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REVIEW ARTICLE

# Effects of probiotic supplementation on natural killer cell function in healthy elderly individuals: a meta-analysis of randomized controlled trials

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## Abstract

To evaluate evidence for the role of probiotic supplementation in enhancing natural killer (NK) cell function in healthy elderly individuals. Five electronic databases were searched, and references of included articles and eligible reviews up to December 2019, with English language and human subject restrictions, were examined. Two independent reviewers identified randomized control trials (RCTs) of probiotic supplementation influencing NK cell function in healthy elderly individuals, assessed the quality of every article, and extracted data for subsequent meta-analysis. We identified six eligible trials including 364 healthy elderly subjects. Trials were heterogeneous in study design and probiotic supplementation (including genus, strain, dose, and duration). Five trials used *Lactobacillus* interventions alone or in combination with *Bifidobacterium*. Only one trial focused on *Bacillus coagulans*. The duration of supplementation ranged from 3 to 12 weeks, and the doses, from $1 \times 10^9$ to $4 \times 10^{10}$ colony-forming units. Pooling data of eligible trials showed that probiotics significantly ($P < 0.05$) increased NK cell activity in healthy elderly individuals (standardized mean difference = 0.777, 95% confidence interval: 0.187–1.366, $P = 0.01$, $I^2 = 84.6\%$). Although we obtained a significant outcome, the data do not provide convincing evidence for associations between probiotic supplementation and enhancement of NK cell function, given the small final number and very large heterogeneity. More RCTs with sufficient sample sizes and long-term follow-up are needed to focus on optimal probiotic dose, species, and duration of supplementation for healthy elderly individuals.

## Introduction

The global aging population is growing very rapidly, with the number of elderly individuals expected to grow by more than 60% over the next 15 years [1]. Aging is accompanied by a decline in immune efficacy [2]. The natural decline of adaptive and innate immunity over time is termed immunosenescence [3, 4], and results in increased vulnerability to infections, diminished responses to vaccination, and susceptibility to age-related systemic chronic inflammation [2, 5]. For example, in December 2019, a novel coronavirus-borne pneumonia occurred in Wuhan, PR China [6–8], and rapidly swept over the world. Studies have established that age and the associated decline in immune function are associated with the disease's severity, ICU enrollment, and mortality [9, 10].

Innate immunity, which represents the first line of defense against pathogens, is known to suffer from age-related changes [4, 11]. Natural killer (NK) cells are an important component of the innate immune system and are

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involved in the elimination of virus-infected cells and tumor cells. They also play a key role in regulating the immune response by producing chemokines and cytokines, which can activate other types of cells associated with both the adaptive and innate immune systems [12–14].

Immunosenescence is greatly influenced by the gut microbiota [15], as gut commensal bacteria influence immune development and function [16]. In this sense, probiotics—living microbes that exert beneficial health effects when administered to a host [17]—have the potential to help elderly individuals maintain immune cells and function [18] and have also been found to improve NK cell function in healthy elderly subjects [19, 20]. A previous meta-analysis of 14 prospective controlled studies showed that probiotic supplementation in healthy elderly individuals increased NK cell activity [19]. However, this meta-analysis included several types of prospective control studies, including six before-after studies and eight randomized control trials (RCTs). Among the eight RCTs, one study focused on immunocompromised elderly subjects, one combined probiotic and prebiotics, and one used heat-killed *Lactobacillus gasseri*, which is not a true probiotic. Considering the study design, which included a variety of subject types and interventions, the resulting clinical heterogeneity made it difficult to draw a conclusion from the results. A more recent RCT was published in 2019 [21]; therefore, we conducted this updated systematic review with a meta-analysis of prospective RCTs to evaluate the effects of probiotics in enhancing NK cell function in healthy elderly individuals.

## Materials and methods

This review was performed in accordance with the PRISMA statement [22].

### Search methods for study identification

The following databases were used in the review: Medline, Embase, Web of Science, The Cochrane Library, and Google Scholar. The search included the following terms: ("Immunity", "Natural killer cell", "NK cell", or "Tumoricidal") and ("Probiotics", "Yogurt", "Lactobacillus", "Fermented milk", "Bifidobacterial", "Nissle", "VSL#3", "HN019" or "HN001") up to December 2019. English language and human subjects were also restrictions used in the search.

