Role of Neutrophils to Determine the Outcome in Traumatic Spinal Cord Injury: A Systematic Review

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Abstract

Background: Based to the National Spinal Cord Injury Statistical Center (NSCISC) of the University of Alabama at Birmingham, USA, there are 15 to 40 cases of Spinal Cord Injury (SCI) per one million population each year (approximately 4,125 to 11,000 new cases) in the world. Neutrophils in SCI have two roles, namely beneficial and detrimental. These roles can be an outcome indicator in Traumatic Spinal Cord Injury, especially in the acute phase of SCI. Therefore, this study aimed to analyze neutrophil's role in SCI outcome indicators.

Methods: This systematic review used secondary data in the form of animal trial studies that were found in the last five years. Five databases were searched, including PubMed, Science Direct, Scopus, Web of Science, and Springer Link, using keywords spinal cord injury, neutrophil, inflammation, and outcome.

Results: There were six studies reviewed in which all studies explained the detrimental role of neutrophils in SCI. Five studies explained that the role could be inhibited by proinflammatory cytokines and chemokines, while four studies explained an increase in locomotor function after observation until the remodeling phase.

Conclusion: The dominant role of neutrophils in SCI is detrimental. This role can be affected by two factors that become resolutions: direct and indirect. These two factors also affect the improvement in the locomotor function of SCI.

Keywords: spinal cord injury, neutrophil, locomotor, beneficial, detrimental

1. Introduction

Spinal Cord Injury (SCI) is a disorder that results in temporary or permanent changes in motor, sensory, or autonomic functions. The most common causes of SCI are traffic accidents, sports trauma, falling from a height, and violence.[1] Traumatized spinal cords cannot regenerate. It inflicts chronic wounds that undergo expansion and persistent demyelination, disrupting the healing process and progressive tissue degeneration.[2]

The yearly incidence rate of SCI in many countries is relatively high. The National Spinal Cord Injury Statistical Centre (NSCISC) of the University of Alabama at Birmingham, USA, notes that there are 15 to 40 SCI cases per one million population (about 4,125 to 11,000 new cases) in the world. Moreover, mortality in SCI is still high. In developed countries, mortality rates range from 3.1% to 22.2%, while in developing countries, it is between 1.4% and 20.0%.[3] Data on SCI in Indonesia is not well recorded because of the difficulty in finding the prevalence of SCI. Based on the data from the International Perspectives on Spinal Cord Injury, it was found that the incidence of SCI in men was 77.8% higher compared to women.

SCI will impact cellular damage and release intracellular proteins, which act as solid inflammatory stimuli. Then, the release of chemokines and cytokines recruit peripheral neutrophils and macrophages to the injured spinal cord. Neutrophils are motile phagocytic cells that play a crucial role in acute inflammation. Neutrophils act as bactericidal and the first line of defence against pathogens that enter the body. Neutrophils have high levels when SCI occurs, but it will decrease within a week due to the increase in the infiltration of macrophages into the spinal cord.[4]

The role of neutrophils in SCI is not entirely understood. However, the role of neutrophils in debris clearance at lesions has been widely known. Zivkovic et al. (2021) revealed a dual role caused by neutrophil infiltration in SCI: detrimental and beneficial.[1] There has been much research on the dual role of neutrophils which used animals, especially rats and mice. Nevertheless, no research on the dual role of neutrophils in humans (clinical trial) has been conducted. Furthermore, systematic review studies discussing neutrophils' dual role are not yet found. Therefore, the researchers conducted a systematic review of animal trials to learn more about neutrophils' detrimental and beneficial roles in SCI.

2. Materials and Methods

Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) were used to conduct this systematic review.[5]

2.1 Research questions and search strategies

Research questions were selected based on characteristic of PICO (Population, Intervention, Comparison, Outcome). The researchers collected studies through five databases, including PubMed, Science Direct, Web of Science, Scopus, and Springer Link, until September 27, 2021. The main search terms were "Neutrophil and "Spinal Cord Injury." The limitations on search studies included 1) Publication year between 2016-2021 on all databases, 2) research articles from science direct, and 3) Springer Link and Scopus. The results were as follows: PubMed (n = 2); Science Direct (n = 247); Web of Science (n = 6); Scopus (n = 20); and Springer Link (n = 271). Fourteen articles were duplicates, resulting in a total of 532 studies **[Figure 1]**.

