eAppendix 1: Systematic Review Protocol

What is the prognostic value of cholesterol measurements, i.e. total cholesterol, HDL-, LDL-cholesterol, ratio total cholesterol/HDL-cholesterol, and triglycerides, in blood of adult patients with amyotrophic lateral sclerosis?

A SYSTEMATIC REVIEW

PROTOCOL

Organization, City, Country:	University of Utrecht, Department of Neurology, ALS Center, UMC Utrecht Hersencentrum, Utrecht, The Netherlands
Prepared by:	Mark Janse van Mantgem, M.D.
Date:	April 2020
Senior supervisor:	Prof. dr. Leonard van den Berg
Project lead:	Prof. dr. Leonard van den Berg
Research team members:	Mark Janse van Mantgem, M.D. and Ruben van Eijk, M.D., PhD.
Advisory group:	Viyanti Orie and Eva de Boer

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1.0 Background

Amyotrophic lateral sclerosis (ALS), also known as 'Lou Gehrig's disease,' is a neurodegenerative disorder of the upper motor neuron (UMN) and the lower motor neuron (LMN). A disease with an incidence of 2.0 to 3.0 per 100.000 person-years in Europe with a peak incidence between 50 and 70 years old (Logroscino et al., 2010). With a ratio of 1.5:1, the incidence and prevalence are greater in man than in women. Depending on the site of symptom onset, defined as spinal, bulbar, respiratory or generalized onset, first symptoms are mainly muscle weakness, fasciculations, dysphagia, weight loss (Janse van Mantgem et al., 2020), and speech/voice changes. However, ALS is a heterogeneous disease with a broad range of symptoms.

The complete pathophysiology has not been clarified. This means ALS is nowadays a fatal disease with a life expectancy of 3 to 5 years after diagnosis (Salameh et al., 2015). Researchers are focusing on finding a cure, but also on predicting survival more accurate. Recently, a prognostic tool has been validated to predict survival in individual patients with ALS (Westeneng et al., 2018). This tool is based on eight clinical predictors of survival in ALS. Besides the prognostic value of these eight clinical factors, other factors might play a crucial role in predicting survival in patients with ALS.

ALS is increasingly recognized as a systemic disease, instead of a pure neurological disease (Bäumer et al., 2014). Growing evidence has been found in an abnormal metabolic state of patients with ALS, because it seems to underpin disease prognosis and progression. Therefore, several predictors related to the metabolic and hormonal state have been described. A hypermetabolic state (Bouteloup et al., 2009; Steyn et al., 2018), lower BMI during the life course (Peter et al., 2017), weight loss at time of diagnosis (Janse van Mantgem et al., 2020, increased creatinine values (Van Eijk et al., 2018), reduced glucose intolerance, and increased values of the cholesterol/lipid spectrum (Ingre et al., 2020) are described as unfavorable predictors. The prognostic value of increased serum cholesterol remains unclear, because studies have different results and, therefore, conclusions. Because lipids are an important source of energy for muscles, the hypothesis arise that high values of serum cholesterol might indicate neuron loss. As neurons die, which might be due to oxidative stress, cholesterol is released. Elevated levels of cholesterol might be, therefore, a biomarker of neurodegeneration (Ingre et al., 2020). The opposite hypothesis also exists: a neuroprotective role of high cholesterol levels (Dorst et al., 2011; Dupuis et al., 2008).

Recently, an overview has been published in Neurology (Ingre et al., 2020) of several studies that have looked at the prognostic value of the cholesterol spectrum on survival in patients with ALS. However, this overview was not systematically performed, and no hazard ratios were presented. Therefore, an overview of all available literature on the predictive value of cholesterol measurements on survival will be helpful. We are pleased to write an updated and more convenient systematic review.

2.0 Objective

To overview available literature on the prognostic value of cholesterol measurements in blood, i.e. total cholesterol, HDL-, LDL-cholesterol, and triglycerides, in adult patients with amyotrophic lateral sclerosis.

3.0 Review Question

The PICOS for this systematic review is presented below.

