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BMJ Open

The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis

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Manuscripts

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The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis

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This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172.

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Word count: 2,921.

25 ABSTRACT

26 **Introduction:** Preterm birth (PTB) is the leading cause of neonatal mortality and short- and
27 long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still
28 unclear, which makes the identification of reliable and accurate predictor markers more
29 difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers
30 have been demonstrated to be potentially accurate biomarkers for many disorders with
31 complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of
32 metabolomics markers associated with spontaneous PTB. Our research question is "What is
33 the performance of metabolomics for predicting spontaneous preterm birth in
34 asymptomatic pregnant women?"

35 **Methods and analysis:** We will focus on studies assessing metabolomics techniques for
36 predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a
37 comprehensive systematic review of the literature from the last 10 years. Only
38 observational cohort and case-control studies will be included. Our search strategy will be
39 carried out by two independent reviewers, who will scan title and abstract before carrying
40 out a full review of the article. The scientific databases to be explored include PubMed,
41 MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.

42 **Ethics and dissemination:** This systematic review protocol does not require ethical approval.
43 We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm
44 SAMBA study open access website, specialists' conferences, and to our funding agencies.

45 **Registration details:** This protocol is registered in PROSPERO platform (code
46 CRD42018100172).

47 **Keywords:** preterm birth, spontaneous preterm birth, metabolomics, biomarkers,
48 prediction.

49 **Strengths and limitations of this study**

- 50 • This systematic review protocol takes into account some important aspects regarding
- 51 conducting a systematic review about spontaneous preterm birth and metabolomics such as
- 52 the criteria used for defining spontaneous preterm birth, different population risk
- 53 stratification, method used to estimate gestational age, and metabolomics techniques
- 54 details.
- 55 • Two independent reviewers are responsible for searching and selecting studies, as also
- 56 extracting data, and a third reviewer will resolve any disagreement.
- 57 • If possible, proper statistical methods will be applied to investigate metabolomics
- 58 accuracy in predicting spontaneous preterm birth.

60 INTRODUCTION

61 Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and
62 long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to
63 spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4].
64 Several pathways and mechanisms linked with preterm birth have been proposed including,
65 neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More
66 specifically, several markers associated with uterine distension/contraction, decidual
67 inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been
68 studied in the past decades [5,6]. However, no single marker or combination of markers has
69 been found to be accurate enough for predicting sPTB [7–10].

70 Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical
71 phase, maternal and fetal interactions, genetic and environmental influences, and adaptive
72 mechanisms [11,12]. These challenging aspects, and the presence of still unknown
73 underlying mechanisms, are the main limitations for the identification of an accurate
74 predictor for sPTB [13–15]. None of the predictors used in clinical practice, such as previous
75 history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and
76 transvaginal ultrasonography cervical length demonstrated exceptional accuracy for
77 predicting spontaneous preterm birth [7]. An exploration of innovative approaches is
78 urgently required.

79 Metabolomics is the study of metabolites, through identification and quantification of low-
80 weight molecular particles, i.e. tens to hundreds thousands of intermediate products and
81 substrates of systems biology chemical reactions [16,17]. This novel approach has been
82 applied for identifying biomarkers and underlying biochemical pathways associated with
83 complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational

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84 diabetes and preterm birth. In contrast to other “*Omics Sciences*” techniques, metabolomics
85 is more closely associated with the phenotype of the disease and might thus identify a more
86 robust and reliable set of predictors [18]. Importantly, implementing an adequate *Omics*
87 experimental design is crucial for metabolomics studies.
88 Using different baseline population (asymptomatic vs symptomatic or low- vs high-risk
89 women for developing sPTB), study designs (prospective cohorts, case-control or cross
90 sectional studies), sources of samples (amniotic fluid, vaginal fluid, blood, urine, hair, etc)
91 and the timing of sample collection each have significant effects on study findings and the
92 consequent interpretation and contribution to the current gap of knowledge [16].
93 Therefore, we aim to conduct a systematic review of the use of metabolomics biomarkers
94 for predicting spontaneous preterm birth in asymptomatic pregnant women. This protocol
95 describes the methods that will be applied in our systematic review.

96 **METHODS AND ANALYSIS**

97 The current systematic review proposal will be conducted, written and published following
98 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)
99 recommendations [19]. Also, it is properly registered at PROSPERO platform – code
100 CRD42018100172.

101 **Review question**

102 What is the performance of metabolomics for predicting spontaneous preterm birth in
103 asymptomatic pregnant women?

104 **Eligibility Criteria**

105 Original cohort or case-control studies involving asymptomatic pregnant women at the
106 moment of sample collection (exposure) and with samples analysed using metabolomics

techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials, editorials, letter to editors, case reports, expert opinions, commentaries, or any type of review; (2) they describes only experimental studies with animals; or (3) they are duplicated data (e.g. data published in conferences proceedings and, then, published again in scientific journals). In this case, only the most complete publication will be considered, after comparing and confirming that the same technique and metabolites were explored. Studies published from 2008 to 2018 will be considered, and there will be no language restriction. Before submitting this systematic review for publication, we will rerun the search strategy to identify new studies that have been published after performing the first round of search.

Participants

The current review is interested in evaluating the performance of metabolomics biomarkers for spontaneous preterm birth in asymptomatic women, which may contribute to clinical practice, potentially providing information regarding onset of preterm labour. Nevertheless, we aim to identify studies addressing only early predictors collected from asymptomatic women (i.e. women who are in an early preclinical stage), which might contribute to a wider window of opportunity for interventions and also to develop a widely reproducible screening test. Asymptomatic pregnant women should not have regular uterine tightening/contractions or signs of rupture of membranes (i.e. watery discharge). In addition, the study should preferably have a standardized definition of spontaneous preterm birth, the outcome of interest.

Information Sources

The search will be held in the following databases: PubMed, EMBASE, ProQuest, Scopus, CINAHL, and Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health Sciences Literature (LILACS), Medline and the Scientific Electronic Library

Online (Scielo). In addition, secondary sources of original studies will be explored such as Google Scholar, hand-held searching of the reference list of eligible studies, conference proceedings, and contact with authors when necessary.

Search Strategy

The following terms will be used in our search strategy for the different scientific databases: (preterm birth, premature birth, premature infant, premature labor, extremely premature infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic*, metabonomic*, metabolit*, lipidomic*, H NMR, proton NMR, proton nuclear magnetic resonance, liquid chromatogra*, gas chromatogra*, UPLC, ultra-performance liquid chromatograph*, ultra performance liquid chromatograph*, HPLC, high performance liquid chromatograph*, high-performance liquid chromatograph*) AND (pregnan*, antenat*, ante nat*, prenat*, pre nat*). Respective adaptations in the syntax of search for each database will be applied accordingly. No filters - such as “research in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded according to eligibility criteria. The complete search strategy, including Boolean terms, is provided as Supplementary Material.

Data Management

We will export search results to a reference manager (Mendeley®). Then, the following information will be collected from each study using an appropriate form, which will be entered in an Excel® spreadsheet: author’s name, year of publication, country, study design, number of participants with and without spontaneous preterm birth, type of metabolomics analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods

for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm birth (spontaneous or pPROM), number of fetuses (singleton vs multiple), gestational age when samples were collected, source of samples (type/site of tissue), low or high-risk for preterm birth (authors criteria used to define the population will be collected) and method applied to estimate gestational age. If possible, additional variables related to spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will be recorded for secondary analyses. Finally, we will check the biochemical class of identified metabolites in Human Metabolome Database (HMDB, version 4.0) to explore and synthesize whether there are common biological pathways associated with spontaneous preterm birth [17].