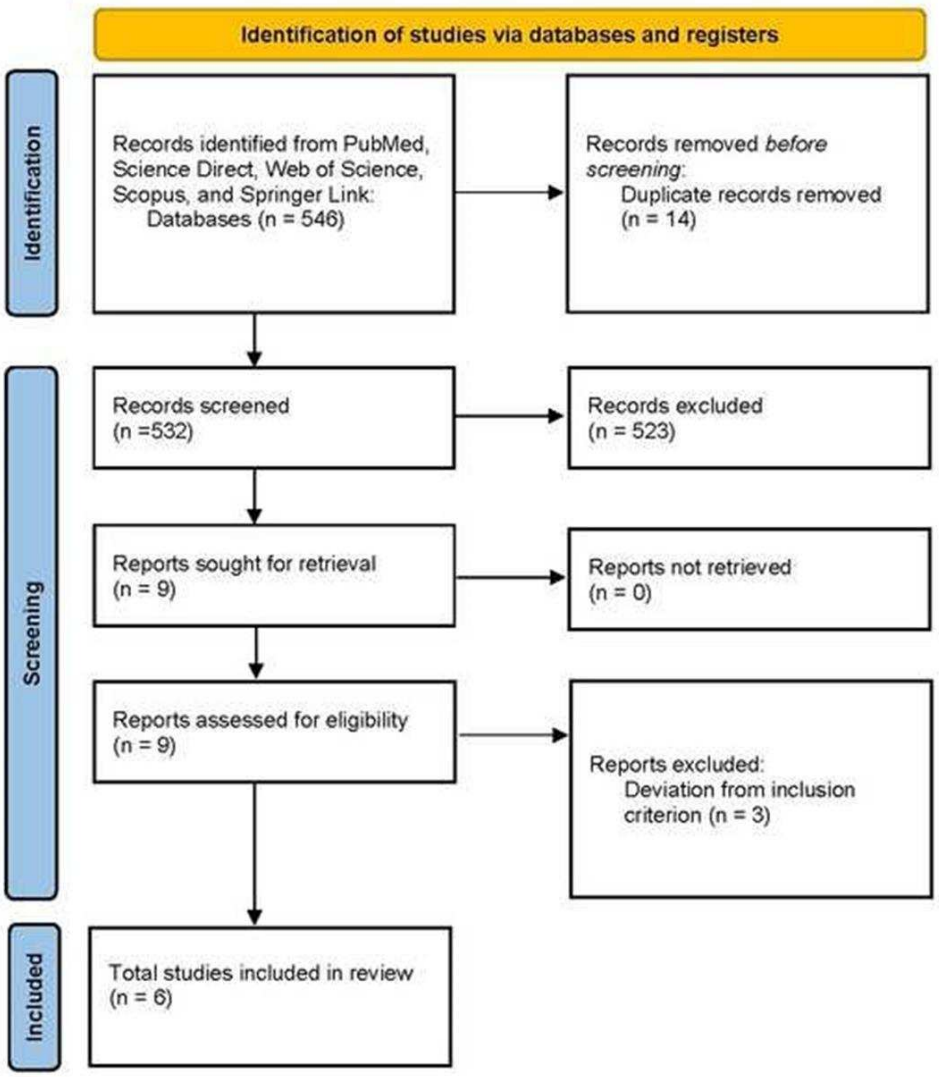

Fig. 1. PRISMA flow diagram of included and excluded studies

2.2 Inclusion and exclusion criteria

The selected studies had to meet the following criteria: (1) using mice as model animals in SCI, (2) the outputs in the form of a state of locomotor function, (3) published in English, (4) published within the last five years (2016- 2021), and (5) published in the form of an entire article. Then, the researchers excluded studies published in the form of abstracts and narrative literature reviews.

