

# BMJ Open

## Glycosylated Haemoglobin as a Predictor of Cardiovascular Events and Mortality: A Protocol for a Systematic Review and Meta-Analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-012229
Article Type:	Protocol
Date Submitted by the Author:	11-Apr-2016
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<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Cardiovascular medicine, Diabetes and endocrinology, Public health
Keywords:	mortality, glycated haemoglobin, cardiovascular disease

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3 1 **Glycosylated Haemoglobin as a Predictor of Cardiovascular Events and Mortality:**  
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5 2 **A Protocol for a Systematic Review and Meta-Analysis**  
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8 3 **Systematic review registration:** PROSPERO CRD42015032552  
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## 26 ABSTRACT

27 **Introduction:** The glycosylated haemoglobin level (HbA1c) is an indicator of the  
28 average blood glucose concentrations over the preceding 2-3 months, which is used as a  
29 convenient and well-known biomarker in clinical practice. Currently, epidemiological  
30 evidence suggests that the HbA1c level is an independent risk factor for cardiovascular  
31 events such as myocardial infarction, stroke, coronary heart disease, and heart failure.  
32 This protocol aim to conduct systematic review and meta-analysis to determine the  
33 relationships between the HbA1c levels with cardiovascular outcomes and cause of  
34 death; and to analyse the range of HbA1c that is a predictor of cardiovascular disease  
35 and/or mortality based on data from published observational studies.

36 **Methods and analysis:** The search will be conducted using the MEDLINE, EMBASE,  
37 Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic  
38 Reviews, and Web of Science databases from their inception. Observational studies  
39 written in Portuguese, Spanish or English will be included. The Quality In Prognosis  
40 Studies tool will be used to assess the risk of bias for the studies included in the  
41 systematic review or meta-analysis. The hazard ratios for the cardiovascular outcomes  
42 and causes of death with 95% confidence intervals will be determined as the primary  
43 outcomes. Subgroup analyses will be performed based on the cardiovascular outcomes,  
44 the cause of death studied, or the type of population included in the studies.

45 **Ethics and dissemination:** This systematic review will synthesise evidence regarding  
46 the potential of using the HbA1c level as a prognostic marker for cardiovascular disease  
47 outcomes and/or mortality. The results will be disseminated by the publication of a  
48 manuscript in a peer-reviewed journal. Ethical approval will not be needed because the  
49 data used for this systematic review will be obtained from published studies and there  
50 will not be any concerns about privacy.

51 **Systematic review registration:** PROSPERO CRD42015032552

### 52 **Strengths and limitations of this study**

- 53 - This review of evidence will be useful to improve future research on HbA1c  
54 level as a prognostic marker for cardiovascular disease outcomes and/or  
55 mortality.

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3 56 - Study selection, data extraction and quality assessment will be performed  
4 57 independently by two researchers.  
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6 58 - Limitations and strengths will be discussed in our review, and the results will be  
7 59 put into context with other studies in the field.  
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9 60 - Different population-based studies can be a source of variable quality and  
10 61 heterogeneity between studies and may limit the quality of the evidence of this  
11 62 meta-analysis and systematic review.  
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## 64 INTRODUCTION

65 Cardiovascular disease (CVD) is a chronic disorder that develops insidiously  
66 throughout an individual's life and usually has progressed to an advanced stage by the  
67 time-symptoms occur<sup>1</sup>. The percentage of all deaths due to CVD before the age of 75  
68 years in Europe represents 42% in women and 38% in men<sup>2</sup>. Cardiovascular disease,  
69 especially coronary heart disease, is the leading cause of premature death worldwide.<sup>3</sup>

70 In 2007 was developed The Reynolds Risk Score for predicting CVD risk, which  
71 incorporates information on glycosylated haemoglobin (HbA1c), but this score was only  
72 used in people with known diabetes<sup>4</sup>. In 2010, the American College of Cardiology  
73 Foundation/American Heart Association Task Force on Practice Guidelines considered  
74 the HbA1c level as an appropriate index for CVD risk assessment in asymptomatic  
75 adults without a diagnosis of diabetes<sup>5</sup>. Finally, the Canadian Cardiovascular Society  
76 proposed that the CVD risk could be stratified by measuring the levels of fasting plasma  
77 glucose, HbA1c, or both<sup>6</sup>.

78 The HbA1c level is an indicator of the average blood glucose concentrations over the  
79 preceding two to three months, which is used as a convenient and well-known  
80 biomarker in clinical practice<sup>7-8</sup>. Epidemiological evidence suggests that the HbA1c  
81 level is an independent risk factor for cardiovascular events<sup>9</sup>. There is also evidence that  
82 the association between the HbA1C level with mortality from all-causes and CVD could  
83 be found at lower levels than the diabetic threshold<sup>10</sup>. A recent meta-analysis showed  
84 that HbA1c level was an independent predictor of mortality in coronary artery disease  
85 patients without but not in patients with established diabetes<sup>11</sup>.

