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Global prevalence of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* coinfection in *Ixodes* tick populations: protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction *Ixodes* ticks are pivotal in transmitting diseases like Lyme disease and human granulocytic anaplasmosis, caused by *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, respectively. These pathogens not only affect humans through single or multiple tick bites but also pose risks to animal hosts, leading to potential coinfections. Despite regional studies indicating significant prevalence, their global coinfection data remain sparse. This study aims to bridge this gap through a systematic review and meta-analysis of *B. burgdorferi* and *A. phagocytophilum* coinfections in *Ixodes* ticks worldwide. Addressing data limitations and study variability, it seeks to provide a nuanced understanding of coinfection patterns, their epidemiological implications and inform targeted prevention strategies.

Methods and analysis Following Preferred Reporting Items for Systematic Review and Meta-analysis Protocols 2015 guidelines and PROSPERO registration, this study will undertake a thorough database search without constraints on language or publication date, using standardised screening and data extraction protocols. The quality and bias of studies will be evaluated using Joanna Briggs Institute tools. In the statistical analysis phase, conducted in R, we will initially determine the use of fixed or random-effects models based on the assessment of data heterogeneity. This choice will guide the framework for subsequent analyses. Within the selected model's framework, we will perform subgroup analyses and meta-regression to investigate the effects of various factors, ensuring that each step is tailored to the initial model selection to maintain analytical consistency.

Ethics and dissemination As this study does not involve clinical research or data collection from subjects, ethical approval is not required. We will uphold ethical standards in synthesising and reporting data. Study outcomes will be published in peer-reviewed journals, communicating findings to the scientific community and contributing to the understanding of *Ixodes* tickborne diseases.

PROSPERO registration number CRD42023449735.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The review employs a comprehensive literature search strategy across all languages and time periods, aiming to minimise selection bias and enhance the generalisability of findings regarding *Borrelia burgdorferi* and *Anaplasma phagocytophilum* coinfections in *Ixodes* ticks.
⇒ The methodology rigorously assesses ecological and environmental factors, offering a comprehensive understanding of the variables affecting these coinfection rates.
⇒ The review's scope is subject to the quality and availability of existing literature, which may influence the findings' generalisability and introduce variability in data synthesis.
⇒ The exclusion of studies with insufficient detail for rigorous quality assessment could limit the breadth of evidence, potentially affecting the review's comprehensiveness.

INTRODUCTION

Ticks play a crucial role in the transmission of diseases in humans and animals, with *Ixodes* ticks garnering particular attention due to their wide host range and ability to transmit a variety of pathogens, including *Borrelia burgdorferi* and *Anaplasma phagocytophilum*.[1] These pathogens can cause Lyme disease (LD) and human granulocytic anaplasmosis (HGA) in humans following one or multiple bites from an infected tick, potentially leading to coinfections.[2–19] Animal hosts are also at risk of similar infections.[1] Two meta-analyses have revealed that the global seropositivity rates for individual infections of *B. burgdorferi* and *A. phagocytophilum* are 14.5%[20] and 8.4%,[21] respectively. Despite the lack of global meta-analyses on their coinfections, regional studies indicate that their coinfections are notably prevalent, especially in patients exhibiting classic symptoms of LD or poor response to antibiotic treatments.[22–28] In animal hosts,

particularly domestic pets and wildlife, infection and coinfection rates with these pathogens are equally widespread.[29 30] For example, one study found that dogs with positive *B. burgdorferi* antibodies were 1.60 times more likely to be *A. phagocytophilum* antibodies positive than those dogs with *B. burgdorferi* antibodies were negative.[31]

Coinfections not only complicate and exacerbate the severity of diseases, manifesting overlapping and intensified symptoms, but they also make diagnosis and differential diagnosis more challenging and often lead to poor responses to conventional antibiotic treatments, resulting in more severe health outcomes.[32] This places a significant burden on public health systems and has a substantial economic impact. In the USA, for instance, the direct diagnosis and treatment costs for LD amount to US$1.3 billion annually, not including losses in productivity and research costs.[33 34] The annual diagnostic expenses for HGA stand at US$1.6 million.[33 34] Infections and coinfections in animals also lead to decreased productivity and mortality, particularly in developing countries, where the estimated economic losses can reach billions of dollars,[35] significantly impacting agriculture and livestock industries.

