The Risk Of Bias In Non-randomized Studies – of Interventions, Version 2 (ROBINS-I V2) assessment tool

(for follow-up studies)

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Outline of ROBINS-I V2

ROBINS-I aims to assess the risk of bias in a specific result from a non-randomized study that examines the effect of an intervention strategy, versus a comparator strategy, on an outcome. This document describes the ROBINS-I V2 tool for **follow-up (cohort) studies**. Throughout ROBINS-I V2, we refer to the "intervention strategy" and "comparator strategy" that are being compared. The comparator may be an alternative active intervention, standard care, or no intervention. Because there is no randomized assignment to the intervention or comparator, assignment is inferred from the intervention prescribed to or received by each participant. We refer to the participants classified into each strategy as the "intervention group" and "comparator group".

Risk-of-bias assessments should relate to risk of **material bias** rather than risk of any bias. Material bias should be interpreted as bias sufficient to cause an important change to the magnitude of the estimated effect, compared with the true value.

Before undertaking a ROBINS-I assessment (or series of assessments, e.g. in the context of a systematic review), users of the tool should specify the important confounding factors that are likely to influence the association between the intervention and the outcome (see section "At planning stage").

A key feature of the ROBINS-I approach is the specification, for each study, of the causal effect estimated by the result under consideration through specification of a hypothetical 'target trial'. This is essential for assessment of risk of bias, because the causal effect defines the result that would be seen (other than the impact of sampling variation) in the absence of bias.

If multiple assessors will implement ROBINS-I independently, the *Preliminary considerations* should be agreed between all assessors before each assessor works individually through evaluation of the confounding factors and bias domains.

ROBINS I V2 includes six domains of bias:

- Domain 1: Risk of bias due to confounding
- Domain 2: Risk of bias in classification of interventions
- Domain 3: Risk of bias in selection of participants into the study (or into the analysis)
- Domain 4: Risk of bias due to missing data
- Domain 5: Risk of bias arising from measurement of the outcome
- Domain 6: Risk of bias in selection of the reported result

Unlike version 1 of ROBINS-I. and RoB 2, ROBINS-I V2 does not include the domain "Risk of bias due to deviations from the intended intervention" (this is renamed as "Risk of bias due to protocol deviations" in the next version of RoB 2). In non-randomized studies, issues related to deviations from the intended intervention (protocol deviations) are relevant for analyses that estimate the 'per protocol' effect (see Guidance note 8). Bias arising in such analyses is addressed in Domain 1 (Variant B), through consideration of whether both baseline and time-varying confounding were adequately controlled for, using an appropriate analysis.

Each bias domain in ROBINS-I is addressed using a series of **signalling questions** that aim to gather important information about the study and the analysis being assessed. Most signalling questions have response options 'Yes', 'Probably yes', 'Probably no', 'No' and 'No information', with 'Yes' and 'Probably yes' having the same implications for risk of bias and similarly for 'No' and 'Probably no'. Some questions have additional response options (a 'weak' and a 'strong' version of 'Yes' or 'No') to help discriminate between higher and lower risk of bias. After the relevant signalling questions have been completed, an algorithm maps the answers to the signalling questions onto a proposed judgement about **risk of bias** in the result that arises from this domain. The judgements and their broad interpretations are as follows.

Judgement	Interpretation
*Low risk of bias**	There is little or no concern about bias with regard to this domain.
Moderate risk of bias	There is some concern about bias with regard to this domain, although it is not clear that there is an important risk of bias.
Serious risk of bias	The study has some important problems in this domain: characteristics of the study give rise to a serious risk of bias.
Critical risk of bias	The study is very problematic in this domain: characteristics of the study give rise to a critical risk of bias, such that the result should generally be excluded from evidence syntheses.

*For Domain 1 (Risk of bias due to confounding), this is referred to as "Low risk of bias (except for concerns about uncontrolled confounding)", in which confounding is very well addressed but cannot be eliminated as a possibility. This is because a risk of bias due to uncontrolled confounding cannot be excluded in an observational study.

ROBINS-I is intended to provide a framework for making informed and reasonable judgements about risk of material bias in studies of the effects of an intervention strategy (versus a comparator strategy) on an outcome. On occasion, answers to the signalling questions may not yield an appropriate risk of bias judgement based on the algorithm. Therefore, suggested risk of bias judgements produced by the algorithms can be overridden, in which case justification should be provided. We aim for transparency and reasonableness rather than mechanistic adherence to every word of the tool's contents.

Optionally, a **predicted direction of bias** may be selected, balancing the various issues addressed within the domain. Response options for this depend on the type of bias being addressed.

After completing all six bias domains, an **overall judgement** is made for the risk of bias (and optionally for the predicted direction of any bias). The risk-of-bias Judgement is derived from the domain-level judgements using an algorithm. As for bias domain-level judgements, justification should be provided when the overall judgement suggested by the algorithm is overridden.

An online implementation of ROBINS-I V2 including automatic selection of relevant signalling questions and algorithm-derived risk-of-bias judgements is being developed and will be made available via www.riskofbias.info.

Index to guidance notes

The ROBINS-I V2 tool

At planning stage: list confounding factors

P1. List the important confounding factors relevant to all or most studies on this topic. Specify whether these are particular to specific intervention-outcome combinations.

Guidance note 1 *: listing confounding factors*

A confounding factor is a prognostic factor that predicts whether participants receive the intervention or the comparator strategy. Important confounding factors are those that have the potential to introduce material bias into an estimated effect. Factors that are expected to have only very weak associations with the intervention or with the outcome, such that failure to account for them in the analysis will not have a material impact on the estimated effect of intervention on outcome, need not be considered here. Important confounding factors should be pre-specified at the planning stage, for example in the protocol of a systematic review that will include studies of the effects of interventions. The identification of potential confounding factors requires content knowledge and may usefully be informed by examination of relevant literature. Important confounding factors should be specified at the level of the broad research question (e.g. using a single list of confounding factors for a systematic review). This broad question may cover several specific interventions and/or outcomes. If confounding factors are specific to particular intervention-outcome combinations, then this should be stated.

Note that ROBINS-I does not address effect modification (the situation in which the effect of an intervention is different in different subsets of the population under study).

The confounding factors possible include:

- uses multiple cell types (SH-SY5Y neuroblastoma cells, MEF+/+ wildtype, MEF PINK1−/− knockout), excludes significance or reasoning why cells were chosen
- Four HA molecular weights (35, 117, 1280, 2190 kDa), excludes significance or reasoning why weights were chosen
- Only male C57BL/6 mice aged 8 weeks were used. Sex, age, and species all impact the outcomes and pathology of PD differently.
- Mice were divided into four groups of three, but no randomization procedure is described
- PINK1 antibody itself could alter mitochondrial membrane dynamics independently of USP30 silencing

There are far more variables, but scope was narrowed to find the most important and relevant ones.

For each study result: preliminary considerations (parts A to D)

Guidance note 2 *: overview of preliminary considerations*

The start point for a ROBINS-I assessment of an individual study is to specify the result from the study that is being assessed for risk of bias. The preliminary considerations questions should be answered in relation to the specific result that is being evaluated for the current ROBINS-I assessment.

In case of multiple alternative analyses being presented, it is important to specify the numeric result (e.g. RR = 1.52 (95% CI 0.83 to 2.77) and/or a reference (e.g. to a table, figure or paragraph) that uniquely defines the result being assessed.

Some characteristics of a study or a result may lead directly to the result being at critical risk of bias, and so make detailed risk-of-bias assessments unnecessary. A series of 'screening' questions in this section aim to identify such situations.

The **target trial specific to the study** is a hypothetical randomized trial, which need not be ethical or feasible, that compares the health effects of the same intervention and comparator strategies, conducted with the same eligibility criteria as the non-randomized study. In general, such target trials will not use blinding of participants or of health professionals administering interventions.

If multiple assessors will implement ROBINS-I independently, the questions in this section should be agreed between all assessors before each assessor works individually through the risk-of-bias assessment itself.

A. Specify the result being assessed for risk of bias

Guidance note 3 *: specifying the numerical result being assessed for risk of bias*

A ROBINS-I assessment of risk of bias is specific to a particular study result. This is because different results from the same study may be at importantly different risks of bias (consider, for example, an unadjusted estimate of intervention effect compared with an estimate that is adjusted for numerous important confounding factors). Consequently, it may be necessary to undertake several ROBINS-I assessments of different results from the same study. If the study presents multiple alternative analyses, specify the numerical result (e.g. RR=1.52 (95% CI 0.83 to 2.77)) and/or a reference (e.g. to a table, figure or paragraph) that uniquely defines the result being assessed.

A1. Specify the numerical result being assessed

Multiple numerical values being assessed after different masses of HA-NP are utilized (in KDa). These include, but are not limited to:

- basic respiratory rate
- maximum respiratory rate
- H+ proton leakage
- ATP production
- SOD activity of the cells
- Oxidative phosphorylation of respiratory metabolism
    - Increased with HA-NPs with 117 kDa and 2190 kDa HA

used PINK1-deficient cells to further verify the repairing effect of different molecular weights of HA on the damaged mitochondria of PD.

- respectively treated MEF+/+ and MEF PINK1− /− cells with HA-NPs and studied the oxidative phosphorylation of cell energy metabolism.
    - showed that the HA-NPs with 117 kDa, 1280 kDa, and 2190 kDa HA had reparative and protective effects on both MEF+/+ and MEF PINK1− /− cells

HA-NPs with 1280 kDa and 2190 kDa HA resulted in a significant increase in the basic respiratory rate, maximum respiration rate, H+ proton leakage, and ATP production of MEF PINK1− /− cells. Meanwhile, the basic respiratory rate, maximum respiratory rate, H+ proton leakage and ATP production in MEF+/+ cells were significantly greater than those in MEF PINK1− /− cell

A2. Provide further details about this result (for example, location in the study report, reason it was chosen) [optional]

“3.2. HA-NPs improve the function of reversibly damaged mitochondria” Chosen because it is most closely related to the conclusions discussed at the end of the paper.

A3. Specify the outcome to which this result relates

“We successfully designed and developed NPs for the treatment of PD that can target different stages of mitochondrial damage. Our HA-NPs with 2190 kDa HA can protect the mitochondria and salvage the mitochondrial function of the mild and limited damage conditions in both SH-SY5Y cells and PINK1 knockout MEF cells. NPs with USP30 siRNA and PINK1 antibodies can selectively target and promote the clearance of irreversibly damaged mitochondria both in vitro and in vivo.”

B. Decide whether to proceed with a risk-of-bias assessment

Guidance note 4 *: deciding whether to proceed with a risk-of-bias assessment*
Some characteristics of a study or a result may lead directly to the result being at critical risk of bias, and so make detailed risk-of-bias assessments unnecessary. The questions in this section aim to identify such situations.

Question	Comments	Response options
B1 Did the authors make any attempt to control for confounding in the result being assessed?	Confounding is a substantial problem in most non-randomized studies, and it is usually important to control for the important confounding factors.	Y / PY / PN / N
B2 If N/PN to B1: Is there sufficient potential for confounding that this result should not be considered further?	If there is sufficient potential for confounding that an unadjusted result should not be considered further, then the result is judged to be at 'Critical risk of bias'.	Y / PY / PN / N
B3 Was the method of measuring the outcome inappropriate?	This question aims to identify methods of outcome measurement (or data collection) that are unsuitable for the outcome they are intended to evaluate. This enables a rapid assessment that a result should be regarded as at 'Critical risk of bias'. The question does not aim to assess whether the choice of outcome being evaluated was *sensible* (e.g. because it is a surrogate or proxy for the main outcome of interest). In most circumstances, for pre-specified outcomes, the answer to this question will be 'N' or 'PN'. Answer 'Y or 'PY' if the method of measuring the outcome is inappropriate, for example because: (1) important ranges of outcome values fall outside levels that are detectable using the measurement method; or (2) the measurement instrument has been demonstrated to have such poor reliability or validity that estimates of the relationship between intervention and the measured outcome are not useful. (3) The measurement method differed substantially between people in the intervention and comparator groups, so that differences between the groups are not interpretable.	Y / PY / PN / N

If the answer to either B2 or B3 is 'Yes' or 'Probably yes', the result should be considered to be at 'Critical risk of bias' and no further assessment is required.

C. Specify the (hypothetical) target trial specific to the study

The target trial is either explicitly described by the primary study investigators or implied by the study's design and analysis. Alongside specifying the target trial, it is necessary to consider whether the analysis estimates the intention-to-treat effect or the per-protocol effect.

Guidance note 5 *: the target trial specific to the study*

Evaluations of risk of bias are facilitated by considering the non-randomized study as an attempt to emulate a pragmatic randomized trial, which we refer to as the **target trial**. This is the hypothetical randomized trial whose results would be the same (other than due to sampling variation) as those from the non-randomized study under consideration, in the absence of bias. Its key characteristics are the eligible participants (specified by the inclusion/exclusion criteria) the intervention strategy and comparator strategy, the start and end of follow-up and the outcome assessed (see Table 1 of Cashin et al. (2025), Hernán and Robins (2016) and Hernán et al. (2025)).