Selection Process

Two independent reviewers (RTS and RBFG) will be responsible for screening and selecting studies initially according to title or abstract. Full text of non-excluded studies will be read to discriminate eligibility. A third reviewer (DFBL) will consider any disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all steps and approving data extraction.

Data Collection Process

We will extract search results to a reference manager where all studies will be stored. Then, included studies will be placed in a new folder. Finally, we will manually extract data of interest from these included studies to an Excel® file. Each reviewer will have their own reference manager account, file and folder and discrepant results will be discussed together with the third reviewer.

Outcomes and Prioritization

177 The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37
178 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of
179 membranes. Secondary outcomes are:

180 1) Spontaneous preterm birth before 28 weeks;

181 2) Spontaneous preterm birth before 34 weeks;

182 The capacity to predict different degrees of sPTB (categories of gestational age) is important
183 as the extreme (<28wks) and non-late preterm (<34wks) newborns have different adverse
184 outcomes compared to non-extreme (≥28wks) or late (≥34 wks) preterm newborns.

185 Ideally, the method of gestational age estimation should be clearly reported. For instance, it
186 can be reported as estimated by last menstrual period (LMP) and confirmed by an early
187 ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

188 **Index test**

189 Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of
190 interest. Metabolomics is a technique to identify and quantify metabolites from biological
191 samples using different type of platforms/equipment. The most common platforms include
192 gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass
193 spectrometer or a proton nuclear magnetic resonance [20]. If possible, the performance of
194 each metabolomics techniques will be assessed through hierarchical summary receiver
195 operator characteristic curve (HSROC) (meta-analysis).

196 **Risk of Bias in individual Studies**

197 We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [21]
198 to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study
199 will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four
200 domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard

(occurrence of preterm birth), and Flow and Timing of participant's inclusion and follow-up. For example, studies will be labelled as "low" risk of bias for Reference Standard when definition of spontaneous preterm birth and gestational age estimation are clear; "high" risk of bias would be considered when the moment of sample collection is not well described.

Data Synthesis

We will report details of identification, screening, eligibility and included studies using a flow diagram, according to PRISMA recommendations [19]. Data from included studies will be synthesized into tables according to the variables of interest. If possible, we will present data meta-analysis according to study design, metabolomics technique and type of samples analysed. We intend to perform subgroup analysis according to:

- Different metabolomics methods applied: gas or liquid chromatography coupled with mass spectrometry or proton nuclear magnetic resonance;
- Singleton and multiple pregnancies;
- Low-risk and high-risk women for developing preterm birth;
- Subtype of preterm birth: Spontaneous preterm birth exclusively due to spontaneous onset of labour with intact membranes or sPTB due to premature rupture of membranes.

Potential anticipated limitations to this review

Firstly, although we have not considered any language restriction, we consider that there might be a limitation in studies published entirely in non-English language. However, in the last decade, more than 95% of scientific biomedical literature has been published in English [22], then we consider this a minor selection bias. Secondly, we intend to stratify the groups according to population risk. However, the characterization of low- or high-risk for spontaneous preterm birth is controversial and lacks standardization, which might limit data

225 comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of
226 labour or pPROM is another topic of potential limitation - the recognition of the main initial
227 mechanism for preterm delivery might not always be possible. Even when specified, it might
228 provoke uncertainty and could limit further considerations regarding preterm phenotypes.

229 Patient and Public Involvement

230 Patients will not be directly involved in the study and no experience or direct impact from
231 their perspective can be discussed.

232 ETHICS AND DISSEMINATION

233 This systematic review does not require ethical approval from the Research Council or Ethics
234 board. We intend to disseminate our findings in scientific peer-reviewed journal, general
235 free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm
236 SAMBA) study, specialists' conferences, and to our funding agencies.

237 DISCUSSION

238 This systematic review will comprise current knowledge related with metabolomics in the
239 context of preterm birth prediction. Metabolomics science, a resourceful innovative field
240 that allows better understanding on pathophysiology of complex syndromes, may address
241 the main compounds associated with the spontaneous preterm delivery and, therefore,
242 motivate further researchers to validate early measurable predictors of preterm birth.

243 Metabolomics performance for predicting sPTB remains unclear and standardized and high-
244 quality studies are needed to clarify the clinical application of metabolites for predicting
245 sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation
246 studies; reproducible methodology is crucial. This systematic review protocol will collate the
247 main potential early biomarkers, subgroup analysis and standardized definition for

spontaneous preterm birth to better understand metabolomics performance in predicting sPTB and also to show its heterogeneity in terms of methodology (samples used, metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of preterm birth will help combat this leading cause of neonatal mortality and morbidity.

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47 309 **Author's Contributions**
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49 310 RTS and RBFG will conduct the systematic review as independent first reviewers. JGC, RPJ
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51 311 and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and
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JGC participated in the systematic review conception, methodology and framework, together with all the others co-authors.

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Competing interests

All authors are carrying original research about metabolomics and presenting conferences about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics Diagnostics Ltd, a company dedicated to develop innovative screening tests using metabolomics technology.

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Ethics approval and consent to participate

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333 This systematic review does not require ethical approval from the Research Council or Ethics
334 board.

For peer review only

Search strategy: #1 AND #2 AND #3

- 1 (OR for each term)
- preterm birth
 - premature birth
 - premature infant
 - premature labor
 - extremely premature infant
 - premature obstetric labor
 - spontaneous preterm birth
 - extreme preterm birth
 - late preterm birth
 - moderate preterm birth
 - preterm premature rupture of membranes
 - preterm delivery
 - PROM
 - sPTB
 - preterm PROM
 - pPROM
 - p-PROM
- 2 (OR for each term)
- metabolomic*
 - metabonomic*
 - metabolit*
 - lipidomic*
 - H NMR
 - proton NMR
 - proton nuclear magnetic resonance
 - liquid chromatogra*
 - UPLC
 - ultra-performance liquid chromatograph*
 - ultra performance liquid chromatograph*
 - HPLC
 - high performance liquid chromatograph*
 - high-performance liquid chromatograph*
- 3 (OR for each term)
- pregnan*
 - antenat*
 - ante nat*
 - prenat*
 - pre nat*

PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 **5**:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Support					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2 , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a

BMJ Open

The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis

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1 The use of metabolomics for predicting spontaneous preterm birth in asymptomatic
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16 This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available
17 from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172.

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ABSTRACT

Introduction: Preterm birth (PTB) is the leading cause of neonatal mortality and short- and long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still unclear, which makes the identification of reliable and accurate predictor markers more difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers have been demonstrated to be potentially accurate biomarkers for many disorders with complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of metabolomics markers associated with spontaneous PTB. Our research question is “What is the performance of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women?”

Methods and analysis: We will focus on studies assessing metabolomics techniques for predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a comprehensive systematic review of the literature from the last 10 years. Only observational cohort and case-control studies will be included. Our search strategy will be carried out by two independent reviewers, who will scan title and abstract before carrying out a full review of the article. The scientific databases to be explored include PubMed, MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.

Ethics and dissemination: This systematic review protocol does not require ethical approval. We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm SAMBA study open access website, specialists’ conferences, and to our funding agencies.

Registration details: This protocol is registered in PROSPERO platform (code CRD42018100172).

Keywords: preterm birth, spontaneous preterm birth, metabolomics, biomarkers, prediction.