86 Currently, the association between chronic hyperglycaemia and cardiovascular  
87 complications is not well defined. Several observational studies have demonstrated that

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3 88 a higher HbA1c level was associated with increased risks of CVD and death<sup>9, 12-13</sup>.  
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5 89 Thus, an elevated HbA1c level might contribute to the development of CVD, but the  
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7 90 association between the HbA1c level with the risk of CVD and mortality in the general  
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9 91 population remains unclear. Therefore, this protocol aims to present a clear and  
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11 92 transparent procedure for systematically review, evaluate and summarize the existing  
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13 93 information on the relationship between the HbA1c levels and CVD and death, which  
14  
15 94 could guide clinical decision making for further treatment strategies and also could  
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17 95 inform and facilitate future intervention research.  
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## 19 97 **OBJECTIVE**

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21 98 The aim of this protocol study is to establish a transparent and clear methodology to  
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23 99 conduct a systematic review and meta-analysis aimed to: i) determine the relationship  
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25 100 between the HbA1c levels with the cause of death and cardiovascular outcomes based  
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27 101 on data from observational studies, and ii) analyse what level of HbA1c is a predictor of  
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29 102 CVD and/or mortality.  
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## 32 104 **METHODS AND ANALYSIS**

### 33 105 **Review design**

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35 106 This protocol was developed based on the Preferred Reporting Items for Systematic  
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37 107 Review and Meta-analysis Protocols (PRISMA-P)<sup>14</sup> and was registered with  
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39 108 PROSPERO (Registration number: CRD42015032552). The MOOSE<sup>15</sup> (Meta-analysis  
40  
41 109 of observational studies in epidemiology: a proposal for reporting), PRISMA<sup>16</sup>  
42  
43 110 (Preferred Reporting Items for Systematic Reviews) and Cochrane Collaboration  
44  
45 111 Handbook<sup>17</sup> will be used to guide the review methods.  
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### 48 112 **Literature review**

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50 113 The literature search will be conducted using the MEDLINE (via PubMed), EMBASE,  
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52 114 the Cochrane Central Register of Controlled Trials, the Cochrane Database of  
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54 115 Systematic Reviews, and the Web of Science databases from their inception.  
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56 116 The following search terms will be combined using Boolean operators: glycosylated  
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58 117 hemoglobin, HbA1c, hemoglobin levels, glycated hemoglobin, hemoglobin A1c,  
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3 118 cardiovascular, cardiovascular disease, coronary heart disease, heart failure, stroke,  
4 119 peripheral arterial disease, cardiovascular events, coronary artery disease, myocardial  
5 120 infarction, cardiovascular outcomes, mortality, all-cause mortality, cardiovascular  
6 121 mortality, cause-specific mortality, death, cardiovascular death, observational study,  
7 122 cohort study and population-based (Table 1).

11 123 Previous systematic reviews and meta-analysis, and relevant references included in the  
12 124 selected studies will be screened as supplemental sources.

### 15 125 **Inclusion/exclusion criteria for study selection**

16 126 Studies regarding on the HbA1c level and cardiovascular outcomes retrieved in the  
17 127 literature search that meet the following criteria will be included: i) prospective or  
18 128 retrospective observational studies; ii) studies that observed the following  
19 129 cardiovascular outcomes: myocardial infarction, stroke, major adverse cardiovascular  
20 130 events (MACE), coronary heart disease, and heart failure; iii) reports of all-cause  
21 131 mortality and/or cardiovascular mortality; iv) outcomes measured using univariate and  
22 132 multivariable Cox proportional hazards models; v) population of adults aged 18 or older  
23 133 with any restriction on the race, gender or diabetic status; and vi) studies published in  
24 134 Portuguese, Spanish or English.

25 135 The process; of identifying, screening of studies and inclusion or exclusion of those  
26 136 studies; is shown in the PRISMA flow chart (Figure 1).

### 27 137 **Study selection and data extraction**

28 138 Two reviewers will independently check titles and abstracts to identify eligible studies  
29 139 according to the inclusion criteria. Then, the full manuscripts of the identified studies  
30 140 will be examined. Finally, two reviewers will check the included and excluded studies  
31 141 and verify the reasons why they were included/excluded. Any discrepancies will be  
32 142 resolved by discussion, a third reviewer will be asked on case of disagreement.

33 143 Two authors will independently extract the data regarding the author information, year  
34 144 of publication, design of the study, country, study project name and year of data  
35 145 collection, number, age of participants, methods used for HbA1c test certified by  
36 146 National Glycohemoglobin Standardization Program (NGSP), number of cardiovascular  
37 147 events, level of HbA1c used as the reference, and the hazard ratio (HR) for each HbA1c  
38 148 level (Table 2)

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3 149 Any disagreement will be resolved by discussion to reach a consensus. When necessary,  
4 150 authors of the potential included studies will be contacted to obtain any missing  
5  
6 151 information.  
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### 8 152 **Assessment of the risk of bias in the included studies**

9  
10 153 After blinding the included studies by author, title and year of publication, two  
11 154 independent researchers to assess the methodological quality will by the Quality in  
12 155 Prognosis Studies tool (QUIPS)<sup>18</sup>. Any disagreement in the assessment of the risk of  
13 156 bias will be discussed to reach a consensus. A third reviewer will make the final  
14 157 decision if a consensus is not reached. The QUIPS tool involves the use of 6 domains  
15 158 for the risk of bias: study participation (sampling bias), study attrition (attrition bias),  
16 159 prognostic factor measurement, outcome measurement (ascertainment bias),  
17 160 confounding measurement and accounting, and analysis and reporting. Studies will be  
18 161 considered to have a low, moderate or high risk of bias, satisfied by scores of 5 to 6, 3  
19 162 to 4, or 1 to 2 for the 6 bias domains, respectively.  
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### 28 163 **Statistical analysis**

