tables

I. Characteristics of the included studies

Study	Region	Study design	Sample size	Evaluation method of microbiota
Study 1 (ref)	XX	XX	XX	XX
Study 2 (ref)	XX	XX	XX	XX
…	…	…	…	…

II. Major findings of the included studies on diversity (primary outcomes)

Study	Change of alpha diversity after treatment	Change of beta diversity after treatment
Study 1 (ref)	XX	XX
Study 2 (ref)	XX	XX
…	…	…

III. Major findings of the included studies on treatment-related differential microbes (primary outcomes)

Kingdom	Phylum	Class	Order	Family	Genus	Species
XX (ref)	XX (ref)	XX (ref)	XX (ref)	XX (ref)	XX (ref)	XX (ref)
XX (ref)	XX (ref)	XX (ref)	XX (ref)	XX (ref)	XX (ref)	XX (ref)
…	…	…	…	…	…	…

IV. Major findings of microbiota functions and microbial interactions (secondary outcomes)

Study	Change of microbiota functions after treatment	Change of microbial interactions after treatment
Study 1 (ref)	XX	XX
Study 2 (ref)	XX	XX
…	…	…