Specifying the target trial based on a published study involves deducing or 'reverse engineering' the research question that the study addressed based on the analysis that was done. This may be difficult when the study itself does not clearly specify the question, or because the implicit intervention and comparator strategies implied by the analysis do not address a clinically relevant question.

Because it is hypothetical, ethics and feasibility need not be considered when specifying the target trial. For example, there would be no objection to a target trial that compared individuals who did and did not start smoking, even though such a trial would be neither ethical nor feasible in practice.

Differences between the target trial for the non-randomized study being considered and the generic research question of the review relate to issues of heterogeneity and/or generalizability rather than risk of bias.

Cashin AG, Hansford HJ, Hernán MA, Swanson SA, Lee H, Jones MD, Dahabreh IJ, Dickerman BA, Egger M, Garcia-Albeniz X, Golub RM, Islam N, Lodi S, Moreno-Betancur M, Pearson SA, Schneeweiss S, Sharp MK, Sterne JAC, Stuart EA, McAuley JH. Transparent reporting of observational studies emulating a target trial: the TARGET Statement. BMJ. 2025 Sep 3;390:e087179. doi: 10.1136/bmj-2025-087179.

Hernán MA, Robins JM. Using big data to emulate a target trial when a randomized trial is not available. *American Journal of Epidemiology* 2016;183:758-64; doi:10.1093/aje/kwv254.

Hernán MA, Dahabreh IJ, Dickerman BA and Swanson SA. The target trial framework for causal inference from observational data: why and when is it helpful? *Annals of Internal Medicine* 2025;178: 402-407. https://doi.org/10.7326/ANNALS-24-01871.

Guidance note 6 *: specifying the participants of the target trial*

The eligible participants for the target trial are determined by the eligibility criteria for inclusion in the study and analysis being assessed for risk of bias. Specification of the eligible participants may require careful consideration. For example, Magid et al (2010) studied the comparative effectiveness of ACE inhibitors compared to beta-blockers as second-line treatments for hypertension. From an initial cohort of 1.6 million patients, they restricted the analysis population to persons (1) with incident hypertension; (2) who were initially treated with a thiazide agent; (3) who had one of the two drugs of interest added as a second agent for uncontrolled hypertension; and (4) who did not have a contraindication to either drug. Their "comparative effectiveness" cohort included 15,540 individuals: less than 1% of the original cohort.

C1. Specify the participants and eligibility criteria	C57BL/6 mice (male, 8 weeks)

Guidance note 7 *: specifying the intervention and comparator strategies in the target trial*

The intervention and comparator strategies may be point interventions that are administered at baseline, or be administered over a period of time rather than on a single occasion. Examples of point interventions include surgical interventions administered at a single time, a single vaccine dose, or starting a drug treatment. Note that, in non-randomized studies, the distinction between intention-to-treat and per-protocol effects (see Guidance note 8) is typically of little relevance for point interventions when participants are assigned (classified) into groups depending on the intervention they receive at baseline.

Examples of intervention strategies that are administered over time include surgery within 3 months of diagnosis, initial vaccination followed by a second dose 12 weeks later, or starting a drug treatment and continuing it for two years.

Analyses that estimate the intention-to-treat effect (see Guidance note 8) do not account for changes to the initial intervention. For such analyses detailed specification of the whole intervention and comparator strategy is not necessary.

For analyses that estimate the per-protocol effect (see Guidance note 8), the intervention and comparator strategies should be those whose effect is compared in the analysis that produced the result being assessed. This implies that the intervention changes that should not occur under the strategy (i.e. the protocol deviations) should be specified. For example:

- Suppose follow-up for individual participants is partitioned into time on drug A (intervention) and time on drug B (comparator). The intervention strategy is "Start on drug A and remain on drug A". The comparator strategy is "Start on drug B and remain on drug B". A protocol deviation occurs when participants stop the initial drug regimen.
- Suppose follow-up of comparison group participants is censored when they start the drug treatment without a clinical indication to do so. The comparator strategy is "Do not start the drug treatment during follow-up unless this is clinically indicated". A protocol deviation occurs when the comparison group participants start the drug treatment when it is not clinically indicated.
- Suppose follow-up of initially vaccinated participants is censored if they do not receive a second vaccine dose within 15 weeks. The intervention strategy is "Receive initial vaccination and then a second vaccine dose within 15 weeks". A protocol deviation occurs if a second vaccine dose is not received within 15 weeks.

C2. Specify the intervention strategy	35, 117, 1280, 2190 kDa HA-NPs; SH-SY5Y, MEF PINK1-/- cells
C3. Specify the comparator strategy	Untreated SH-SY5Y/MEF+/+ cells, H2O2 treated cells without HA-NPs, MEF PINK1-/- without HA-NPs

Guidance note 8 *: whether the analysis estimates the intention-to-treat effect or the per-protocol effect*

ROBINS-I assessments of risk of bias due to confounding are tailored to whether the analysis estimates the 'intention-to-treat effect' or the 'per-protocol' effect. The intention-to-treat effect is the effect of assignment to the intervention versus the comparator, regardless of subsequent changes to the intervention received by participants. In non-randomized studies, assignment is often unknown, so it is usually inferred from the intervention started by or prescribed to each participant. If the analysis is estimating the intention-to-treat effect, Variant A of Domain 1 (confounding) is used.

Estimation of the intention-to-treat effect requires that participants' follow-up is not censored because of post-baseline changes to the initial intervention, for example if participants assigned to first-line cancer chemotherapy are censored when they start second-line therapy because of cancer recurrence.

It is sometimes necessary, or of interest, to account for changes to the intervention received by study participants over time because such changes are inconsistent with the intervention or comparator strategy. We refer to such changes as **protocol deviations**. For example, participants classified in a comparator "no intervention" group because they did not receive the intervention at baseline may deviate from the protocol by starting intervention during follow-up.

Changes to participants' initial intervention can be consistent with the intervention or comparator strategy. For example, stopping initial intervention because of intervention-related toxicities or switching to second-line treatment because of cancer progression are generally not considered to be protocol deviations so should not be accounted for in analyses.

The 'per-protocol' effect is the effect of receiving the intervention strategy versus the comparator strategy over time. Therefore, protocol deviations should be accounted for by analyses that estimate the per-protocol effect and, correspondingly, if the analysis accounts for protocol deviations then we can infer that it estimates the per-protocol effect. Examples include: (i) interventions varied over time and follow-up for individual participants is partitioned according to the intervention received; or (ii) follow-up is censored when participants deviate from their initial intervention (e.g. participants assigned to a no-intervention comparison group start the intervention or those assigned to active intervention stop the intervention without a clinical indication).

When the analysis accounts for protocol deviations, it is necessary to account for 'time varying confounding', which is addressed in Variant B of Domain 1. Time-varying confounding occurs when time-varying prognostic factors influence changes to intervention after the start of follow-up. For example, in a cohort study of the effect of antiretroviral therapy (ART) on rates of AIDS and death in people with HIV, follow-up time for each participant was partitioned according to whether participants were receiving or not receiving ART. Because CD4 counts during follow-up influenced the decision to start ART, CD4 count was a time-varying confounder. Specialized analysis methods ('g-methods') should be used to estimate per-protocol effects, because standard regression models including time-varying confounders are likely to produce biased estimates of the effect of the intervention (versus the comparator) on the outcome.

C4. Did the analysis account for switches during follow-up between the intervention strategies being compared, or for other protocol deviations during follow-up?

X No (the analysis is estimating the intention-to-treat effect)

⬜ Yes (the analysis is estimating the per-protocol effect)

D. Information sources

Guidance note 9 *: information sources*
Evaluation of a study should be based on the maximum possible amount of available information. In addition to published articles describing a study's methods and results, such information may be derived from the study protocol, unpublished reports or through correspondence with the study investigators.

Which of the following sources have you obtained to help you inform your risk of bias judgements (tick as many as apply)?

- € Journal article(s)
- € Study protocol
- € Statistical analysis plan (SAP)
- € Non-commercial registry record (e.g. ClinicalTrials.gov record)
- € Company-owned registry record (e.g. GSK Clinical Study Register record)
- € "Grey literature" (e.g. unpublished thesis)
- € Conference abstract(s)
- € Regulatory document (e.g. Clinical Study Report, Drug Approval Package)
- € Individual participant data
- € Research ethics application
- € Grant database summary (e.g. NIH RePORTER, Research Councils UK Gateway to Research)
- € Personal communication with investigator
- € Personal communication with sponsor

Please specify any additional sources not listed above

n/a

Risk-of-bias assessment

Evaluation of confounding factors

Complete a row for each important confounding factor listed in advance (subsection (i) below); and either relevant to the setting of this particular study or identified by the study authors (subsection (ii)). **"Important" confounding factors are those for which, in the context of this study, adjustment is expected to lead to a meaningful change in the estimated effect of the intervention.**

Guidance note 10 *: evaluating confounding factors*

Confounding is of fundamental importance to the analysis and interpretation of non-randomized studies of the effect of interventions on outcomes. It occurs when prognostic factors also predict receipt of the intervention or comparator strategy: we call these '**confounding factors**'. ROBINS-I addresses two types of confounding: baseline confounding and time-varying confounding. **Baseline confounding** occurs when one or more prognostic factors, present before the start of follow-up, predict whether participants start the intervention or comparator strategy. **Time-varying confounding** needs to be considered in analyses that estimate the per-protocol effect (see Guidance note 8) by allowing for time-varying interventions. Examples include analyses that partition follow-up time for individual participants according to intervention received, or that censor participants' follow-up when they deviate from their assigned intervention strategy. The time-varying confounding factors are prognostic factors that vary over time and predict post-baseline protocol deviations.

All the important confounding factors should be listed in this section. This includes both baseline confounding factors and, if relevant (if yes to C4), time-varying confounding factors.

It may not be possible to measure a confounding factor well, and we distinguish between the confounding factor and the **variables** used to measure it. These variables may be used, for example, as covariates in a regression analysis.

In the context of a particular study, variables need not be included in the analysis: (a) if they are not associated with the outcome, conditional on intervention received (noting that lack of a statistically significant association is not evidence of a lack of association); (b) if they are not associated with intervention; (c) if adjustment makes no or minimal difference to the estimated effect of intervention on outcome; (d) because the confounder was addressed in the study design, for example by restricting to individuals with the same value of the confounder; (e) because a negative control demonstrates that there was unlikely to have been confounding due to this variable or that uncontrolled confounding was likely to be minimal; or (f) because external evidence suggests that controlling for the variable is not necessary in the context of the study being assessed.

In some studies, researchers may include a very large set of potential confounding variables in an analysis without considering their associations with outcome and intervention. Users of ROBINS-I should focus on (i) the confounding factors they determined a priori to be important and (ii) other factors for which adjustment is expected to lead to an important change in the estimated effect of the intervention on the outcome in the context of the current study.

Users of ROBINS-I should evaluate the confounding factors that they prespecified as important for the intervention-outcome relationship under study. The tool also allows the user to evaluate a second list of any further confounding factors that are either relevant to the setting of this particular study or which the study authors identified as potentially important. It is likely that new ideas relating to confounding and other potential sources of bias will be identified after the drafting of the review protocol, and even after piloting data collection from studies selected for inclusion in the systematic review. For example, such issues may be identified because they are mentioned in

the introduction and/or discussion of one or more articles. This could be addressed in practice by explicitly recording whether potential confounders or other sources of bias are mentioned in the article.

In very rare situations it is possible that no confounding factors are present, either because interventions received are known to be unrelated to any prognostic factors for the outcome of interest, or because no such prognostic factors exist. In such situations, the risk of bias due to confounding may be assessed as low.

The purpose of this preliminary assessment of confounding factors is to review the extent to which the result being assessed was controlled for confounding, considering both the prespecified confounding factors and any further confounding factors identified as important in the context of the study being assessed. This enables users of ROBINS-I to answer the signalling questions for the Domain 1 assessment (Risk of bias due to confounding). “Important” confounding factors are those for which, in the context of this study, adjustment is expected to lead to an important change in the estimated effect of the intervention.

The preliminary assessment consists of the following steps for each confounding factor.

- determine which variables (if any) were measured for the factor;
- determine which of these variables were controlled for in the analysis;
- for variables that were not controlled for, look for evidence that controlling for the variable was not necessary in this particular study;
- determine whether the confounding factor was measured validly and reliably by the variables used to measure it (this is assessed at the level of the confounding factor rather than the level of the individual variables used to measure the factor);
- determine the likely direction of bias if the analysis fails to adjust for this variable (alone).

The direction of bias, if the analysis fails to adjust for a particular variable (alone), will be that the effect estimate is biased *upwards* or biased *downwards*. For example, if older age predicts that a particular intervention is more likely to be received and the outcome is mortality, then this confounding would bias the estimated effect downwards: unless we adjust for age the intervention will appear more positively associated with higher mortality than it should. In the presence of *positive confounding* (the confounder is positively associated with both intervention and outcome, or negatively associated with both intervention and outcome), the bias will be upwards. In the presence of *negative confounding* (the confounder is positively associated with intervention and negatively associated with outcome, or vice versa), the bias will be downwards.