Population (P)	Intervention (I) (as predictor)	Comparison (C)	Outcome (O)	Studies (S)
Adult patients with	Abnormal values of cholesterol	Cholesterol measurements	Survival rate	- Observational/longitudinal studies

amyotrophic lateral sclerosis (ALS)	measurements	within normal ranges		- Randomized controlled trials
				15-04-2020

3.1. Search terms

Terms are carefully chosen and discussed with the outreach librarian at the UMC Utrecht hospital, Utrecht, The Netherlands. Relevant prognostic factors were chosen.

1. “Amyotrophic lateral sclerosis”[Mesh]
2. “Amyotrophic Lateral Sclerosis”*ti,ab
3. ALS.ti,ab
4. “Motor Neuron Disease”[Mesh]
5. MND.ti,ab
6. Neuron.ti,ab
7. Gehrig*.ti,ab
8. (1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7)

9. “Cholesterol”[Mesh]
10. “Cholesterol, LDL”[Mesh]
11. “Cholesterol, HDL”[Mesh]
12. “Triglycerides”[Mesh]
13. Cholesterol*.ti,ab
14. LDL*.ti,ab
15. HDL*.ti,ab
16. Triglyceride*.ti,ab
17. Lipid*.ti,ab
18. (9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17)

19. “Prognosis”[Mesh]
20. Prognos*.ti,ab
21. “Survival”[Mesh]
22. Survival*.ti,ab
23. “Mortality”[Mesh]
24. Mortalit*.ti,ab
25. “Kaplan-Meijer Estimate”[Mesh]
26. Kaplan*.ti,ab
27. “Proportional Hazard Models”[Mesh]
27. Cox.ti,ab
28. (19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27)
29. 8 AND 18 AND 28

Example Pubmed search:
(“Amyotrophic Lateral Sclerosis”[Mesh] OR “Amyotrophic Lateral Sclerosis”[tiab] OR ALS[tiab] OR “Motor Neuron Disease”[Mesh] OR MND[tiab] OR Neuron[tiab] OR Gehrig*[tiab]) AND (“Cholesterol”[Mesh] OR “Cholesterol, LDL”[Mesh] OR “Cholesterol, HDL”[Mesh] OR

"Triglycerides"[Mesh] OR Cholesterol*[tiab] OR LDL*[tiab] OR HDL*[tiab] OR Triglyceride*[tiab] OR Lipid*[tiab]) AND ("Prognosis"[Mesh] OR Prognos*[tiab] OR "Survival"[Mesh] OR Survival*[tiab] OR "Mortality"[Mesh] OR Mortalit*[tiab] OR "Kaplan-Meier Estimate"[Mesh] OR Kaplan*[tiab] OR "Proportional Hazards Models"[Mesh] OR Cox[tiab])

4.0 Evidence gathering and study selection

We will perform our search to the following search engines:

	Evidence based databases	Other resources
	Cochrane Library	Hand-searching
	The Database of Abstracts of Reviews of Effects (DARE)	
	PubMed	
	EMBASE	
	Medline	

4.1 Eligibility criteria

After gathering the evidence, the following eligibility criteria will be applied to the results and all identified references screened independently by two reviewers (Mark Janse van Mantgem and Ruben van Eijk).

4.1.1 Types of studies

- Longitudinal studies:
  - Prospective studies
  - Retrospective studies
  - Cohort studies
  - Case-control studies
- Randomized controlled trials where two (or more) interventions are compared with a positive effect on our research question.
- Studies without follow-up time (i.e. cross-sectional) will not be selected.

4.1.2 Types of participants

This literature review will include all studies which have recruited adult patients with ALS. The diagnosis 'amyotrophic lateral sclerosis' has to be officially diagnosed by a neurologist according to the El Escorial criteria.

4.1.3 Types of intervention and comparison

There are no interventions and comparisons involved in this literature review.

4.1.4 Type of outcome measures

The outcome of interest is the survival rate, preferably defined in months. The survival rate is defined as the percentage of ALS patients still alive after the time of diagnosis.

4.2 Inclusion criteria

Studies are included if they fulfil the following criteria:

1. Participants have been officially diagnosed with ALS according to the El Escorial criteria;
2. Studies have to be performed on human beings.
3. Determination of cholesterol values in blood of adult patients with ALS at or after their date of diagnosis;
4. At least one of the following cholesterol measurements have to be determined:
    a. Total cholesterol
    b. HDL-cholesterol
    c. LDL-cholesterol
    d. Triglycerides
    e. Ratio total cholesterol/HDL-cholesterol
5. Articles has to present original researches only
6. Written in English or Dutch;
7. Measured survival time.