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30 164 The researchers will create tables to summarize the characteristics of the included  
31 165 studies and any important questions related to the aim of this systematic review. The  
32 166 reviewers will determine whether a meta-analysis is possible after the data have been  
33 167 extracted. At least, five observations addressing HR for cardiovascular outcomes and  
34 168 mortality will be required to conduct a meta-analysis. If it is possible to carry out a  
35 169 meta-analysis, the STATA 14 software will be used to combine the extracted HR with  
36 170 95% CIs using an inverse variance model. We will compare adjusted and unadjusted  
37 171 estimates separately for each outcome. A fixed-effects model will be used if there is no  
38 172 evidence of heterogeneity; otherwise, a random-effects model will be used<sup>19</sup>. For the  
39 173 HbA1c levels, we will group studies by similar cut-off points to obtain meta-analysis  
40 174 results for each cut-off point whenever possible. We will used generalized least squares  
41 175 regression models to assess the pooled dose-response relation between HbA1c and CVD  
42 176 outcomes across prospective cohort studies that have heterogeneous categorizations of  
43 177 HbA1c<sup>20</sup>. Each meta-analysis will be summarized by the pooled HR and 95%  
44 178 confidence intervals. Studies providing insufficient data to perform the analyses will be  
45 179 omitted from the data synthesis. The heterogeneity of the studies will be assessed with  
46 180 an  $I^2$  statistic. Usually,  $I^2$  values of <25, 25-50, and >50% are considered to represent  
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3 181 small, medium, and large amounts of heterogeneity, respectively<sup>21</sup>. If a meta-analysis is  
4 182 not possible, we will undertake a narrative synthesis. Finally, publication bias was  
5 183 visually evaluated using a funnel plot, as well as with the method proposed by Egger<sup>22</sup>.  
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7 184 The strength of the body evidence will be evaluated by Grading of Recommendations  
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9 185 Assessment, Development and Evaluation tool (GRADE)<sup>23</sup>.  
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### 11 186 *Subgroup analyses and meta-regression*

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14 187 Subgroup analyses and meta-regression will be performed based on the cardiovascular  
15 188 outcomes (myocardial infarction, stroke, MACE, coronary heart disease, heart failure),  
16 189 cause of death studied (all causes of mortality or cardiovascular mortality), or on the  
17 190 type of population included in the studies (diabetic, prediabetic or non-diabetic),  
18 191 because these may be major factors causing heterogeneity. Furthermore, the age of the  
19 192 study participants, design of the study, and QUIPS score will be considered for  
20 193 additional subgroup analyses.  
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### 22 194 *Sensitivity analysis*

23 195 Excluding the included studies from the analysis one by one and comparing the results  
24 196 will perform sensitivity analyses.  
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## 26 197 **DISCUSSION**

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29 198 The utility of the HbA1c level as a prognostic marker for CVD outcomes and/or  
30 199 mortality is currently a source of controversy in the medical literature. Therefore, we  
31 200 will conduct a systematic review to identify what HbA1c level might be able to predict  
32 201 the CVD outcomes and mortality.  
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35 202 There is currently no consensus on what percentages should be used to determine the  
36 203 level of heterogeneity in categorical terms. Therefore, in this study, we will use the  
37 204 definition suggested by Higgins et al. to indicate that there is heterogeneity when the  $I^2$   
38 205 value is greater than 50%<sup>21</sup>.  
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42 206 Possible limitations that can be found in this research are: publication bias, information  
43 207 bias, poor statistical analyses, and inadequate reporting of methods and findings of the  
44 208 primary studies<sup>24</sup>. However, it is important to summarize the information available on  
45 209 this issue. To overcome these limitations, we will follow the recommendations included  
46 210 in the MOOSE, PRISMA and Cochrane Collaboration Handbook. According to the  
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3 211 Cochrane Prognosis Methods Group, we will use the QUIPS tool to assess the quality of  
4 212 the included studies<sup>18</sup>.

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7 213 There have already been numerous studies on the use of the HbA1c level as a  
8 214 prognostic marker for CVD outcomes and mortality, but the individual studies have  
9 215 been controversial, so there is uncertainty regarding its use. This makes it necessary to  
10 216 conduct this systematic review to provide a global overview of the current literature and  
11 217 to improve future research on this topic. It is therefore this protocol provide a clear and  
12 218 structured procedure for maximizing the extraction of relevant information and provide  
13 219 summarized information regarding the importance of HbA1c levels for controlling the  
14 220 risk of CVD outcomes and mortality.

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17 221 **Authors' contributions:** VMV and ICR designed the study. VMV was the principal  
18 222 investigator and guarantor. ICR and VMV were the main coordinators of the study. BP,  
19 223 CAB, FRA, and VMV conducted the study. ICR, BP and FRA gave statistical and  
20 224 epidemiological support. ICR wrote the article with the support of CAB and ICR. All  
21 225 authors revised and approved the final version of the manuscript.

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24 226 **Funding statement:** This research received no specific grant from any funding agency  
25 227 in the public, commercial or not-for-profit sectors.