Notably, pathogen coinfections within *Ixodes* ticks are widespread globally, yet there is a marked geographical variability, with coinfection rates as low as 0.5%[24] in some regions and up to 20.2%[35] in others. Additionally, climate change and increasing human activities have expanded tick habitats and extended their active periods, further increasing the chances of human-tick encounters and, consequently, the risk of coinfections. Although regional studies on coinfections between *B. burgdorferi* and *A. phagocytophilum* in ticks exist,[24 35–38] a comprehensive analysis of global data is lacking, underscoring the importance of integrating global data to fully understand the coinfection patterns of these pathogens and their impact on public health.

This study employs systematic review and meta-analysis methodologies to thoroughly investigate the natural coinfections of *B. burgdorferi* and *A. phagocytophilum* in global *Ixodes* tick populations, aiming to address the gaps in current research and unveil the transmission dynamics and potential health impacts of these coinfections, thereby providing a scientific basis for the development of effective prevention and control strategies. By overcoming challenges such as limitations in data sources and variability in study quality, we aim to ensure comprehensive and accurate data collection and analysis. This study is expected to not only reveal the distribution patterns and trends of coinfections between *B. burgdorferi* and *A. phagocytophilum* in *Ixodes* ticks worldwide, but also to enhance our understanding of their ecological and epidemiological aspects, including but not limited to geographical distribution differences, seasonal variations and the effects of host species. These insights are anticipated to inform improvements in existing prevention and treatment strategies, particularly in regions or seasons with significantly high coinfection rates, by providing evidence for targeted public health interventions and serving as a valuable reference for future research and policy-making.

METHODS AND ANALYSIS

Registration

The study protocol adhered to the guidelines of the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols 2015 (PRISMA-P 2015).[39 39 39] The protocol for this systematic review was registered with the International Prospective Register of Systematic Reviews (PROSPERO) and received the trial registration number CRD42023449735.

Patient and public involvement

No patients or other individuals will be involved in the design, conduct, reporting or dissemination of this research.

Eligibility criteria

Inclusion criteria

1. Observational studies, without language or time restrictions, on natural coinfections of *B. burgdorferi* and *A. phagocytophilum* in *Ixodes* ticks, including cross-sectional, cohort and case–control studies, that report on the prevalence and distribution of these coinfections.
2. Studies that examine the impact of ecological and environmental factors, such as tick developmental stages, gender, detection methods, climatic conditions and land use patterns, on these coinfection rates.

Exclusion criteria

1. Studies not specifically examining the natural coinfection of *B. burgdorferi* and *A. phagocytophilum* in *Ixodes* ticks, or those only examining single infections.
2. Non-observational studies (such as randomised controlled trials, interventional studies, animal model studies), editorials, letters and conference abstracts lacking detailed data for quality assessment.
3. Studies that lack detailed and transparent reporting of their research design, methodology and results, which are essential for a comprehensive and rigorous quality assessment. This includes studies with inadequate descriptions of sample selection, data collection methods, statistical analyses and outcome reporting.
4. Studies that do not provide quantitative data on natural coinfection rates or fail to distinguish between natural and laboratory-induced coinfections.
5. Studies with inaccessible full-text articles that cannot be resolved by contacting authors, or those containing duplicate data or findings.

Search strategy

To ensure the comprehensiveness of the literature search, this systematic review will be independently conducted by two reviewers, WeijiangM and WeijieM, using key search terms such as "*Ixodes*", "*Ixodidae*", "Tick", "Ticks", "*Borrelia burgdorferi*", "*Anaplasma phagocytophilum*"

Table 1 Search strategy for PubMed

Step	Search algorithm
#1	"ixodes"(MeSH Terms)
#2	"Ixodes"(Title/Abstract)
#3	"ticks"(MeSH Terms)
#4	"tick*"(Title/Abstract)
#5	"ixodidae"(MeSH Terms)
#6	"ixodidae*"(Title/Abstract)
#7	#1 OR #2 OR #3 OR #4 OR #5 OR #6
#8	"borrelia burgdorferi"(MeSH Terms)
#9	"borrelia burgdorferi*"(Title/Abstract)
#10	"lyme disease spirochete"(Title/Abstract)
#11	"borrelia burgdorferi sensu stricto"(Title/Abstract)
#12	#8 OR #9 OR #10 OR #11
#13	"anaplasma phagocytophilum"(MeSH Terms)
#14	"anaplasma phagocytophilum*"(Title/Abstract)
#15	#13 OR #14
#16	"coinfection"(MeSH Terms)
#17	"coinfection*"(Title/Abstract)
#18	"dual infection*"(Title/Abstract)
#19	"mixed infection*"(Title/Abstract)
#20	#16 OR #17 #18 OR #19
Final Combination	#7 AND #12 AND #15 AND #20