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Strengths and limitations of this study

- This systematic review protocol takes into account some important aspects regarding conducting a systematic review about spontaneous preterm birth and metabolomics such as the criteria used for defining spontaneous preterm birth, different population risk stratification, method used to estimate gestational age, and metabolomics techniques details.
- Two independent reviewers are responsible for searching and selecting studies, as also extracting data, and a third reviewer will resolve any disagreement.
- If possible, proper statistical methods will be applied to investigate metabolomics accuracy in predicting spontaneous preterm birth.
- Possible limitations to this review include the different criteria applied for defining spontaneous preterm birth, and the diverse population risk stratification.

INTRODUCTION

Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4]. Several pathways and mechanisms linked with preterm birth have been proposed including, neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More specifically, several markers associated with uterine distension/contraction, decidual inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been studied in the past decades [5,6]. However, no single marker or combination of markers has been found to be accurate enough for predicting sPTB [7–10]. History of previous preterm birth, cervical length at second trimester and cervico-vaginal fetal fibronectin are the most promising clinical tests for predicting spontaneous preterm, but they seem not to be clinically useful for asymptomatic women. Sensitivity of short cervical length (<25mm) and high cervico-vaginal fFN (>50ng/ml) are around 33-36% and 46%, respectively [11–13].

Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical phase, maternal and fetal interactions, genetic and environmental influences, and adaptive mechanisms [14,15]. These challenging aspects, and the presence of still unknown underlying mechanisms, are the main limitations for the identification of an accurate predictor for sPTB [16–18]. None of the predictors used in clinical practice, such as previous history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and transvaginal ultrasonography cervical length demonstrated exceptional accuracy for predicting spontaneous preterm birth [7]. An exploration of innovative approaches is urgently required.

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Metabolomics is the study of metabolites, through identification and quantification of low-weight molecular particles, i.e. tens to hundreds thousands of intermediate products and substrates of systems biology chemical reactions [19,20]. This novel approach has been applied for identifying biomarkers and underlying biochemical pathways associated with complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational diabetes and preterm birth. In contrast to other “*Omics Sciences*” techniques, metabolomics is more closely associated with the phenotype of the disease and might thus identify a more robust and reliable set of predictors [21]. Importantly, implementing an adequate *Omics* experimental design is crucial for metabolomics studies. Using different baseline population (asymptomatic vs symptomatic or low- vs high-risk women for developing sPTB), study designs (prospective cohorts, case-control or cross sectional studies), sources of samples (amniotic fluid, vaginal fluid, blood, urine, hair, etc) and the timing of sample collection each have significant effects on study findings and the consequent interpretation and contribution to the current gap of knowledge [19].

Different reviews collating scientific knowledge regarding preterm birth biomarkers/predictors has been conducted. Different methodology approaches has been applied so far, including narrative, systematic and umbrella reviews, a more comprehensive review that includes not only original studies but also other reviews [7,19,22,23]. At the best of our knowledge, there is no systematic review on metabolomics markers. Therefore, we aim to conduct a systematic review of original studies investigating the use of metabolomics biomarkers for predicting spontaneous preterm birth in asymptomatic pregnant women. This protocol describes the methods that will be applied in our systematic review.

METHODS AND ANALYSIS

108 The current systematic review proposal will be conducted, written and published following
109 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)
110 recommendations [24]. Also, it is properly registered at PROSPERO platform – code
111 CRD42018100172.

112 **Review question**

113 What is the performance of metabolomics for predicting spontaneous preterm birth in
114 asymptomatic pregnant women?

115 **Eligibility Criteria**

116 Original cohort or case-control studies involving asymptomatic pregnant women at the
117 moment of sample collection (exposure) and with samples analysed using metabolomics
118 techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials,
119 editorials, letter to editors, case reports, expert opinions, commentaries, or any type of
120 review; (2) they describes only experimental studies with animals; or (3) they are duplicated
121 data (e.g. data published in conferences proceedings and, then, published again in scientific
122 journals). In this case, only the most complete publication will be considered, after
123 comparing and confirming that the same technique and metabolites were explored. Studies
124 published from 2008 to 2018 will be considered, and there will be no language restriction.
125 Before submitting this systematic review for publication, we will rerun the search strategy to
126 identify new studies that have been published after performing the first round of search.

127 **Participants**

128 The current review is interested in evaluating the performance of metabolomics biomarkers
129 for spontaneous preterm birth in asymptomatic pregnant women, which may contribute to
130 clinical practice, potentially providing information regarding onset of preterm labour.
131 Nevertheless, we aim to identify studies addressing only early predictors collected from

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women who are in an early preclinical stage, which might contribute to a wider window of opportunity for interventions and also to develop a widely reproducible screening test. Asymptomatic pregnant women should not have regular uterine tightening/contractions or signs of rupture of membranes (i.e. watery discharge). In addition, the study should preferably have a standardized definition of spontaneous preterm birth, the outcome of interest.

Information Sources

The search will be held in the following databases: PubMed, EMBASE, Scopus, CINAHL, and Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health Sciences Literature (LILACS), Medline and the Scientific Electronic Library Online (SciELO). In addition, secondary sources of original studies will be explored such as Google Scholar, hand-held searching of the reference list of eligible studies, conference proceedings, and contact with authors when necessary.

Search Strategy

The following terms will be used in our search strategy for the different scientific databases: (preterm birth, premature birth, premature infant, premature labor, extremely premature infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic*, metabonomic*, metabolit*, lipidomic*, H NMR, proton NMR, proton nuclear magnetic resonance, liquid chromatogra*, gas chromatogra*, UPLC, ultra-performance liquid chromatograph*, ultra-performance liquid chromatograph*, HPLC, high performance liquid chromatograph*, high-performance liquid chromatograph*) AND (pregnan*, antenat*, ante nat*, prenat*, pre nat*) (Supplementary Material). Respective adaptations in the

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3 156 syntax of search for each database will be applied accordingly. No filters - such as “research
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6 157 in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded
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8 158 according to eligibility criteria. The complete search strategy, including Boolean terms, is
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11 159 provided as Supplementary Material.

12 13 160 **Data Management**

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15 161 We will export search results to a reference manager (Mendeley®). Then, the following
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17 162 information will be collected from each study using an appropriate form, which will be
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20 163 entered in an Excel® spreadsheet: author’s name, year of publication, country, study design,
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22 164 number of participants with and without spontaneous preterm birth, type of metabolomics
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24 165 analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods
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26 166 for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm
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28 167 birth (spontaneous preterm labour or pPROM), number of fetuses (singleton vs multiple),
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30 168 gestational age when samples were collected, source of samples (type/site of tissue), low or
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32 169 high-risk for preterm birth (authors criteria used to define the population will be collected)
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35 170 and method applied to estimate gestational age. If possible, additional variables related to
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37 171 spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will
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39 172 be recorded for secondary analyses. Original authors will be contacted to clarify data, when
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41 173 needed. Finally, we will check the biochemical class of identified metabolites in Human
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43 174 Metabolome Database (HMDB, version 4.0) to explore and synthesize whether there are
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45 175 common biological pathways associated with spontaneous preterm birth [20].

46 47 176 **Selection Process**

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49 177 Two independent reviewers (RTS and RBFG) will be responsible for screening and selecting
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51 178 studies initially according to title or abstract. Both researchers will read the full text of non-
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53 179 excluded studies to discriminate eligibility. A third reviewer (DFBL) will consider any
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disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all steps and approving data extraction.

Data Collection Process

We will extract search results to a reference manager where all studies will be stored. Then, included studies will be placed in a new folder. Finally, we will manually extract data of interest from these included studies to an Excel® file. Each reviewer will have their own reference manager account, file and folder and discrepant results will be discussed together with the third reviewer.