26  
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28 228 **Competing interests:** None declared

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**Table 1.** Search strategy for MEDLINE

		cardiovascular		
		OR		
		“cardiovascular disease”		
		OR		
		“coronary heart disease”		
		OR		
		“heart failure”		
		OR		
		Stroke		
		OR		
		“peripheral arterial disease”		
		OR		
“glycosylated haemoglobin”		“cardiovascular events”		“observational study”
OR		OR		OR
“HbA1c”	AND	“coronary artery disease”	AND	“cohort study”
OR		OR		OR
“hemoglobin levels”		“myocardial infarction”		“population-based
OR		OR		
“glycated haemoglobin”		“cardiovascular outcomes”		
OR		OR		
“hemoglobin A1c”		mortality		
		OR		
		“all-cause mortality”		
		OR		
		“cardiovascular mortality”		
		OR		
		“cause-specific mortality”		
		OR		
		death		
		OR		
		“cardiovascular death”		

**Table 2.** Characteristics of studies included in the systematic review and/or meta-analysis.

Reference	Design	Country	Study/Year data collection	Age	n	n cardiovascular events	HbA1c method	HbA1c reference level	HR for Hba1c levels
<i>Author information of the and year of study publication</i>	<i>Design of the study</i>	<i>Country</i>	<i>Study project name and year of data collection</i>	<i>Age of participants</i>	<i>Number of participants</i>	<i>Number of cardiovascular events</i>	<i>Methods used for HbA1c test certified by NGSP</i>	<i>Level of HbA1c used as the reference</i>	<i>HR for each HbA1c level</i>

Hba1c: glycosilated haemoglobin; NGSP: National Glycohemoglobin Standardization Program; HR: Hazard ratio

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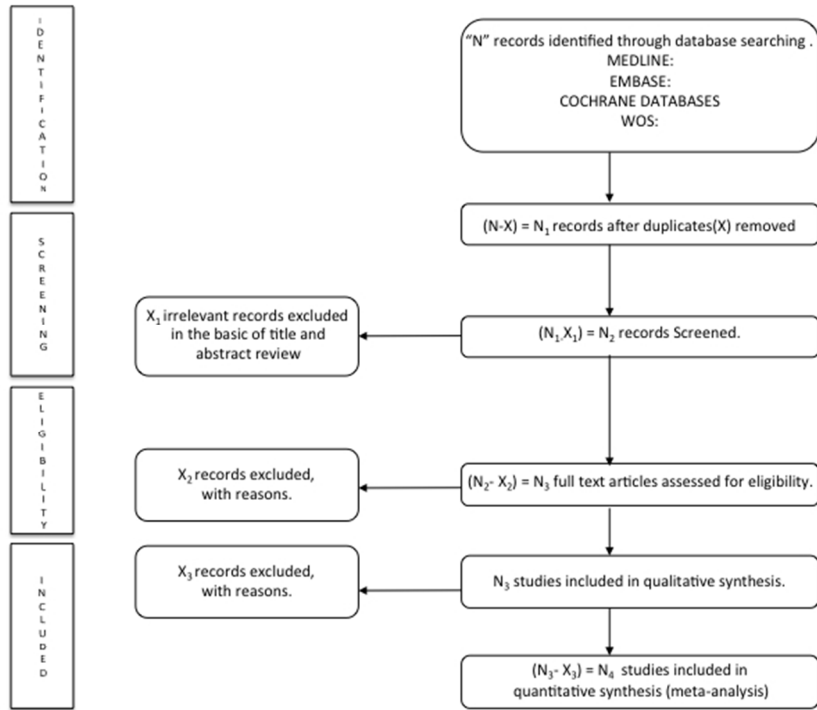


Figure 1. Literature search PRISMA consort diagram.  
254x190mm (72 x 72 DPI)

**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol\***

Section and topic	Item No	Checklist item	Page number
<b>ADMINISTRATIVE INFORMATION</b>			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Page 1; line 1-2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	NA
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Page 1; line 3
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Page 1; line 4-11
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 8; line 217-221
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	NA
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Page 8; line 222-223
Sponsor	5b	Provide name for the review funder and/or sponsor	NA
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	NA
<b>INTRODUCTION</b>			
Rationale	6	Describe the rationale for the review in the context of what is already known	Page 3-4; line 63-94
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 4; line 96-101
<b>METHODS</b>			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 5; line 124-136
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other	Page 4-5; line



**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol\***

		grey literature sources) with planned dates of coverage	111-123 Figure 1
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	Table 1
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 5-6; line 136-150
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 5-6; line 136-150
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 5-6; line 136-150
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 5-6; line 136-150 Table 2
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Table 2
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	Page 6; line 151-161
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Page 6-7; line 162-182
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as $I^2$ , Kendall's $\tau$ )	Page 6-7; line 162-182
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 7; line 183-193
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 6; line 163-167
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Page 7; line 181-182
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 7; line 183-184

\* It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.