Search quary for PubMed: ("Ixodes"(MeSH Terms) OR "Ixodes"[Title/Abstract] OR "ticks"(MeSH Terms) OR "tick*"[Title/Abstract] OR "ixodidae"(MeSH Terms) OR "ixodidae*"[Title/Abstract]) AND ("borrelia burgdorferi"(MeSH Terms) OR "borrelia burgdorferi*"[Title/Abstract] OR "lyme disease spirochete"[Title/Abstract] OR "borrelia burgdorferi sensu stricto"[Title/Abstract]) AND ("anaplasma phagocytophilum"(MeSH Terms) OR "anaplasma phagocytophilum*"[Title/Abstract]) AND ("coinfection"(MeSH Terms) OR "coinfection*"[Title/Abstract] OR "dual infection*"[Title/Abstract] OR "mixed infection*"[Title/Abstract]).

and 'co-infection'. The searches will cover electronic databases including PubMed, EMBASE, Web of Science and Scopus. To enhance the geographical diversity and inclusiveness of our literature search, we will also include databases such as the Global Index Medicus (GIM) and SinoMed, along with other relevant databases as identified to ensure a comprehensive coverage of global research. Searches in all databases will be conducted from their inception to the present, ensuring thorough coverage of the literature, with no restrictions on publication date or language.

In addition to these database searches, reference lists of identified articles and grey literature sources will be manually reviewed to identify any additional studies not indexed in the databases. Relevant experts in the field will also be consulted to ensure the inclusion of all pertinent studies.

The specific search strategies for each database, including combinations of these search terms, will be detailed in table 1 and in online supplemental table 1–14 and online supplemental file of this protocol. Any discrepancies will be resolved through discussion and, if necessary, arbitrated by a third reviewer, BxL, to make the final decision.

To balance the comprehensiveness of the search with the feasibility of the practical operation, we will adopt a phased search strategy. Initially, preliminary searches will be conducted in primary databases such as PubMed, EMBASE, Web of Science and Scopus. Based on the coverage and research gaps identified from these preliminary results, we will evaluate the necessity of expanding the search to other specific or regional databases. Additionally, a pilot screening will be conducted to estimate the number and quality of relevant literature, thereby better assessing the need to further expand the database range, including Sinomed, GIM database and other regional databases. This flexible search strategy aims to ensure the comprehensiveness of the study while considering the limitations of project resources and time frames.

Our team includes multilingual experts who will be responsible for retrieving and reviewing non-English literature to expand the coverage of our literature search and reduce language bias. In the event of specific language or technical challenges, we may seek collaboration with external language experts or purchase professional translation services to ensure the accuracy and comprehensiveness of literature retrieval and analysis. Moreover, to assist in the preliminary screening and identification of potential non-English literature, we may use translation tools to aid in understanding the titles and abstracts of documents. However, for all literature ultimately included in the analysis, we will rely on our language experts or external translation services for comprehensive and accurate translation and review.

Study selection, data extraction, quality and bias assessment

Articles identified from systematic searches will be imported into EndNote for reference management and duplicate removal. After removing duplicates, two reviewers, LyZ and XH, will independently screen study titles and abstracts for eligibility. The selection process and results will be presented in a PRISMA flow chart (figure 1), offering a transparent overview.

Studies clearing initial screening will undergo full-text review against eligibility criteria, with selections cross-checked by LyZ and XH. Disagreements will be resolved through discussion with RY, or arbitrated by BxL if needed. For multiple articles on the same trial, only the most comprehensive study will be included.