4.3 Exclusion criteria

Studies are excluded if they fulfil the following criteria:

1. Grey literature, incomplete articles, such as: no full text available, posters, commentaries, hypothesis articles, not peer reviewed. If necessary we will get in touch with the authors.
2. Studies who performed a systematic review only.
3. If no information is given on the diagnostic procedure of ALS.
4. If patients <18 years old were included.
5. Studies who do not report survival time will be excluded (e.g. solely evaluated rate of ALSFRS-R decline and/or forced vital capacity (FVC)).
6. Studies with subjects other than human beings will be excluded.

5.0 Analysis

5.1 Study extraction

The search results will be initially selected based on the title and abstract by two reviewers (Mark Janse van Mantgem and Ruben van Eijk), working separately using the above mentioned in- and exclusion criteria to select articles. Any potential disagreement will be resolved by further discussion and if necessary by consulting our advisory group.

The final selected articles will be read in full. The first 5 articles will be read and data extracted by both reviewers. After the first 10 articles the remaining articles will be divided over both reviewers in randomized order.

The articles information will be imported in Mendeley and/or EndNote, where the study and data extraction will be performed. Which reference manager is used, is carefully balanced beforehand.

5.2 Data extraction

5.2.1 Design

Data will be extracted from the included articles. A standard sheet will be provided to note all data. Data of interest will be described in section 5.2.2.
All articles found with our search terms will be coded in the following format: ALS/SR/000. 'ALS' stands for Amyotrophic Lateral Sclerosis, 'SR' for Systematic Review, followed by a 3 digit number.

5.2.2 Content

We have made a list of information requirements that is necessary for our data extraction:

- **General information**. Title of study, author, type of publication, country of origin, source of funding, researcher performing data extraction, date of data extraction. Authors will be contacted if necessary.
- **Study characteristics**. Aim/objectives of the study, study design, in- and exclusion criteria, number of participants. If multiple articles from the same study team are available, we will include the latest published article, unless the quality of the latest article is less than the previous one.
- **Participants**. Age at onset and diagnosis, gender, site of symptom onset, El Escorial criterium, forced vital capacity, disease duration when blood is drawn, ALSFRS-R score when blood is drawn, presence of genetic mutations related to ALS, and presence of frontotemporal dementia (FTD).
- **Intervention**. Description of measured cholesterol values, i.e. total cholesterol, HDL-, LDL-cholesterol, ratio total cholesterol/HDL- cholesterol, and triglycerides. Cut-off scores of normal/abnormal ranges.
- **Comparison**. Description of measured cholesterol values, i.e. total cholesterol, HDL-, LDL-cholesterol, ratio total cholesterol/HDL-cholesterol, and triglycerides. Cut-off scores of normal/abnormal ranges.
- **Outcome.** Survival time, hazard ratio.

5.3 Meta-analysis

A meta-analysis will be considered. We expect a lot of heterogeneity between the studies, so we will assess whether a meta-analysis will produce a good overview.