**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol\***

*From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.*

For peer review only

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# BMJ Open

## Glycosylated Haemoglobin as a Predictor of Cardiovascular Events and Mortality: A Protocol for a Systematic Review and Meta-Analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-012229.R1
Article Type:	Protocol
Date Submitted by the Author:	13-Jun-2016
Complete List of Authors:	Cavero-Redondo, Iván; Universidad de Castilla-La Mancha, Health and Social Research Center Peleteiro, Barbara; University of Porto, EPIUnit - Institute of Public Health Álvarez-Bueno, Celia; Universidad de Castilla-La Mancha, Health and Social Research Center Rodríguez-Artalejo, Fernando; Universidad Autónoma de Madrid, Department of Preventive Medicine and Public Health Martínez-Vizcaino, Vicente; Universidad de Castilla-La Mancha, Centro de Estudios Sociosanitarios
<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Cardiovascular medicine, Diabetes and endocrinology, Public health
Keywords:	mortality, glycated haemoglobin, cardiovascular disease

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3 1 **Glycosylated Haemoglobin as a Predictor of Cardiovascular Events and Mortality:**  
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5 2 **A Protocol for a Systematic Review and Meta-Analysis**  
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8 3 **Systematic review registration:** PROSPERO CRD42015032552  
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10 4 Cavero-Redondo I<sup>1</sup>, Peleteiro B<sup>2</sup>, Álvarez-Bueno C<sup>1</sup>, Rodríguez-Artalejo F<sup>3</sup>, Martínez-  
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## 26 ABSTRACT

27 **Introduction:** The glycosylated haemoglobin level (HbA1c) is an indicator of the  
28 average blood glucose concentrations over the preceding 2-3 months, which is used as a  
29 convenient and well-known biomarker in clinical practice. Currently, epidemiological  
30 evidence suggests that the HbA1c level is an independent risk factor for cardiovascular  
31 events such as myocardial infarction, stroke, coronary heart disease, and heart failure.  
32 This protocol aim to conduct systematic review and meta-analysis to determine the  
33 relationships between the HbA1c levels with cardiovascular outcomes and cause of  
34 death; and to analyse the range of HbA1c that is a predictor of cardiovascular disease  
35 and/or mortality based on data from published observational studies.

36 **Methods and analysis:** The search will be conducted using the MEDLINE, EMBASE,  
37 Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic  
38 Reviews, and Web of Science databases from their inception. Observational studies  
39 written in Portuguese, Spanish or English will be included. The Quality In Prognosis  
40 Studies tool will be used to assess the risk of bias for the studies included in the  
41 systematic review or meta-analysis. The hazard ratios for the cardiovascular outcomes  
42 and causes of death with 95% confidence intervals will be determined as the primary  
43 outcomes. Subgroup analyses will be performed based on the cardiovascular outcomes,  
44 the cause of death studied, or the type of population included in the studies.

45 **Ethics and dissemination:** This systematic review will synthesise evidence regarding  
46 the potential of using the HbA1c level as a prognostic marker for cardiovascular disease  
47 outcomes and/or mortality. The results will be disseminated by the publication of a  
48 manuscript in a peer-reviewed journal. Ethical approval will not be needed because the  
49 data used for this systematic review will be obtained from published studies and there  
50 will not be any concerns about privacy.

51 **Systematic review registration:** PROSPERO CRD42015032552

### 52 **Strengths and limitations of this study**

- 53 - This review of evidence will be useful to improve future research on HbA1c  
54 level as a prognostic marker for cardiovascular disease outcomes and/or  
55 mortality.

- 1  
2  
3 56 - Study selection, data extraction and quality assessment will be performed  
4 57 independently by two researchers.  
5  
6 58 - Limitations and strengths will be discussed in our review, and the results will be  
7 59 put into context with other studies in the field.  
8  
9 60 - Different population-based studies can be a source of variable quality and  
10 61 heterogeneity between studies and may limit the quality of the evidence of this  
11 62 meta-analysis and systematic review.  
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## 64 INTRODUCTION

65 Cardiovascular disease (CVD) is a chronic disorder that develops insidiously  
66 throughout an individual's life and usually has progressed to an advanced stage by the  
67 time-symptoms occur<sup>1</sup>. The percentage of all deaths due to CVD before the age of 75  
68 years in Europe represents 42% in women and 38% in men<sup>2</sup>. Cardiovascular disease,  
69 especially coronary heart disease, is the leading cause of premature death worldwide.<sup>3</sup>

70 In 2007 was developed The Reynolds Risk Score for predicting CVD risk, which  
71 incorporates information on glycosylated haemoglobin (HbA1c), but this score was only  
72 used in people with known diabetes<sup>4</sup>. In 2010, the American College of Cardiology  
73 Foundation/American Heart Association Task Force on Practice Guidelines considered  
74 the HbA1c level as an appropriate index for CVD risk assessment in asymptomatic  
75 adults without a diagnosis of diabetes<sup>5</sup>. Finally, the Canadian Cardiovascular Society  
76 proposed that the CVD risk could be stratified by measuring the levels of fasting plasma  
77 glucose, HbA1c, or both<sup>6</sup>.

78 The HbA1c level is an indicator of the average blood glucose concentrations over the  
79 preceding two to three months, which is used as a convenient and well-known  
80 biomarker in clinical practice<sup>7-8</sup>. Epidemiological evidence suggests that the HbA1c  
81 level is an independent risk factor for cardiovascular events<sup>9</sup>. There is also evidence that  
82 the association between the HbA1C level with mortality from all-causes and CVD could  
83 be found at lower levels than the diabetic threshold<sup>10</sup>. A recent meta-analysis showed  
84 that HbA1c level was an independent predictor of mortality in coronary artery disease  
85 patients without but not in patients with established diabetes<sup>11</sup>.

