BMJ Open

Application of probiotics, prebiotics and synbiotics in patients with breast cancer: a systematic review and meta-analysis protocol for randomised controlled trials

Dan Duan ,[1,2] Maojun Chen,[1,2] Wenyao Cui,[1,2] Wenjie Liu,[1,2] Xinrong Chen[2]

To cite: Duan D, Chen M, Cui W, *et al*. Application of probiotics, prebiotics and synbiotics in patients with breast cancer: a systematic review and meta-analysis protocol for randomised controlled trials. *BMJ Open* 2022;**12**:e064417. doi:10.1136/bmjopen-2022-064417

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-064417).

Received 02 May 2022
Accepted 21 October 2022

[1]Department of Neurosurgery, Sichuan University West China Hospital, Chengdu, Sichuan, China
[2]West China School of Nursing, Sichuan University, Chengdu, Sichuan, China

Correspondence to
Ms Maojun Chen;
chenmaojunlh@163.com

ABSTRACT

Introduction Breast cancer has become a common tumour that threatens women's physical and mental health. Microbial agents play an important role in maintaining the balance of gut microbiota and modulating intestinal immunity, anti-inflammatory and antioxidant effects. Available evidence points to a strong association between them and breast cancer. However, there has been no systematic review of the effects of microbial agents in patients with breast cancer. This protocol aims to explore the effectiveness and safety of probiotics, prebiotics and synbiotics in patients with breast cancer.

Methods and analysis We will search the following electronic databases for relevant randomised controlled trials: PubMed, EMBASE, Cochrane Library and Web of Science. Grey literature and reference lists of original studies will also be searched to avoid omissions. We will use the Cochrane Collaboration's Risk of Bias tool to assess the quality of the included studies. The primary outcomes include patients' arm oedema volume, changes in gut microbiota composition and anthropometric parameters. Two independent reviewers will perform literature screening, data extraction and risk of bias assessment. Data synthesis will be performed using descriptive analysis or meta-analysis. The quality of the evidence for each outcome will be assessed using the Grading of Recommendations Assessment, Development and Evaluation tool.

Ethics and dissemination The data for systematic reviews are derived from published original studies and do not require review and approval by the ethics committee. The results will be disseminated through a peer-reviewed journal and conferences.

PROSPERO registration number CRD42022311502.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This protocol will strictly follow the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols guidelines.
⇒ This protocol will be conducted in strict accordance with the recommendations of the Cochrane Handbook.
⇒ In order to provide a comprehensive analysis of the research results as possible, grey literature will be searched for this study.
⇒ Study heterogeneity may affect pooled effects.

INTRODUCTION

The 2020 global cancer burden data show that there are approximately 19.3 million new cancer cases and more than 9.95 million deaths worldwide, including more than 2.26 million new breast cancer cases and more than 680 000 deaths, accounting for 11.7% and 6.9% of all cancer cases, respectively.[1] The incidence of breast cancer has surpassed that of lung cancer for the first time and ranks as the top cancer in the world. In addition, among women, breast cancer ranks first in the incidence and mortality of most countries in the world, accounting for 24.5% of new cancer cases and 15.5% of deaths among women, respectively.[1] In recent years, the incidence of breast cancer in the USA has shown an upward trend, with an annual increase in approximately 0.5%.[2] Breast cancer has become a common tumour that threatens women's physical and mental health. Although studies have reported a declining trend in breast cancer mortality, the rate of decline has slowed in recent years, and it remains the leading cause of cancer death in women aged 20–59.[2] According to a survey of 195 countries and regions, breast cancer has a disability-adjusted life-year of 17.7 million, and it has become one of the most serious cancer burdens in the world.[3] Therefore, new prevention and treatment strategies are needed to alleviate the burden of breast cancer.