2.3 Assessment of quality and risk of bias in the included study

Two independent reviewers assessed the quality of the study by using SYRCLE's Risk of Bias Tool based on the Cochrane Risk of Bias Tool criteria advised by Hoojimans et al., 2014.[3] The areas assessed included selection, performance, detection, attrition, reporting, and other biases such as small sample size, ethical considerations, and whether funding is included or not. If each area had a low risk of bias, it showed "Yes." On the other hand, if each area had a low risk of bias, it showed "No." If the risk of bias was unclear due to short descriptions in the studies, it showed "Unclear" **[Table 1]**.

Table 1. Risk of bias assessment

Bias	Domain	Coll-Miró et al., 2016	Rudman et al., 2018	Brennan et al., 2019	Li et al., 2019	Khajoueinejad et al., 2019	Francos-Quijorna et al., 2017
Selection bias	Sequence generation	No	No	No	No	No	No
	Baseline characteristic	No	Yes	No	Yes	Yes	No
	Allocation concealment	Unclear	No	No	No	No	No
Performance bias	Random housing	Unclear	Yes	Yes	No	Yes	Unclear
	Blinding	No	No	Yes	No	No	Yes
Detection bias	Random outcome assessment	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
	Blinding	Yes	Yes	Yes	Yes	Yes	Yes
Attrition bias	Incomplete outcome data	Yes	Yes	Yes	Yes	Yes	Yes
Reporting bias	Selective outcome reporting	Yes	Yes	Yes	Yes	Yes	Yes
Other	Other source of bias	Yes	Yes	Yes	Yes	No	No

2.4 Data collection process and extraction

The same two review authors who performed the risk of bias assessment conducted data extraction independently from one another. The data extracted was the secondary data from studies published in a predetermined database. The search for data on the database was carried out using Boolean Operators covering or/and with search terms or keywords including ("Spinal Cord Injury" or "Spinal Cord Trauma") and

("Neutrophil" or "Polymorphonuclear") and ("Outcome" or "Treatment Outcome") and ("Inflammation" or "Neuroinflammation"). The keywords were entered simultaneously into the electronic database search engine using the advanced search. Then they were selected using the PRISMA flow according to the predetermined inclusion and exclusion criteria. After obtaining the desired studies, the studies were assessed using the SRYCLE risk of bias tool.

3. Result

A total of 532 studies entered the screening stage through reading titles and abstracts after eliminating the duplication. A total of 520 studies did not meet PICO and sample criteria such as 1) the main topic of study was not SCI (n: 404), 2) the interventions were not neutrophils (n: 56), 3) the studies conducted were not only on acute SCI (n: 60). A total of 12 studies were obtained after going through an appropriate screening process and had the potential to be involved in this review. Then, the studies were reviewed for eligibility based on the predetermined inclusion and exclusion criteria. A total of six studies did not meet the inclusion criteria because they were not animal experiments (n: 4) and they were non-rat studies (n: 2). Before entering the review stage, the studies were assessed for quality and validity using SYRCLE's Risk of Bias Tool **[Table 1]**. After validity assessment, these six studies were reviewed to observe their interventions, controls, and results.

The characteristics of these six studies are presented in **Table 2**. Studies using the interventions' precursor interleukin-37 (rIL-37) protein were divided into two groups. The first group showed a significant improvement in locomotor function, which was indicated by the increase in Basso Mouse Scale (BMS) scores starting from 7-day post injury (dpi). The second group showed that injections of RIL-371- 218 and rIL-37V46-218 also significantly improved locomotor recovery, which was perceived by the increase in BMS scores starting from 10 dpi. The study also explained that IL-37 attenuated protein levels from proinflammatory cytokines observed from SCI in model animals. The capacity of IL-37 to suppress cytokine production following SCI could alter immune cell infiltration and activation and recruit such cells as neutrophils and macrophages into the SCI. [6]

Another study mentioned that JQ1 significantly decreased the expression of some groups of cytokines and chemokines. One of the chemokines that level was lowered by JQ1 was Ccl2, a signal for macrophage infiltration after SCI occurred. Among cytokines weakened by JQ1, a small percentage were proinflammatory cytokines, including IL-1β, rapidly regulated after SCI and played a vital role in the secondary damage. The administration of JQ1 increased the anti-inflammatory cytokine IL-13, which inhibited the production of proinflammatory cytokines and chemokines.[7] One of the studies performed genetic modifications to assess the improvements in locomotor function in mice by removing the C3aR1 receptor as a C3a complement

receptor. This complement regulated the mobilization of neutrophils in the bone marrow. The results showed that the C3aR1-/- sample experienced significantly fewer improvements in locomotor function compared to the WT control group. These observations were made on 14, 21, 28, and 35 days after SCI modeling ($p < 0.05$).[8]