86 Currently, the association between chronic hyperglycaemia and cardiovascular  
87 complications is not well defined. Several observational studies have demonstrated that

1  
2  
3 88 a higher HbA1c level was associated with increased risks of CVD and death<sup>9, 12-13</sup>.  
4  
5 89 Thus, an elevated HbA1c level might contribute to the development of CVD, but the  
6  
7 90 association between the HbA1c level with the risk of CVD and mortality in the general  
8  
9 91 population remains unclear. Therefore, this protocol aims to present a clear and  
10  
11 92 transparent procedure for systematically review, evaluate and summarize the existing  
12  
13 93 information on the relationship between the HbA1c levels and CVD and death, which  
14  
15 94 could guide clinical decision making for further treatment strategies and also could  
16  
17 95 inform and facilitate future intervention research.  
18

## 19 97 **OBJECTIVE**

20  
21 98 The aim of this protocol study is to establish a transparent and clear methodology to  
22  
23 99 conduct a systematic review and meta-analysis aimed to: i) determine the relationship  
24  
25 100 between the HbA1c levels with the cause of death and cardiovascular outcomes based  
26  
27 101 on data from observational studies, and ii) analyse what level of HbA1c is a predictor of  
28  
29 102 CVD and/or mortality.  
30

31 103

## 32 104 **METHODS AND ANALYSIS**

### 33 105 **Review design**

34  
35 106 This protocol was developed based on the Preferred Reporting Items for Systematic  
36  
37 107 Review and Meta-analysis Protocols (PRISMA-P)<sup>14</sup> and was registered with  
38  
39 108 PROSPERO (Registration number: CRD42015032552). The MOOSE<sup>15</sup> (Meta-analysis  
40  
41 109 of observational studies in epidemiology: a proposal for reporting), PRISMA<sup>16</sup>  
42  
43 110 (Preferred Reporting Items for Systematic Reviews) and Cochrane Collaboration  
44  
45 111 Handbook<sup>17</sup> will be used to guide the review methods.  
46  
47

### 48 112 **Literature review**

49  
50 113 The literature search will be conducted using the MEDLINE (via PubMed), EMBASE,  
51  
52 114 the Cochrane Central Register of Controlled Trials, the Cochrane Database of  
53  
54 115 Systematic Reviews, and the Web of Science databases from the date of their inception  
55  
56 116 until August 2016. Study records will be managed by means of the Mendeley reference  
57  
58 117 manager (Mendeley Ltd. 2016, United Kingdom).  
59  
60



118

119 The following search terms will be combined using Boolean operators: glycosylated  
120 hemoglobin, HbA1c, hemoglobin levels, glycated hemoglobin, hemoglobin A1c,  
121 cardiovascular, cardiovascular disease, coronary heart disease, heart failure, stroke,  
122 peripheral arterial disease, cardiovascular events, coronary artery disease, myocardial  
123 infarction, cardiovascular outcomes, mortality, all-cause mortality, cardiovascular  
124 mortality, cause-specific mortality, death, cardiovascular death, observational study,  
125 cohort study and population-based (Table 1).

126 Previous systematic reviews and meta-analysis, and relevant references included in the  
127 selected studies will be screened as supplemental sources.

### 128 **Inclusion/exclusion criteria for study selection**

129 Studies regarding on the HbA1c level and cardiovascular outcomes retrieved in the  
130 literature search that meet the following criteria will be included: i) prospective or  
131 retrospective observational studies; ii) studies that observed the following  
132 cardiovascular outcomes: myocardial infarction, stroke, major adverse cardiovascular  
133 events (MACE), coronary heart disease, and heart failure; iii) reports of all-cause  
134 mortality and/or cardiovascular mortality; iv) outcomes measured using univariate and  
135 multivariable Cox proportional hazards models; v) population of adults aged 18 or older  
136 with any restriction on the race, gender or diabetic status; and vi) studies published in  
137 Portuguese, Spanish or English.

138 The process; of identifying, screening of studies and inclusion or exclusion of those  
139 studies; is shown in the PRISMA flow chart (Figure 1).

### 140 **Study selection and data extraction**

141 Two reviewers will independently check titles and abstracts to identify eligible studies  
142 according to the inclusion criteria. Then, the full manuscripts of the identified studies  
143 will be examined. Finally, two reviewers will check the included and excluded studies  
144 and verify the reasons why they were included/excluded. Any discrepancies will be  
145 resolved by discussion, a third reviewer will be asked on case of disagreement.

146 Two authors will independently extract the data regarding the author information, year  
147 of publication, design of the study, country, study project name and year of data  
148 collection, number, age of participants, population characteristics (diabetic or non-

1  
2  
3 149 diabetic), methods used for HbA1c test certified by National Glycohemoglobin  
4  
5 150 Standardization Program (NGSP), number of cardiovascular events, level of HbA1c  
6  
7 151 used as the reference, and the hazard ratio (HR) for each HbA1c level (Table 2)

8  
9 152 Any disagreement will be resolved by discussion to reach a consensus. When necessary,  
10  
11 153 authors of the potential included studies will be contacted to obtain any missing  
12  
13 154 information.