We reviewed the references of the included articles and relevant reviews. In addition, we used unpublished and ongoing trials registered in the International Clinical Trials Registry Platform. We attempted to contact the study investigators for more information or data on trials.

### Criteria for considering studies

This meta-analysis matched the following five criteria: (1) participants: healthy elderly (≥65 years old), excluding adults, children, pregnant women, athletes, people under psychological stress, inpatients, and immunocompromised elderly subjects. (2) Interventions: probiotics (any strain or dose), excluding prebiotics, synbiotics, and heat-killed probiotics. (3) Comparisons: placebo control group, excluding prebiotics and synbiotics or other probiotic control groups. (4) Outcome: NK cell activity; secondary outcome: cytokines and chemokines secreted by NK cells including interferon γ (IFN-γ), granulocyte macrophagocyte colony stimulating factor (GMCSF), interleukin (IL)-5, IL-13, macrophage inflammatory protein-1 (MIP-1), and regulated upon activation normal T cell expressed and secreted factor (RANTES) [23–25]. (5) Study design: human randomized controlled study (both crossover and parallel group study), excluding observation and semi-randomized studies, as well as in vitro and animal studies.

### Data extraction and management

Two reviewers (GQF and WAG) independently reviewed the titles and abstracts for all retrieved literature and excluded all irrelevant studies. The same two reviewers independently evaluated eligible studies to identify analysis data based on full-text review; a third reviewer (TZJ) resolved any differences [26].

The two reviewers also independently extracted data from all selected studies using a standardized reporting form. We recorded information for both the probiotic and control groups, including the first author, publication time, region, age, study design, sample size, probiotic used (genus, strain, dose, and duration), and NK cell marker/effect. Any disagreement between the two reviewers was resolved through discussion and a third reviewer (TZJ).

### Assessment of bias in included studies

Both reviewers (GQF and WAG) independently assessed the risk of bias for each study using the "Risk of Bias" tool, following the Cochrane Handbook for Systematic Reviews of Interventions (version 6, http://handbook.cochrane.org). The third reviewer (TZJ) resolved disagreements. Bias was assessed on selection (randomization, allocation concealment), performance (blinding of participants and personnel), detection (blinding of outcome assessment), attrition (incomplete outcome data), reporting (selective reporting), and others. Each criterion was categorized as "yes" (low risk of bias), "unclear" (unclear risk of bias), or "no" (high risk of bias)." We presented our assessment of risk of bias using two summary figures: (1) a summary of bias for each

item across all included trials, and (2) a summary of each risk of bias item for each included trial.

### Data synthesis and analysis

Meta-analyses were performed by Review Manager Version 5.3 (The Cochrane Collaboration, Oxford, UK) and STATA software Version 15.1 (Stata, College Station, TX, USA). Review Manager Version 5.3 was used for assessing the risk of bias for the included trials and to prepare the risk of bias chart. All statistical analyses were conducted using STATA 15.1. For continuous outcomes, standardized mean difference (SMD) was calculated using Cohen statistics and 95% confidence intervals (CI) for each study. Heterogeneity was examined using $I^2$ statistics, and we explored potential sources of heterogeneity using subgroup and sensitivity analyses. A fixed-effect model was estimated when $I^2$ values of less than 50% were present. Otherwise, a random effects model was estimated. Publication bias was assessed by Begg's test ($P < 0.05$ was considered significant). All analyses used two-sided tests, and $P < 0.05$ was considered statistically significant.

## Results

### Description of studies

Our search for studies involving probiotic supplementation identified 648 articles, of which 146 duplicated studies were excluded. The remaining 502 articles went through title and abstract screening, resulting in the exclusion of further 435 articles. A total of 67 articles were selected and assessed in full; 61 articles were excluded after full-text reading for the following reasons: not RCTs, participants were not elderly or their age was unknown, multiple probiotic doses were administered, the study had no placebo control group, participants were not healthy elderly, experiments were performed in vitro or using animal models, or dead probiotics were used. Finally, six articles were included in the review for meta-analysis [21, 27–31] (Fig. 1).