(i) Important confounding factors listed in advance						
Confounding factor	Measured variable(s) for this factor, if any	Was this variable (or were these variables) controlled for in the analysis? (Y / N)	If this confounding factor was controlled for, was it measured validly and reliably by this variable (or these variables)?* (NA / Y / PY / PN / N / NI)	If this confounding factor was not controlled for, is there evidence that controlling for it was unnecessary?** (NA / Y / PY / PN / N)	OPTIONAL: Is failure to adjust for this confounding factor expected to bias the effect estimate upwards or downwards? (Upward bias (overestimate the intervention effect) / Downward bias (underestimate the intervention effect) / No information or unpredictable)	Comments
Multiple cell types (SH-SY5Y, MEF +/+, MEF PINK1-/-	Cell type identity	N	NA	N	Upward bias	No justification given for selections
Four HA-NP molecular weights (35, 117, 1280, 2190 kDa)	Molecular weight	N	NA	N	Upward bias	No justification given for selections
Only male C57BL/6 Mice under 8 weeks	Sex, age, species	N	NA	N	Upward bias	Sex hormones and age alter the physiology of the mice, therefore rendering unable to be generalized to a larger population

(ii) Additional important confounding factors relevant to the setting of this particular study, or identified by the study authors						
Confounding factor	Measured variable(s) for this factor, if any	Was this variable (or were these variables) controlled for in the analysis? (Y / N)	If this confounding factor was controlled for, was it measured validly and reliably by this variable (or these variables)?* (NA / Y / PY / PN / N / NI)	If this confounding factor was not controlled for, is there evidence that controlling for it was unnecessary?** (NA / Y / PY / PN / N)	OPTIONAL: Is failure to adjust for this confounding factor expected to bias the effect estimate upwards or downwards? (Upward bias (overestimate the intervention effect) / Downward bias	Comments

					(underestimate the intervention effect) / No information or unpredictable)	
H2O2 concentration	H2O2 concentration	N	NA	N	Upward bias	Inconsistent across outcome measures, makes it unreliable
No blinding of investigators during outcome assessment	None reported	N	NA	N	Upward bias	Subject to observer bias upon outcomes

* “Validity” refers to whether the confounding variable or variables accurately measure the confounding factor, while “reliability” refers to the precision of the measurement (more measurement error means less reliability).

** In the context of a particular study, variables need not be included in the analysis: (a)) if they are measured validly and reliably and are not associated with the outcome, conditional on intervention (noting that lack of a statistically significant association is not evidence of a lack of association; (b) if they are measured validly and reliably and are not associated with intervention; (c) if they are measured validly and reliably and adjustment makes no or minimal difference to the estimated effect of the primary parameter; (d) because the confounder was addressed in the study design, for example by restricting to individuals with the same value of the confounder; (e) because a negative control demonstrates that there was unlikely to have been confounding due to this variable or that uncontrolled confounding was likely to be minimal; or (f) because external evidence suggests that controlling for the variable is not necessary in the context of the study being assessed”.

Signalling questions and risk-of-bias judgements

Responses underlined in green are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to sign posts to other questions, no formatting is used.

1. Bias due to confounding

Guidance note 11 *: bias due to confounding*

The questions in this domain focus on the confounding factors (see Guidance note 10) that were identified as important in the preliminary evaluation in section E.

We use the term **uncontrolled confounding** to refer to confounding that was not controlled by the design or analysis of the study – and is therefore likely to bias the estimated effect of intervention. This may arise because (i) confounding factors were not (or could not) be measured; (ii) variables used to measure confounding factors were insufficient to characterize the confounding factor; or (iii) variables that characterize the confounding factor were measured but not included in the analysis.

The answers to the signalling questions are based on contents of the completed table 'Evaluation of confounding factors'.

There are two variants of this domain.

Variant A is used when the analysis is estimating the intention-to-treat effect, when only baseline confounding needs to be addressed. Baseline confounding occurs when one or more prognostic factors, present before the start of follow-up, predict whether participants start the intervention or comparator strategy. Appropriate methods to control for baseline confounding include stratification, regression, matching, standardization, and inverse probability weighting. The analysis may control for individual variables or for estimated propensity scores. Inverse probability weighting is based on a function of the propensity score.

Variant B is used when the analysis is estimating the per-protocol effect, that is, the effect of receiving a specified intervention strategy versus a comparator strategy over time. This is the case when the analysis accounted for protocol deviations (switches during follow-up between the intervention strategies being compared, or other protocol deviations during follow-up). Such an analysis might be performed, for example, by partitioning follow-up for individual participants according to the intervention received, or by censoring follow-up when participants deviated from their initial intervention. When estimating the per protocol effect, both baseline confounding and time-varying confounding need to be addressed. It is usually **not appropriate** to attempt to control for time-varying confounding by including the time-varying confounders in regression models. Special methods ('g methods') that avoid 'conditioning on' time-varying confounders should be used. For example, in analyses that censor follow-up when participants deviate from their initial intervention strategy, one appropriate approach to analysis is to model the probability of such deviations over time, based on the baseline- and time—varying confounding factors, and then to adjusted for the censoring using inverse probability of censoring weights.

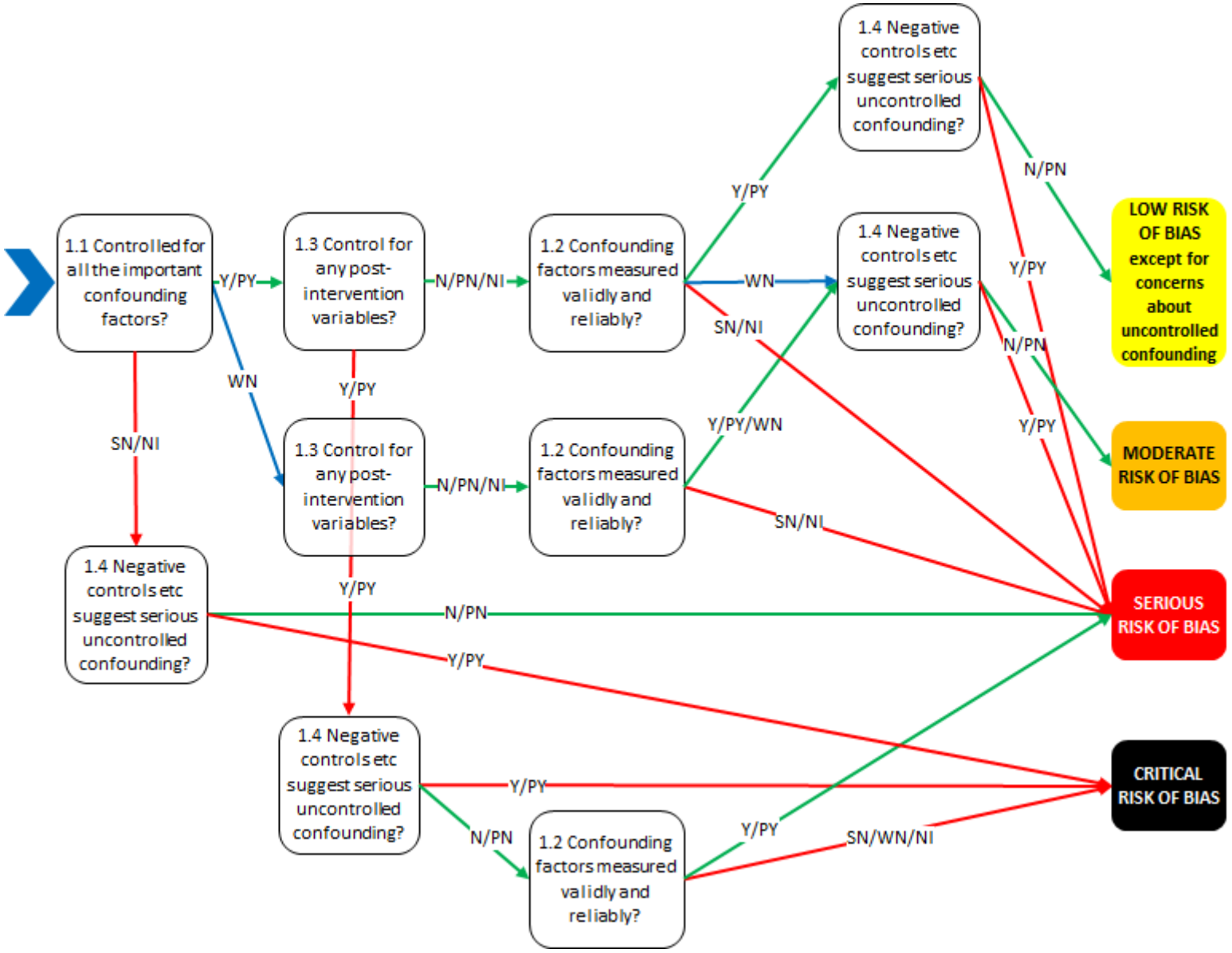
Domain 1, Variant A (the analysis is estimating the intention-to-treat effect so only baseline confounding needs to be addressed – if No to C4)

Signalling questions	Elaboration	Response

1.1 Did the authors control for all the important confounding factors for which this was necessary?	The important confounding factors are those specified in the *Preliminary consideration of confounding factors*. The preliminary assessment will have determined whether there were important confounding factors that were not controlled for and should have been (because there was no evidence that controlling for the variable was unnecessary). Failure to control for all important confounding factors may lead to bias. The analysis should attempt to control for these confounding factors using an appropriate method, for example using stratification, regression, matching, standardization or inverse probability weighting (control may be for individual variables or for estimated propensity scores). Answer 'Y' or 'PY' if all the important confounding factors for which it is was deemed necessary to control (under *Preliminary consideration of confounding factors*) were indeed controlled for appropriately. Also answer 'Y' or 'PY' in the (very rare) situation that there are no confounding factors and an unadjusted analysis is presented. Answer 'WN' if most of the important confounding factors for which it was deemed necessary to control (under *Preliminary consideration of confounding factors*) were controlled for using appropriate methods, and any uncontrolled confounding (because not all important confounding factors were controlled for) was not likely to be substantial. This would be the case, for example, if the factors that were not controlled for were likely to be highly correlated with factors that were controlled for. Answer 'SN' if there is at least one important confounding factor that should have been controlled for but was not, and the failure to control for this factor is likely to have a material impact on the estimated effect of intervention.	Y / PY / WN (no, but uncontrolled confounding was probably not substantial) / SN (no, and uncontrolled confounding was probably substantial) / NI
1.2 If Y/PY/WN to 1.1: Were confounding factors that were controlled for (and for which control was necessary) measured validly and reliably by the variables available in this study?	Appropriate control of confounding requires that the variables adjusted for are valid and reliable measures of the confounding factors. For some topics, a list of valid and reliable measures of confounding factors will be specified in the review protocol but for others such a list may not be available. Study authors may cite references to support the use of a particular measure. If authors control for confounding variables with no indication of their validity or reliability, then subjectivity of the measure should be evaluated. In the (very rare) situation that there are no confounding factors and an unadjusted analysis is presented, answer 'Y' or 'PY'.	NA / Y / PY / WN (no, but the extent of measurement error in confounding factors was probably not substantial) / SN (no, and the extent of measurement error in confounding factors was probably substantial) / NI

1.3 If Y/PY/WN to 1.1: Did the authors control for any post-intervention variables that could have been affected by the intervention?	Controlling for post-intervention variables that are affected by intervention is not appropriate (this is sometimes called 'over-adjustment'). Controlling for mediating variables estimates the direct effect of intervention and may introduce bias. Controlling for common effects of intervention and outcome (sometimes referred to as 'colliders') introduces bias.	NA / Y / PY / PN / N / NI
1.4. Did the use of negative controls, quantitative bias analysis, or other considerations, suggest serious uncontrolled confounding?	Use of a "negative control" – exploration of an alternative analysis in which no association should be observed – can sometimes suggest that the result is subject to uncontrolled confounding, if similar associations are identified for the result being assessed and the negative control. If the study did not use negative controls and no other considerations suggest uncontrolled confounding, answer 'N'. Answer 'Y' or 'PY' if negative controls indicate that the result being assessed suffers from material bias due to confounding.	Y / PY / PN / N
Risk of bias judgement	See algorithm.	Low (except for concerns about uncontrolled confounding) / Moderate / Serious / Critical
Optional: What is the predicted direction of bias due to confounding?	If the likely direction of bias can be predicted, it is helpful to state this. A judgement about the predicted direction of bias should take into account all uncontrolled confounding from omitted important confounders and may therefore require judgements about the relative impact of confounding factors operating in different directions. The effect of confounding is to bias the estimated effect upwards or downwards – to overestimate or to underestimate the intervention effect. If the true intervention effect is above 0 (above 1 for a ratio measure) then an upward bias will also represent a bias away from the null; if the true intervention effect is below 0 (below 1 for a ratio measure) then a downward bias will also represent a bias away from the null. The situation is not always so clear. For example, if the true effect is above 0 then a downward bias may bring the estimate towards the null or beyond it (to a value less than 0).	Upward bias (overestimate the effect) / Downward bias (underestimate the effect) / Unpredictable

Algorithm for reaching default risk of bias judgement:



Domain 1, Variant B (the analysis is estimating the per-protocol effect so both baseline and time-varying confounding need to be addressed – if Yes to C4)

Variant B of Domain 1 is used when the analysis accounted for protocol deviations such as switches during follow-up between the intervention strategies being compared, or stopping active intervention without a clinical indication to do so. Accounting for protocol deviations might be done for example by partitioning follow-up for individual participants according to the intervention received, or by censoring follow-up when participants deviated from their initial intervention. It is then necessary to control for both baseline and time—varying confounding.