MicroRNA-210 (miR-210) produced by adenoviruses had an essential role in improving neurological function. According to the observations, neurological function improved 3 and 7 days after the damage. In addition to improving neurological function, miR-210 also lowered inflammatory serums levels such as IL-1β and Tumor Necrosis Factor (TNF)-α, whose levels decreased after three dpi.[9] Calcitriol was a form of biologically active vitamin D with potent neuroprotective properties and improved locomotor function. The improvement of this function was observed through the increase in the Basso-Beattie-Bresnahan (BBB) score as an indicator of locomotor function improvement.[10] Recent studies explained that a specialized pro-resolving mediator (SPM), maresin-1 (MaR1), had potent anti-inflammatory and recovery properties. When observed at 28 dpi, there was an improvement in locomotor function in the MaR1 intervention group. The study also explained that MaR1 significantly lowered levels of proinflammatory cytokines and chemokines ($p < 0.05$).[11]

Table 2. Characteristic of selected studies

No	Author and year of publication	Animal Model	Intervention	Control	Outcome	Findings
1	**Coll-Miró et al., 2016**	Adult 8-10 weeks female CB57BL (WT) / 6 (n = 40) and hIL-37tg mice (n = 8)	rIL-37	WT mice	Functional deficit, Locomotor movement	- Reduction of functional deficit after SCI - Improvement of functional /locomotor outcomes after SCI
2	**Rudman et al., 2018**	8–10 weeks old female CB57BL / 6 mice (n=16)	JQ1 (BET inhibitor)	WT mice	Pro inflammatory cytokine and chemokine expression, Leukocyte infiltration, Locomotor recovery	- Decreasing of pro inflammatory cytokine and chemokine expression - Decreasing of leukocyte infiltration after SCI - Unimprovement of locomotor recovery

3	**Brennan et al., 2019**	Adult female C57BL/6J (WT, n = 271), C3ar1$^{-/-}$ (n = 123), C5ar1$^{-/-}$ (n = 14), and Cx3cr1$^{gfp/+}$ mice (n = 3)	C3a / C3aR1	WT mice	Cytokine production, Locomotor outcomes	- Genetic ablation of C3aR1 worsens SCI outcomes - C3aR1 regulates BM cytokine production
4	**Li et al., 2019**	16-18 weeks male Sprague-Dawley rats (n=34)	MiR-210	Model groups	Neurologic function, Serum inflammation level	- Improvement of neurologic function scores - Regulation of serum inflammation level by MiR-210
5	**Khajoueinejad et al., 2019**	11 weeks female Sprague-Dawley rats (n = 36)	Calcitriol	Control SCI and SO groups	Functional recovery, Leukocyte infiltration, Cytokine and chemokine secretion	- Improvement of motor function recovery - Decreasing secretion level of IFN-γ - Decreasing secretion level of IL-17A - Decreasing recruitment of leukocyte at lesion area
6	**Francos-Quijorna et al., 2017**	8-10 weeks old female C57BL / 6 mice (n = 142)	MaR1	WT mice	Population of inflammation cell, Cytokine expression, and locomotor recovery	- The clearance of inflammation cell - Attenuation of pro-inflammatory cytokines - Improvement locomotor recovery

BET: Bromodomain and extra terminal domain containing protein; BM: Bone Marrow; IFN: Interferon; IL: Interleukin; MaR1: Maresin-1; MiR-210: MicroRNA-210; SCI: Spinal Cord Injury; SO: Sham Operation; WT: Wild Type