Outcomes and Prioritization

The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of membranes. Secondary outcomes are:

- 1. Spontaneous preterm birth before 28 weeks;
- 2. Spontaneous preterm birth before 32 weeks;
- 3. Spontaneous preterm birth before 34 weeks;

The capacity to predict different degrees of sPTB (categories of gestational age) is important as the extreme (<28wks), moderate (<32 weeks) and non-late preterm (<34wks) newborns have different adverse outcomes compared to non-extreme (≥28wks); non-moderate (≥32wks) or late (≥34 wks) preterm newborns.

Ideally, the method of gestational age estimation should be clearly reported. For instance, it can be reported as estimated by last menstrual period (LMP) and confirmed by an early ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

Index test

Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of interest. Metabolomics is a technique to identify and quantify metabolites from biological samples using different type of platforms/equipment. The most common platforms include gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass spectrometer or a proton nuclear magnetic resonance [25]. If possible, the performance of each metabolomics techniques will be assessed through hierarchical summary receiver operator characteristic curve (HSROC) (meta-analysis).

Risk of Bias in individual Studies

We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [26] to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard (occurrence of preterm birth), and Flow and Timing of participant’s inclusion and follow-up. For example, studies will be labelled as “low” risk of bias for Reference Standard when definition of spontaneous preterm birth and gestational age estimation are clear; “high” risk of bias would be considered when the moment of sample collection is not well described.

Data Synthesis

We will report details of identification, screening, eligibility and included studies using a flow diagram, according to PRISMA recommendations [24]. Data from included studies will be synthesized into tables according to the variables of interest. If possible, we will present data meta-analysis according to study design, metabolomics technique and type of samples analysed. We intend to perform subgroup analysis according to:

- Different metabolomics methods applied: gas or liquid chromatography coupled with mass spectrometry or proton nuclear magnetic resonance;

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- Singleton and multiple pregnancies;
- Low-risk and high-risk women for developing preterm birth;
- Subtype of preterm birth: Spontaneous preterm birth exclusively due to spontaneous onset of labour with intact membranes or sPTB due to premature rupture of membranes.

Heterogeneity will be assessed by Cochran’s Q, Hotelling’s T-squared (τ^2) and I^2 tests. Funnel plots and sensitivity and cumulative analyses will be applied for detection of temporal trends and publication bias.

Potential anticipated limitations to this review

Firstly, although we have not considered any language restriction, we consider that there might be a limitation in studies published entirely in non-English language. However, in the last decade, more than 95% of scientific biomedical literature has been published in English [27], then we consider this a minor selection bias. Secondly, we intend to stratify the groups according to population risk. However, the characterization of low- or high-risk for spontaneous preterm birth is controversial and lacks standardization, which might limit data comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of labour or pPROM is another topic of potential limitation - the recognition of the main initial mechanism for preterm delivery might not always be possible. Even when specified, it might provoke uncertainty and could limit further considerations regarding preterm phenotypes. In addition, another limitation is that individual patient data will not be collected.

Patient and Public Involvement

Patients will not be directly involved in the study and no experience or direct impact from their perspective can be discussed.

251 ETHICS AND DISSEMINATION

252 This systematic review does not require ethical approval from the Research Council or Ethics
253 board. We intend to disseminate our findings in scientific peer-reviewed journal, general
254 free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm
255 SAMBA) study, specialists' conferences, and to our funding agencies.

256 DISCUSSION

257 This systematic review will comprise current knowledge related with metabolomics in the
258 context of preterm birth prediction. Metabolomics science, a resourceful innovative field
259 that allows better understanding on pathophysiology of complex syndromes, may address
260 the main compounds associated with the spontaneous preterm delivery and, therefore,
261 motivate further researchers to validate early measurable predictors of preterm birth.
262 Metabolomics performance for predicting sPTB remains unclear and standardized and high-
263 quality studies are needed to clarify the clinical application of metabolites for predicting
264 sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation
265 studies; reproducible methodology is crucial. This systematic review protocol will collate the
266 main potential early biomarkers, subgroup analysis and standardized definition for
267 spontaneous preterm birth to better understand metabolomics performance in predicting
268 sPTB and also to show its heterogeneity in terms of methodology (samples used,
269 metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of
270 preterm birth will help combat this leading cause of neonatal mortality and morbidity.

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Author’s Contributions

RTS and RBFG will conduct the systematic review as independent first reviewers. JGC, RPJ and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and JGC participated in the systematic review conception, methodology and framework, together will all the others co-authors.

Funding

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Competing interests

All authors are carrying original research about metabolomics and presenting conferences about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics Diagnostics Ltd, a company dedicated to develop innovative screening tests using metabolomics technology.

Acknowledgements

366 Ana Paula de Moraes e Oliveira, librarian of University of Campinas – Unicamp, Brazil, for
367 collaborating in developing search strategy and Rachel Hanisch for her suggestions to some
368 sections of the paper.

369 **Ethics approval and consent to participate**

370 This systematic review does not require ethical approval from the Research Council or Ethics
371 board.

For peer review only

Search strategy: #1 AND #2 AND #3

- 1 (OR for each term)
- preterm birth

premature birth

premature infant

premature labor

extremely premature infant

premature obstetric labor

spontaneous preterm birth

extreme preterm birth

late preterm birth

moderate preterm birth

preterm premature rupture of membranes

preterm delivery

PROM

sPTB

preterm PROM

pPROM

p-PROM
- 2 (OR for each term)
- metabolomic*

metabonomic*

metabolit*

lipidomic*

H NMR

proton NMR

proton nuclear magnetic resonance

liquid chromatogra*

UPLC

ultra-performance liquid chromatograph*

ultra performance liquid chromatograph*

HPLC

high performance liquid chromatograph*

high-performance liquid chromatograph*
- 3 (OR for each term)
- pregnan*

antenat*

ante nat*

prenat*

pre nat*

PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 **5**:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Support					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2 , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a

BMJ Open

The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-026033.R2
Article Type:	Protocol
Date Submitted by the Author:	20-Dec-2018
Complete List of Authors:	Souza, Renato; Universidade Estadual de Campinas, Obstetrics and Gynecology Galvão, Rafael; Universidade Estadual de Campinas, Obstetrics and Gynecology Leite, Debora; Campinas' State University, Department of Tocogynecology; Universidade Federal de Pernambuco, Passini Jr, Renato; Universidade Estadual de Campinas Faculdade de Ciencias Medicas Baker, Philip ; University of Leicester, College of Medicine Cecatti, Jose; University of Campinas, Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Diagnostics
Keywords:	preterm birth, metabolomics, biomarkers, prediction, metabolome

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The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis

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³ College of Life Sciences, University of Leicester, England, United Kingdom.

This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172.

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Word count: 2,233.

ABSTRACT

Introduction: Preterm birth (PTB) is the leading cause of neonatal mortality and short- and long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still unclear, which makes the identification of reliable and accurate predictor markers more difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers have been demonstrated to be potentially accurate biomarkers for many disorders with complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of metabolomics markers associated with spontaneous PTB. Our research question is “What is the performance of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women?”

Methods and analysis: We will focus on studies assessing metabolomics techniques for predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a comprehensive systematic review of the literature from the last 10 years. Only observational cohort and case-control studies will be included. Our search strategy will be carried out by two independent reviewers, who will scan title and abstract before carrying out a full review of the article. The scientific databases to be explored include PubMed, MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.

Ethics and dissemination: This systematic review protocol does not require ethical approval. We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm SAMBA study open access website, specialists’ conferences, and to our funding agencies.

Registration details: This protocol is registered in PROSPERO platform (code CRD42018100172).