Cancer is one of the leading causes of death worldwide. It has been reported that 20% of cancers are closely associated with gut microbes.[4] The gut microbiota is involved in many areas of human health, including providing nutrients, participating in metabolism, defending against pathogens and promoting the development of the immune

system and epithelial mucosal homeostasis.[5] It has been noted that gut microbes play an important and decisive role in health or pathological states, including cancer.[6] They are involved in cancer occurrence, progression and spread by regulating inflammation as well as immune and cellular responses.[7] Probiotics are live microorganisms that are beneficial to host health when ingested in a certain amount.[8] Prebiotics are substrates selectively used by host microorganisms to induce the growth and activity of beneficial microorganisms with health benefits.[8] Synbiotics are combinations of prebiotics and probiotics that have a synergistic effect on the survival and growth of probiotics.[9] Recently, there has been increasing interest in the potential role of these microbial agents in altering the gastrointestinal microbiota to promote health.

In the field of breast cancer, available evidence suggests a strong association between microbial agents and breast cancer. Lifestyles such as dietary habits and obesity have been shown to be modifiable components that may influence the development of breast cancer. Diet is also an influential factor in gut microbial diversity. Despite significant progress in breast cancer treatment, patients may still experience problems such as arm lymphedema and decreased quality of life after surgery. Patients under chemotherapy may also suffer from side effects such as diarrhoea, nausea and vomiting. Microbial agents are generally considered safe, and appropriate supplementation may be beneficial in the treatment of breast cancer. In human studies, previous studies have suggested that the microbiota of breast cancer patients are different from that of healthy people and that the diversity and composition of the gut microbiota in patients with breast cancer is less diverse.[10] A case–control study investigated the relationship between probiotic intake and the risk of breast cancer and found that daily probiotic supplementation from adolescence was negatively associated with the incidence of breast cancer.[11] Researchers believe that inflammation is one of the main risk factors for lymphedema in patients with breast cancer, and collateral lymphatic vessels are regulated by inflammatory cytokines and growth factors.[12] Synbiotics can modulate the gut microbiota, inhibit the production of proinflammatory cytokines and cell proliferation and reduce the volume of oedema by exerting their anti-inflammatory effects.[13] Besides, synbiotics may enhance the activity of antioxidant enzymes in patients with breast cancer and exert their cytotoxic effects, thus potentially ameliorating the physical and functional impairments associated with lymphedema.[14] On the other hand, in vitro cell experiments have shown that probiotics can induce breast cancer cell apoptosis, thus showing cytotoxicity and ultimately inhibiting the growth of breast cancer cells.[15] Animal experiments have also shown the benefits of probiotics for breast cancer prevention and treatment. Probiotics inhibit breast tumour cell growth and reduce tumour volume mainly through their immunomodulatory effects.[15] A recent study reported that probiotics can reduce the adverse reactions of chemotherapy drugs while maintaining the anticancer effect of capecitabine.[16] Therefore, these microbial agents can provide new ideas for anticancer therapy or adjuvant therapy of breast cancer.

To date, several clinical trials have investigated the effects of probiotics, prebiotics or synbiotics on many outcomes in patients with breast cancer, including gut microbiota, lymphedema and anthropometric and metabolic parameters. However, to the best of our knowledge, there are currently no relevant systematic reviews. In addition, existing studies are inconsistent in their conclusions about the effects of interventions. For example, a

Table 1 Parameters associated with primary and secondary outcomes

Primary outcomes	
Arm oedema volume Changes in gut microbiota composition Anthropometric parameters (weight, body mass index (BMI), waist circumference, etc.)	
Secondary outcomes	
Laboratory indicators	Inflammatory markers[24 25]: tumour necrosis factor-α (TNF-α), high-sensitivity C reactive protein (hs-CRP), interleukin-1β (IL-1β) and interleukin-6 (IL-6)
	Oxidative markers[26]: serum total antioxidant capacity (TAC), malondialdehyde (MDA), glutathione peroxidase (GPx) and superoxide dismutase (SOD)
	Sex hormones[18]: estradiol, testosterone and dehydroepiandrosterone sulfate (DHEA-S)
	Blood glucose parameters[17 18]: fasting glucose, serum insulin, insulin resistance (HOMA-IR), glycated haemoglobin (HbA1c)
Psychological function	Anxiety[25]: measured by Self-Rating Anxiety Scale (SAS), Hamilton Anxiety Rating Scale (HAMA), Beck Anxiety Inventory (BAI) or other validated scales.
	Depression[25]: measured by Self-Rating Depression Scale (SDS), Hamilton Depression Rating Scale (HAMD), Beck Depression Inventory (BDI) or other validated scales.
Incidence of adverse events	Such as abdominal pain, bloating, soft stools, diarrhoea, nausea, taste disorder, infection, etc.

