SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Experimental design in methotrexate efficacy studies for rheumatoid arthritis	
2.	Authors (names, affiliations, contributions)	CHC Leenaars DH de Jong T Coenen FR Stafleu RBM de Vries M Ritskes-Hoitinga	
3.	Other contributors (names, affiliations, contributions)	Reumazorg Nederland HU Schrerer JB Prins FLB Meijboom	
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5.	Funding sources/sponsors	NWO	
6.	Conflicts of interest	-	
7.	Date and location of protocol registration	19-02-2016 SYRCLE website	
8.	Registration number (if applicable)	-	
9.	Stage of review at time of registration	Planned	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	When developing new medication for rheumatoid arthritis (RA), complementary animal models are used to predict clinical effects. These models cause substantial discomfort to the experimental animals. It is unknown how effective the animal models are in predicting clinical efficacy. The clinical trials and animal studies for the classical disease-modifying anti-rheumatic drugs (DMARDs) are accepted as a viable research tool. Methotrexate is a widely used DMARD. Presently a clear overview of the used experimental designs and the differences between designs in clinical trials and animal studies is lacking. It will be provided by this SR.	
	Research question		
11.	Specify the disease/health problem of interest	Rheumatoid arthritis (RA)	
12.	Specify the population/species studied	Animals (including humans)	
13.	Specify the intervention/exposure	Methotrexate	
14.	Specify the control population	Untreated, placebo or other control	
15.	Specify the outcome measures	Any	
16.	State your research question (based on items 11-15)	1.) Are the experimental designs of the pre-clinical animal studies comparable with those of the	

		clinical trials? 2.) Are the improvements (in swelling, pain, bone-and cartilage damage) found in RA animal models comparable with the improvements found in patients?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	X MEDLINE via PubMed ☐ Web of Science ☐ SCOPUS X EMBASE ☐ Other, namely: ☐ Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	Search strategy provided below.	
19.	Identify other sources for study identification	X Reference lists of included studies ☐ Books X Reference lists of relevant reviews ☐ Conference proceedings, namely: ☐ Contacting authors/ organisations, namely: ☐ Other, namely:	
20.	Define search strategy for these other sources	Titles in reference lists of included studies and retrieved reviews will be screened according to the inclusion and exclusion criteria specified below.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	Phase 1: screening based on title and abstract Phase 2: full-text screening of the eligible articles	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Phase 1: 2 → Discrepancies will be resolved by a third reviewer. Phase 2: 2 → Discrepancies will be resolved by a third reviewer.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: • Any full paper describing an efficacy study to methotrexate. Exclusion criteria: • Study not addressing rheumatoid arthritis • No methotrexate • Not an efficacy study • Abstracts (without a full description of materials and methods) • Not a primary study/no new data	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: • Any animal (including humans) Exclusion criteria: other type of study.	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Methotrexate Exclusion criteria: Not methotrexate	
26.	Outcome measures	Inclusion criteria: Any	

		Exclusion criteria:	
27.	Language restrictions	Inclusion criteria: Any language Exclusion criteria: -	
28.	Publication date restrictions	Inclusion criteria: Any date Exclusion criteria: -	
29.	Other	Inclusion criteria:- Exclusion criteria:-	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase 1: 1.Not rheumatoid arthritis 2. Not an animal model or a clinical trial 3. No methotrexate 4. Not an efficacy study Selection phase 2: 1. Not rheumatoid arthritis 2. Not an animal model or a clinical trial 3. No methotrexate 4. Not efficacy 5. Not a primary study/no new data	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	• 1st author • Year • Title • Journal • Language	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Animal studies: • Number of animals • Control group • Laboratory temperature • Laboratory humidity • Laboratory lighting regime • Habituation period (after arrival) • Handling (Y/N/?) • Cage size • Number of animals per cage • Randomisation • Blinding • Power calculations • Comorbidities Human studies: • Number of patients • Diagnostic criteria (used for inclusion) • Control group • Time zone • Geographic location • Randomisation • Blinding • Power calculations • Number of centres • Patient population	

		• Disease status • Treatment status • Comorbidities • Comedication	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	• Animal • Strain, substrain • Line • Sex • Age (/ weight) • Disease model	

34.	Intervention characteristics (*e.g.* intervention, timing, duration)	• Method of model induction +
		  o Time of model induction
		  o administration route
		  o Dose
		  o Frequency
		• Type of treatment
		• Time of treatment
		• Administration route	