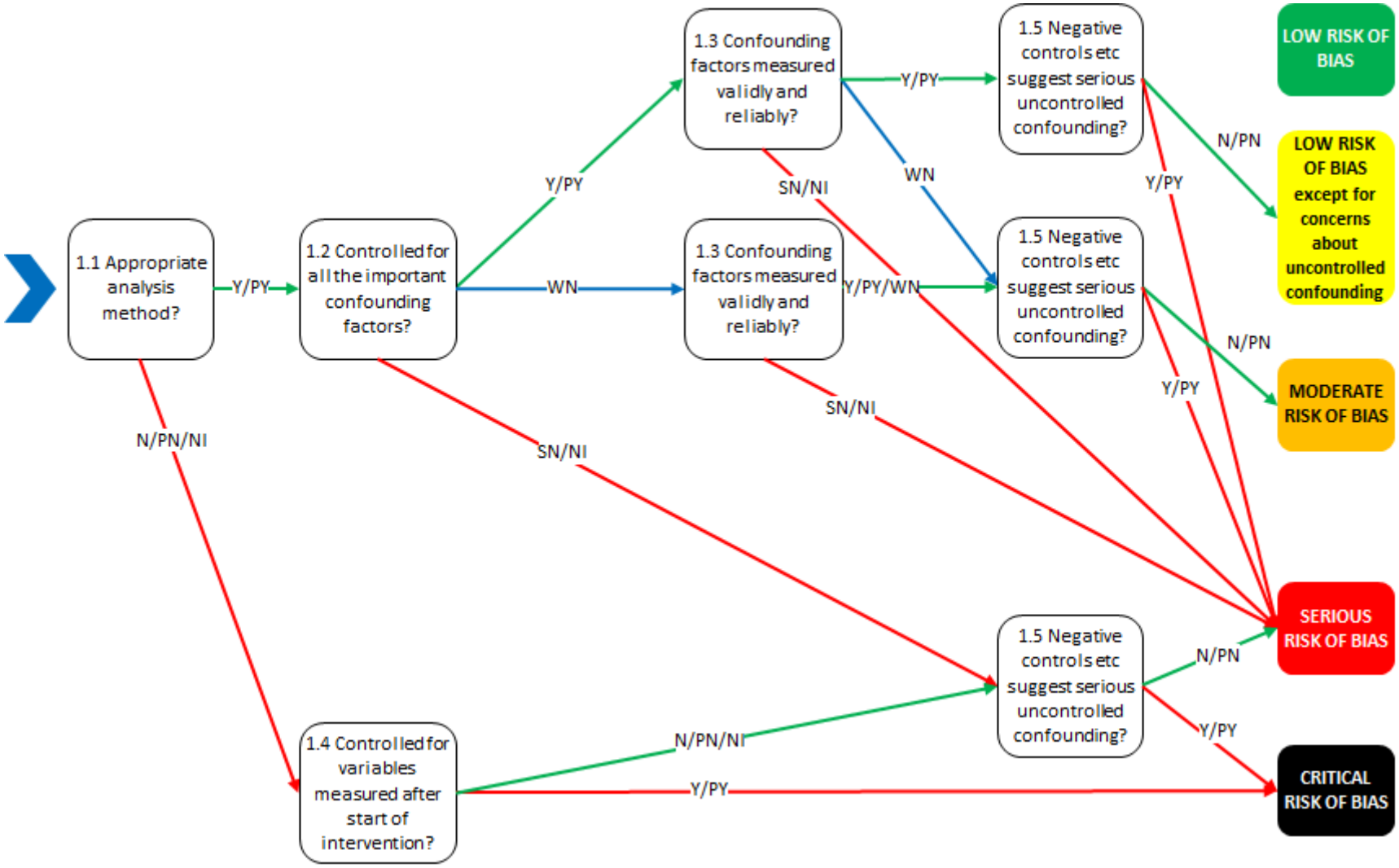
Signalling questions	Elaboration	Response
1.1 Did the authors use an analysis method that was appropriate to control for time-varying as well as baseline confounding?	Appropriate methods to control for time-varying confounding ('g methods') include those based on inverse probability weighting. Standard regression models that include time-varying confounders may be problematic when the time-varying confounders are affected by prior intervention (this is also known as treatment-confounder feedback).	Y / PY / PN / N / NI

1.2 If Y/PY to 1.1: Did the authors control for all the important baseline and time-varying confounding factors for which this was necessary?	The important confounding factors are those specified in the *Preliminary consideration of confounding factors*. For studies that allow for time-varying interventions, for example by partitioning follow up time according to intervention received or by censoring participants' follow-up when they deviate from their assigned intervention strategy, the analysis should control for both the baseline confounding factors and for time-varying confounders (prognostic factors that predict changes to intervention received). The preliminary assessment will have determined whether there were important confounding factors that were not controlled for and should have been (because there was no evidence that controlling for the variable was unnecessary). Failure to control for all important confounding factors may lead to bias. Answer 'Y' or 'PY' if all the important confounding factors for which it is was deemed necessary to control (under *Preliminary consideration of confounding factors*) were indeed controlled for. Also answer 'Y' or 'PY' in the (very rare) situation that there are no confounding factors and an unadjusted analysis is presented. Answer 'WN' if most of the important confounding factors for which it was deemed necessary to control (under *Preliminary consideration of confounding factors*) were controlled for, and the any uncontrolled confounding (because not all important confounding factors were controlled for) was not likely to be substantial. This would be the case, for example, if the factors that were not controlled for were likely to be highly correlated with factors that were controlled for. Answer 'SN' if there is at least one important confounding factor that should have been controlled for but was not, and the failure to control for this factor is likely to have a material impact on the estimated effect of intervention.	NA / Y / PY / WN (no, but uncontrolled confounding was probably not substantial) / SN (no, and uncontrolled confounding was probably substantial) / NI

1.3 If Y/PY/WN to 1.2: Were confounding factors that were controlled for (and for which control was necessary) measured validly and reliably by the variables available in this study?	Appropriate control of confounding requires that the variables adjusted for are valid and reliable measures of the confounding factors. For some topics, a list of valid and reliable measures of confounding factors will be specified in the review protocol but for others such a list may not be available. Study authors may cite references to support the use of a particular measure. If authors control for confounding variables with no indication of their validity or reliability, then subjectivity of the measure should be evaluated. In the (very rare) situation that there are no confounding factors and an unadjusted analysis is presented, answer 'Y' or 'PY'.	NA / Y / PY / WN (no, but the extent of measurement error in confounding factors was probably not substantial) / SN (no, and the extent of measurement error in confounding factors was probably substantial) / NI
1.4 If N/PN/NI to 1.1: Did the authors control for time-varying factors or other variables measured after the start of intervention?	This question is asked if an inappropriate analysis method has been used to control for time-varying confounding factors. In such a situation, controlling for (conditioning on) time-varying factors measured after the start of intervention is likely to lead to bias if these are also on the causal pathway from the intervention to the outcome.	NA / Y / PY / PN / N / NI
1.5 Did the use of negative controls, or other considerations, suggest serious uncontrolled confounding?	Use of a "negative control" – exploration of an alternative analysis in which no association should be observed – can sometimes suggest that the result is subject to uncontrolled confounding, if similar associations are identified for the result being assessed and the negative control. If the study did not use negative controls and no other considerations suggest uncontrolled confounding, answer 'N'. Answer 'Y' or 'PY' if negative controls indicate that the result being assessed suffers from material bias due to confounding.	Y / PY / PN / N
Risk of bias judgement	See algorithm.	Low (except for concerns about uncontrolled confounding) / Moderate / Serious / Critical

Optional: What is the predicted direction of bias due to confounding?	If the likely direction of bias can be predicted, it is helpful to state this. A judgement about the predicted direction of bias should take into account all uncontrolled confounding from omitted important confounders and may therefore require judgements about the relative impact of confounding factors operating in different directions. The effect of confounding is to bias the estimated effect upwards or downwards – to overestimate or to underestimate the intervention effect. If the true intervention effect is above 0 (above 1 for a ratio measure) then an upward bias will also represent a bias away from the null; if the true intervention effect is below 0 (below 1 for a ratio measure) then a downward bias will also represent a bias away from the null. The situation is not always so clear. For example, if the true effect is above 0 then a downward bias may bring the estimate towards the null or beyond it (to a value less than 0).	Upward bias (overestimate the effect) / Downward bias (underestimate the effect) / Unpredictable

Algorithm for reaching default risk of bias judgement:



2. Bias in classification of interventions

Guidance note 12 *: bias in classification of interventions*

This domain addresses bias arising from misclassification of the intervention strategies being compared. The first three questions in the domain address a type of misclassification bias arising from **immortal time**, time during which outcome events are prevented from happening by the way the analysis was done. Such analytical problems generally occur when the intervention and comparator strategies cannot be distinguished at the time when follow-up would have started in the target trial, making it difficult or impossible to know which intervention strategy participant were following. This is the first of two types of bias arising from immortal time that are addressed in ROBINS-I V2 (the second type, which is usually less serious, is addressed in *Domain 3, Bias in selection of participants into the study*).

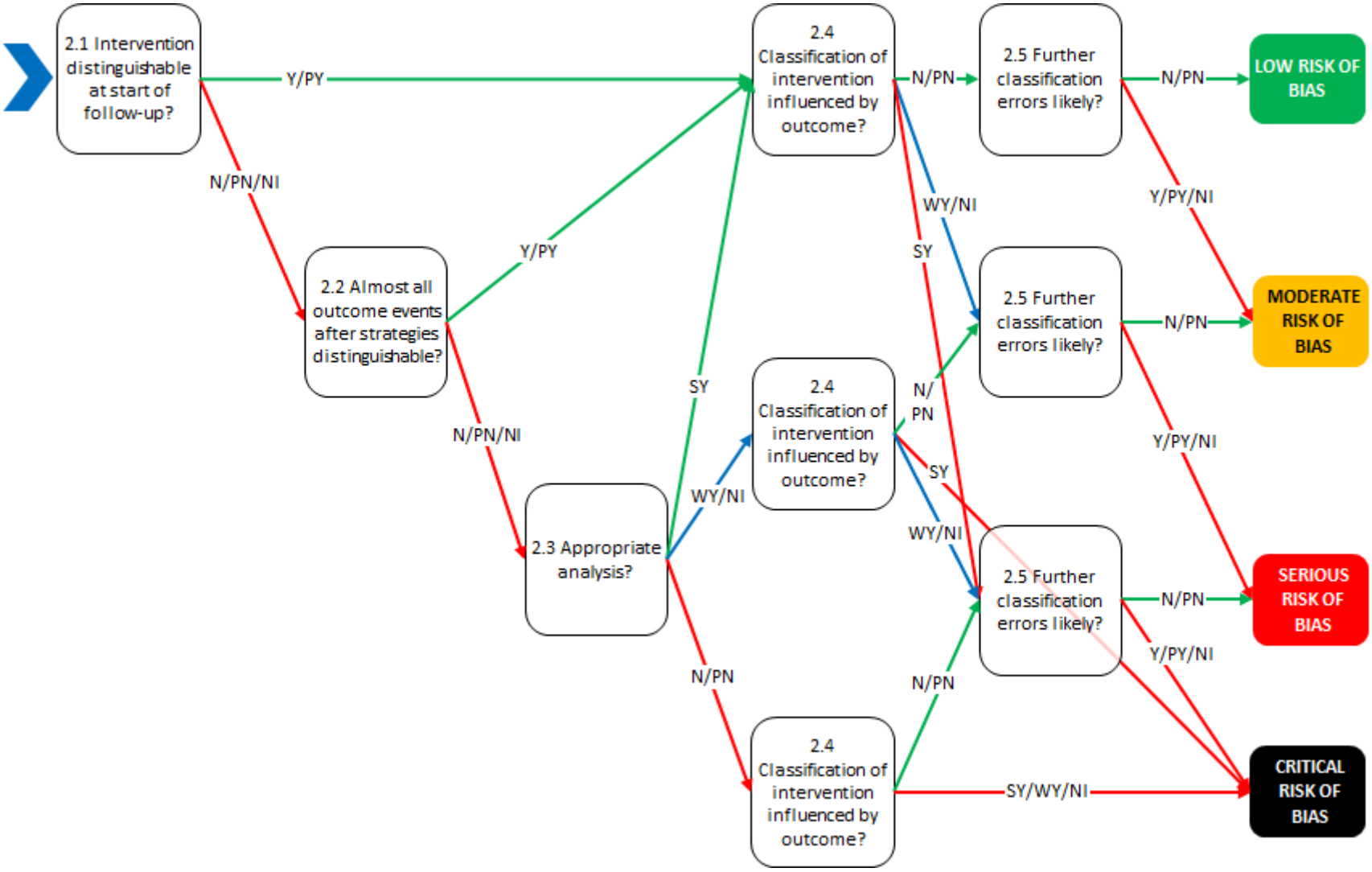
Misclassification is generally considered to be **differential** if it is related to subsequent outcomes; or **non differential** if it is unrelated to subsequent outcomes. The fourth and fifth signalling questions in the domain address differential misclassification and non-differential misclassification, respectively. Differential misclassification, which is generally considered to be more serious than non-differential misclassification, may occur when classification of intervention status is influenced by knowledge of the outcome or risk of the outcome.