#### 14 155 **Assessment of the risk of bias in the included studies**

15  
16  
17 156 After blinding the included studies by author, title and year of publication, two  
18  
19 157 independent researchers to assess the methodological quality will by the Quality in  
20  
21 158 Prognosis Studies tool (QUIPS)<sup>18</sup>. Any disagreement in the assessment of the risk of  
22  
23 159 bias will be discussed to reach a consensus. A third reviewer will make the final  
24  
25 160 decision if a consensus is not reached. The QUIPS tool involves the use of 6 domains  
26  
27 161 for the risk of bias: study participation (sampling bias), study attrition (attrition bias),  
28  
29 162 prognostic factor measurement, outcome measurement (ascertainment bias),  
30  
31 163 confounding measurement and accounting, and analysis and reporting. Studies will be  
32  
33 164 considered to have a low, moderate or high risk of bias, satisfied by scores of 5 to 6, 3  
34  
35 165 to 4, or 1 to 2 for the 6 bias domains, respectively.

#### 36 166 **Statistical analysis**

37  
38 167 The researchers will create tables to summarize the characteristics of the included  
39  
40 168 studies and any important questions related to the aim of this systematic review. The  
41  
42 169 reviewers will determine whether a meta-analysis is possible after the data have been  
43  
44 170 extracted. At least, five observations addressing HR for cardiovascular outcomes and  
45  
46 171 mortality will be required to conduct a meta-analysis. If it is possible to carry out a  
47  
48 172 meta-analysis, the STATA 14 software will be used to combine the extracted HR with  
49  
50 173 95% CIs using an inverse variance model. We will compare adjusted and unadjusted  
51  
52 174 estimates separately for each outcome. A fixed-effects model will be used if there is no  
53  
54 175 evidence of heterogeneity; otherwise, a random-effects model will be used<sup>19</sup>. For the  
55  
56 176 HbA1c levels, we will group studies by similar cut-off points to obtain meta-analysis  
57  
58 177 results for each cut-off point whenever possible. We will used generalized least squares  
59  
60 178 regression models to assess the pooled dose-response relation between HbA1c and CVD  
179  
180 179 outcomes across prospective cohort studies that have heterogeneous categorizations of  
HbA1c<sup>20</sup>. Each meta-analysis will be summarized by the pooled HR and 95%

1  
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3 181 confidence intervals. Studies providing insufficient data to perform the analyses will be  
4 182 omitted from the data synthesis. The heterogeneity of the studies will be assessed with  
5 183 an  $I^2$  statistic. Usually,  $I^2$  values of <25, 25-50, and >50% are considered to represent  
6 184 small, medium, and large amounts of heterogeneity, respectively<sup>21</sup>. If a meta-analysis is  
7 185 not possible, we will undertake a narrative synthesis. Finally, publication bias was  
8 186 visually evaluated using a funnel plot, as well as with the method proposed by Egger<sup>22</sup>.  
9  
10 187 The strength of the body evidence will be evaluated by Grading of Recommendations  
11 188 Assessment, Development and Evaluation tool (GRADE)<sup>23</sup>.

### 17 189 *Subgroup analyses and meta-regression*

19 190 Subgroup analyses and meta-regression will be performed based on the cardiovascular  
20 191 outcomes (myocardial infarction, stroke, MACE, coronary heart disease, heart failure),  
21 192 cause of death studied (all causes of mortality or cardiovascular mortality), on the type  
22 193 of population included in the studies (diabetic, pre-diabetic or non-diabetic), or on the  
23 194 age of the study participants (young adults aged 18–35 years, middle-aged adults aged  
24 195 36–55 years, or older adults aged older than 55 years), because these may be major  
25 196 factors causing heterogeneity. Furthermore, design of the study and QUIPS score will  
26 197 be considered for additional subgroup analyses.

### 33 198 *Sensitivity analysis*

35 199 Excluding the included studies from the analysis one by one and comparing the results  
36 200 will perform sensitivity analyses.

## 39 201 **DISCUSSION**

42 202 The utility of the HbA1c level as a prognostic marker for CVD outcomes and/or  
43 203 mortality is currently a source of controversy in the medical literature. Therefore, we  
44 204 will conduct a systematic review to identify what HbA1c level might be able to predict  
45 205 the CVD outcomes and mortality.

49 206 There is currently no consensus on what percentages should be used to determine the  
50 207 level of heterogeneity in categorical terms. Therefore, in this study, we will use the  
51 208 definition suggested by Higgins et al. to indicate that there is heterogeneity when the  $I^2$   
52 209 value is greater than 50%<sup>21</sup>.

56 210 Possible limitations that can be found in this research are: publication bias, information  
57 211 bias, poor statistical analyses, and inadequate reporting of methods and findings of the

primary studies<sup>24</sup>. However, it is important to summarize the information available on this issue. To overcome these limitations, we will follow the recommendations included in the MOOSE, PRISMA and Cochrane Collaboration Handbook. According to the Cochrane Prognosis Methods Group, we will use the QUIPS tool to assess the quality of the included studies<sup>18</sup>.

There have already been numerous studies on the use of the HbA1c level as a prognostic marker for CVD outcomes and mortality, but the individual studies have been controversial, so there is uncertainty regarding its use. This makes it necessary to conduct this systematic review to provide a global overview of the current literature and to improve future research on this topic. It is therefore this protocol provide a clear and structured procedure for maximizing the extraction of relevant information and provide summarized information regarding the importance of HbA1c levels for controlling the risk of CVD outcomes and mortality.