6.0 Quality assessment

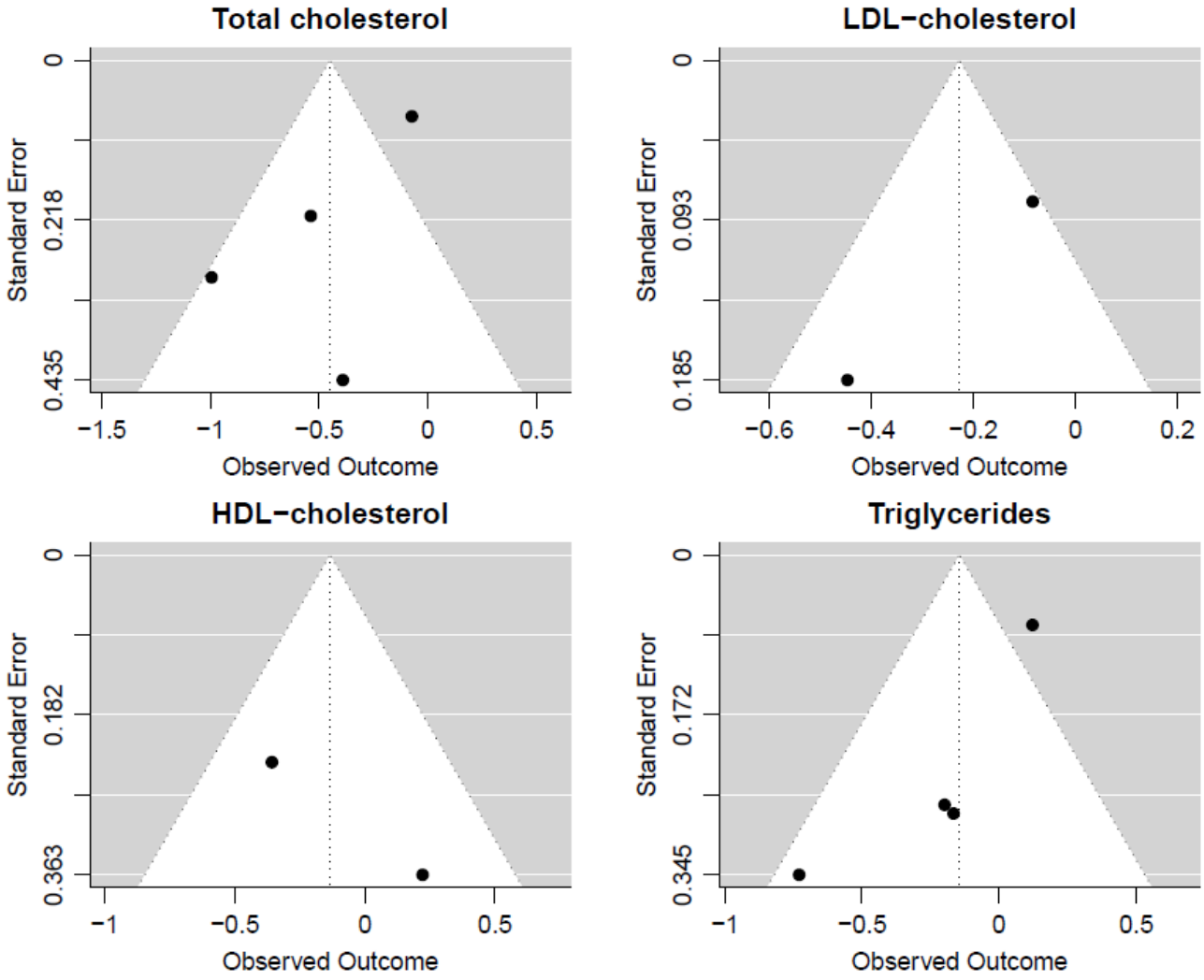
The QUIPS (Quality in Prognosis Studies tool) will be used to evaluate the quality and bias of our included articles. See Appendix A.

APPENDIX A: Quality Assessment

Domains	Prompting items for Consideration	Ratings
Study Participation	a. Adequate participation in the study by eligible persons b. Description of the source population or population of interest c. Description of the baseline study sample d. Adequate description of the sampling frame and recruitment e. Adequate description of the period and place of recruitment f. Adequate description of inclusion and exclusion criteria	**High bias:** The relationship between the PF and outcome is very likely to be different for participants and eligible nonparticipants **Moderate bias**: The relationship between the PF and outcome may be different for participants and eligible nonparticipants **Low bias**: The relationship between the PF and outcome is unlikely to be different for participants and eligible nonparticipants
Study Attrition	a. Adequate response rate for study participants b. Description of attempts to collect information on participants who dropped out c. Reasons for loss to follow-up are provided d. Adequate description of participants lost to follow-up e. There are no important differences between participants who completed the study and those who did not	**High bias**: The relationship between the PF and outcome is very likely to be different for completing and non-completing participants **Moderate bias**: The relationship between the PF and outcome may be different for completing and non-completing participants **Low bias**: The relationship between the PF and outcome is unlikely to be different for completing and non-completing participants
Prognostic Factor Measurement	a. A clear definition or description of the PF is provided b. Method of PF measurement is adequately valid and reliable c. Continuous variables are reported or appropriate cut points are used d. The method and setting of measurement of PF is the same for all study participants e. Adequate proportion of the study sample has complete data for the PF f. Appropriate methods of imputation are used	**High bias**: The measurement of the PF is very likely to be different for different levels of the outcome of interest **Moderate bias**: The measurement of the PF may be different for different levels of the outcome of interest **Low bias**: The measurement of the PF is unlikely to be different for different levels of the outcome of interest