Three reviewers, LP, LG and XyW, will independently extract data into two copies of a predefined form, ensuring data accuracy. The data will cover authorship, publication year, study design, sample size, data collection period, pathogens, detection methods, tick species and characteristics, tick developmental stages, geographical origin, coinfection rates and ecological/environmental factors. We will contact authors twice for missing or unclear data. Persistent data gaps across studies will be

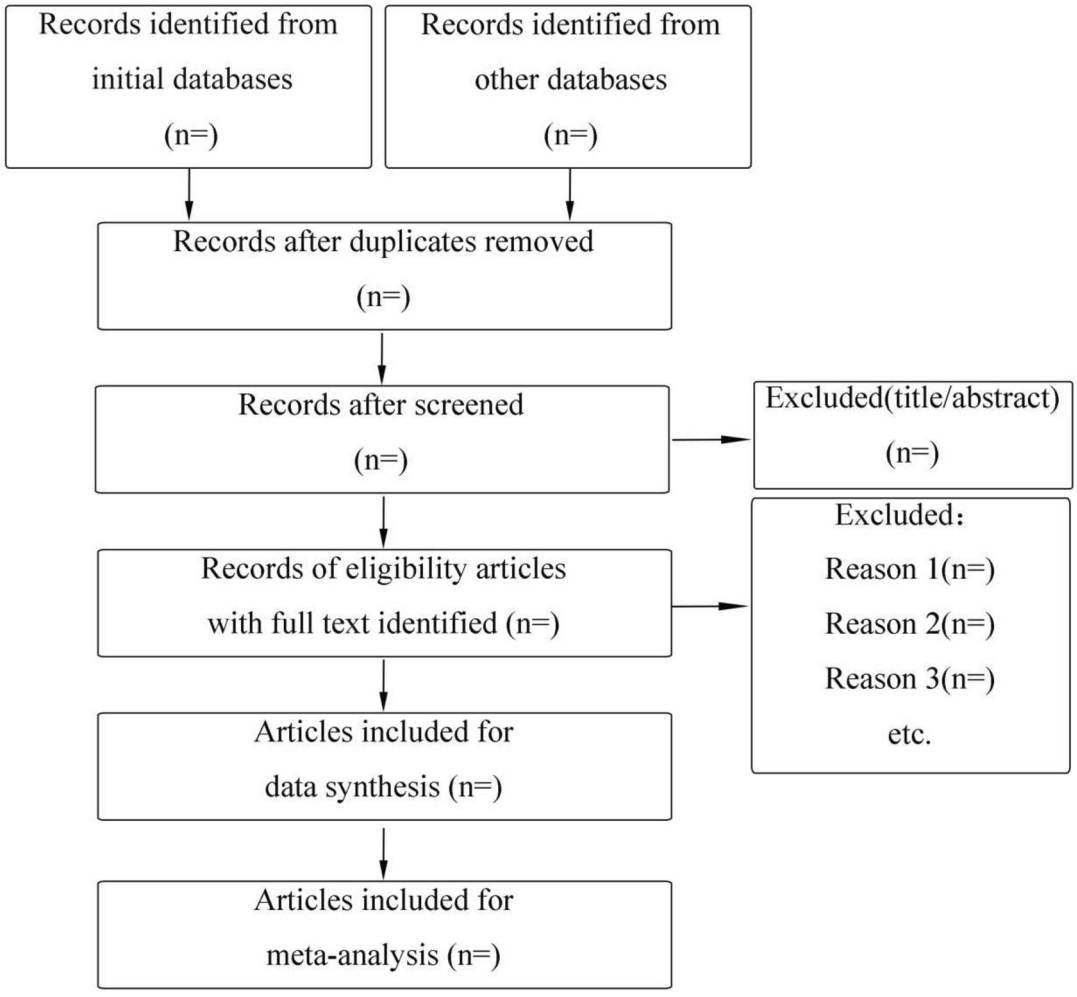

Figure 1 Flow diagram illustrating the study selection process.

noted as limitations, with their potential impact on results discussed. Data accuracy will be cross-checked, resolving discrepancies through discussion or with a third reviewer, FkB.

The risk of bias and quality of the included studies will be assessed using the Joanna Briggs Institute critical appraisal tools, focusing on aspects like sampling methodology, data analysis and outcome reporting. SyL and HxW will independently conduct these assessments. Discrepancies will be resolved through discussion and, if necessary, arbitrated by AhL. The results from assessments will be categorised as 'low,' 'moderate' or 'high' risk and summarised in a table. This method will ensure the reliability of our findings and provide a comprehensive overview of the credibility and impact of the included studies.