**Authors' contributions:** VMV and ICR designed the study. VMV was the principal investigator and guarantor. ICR and VMV were the main coordinators of the study. BP, CAB, FRA, and VMV conducted the study. ICR, BP and FRA gave statistical and epidemiological support. ICR wrote the article with the support of CAB and ICR. All authors revised and approved the final version of the manuscript.

**Funding statement:** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests:** None declared

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**Table 1.** Search strategy for MEDLINE

		cardiovascular		
		OR		
		“cardiovascular disease”		
		OR		
		“coronary heart disease”		
		OR		
		“heart failure”		
		OR		
		Stroke		
		OR		
		“peripheral arterial disease”		
		OR		
“glycosylated haemoglobin”		“cardiovascular events”		“observational study”
OR		OR		OR
“HbA1c”	AND	“coronary artery disease”	AND	“cohort study”
OR		OR		OR
“hemoglobin levels”		“myocardial infarction”		“population-based
OR		OR		
“glycated haemoglobin”		“cardiovascular outcomes”		
OR		OR		
“hemoglobin A1c”		mortality		
		OR		
		“all-cause mortality”		
		OR		
		“cardiovascular mortality”		
		OR		
		“cause-specific mortality”		
		OR		
		death		
		OR		
		“cardiovascular death”		



**Table 2.** Characteristics of studies included in the systematic review and/or meta-analysis.

Reference	Design	Country	Study/Year data collection	Age	n	n cardiovascular events	HbA1c method	HbA1c reference level	HR for Hba1c levels
<i>Author information of the and year of study publication</i>	<i>Design of the study</i>	<i>Country</i>	<i>Study project name and year of data collection</i>	<i>Age of participants</i>	<i>Number of participants</i>	<i>Number of cardiovascular events</i>	<i>Methods used for HbA1c test certified by NGSP</i>	<i>Level of HbA1c used as the reference</i>	<i>HR for each HbA1c level</i>

Hba1c: glycosilated haemoglobin; NGSP: National Glycohemoglobin Standardization Program; HR: Hazard ratio

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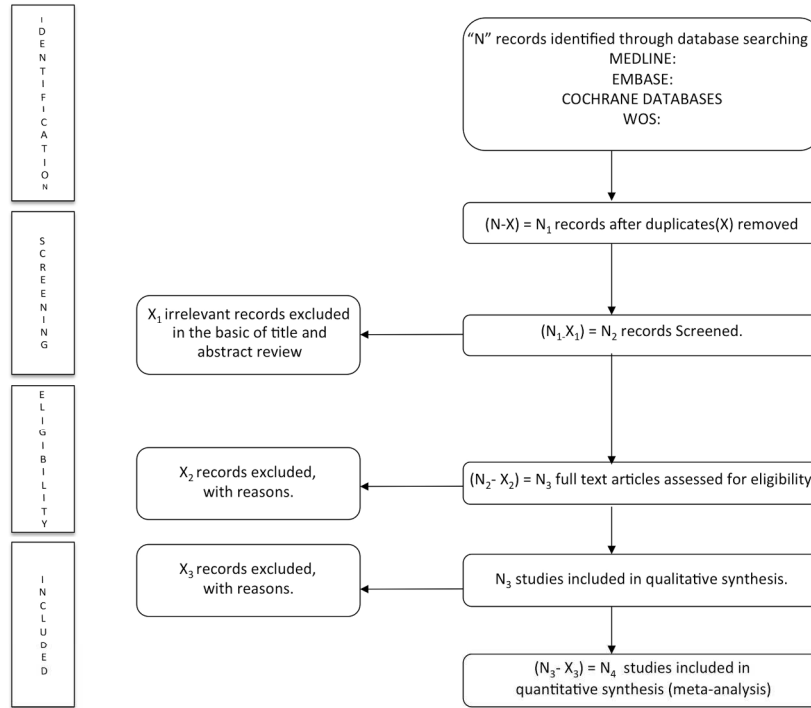


Figure 1. Literature search PRISMA consort diagram.  
299x225mm (300 x 300 DPI)

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**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol\***

Section and topic	Item No	Checklist item	Page number
<b>ADMINISTRATIVE INFORMATION</b>			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Page 1; line 1-2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	NA
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Page 1; line 3
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Page 1; line 4-11
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Page 8; line 217-221
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	NA
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Page 8; line 222-223
Sponsor	5b	Provide name for the review funder and/or sponsor	NA
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	NA
<b>INTRODUCTION</b>			
Rationale	6	Describe the rationale for the review in the context of what is already known	Page 3-4; line 63-94
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 4; line 96-101
<b>METHODS</b>			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 5; line 124-136
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other	Page 4-5; line

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**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol\***

		grey literature sources) with planned dates of coverage	111-123 Figure 1
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	Table 1
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 5-6; line 136-150
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 5-6; line 136-150
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 5-6; line 136-150
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 5-6; line 136-150 Table 2
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Table 2
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	Page 6; line 151-161
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Page 6-7; line 162-182
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as $I^2$ , Kendall's $\tau$ )	Page 6-7; line 162-182
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Page 7; line 183-193
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 6; line 163-167
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Page 7; line 181-182
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Page 7; line 183-184

**\* It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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**PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol\***

*From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.*

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