4. Discussion

This systematic review identified and analyzed six research studies explaining mice's intervention in acute SCI. It then observed the impact of neutrophils in responding to these interventions on the location of wounds in SCI and improvements in their locomotor function. All studies showed neutrophils acted more like "bad guys" in acute SCI, as Neirinckx et al., (2014).[12] However, some factors can be used to address neutrophil's detrimental role. These factors are grouped into two: direct and indirect. Direct factors affect the role of the neutrophils themselves. Brennan et al., (2019) mentioned that the mobilization of neutrophils had a detrimental role at the SCI wound site, so the role could be prevented by regulating physiological receptors.[8] Indirect factors affect the role of proinflammatory cytokines and chemokines. These cytokines and chemokines are neutrophil recruiters heading to the wound site in the SCI. There are 5 studies that discuss

indirect factors: Coll-Miró et al., (2016); Rudman et al., (2018); Li et al., (2019); Khajoueinejad et al., (2019); and Francos-Quijorna et al., (2017).[6,7,9–11] Research by Coll-Miró et al., (2016); Khajoueinejad et al., (2019) explained that the inhibition of proinflammatory cytokines indirectly inhibited the recruitment of neutrophils to the wound area in SCI.[6,10] This statement is related to the explanations of Anwar et al., (2016) and Dinarello, (2000).[13,14] They mentioned that proinflammatory cytokines recruited neutrophil cells, resulting in fever, inflammation, and even tissue damage.[6,10,13,14] Three other studies (Francos-Quijorna et al., 2017; Li et al., 2019 and Rudman et al., 2018) explained that in addition to attenuating the levels of proinflammatory cytokines, the increase in anti-inflammatory cytokines, such as IL-13 and IL-10, indirectly harm cytokine production which causes inflammation.[7,9,11,15]

The decrease in proinflammatory cytokines and the increase in anti-inflammatory cytokines and chemokines lower the cells that play roles in inflammation, repair damaged tissues, and improve neurological /locomotor function after SCI. [16] However, all studies do not show a decrease in cytokines that can improve locomotor function [Table 2]. Rudman et al., (2018) explained that with decreased levels of proinflammatory cytokines, there was no improvement in locomotor function after SCI occurred.[7] This may be due to the late administration of the intervention so that its ability to suppress the production of proinflammatory cytokines does not work optimally shortly after the SCI occurs. Brennan et al., (2019) mentioned that the inhibition of physiological receptors, which limited the mobilization of neutrophils, did not experience a significant improvement in neurological function.[8]

All studies monitor locomotor movements up to the SCI wound healing remodeling stage, which starts from 2-3 weeks after the SCI occurs and can continue for the next few months. [17,18] However, Li et al., (2019) conducted neurological function assessments at 3 and 7 dpi. During the period, time healing in SCI was still in its early stages.[9] It can be interpreted that the early stages of SCI healing may be inhibited mainly due to improved neurological function before the remodeling stage.

Based on this explanation, one crucial finding is found and can be researched for further theory development about SCI. The finding is about the effective therapy used for acute SCI. It is still an obstacle in acute SCI studies. This systematic review is written to collect, summarize, and analyze the dual role of neutrophils to be used as indicators in observing the output of SCI.

5. Conclusion

The dominant neutrophil role in acute SCI is the detrimental role. This role can be prevented directly, which impacts the neutrophils themselves, and indirectly, which impacts the signals of neutrophil callers, namely chemokines and proinflammatory cytokines. The beneficial role of neutrophils in SCI is not very

visible in experimental, but in theory, it could play a role in SCI. There are no driving factors for the beneficial role of neutrophils in SCI. The role of detrimental neutrophils in SCI is perceived in the experimental studies, so many factors are resolved in addressing this role in SCI. Factors that become resolutions in overcoming the detrimental role of neutrophils are divided into direct and indirect factors. Direct factors affect the neutrophils themselves, while indirect factors affect the caller's signal from the neutrophils themselves.

Conflict of interest

The authors declare that they have no conflict of interest

Funding

None

Consent

Not required

Ethical approval

Not required

Abbreviations

BBB	: Basso-Beattie-Bresnahan
BMS	: Basso Mouse Scale
dpi	: day post injury
IL	: interleukin
MaR	: Maresin
miR	: micro-RNA
NSCISC	: National Spinal Cord Injury Statistical Center
PICO	: Population, Intervention, Comparison, Outcome
PRISMA	: Preferred Reporting Items for Systematic Review and Meta Analysis
rIL	: precursor interleukin
SCI	: Spinal Cord Injury
SPM	: Specialized Presolving Mediator
TNF	: Tumor Necrosing Factor

Acknowledgements

The authors would like to thank all who have contributed to the process and completion of this systematic review, including all the teaching staffs of Faculty of Medicine, Department of Anesthesiology and Reanimation, Neurosurgery, and Pulmonary and Health Respiration of Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

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Appendix A.