Signalling questions	Elaboration	Response options
2.1 Were the intervention strategies distinguishable at the time when follow-up would have started in the target trial?	In the target trial, follow-up starts when participants meet eligibility criteria and are assigned to a strategy. In most non-randomized studies, there is no formal assignment and so participants are classified according to information on interventions that were prescribed or received. However, some strategies cannot be distinguished at the start of follow-up. For example, for the strategies "Surgery within 6 months of diagnosis" and "Delay surgery until clinical progression of disease", then follow-up starts at the time of diagnosis and patients who do not undergo surgery and do not experience disease progression have adhered to both strategies until 6 months after diagnosis. Assigning participants to intervention strategies based on information after the start of follow-up may lead to participants in one of the groups having a period of 'immortal time' during which the outcome cannot occur. In the example above, participants who are assigned to the intervention group because they have surgery within 6 months of diagnosis cannot experience the outcome during the period between diagnosis and surgery.	Y / PY / PN / N / NI
2.2 If N/PN/NI to 2.1: Did all or nearly all outcome events occur after the intervention and comparator strategies could be distinguished?	If the period during which the intervention and control strategies cannot be distinguished is short in relation to the total follow-up, then the proportion of outcome events occurring during that period may be low. This limits the risk of bias due to misclassification of participants into intervention groups based on events that occurred after the start of follow-up.	NA / Y / PY / PN / N / NI

2.3 If N/PN/NI to 2.2: Did the analysis avoid problems arising from intervention strategies that are not distinguishable at the start of follow-up?	Analyses using, for example, the 'clone-censor-weighting method' (https://www.bmj.com/content/360/bmj.k182.long) or the g-formula can overcome problems associated with intervention strategies that are not distinguishable at the start of follow-up. For analyses using these approaches, answer 'SY' if predictors of treatment during follow-up were measured and used appropriately to derive inverse-probability weights. Answer 'WY' if the analysis used these methods or another appropriate method but is unlikely to have fully adjusted for prognostic factors that predict treatment after the start of follow-up. Answer 'SY' if the study used a 'landmark' analysis, which avoids these problems by starting follow-up after the intervention strategies can be distinguished. However, note that a 'landmark' analysis will be at risk of selection bias, which is addressed in the domain 'Bias in selection of participants into the study'.	NA / SY (yes, fully) / WY (yes, partially) / PN / N / NI
2.4 Was classification of intervention status influenced by knowledge of the outcome or risk of the outcome?	Differential misclassification arises if the outcome, or the factors influencing the outcome (other then the interventions), influence the classification. If intervention classification is based on information that was collected after follow-up started, this information may be influenced by the outcome or the risk of the outcome, for example if participants were asked to recall past interventions. Differential misclassification is less likely if all the information used to classify the intervention and comparator groups came from sources that were recorded at or before the time that interventions were assigned and follow-up started. It is possible for outcome events to affect the availability of information used to classify interventions in a non-randomized study. For example, if records of receipt of an intervention are destroyed if a participant dies (leading to misclassification of that participant into the comparator group) then analyses investigating the effect of the intervention on mortality will be biased due to differential misclassification depending on the outcome. Response options 'WY' and 'SY' are used to distinguish between different risk-of-bias judgements. Answer 'SY' if the impact of knowledge of the outcome or risk of bias outcome was likely to be substantial.	SY (yes, and the impact was substantial) / WY (yes, but the impact was not substantial) / PN / N / NI
2.5 Were further classification errors (not influenced by knowledge of the outcome or risk of the outcome) likely?	This question relates to non-differential misclassification (that is, unrelated to the subsequent outcome) of intervention status. For example, intervention status may be misclassified for some participants if receipt of (or assignment to) the intervention is not recorded in the information source used to classify intervention status. Similarly, some participants may receive (or be assigned to)	Y / PY / PN / N / NI

	the intervention without it being recorded. Misclassification errors of this nature usually bias the result towards the null. Criteria for considering individuals to have received each intervention should be clear and explicit, covering issues such as type, setting, dose, frequency, intensity and timing of intervention. A pre-requisite for correct classification of interventions is that the interventions are well defined. Ambiguity in the definition may lead to misclassification of participants, and so is likely to lead to a response of 'N' or 'PN' to this question. It may be helpful to think separately about (i) whether all people who were assigned to (or who received) the intervention were correctly classified, and (ii) whether all people who were assigned to (or who received) the comparator were correctly classified. "Nearly all" should be interpreted as "enough to be confident of the findings", and a suitable proportion will depend on the context. In many situations, correct classification for 95% of the participants may be sufficient.	
Risk of bias judgement	See algorithm.	Low / Moderate / Serious / Critical
Optional: What is the predicted direction of bias in classification of interventions?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized as being in favour of the intervention, as being in favour of the comparator, or as towards (or away from) the null. Non-differential misclassification will often bias results towards the null.	Favours intervention / Favours comparator / Towards null /Away from null / Unpredictable

Algorithm for reaching default risk of bias judgement:



3. Bias in selection of participants into the study (or into the analysis)

Guidance note 13 *: bias in selection of participants into the study or into the analysis*

In their book *Causal Inference: What If*, Robins and Hernán define the term 'selection bias' as encompassing "various biases that arise from the procedure by which individuals are selected into the analysis". ROBINS-I focusses on selection biases that affect estimates of the effect of an intervention (versus a comparator) on an outcome: these arise from 'conditioning on a common effect of the intervention and the outcome'. 'Conditioning' on a variable happens when we restrict the analysis to people with a chosen value or range of values of the variables, or if we include the variable in a regression model for the effect of the intervention on the outcome.

A well-known example of selection bias is from studies of the effect of folic acid supplementation on the risk of neural tube defects in infancy. Many such studies were restricted, for pragmatic reasons, to babies born alive. However, both folic acid supplementation and foetal neural tube defects affect whether a pregnancy ends in live birth. The analysis was affected by selection bias because it was restricted to live births and excluded pregnancies that did not end in a live birth.

Selection bias is addressed in two ROBINS-I bias domains. This domain (Domain 3, Bias in selection of participants into the study (or into the analysis) addresses selection biases arising because participants, periods of follow-up or outcome events were omitted from the analysis because of participants characteristics that were measured, or events that occurred, after the start of the intervention strategies.

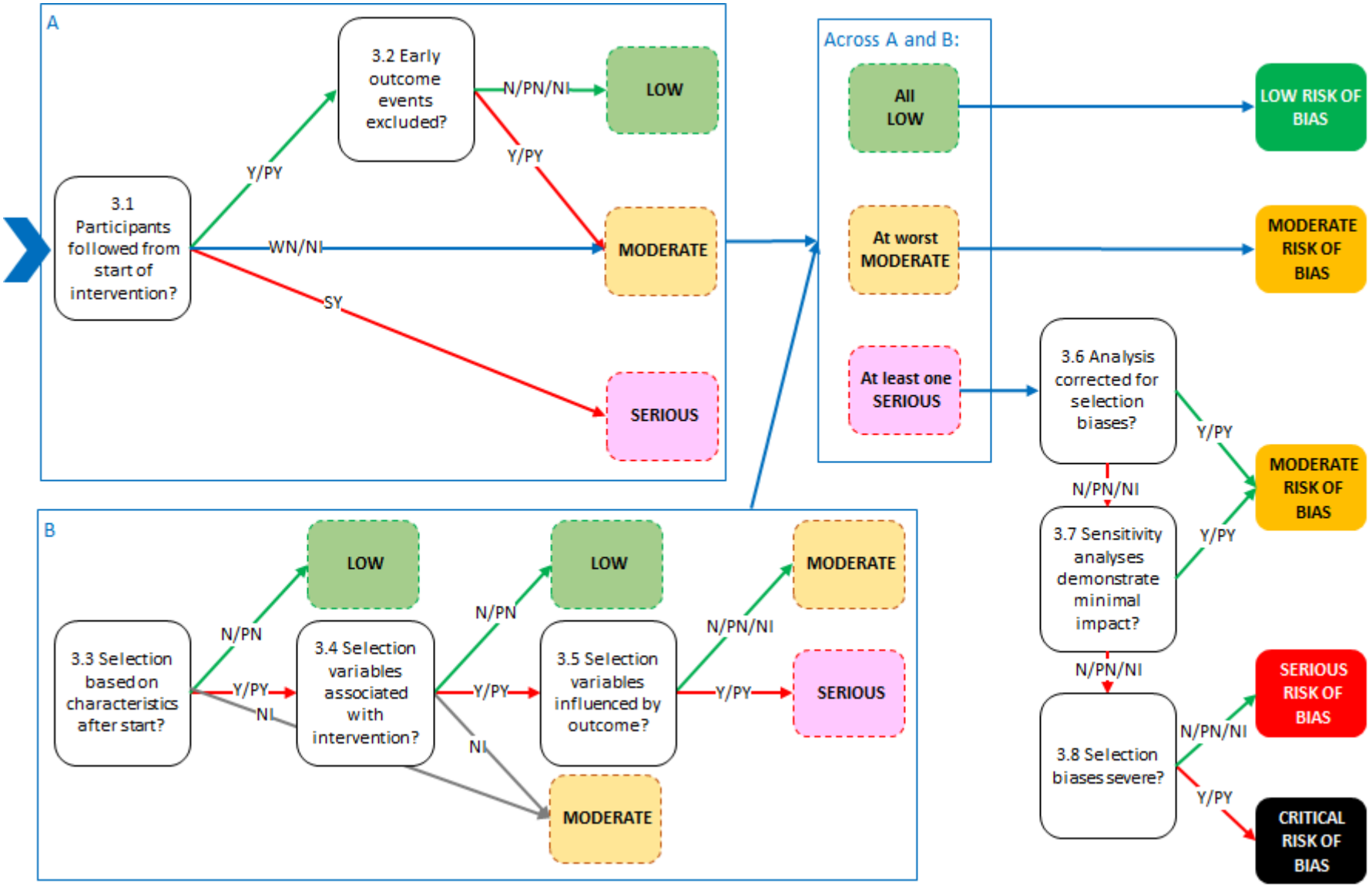
Domain 5 (Bias due to missing data) addresses selection biases that arise when because participants are excluded from the analysis because of missing data, or when analyses accounting for missing data cannot correct for the selection bias.