### Basic characteristics of the selected studies

A total of 364 subjects were enrolled in the studies described in the six selected articles. Three studies were conducted in Western countries [21, 28, 31] and the remaining three in Eastern countries [27, 29, 30]. Three trials were crossover studies [27, 28, 31], whereas the others were parallel group studies [21, 29, 30]. The probiotic interventions were primarily *Lactobacillus*-based, either used alone (three of six studies) [27, 29, 31] or combined with *Bifidobacterium* (two of six studies) [21, 30], with only one study primarily using *Bacillus coagulans* [28]. Daily probiotic dosages ranged from $1 \times 10^9$ to $4 \times 10^{10}$ colony-forming units (CFU), and the duration of treatments ranged from 3 to 12 weeks. NK cell activity was reported in all studies [21, 27–31], but in one study, the assay result was processed [28], and thus excluded from our meta-analysis evaluating NK cell function. IFN- γ was also assessed in three studies [27, 30, 31], but the methods used for its determination of were different. Only a single study reported GMCSF, MIP-1a, MIP-1b, and RANTES levels [31]; thus, we could not perform a meta-analysis of these cytokines (Table 1).

### Risk of bias in included studies

The analyses associated with the quality of each study and risk of bias are described in Figs. 2 and 3. Four studies did not mention any method of randomization [21, 27–29], and all but one study mentioned a method of allocation concealment [31]. Three studies had a high risk of bias because study participants were not blinded to either the intervention or placebo groups [29–31]. In two studies, an imbalance in either the number of or reasons for missing data between experimental and control groups was observed [21, 28]. Finally, three authors did not publish all of the targeted and measured outcomes [28, 29, 31].

### Effects of interventions

The result of the meta-analysis on NK cell activity is displayed in Fig. 4. The higher NK cell activity observed in the probiotic group in comparison with the control group was deemed to be statistically significant. The pooled SMD of NK cell activity was 0.777 (95% CI: 0.187–1.366, $P = 0.01$, random Cohen), exhibiting a significant favorable result for probiotic supplementation (Fig. 4). A significant heterogeneity in NK cell activity was found among these studies ($I^2 = 84.6\%$, $P < 0.001$). Begg's test for publication bias was not evident ($P = 0.806$, Fig. 5).

## Discussion

In this meta-analysis of RCTs, we found evidence that supports a favorable association between probiotic supplementation in healthy elderly individuals and increasing NK cell activity. Our results are similar to those of previous meta-analysis studies [19, 20]. In 2017, Miller et al. [20] found that *B. lactis* HN019 could enhance NK cell function in healthy elderly individuals. In 2019, another meta-analysis showed that short-term probiotic supplementation can also enhance NK cell function in healthy elderly individuals [19]. Among the cytokines and chemokines secreted

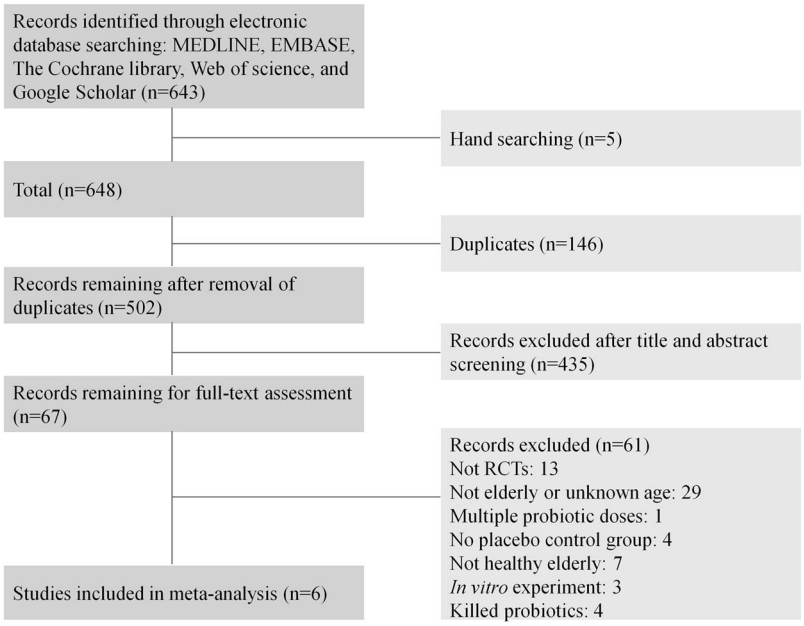


**Fig. 1** Flow diagram of the literature search process.

by NK cells, IFN- γ, GMCSF, MIP-1, and RANTES were reported in these RCTs; however, we could not conduct a meta-analysis of these cytokines owing to methodological heterogeneity and an insufficient number of studies.

