



BMJ Open 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.

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,

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.

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. dermatitis* 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 States¹⁸ 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-IJ 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;

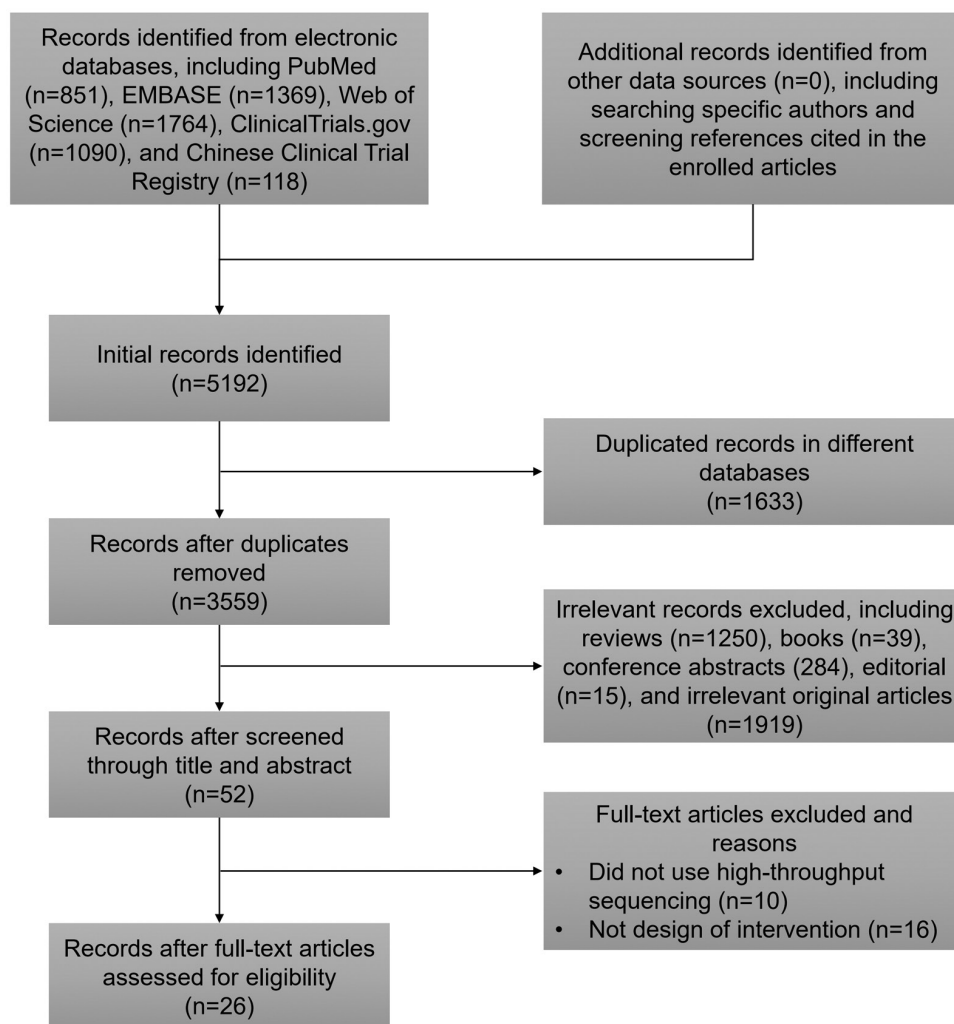


Figure 1 PRISMA flow diagram for study selection. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

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-IJ 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 tool²¹ 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 tool²² 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-IJ 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.

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Data availability statement Data are available upon reasonable request.

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