Signalling questions	Elaboration	Response options
A. Questions about prevalent user bias and immortal time		
3.1 Did follow up in the analysis begin at the start of the intervention strategies being compared?	Answer this question by considering the analysis of the study under consideration in relation to the target trial. Answer ''Y' or 'PY' if all outcome events and follow-up time after the start of the intervention and comparator strategies were included in the analysis that led to the study result under consideration. If the comparison strategy is "no intervention" then follow up can start at any point at which participants remain eligible to start the intervention. Analyses in which follow-up starts after the start of the intervention are sometimes called 'landmark' analyses. In such analyses, a follow-up period that would have been observed in the target trial is missing and participants who experienced the outcome soon after the start of the intervention will be missing from analyses. This problem may occur when prevalent, rather than new (incident), users of the intervention are included in analyses. The bias introduced by this is sometimes referred to as prevalent user bias. Answer 'SY' if either: (i) the effect of intervention is likely to differ markedly between the excluded and included periods of follow-up; or (ii) a substantial	Y / PY / WN (no, but the risk of bias was not substantial) / SN (no, leading to a substantial risk of bias) / NI

	proportion of follow-up or outcomes after the start of the interventions were excluded.	
3.2 If Y/PY to 3.1: Were outcome events during a period of follow-up after the start of the interventions excluded from the analysis?	If follow-up in the study begins at the start of the interventions but there was a period after the start of intervention during which outcomes were not ascertained or the events were excluded from the analysis, then this period is 'immortal time' during which events cannot occur.	Y / PY / PN / N / NI
B. Questions about other types of selection bias		
3.3 Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention (additional to the situations addressed in 3.1 and 3.2)?	Answer 'Y' or 'PY' to this question if selection into the study was based on participant characteristics observed *after* the start of intervention, for reasons additional to those addressed in parts A and B of this domain. Answer 'N' or 'PN' if selection was based only on characteristics observed *before* the start of intervention. Such selection can be addressed by controlling for imbalances between intervention and comparator groups in baseline characteristics that are prognostic for the outcome (baseline confounding). This domain *does not* address exclusion because of missing data (incomplete information on a variable measured in some participants).	Y / PY / PN / N / NI
3.4 If Y/PY to 3.3: Were the post-intervention variables that influenced selection likely to be associated with intervention?	Selection bias occurs when selection is related to an effect of either intervention or a cause of intervention **and** an effect of either the outcome or a cause of the outcome. Therefore, the result is at risk of selection bias if selection into the study is related to both the intervention and the outcome.	NA / Y / PY / PN / N / NI
3.5 If Y/PY to 3.4: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
C. Questions about analysis, sensitivity analyses and severity of the problem		
3.6 If SN to 3.1 or Y/PY to 3.5: Is it likely that the analysis corrected for all of the potential selection biases identified above?	In some circumstances, for example when data for a period of follow-up are completely unavailable, then it is not possible to correct for the resulting selection bias. In other situations, it may be possible to correct for selection biases, for example by using inverse probability weights to create a pseudo-population in which the selection bias has been removed or by modelling the distributions of the missing follow up times and outcome events in prevalent users of intervention and including them using missing data methodology. Answer 'Y' or 'PY' if appropriate methods were used and the assumptions required for their validity were likely to be justified. Otherwise, the answer to this question will usually be 'N' or 'PN'.	NA / Y / PY / PN / N / NI

3.7 If N/PN/NI to 3.6: Did sensitivity analyses demonstrate that the likely impact of the potential selection biases identified above was minimal?		NA / Y / PY / PN / N / NI
3.8 If N/PN/NI to 3.7: Were potential selection biases identified above sufficiently severe that the result should not be included in a quantitative synthesis?	This question is used to distinguish between 'Serious' and 'Critical' risk of selection bias. Answer 'N', 'PN' or 'NI' unless there is clear evidence that the selection biases identified were severe.	NA / Y / PY / PN / N / NI
Risk of bias judgement	See algorithm.	Low / Moderate / Serious / Critical
Optional: What is the predicted direction of bias in selection of participants into the study?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized as being in favour of the intervention, as being in favour of the comparator, or as towards (or away from) the null.	Favours intervention / Favours comparator / Towards null /Away from null / Unpredictable

Algorithm for reaching default risk of bias judgement



4. Bias due to missing data

Guidance note 14 *: bias due to missing data*

Missing outcome data may arise, among other reasons, through attrition (loss to follow up), missed appointments and incomplete data collection. Additionally, in non-randomized studies data may be missing for characteristics including interventions received and confounders.

A general rule for consideration of bias due to missing data is that we should consider biases introduced by the missing data, compared with the effect estimate from an analysis in which all the data we intended to collect were available. Unfortunately, a single threshold for an acceptable proportion of missing data cannot meaningfully be defined. For example, a result based on 95% complete outcome data might be biased if the outcome was rare and if reasons for missing outcome data were strongly related to intervention group. Therefore, the potential for bias due to missing data should be assessed unless complete data on intervention status, the outcome and confounding variables were available all, or nearly all, participants.

Considerations of bias due to missing data depend on how the analysis accounted for the missing data. Different signalling questions should be answered depending on three types of analysis. The first is that a **complete case analysis**, restricted to participants with complete data on all the intervention, outcome and confounding variables, was performed. In this situation, an important consideration is whether missingness of individual participants from the analysis is related to the true value of the outcome for those participants. The second is that missing data were **imputed**, which means that estimated or assumed values were assigned to participants with missing data. Imputed data should not lead to bias if the data are 'missing at random' (see the elaboration for signalling question 4.8) and an appropriate imputation method is applied. Other types of analysis are addressed by a separate, general, signalling question. The final signalling question asks whether sensitivity analyses were performed that demonstrated that the impact of missing data is minimal.

Further discussion of many of these issues can be found in Lee et al (J Clin Epidemiol 2021;134:79-88) and Hughes et al (IJE 2019;48:1294-1304).

Signalling questions	Elaboration	Response options
4.1 Were complete data on intervention status available for all, or nearly all, participants?	"Nearly all" should be interpreted as that the number of participants excluded from the analysis due to missing data is so small that they could have made no important difference to the estimated effect of the intervention. Only answer 'NI' if the study report provides no information about the extent of missing data. This situation will usually lead to a judgement that there is a high risk of bias due to missing data. Note that this question refers to data actually recorded in the study. Imputed data (see question 4.7) should be regarded as missing data in the context of this question.	Y / PY / PN / N / NI
4.2 Were complete data on the outcome available for all, or nearly all, participants?	"Nearly all" should be interpreted as that the number of participants excluded from the analysis due to missing data is so small that they could have made no important difference to the estimated effect of the intervention. For continuous outcomes, complete data for 95% (or possibly 90%) of the participants would often be sufficient. For dichotomous outcomes, the proportion required is directly	Y / PY / PN / N / NI

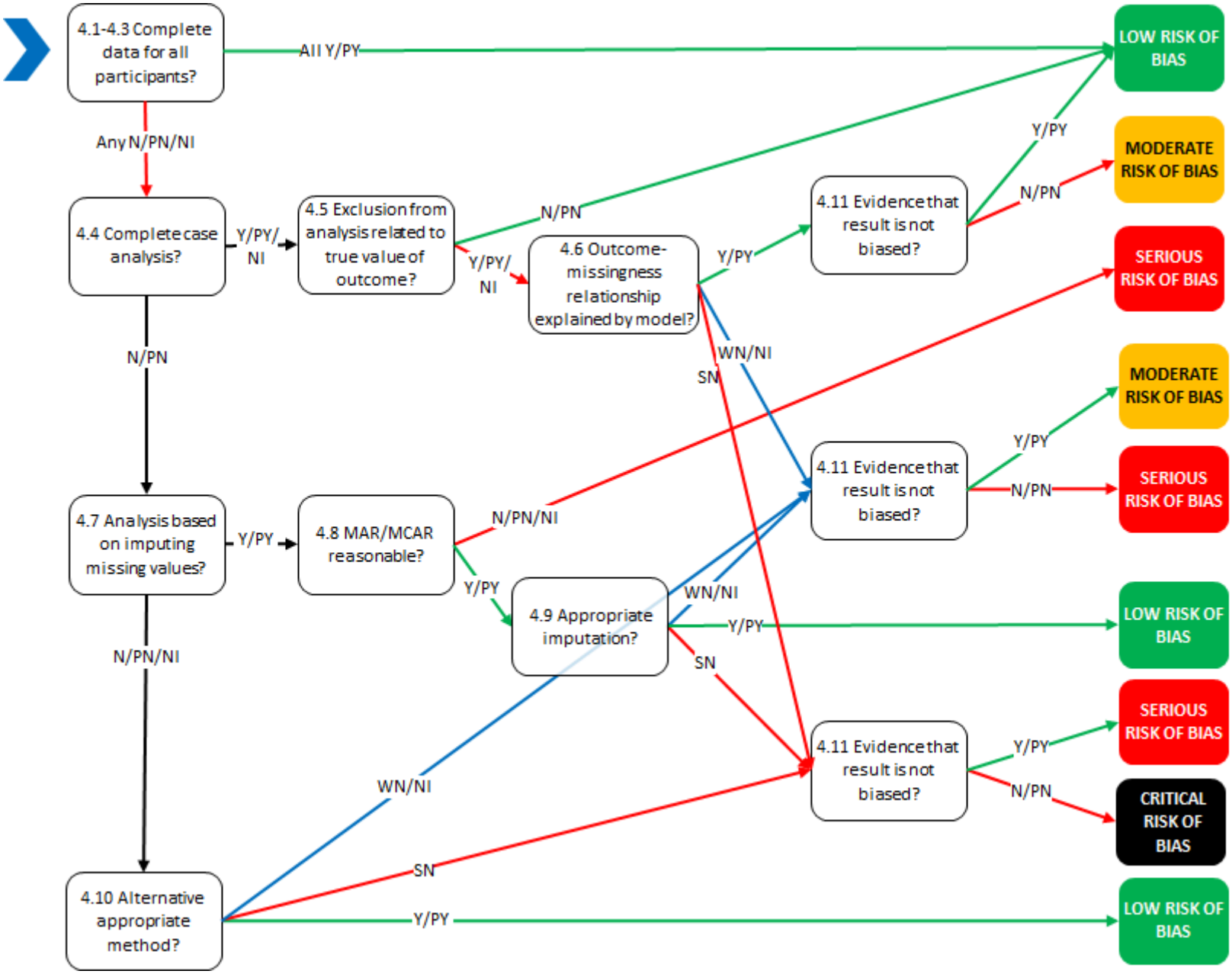
	linked to the risk of the outcome event. If the observed number of outcome events is much greater than the number of participants with missing data, the bias would necessarily be small. Only answer 'NI' if the study report provides no information about the extent of missing data. This situation will usually lead to a judgement that there is a high risk of bias due to missing data. Note that this question refers to data actually recorded in the study. Imputed data should be regarded as missing data in the context of this question.	
4.3 Were complete data on important confounding variables available for all, or nearly all, participants?	"Nearly all" should be interpreted as that the number of participants excluded from the analysis due to missing data is so small that they could have made no important difference to the estimated effect of the intervention. Only answer 'NI' if the study report provides no information about the extent of missing data. This situation will usually lead to a judgement that there is a high risk of bias due to missing data. Note that this question refers to data actually recorded in the study. Imputed data should be regarded as missing data in the context of this question.	Y / PY / PN / N / NI
4.4 If N/PN/NI to 4.1, 4.4 or 4.4: Is the result based on a complete case analysis?	How risk of bias is assessed depends on whether a complete case analysis has been done. A complete case analysis is one that is restricted to participants with complete data on all the intervention, outcome and confounding variables.	NA / Y / PY / PN / N / NI
4.5 If Y/PY/NI to 4.4: Was exclusion from the analysis because of missing data (in intervention, confounders or the outcome) likely to be related to the true value of the outcome?	This question aims to identify situations in which a "complete case" analysis will be at risk of bias due to missing data. A complete case analysis is one that includes all participants who provide full data for the variables involved in the analysis. A complete case analysis may be biased if missingness (in the intervention, outcome or confounders) is related to the outcome. For example, if it is likely that participants with underlying health problems missed a visit at which baseline intervention status or confounders should have been measured, their consequent exclusion from the analysis may be related to their eventual outcome. Four reasons for answering 'Y' or 'PY' are: (1) There are differences between intervention groups or confounder groups/levels in the proportions of participants excluded from the analysis due to missing outcome data. For time-to-event-data, the analogue is that rates of censoring (loss to follow-up) depend on the intervention group. (2) There are differences between outcome groups/levels in the proportions of participants excluded from the analysis due to missing intervention/confounder data.	NA / Y / PY / PN / N / NI

	(3) Reported reasons for missing outcome data provide evidence that missingness in the outcome depends on the true outcome or a cause of the outcome; (4) It is reasonable to assume that the circumstances of the study make it likely that missingness in the outcome depends on its true value. For example, if the outcome is severe depression, it may be likely that participants experiencing the outcome miss appointments at which it would have been recorded. Answer 'N' or 'PN' if missing data, loss to follow up or withdrawal occurred for documented reasons that are unrelated to the outcome, in which case the risk of bias due to missing data will be low.	
4.6 If Y/PY/NI to 4.5: Is the relationship between the outcome and missingness likely to be explained by the variables in the analysis model?	If all the variables that plausibly explain the relationship between the outcome and missingness (in intervention, confounders or the outcome) are included in the complete case analysis, then the risk of bias will be low. For example, in a regression of blood pressure at age 55 (outcome) on reducing salt intake (intervention) that adjusts for the confounders sex, education level and blood pressure measured at age 25, if women were more likely to have blood pressure measured at age 25 and if sex is the only variable plausibly related to missingness, this would not cause bias because sex is adjusted for in the analysis model. Hence blood pressure at age 55 is not related to missingness in blood pressure at age 25, once sex is adjusted for. Technical note: If a mediator (a variable on the causal pathway from intervention to outcome) affects missingness, than adjusting for this variable would be appropriate to avoid bias due to missing data in a complete case analysis, but would change the intervention effect being estimated. In the presence of such variables, multiple imputation (see question 4.7) should be used to address bias due to missing data. Adjusting for a mediator will increase the risk of bias due to confounding (domain 1).	NA / Y / PY / WN (No, but not leading to substantial bias) / SN (No, and bias is likely to be substantial) / NI
4.7 If N/PN to 4.4: Was the analysis based on imputing missing values?	Imputing missing values is the process of assigning estimated or assumed values to them for use in the main analysis. Answer 'Y' or 'PY' if the analysis was based on either single or multiple imputation.	NA / Y / PY / PN / NI

