SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/ item	Description	Check for approval
	General		
1.	Title of the review	A Systematic Review of the Modifying Effect of Anaesthetic Drugs on Metastasis in Animal Models for Cancer	
2.	Authors (names, affiliations, contributions)	Carlijn R Hooijmans [12]; designing and performing research, analysing data, writing paper Merel Ritskes-Hoitinga [1]; designing research, writing paper Gert-Jan Scheffer [2]; designing research, writing paper Florentine J Geessink [12]; performing research: data extraction, Quality assessment Departments of [1]SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) [2] Anesthesiology, [3] Medical Library, Radboud UMC Nijmegen, The Netherlands.	
3.	Other contributors (names, affiliations, contributions)	Alice Tillema [3], search strategy design Moira Bruintjes [12]; performing research; In- and exclusion Marleen Egberink [12]; performing research; in and exclusion and data extraction Sandra de Groot [2] ; performing research ; data extraction and quality assessment Marieke Schouten [2]; performing research ; data extraction and quality assessment	
4.	Contact person + e-mail address	Carlijn R Hooijmans; Carlijn.Hooijmans@radboudumc.nl	
5.	Date of protocol registration		
	Background		
6.	What is already known about this disease/ model/ intervention? Why is it important to do this review?	Despite the progress made in cancer treatment, distant metastasis or local recurrence after primary tumour resection remain a major clinical problem. As a consequence, a lot of research concentrates on exploring factors that might influence the metastatic process. One of the factors that has been suggested to influence tumour reoccurrence or metastasis is the anaesthetic technique applied during or immediately after oncologic surgery. Many published clinical studies are retrospective and suffer from confounding, most studies investigate combinations of anaesthetic and analgesic drugs, which makes it a challenge to isolate the contribution of a specific drug. Large-scale RCTs are needed to prove a causal link between anaesthetic techniques and metastasis. Some multicentre trials have been launched, but while we await their results, we could further evaluate this link in animal studies We will conduct the first SR and MA of the effect of anaesthetic drugs on metastasis in experimental cancer models. We will provide: 1) a complete and systematic overview of all animal studies on this topic; 2) insight into	

		the efficacy of anaesthetic techniques overall and in subgroups; and 3) an overview of various factors that modify the efficacy of in experimental cancer models	
	Objectives of this SR		
7.	Specify the disease / health problem of interest	Metastasis/ metastatic spread in experimental cancer	
8.	Specify the population /species studied	All species	
9.	Specify the intervention/exposure	Anesthetic drugs (used in the clinical setting)	
10.	Specify the control population	No anesthetic drugs (placebo or sham or no intervention)	
11.	Specify the outcome measures	1) Number of metastasis 2) Metastasis incidence	
12.	State your research question (based on points 7-11)	Does analgesic treatment reduce the number or incidence of metastasis in experimental cancer	
	Methods:		
	Search and study identification		
13.	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:	
14.	Define electronic search strategies (*e.g.* use the step by step search guide [1] and animal search filters [2, 3])	When available, please add a supplementary file containing your search strategy: see *supplementary file*	
15.	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:	
16.	Define search strategy for these other sources	Screening the reference lists for relevant titles and screening the abstracts of these relevant titles	
	Study selection phases		
17.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1) screening based on title and abstract 2) full-text screening of the eligible articles	
18.	Specify number of reviewers per screening phase	Each phase: 2 independent observers per article. Phase 1 : CH and MB screen all papers. Phase 2: CH and ME screen all papers. Differences will be solved through discussion or by consulting a fourth investigator	
	Study selection criteria. Define all inclusion and exclusion criteria based on:		
19.	Type of study (design)	Inclusion criteria: Comparison of anaesthetic drug used in clinical practice versus no anaesthetic drug on number of metastasis or metastasis incidence in animals with experimental cancer Exclusion criteria: Co-interventions/ contamination	
20.	Type of animals/ population (*e.g.* age, gender, disease model)	Inclusion criteria: animals with experimental cancer in which metastasis can develop Exclusion criteria: Co-morbidities, ex vivo, in vitro, in silico, experimental cancer without metastasis.	