Keywords: preterm birth, spontaneous preterm birth, metabolomics, biomarkers, prediction, metabolome.

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Strengths and limitations of this study

- This systematic review protocol takes into account some important aspects regarding conducting a systematic review about spontaneous preterm birth and metabolomics such as the criteria used for defining spontaneous preterm birth, different population risk stratification, method used to estimate gestational age, and metabolomics techniques details.
- Two independent reviewers are responsible for searching and selecting studies, as also extracting data, and a third reviewer will resolve any disagreement.
- If possible, proper statistical methods will be applied to investigate metabolomics accuracy in predicting spontaneous preterm birth.
- Possible limitations to this review include the different criteria applied for defining spontaneous preterm birth, and the diverse population risk stratification.

INTRODUCTION

Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4]. Several pathways and mechanisms linked with preterm birth have been proposed including, neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More specifically, several markers associated with uterine distension/contraction, decidual inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been studied in the past decades [5,6]. However, no single marker or combination of markers has been found to be accurate enough for predicting sPTB [7–10]. History of previous preterm birth, cervical length at second trimester and cervico-vaginal fetal fibronectin are the most promising clinical tests for predicting spontaneous preterm, but they seem not to be clinically useful for asymptomatic women. Sensitivity of short cervical length (<25mm) and high cervico-vaginal fFN (>50ng/ml) are around 33-36% and 46%, respectively [11–13].

Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical phase, maternal and fetal interactions, genetic and environmental influences, and adaptive mechanisms [14,15]. These challenging aspects, and the presence of still unknown underlying mechanisms, are the main limitations for the identification of an accurate predictor for sPTB [16–18]. None of the predictors used in clinical practice, such as previous history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and transvaginal ultrasonography cervical length demonstrated exceptional accuracy for predicting spontaneous preterm birth [7]. An exploration of innovative approaches is urgently required.

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Metabolomics is the study of metabolites, through identification and quantification of low-weight molecular particles, i.e. tens to hundreds thousands of intermediate products and substrates of systems biology chemical reactions [19,20]. This novel approach has been applied for identifying biomarkers and underlying biochemical pathways associated with complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational diabetes and preterm birth. In contrast to other “*Omics Sciences*” techniques, metabolomics is more closely associated with the phenotype of the disease and might thus identify a more robust and reliable set of predictors [21]. Importantly, implementing an adequate *Omics* experimental design is crucial for metabolomics studies. Using different baseline population (asymptomatic vs symptomatic or low- vs high-risk women for developing sPTB), study designs (prospective cohorts, case-control or cross sectional studies), sources of samples (amniotic fluid, vaginal fluid, blood, urine, hair, etc) and the timing of sample collection each have significant effects on study findings and the consequent interpretation and contribution to the current gap of knowledge [19].

Different reviews collating scientific knowledge regarding preterm birth biomarkers/predictors has been conducted. Different methodology approaches has been applied so far, including narrative, systematic and umbrella reviews, a more comprehensive review that includes not only original studies but also other reviews [7,19,22,23]. At the best of our knowledge, there is no systematic review on metabolomics markers. Therefore, we aim to conduct a systematic review of original studies investigating the use of metabolomics biomarkers for predicting spontaneous preterm birth in asymptomatic pregnant women. This protocol describes the methods that will be applied in our systematic review.

METHODS AND ANALYSIS

108 The current systematic review proposal will be conducted, written and published following
109 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)
110 recommendations [24]. Also, it is properly registered at PROSPERO platform – code
111 CRD42018100172.

112 **Review question**

113 What is the performance of metabolomics for predicting spontaneous preterm birth in
114 asymptomatic pregnant women?

115 **Eligibility Criteria**

116 Original cohort or case-control studies involving asymptomatic pregnant women at the
117 moment of sample collection (exposure) and with samples analysed using metabolomics
118 techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials,
119 editorials, letter to editors, case reports, expert opinions, commentaries, or any type of
120 review; (2) they describes only experimental studies with animals; or (3) they are duplicated
121 data (e.g. data published in conferences proceedings and, then, published again in scientific
122 journals). In this case, only the most complete publication will be considered, after
123 comparing and confirming that the same technique and metabolites were explored. Studies
124 published from 2008 to 2018 will be considered, and there will be no language restriction.
125 Before submitting this systematic review for publication, we will rerun the search strategy to
126 identify new studies that have been published after performing the first round of search.

127 **Participants**

128 The current review is interested in evaluating the performance of metabolomics biomarkers
129 for spontaneous preterm birth in asymptomatic pregnant women, which may contribute to
130 clinical practice, potentially providing information regarding onset of preterm labour.
131 Nevertheless, we aim to identify studies addressing only early predictors collected from

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women who are in an early preclinical stage, which might contribute to a wider window of opportunity for interventions and also to develop a widely reproducible screening test. Asymptomatic pregnant women should not have regular uterine tightening/contractions or signs of rupture of membranes (i.e. watery discharge). In addition, the study should preferably have a standardized definition of spontaneous preterm birth, the outcome of interest.

Information Sources

The search will be held in the following databases: PubMed, EMBASE, Scopus, CINAHL, and Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health Sciences Literature (LILACS), Medline and the Scientific Electronic Library Online (Scielo). In addition, secondary sources of original studies will be explored such as Google Scholar, hand-held searching of the reference list of eligible studies, conference proceedings, and contact with authors when necessary.

Search Strategy

The following terms will be used in our search strategy for the different scientific databases: (preterm birth, premature birth, premature infant, premature labor, extremely premature infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic*, metabonomic*, metabolit*, lipidomic*, H NMR, proton NMR, proton nuclear magnetic resonance, liquid chromatogra*, gas chromatogra*, UPLC, ultra-performance liquid chromatograph*, ultra-performance liquid chromatograph*, HPLC, high performance liquid chrormatograph*, high-performance liquid chrormatograph*) AND (pregnan*, antenat*, ante nat*, prenat*, pre nat*) (Supplementary Material). Respective adaptations in the

1
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3 156 syntax of search for each database will be applied accordingly. No filters - such as “research
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6 157 in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded
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8 158 according to eligibility criteria. The complete search strategy, including Boolean terms, is
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11 159 provided as Supplementary Material.

12 13 160 **Data Management**

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15 161 We will export search results to a reference manager (Mendeley®). Then, the following
16
17 162 information will be collected from each study using an appropriate form, which will be
18
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20 163 entered in an Excel® spreadsheet: author’s name, year of publication, country, study design,
21
22 164 number of participants with and without spontaneous preterm birth, type of metabolomics
23
24 165 analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods
25
26 166 for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm
27
28 167 birth (spontaneous preterm labour or pPROM), number of fetuses (singleton vs multiple),
29
30 168 gestational age when samples were collected, source of samples (type/site of tissue), low or
31
32 169 high-risk for preterm birth (authors criteria used to define the population will be collected)
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35 170 and method applied to estimate gestational age. If possible, additional variables related to
36
37 171 spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will
38
39 172 be recorded for secondary analyses. Original authors will be contacted to clarify data, when
40
41 173 needed. Finally, we will check the biochemical class of identified metabolites in Human
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43 174 Metabolome Database (HMDB, version 4.0) to explore and synthesize whether there are
44
45 175 common biological pathways associated with spontaneous preterm birth [20].
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51 176 **Selection Process**

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53 177 Two independent reviewers (RTS and RBFG) will be responsible for screening and selecting
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55 178 studies initially according to title or abstract. Both researchers will read the full text of non-
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57 179 excluded studies to discriminate eligibility. A third reviewer (DFBL) will consider any
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180 disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all
181 steps and approving data extraction.