randomised controlled trial in Italy showed that probiotics reduced fasting glucose in patients with breast cancer.[17] However, the results of Raji *et al* were not significantly different.[18] Therefore, it is necessary to conduct a comprehensive systematic review of the existing clinical practice evidence to explore the effect of probiotics, prebiotics and synbiotics in patients with breast cancer.

Objectives

To explore the effects of probiotics, prebiotics and synbiotics in patients with breast cancer by systematically reviewing, summarising and interpreting clinical randomised controlled trials (RCTs). We will attempt to answer the following questions: What is the effect of probiotics, prebiotics and synbiotics on clinical outcomes in patients with breast cancer (arm oedema volume, gut microbiota composition, anthropometric parameters, laboratory indicators, psychological function)? Do the effects of probiotics, prebiotics and synbiotics in breast cancer patients vary by intervention characteristics (eg, type of probiotic, strain, dose)?

METHODS AND ANALYSIS

Patient and public involvement

Patients and the public will not be involved in this review.

Protocol and registration

This systematic review protocol will strictly follow the Preferred Reporting Items for Systematic Review and

Table 2 Details of search strategies for PubMed

women, respe	
women, respe	"Breast Neoplasms"(MeSH Terms]
women, respe	(breast(Title/Abstract)OR mammary(Title/Abstract)) AND (cancer(Title/Abstract)OR neopla*(Title/Abstract)OR tumo*(Title/Abstract)OR carcinoma(Title/Abstract)OR malignan*(Title/Abstract)OR oncolog*(Title/Abstract))
women, respe	#1 OR #2
women, respe	"Probiotics"(MeSH Terms]
women, respe	"Prebiotics"(MeSH Terms]
women, respe	"Synbiotics"(MeSH Terms]
women, respe	"Lactobacillus"(MeSH Terms]
women, respe	"Bifidobacterium"(MeSH Terms]
women, respe	"Gastrointestinal microbiome"(MeSH Terms]
women, respe	"Saccharomyces"(MeSH Terms]
women, respe	"Escherichia"(MeSH Terms]
women, respe	"Yogurt"(MeSH Terms]
women, respe	"Cultured Milk Products"(MeSH Terms]
women, respe	probiotic*(Title/Abstract)OR prebiotic*(Title/Abstract)OR synbiotic*(Title/Abstract)OR bifidobacteria(Title/Abstract)OR lactobailli(Title/Abstract)
women, respe	gastrointestinal microb*(Title/Abstract)OR gastrointestinal microflora*(Title/Abstract)OR gastrointestinal flor*(Title/Abstract)OR gastric microb*(Title/Abstract)OR gastric microflor*(Title/Abstract)OR gastric flor*(Title/Abstract)OR gut microb*(Title/Abstract)OR gut microflor*(Title/Abstract)OR gut bacteria*(Title/Abstract)OR gut flor*(Title/Abstract)OR intestinal microb*(Title/Abstract)OR intestinal microflor*(Title/Abstract)OR intestinal flor*(Title/Abstract)OR intestinal bacteri*(Title/Abstract)OR intestine bacteri*(Title/Abstract)OR intestine microbial flor*(Title/Abstract)OR intestine microflor*(Title/Abstract)OR intestine microb* OR enteric flor*(Title/Abstract)OR enteric microb*(Title/Abstract)OR enteric microflor*(Title/Abstract)
women, respe	yoghurt(Title/Abstract)OR yeast(Title/Abstract)OR fermented milk(Title/Abstract)OR sour milk(Title/Abstract)
women, respe	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16
women, respe	randomized controlled trial [Publication Type]
women, respe	controlled clinical trial [Publication Type]
women, respe	randomized(Title/Abstract)OR randomised(Title/Abstract)OR randomly(Title/Abstract)
women, respe	placebo(Title/Abstract)
women, respe	trial(Title/Abstract)
women, respe	#18 OR #19 OR #20 OR #21 OR #22
women, respe	animals(MeSH Terms] NOT humans(MeSH Terms]
women, respe	#23 NOT #24
women, respe	#3 AND #17 AND#25

Meta-Analysis Protocols guidelines (online supplemental file 1) and the general guidelines of the Cochrane Collaboration.[19 20] We have registered this study on the PROSPERO website.