35.	Outcome measures	Any	
36.	Other (*e.g.* drop-outs)	% survival, humane endpoints, drop-out (+ reason)	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	2	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	X By use of SYRCLE's Risk of Bias tool[4] ☐ By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐ By use of CAMARADES' study quality checklist, e.g [22] ☐ By use of CAMARADES' study quality checklist, adapted as follows: X Other criteria, namely: **Cochrane risk of bias tool**	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Any as provided	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1. Data extraction from test, tables, and figures 2. In case of graphic data digital image software will be used to obtain these data.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	A random sample of at least 5% of the included papers will be checked by an independent observer for accuracy of data-extraction.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Descriptive summary + tabulation	

43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	For the following outcome measures a meta-analysis is considered: Degree of inflammation, cartilage-and bone destruction, mobility and anti-citruline data. Meta-analysis will be performed if at least 3 informative human and 3 informative animal papers on the outcome measures are retrieved by our searches.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	(Standardized) mean difference	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Considered: Species and model type	
48.	Any sensitivity analyses you propose to perform	-	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	-	
50.	The method for assessment of publication bias	Funnel plots	
Final approval by (names, affiliations): DH de Jong, CHC Leenaars, RBM de Vries (Syrcle)		Date:	

Search strategies Pubmed:

Rheumatoid arthritis:

Arthritis, Rheumatoid [MeSH] OR Rheumatoid Arthritis [tiab] OR (Rheumatoid [tiab] AND Nodul* [tiab]) OR (Rheumatoid [tiab] AND Vasculiti* [tiab]) OR Arthritis, Experimental [MeSH] OR RA model* [tiab] OR rheumatic arthritis [tiab] OR

((Collagen-Induced Arthritides [tiab] OR Collagen-Induced Arthritis [tiab] OR (Arthritides [tiab] AND collagen [tiab]) OR (arthritis[tiab] AND (collagen[tiab] OR (Collagen [tiab] AND antibody [tiab]AND induced [tiab] AND arthritis [tiab]) OR collagens[tiab] OR adjuvant*[tiab] OR experimental[tiab])) OR Arthritides [tiab] OR Collagen type II [MeSH] OR (Type II [tiab] AND (Collagen [tiab] OR Procollagen [tiab] OR Col2a1 [tiab] OR chondrocalcin [tiab])) OR
CIA [tiab] OR Proteoglycans [Mesh] OR Proteoglycans [tiab] OR Proteoglycan [tiab] OR PGIA [tiab] OR HSPG [tiab] OR Proteoheparan Sulfate* [tiab] OR glypican* [tiab] OR syndecan* [tiab] OR CD138 Antigens [tiab] OR CD138 Antigen [tiab] OR Fibroglycan [tiab] OR Ryudocan [tiab] OR Amphiglycan [tiab] OR Proteochondroitin Sulfate [tiab] OR Proteochondroitin Sulfates [tiab] OR Aggrecans [tiab] OR Aggrecan [tiab] OR Versicans [tiab] OR Versican [tiab] OR Biglycan [tiab] OR Decorin [tiab] OR DSPG-II [tiab] OR Hyalectins [tiab] OR Brevican [tiab] OR Neurocan [tiab] OR Lectins, C-Type [tiab] OR Nerve Tissue Proteins [tiab] OR Citrulline [MeSH] OR Citrul* [tiab] OR Freund's Adjuvant [MeSH] OR adjuvant [tiab] OR Freund* [tiab]) OR Mycobacterium tuberculosis

[MeSH] OR Mycobacterium tuberculosis [tiab] OR Mycobacterium butyricum [tiab] OR Antigen induced arthritis [tiab] OR AIA [tiab] OR (Streptococcal [tiab] AND induced [tiab] AND arthritis [tiab]) OR SCW-A [tiab] OR CAIA [tiab] OR K/BxN model [tiab] OR G6PI-induced arthritis [tiab] OR SKG [tiab] OR TNF transgenic [tiab] OR gp130 arthritis model [tiab] OR IL-1 transgenic [tiab] OR pristane induced arthritis [tiab] OR PIA [tiab] OR oil induced arthritis [tiab] OR OIA [tiab]) AND
(RA [tiab] OR rheumatism [tiab]))

Human
clinical study [pt] OR clinical trial [MeSH] OR clinical trial [tiab] OR intervention study [tiab] OR controlled clinical trial [MeSH] OR clinical trial as topic [MeSH] OR first in man [tiab] OR proof of concept [tiab] OR randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab]

Animal: standard Syrcle animal filter

Methotrexate
methotrexate [MeSH] OR methotrexate [tiab] OR MTX [tiab] OR Ametopterine [tiab] OR Mexate [tiab] OR Abitrexate [tiab] OR Emtexate [tiab] OR Emthexate [tiab] OR Farmitrexate [tiab] OR Folex [tiab] OR Ledertrexate [tiab] OR Methoblastin [tiab] OR Methohexate [tiab] OR Methotrate [tiab] OR Methylaminopterin [tiab] OR Methotrexate [tiab] OR Novatrex [tiab] OR Rheumatrex [tiab] OR metoject [tiab] OR maxtrex [tiab]