182 **Data Collection Process**

183 We will extract search results to a reference manager where all studies will be stored. Then,
184 included studies will be placed in a new folder. Finally, we will manually extract data of
185 interest from these included studies to an Excel® file. Each reviewer will have their own
186 reference manager account, file and folder and discrepant results will be discussed together
187 with the third reviewer.

188 **Outcomes and Prioritization**

189 The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37
190 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of
191 membranes. Secondary outcomes are:

- 192 1. Spontaneous preterm birth before 28 weeks;
- 193 2. Spontaneous preterm birth before 32 weeks;
- 194 3. Spontaneous preterm birth before 34 weeks;

195 The capacity to predict different degrees of sPTB (categories of gestational age) is important
196 as the extreme (<28wks), moderate (<32 weeks) and non-late preterm (<34wks) newborns
197 have different adverse outcomes compared to non-extreme (≥28wks); non-moderate
198 (≥32wks) or late (≥34 wks) preterm newborns.

199 Ideally, the method of gestational age estimation should be clearly reported. For instance, it
200 can be reported as estimated by last menstrual period (LMP) and confirmed by an early
201 ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

202 **Index test**

Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of interest. Metabolomics is a technique to identify and quantify metabolites from biological samples using different type of platforms/equipment. The most common platforms include gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass spectrometer or a proton nuclear magnetic resonance [25]. The performance of the different thresholds of each metabolite will be compared and summarized through hierarchical summary receiver operator characteristic curve (HSROC) (meta-analysis) according to the subgroups described above.

Risk of Bias in individual Studies

We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [26] to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard (occurrence of preterm birth), and Flow and Timing of participant’s inclusion and follow-up. For example, studies will be labelled as “low” risk of bias for Reference Standard when definition of spontaneous preterm birth and gestational age estimation are clear; “high” risk of bias would be considered when the moment of sample collection is not well described.

Data Synthesis

We will report details of identification, screening, eligibility and included studies using a flow diagram, according to PRISMA recommendations [24]. Data from included studies will be synthesized into tables according to the variables of interest. If possible, we will present data meta-analysis according to study design, metabolomics technique and type of samples analysed. We intend to perform subgroup analysis according to:

- Different metabolomics methods applied: gas or liquid chromatography coupled

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- with mass spectrometry or proton nuclear magnetic resonance;
- Singleton and multiple pregnancies;
- Low-risk and high-risk women for developing preterm birth;
- Subtype of preterm birth: Spontaneous preterm birth exclusively due to spontaneous onset of labour with intact membranes or sPTB due to premature rupture of membranes.
- Gestational age interval when samples were collected: 1st trimester, 2nd trimester and 3rd trimester.

Heterogeneity will be assessed by Cochran’s Q, Hotelling’s T-squared (τ^2) and I^2 tests. Funnel plots and sensitivity and cumulative analyses will be applied for detection of temporal trends and publication bias.

Potential anticipated limitations to this review

Firstly, although we have not considered any language restriction, we consider that there might be a limitation in studies published entirely in non-English language. However, in the last decade, more than 95% of scientific biomedical literature has been published in English [27], then we consider this a minor selection bias. Secondly, we intend to stratify the groups according to population risk. However, the characterization of low- or high-risk for spontaneous preterm birth is controversial and lacks standardization, which might limit data comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of labour or pPROM is another topic of potential limitation - the recognition of the main initial mechanism for preterm delivery might not always be possible. Even when specified, it might provoke uncertainty and could limit further considerations regarding preterm phenotypes. In addition, another limitation is that individual patient data will not be collected.

Patient and Public Involvement

Patients will not be directly involved in the study and no experience or direct impact from their perspective can be discussed.

ETHICS AND DISSEMINATION

This systematic review does not require ethical approval from the Research Council or Ethics board. We intend to disseminate our findings in scientific peer-reviewed journal, general free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm SAMBA) study, specialists' conferences, and to our funding agencies.

DISCUSSION

This systematic review will comprise current knowledge related with metabolomics in the context of preterm birth prediction. Metabolomics science, a resourceful innovative field that allows better understanding on pathophysiology of complex syndromes, may address the main compounds associated with the spontaneous preterm delivery and, therefore, motivate further researchers to validate early measurable predictors of preterm birth. Metabolomics performance for predicting sPTB remains unclear and standardized and high-quality studies are needed to clarify the clinical application of metabolites for predicting sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation studies; reproducible methodology is crucial. This systematic review protocol will collate the main potential early biomarkers, subgroup analysis and standardized definition for spontaneous preterm birth to better understand metabolomics performance in predicting sPTB and also to show its heterogeneity in terms of methodology (samples used, metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of preterm birth will help combat this leading cause of neonatal mortality and morbidity.

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349 Author's Contributions

350 RTS and RBFG will conduct the systematic review as independent first reviewers. JGC, RPJ
351 and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and
352 JGC participated in the systematic review conception, methodology and framework,
353 together will all the others co-authors.

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362 Competing interests

363 All authors are carrying original research about metabolomics and presenting conferences
364 about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes
365 mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics
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367 metabolomics technology.

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370 collaborating in developing search strategy and Rachel Hanisch for her suggestions to some
371 sections of the paper.

372 **Ethics approval and consent to participate**

373 This systematic review does not require ethical approval from the Research Council or Ethics
374 board.

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Search strategy: #1 AND #2 AND #3

- 1 (OR for each term)
- preterm birth
 - premature birth
 - premature infant
 - premature labor
 - extremely premature infant
 - premature obstetric labor
 - spontaneous preterm birth
 - extreme preterm birth
 - late preterm birth
 - moderate preterm birth
 - preterm premature rupture of membranes
 - preterm delivery
 - PROM
 - sPTB
 - preterm PROM
 - pPROM
 - p-PROM
- 2 (OR for each term)
- metabolomic*
 - metabonomic*
 - metabolit*
 - lipidomic*
 - H NMR
 - proton NMR
 - proton nuclear magnetic resonance
 - liquid chromatogra*
 - UPLC
 - ultra-performance liquid chromatograph*
 - ultra performance liquid chromatograph*
 - HPLC
 - high performance liquid chromatograph*
 - high-performance liquid chromatograph*
- 3 (OR for each term)
- pregnan*
 - antenat*
 - ante nat*
 - prenat*
 - pre nat*

PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 **5**:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Support					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2 , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a

BMJ Open

The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-026033.R3
Article Type:	Protocol
Date Submitted by the Author:	09-Jan-2019
Complete List of Authors:	Souza, Renato; Universidade Estadual de Campinas, Obstetrics and Gynecology Galvão, Rafael; Universidade Estadual de Campinas, Obstetrics and Gynecology Leite, Debora; Campinas' State University, Department of Tocogynecology; Universidade Federal de Pernambuco, Passini Jr, Renato; Universidade Estadual de Campinas Faculdade de Ciencias Medicas Baker, Philip ; University of Leicester, College of Medicine Cecatti, Jose; University of Campinas, Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Diagnostics
Keywords:	preterm birth, metabolomics, biomarkers, prediction, metabolome

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1 The use of metabolomics for predicting spontaneous preterm birth in asymptomatic
2 pregnant women: protocol for a systematic review and meta-analysis

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15
16 This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available
17 from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172.

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24 Word count: 2,233.

ABSTRACT

Introduction: Preterm birth (PTB) is the leading cause of neonatal mortality and short- and long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still unclear, which makes the identification of reliable and accurate predictor markers more difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers have been demonstrated to be potentially accurate biomarkers for many disorders with complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of metabolomics markers associated with spontaneous PTB. Our research question is “What is the performance of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women?”