Data synthesis and analysis

A team proficient in data science, including SyL and HxW, will conduct the statistical analysis. The meta-analysis will be conducted using R software, leveraging packages such as 'meta' and 'metafor', chosen for their suitability to the analyses. This team will ensure data analysis integrity and accuracy, focusing on estimating the prevalence of *B. burgdorferi* and *A. phagocytophilum* coinfection in *Ixodes* ticks.

In this systematic review and meta-analysis, our primary aim is to synthesise existing literature to determine the global prevalence rates of *B. burgdorferi* and *A. phagocytophilum* coinfections in *Ixodes* ticks, addressing our central research question and shedding light on the epidemiological patterns of these diseases. Geographical distribution will be analysed as it contributes to understanding variations in prevalence rates across different regions. We will also examine how ecological and environmental factors, including tick developmental stages and climatic conditions, influence these prevalence rates. The methodological variability across studies will be assessed to ensure the robustness and reliability of our findings.

Our data synthesis approach will be methodical and structured, using quantitative methods where data are consistent and comprehensive, and narrative synthesis where heterogeneity or data limitations preclude a purely quantitative analysis. This dual approach ensures that all available data contributes to our overall understanding, with the synthesis type chosen based on data uniformity

and quality, leading to informed interpretations and actionable conclusions.

Heterogeneity across studies will be initially assessed using χ^2 tests (significance at p<0.10), followed by the I^2 statistic to measure heterogeneity extent. A fixed-effects model will be adopted for low I^2 values (less than 50%), indicating minimal variability. For high I^2 values (50% or greater), or when factors like study design differences or inconsistent sample sizes indicate significant heterogeneity, a random-effects model will be used. This model considers variability in outcomes due to different study designs or methodologies.

Unadjusted prevalence will be recalculated from the crude numerator and denominator in each study. We plan to use the Freeman-Tukey double arcsine transformation to stabilise variances and calculate pooled prevalence and 95% CIs for coinfection rates.[40] However, we will conduct preliminary normality tests, such as the Shapiro-Wilk or Kolmogorov-Smirnov tests, based on the characteristics of our collected data, to ensure the transformation's appropriateness. If the data do not meet the Freeman-Tukey transformation's assumptions, we will consider alternative methods, like the logit transformation or adding a small constant to all proportions before transformation. This flexible approach will ensure our methodology's rigour, adapting to the specific requirements of our data. Methodological disagreements during analysis will be resolved through team discussion, with final decisions made by the lead analyst (SyL).

Advanced analytical procedures

The data analysis team, including SyL and HxW, will undertake advanced analytical procedures such as subgroup analyses and meta-regression, sensitivity analyses and the assessment of publication bias. Our approach aims to provide a nuanced understanding of the variations in coinfection rates and the underlying factors contributing to these variations, enhancing the significance of our findings.

Subgroup analyses and meta-regression

To deepen our understanding of the potential impact of factors such as geographic diversity, host species, tick developmental stages, climate and land use on coinfection rates, we plan to conduct detailed subgroup analyses. These factors are expected to significantly influence coinfection rates. Through subgroup analysis, we can not only reveal heterogeneity among study results but also assess the impact of specific factors on coinfection rates, providing a scientific basis for future research directions and targeted intervention measures. We will used the meta package in R software for this task, particularly employing its metagen function to compare effect size differences across different subgroups. The results will be presented through forest plots to facilitate a visual understanding of the effect sizes and their 95% CIs across subgroups.

Furthermore, we will assess the impact of different covariates on effect sizes through meta-regression analysis, including both continuous and categorical variables. This will be implemented using the metafor package in R software, whose rma function is well suited for such analyses. For categorical data, such as different categories of host species, we will employ dummy variables.

Considering the potentially limited number of studies that can be included in the analysis, we will maintain flexibility in implementing these analyses. Especially in conducting subgroup and meta-regression analyses, we will adjust our analysis strategy based on the number and quality of studies ultimately included. This may mean simplifying subgroup analyses or limiting the complexity of meta-regression analyses in some cases.

In our analyses, we will also consider the impact of factors such as climate, and land use on tick behaviour and coinfection rates. Given that data on these variables may be limited in the existing literature, we will conduct a preliminary assessment of data availability in the studies included. For variables with sparse data, we will undertake exploratory or descriptive analyses and clearly denote the exploratory nature and limitations of these analyses in our research report. Finally, in the discussion section, we will delve into the potential impact of data limitations on our analysis results and propose directions for future research, especially emphasising the importance of collecting and reporting data on key variables to facilitate more in-depth analyses.