Criteria for study selection

Participants

This protocol will include patients diagnosed with breast cancer, which will include patients who have undergone surgery as well as patients who have received or are receiving radiation therapy, chemotherapy, endocrine therapy or targeted therapy, whether or not. We will not limit the age, ethnicity, clinical stage and pathological type of the patients.

Intervention

We will consider RCTs of patients with breast cancer treated with probiotics, prebiotics or synbiotics administered orally in any form (eg, drink, powder, capsule). There will be no limitation on the type, dose, frequency or duration of probiotics, prebiotics or synbiotics.

Control

The control group will be given a placebo or usual care or no intervention. Usual care refers to the standard of care that patients receive in a hospital setting.

Outcomes

The primary and secondary outcomes are shown in table 1.

Study design

RCTs are eligible for this review.

Other exclusion criteria

- Articles not in English language.
- In vitro studies or animal studies.
- The control group was healthy people or patients without breast cancer.

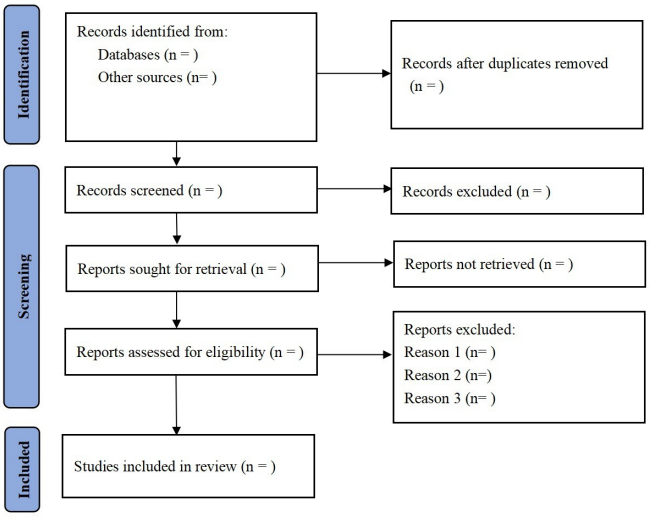

Figure 1 Flow diagram of the study screening process.

- Quasi-RCTs, controlled before-and-after trials, controlled clinical trials, crossover RCTs or cluster RCTs.
- RCTs with probiotics, prebiotics or synbiotics in both groups.
- Trials that did not report primary or secondary outcomes.

Literature searches

We will search the following electronic databases for relevant RCTs: PubMed, EMBASE, the Cochrane Central Register of Controlled Trials and Web of Science. The European Grey Literature (OpenSIGLE) Database and Google Scholar will also be searched to identify grey literature. In addition, reference lists of original studies will be manually searched to identify articles that may have been missed during the electronic search process. The search date is until 10 May 2022, and the search language will be limited to English. A combination of medical subject headings and free text terms related to breast cancer, probiotics and RCTs will be searched. The detailed search strategy in PubMed is presented in table 2.

Selection process

Literature screening will be performed independently by two reviewers, who will import all retrieved original literature into Endnote V.X9 Literature Manager. After removing duplicate literature, two reviewers will independently screen titles and abstracts, read the full text of the articles that meet the inclusion criteria and finally determine the literature to be included. For studies excluded after full-text review, we will record the number and reasons for excluded articles. Disagreements will be resolved during the literature screening stage through discussion or consultation with a third party if necessary. The process of study selection is illustrated in figure 1.