Methods and analysis: We will focus on studies assessing metabolomics techniques for predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a comprehensive systematic review of the literature from the last 10 years. Only observational cohort and case-control studies will be included. Our search strategy will be carried out by two independent reviewers, who will scan title and abstract before carrying out a full review of the article. The scientific databases to be explored include PubMed, MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.

Ethics and dissemination: This systematic review protocol does not require ethical approval. We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm SAMBA study open access website, specialists’ conferences, and to our funding agencies.

Registration details: This protocol is registered in PROSPERO platform (code CRD42018100172).

Keywords: preterm birth, spontaneous preterm birth, metabolomics, biomarkers, prediction, metabolome.

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Strengths and limitations of this study

- This systematic review protocol takes into account some important aspects regarding conducting a systematic review about spontaneous preterm birth and metabolomics such as the criteria used for defining spontaneous preterm birth, different population risk stratification, method used to estimate gestational age, and metabolomics techniques details.
- Two independent reviewers are responsible for searching and selecting studies, as also extracting data, and a third reviewer will resolve any disagreement.
- If possible, proper statistical methods will be applied to investigate metabolomics accuracy in predicting spontaneous preterm birth.
- Possible limitations to this review include the different criteria applied for defining spontaneous preterm birth, and the diverse population risk stratification.

INTRODUCTION

Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4]. Several pathways and mechanisms linked with preterm birth have been proposed including, neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More specifically, several markers associated with uterine distension/contraction, decidual inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been studied in the past decades [5,6]. However, no single marker or combination of markers has been found to be accurate enough for predicting sPTB [7–10]. History of previous preterm birth, cervical length at second trimester and cervico-vaginal fetal fibronectin are the most promising clinical tests for predicting spontaneous preterm, but they seem not to be clinically useful for asymptomatic women. Sensitivity of short cervical length (<25mm) and high cervico-vaginal fFN (>50ng/ml) are around 33-36% and 46%, respectively [11–13].

Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical phase, maternal and fetal interactions, genetic and environmental influences, and adaptive mechanisms [14,15]. These challenging aspects, and the presence of still unknown underlying mechanisms, are the main limitations for the identification of an accurate predictor for sPTB [16–18]. None of the predictors used in clinical practice, such as previous history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and transvaginal ultrasonography cervical length demonstrated exceptional accuracy for predicting spontaneous preterm birth [7]. An exploration of innovative approaches is urgently required.

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Metabolomics is the study of metabolites, through identification and quantification of low-weight molecular particles, i.e. tens to hundreds thousands of intermediate products and substrates of systems biology chemical reactions [19,20]. This novel approach has been applied for identifying biomarkers and underlying biochemical pathways associated with complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational diabetes and preterm birth. In contrast to other “*Omics Sciences*” techniques, metabolomics is more closely associated with the phenotype of the disease and might thus identify a more robust and reliable set of predictors [21]. Importantly, implementing an adequate *Omics* experimental design is crucial for metabolomics studies. Using different baseline population (asymptomatic vs symptomatic or low- vs high-risk women for developing sPTB), study designs (prospective cohorts, case-control or cross sectional studies), sources of samples (amniotic fluid, vaginal fluid, blood, urine, hair, etc) and the timing of sample collection each have significant effects on study findings and the consequent interpretation and contribution to the current gap of knowledge [19].

Different reviews collating scientific knowledge regarding preterm birth biomarkers/predictors has been conducted. Different methodology approaches has been applied so far, including narrative, systematic and umbrella reviews, a more comprehensive review that includes not only original studies but also other reviews [7,22–24]. At the best of our knowledge, there is no systematic review on metabolomics markers. Therefore, we aim to conduct a systematic review of original studies investigating the use of metabolomics biomarkers for predicting spontaneous preterm birth in asymptomatic pregnant women. This protocol describes the methods that will be applied in our systematic review.

METHODS AND ANALYSIS

108 The current systematic review proposal will be conducted, written and published following
109 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)
110 recommendations [25]. Also, it is properly registered at PROSPERO platform – code
111 CRD42018100172.

112 **Review question**

113 What is the performance of metabolomics for predicting spontaneous preterm birth in
114 asymptomatic pregnant women?

115 **Eligibility Criteria**

116 Original cohort or case-control studies involving asymptomatic pregnant women at the
117 moment of sample collection (exposure) and with samples analysed using metabolomics
118 techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials,
119 editorials, letter to editors, case reports, expert opinions, commentaries, or any type of
120 review; (2) they describes only experimental studies with animals; or (3) they are duplicated
121 data (e.g. data published in conferences proceedings and, then, published again in scientific
122 journals). In this case, only the most complete publication will be considered, after
123 comparing and confirming that the same technique and metabolites were explored. Studies
124 published from 2008 to 2018 will be considered, and there will be no language restriction.
125 Before submitting this systematic review for publication, we will rerun the search strategy to
126 identify new studies that have been published after performing the first round of search.

127 **Participants**

128 The current review is interested in evaluating the performance of metabolomics biomarkers
129 for spontaneous preterm birth in asymptomatic pregnant women, which may contribute to
130 clinical practice, potentially providing information regarding onset of preterm labour.
131 Nevertheless, we aim to identify studies addressing only early predictors collected from

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women who are in an early preclinical stage, which might contribute to a wider window of opportunity for interventions and also to develop a widely reproducible screening test. Asymptomatic pregnant women should not have regular uterine tightening/contractions or signs of rupture of membranes (i.e. watery discharge). In addition, the study should preferably have a standardized definition of spontaneous preterm birth, the outcome of interest.

Information Sources

The search will be held in the following databases: PubMed, EMBASE, Scopus, CINAHL, and Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health Sciences Literature (LILACS), Medline and the Scientific Electronic Library Online (SciELO). In addition, secondary sources of original studies will be explored such as Google Scholar, hand-held searching of the reference list of eligible studies, conference proceedings, and contact with authors when necessary.

Search Strategy

The following terms will be used in our search strategy for the different scientific databases: (preterm birth, premature birth, premature infant, premature labor, extremely premature infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic*, metabonomic*, metabolit*, lipidomic*, H NMR, proton NMR, proton nuclear magnetic resonance, liquid chromatogra*, gas chromatogra*, UPLC, ultra-performance liquid chromatograph*, ultra-performance liquid chromatograph*, HPLC, high performance liquid chromatograph*, high-performance liquid chromatograph*) AND (pregnan*, antenat*, ante nat*, prenat*, pre nat*) (Supplementary Material). Respective adaptations in the

syntax of search for each database will be applied accordingly. No filters - such as “research in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded according to eligibility criteria. The complete search strategy, including Boolean terms, is provided as Supplementary Material.

Data Management

We will export search results to a reference manager (Mendeley®). Then, the following information will be collected from each study using an appropriate form, which will be entered in an Excel® spreadsheet: author’s name, year of publication, country, study design, number of participants with and without spontaneous preterm birth, type of metabolomics analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm birth (spontaneous preterm labour or pPROM), number of fetuses (singleton vs multiple), gestational age when samples were collected, source of samples (type/site of tissue), low or high-risk for preterm birth (authors criteria used to define the population will be collected) and method applied to estimate gestational age. If possible, additional variables related to spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will be recorded for secondary analyses. Original authors will be contacted to clarify data, when needed. Finally, we will check the biochemical class of identified metabolites in Human Metabolome Database (HMDB, version 4.0) to explore and synthesize whether there are common biological pathways associated with spontaneous preterm birth [20].