21.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: anaesthetic drug used Exclusion criteria: anesthetic drugs not used in the clinical setting	
22.	Outcome measures	Inclusion criteria: number of metastasis or metastasis incidence Exclusion criteria: weight of metastasis, surface covered with metastasis, number of occupied bones, number of invading cells	
23.	Language restrictions	Inclusion criteria: all languages Exclusion criteria: none	
24.	Publication date restrictions	Inclusion criteria: all publication dates Exclusion criteria: none	
25.	Other	Inclusion criteria: Exclusion criteria: Reviews or non original papers	
26.	Sort and prioritize your exclusion criteria per selection phase	Selection phase 1: 1. Review 2. Human study 3. Not in vivo 4. No metastases/ only primary tumor 5. No control group 6. Combination therapy or contamination 7. Not about anaesthetics used in the clinic Selection phase 2: 1. Review 2. Human study 3. Not in vivo 4. No metastases/ only primary tumor 5. No control group 6. Combination therapy or contamination 7. Not about analgesics used in the clinic 8. No relevant outcome measure	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
27.	Study ID (*e.g.* authors, year)	Authors, title, year, language, contact author e-mail	
28.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of animals in experimental and control groups, presence of control group.	
29.	Animal model characteristics (*e.g.* species, gender, disease induction)	Animal species, strain, age or weight, gender, cancer model (transgenic or induced), type of cells/ drugs used to induce cancer, type of cancer, amount of cells, location of injection of tumor cells, type of anesthetics used to create model.	
30.	Intervention characteristics (*e.g.* intervention, timing, duration)	Type of drugs, Route of administration, dose, frequency, timing relative to tumor cell injection, duration of treatment, type of control group	
31.	Outcome measures	Number of metastasis, incidence of metastasis	
32.	Other (*e.g.* drop-outs)	Age of sacrificing animals, anesthetics used for sacrificing, region of metastasis count	
	Risk of bias assessment (internal validity)		
33.	Specify the number of reviewers assessing the risk of bias in each study	2	

34.	Define criteria to assess the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias)	☐By use of SYRCLE's Risk of Bias tool [4] X By use of SYRCLE's Risk of Bias tool, adapted as follows: addition of 2 reporting items; 1) reporting of randomisation at any level 2) reporting of blinding at any level. ☐By use of CAMARADES' study quality checklist, e.g. [5] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other, namely:
	Collection of outcome data	
35.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/ dichotomous, unit of measurement)	Number of metastasis: continuous Incidence of metastasis: Continuous (% or number of animals in control and experimental group with metastasis)
36.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	First extraction from graphs using universal desktop ruler software (http://avpsoft.com/products/udruler/) by two independent reviewers. If data could not be extracted from text or figures authors will be contacted via e-mail (max. 3 e-mails).
	Data analysis/synthesis. Specify (per outcome measure):	
37.	How you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Meta-analysis with subgroup analysis and sensitivity analysis for all outcome measures
38.	How the decision as to whether a meta-analysis will be performed will be made	A minimum of 2 articles per outcome measure is required No restrictions in terms of heterogeneity will be applied, instead, sources of heterogeneity will be investigated through sensitivity and subgroup analysis.
	If a meta-analysis seems feasible/sensible, specify for each outcome measure:	
39.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Number of metastases: SMD Incidence of metastasis: RR
40.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model
41.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2
42.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Anesthetic technique Type of drug (volatile, barbiturates, ketamin, propofol) Species Gender Region of metastasis Timing and duration of treatment ** Subgroup analyses are only performed when a minimum of 3 studies or 5 independent comparisons are available*
43.	The method for assessment of publication bias	Funnel plots, performing Duval and Tweedie's trim and fill analysis
44.	Any sensitivity analyses you propose to perform	Impact of single or multiple use of anaesthetic drug treatment;

		impact of excluding other species than rodents; impact of recalculating median and ranges into means and SDs	
Final approval by (names, affiliations):	Carlijn Hooijmans		Date: 01-09-2014