Unlike two previously published meta-analyses [19, 20], which included prospective controlled studies (including RCTs and before-after studies) and cellular immune function (including polymorphonuclear cell phagocytic capacity and NK cell function), our meta-analysis is the first to only address RCTs and markers of NK cell function. Our decision to focus on NK cell function and RCTs was based on two major considerations. First, choosing RCTs only, and not all prospective control studies, reduces clinical confounding and bias, allowing us to obtain more convincing results for clinical outcomes. Second, NK cell function is an important component of innate immunity and plays a key role in the immune system of elderly individuals [32–34], and there was a sufficient number of RCTs published in the recent years for performing a meta-analysis [21, 27–31]. However, polymorphonuclear cell phagocytic capacity, which represents another important component of innate immunity [4, 19, 20], is not suitable for meta-analysis as only two RCTs [31, 35] have been published on this subject, providing an insufficient amount of data.

Immune function decline affects the quality of life and the life span of elderly individuals. Among cells of the immune system, NK cells are separate effector lymphocytes with both cytotoxicity and cytokine-producing effector functions [12, 36]. In humans, NK cells appear to play an essential role in viral infection, cancers, transplantation, autoimmunological disorders, and pregnancy [37]. NK cells readily produce IFN-γ [12], but also produce other cytokines and chemokines, including GMCSF, IL-5, IL-13, MIP-1, and RANTES [23–25]. In this review, we not only determined NK cell function, but also determined the presence of cytokines and chemokines.

A decrease in the diversity and stability of gut microbiota in the elderly can influence chronic inflammation and age-related disorders [38]. Aging represents a major factor impacting the composition and activity of the gut microbiota, which is important for maintenance of good health and immune system function [39]. Finally, the stability of gut microbiota is critical to maintaining the integrity of the intestinal epithelial barrier and immunological homeostasis [39].

The relationship between gut microbiota dysbiosis and immune function decline provides a solid theoretical basis for the application of probiotics in order to slow immunosenescence. Probiotics are known to be beneficial in improving immune function in both human elderly subjects [40–43] and aging animal models [44–46]. The mechanism by which probiotics enhance NK cell function has been revealed in previous researches [47–50]. One study [49] suggested that NK cell activation is exopolysaccharide-dependent, occurs via IL-18- and IL-12-mediated IFN-γ production, and requires myeloid differentiation factor 88. Similar results were reported by another study, where it was shown that lactic acid bacteria can elicit NK cell activities via IL-12 induction [50]. Similarly, dendritic cells play a vital role in activating NK cells [47, 48]. In addition, direct stimulation of NK cells by probiotics has been reported [48].

Although we have not observed a change in IFN-γ levels, significant increases in IFN-γ after probiotic use have been

**Table 1** Characteristics of the included trials.

| Ref. | Region | Study design | Sample size (probiotic: control) | Age (mean, range) | Probiotics (genus, strain, dose, and duration) | NK cell marker/effect |
|---|---|---|---|---|---|---|
| Dong et al. [31] | UK | Crossover | 30 | –(55–74) | Low-fat milk with *L. casei Shirota*, $1.3 \times 10^{10}$ cfu for 4 weeks | NK cell activity, IFN- γ, GMCSF, MIP-1, RANTES |
| Finamore et al. [21] | Italy | Parallel group | 79 (45:34) | 84.6 (75–) | Biscuit containing *B. longum Bar33* and *L. helveticus Bar13*, $1 \times 10^9$ cfu for 30 days | NK cell activity |
| Lee et al. [30] | Korea | Parallel group | 152 (73:79) | 66 (60–) | Yogurt with *L. paracasei (L. casei 431)*, $12.0 \times 10^8$ cfu; *B. lactis (BB-12)*, $12.0 \times 10^8$ cfu and heat-treated *L. plantarum (nF1)*, 0.0175% for 12 weeks | NK cell activity, IFN- γ |
| Makinoet al.[a] [29] | Japan | Parallel group | 57 (29:28) | 74·5 (69–80) | Yogurt with *L. bulgaricus OLL1073R-1*, 1.8–$3.15 \times 10^{10}$ cfu for 8 weeks[a] | NK cell activity |
| Nyangale et al. [28] | UK | Crossover | 36 | –(65–80) | Capsules contained *Bacillus coagulans BC30*, $1 \times 10^9$ cfu for 4 weeks | NK cell activity |
| Takeda et al. [27] | Japan | Crossover | 10 | –(69–97) | Fermented milk with *L. casei Shirota*, $4 \times 10^{10}$ cfu for 3 weeks | NK cell activity, IFN- γ |

*CFUs* colony-forming units.