Search strategies Embase:

Rheumatoid arthitis
Rheumatoid arthritis/ OR rheumatoid arthritis.ti,ab,kw. OR

((Type II and (Collagen OR Procollagen or Col2a1 OR chondrocalcin)).ti,ab. OR exp experimental arthritis/ OR exp adjuvant arthritis/ OR exp collagen type 2/ OR exp proteoglycan/ OR exp proteoheparan sulfate/ OR exp aggrecan/ OR exp citrulline/ OR exp freund adjuvant/ OR Glypican$2.ti,ab,kw. OR Syndecan$2.ti,ab,kw. OR CD138 Antigens.ti,ab,kw. OR CD138 Antigen.ti,ab,kw. OR Heparan Sulfate$.ti,ab,kw. OR Chondroitin Sulfate Proteoglycans.ti,ab,kw. OR Chondroitin Sulfate Proteoglycan.ti,ab,kw. OR Proteochondroitin Sulfate.ti,ab,kw. OR Proteochondroitin Sulfates.ti,ab,kw. OR DSPG-II.ti,ab,kw. OR Lectins, C-Type.ti,ab,kw. OR Nerve Tissue Proteins.ti,ab,kw. OR Citrul$.ti,ab,kw. OR adjuvant induced arthritis.ti,ab,kw. OR Mycobacterium tuberculosis.ti,ab,kw. OR Mycobacterium tuberculosis H37Rv.ti,ab,kw. OR Mycobacterium butyricum.ti,ab,kw. OR Antigen induced arthritis.ti,ab,kw. OR AIA.ti,ab,kw. OR (Streptococcal AND induced AND arthritis).ti,ab,kw. OR SCW-A.ti,ab,kw. OR (Collagen AND antibody AND induced AND arthritis).ti,ab,kw. OR BxN model.ti,ab,kw. OR G6PI-induced arthritis.ti,ab,kw. OR TNF transgenic.ti,ab,kw. OR gp130 arthritis model.ti,ab,kw. OR IL-1 transgenic.ti,ab,kw. OR pristane induced arthritis.ti,ab,kw. OR oil induced arthritis.ti,ab,kw. OR ((CIA OR PGIA OR HSPG OR Glypicans OR Glypican OR Syndecans OR Syndecan OR Fibroglycan OR Ryudocan OR Amphiglycan OR Aggrecan OR Aggrecans OR Versicans OR Versican OR Biglycan OR Decorin OR Hyalectins OR Brevican OR neurocan OR SKG OR PIA OR OIA OR CAIA).ti,ab,kw.) AND
(RA.ti,ab,kw. OR rheumatism.ti,ab,kw.))

Human
exp clinical trial/ OR clinical study/ OR human subject.ti,ab,kw. OR clinical drug trial.ti,ab,kw. OR major clinical trial.ti,ab,kw. OR trial, clinical.ti,ab,kw. OR clinical study.ti,ab,kw. OR phase 1 clinical trial.ti,ab,kw. OR phase 2 clinical trial.ti,ab,kw. OR phase 3 clinical trial.ti,ab,kw. OR clinical trial, controlled.ti,ab,kw. OR clinical trial, phase 1.ti,ab,kw. OR clinical trial, phase 2.ti,ab,kw. OR clinical trial, phase 3.ti,ab,kw. OR clinical trials.ti,ab,kw. OR clinical trial, phase I.ti,ab,kw. OR clinical trial, phase II.ti,ab,kw. OR clinical trial, phase III.ti,ab,kw. OR intervention study.ti,ab,kw.

Animal: standard Syrcle animal filter

Methotrexate:
methotrexate/ OR methotrexate.ti,ab,kw. OR MTX.ti,ab,kw. OR Ametopterine.ti,ab,kw. OR Mexate.ti,ab,kw. OR Abitrexate.ti,ab,kw. OR Emtexate.ti,ab,kw. OR emthexate.ti,ab,kw. OR Farmitrexate.ti,ab,kw. OR Folex.ti,ab,kw. OR Ledertrexate.ti,ab,kw. OR Methoblastin.ti,ab,kw. OR Methohexate.ti,ab,kw. OR Methotrate.ti,ab,kw. OR Methylaminopterin.ti,ab,kw. OR Methotrexate.ti,ab,kw. OR Novatrex.ti,ab,kw. OR Rheumatrex.ti,ab,kw. OR metoject.ti,ab,kw. OR maxtrex.ti,ab,kw.