Selection Process

Two independent reviewers (RTS and RBFG) will be responsible for screening and selecting studies initially according to title or abstract. Both researchers will read the full text of non-excluded studies to discriminate eligibility. A third reviewer (DFBL) will consider any

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180 disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all
181 steps and approving data extraction.

182 **Data Collection Process**

183 We will extract search results to a reference manager where all studies will be stored. Then,
184 included studies will be placed in a new folder. Finally, we will manually extract data of
185 interest from these included studies to an Excel® file. Each reviewer will have their own
186 reference manager account, file and folder and discrepant results will be discussed together
187 with the third reviewer.

188 **Outcomes and Prioritization**

189 The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37
190 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of
191 membranes. Secondary outcomes are:

- 192 1. Spontaneous preterm birth before 28 weeks;
- 193 2. Spontaneous preterm birth before 32 weeks;
- 194 3. Spontaneous preterm birth before 34 weeks;

195 The capacity to predict different degrees of sPTB (categories of gestational age) is important
196 as the extreme (<28wks), moderate (<32 weeks) and non-late preterm (<34wks) newborns
197 have different adverse outcomes compared to non-extreme (≥28wks); non-moderate
198 (≥32wks) or late (≥34 wks) preterm newborns.

199 Ideally, the method of gestational age estimation should be clearly reported. For instance, it
200 can be reported as estimated by last menstrual period (LMP) and confirmed by an early
201 ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

202 **Index test**

Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of interest. Metabolomics is a technique to identify and quantify metabolites from biological samples using different type of platforms/equipment. The most common platforms include gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass spectrometer or a proton nuclear magnetic resonance [26]. The performance of the different thresholds of each metabolite will be compared and summarized through hierarchical summary receiver operator characteristic curve (HSROC) (meta-analysis) according to the subgroups described above. Considering that the raw data is not available in the majority of the diagnostic test accuracy studies [27] and that metabolites levels are usually reported as continuous variables, we intend to use a meta-analysis model based on ROC curves [28]. Briefly, a two-parameter model, based on the estimation of α and β parameters (using standard errors or maximum likelihood), will be applied as reported by Kester & Buntinx [28]. Therefore, pooled ROC curves can be converted to a estimated ROC curve with 95% confidence interval. This method can also be applied in categorical-ordinal variables tests.

Risk of Bias in individual Studies

We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [29] to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard (occurrence of preterm birth), and Flow and Timing of participant’s inclusion and follow-up. For example, studies will be labelled as “low” risk of bias for Reference Standard when definition of spontaneous preterm birth and gestational age estimation are clear; “high” risk of bias would be considered when the moment of sample collection is not well described.

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Data Synthesis

We will report details of identification, screening, eligibility and included studies using a flow diagram, according to PRISMA recommendations [25]. Data from included studies will be synthesized into tables according to the variables of interest. If possible, we will present data meta-analysis according to study design, metabolomics technique and type of samples analysed. We intend to perform subgroup analysis according to:

- Different metabolomics methods applied: gas or liquid chromatography coupled with mass spectrometry or proton nuclear magnetic resonance;
- Singleton and multiple pregnancies;
- Low-risk and high-risk women for developing preterm birth;
- Subtype of preterm birth: Spontaneous preterm birth exclusively due to spontaneous onset of labour with intact membranes or sPTB due to premature rupture of membranes.
- Gestational age interval when samples were collected: 1st trimester, 2nd trimester and 3rd trimester.

Heterogeneity will be assessed by Cochran’s Q, Hotelling’s T-squared (τ^2) and I^2 tests. Funnel plots and sensitivity and cumulative analyses will be applied for detection of temporal trends and publication bias.

Potential anticipated limitations to this review

Firstly, although we have not considered any language restriction, we consider that there might be a limitation in studies published entirely in non-English language. However, in the last decade, more than 95% of scientific biomedical literature has been published in English [30], then we consider this a minor selection bias. Secondly, we intend to stratify the groups according to population risk. However, the characterization of low- or high-risk for

spontaneous preterm birth is controversial and lacks standardization, which might limit data comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of labour or pPROM is another topic of potential limitation - the recognition of the main initial mechanism for preterm delivery might not always be possible. Even when specified, it might provoke uncertainty and could limit further considerations regarding preterm phenotypes. In addition, another limitation is that individual patient data will not be collected.

Patient and Public Involvement

Patients will not be directly involved in the study and no experience or direct impact from their perspective can be discussed.

ETHICS AND DISSEMINATION

This systematic review does not require ethical approval from the Research Council or Ethics board. We intend to disseminate our findings in scientific peer-reviewed journal, general free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm SAMBA) study, specialists' conferences, and to our funding agencies.

DISCUSSION

This systematic review will comprise current knowledge related with metabolomics in the context of preterm birth prediction. Metabolomics science, a resourceful innovative field that allows better understanding on pathophysiology of complex syndromes, may address the main compounds associated with the spontaneous preterm delivery and, therefore, motivate further researchers to validate early measurable predictors of preterm birth. Metabolomics performance for predicting sPTB remains unclear and standardized and high-quality studies are needed to clarify the clinical application of metabolites for predicting

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274 SPTB. Nevertheless, metabolomics discovery studies commonly requires further validation
275 studies; reproducible methodology is crucial. This systematic review protocol will collate the
276 main potential early biomarkers, subgroup analysis and standardized definition for
277 spontaneous preterm birth to better understand metabolomics performance in predicting
278 SPTB and also to show its heterogeneity in terms of methodology (samples used,
279 metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of
280 preterm birth will help combat this leading cause of neonatal mortality and morbidity.
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For peer review only

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366 Author's Contributions

367 RTS and RBFG will conduct the systematic review as independent first reviewers. JGC, RPJ
 368 and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and
 369 JGC participated in the systematic review conception, methodology and framework,
 370 together will all the others co-authors.

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 373 401636/2013-5) and Bill and Melinda Gates Foundation (grant number OPP1107597- Grand
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 378 respectively. The sponsors played no role on the study design or manuscript writing.

379 Competing interests

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380 All authors are carrying original research about metabolomics and presenting conferences
381 about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes
382 mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics
383 Diagnostics Ltd, a company dedicated to develop innovative screening tests using
384 metabolomics technology.

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386 Ana Paula de Moraes e Oliveira, librarian of University of Campinas – Unicamp, Brazil, for
387 collaborating in developing search strategy and Rachel Hanisch for her suggestions to some
388 sections of the paper.

389 **Ethics approval and consent to participate**

390 This systematic review does not require ethical approval from the Research Council or Ethics
391 board.

Search strategy: #1 AND #2 AND #3

- 1 (OR for each term)
- preterm birth
 - premature birth
 - premature infant
 - premature labor
 - extremely premature infant
 - premature obstetric labor
 - spontaneous preterm birth
 - extreme preterm birth
 - late preterm birth
 - moderate preterm birth
 - preterm premature rupture of membranes
 - preterm delivery
 - PROM
 - sPTB
 - preterm PROM
 - pPROM
 - p-PROM
- 2 (OR for each term)
- metabolomic*
 - metabonomic*
 - metabolit*
 - lipidomic*
 - H NMR
 - proton NMR
 - proton nuclear magnetic resonance
 - liquid chromatogra*
 - UPLC
 - ultra-performance liquid chromatograph*
 - ultra performance liquid chromatograph*
 - HPLC
 - high performance liquid chromatograph*
 - high-performance liquid chromatograph*
- 3 (OR for each term)
- pregnan*
 - antenat*
 - ante nat*
 - prenat*
 - pre nat*

PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 **5**:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Support					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2 , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a