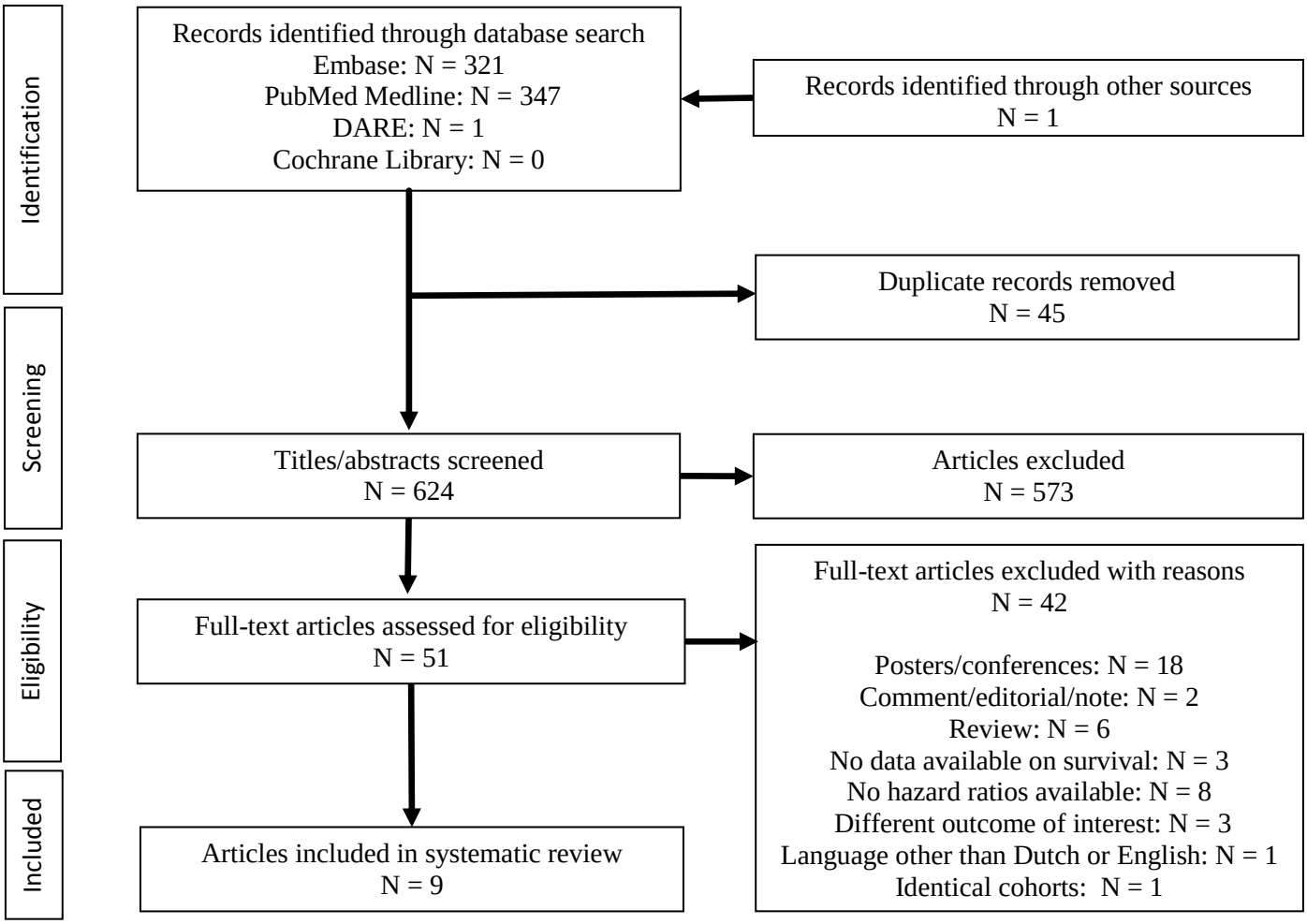
https://static-content.springer.com/esm/art%3A10.1186%2F1546-0096-12-19/MediaObjects/12969_2014_1722_MOESM1_ESM.pdf

eFigure 1. Publication bias and study heterogeneity



Funnel plots show the publication bias assessment for each cholesterol variable.

eFigure 2. Flowchart of the selection process



eFigure 3. Risk of bias in included studies.