Sensitivity analyses

To assess the stability and robustness of our subgroup analysis and meta-analysis results, we will conduct a comprehensive sensitivity analysis. We plan to use the metainf function of the meta package to sequentially exclude studies and observe the impact on the overall effect size estimate, thus evaluating the sensitivity of individual studies to the meta-analysis results. At the same time, we will also consider using methods provided by the robust package to assess the robustness of results under different statistical assumptions to ensure that our findings do not change with minor variations in assumptions.

In conducting sensitivity analysis to evaluate the stability and robustness of our subgroup analysis and meta-regression analysis results, we will continue to consider the potential limitations related to the number of included studies previously discussed. As pointed out in the subgroup analysis and meta-regression sections, we will maintain flexibility in our analysis strategy to adapt to the number and quality of studies ultimately included. This means that our sensitivity analysis may need to be appropriately simplified in certain situations to ensure the reliability and validity of the analysis.

Our sensitivity analysis will be multifaceted: first, by excluding low-quality studies, we will compare the meta-analysis results that include all studies with those that only include high-quality or medium-quality studies, as well as the changes in results after excluding studies with missing or unclear data. Additionally, we will use meta-regression analysis to assess the impact of factors such as detection

techniques, study size, publication year and geographical region on the meta-analysis results.

If sensitivity analysis shows that our main conclusions remain stable even after excluding some low-quality studies, this will indicate that our findings are resistant to variations in study design. Conversely, if we find that studies with smaller sample sizes have a significant impact on the overall effect size estimate, this will prompt us to pay special attention to this aspect in future research.

Through these comprehensive sensitivity analyses, we aim to gain a deeper understanding of the impact of various factors on the stability of research results and ensure that our conclusions are highly credible.

Publication bias

Publication bias will be assessed using funnel plots and Egger's test if the systematic review includes at least 10 studies that meet our inclusion criteria.[41] Trim-and-fill analyses will be conducted if bias is detected.

Study timeline

This systematic review and meta-analysis is scheduled to commence on 24 May 2024 and is expected to be completed by 24 June 2026.

DISCUSSION

This study aims to globally assess the coinfection rates of *B. burgdorferi* and *A. phagocytophilum* in *Ixodes* ticks, potentially filling a significant gap in epidemiological data. Employing a systematic review and meta-analysis approach, we hope to offer insights that could inform public health strategies and address economic losses in agriculture. Anticipated findings might suggest areas for future research, such as exploring ecological factors and the impact of climate change on disease transmission, enhancing our understanding of tickborne diseases and their broader implications.

Ethics and dissemination

This systematic review and meta-analysis do not involve primary data collection from human subjects, and thus formal ethical approval is not required. Nevertheless, we pledge to uphold the highest ethical standards during the research process, particularly during the synthesis and reporting of literature, in accordance with *BMJ Open*'s ethical guidelines. For dissemination, our goal is to broadly share the study's findings with the scientific community via high-impact, peer-reviewed journal publications and conference presentations. Necessary protocol amendments that do not affect the primary outcomes or objectives will be thoroughly documented and updated in the PROSPERO registry. This ensures continued transparency and integrity of our research. Furthermore, we will deposit our compiled dataset in a recognised public repository following strict data curation principles, thereby enabling and supporting future research endeavours.

Contributors FkB and AhL are the guarantors of this study, having significantly contributed to its conception. The protocol manuscript was initially drafted by SyL and underwent subsequent revisions by FkB and AhL. LyZ, XH, RY, BxL, LP, LG and XyW will handle literature search, data extraction and management. Designated team members will resolve any disputes or methodological issues under the overall guidance of SyL, the lead researcher. SyL and HxW will be responsible for statistical modelling, data analysis, literature quality and bias risk assessment. They will also conduct data synthesis focusing on both quantitative and narrative methods based on the data's nature. FkB and AhL will oversee data management and security, ensuring the integrity and confidentiality of the data. All authors have committed to maintaining integrity and accuracy and have collectively approved the protocol for publication. Each contributes significantly to their designated roles, ensuring the success of the study. Both SyL and FkB are recognised as co-first authors due to their significant and equal contributions to this protocol.

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Competing interests None declared.

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

Patient consent for publication Not applicable.

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