A.1. PRISMA 2020 Checklist

A.2. AMSTAR 2 Checklist

Section and Topic	Item #	Checklist item
TITLE		
Title	1	Identify the report as a systematic review.
ABSTRACT		
Abstract	2	See the PRISMA 2020 for Abstracts checklist.
INTRODUCTION		
Rationale	3	Describe the rationale for the review in the context of existing knowledge.
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.
METHODS		
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to date when each source was last searched or consulted.
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many rev and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each repor independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of a process.
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which result
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, fundin assumptions made about any missing or unclear information.
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how ma study and whether they worked independently, and if applicable, details of automation tools used in the process.
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentatio
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study inter comparing against the planned groups for each synthesis (item #5)).
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summ conversions.
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was perf model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysi
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.

Section and Topic	Item #	Checklist item
RESULTS		
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the n the review, ideally using a flow diagram.
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were ex
Study characteristics	17	Cite each included study and present its characteristics.
Risk of bias in studies	18	Present assessments of risk of bias for each included study.
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effec (e.g. confidence/credible interval), ideally using structured tables or plots.
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estir confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction o
	20c	Present results of all investigations of possible causes of heterogeneity among study results.
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assesse
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.
DISCUSSION		
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.
	23b	Discuss any limitations of the evidence included in the review.
	23c	Discuss any limitations of the review processes used.
	23d	Discuss implications of the results for practice, policy, and future research.
OTHER INFORMATION		
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the revi
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.
	24c	Describe and explain any amendments to information provided at registration or in the protocol.
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the re
Competing interests	26	Declare any competing interests of review authors.
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; dat studies; data used for all analyses; analytic code; any other materials used in the review.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporti 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both

1. Did the research questions and inclusion criteria for the review include the components of PICO?

For Yes:
- ☑ Population
- ☑ Intervention
- ☑ Comparator group
- ☑ Outcome

Optional (recommended)
- ☐ Timeframe for follow-up

☑ Yes
☐ No

2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?

For Partial Yes:
The authors state that they had a written protocol or guide that included ALL the following:
- ☑ review question(s)
- ☑ a search strategy
- ☑ inclusion/exclusion criteria
- ☑ a risk of bias assessment

For Yes:
As for partial yes, plus the protocol should be registered and should also have specified:
- ☐ a meta-analysis/synthesis plan, if appropriate, and
- ☐ a plan for investigating causes of heterogeneity
- ☐ justification for any deviations from the protocol

☑ Yes
☐ Partial Yes
☐ No

3. Did the review authors explain their selection of the study designs for inclusion in the review?

For Yes, the review should satisfy ONE of the following:
- ☑ Explanation for including only RCTs
- ☐ OR Explanation for including only NRSI
- ☐ OR Explanation for including both RCTs and NRSI

☑ Yes
☐ No

4. Did the review authors use a comprehensive literature search strategy?

For Partial Yes (all the following):
- ☐ searched at least 2 databases (relevant to research question)
- ☐ provided key word and/or search strategy
- ☐ justified publication restrictions (e.g. language)

For Yes, should also have (all the following):
- ☑ searched the reference lists / bibliographies of included studies
- ☑ searched trial/study registries
- ☑ included/consulted content experts in the field
- ☑ where relevant, searched for grey literature
- ☑ conducted search within 24 months of completion of the review

☑ Yes
☐ Partial Yes
☐ No

5. Did the review authors perform study selection in duplicate?

For Yes, either ONE of the following:
- ☑ at least two reviewers independently agreed on selection of eligible studies and achieved consensus on which studies to include
- ☐ OR two reviewers selected a sample of eligible studies and achieved good agreement (at least 80 percent), with the remainder selected by one reviewer.

☑ Yes
☐ No

6. Did the review authors perform data extraction in duplicate?

For Yes, either ONE of the following:

- ☑ at least two reviewers achieved consensus on which data to extract from included studies
- ☐ OR two reviewers extracted data from a sample of eligible studies and achieved good agreement (at least 80 percent), with the remainder extracted by one reviewer.