4.8 If Y/PY to 4.7: Is it reasonable to assume that data were 'missing at random' (MAR) or 'missing completely at random' (MCAR)?	In their book *Statistical analysis with missing data* (Wiley 2002), Little and Rubin proposed commonly-used categorizations of missing data. These were summarized by Sterne et al (BMJ 2009; **338**: b2393) as follows: *Missing completely at random* (MCAR): There are no systematic differences between the missing values and the observed values. For example, blood pressure measurements may be missing because of breakdown of an automatic sphygmomanometer. *Missing at random* (MAR): any systematic difference between the missing values and the observed values can be explained by differences in observed data. For example, missing blood pressure measurements may be lower than measured blood pressures but only because younger people may be more likely to have missing blood pressure measurements. *Missing not at random* (MNAR): Even after the observed data are taken into account, systematic differences remain between the missing values and the observed values. For example, people with high blood pressure may be more likely to miss clinic appointments because they have headaches. Analyses based on multiple imputation can avoid bias due to missing data provided that the incomplete variables for which data are imputed are MAR or MCAR, but not if data are MNAR. Answer 'N' or 'PN' if there is a reason to believe that data are missing not at random (MNAR). Otherwise, answer 'Y' or 'PY'.	NA / Y / PY / PN / N / NI
4.9 If Y/PY to 4.8: Was imputation performed appropriately?	Answer 'SN' or 'WN' if simple imputation methods such as last observation carried forward or imputing a mean value are used. The amount of bias likely to be introduced by this will depend on the proportion of participants with missing data. Answer 'Y' or 'PY' if multiple imputation was used and (i) all the predictors of missingness in any variable were included in the imputation models; and (ii) all the variables in the model used for the main analysis were included in the imputation models.	NA / Y / PY / WN (no, but not leading to substantial bias) / SN (no, such that bias would not be substantially reduced) / NI
4.10 If N/PN/NI to 4.7: Was an appropriate alternative method used to correct for bias due to missing data?	This signalling question covers situations in which the analysis was neither a complete case analysis nor based on imputing missing values. Examples of such analyses include inverse probability weighting and full information maximum likelihood. If weighting is used, its validity depends on the weighting model being correctly specified (see Seaman and White, Stat Methods Med Res 2013; 22: 278-95). In these situations, the ROBINS-I assessor (possibly in conjunction with a statistician knowledgeable about missing data methods) should attempt to determine whether the analysis was appropriate to correct for any biases.	NA / Y / PY / WN (no, but not leading to substantial bias) / SN (no, such that bias would not be substantially reduced) / NI

4.11 If PN/N/NI to 4.1, 4.2 or 4.3 AND (Y/PY/NI to 4.5 OR WN/SN/NI to 4.9 OR WN/SN/NI to 4.10): Is there evidence that the result was not biased by missing data?	Evidence that the result was not biased by missing data may come from: (1) analysis methods that would not be biased under plausible relationships between the missing values and the likelihood that data are missing; or (2) sensitivity analyses showing that results are little changed under a range of plausible assumptions about the missing values. Note that multiple imputation based only on outcome, intervention and confounder information should not be assumed to correct for bias due to missing data, so similarity between results with and without such imputation should not be taken as reassurance when answering this question. Similarly, a weighted analysis should not be assumed to correct for bias due to missing data without further consideration of (1) and (2) above, or sensitivity analyses.	NA / Y / PY / PN / N
Risk of bias judgement	See algorithm.	Low / Moderate / Serious / Critical
Optional: What is the predicted direction of bias due to missing data?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized as being in favour of the intervention, as being in favour of the comparator, or as towards (or away from) the null.	Favours intervention / Favours comparator / Towards null /Away from null / Unpredictable

Algorithm for reaching default risk of bias judgement:


4.1-4.3 Complete data for all participants?
All Y/PY
LOW RISK OF BIAS
Any N/PN/NI
4.4 Complete case analysis?
Y/PY/ NI
4.5 Exclusion from analysis related to true value of outcome?
N/PN
Y/PY/ NI
4.6 Outcome-missingness relationship explained by model?
Y/PY
4.11 Evidence that result is not biased?
Y/PY
N/PN
MODERATE RISK OF BIAS
SERIOUS RISK OF BIAS
WN/NI
SN
N/PN
4.7 Analysis based on imputing missing values?
Y/PY
4.8 MAR/MCAR reasonable?
N/PN/NI
4.11 Evidence that result is not biased?
Y/PY
N/PN
MODERATE RISK OF BIAS
SERIOUS RISK OF BIAS
Y/PY
4.9 Appropriate imputation?
WN/NI
Y/PY
LOW RISK OF BIAS
SN
N/PN/NI
4.11 Evidence that result is not biased?
Y/PY
N/PN
SERIOUS RISK OF BIAS
CRITICAL RISK OF BIAS
WN/NI
SN
4.10 Alternative appropriate method?
Y/PY
LOW RISK OF BIAS

5. Bias in measurement of the outcome

Guidance note 15 *: bias in measurement of the outcome*

Bias may be introduced if outcomes are misclassified or measured with error. Misclassification or measurement error of outcomes may be non-differential or differential.

Non-differential measurement error is unrelated to the intervention received. It can be systematic (for example when measurement of blood pressure is consistently 5 units too high in every participant) – in which case it will not affect precision or cause bias; or it can be random (for example when measurement of blood pressure is sometimes too high and sometimes too low in a manner that does not depend on the intervention or the outcome) – in which case it will affect precision without causing bias.

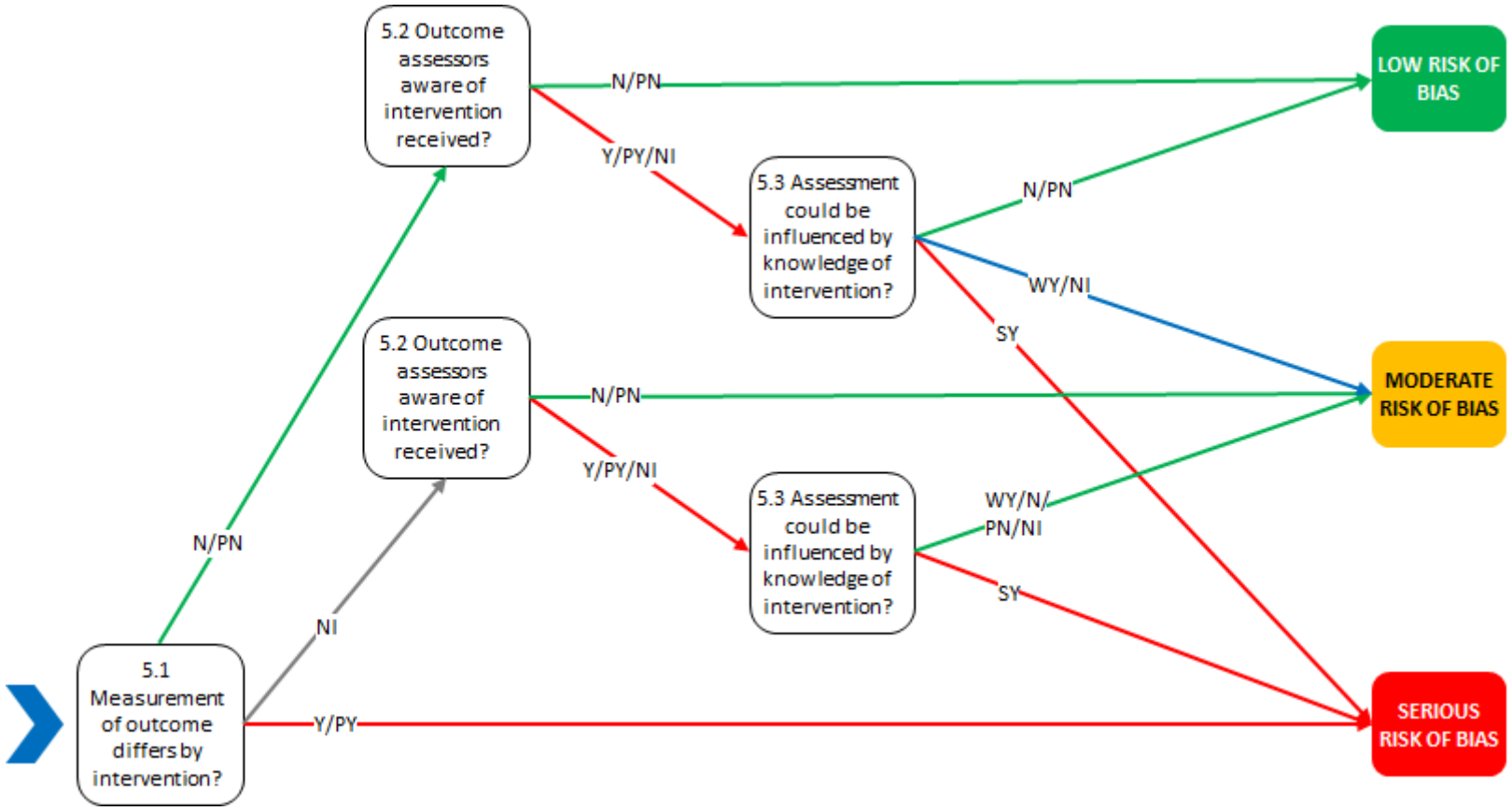
Differential measurement error is measurement error related to intervention received. It will bias the intervention-outcome relationship. This is often referred to as detection bias. Examples of situations in which detection bias can arise are (i) if outcome assessors are aware of intervention received (particularly when the outcome is subjective); (ii) different methods (or intensities of observation) are used to assess outcomes of participants receiving different interventions; and (iii) measurement errors are related to intervention received (or to a confounder of the intervention-outcome relationship).

Blinding of outcome assessors aims to prevent systematic differences in measurements according to intervention received. However, blinding is frequently not possible or not performed for practical reasons.

Signalling questions	Elaboration	Response options
5.1 Could measurement or ascertainment of the outcome have differed between intervention groups?	Comparable methods of outcome measurement (data collection) involve the same measurement methods and thresholds, used at comparable time points. Differences between intervention groups may arise because of 'diagnostic detection bias' in the context of passive collection of outcome data, or if an intervention involves additional visits to a healthcare provider, leading to additional opportunities for outcome events to be identified.	Y / PY / PN / N / NI
5.2 Were outcome assessors aware of the intervention received by study participants?	Answer 'N' if outcome assessors were blinded to intervention status. In other situations, outcome assessors may be unaware of the interventions being received by participants despite there being no active blinding by the study investigators; the answer this question would then also be 'N'. In studies where participants report their outcomes themselves, for example in a questionnaire, the outcome assessor is the study participant. In an observational study, the answer to this question will usually be 'Y' when the participants report their outcomes themselves.	Y / PY / PN / N / NI

5.3 If Y/PY/NI to 5.2: Could assessment of the outcome have been influenced by knowledge of the intervention received?	Knowledge of the assigned intervention could influence participant-reported outcomes (such as level of pain), observer-reported outcomes involving some judgement, and intervention provider decision outcomes. Knowledge of the assigned intervention is unlikely to influence observer-reported outcomes that do not involve judgement, for example all-cause mortality or laboratory measures of levels of substances in the blood. The response options distinguish between situations in which (i) knowledge of intervention status could have influenced outcome assessment but there is no reason to believe that it did from those in which (ii) knowledge of intervention status was likely to influence outcome assessment. When there are strong levels of belief in or preference for either beneficial or harmful effects of the intervention, it is more likely that the outcome was influenced by knowledge of the intervention received. Examples justifying the answer 'SY' may include patient-reported symptoms in studies of homeopathy, or assessments of recovery of function by a physiotherapist.	NA / SY (yes, to a large extent) / WY (yes, to a small extent) / PN / N / NI
Risk of bias judgement	See algorithm.	Low / Moderate / Serious / Critical
Optional: What is the predicted direction of bias in measurement of outcomes?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized as being in favour of the intervention, as being in favour of the comparator, or as towards (or away from) the null.	Favours intervention / Favours comparator / Towards null /Away from null / Unpredictable

Algorithm for reaching default risk of bias judgement:



6. Bias in selection of the reported result

Guidance note 16 *: bias in selection of the reported result*

Selective reporting can arise for both harms and benefits of an intervention, although the motivations (and direction of bias) underlying selective reporting of effect estimates for harms and benefits may differ. Selective reporting may arise, for example, from a desire for findings to be newsworthy (or sufficiently noteworthy to merit publication), or from commercial considerations, or from a desire to demonstrate that there is not evidence of a harmful effect of an intervention.

Selective outcome reporting occurs when the effect estimate for an outcome measurement was selected from among analyses of multiple outcome measurements for the outcome domain. Examples include: use of multiple measurement instruments (e.g. pain scales) and reporting only the most favourable result; reporting only the most favourable subscale (or a subset of subscales) for an instrument when measurements for other subscales were available; reporting only one or a subset of time points for which the outcome was measured.