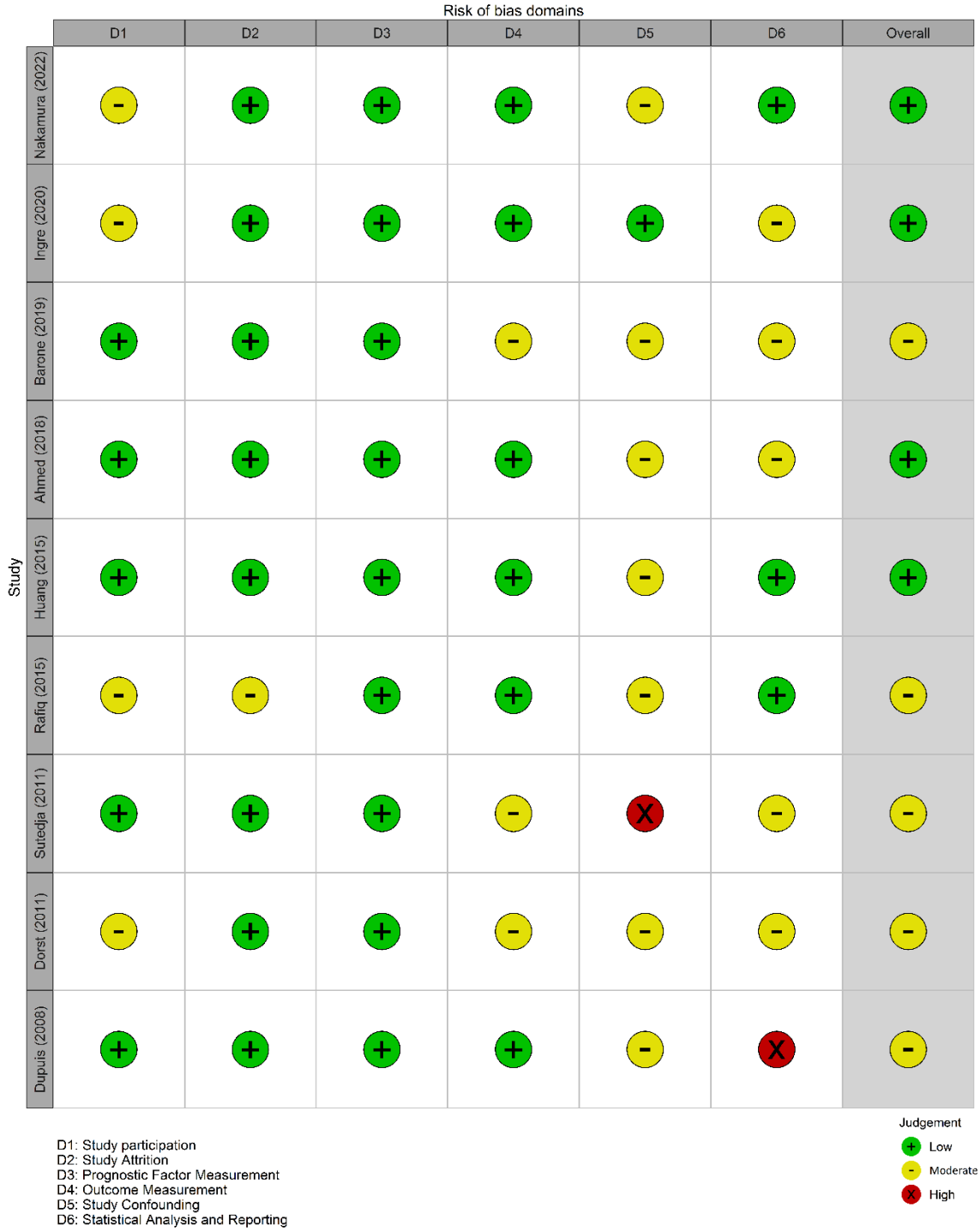


Figure provides an overview of the risk of bias using the QUIPS tool.[17] Green ('+' sign) represents low bias, yellow ('-' sign) represents moderate bias, and red ('X' sign) represents high bias. The rows reflect the eight included studies in chronological order by publication date.