[a]Study describes the results of two separate studies: one was an RCT and the other was not; this table presents information from the RCT only.

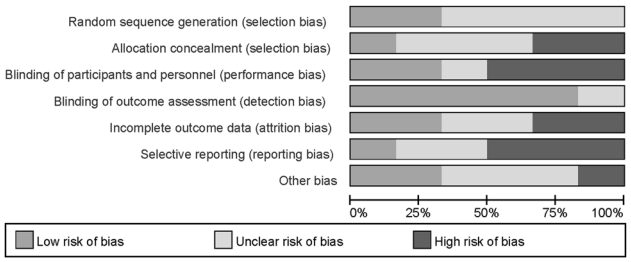


**Fig. 2 Risk of bias graph representing the review authors' judgements for each risk of bias item.** Data are presented as percentages across all included studies.

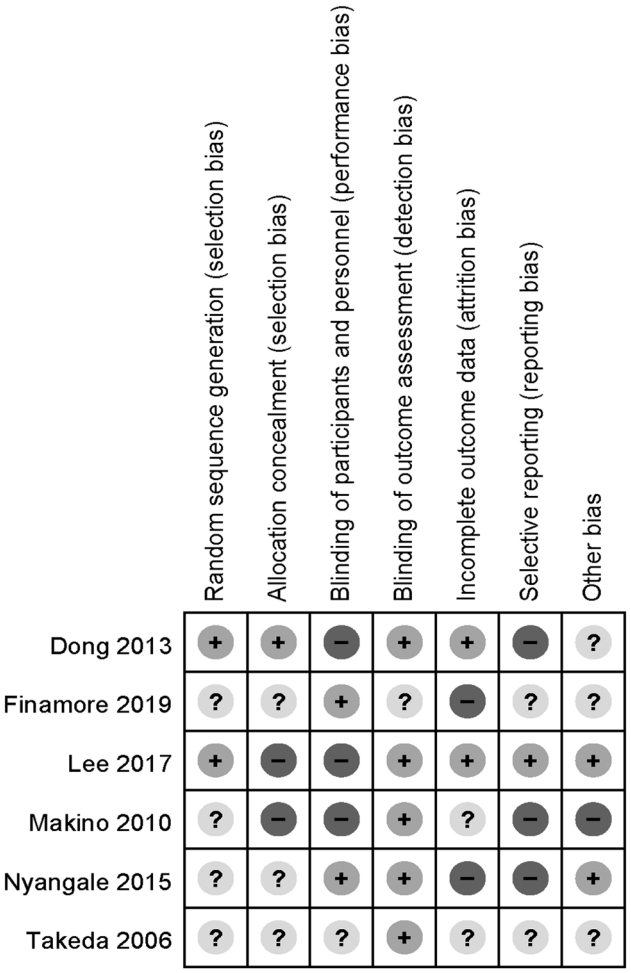


**Fig. 3 Risk of bias summary.** Review authors' judgements about each risk of bias item for each included study.

found in some of the articles included in our review [30, 31], which is consistent with previous studies that reported that NK cells secrete IFN-γ and may represent a major source of cellular IFN-γ [51]. The tumoricidal activity of NK cells is known to be involved in the production of IFN-γ [52]. Therefore, an increase in IFN-γ accompanied by an increase in NK cell tumoricidal activity after probiotic

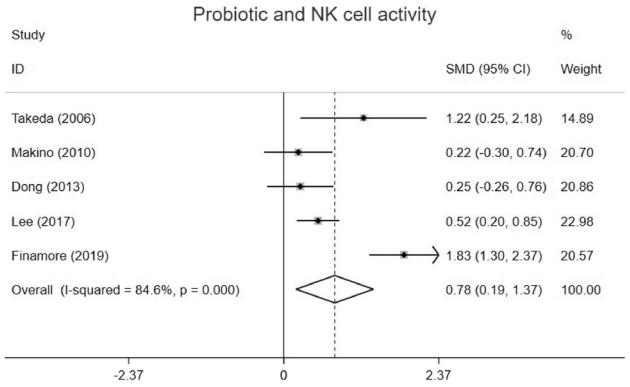


**Fig. 4 Forest plot obtained after pooling data for NK cell activity from eligible studies.** Random-effects meta-analysis was performed using SMD statistics. ID identification, NK cell natural killer cell, SMD standard mean difference.