Data extraction

Data extraction will be carried out independently using predesigned standardised forms by two reviewers participating in study screening. They will extract all the data into Microsoft Excel. Data collected will include study characteristics (first author, title, year of publication, country, design), participant characteristics (number of patients per group, age, clinical stage, pathological type), intervention information (type of probiotics, strains, route of administration, dose, frequency, duration), comparative measures and outcomes (primary and secondary outcomes). If the data are incomplete or unclear, the original author will be contacted by email. Discrepancies in the data extraction process will be discussed or consulted with a third party.

Risk of bias assessment

Two authors will independently assess the risk of bias for each study using the Cochrane Collaboration's Risk of Bias tool, which assesses the following seven domains: random sequence generation, allocation concealment, blinding of participants and researchers, blinding of

outcome assessment, incomplete outcome data, selective outcome reporting and other biases.[20] Each domain is judged and classified as 'high risk', 'uncertain risk of bias' or 'low risk' for research quality. Other study members will join the discussion when the evaluation results cannot be agreed on.

Data synthesis

The protocol will plan to use RevMan V.5.4 software for data analysis. Where possible, at least two studies are required to perform a meta-analysis for each outcome measure.[21] The mean difference (MD) or standardised MD and 95% CI will be used to display continuous data. Relative risk and 95% CI will be used to show dichotomous data.

We will use the χ^2 test and the I^2 statistic to determine whether there is heterogeneity among studies. If the data are homogeneous, we plan to choose to combine effect sizes using a random effects model. When $I^2 > 50\%$ or $p<0.10$, it indicates the existence of heterogeneity. In order to explore the source of significant heterogeneity with sufficient available data, we will attempt to perform subgroup analysis and meta-regression analysis,[22] taking into account breast cancer clinical stage, sample size, type of microbial agents, route of administration, dose, duration and other factors. However, if the heterogeneity is too obvious to resolve or the number of RCTs eventually included is small, a descriptive analysis will be performed. If necessary, sensitivity analyses can be performed to test the robustness of the results by removing studies with a high risk of bias or missing data.

When more than 10 eligible trials are included in this review, we will detect publication bias by looking at funnel plot symmetry or using Egger's test.[20]

Strength of evidence

This review will plan to use the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to assess the quality of evidence for the included outcomes.[23] GRADE downgrades RCTs based on risk of bias, inconsistency, imprecision, indirectness and publication bias and classifies the quality of evidence into four grades: high, medium, low and very low. We will eventually generate a summary of the findings table and a GRADE evidence profile.

Ethics and dissemination

The data for systematic reviews are derived from published original studies and do not require review and approval by the ethics committee. The results will be disseminated through a peer-reviewed journal and conferences.

Contributors MC is the guarantor for this manuscript. DD and MC contributed to the research conception. DD, MC and WC were responsible for the research design. Research screening was completed by WC and WL. Data extraction and analysis were performed by WL and XC. The first draft was written by DD. The final manuscript was read and approved by all authors.

Funding This work was supported by the Department of Science and Technology of Sichuan Province (Grant Number 2021YFS0206). The funder was not involved in the design of the protocol.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD

Dan Duan http://orcid.org/0000-0003-1733-4974

REFERENCES

1 Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209–49.
2 Siegel RL, Miller KD, Fuchs HE, *et al*. Cancer statistics, 2022. *CA Cancer J Clin* 2022;72:7–33.
3 Li N, Deng Y, Zhou L, *et al*. Global burden of breast cancer and attributable risk factors in 195 countries and territories, from 1990 to 2017: results from the global burden of disease study 2017. *J Hematol Oncol* 2019;12:140.
4 Pevsner-Fischer M, Tuganbaev T, Meijer M, *et al*. Role of the microbiome in non-gastrointestinal cancers. *World J Clin Oncol* 2016;7:200–13.
5 Zitvogel L, Daillère R, Roberti MP, *et al*. Anticancer effects of the microbiome and its products. *Nat Rev Microbiol* 2017;15:465–78.
6 Zhang Y-J, Li S, Gan R-Y, *et al*. Impacts of gut bacteria on human health and diseases. *Int J Mol Sci* 2015;16:7493–519.
7 Lofgren JL, Whary MT, Ge Z, *et al*. Lack of commensal flora in Helicobacter pylori-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology* 2011;140:210–20.
8 Gibson GR, Hutkins R, Sanders ME, *et al*. Expert consensus document: the International scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017;14:491–502.
9 Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients* 2017;9:1021.
10 Goedert JJ, Jones G, Hua X, *et al*. Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: a population-based case-control pilot study. *J Natl Cancer Inst* 2015;107. doi:10.1093/jnci/djv147. [Epub ahead of print: 01 06 2015].
11 Toi M, Hirota S, Tomotaki A, *et al*. Probiotic beverage with soy isoflavone consumption for breast cancer prevention: a case-control study. *Curr Nutr Food Sci* 2013;9:194–200.
12 Ly CL, Kataru RP, Mehrara BJ. Inflammatory manifestations of lymphedema. *Int J Mol Sci* 2017;18:171.
13 McLoughlin RF, Berthon BS, Jensen ME, *et al*. Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. *Am J Clin Nutr* 2017;106:ajcn156265–945.
14 Vafa S, Zarrati M, Malakootinejad M, *et al*. Calorie restriction and synbiotics effect on quality of life and edema reduction in breast cancer-related lymphedema, a clinical trial. *Breast* 2020;54:37–45.
15 Ranjbar S, Seyednejad SA, Azimi H, *et al*. Emerging roles of probiotics in prevention and treatment of breast cancer: a comprehensive review of their therapeutic potential. *Nutr Cancer* 2019;71:1–12.
16 Méndez Utz VE, Pérez Visñuk D, Perdigón G, *et al*. Milk fermented by Lactobacillus casei CRL431 administered as an immune adjuvant in

models of breast cancer and metastasis under chemotherapy. *Appl Microbiol Biotechnol* 2021;105:327–40.
17 Pellegrini M, Ippolito M, Monge T, *et al*. Gut microbiota composition after diet and probiotics in overweight breast cancer survivors: a randomized open-label pilot intervention trial. *Nutrition* 2020;74:110749.
18 Raji Lahiji M, Najafi S, Janani L, *et al*. The effect of synbiotic on glycemic profile and sex hormones in overweight and obese breast cancer survivors following a weight-loss diet: a randomized, triple-blind, controlled trial. *Clin Nutr* 2021;40:394–403.
19 Moher D, Shamseer L, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4:1.
20 Higgins JPT, Thomas J, Chandler J. Cochrane Handbook for systematic reviews of interventions version 6.3 (updated February 2022), 2022. Cochrane. Available: www.training.cochrane.org/handbook
21 Bae S, Kamynina E, Guetterman HM, *et al*. Provision of folic acid for reducing arsenic toxicity in arsenic-exposed children and adults. *Cochrane Database Syst Rev* 2021;10:CD012649.
22 Cordero CP, Dans AL. Key concepts in clinical epidemiology: detecting and dealing with heterogeneity in meta-analyses. *J Clin Epidemiol* 2021;130:149–51.
23 Guyatt G, Oxman AD, Akl EA, *et al*. Grade guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol* 2011;64:383–94.
24 Vafa S, Haghighat S, Janani L, *et al*. The effects of synbiotic supplementation on serum inflammatory markers and edema volume in breast cancer survivors with lymphedema. *Excli J* 2020;19:1–15.
25 Juan Z, Chen J, Ding B, *et al*. Probiotic supplement attenuates chemotherapy-related cognitive impairment in patients with breast cancer: a randomised, double-blind, and placebo-controlled trial. *Eur J Cancer* 2022;161:10–22.
26 Navaei M, Haghighat S, Janani L, *et al*. The effects of synbiotic supplementation on antioxidant capacity and arm volumes in survivors of breast cancer-related lymphedema. *Nutr Cancer* 2020;72:62–73.

PRISMA-P checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Reported on 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	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2,7
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	15
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	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	15
Sponsor	5b	Provide name for the review funder and/or sponsor	15
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	15
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3-6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	7
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	7-9
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	10
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	10-12 Table 2

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	12
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)	12
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	12-13
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	12
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8,9
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	13
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	13,14
	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)	13,14
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	13,14
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	14
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	14
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	14

*** 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.