Selective analysis reporting occurs when results are selected from effects estimated in multiple ways: e.g. carrying out analyses of both change scores and post-intervention scores adjusted for baseline; multiple analyses of a particular measurement with and without transformation; multiple analyses of a particular outcome with and without adjustment for potential confounders (or with adjustment for different sets of potential confounders); multiple analyses of a particular outcome with and without, or with different, methods to take account of missing data; a continuously scaled outcome converted to categorical data with different cut-points; multiple composite outcomes analysed for one outcome domain, but results were reported only for one (or a subset) of the composite outcomes. (Reporting an effect estimate for an unusual composite outcome might be evidence of such selective reporting.)

Selection of a subgroup from a larger cohort: The cohort for analysis may have been selected from a larger cohort for which data were available on the basis of a more interesting finding. Subgroups defined in unusual ways (e.g. an unusual classification of subgroups by dose or dose frequency) may provide evidence of such selective reporting.

The best evidence that results were not selectively reported is available if a pre-specified, publicly available analysis plan is available (e.g. from a link in a publication or from an online platform) and is in line with the reported results. Protocols for non-randomized studies are increasingly being registered, although there is inconsistency across platforms (Malmsiø et al, 2022). An analysis plan that is sufficiently detailed to permit full assessment of selective reporting may seldom be available for observational studies. In the absence of a protocol or analysis plan, clues can sometimes be gained by comparing Methods sections with Results sections.

Malmsiø D, Frost A, Hróbjartsson A. A scoping review finds that guides to authors of protocols for observational epidemiological studies varied highly in format and content. J Clin Epidemiol. 2022 Dec 20;154:156-166. doi: 10.1016/j.jclinepi.2022.12.012.

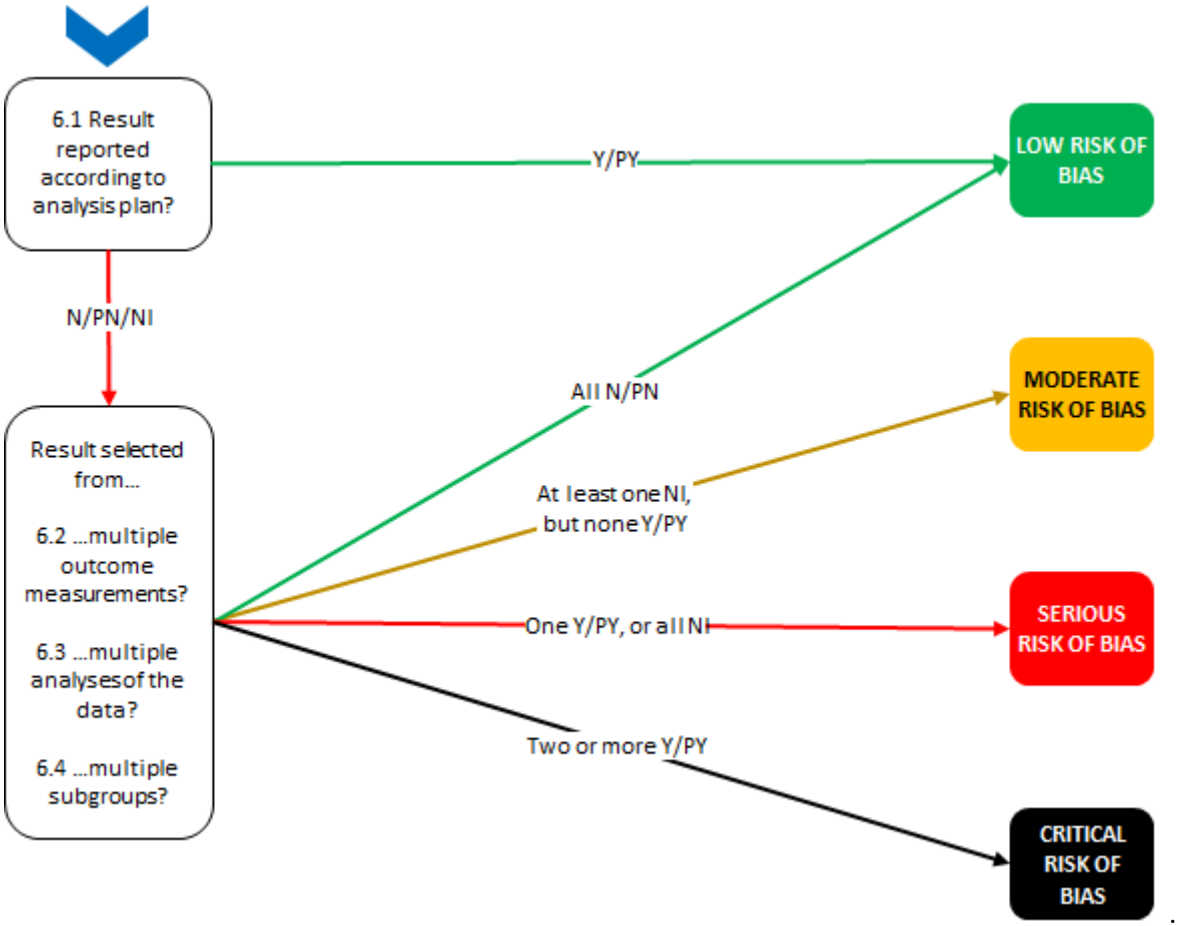
Signalling questions	Elaboration	Response options
6.1 Was the result reported in accordance with an available, pre-determined analysis plan?	If the researchers' pre-specified intentions are available in sufficient detail, then planned outcome measurements and analyses can be compared with those presented in the published report(s). To avoid the possibility of selection of the reported result, finalization of the analysis intentions must precede availability of unblinded outcome data to the study authors. Such analysis plans are rarely publicly available for non-randomized studies, so it is unlikely that a study will be assessed as at low risk of bias for this domain.	Y / PY / PN / N / NI

Is the numerical result being assessed likely to have been selected, on the basis of the results, from...		
6.2 ... multiple outcome *measurements* (e.g. scales, definitions, time points) within the outcome domain?	A particular outcome domain (i.e. a true state or endpoint of interest) may be **measured** in multiple ways. For example, the domain pain may be measured using multiple scales (e.g. a visual analogue scale and the McGill Pain Questionnaire), each at multiple time points (e.g. 3, 6 and 12 weeks post-treatment). If multiple measurements were made, but only one or a subset is reported on the basis of the results (e.g. statistical significance), there is a high risk of bias in the fully reported result. Answer 'Y' or 'PY' if**:** There is clear evidence (usually through examination of a study protocol or statistical analysis plan) that a domain was measured in multiple ways, but data for only one or a subset of measures is fully reported (without justification), and the fully reported result is likely to have been selected on the basis of the results. Selection on the basis of the results arises from a desire for findings to be newsworthy, sufficiently noteworthy to merit publication, or to confirm a prior hypothesis. For example, analysts who have a preconception, or vested interest in showing, that an intervention is beneficial may be inclined to report outcome measurements selectively that are favourable to the intervention. Answer 'N' or 'PN' if: There is clear evidence (usually through examination of a study protocol or statistical analysis plan) that all reported results for the outcome domain correspond to all intended outcome measurements. or There is only one possible way in which the outcome domain can be measured (hence there is no opportunity to select from multiple measures). or Outcome measurements are inconsistent across different reports on the same study, but the analysts have provided the reason for the inconsistency and it is not related to the nature of the results. Answer 'NI' if: Analysis intentions are not available, or the analysis intentions are not reported in sufficient detail to enable an assessment, and there is more than one way in which the outcome domain could have been measured.	Y / PY / PN / N / NI

6.3 ... multiple *analyses* of the data?	Because of the limitations of using data from non-randomized studies for analyses of effectiveness (need to control confounding, substantial missing data, etc), analysts may implement different analytic methods to address these limitations. Examples include unadjusted and adjusted models; use of final value vs change from baseline vs analysis of covariance; exploration of different ways of defining intervention and control groups; transformations of variables; a continuously scaled outcome converted to categorical data with different cut-points; different sets of covariates for adjustment; and different strategies for dealing with missing data. Application of multiple methods generates multiple effect estimates for a specific outcome domain. If multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results (e.g. statistical significance). Answer 'Y' or 'PY' if : There is clear evidence (usually through examination of a study protocol or statistical analysis plan) that a domain was analysed in multiple ways, but data for only one or a subset of analyses is fully reported (without justification), and the fully reported result is likely to have been selected on the basis of the results. Selection on the basis of the results arises from a desire for findings to be newsworthy, sufficiently noteworthy to merit publication, or to confirm a prior hypothesis. For example, analysts who have a preconception or vested interest in showing that an intervention is beneficial may be inclined to report analyses selectively that are favourable to the intervention. Answer 'N' or 'PN' if : There is clear evidence (usually through examination of a study protocol or statistical analysis plan) that all reported results for the outcome domain correspond to all intended analyses. or There is only one possible way in which the outcome domain can be analysed (hence there is no opportunity to select from multiple analyses). or Analyses are inconsistent across different reports on the same study, but the analysts have provided the reason for the inconsistency and it is not related to the nature of the results. Answer 'NI' if : Analysis intentions are not available, or the analysis intentions are not reported in sufficient detail to enable an assessment, and there is more than one way in which the outcome domain could have been analysed.	Y / PY / PN / N / NI

6.4 ... multiple *subgroups*?	Particularly with large cohorts often available from routinely collected data sources, it is possible to generate multiple effect estimates for different subgroups or simply to omit varying proportions of the original cohort. If multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results (e.g. statistical significance). Answer 'Y' or 'PY' if : There is clear evidence (usually through examination of a study protocol or statistical analysis plan) that different subgroups were analysed, but data for only one or a subset of analyses is fully reported (without justification), and the fully reported result is likely to have been selected on the basis of the results. Selection on the basis of the results arises from a desire for findings to be newsworthy, sufficiently noteworthy to merit publication, or to confirm a prior hypothesis. For example, analysts who have a preconception or vested interest in showing that an intervention is beneficial may be inclined to report results selectively for subgroups that are favourable to the intervention. Answer 'N' or 'PN' if : There is clear evidence (usually through examination of a study protocol or statistical analysis plan that was date-stamped before the analyst had access to the collected data) that all reported results for the subgroups correspond to all intended analyses. or Analyses are inconsistent across different reports on the same study, but the analysts have provided the reason for the inconsistency and it is not related to the nature of the results. Answer 'NI' if : Analysis intentions are not available, or the analysis intentions are not reported in sufficient detail to enable an assessment, and there is more than one way in which subgroups could have been analysed.	Y / PY / PN / N / NI
Risk of bias judgement	See algorithm.	Low / Moderate / Serious / Critical
Optional: What is the predicted direction of bias in selection of the reported result?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized as being in favour of the intervention, as being in favour of the comparator, or as towards (or away from) the null.	Favours intervention / Favours comparator / Towards null /Away from null / Unpredictable

Algorithm for reaching default risk of bias judgement:



Overall risk of bias

Guidance note 17 *: overall risk of bias*

ROBINS-I defaults to setting the overall risk of bias for a result to be equal to the risk-of-bias judgement for the domain with the greatest risk of bias. For example, if the 'worst' judgement across domains is of serious risk of bias, then the result would be judged as at serious risk of bias overall. However, the user may override this to judge the result to be at greater risk of bias if there are problems in several domains. For example, if several domains are assessed to be at serious risk of bias, and it is considered that these problems are likely to be compounded, then it may be reasonable to judge the result to be at critical risk of bias overall.

Predicting the direction of bias overall may be difficult. Risk-of-bias judgements for the individual domains might be used to inform the influence of that domain to the likely direction of bias overall.

Overall risk of bias	See algorithm.	Low risk of bias except for concerns about uncontrolled confounding / Moderate risk / Serious risk / Critical risk
What is the predicted direction of bias?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized as being in favour of the intervention, as being in favour of the comparator, or as towards (or away from) the null. Alternatively, if there direction is driven by bias due to confounding, the direction may be an upwards bias (overestimate the effect) or a downward bias (underestimate the effect).	Upward bias (overestimate the effect) / Downward bias (underestimate the effect) / Favours intervention / Favours comparator / Towards null /Away from null / Unpredictable

Algorithm for reaching overall risk of bias judgement:

Judgement	Interpretation	How reached
Low risk of bias except for concerns about uncontrolled confounding	There is the possibility of uncontrolled confounding that has not been controlled for (given the observational nature of the study), but otherwise little or no concern about bias in the result	*Low risk of bias except for concerns about uncontrolled confounding* in Domain 1 and *Low risk of bias* in all other domains
Moderate risk of bias	There is some concern about bias in the result, although it is not clear that there is an important risk of bias	At least one domain is at *Moderate risk of bias*, but no domains are at *Serious risk of bias* or *Critical risk of bias*
Serious risk of bias	The study has some important problems: characteristics of the study give rise to a serious risk of bias in the result	At least one domain is at *Serious risk of bias*, but no domains are at *Critical risk of bias* OR Several domains are at *Moderate*, leading to an additive judgement of *Serious risk of bias*
Critical risk of bias	The study is very problematic: characteristics of the study give rise to a critical of bias in the result, such that the result should generally be excluded from evidence syntheses.	At least one domain is at *Critical risk of bias* OR Several domains are at *Serious risk of bias*, leading to an additive judgement of *Critical risk of bias*