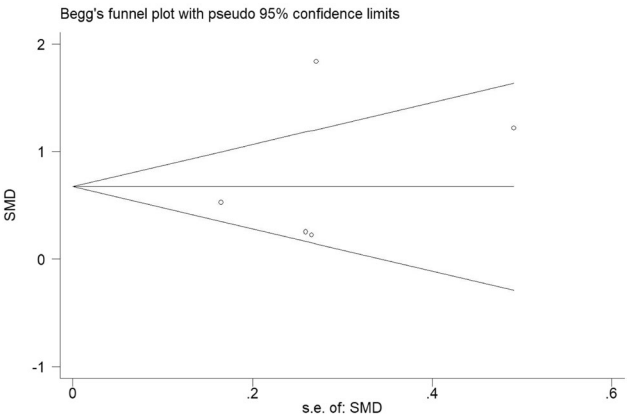


**Fig. 5 Begg's funnel plot for assessment of publication bias for NK cell activity.** SMD standard mean difference.

supplementation could contribute to immunopotentiation effects.

NK cells can control microbial infectious diseases [12, 53], and studies have shown that low NK cell activity is related to the development of infection in elderly subjects [54]. Several studies have shown that probiotics can reduce the incidence of infections in elderly subjects by augmenting NK cell activity [29] or enhancing T cell-mediated natural immune defense [55], although some authors disagree with this hypothesis [56, 57]. In our review, we have not discussed infections because only one study had reported on this issue [29], preventing a systematic statistical analysis. In addition, only short-term effects of probiotic applications were evaluated, and this study duration may not reveal any obvious influence on infection. For example, in a Japanese study, *L. casei* strain Shirota was administered to residents, staff of housing facilities, and the elderly for up to 6 months to evaluate the effectiveness in infection control, indicating the need for a long-term analysis [58].

This systematic review has several limitations. First, only six studies were included, with only five studies used to evaluate NK cell activity; this number is too small to provide robust conclusions. Second, most studies consisted of small sample sizes, which can influence the outcomes when including such studies. Third, long-term effects of probiotic use on NK cell function could not be determined in our work owing to short-term follow-ups, ranging from 3 to 12 weeks. The included studies only assessed the short-term effect of probiotic use on NK cell function, thus the long-term effects such as fighting infection and improving vaccine response could not be evaluated. Finally, probiotic strains and doses were different among the studies. For example, three probiotic interventions were primarily *Lactobacillus* alone, two combined *Lactobacillus* with *Bifidobacterium*, and one was primarily *B. coagulans*-based. A previous study has shown that the relative efficacy of probiotic supplementation may be strain-specific [59]. In addition, the doses employed were also different, ranging from $1 \times 10^9$ to $4 \times 10^{10}$ CFU. However, there were not enough studies for a subgroup meta-analysis, and we have no evidence that the effectiveness of probiotic supplementation for NK cell function in healthy elderly individuals varies according to probiotic genus and dose.

In conclusion, only RCTs were included in our meta-analysis for investigating the effect of probiotic use on NK cell function in healthy elderly individuals. These studies represent the highest level of evidence, according to the Cochrane Handbook. We found that short-term probiotic supplementation can in fact improve NK cell function in healthy elderly individuals. The main strength of this analysis is that it provides a compilation of the available high-quality RCTs evaluating probiotic interventions in healthy elderly individuals and gives an overview of the current clinical research. Although we obtained a significant outcome, the data still do not provide convincing evidence, given the small final number of RCTs and very large heterogeneity. Future studies on a larger scale, including long-term follow-up and a multicenter investigation, are still required to establish a more robust conclusion.

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**Author contributions** Conceptualization: YY, CX. Literature search: QG, AW. Data collection: QG, AW, ZT. Analysis and interpretation of

data: XZ, SH. Writing—original draft: QG. Writing—review and editing: YY, CX.

## Compliance with ethical standards

**Conflict of interest** CX is the first corresponding author of this work. YY is the second corresponding author of this work. All the authors reviewed and approved the submitted version of the paper. The authors declare that they have no conflict of interest.

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