☑ Yes
☐ No

7. Did the review authors provide a list of excluded studies and justify the exclusions?

For Partial Yes:

- ☑ provided a list of all potentially relevant studies that were read in full-text form but excluded from the review

For Yes, must also have:

- ☑ Justified the exclusion from the review of each potentially relevant study

☑ Yes
☐ Partial Yes
☐ No

8. Did the review authors describe the included studies in adequate detail?

For Partial Yes (ALL the following):

- ☑ described populations
- ☑ described interventions
- ☑ described comparators
- ☑ described outcomes
- ☑ described research designs

For Yes, should also have ALL the following:

- ☑ described population in detail
- ☑ described intervention in detail (including doses where relevant)
- ☑ described comparator in detail (including doses where relevant)
- ☑ described study's setting
- ☑ timeframe for follow-up

☑ Yes
☐ Partial Yes
☐ No

9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?

RCTs

For Partial Yes, must have assessed RoB from

- ☑ unconcealed allocation, and
- ☑ lack of blinding of patients and assessors when assessing outcomes (unnecessary for objective outcomes such as all-cause mortality)

For Yes, must also have assessed RoB from:

- ☑ allocation sequence that was not truly random, and
- ☑ selection of the reported result from among multiple measurements or analyses of a specified outcome

☑ Yes
☐ Partial Yes
☐ No
☐ Includes only NRSI

NRSI

For Partial Yes, must have assessed RoB:

- ☑ from confounding, and
- ☑ from selection bias

For Yes, must also have assessed RoB:

- ☐ methods used to ascertain exposures and outcomes, and
- ☐ selection of the reported result from among multiple measurements or analyses of a specified outcome

☐ Yes
☐ Partial Yes
☐ No
☐ Includes only RCTs

10. Did the review authors report on the sources of funding for the studies included in the review?

For Yes

- ☐ Must have reported on the sources of funding for individual studies included in the review. Note: Reporting that the reviewers looked for this information but it was not reported by study authors also qualifies

☐ Yes
☑ No

11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?

RCTs

For Yes:

- The authors justified combining the data in a meta-analysis
  - AND they used an appropriate weighted technique to combine study results and adjusted for heterogeneity if present.
  - AND investigated the causes of any heterogeneity

☐ Yes
☐ No
☑ No meta-analysis conducted

For NRSI

For Yes:

- The authors justified combining the data in a meta-analysis
  - AND they used an appropriate weighted technique to combine study results, adjusting for heterogeneity if present
  - AND they statistically combined effect estimates from NRSI that were adjusted for confounding, rather than combining raw data, or justified combining raw data when adjusted effect estimates were not available
  - AND they reported separate summary estimates for RCTs and NRSI separately when both were included in the review

☐ Yes
☐ No
☐ No meta-analysis conducted

12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?

For Yes:

- included only low risk of bias RCTs
- OR, if the pooled estimate was based on RCTs and/or NRSI at variable RoB, the authors performed analyses to investigate possible impact of RoB on summary estimates of effect.

☐ Yes
☐ No
☑ No meta-analysis conducted

13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?

For Yes:

- ☑ included only low risk of bias RCTs
- OR, if RCTs with moderate or high RoB, or NRSI were included the review provided a discussion of the likely impact of RoB on the results

☑ Yes
☐ No

14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?

For Yes:

- There was no significant heterogeneity in the results
- ☑ OR if heterogeneity was present the authors performed an investigation of sources of any heterogeneity in the results and discussed the impact of this on the results of the review

☑ Yes
☐ No

15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?

For Yes:

- ☐ performed graphical or statistical tests for publication bias and discussed the likelihood and magnitude of impact of publication bias

☐ Yes
☐ No
☑ No meta-analysis conducted

16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	
For Yes:	
☐ The authors reported no competing interests OR	☐ Yes
☐ The authors described their funding sources and how they managed potential conflicts of interest	☑ No

To cite this tool: Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, Moher D, Tugwell P, Welch V, Kristjansson E, Henry DA. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ. 2017 Sep 21;358:j4008.